1148–1154 *Nucleic Acids Research, 1997, Vol. 25, No. 6*

Distribution of both lengths and 5′ terminal nucleotides of mammalian pre-tRNA 3′ trailers reflects properties of 3′ processing endoribonuclease

Masayuki Nashimoto*

Life Science Research Laboratory, Japan Tobacco Inc., 6-2 Umegaoka, Aoba-ku, Yokohama, Kanagawa 227, Japan

Received December 16, 1996; Revised and Accepted January 24, 1997

ABSTRACT

Mammalian tRNA 3′ processing endoribonuclease (3′ tRNase) removes 3′ extra nucleotides after the discriminator from tRNA precursors. Here I examined how the length of a 3′ trailer and the nucleotides on each side of the cleavage site affected 3′ processing efficiency. I performed *in vitro* 3′ processing reactions of pre-tRNA^{Arg}s with various 3′ trailers or various discriminator nucleotides using 3′ tRNase purified from mouse FM3A cells or pig liver. On the whole, the efficiency of pre- tRNA^{Arg} 3′ processing by mammalian 3′ tRNase decreased as the 3′ trailer became longer, except in the case of a 3′ trailer composed of CC, CCA or CCA plus 1 or 2 nucleotides, which was not able to be removed at all. The distribution of 3′ trailer lengths deduced from mammalian nuclear tRNA genomic sequences reflects this property of 3′ tRNase. The cleavage efficiency of pre-tRNA^{Arg}s varied depending on the 5′ end nucleotide of a 3′ trailer in the order G ~ A > U > C. This effect of the 5′ end nucleotide was independent of the discriminator nucleotides. The distribution of the 5′ end nucleotides of mammalian pre-tRNA 3′ trailers reflects this differential 3′ processing efficiency.

INTRODUCTION

Eukaryotic tRNAs transcribed as larger precursors must be processed through a series of steps to yield functional mature molecules. These processing reactions include removal of extra 5′ and 3′ sequences, the addition of CCA to the 3′ terminus, nucleotide modifications at specific residues, and in a subset of the tRNA gene transcripts, RNA splicing (1,2). Recently, the editing of tRNA has also been found in mitochondria of various species (3).

The 5′ processing event in eukaryotic cells that generates the correct 5′ ends of mature tRNAs is carried out by an endoribonuclease similar to the well-studied *Escherichia coli* RNase P (4). On the other hand, the 3′ processing in eukaryotes is essentially different from prokaryotic 3′ processing in which the 3′ extra residues following the CCA sequence of a tRNA precursor are removed (1). 3′ extra sequences, after the discriminator nucleotides, of eukaryotic tRNA precursors must be removed precisely prior to the addition of the 3′ terminal CCA sequence, because eukaryotic tRNA genes do not encode the CCA residues (1). A general 3′ processing model for *E.coli* tRNA precursors is proposed, in which the mature 3′ terminus is generated by the first endonucleolytic cleavage in the 3′ trailer, followed by at least two exonucleolytic trimming steps (5). Six exoribonucleases, RNase II, RNase D, RNase BN, RNase T, RNase PH and RNPase, implicated in the tRNA 3′ processing have been identified in *E.coli* by both biochemical and genetic studies (5).

There seems to be no general model for eukaryotic 3′ processing because the mode of processing nucleases varies in different systems. It has been demonstrated that 3′ processing is achieved by the action of an endoribonuclease in many studies using *in vitro* 3′ processing systems with cell extracts from human (6), pig (7), *Xenopus laevis* (8), *Drosophila* (9,10) and wheat (11); mitochondrial extracts from human (12), rat (13) and yeast (14); and chloroplast extracts from spinach (11). On the other hand, both endonuclease and exonuclease activities that can act on the 3′ termini of artificial tRNA precursors have been shown in germinal vesicle extracts from *X.laevis* (15). Extracts from yeast nuclei and the silk gland of *Bombyx mori* contain a 3′→5′ exonuclease that removes the 3′ trailer of precursor tRNA (16,17). Despite these studies on many species, only two reports have described the purification of 3′ processing endoribonucleases, which cleave precursor tRNAs after the discriminator nucleotide. A 3′ processing endonuclease that accurately processes the 3′ terminus of human pre-tRNA^{Met} has been purified from *X.laevis* ovaries (8). It appears to function as a single polypeptide of ~97 kDa. Recently, I have purified 3′ tRNase from pig liver, which appears to function as a dimer of ~45 kDa protein(s) (7).

Major determinants for substrate recognition by 3′ tRNase probably reside in the mature tRNA domain which forms the well-conserved L-shape, because neither the sequences nor the structures of 3′ trailers are conserved. This is supported by the following two reports. In an *in vitro Drosophila* system, more than half the pre-tRNA^{His-48} variants containing a single mutation in secondary or tertiary base-pairs were 3′-processed less efficiently than the wild type (10). In a two half-tRNA mammalian system, pre-tRNA^{Arg} variants with base changes in the T stem–loop region were cleaved by 3′ tRNase less efficiently

*Correspondence should be addressed to present address: Department of Chemistry and Biochemistry, Brigham Young University, C100 Benson Science Building, PO Box 25700, Provo, UT 84602-5700, USA. Tel: +1 801 378 4845; Fax: +1 801 378 5474; Email: mnashimoto@chemgate.byu.edu

