Co-evolution of tRNA 3' trailer sequences with 3' processing enzymes in bacteria

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ABSTRACT

Maturation of the tRNA 3' terminus is a complicated process in bacteria. Usually, it is initiated by an endonucleolytic cleavage carried out by RNase E and Z in different bacteria. In *Escherichia coli*, RNase E cleaves AU-rich sequences downstream of tRNA, producing processing intermediates with a few extra residues at the 3' end; these are then removed by exoribonuclease trimming to generate the mature 3' end. Here we show that essentially all *E. coli* tRNA precursors contain a potential RNase E cleavage site, the AU-rich sequence element (AUE), in the 3' trailer. This suggests that RNase E cleavage and exonucleolytic trimming is a general pathway for tRNA maturation in this organism. Remarkably, the AUE immediately downstream of each tRNA is selectively conserved in bacteria having RNase E and tRNA-specific exoribonucleases, suggesting that this pathway for tRNA processing is also commonly used in these bacteria. Two types of RNase E-like proteins are identified in actinobacteria and the α -subdivision of proteobacteria. The tRNA 3' proximal AUE is conserved in bacteria with only one type of E-like protein. Selective conservation of the AUE is usually not observed in bacteria without RNase E. These results demonstrate a novel example of co-evolution of RNA sequences with processing activities.

Keywords: tRNA; processing; RNase E; AU-rich; 3' trailer

INTRODUCTION

Maturation of tRNA is a challenging problem in all living systems. In bacteria, the number of tRNA genes varies from ~30 to >100 in each genome (Lowe and Eddy 1997; Minagawa et al. 2004). tRNAs vary in their primary structures, but they conserve similar secondary and tertiary folds. It is common for bacterial tRNAs to be encoded by multicistronic operons. A multicistronic tRNA transcript may contain more than one tRNA, or rRNA and mRNA in addition to tRNA (Wawrousek and Hansen 1983; Komine et al. 1990; Sedlmeier et al. 1994). Some bacteria have genetically encoded 3' terminal CCA for all tRNAs, whereas others only encode CCA for fractions of their total tRNAs (Deutscher 1984; Minagawa et al. 2004). In all cases, extra sequences present at both the 5' and 3' termini of a tRNA are removed during tRNA maturation (Deutscher 1984).

Removal of the 5' leader sequences from tRNA precursors is catalyzed by a single endonucleolytic reaction catalyzed by RNase P (Frank and Pace 1998; Altman and Kirsebom 1999), a ribozyme found in all bacteria with few exceptions (Willkomm et al. 2002; Li and Altman 2004).

Processing at the 3' end is more complicated. Our knowledge of tRNA 3' maturation is most advanced in *Escherichia coli*, an organism in which most, if not all, ribonuclease activities have been identified and studied in detail (Li and Deutscher 2004). A total of 86 tRNAs are identified in *E. coli*, with 3' terminal CCA encoded for all of them. The current model suggests that the endoribonuclease RNase E carries out the first step of tRNA maturation. RNase E has been shown to cleave a number of primary tRNA transcripts at positions usually a few nucleotides downstream of the 3' end of tRNAs (Li and Deutscher 2002; Ow and Kushner 2002). The intermediates with short 3' ends are then cleaved efficiently by RNase P to generate the mature 5' end. The few extra residues at the 3' end are removed by exoribonucleases, mainly RNase T and PH (Reuven and Deutscher 1993; Li and Deutscher 1994, 1996), simultaneously with or after RNase P action (Li et al. 1998; Li and Deutscher 2002). Other exoribonucleases, including the tRNA-specific RNases D and BN, and the nonspecific RNase II, remove the 3' extra residues less efficiently

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(Reuven and Deutscher 1993; Li and Deutscher 1994, 1996). In the absence of RNase T and PH activities, tRNA 3' maturation is greatly impaired and cell growth slows down (Kelly and Deutscher 1992; Li and Deutscher 1994, 1996). In a few cases, RNase E cleavage occurs further downstream (Li and Deutscher 2002). The resulting longer 3' trailer sequences are shortened by the nonspecific exoribonucleases, RNase II and polynucleotide phosphorylase (PNPase), prior to the actions of RNase P, T, and PH (Li and Deutscher 1994, 2002).