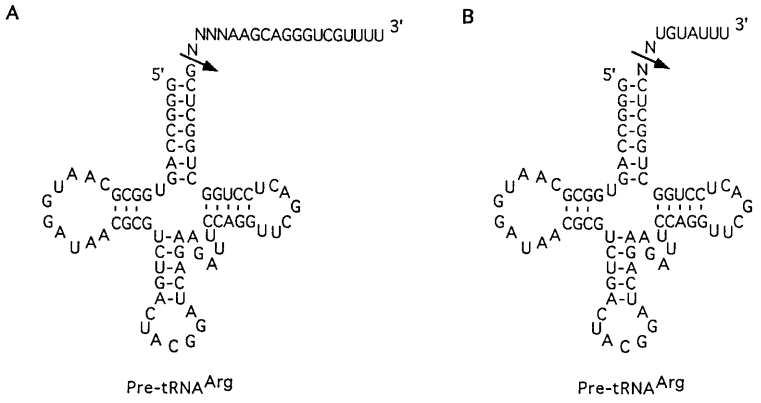

Figure 1. Secondary structures of human pre-tRNA^{Arg}s with various 3′ trailers. The pre-tRNA^{Arg}s were synthesized *in vitro* with T7 RNA polymerase and used as RNA substrates for 3′ tRNase. Ns represent varied bases; arrows the 3′ processing site. (**A**) The pre-tRNA^{Arg}s used for assays shown in Tables 1 and 2. In some substrates, 3′ residues are deleted. (**B**) The pre-tRNA^{Arg}s used for tests shown in Figures 5 and 6.

than the wild type, and extensive deletions of the T stem–loop and extra loop regions abolished the substrate activity (18).

Here I investigated interactions between 3′ tRNase and 3′ trailers plus discriminator nucleotides. To see what effect 3′ trailer length and the nucleotides on both sides of the cleavage site had on 3′ processing efficiency, I performed *in vitro* 3′ processing reactions of pre-tRNA^{Arg}s with various 3′ trailers or various discriminator nucleotides using 3′ tRNase purified from mouse FM3A cells or pig liver. From the results, I discovered that both the length and the 5′ end nucleotide of pre-tRNA 3′ trailers affect the 3′ processing efficiency.

MATERIALS AND METHODS

Precursor tRNAs

Wild type human pre-tRNA^{Arg}, which is 5′-processed and has a 19 nt 3′ trailer (7), and its variants were synthesized *in vitro* with T7 RNA polymerase (Takara Shuzo) from synthetic double-stranded DNA templates containing the T7 promoter. The transcription reactions were performed in the presence or absence of [α-^{32}P]UTP (Amersham Japan) under the conditions specified by the manufacturer (Takara Shuzo). The transcripts were purified by denaturing polyacrylamide gel electrophoresis.

Preparation of mouse 3′ tRNase

Mouse FM3A cells were cultured on a large scale (a total of 192 l) in ES medium (Nissui) containing 3% fetal calf serum and harvested at a density of 5×10^5 cells/ml. Cytosolic S100 extracts were prepared according to Nashimoto (19). 3′ tRNase was purified from the S100 extracts basically in the same way as it was from pig liver (7). Briefly, after precipitation of the extracts with ammonium sulfate (50% saturation) and subsequent 55°C treatment, the sample was fractionated by a series of column chromatographies with Q Sepharose Fast Flow, Blue Sepharose (twice), Heparin Sepharose and Mono Q (Pharmacia). The purified 3′ tRNase was aliquoted and frozen at –80°C.

tRNA 3′ processing assay

The pre-tRNA 3′ processing reaction was performed at 37°C for the indicated time in a reaction mixture (10 µl) containing 10 mM Tris–HCl, pH 7.5, 1 mM dithiothreitol, 3.2 mM spermidine, 0.1 pmol of pre-tRNA^{Arg} and 0.2 U of 3′ tRNase purified from mouse FM3A cells or 0.4 U of 3′ tRNase purified from pig liver (7). One unit of the enzyme is defined as the amount which converts 50% of R-G19 (0.1 pmol) to the 3′ processed product in 10 min under the above conditions (7). After incubation, the processing products were resolved on a 10% polyacrylamide–8 M urea gel, and quantitated with a Bio-Image Analyzer BA100 (FUJIX). In some cases, the gel was also autoradiographed.

RNA sequencing

The 73 nt processing product of a cold pre-tRNA^{Arg}, R-G19, was 3′-end-labeled with [5′-^{32}P]pCp by T4 RNA ligase (Takara Shuzo), and gel-purified (20). The ^{32}P-labeled product was subjected to chemical RNA sequencing reactions (21) and resolved on a 20% polyacrylamide–8 M urea gel to determine the 3′ terminal sequence.

Kinetic analysis

3′ processing of pre-tRNAs by 3′ tRNase was examined at various concentrations of substrate to obtain kinetic parameters. A reaction mixture (6 µl) contained 10 mM Tris–HCl (pH 7.5), 0.5 mM dithiothreitol, 3.2 mM spermidine, 0.017–2.5 µM ^{32}P-labeled pre-tRNA, and pig 3′ tRNase fraction (0.2 ng) after Mono Q column chromatography (7). After incubation at 37°C for 1 min, the reaction products were resolved on a 10% polyacrylamide–8 M urea gel and then quantitated with a Molecular Imager (BioRad). Values of K_m and V_{max} were obtained from Lineweaver–Burk plots.

Analysis of tRNA genomic sequences

Mammalian nuclear tRNA genomic sequences were obtained from the GenBank/EMBL data base by using a computer program, Findget (N. Fujita, National Institute of Genetics, Mishima). The locations of tRNA genes were identified from the GenBank/EMBL features table. Pre-tRNAs terminate with a uridine stretch of various lengths, such as UU, UUU or UUUU (22,23). Therefore, the length of a pre-tRNA 3′ trailer was defined as the distance from the end of a tRNA gene to the third thymidine (including this nucleoside) in the four or more

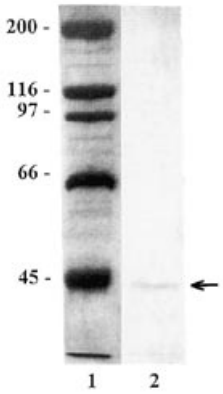

Figure 2. 3′ tRNase from mouse FM3A cells. The fraction after Mono Q column chromatography (lane 2) was electrophoresed on an SDS–7.5% polyacrylamide gel, and stained with Coomassie brilliant blue R-250. Size markers (lane 1) are indicated in kilodaltons. Arrow denotes the 45 kDa protein.

thymidine stretch. Transcription termination also occurs at anomalous signals, TTCTT, GTCTT or ATCTT (24). In this case the 3′ trailer length was defined as the distance from the end of a tRNA gene to the fourth nucleotide of such a signal. Rat mitochondrial tRNA genomic sequences were likewise obtained from the data base; and human, bovine and mouse mitochondrial tRNA genomic sequences were from published sources (25–27).

RESULTS

Length of 3′ trailers affects 3′ processing efficiency

To analyze the influence of the length of 3′ trailers of pre-tRNAs on 3′ processing efficiency, I synthesized human pre-tRNA^{Arg} (R-G19) with a 19 nt 3′ trailer (7) and its six derivatives (R-G15, R-G13, R-G10, R-G8, R-G3 and R-G1) with shorter 3′ trailers using an *in vitro* T7 transcription system (Fig. 1A and Table 1). The tRNA 3′ processing reaction was performed with a mouse 3′ tRNase fraction after Mono Q column chromatography. A protein of ~45 kDa was detected in this fraction on an SDS–polyacrylamide gel (Fig. 2). The size of this mouse enzyme was the same as that of the pig enzyme, though the pig enzyme fraction contained two 45 kDa proteins (7). Figure 3A shows cleavage of those pre-tRNAs. On the whole, the cleavage efficiency increased as the 3′ trailers became shorter (Table 1 and Fig. 4A). Kinetic parameters were also determined using 3′ tRNase purified from pig liver. The values of V_{max}/K_m increased with the decrease in length of the 3′ trailers (Table 1).

The 3′ terminal sequence of an ~73 nt processing product of R-G19 was determined by the chemical RNA sequencing method (21). The sequence indicated that the cleavage occurred after the discriminator (Fig. 3C). The cleavage site of the other pre-tRNA^{Arg}s studied in this paper was determined on a sequencing gel with the 73 nt product of R-G19 as a reference standard (data not shown). In four cases, cleavage was detected both after the discriminator and after the nucleotide 3′ of the discriminator (Table 1). The data presented for those substrates are for total cleavage. Although multi-site cleavage has also been observed in the two half-tRNA system (18), the mechanism has not been elucidated.

I tested another pre-tRNA^{Arg} (R-CCA19) with a 19 nt 3′ trailer beginning with CCA that was substituted for GUG in R-G19, and its seven derivatives (R-CCA8, R-CCA6, R-CCA5, R-CCA4, R-CCA3, R-CC2 and R-C1) with various shorter 3′ trailers (Fig. 1A and Table 1) for 3′ processing efficiency. No 3′ processing was detected for the four pre-tRNAs R-CC2, R-CCA3, R-CCA4 and R-CCA5 with 3′ trailers of CC, CCA, CCAG and CCAGN (N represents a mixture of A, U, G and C), respectively (Table 1). Cleavage of R-CCA19, R-CCA8 and R-C1 was much less efficient than cleavage of the R-G series pre-tRNAs with 3′ trailers of the same length (Table 1 and Fig. 4A). Consequently, the dependence of the cleavage efficiency on the length of the 3′ trailers beginning with CCA was very different from the dependence with regard to the R-G series (Fig. 4A).