As described above, it is apparent that RNase E is a key enzyme for the maturation of tRNA precursors in *E. coli*. Its cleavage initiates maturation pathways. The position of cleavage determines the reactions to follow. RNase E has also been shown to cleave mRNA, antisense RNAs, and other noncoding small RNAs, and precursors of rRNA (for review, see Cohen and McDowall 1997; Kushner 2002; Li and Deutscher 2004). It has been shown that RNase E recognizes AU-rich sequences as cleavage sites for various substrates (for review, see Cohen and McDowall 1997). However, a sequence consensus has not been found (McDowall et al. 1994), and nearby secondary structures play controversial roles in determining cleavage sites (Bouvet and Belasco 1992; Mackie and Genereaux 1993; McDowall et al. 1995). These have prevented accurate predictions of RNase E cleavage sites in other *E. coli* tRNA precursors, which are important to decipher the global role of RNase E in tRNA maturation.

RNase E is an essential enzyme in *E. coli* (Ghora and Apirion 1978). Despite its importance in RNA metabolism, its distribution is limited to a relatively small group of bacteria, including actinobacteria and proteobacteria α , β , and γ subdivisions (Condon and Putzer 2002). Interestingly, a similar enzyme, RNase G, exists in *E. coli* (Li et al. 1999; Wachi et al. 1999). RNase G is smaller than RNase E and is homologous to the N-terminal catalytic domain of RNase E. RNase G is responsible for the maturation of the 5' end of 16S rRNA and for the degradation of a number of mRNAs (for review, see Li and Deutscher 2004). RNase G, like RNase E, cleaves at AU-rich sites of RNA (Li et al. 1999; Jiang et al. 2000; Tock et al. 2000). However, the substrate spectrum of RNase G seems to be much narrower than that of RNase E (Lee et al. 2002). Preliminary studies suggest that RNase G may have a limited function in tRNA maturation in the absence of RNase E (J. Rubine and Z. Li, unpubl.). Overexpression of RNase G causes the formation of cytoplasmic axial filaments (Okada et al. 1994). Interruption of the *rng* gene encoding RNase G does not affect growth, but results in accumulation of a precursor to 16S rRNA (Li et al. 1999; Wachi et al. 1999). RNase G is present in a broader range of bacteria (Condon and Putzer 2002). Some bacteria have enzymes whose sequence features are similar to both RNase E and G. It has been shown that such an RNase E/G enzyme in *Aquifex aeolicus* cleaves tRNA precursors in vitro at AU-rich sequences (Willkomm et al. 2002).

Endonucleolytic activities that are responsible for eukaryotic tRNA 3' processing (3' tRNase or RNase Z) have been described in various organisms (Castano et al. 1985; Oommen et al. 1992; Nashimoto 1997; Kunzmann et al. 1998; Levinger et al. 1998). Recently, such activities were purified and cloned from plant and archaea, and are shown to be members of the ELAC1/2 family of proteins (Schiffer et al. 2002). RNase Z cleaves after the discriminator nucleotide of eukaryotic tRNA precursors that are generally CCA-less. The addition of CCA to CCA-less tRNAs is catalyzed by tRNA nucleotidyl transferase (Deutscher 1990; Schurer et al. 2001). RNase Z is widely present in bacteria (Condon and Putzer 2002). Recent work has demonstrated that RNase Z from *Bacillus subtilis* participates in the maturation of CCA-less tRNAs in the same way as its archaeal counterpart (Pellegrini et al. 2003). This reaction is less efficient if a long 5' leader sequence has not been previously removed. The presence of a residue of C after the discriminator inhibits the activity of *B. subtilis* RNase Z, suggesting that this enzyme is probably not responsible for the maturation of tRNAs with encoded CCA in this organism. In contrast, RNase Z (3' tRNase) in *Thermotoga maritima* works very differently from that in *B. subtilis*. The tRNase of *T. maritima* cleaves after the encoded CCA sequence, generating mature 3' termini directly (Minagawa et al. 2004). In this case, the terminal CA residues are required for cleavage at the correct site. Interestingly, *E. coli* also harbors a RNase Z homolog encoded by the *elaC* gene. The *E. coli* RNase Z is nonessential (Schilling et al. 2004). Its role in the processing of *E. coli* tRNAs remains elusive, although the enzyme cleaves human pre-tRNA after the discriminator residue in vitro (Minagawa et al. 2004). Various mechanisms of tRNA processing are described in Figure 1.