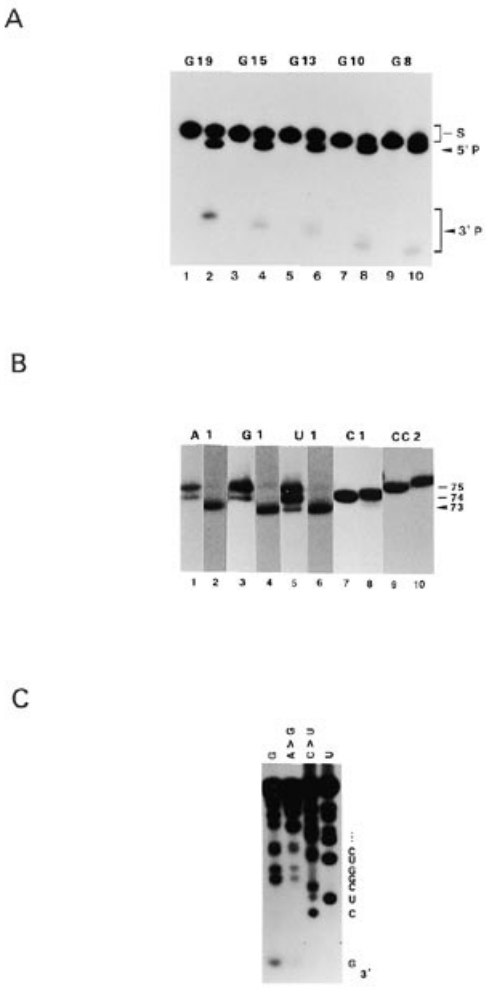

Figure 3. *In vitro* tRNA 3′ processing reactions by mouse 3′ tRNase and 5′ product analysis. (**A**) The 3′ processing reactions were performed under the standard conditions using the pre-tRNA^{Arg}s R-G19, R-G15, R-G13, R-G10 and R-G8. Input substrates (odd lanes) and reaction products (even lanes) were analyzed on a 10% polyacrylamide–8 M urea gel. S, substrates; 5′ P, 5′ products; 3′ P, 3′ products. (**B**) The 3′ processing reactions were performed under the standard conditions using the pre-tRNA^{Arg}s R-A1, R-G1, R-U1, R-C1 and R-CC2. Input substrates (odd lanes) and reaction products (even lanes) were analyzed on a 10% polyacrylamide–8 M urea gel. Bars and an arrowhead with a nucleotide length denote substrates and the 5′ product, respectively. R-A1, R-G1 and R-U1 contained 1 nt longer substrates (75 nt) produced by the addition of nontemplate-encoded nucleotides by T7 RNA polymerase (33), and R-U1 also contained a 1 nt shorter substrate (73 nt). (**C**) The 3′ terminal sequence of the 5′ product generated by the 3′ processing reaction of R-G19 was determined by a chemical RNA sequencing method (21).

Table 1. 3′ processing assays of pre-tRNA^{Arg} variants

Pre-tRNA	Sequence[a]	Cleavage[b] (%)	K_m[c] (μM)	V_{max}[c] (pmol/min)	V_{max}/K_m[c]
	5′ ↓ 3′				
R-G19	***CUCG GUGUAAGCAGGGUCGUUUU	31.2 ± 1.5	1.2	0.24	0.20
R-G15	***CUCG GUGUAAGCAGGGUCG	34.4 ± 1.2	1.0	0.25	0.25
R-G13	***CUCG GUGUAAGCAGGGU	38.0 ± 2.2	1.4	0.39	0.28
R-G10	***CUCG GUGUAAGCAG	64.3 ± 3.9	0.73	0.67	0.92
R-G8[d]	***CUCG GUGUAAGC	55.8 ± 4.3	0.78	0.95	1.2
R-G3	***CUCG GUG	73.7 ± 2.5	0.85	1.4	1.6
R-G1	***CUCG G	99.2 ± 0.6	–	–	–
R-CCA19	***CUCG CCAUAAGCAGGGUCGUUUU	10.1 ± 1.5	–	–	–
R-CCA8	***CUCG CCAUAAGC	19.2 ± 0.9	–	–	–
R-CCA6	***CUCG CCAUAA	11.5 ± 1.6	–	–	–
R-CCA5	***CUCG CCAGN	ND	–	–	–
R-CCA4	***CUCG CCAG	ND	–	–	–
R-CCA3	***CUCG CCA	ND	–	–	–
R-CC2	***CUCG CC	ND	–	–	–
R-C1	***CUCG C	7.5 ± 1.4	–	–	–

[a]An arrow denotes the 3′ processing site. The whole sequences are shown in Figure 1A.
[b]The assays were performed using mouse 3′ tRNase at 37°C for 30 min. Values are averages for three experiments. ND, not detected.
[c]The kinetic parameters were obtained from the assays using pig 3′ tRNase fraction (0.2 ng) after Mono Q column chromatography (7). Each measurement was from averages of three trials with a standard deviation of 5–10%.
[d]Cleavage was detected both after the discriminator (60%) and after the nucleotide 3′ of the discriminator (40%).

Length distribution of mammalian pre-tRNA 3′ trailers

To examine the biological significance of the dependence of the 3′ processing efficiency on the 3′ trailer length, 80 different mammalian nuclear tRNA genomic sequences were obtained from the GenBank/EMBL data base, and their 3′ trailer lengths were deduced from the position of transcription terminators of RNA polymerase III. A histogram of the 3′ trailer lengths is shown in Figure 4B. The peak is at 10–11 nt, and 72.5% of the tRNA genes have a 3′ trailer with a length from 8 to 15 nt. The length distribution patterns below and above 7 nt agree well with the graphic patterns of the cleavage efficiency of the R-CCA and R-G series, respectively (Fig. 4B). The implications of these apparent correlations are discussed below.

The 5′ end nucleotide of 3′ trailers also determines 3′ processing efficiency

The above experimental results indicated that 3′ processing efficiency is affected by the 5′ 3 nt of a 3′ trailer. To analyze this sequence effect, I synthesized another five derivatives of the pre-tRNA^{Arg} (R-CCA19): R-CCG, R-CUA, R- UCA, R-ACA19 and R-GCA (the 5′ 3 nt of the 3′ trailer shown after the hyphen). They each have a 19 nt 3′ trailer with another nucleotide substituted for one of the 5′ 3 nt CCA. Although the 3′ processing efficiency of R-CCG and R-CUA was as low as that of R-CCA19, the other three pre-tRNAs were cleaved in the order R-ACA19 > R-GCA > R-UCA more than twice as well as R-CCA19 (Table 2). R-G19 with a 19 nt 3′ trailer beginning with GUG was also cleaved three times as well as R-CCA19. These results show that the 5′ 3 nt, especially the 5′ end nucleotide, of a 3′ trailer are critical for determining tRNA 3′ processing efficiency, and that cytidine at the 5′ end reduces the efficiency.

Table 2. Cleavage efficiency of pre-tRNA^{Arg} variants

Pre-tRNA	Sequence[a]	Cleavage (%)[b]
	5′ ↓ 3′	
R-CCA19	***CUCG CCAUAAGCAGGGUCGUUUU	10.1 ± 1.5
R-CCG	***CUCG CCGUAAGCAGGGUCGUUUU	8.9 ± 1.2
R-CUA[c]	***CUCG CUAUAAGCAGGGUCGUUUU	11.8 ± 0.8
R-UCA	***CUCG UCAUAAGCAGGGUCGUUUU	21.2 ± 1.5
R-ACA19	***CUCG ACAUAAGCAGGGUCGUUUU	37.9 ± 2.9
R-GCA	***CUCG GCAUAAGCAGGGUCGUUUU	22.6 ± 2.7
R-CCA3	***CUCG CCA	ND
R-ACA3[d]	***CUCG ACA	81.9 ± 4.8
R-G3	***CUCG GUG	73.7 ± 2.5
R-C1	***CUCG C	7.5 ± 1.4
R-U1	***CUCG U	99.0 ± 0.5
R-A1	***CUCG A	99.8 ± 0.1
R-G1	***CUCG G	99.2 ± 0.6

[a]An arrow denotes the 3′ processing site. The whole sequences are shown in Figure 1A.
[b]The assays were performed using mouse 3′ tRNase at 37°C for 30 min. Values are averages for three experiments. ND, not detected.
[c]Cleavage was detected both after the discriminator (50%) and after the nucleotide 3′ of the discriminator (50%).
[d]Cleavage was detected both after the discriminator (90%) and after the nucleotide 3′ of the discriminator (10%).

I further analyzed the effect of 5′ nucleotides on 3′ processing efficiency using three additional pre-tRNAs: R-ACA3, R-U1 and R-A1, which have 3′ trailers consisting of ACA, U and A, respectively. Among the three tested pre-tRNAs with 3 nt trailers, the cleavage of R-ACA3 and R-G3 was very efficient, while the cleavage of R-CCA3 was not detected (Table 2). With regard to

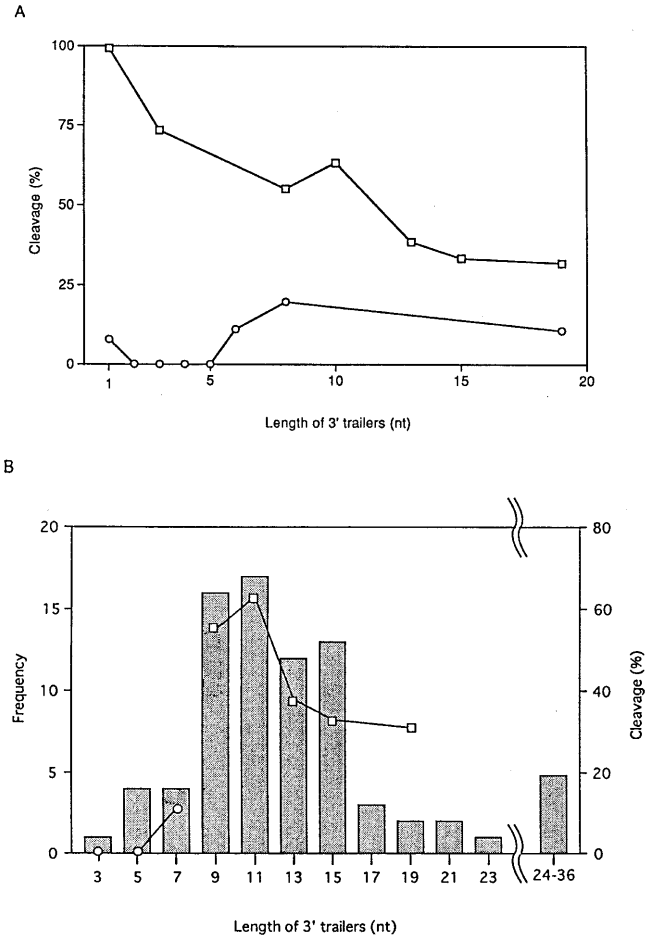

Figure 4. Dependence of 3′ processing efficiency on length of 3′ trailers, and length distribution of the 3′ trailers. (**A**) The percent cleavage (Table 1) of pre-tRNA^{Arg}s, R-G series (squares) and R-CCA series (circles), was plotted against the length of 3′ trailers. (**B**) Length distribution of 3′ trailers deduced from mammalian nuclear tRNA genomic sequences, and overlaid plots of the 3′ processing efficiency are shown. Odd numbers on the abscissa represent 2 nt ranges, 2–3, 4–5, and so on. The plots of the cleavage efficiency against 2–7 nt and >7 nt 3′ trailers were from the R-CCA (circles) and R-G series (squares), respectively.

the four pre-tRNAs with 1 nt trailers, U, A and G were removed very efficiently, but R-C1 was hardly cleaved (Fig. 3B and Table 2). These 3′ processing analyses using various pre-tRNA^{Arg}s with systematically mutated 3′ trailers led me to conclude that, for pre-tRNAs with 3′ trailers of the same length, the 5′ end nucleotide of the 3′ trailer is the key factor determining cleavage efficiency.

The 5′ end nucleotide effect is independent of the discriminator nucleotides

I demonstrated above that the 5′ terminal nucleotide of a 3′ trailer greatly affects the tRNA 3′ processing reaction. This conclusion raised a question as to whether a nucleotide 5′ of the 3′ processing site, the discriminator, also affects the cleavage efficiency. To answer this question, I tested 16 species of pre-tRNA^{Arg}s, each of which contains one of the dinucleotides on both sides of the 3′ processing site, for cleavage using 3′ tRNase purified from mouse FM3A cells or pig liver. The enhancing effect of the 5′ end

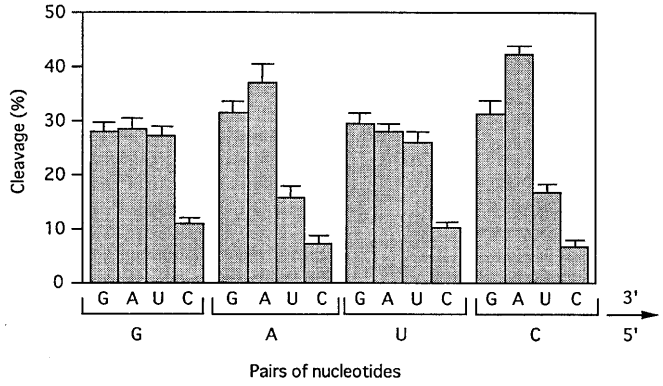

Figure 5. Effect of substitutions of nucleotides on both sides of the cleavage site on 3′ processing efficiency. 3′ processing efficiency of 16 species of pre-tRNA^{Arg}s with an 8 nt 3′ trailer, each of which contains one of the dinucleotides on both sides of the cleavage site (Fig. 1B). The assays were performed using mouse 3′ tRNase at 37°C for 15 min. 3′ and 5′ denote nucleotides 3′ and 5′ of the cleavage site, respectively. Values are averages for two experiments.

nucleotide of a 3′ trailer on cleavage efficiency was in the order G ~ A > U > C (Figs 5 and 6 and Table 3). On the other hand, although there were differences in 3′ processing efficiency among the four pre-tRNAs with the same 5′ end base of the 3′ trailer, there seemed to be no general rule governing the effect of the discriminator nucleotide on 3′ trailer cleavage (Figs 5 and 6).

Table 3. Kinetic parameters of 3′ processing of pre-tRNA^{Arg} variants by pig 3′ tRNase

Sequence[a]	K_m[b] (μM)	V_{max}[b] (pmol/min)	V_{max}/K_m
5′ ↓ 3′			
***CUCG GUGUAUUU	0.63	0.92	1.46
***CUCG AUGUAUUU	0.51	0.71	1.39
***CUCG UUGUAUUU	0.58	0.60	1.03
***CUCG CUGUAUUU[c]	0.76	0.39	0.51

[a]An arrow denotes the 3′ processing site. The whole sequences are shown in Figure 1B.
[b]The kinetic parameters were obtained from the assays using pig 3′ tRNase fraction (0.2 ng) after Mono Q column chromatography (7). Each measurement was from averages of three trials with a standard deviation of 3–9%.
[c]Cleavage was detected both after the discriminator (70%) and after the nucleotide 3′ of the discriminator (30%).

Distribution of 5′ end nucleotides of pre-tRNA 3′ trailers

Table 4 shows the distribution of the 5′ end nucleotides of pre-tRNA 3′ trailers deduced from mammalian nuclear and mitochondrial tRNA genomic sequences. The nuclear distribution differs considerably from the mitochondrial one: each purine occurs more frequently than each pyrimidine in the nuclear distribution, and A occurs prominently in the mitochondrial one (Table 4). The nuclear and mitochondrial frequencies were plotted against the values of V_{max}/K_m of pre-tRNAs with a 3′ trailer beginning with the corresponding nucleotide (Fig. 7). The nuclear frequency correlates well with the 3′ processing efficiency, while the mitochondrial frequency does not.

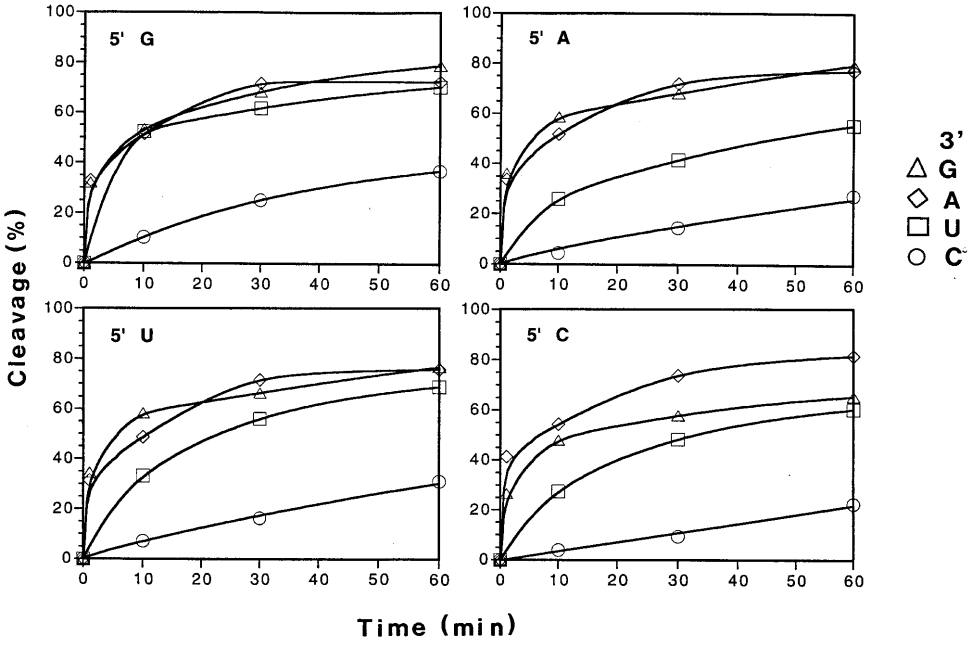

Figure 6. Time courses of 3′ processing of 16 species of pre- tRNAs with an 8 nt 3′ trailer, each of which contains one of the dinucleotides on both sides of the cleavage site (Fig. 1B). The assays were performed using pig 3′ tRNase. Data are averaged from three separate experiments with a standard deviation of 3–8%. 3′ and 5′ denote nucleotides 3′ and 5′ of the cleavage site, respectively.

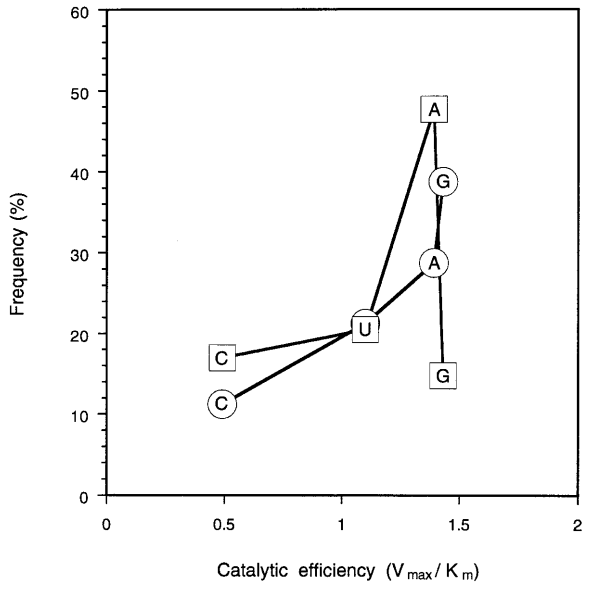

Figure 7. Correlation between catalytic efficiency and frequencies of 5′ terminal nucleotides of 3′ trailers. The nuclear (circles) and mitochondrial (squares) frequencies of 5′ terminal nucleotides of 3′ trailers (Table 4) are plotted against the catalytic efficiency (V_{max}/K_m) of pre-tRNAs with 3′ trailers beginning with the corresponding nucleotides (Table 3).

DISCUSSION

Intracellular location of 3′ tRNase

Here I demonstrated that 3′ tRNase purified from cytosolic extracts has the property that the rate of 3′ processing depends on both the length and the 5′ end base of the 3′ trailer. This property was also shared by 3′ tRNase from nuclear extracts of mouse FM3A cells (data not shown). This suggests that the enzymatic property of cytosolic 3′ tRNase is common at least to mammalian cells, and that cytosolic 3′ tRNase is identical to the nuclear one. Melton *et al.* (28) demonstrated that tRNA 3′ processing occurs in nuclei in *Xenopus* oocytes. Thus, cytosolic 3′ tRNase may have leaked out of the nuclei in the course of the enzyme preparation. Alternatively, 3′ tRNase in the cytosol might be involved in RNase 65 activities (7) or function as an error-correcting enzyme coupled with tRNA nucleotidyltransferase (see below). RNase 65 is a relatively stable complex between 3′ tRNase and a 3′-truncated tRNA of ~65 nt, though little is known about its physiological role and its substrate (7).

Table 4. Distribution of 5′ end nucleotides of 3′ trailers

Organelle	5′ end nucleotide				
	G	A	U	C	Total
Nucleus	31	23	17	9	80
	38.75	28.75	21.25	11.25	100%
Mitochondrion	13	42	18	15	88
	14.8	47.7	20.5	17.0	100%

Short 3′ trailers are potentially deleterious

The length distribution of nuclear pre-tRNA 3′ trailers correlates well with the 3′ processing efficiency of the R-G series pre-tRNAs for 3′ trailers with a length of >7 nt (Fig. 4B). This may reflect a demand for efficient tRNA synthesis in cells. However, the correlation does not hold with regard to smaller 3′ trailers and the distribution agrees rather with the cleavage efficiency of the R-CCA series pre-tRNAs (Fig. 4B). RNA

polymerase III usually terminates transcription within the four thymidine stretch terminator, generating a transcript with a two to four uridine stretch corresponding to the terminator at its 3′ terminus. Thus, it is possible that the polymerase produces pre-tRNAs with trailers that are several nucleotides long. Among them 3′ trailers beginning with C, CC or CCA should be hard for 3′ tRNase to remove. In particular, pre-tRNAs with the 3′ trailer CCAUU, which probably cannot be removed by 3′ tRNase, may be deleterious to cells. The low frequencies of short 3′ trailers may reflect a demand for the prevention of deleterious tRNA synthesis in cells.

Does 3′ tRNase eliminate nucleotides misincorporated by tRNA nucleotidyltransferase *in vivo*?

In eukaryotic cells, the CCA terminal residues must be added to the 3′ end of pre-tRNAs from which a 3′ trailer was removed, to generate functional tRNAs, since eukaryotic tRNA genes do not encode the CCA sequence. This reaction is catalyzed by tRNA nucleotidyltransferase, which has the activity to incorporate not only CMP and AMP residues, but also UMP incorrectly, into tRNA molecules (29). Under certain conditions, this enzyme produces anomalous tRNAs with 3′ terminal residues other than the CCA (29). The CCA end of tRNA is critical for both aminoacylation (30,31) and binding to large rRNA in the peptidyl transferase center of the ribosome (32) in *E.coli* and probably also in eukaryotes. Therefore, some error-correcting mechanism may exist to remove non-CCA residues from those anomalous tRNAs. 3′ tRNase might be one of the enzymes involved in such a mechanism since this endoribonuclease can remove any 3′ trailer except CC, CCA and CCA plus 1 or 2 nt from pre-tRNAs.

Discrimination of CCA residues by 3′ tRNase

In this study I demonstrated that 3′ tRNase recognizes not only the L-shaped mature tRNA domain but also the 3′ trailer to determine cleavage efficiency. 3′ tRNase clearly discriminates the nucleotide C at the 5′ termini of a 3′ trailer from the others (Table 2 and Figs 5 and 6). Besides that, the very short 3′ trailers CC, CCA and CCA plus 1 or 2 additional 3′ nt can be distinguished by this enzyme from the other 3′ trailers (Table 1). 3′ tRNase may have a binding domain for CCA residues. The CCA-binding domain may be composed of two cytidine binding sites and an adenosine binding site. The cleavage efficiency of pre-tRNA^{Arg}s varies depending on the 5′ end nucleotide of a 3′ trailer in the order G ~ A > U > C (Figs 5 and 6 and Table 3). The differential 3′ processing efficiency might reflect the affinity of the 5′ end nucleotide of a 3′ trailer to the first cytidine binding site in the order C > U > A ~ G. It may as well be possible that CCA serves as a negative determinant and that the substrate binding site of 3′ tRNase preferentially accommodates other sequences. 3′ trailers beginning with CCA residues can be removed by 3′ tRNase if they are longer than 5 nt (Table 1). This suggests that mammalian 3′ tRNase may also grip a certain number of distal nucleotides of a 3′ trailer.

ACKNOWLEDGEMENTS

I thank K. Maeda and N. Teramachi for excellent technical assistance and S. Takano for assistance in preparing the figures.

REFERENCES

1 Deutscher,M.P. (1984) *Crit. Rev. Biochem.*, **17,** 45–71.
2 Westaway,S.K. and Abelson,J. (1995) In Söll,D. and RajBhandary,U.L. (eds), *tRNA: Structure, Biosynthesis, and Function.* American Society for Microbiology Press, Washington, DC, pp 79–92.
3 Morl,M., Dorner,M. and Paabo,S. (1995) *Nucleic Acids Res.*, **23,** 3380–3384.
4 Altman,S., Kirsebom,L. and Talbot,S. (1995) In Söll,D. and RajBhandary,U.L. (eds), *tRNA: Structure, Biosynthesis, and Function.* American Society for Microbiology Press, Washington, DC, pp. 67–78.
5 Deutscher,M.P. (1995) In Söll,D. and RajBhandary,U.L. (eds), *tRNA: Structure, Biosynthesis, and Function.* American Society for Microbiology Press, Washington, DC, pp. 51–65.
6 Zasloff,M., Santos,T., Romeo,P. and Rosenberg,M. (1982) *J. Biol. Chem.*, **257,** 7857–7863.
7 Nashimoto,M. (1995) *Nucleic Acids Res.*, **23,** 3642–3647.
8 Castano,J.G., Tobian,J.A. and Zasloff,M. (1985) *J. Biol. Chem.*, **260,** 9002–9008.
9 Frendewey,D., Dingerman,T., Cooley,L. and Soll,D. (1985) *J. Biol. Chem.*, **260,** 449–454.
10 Levinger,L., Vasisht,V., Greene,V., Bourne,R., Birk,A. and Kolla,S. (1995) *J. Biol. Chem.*, **270,** 18903–18909.
11 Oommen,A., Li,X. and Gegenheimer,P. (1992) *Mol. Cell. Biol.*, **12,** 865–875.
12 Rossmanith,W., Tullo,A., Potuschak,T., Karwan,R. and Sbisa,E. (1995) *J. Biol. Chem.*, **270,** 12885–12891.
13 Manam,S. and Van Tuyle,G.C. (1987) *J. Biol. Chem.*, **262,** 10272–10279.
14 Chen,J.Y. and Martin,N.C. (1988) *J. Biol. Chem.*, **263,** 13677–13682.
15 Solari,A. and Deutscher,M.P. (1983) *Mol. Cell. Biol.*, **3,** 1711–1717.
16 Engelke,D.R., Gegenheimer,P. and Abelson,J. (1985) *J. Biol. Chem.*, **260,** 1271–1279.
17 Garber,R.L. and Altamn,S. (1979) *Cell*, **17,** 389–397.
18 Nashimoto,M. (1996) *RNA*, **2,** 523–534.
19 Nashimoto,M. (1992) *Nucleic Acids Res.*, **20,** 3737–3742.
20 Nashimoto,M. (1993) *Nucleic Acids Res.*, **21,** 4696–4702.
21 Peattie,D.A. (1979) *Proc. Natl. Acad. Sci. USA*, **76,** 1760–1764.
22 Hagenbuchle,O., Larson,D., Hall,G.I. and Sprague,K.U. (1979) *Cell*, **18,** 1217–1229.
23 Koski,R.A. and Clarkson,S.G. (1982) *J. Biol. Chem.*, **257,** 4514–4521.
24 Thomann,H.-U., Schmutzler,C., Hudepohl,U., Blow,M. and Gross,H.J. (1989) *J. Mol. Biol.*, **209,** 505–523.
25 Anderson,S., Bankier,A.T., Barrell,B.G., de Bruijn,M.H.L., Coulson,A.R., Drouin,J., Eperon,I.C., Nierlich,D.P., Rpe,B.A., Sanger,F., *et al.* (1981) *Nature (London)*, **290,** 457–465.
26 Anderson,S., de Bruijn,M.H.L., Coulson,A.R., Eperon,I.C., Sanger,F. and Young,I.G. (1982) *J. Mol. Biol.*, **156,** 683–717.
27 Bibb,M.J., Van Etten,R.A., Wright,C.T., Walberg,M.W. and Clayton,D.A. (1981) *Cell*, **26,** 167–180.
28 Melton,D.A., De Robertis,E.M. and Cortese,R. (1980) *Nature (London)*, **284,** 143–148.
29 Deutscher,M.P. (1972) *J. Biol. Chem.*, **247,** 469–480.
30 Reuven,N.B. and Deutscher,M.P. (1993) *Proc. Natl. Acad. Sci. USA*, **90,** 4350–4353.
31 Tamura,K., Nameki,N., Hasegawa,T., Shimizu,M. and Himeno,H. (1994) *J. Biol. Chem.*, **269,** 22173–22177.
32 Samaha,R.R., Green,R. and Noller,H.F. (1995) *Nature (London)*, **377,** 309–314.
33 Milligan,J.F. and Uhlenbeck,O.C. (1989) *Methods Enzymol.*, **180,** 51–62.