To better understand the mechanisms that are used by various bacteria for the 3' processing of tRNA, we have analyzed completely sequenced bacterial genomes for tRNA 3' trailer sequences and the presence of tRNA-processing enzymes. Here we show that RNase E is present in organisms that also have the processing exoribonucleases, and that the potential RNase E cleavage sites, AU-rich elements, are selectively enriched immediately downstream of tRNAs in only these organisms. The results suggest co-evolution of tRNA 3' trailer sequences with 3' processing activities.

RESULTS

RNase E cleaves at AU-rich sequences that are abundant in tRNA 3' trailers in *E. coli*

It has been shown previously that in *E. coli*, RNase E cleaves several primary tRNA transcripts. This cleavage is the initial processing step for those tRNAs. As shown in Figure 2, it is apparent that the sequences surrounding RNase E cleavage

FIGURE 2. RNase E cleavage sites of *E. coli* tRNA transcripts. Cleavage sites on two tRNA transcripts were determined by Northern blotting and primer extension (Li and Deutscher 2002). Some well-defined cleavage sites are shown by “E” with vertical arrows above the RNA sequence. Marks with only “E” indicate cleavages observed by Northern blotting, but the exact locations were not shown by primer extension. AU-rich sequences are shown in bold letters.

FIGURE 3. Nucleotide frequency of the 3' trailers of *E. coli* tRNA. Nucleotide percentages at each of 20 positions were calculated by dividing the number of occurrences of a nucleotide by the total number counted at that position.

Maturation of the 5' termini of the downstream tRNAs by RNase P generates precursors of the AUE-less tRNAs with only two to four extra residues at the 3' termini, which can be removed by solely exonucleolytic trimming reactions.

Most tRNA precursors (73 out of 82) contain at least one AUE within the first 10 nt of the 3' trailers. It should be noted that the majority of AUEs are quite long and may harbor more than one cleavage site for RNase E. It is common that multiple AUEs are present in the long 3' trailer sequences. Altogether, the results clearly demonstrate that potential RNase E cleavage sites are present in the 3' trailer sequences of almost all tRNA precursors in *E. coli*, and are highly abundant in the region close to the 3' end. This suggests that tRNA-processing initiated by RNase E cleavage is a common mechanism in this organism.

Distribution of AU-rich elements in tRNA 3' trailers in 51 bacterial genomes

After observing the high occurrence of AUEs in the 3' trailers of *E. coli* tRNAs, we set up a search of the AUEs in other bacteria. The purpose is to obtain information regarding AUE abundance in 3' trailers and to understand the relationship, if any, between the presence of AUE and tRNA 3' maturation in other bacteria.

To do this, we selected 51 genomes that are well annotated and have coordinates for all tRNA genes as well as downstream genes. Complete genome sequences and annotation data (GenBank format) for all bacteria were obtained from NCBI (http://www.ncbi.nlm.nih.gov/genomes/static/eub_g.html). Using procedures described above, tRNA 3' trailer sequences were extracted and AUEs were searched within the 3' trailers for all bacteria. Annotation errors present in some genomes were fixed manually. Perl scripts were developed to automate the search.

In order to compare the abundance of AUEs among all

genomes, we have developed a numeric system to record the number of AUEs in different regions of the 3' trailers. We divided the 100-nt 3' trailer sequences into 10 regions, each containing 10 nt. The number of AUEs in each of the 10 regions is counted as described below. This enabled us to compare AUE abundance among different regions of the 3' trailers, as well as across genomes.

The presence of an AUE in a region is defined as follows. (1) At least 3 nt of the AUE occupies this region; (2) one AUE can be counted in more than one region if it is long enough; and (3) one 3' trailer sequence may contain 0–2 AUEs in a region. If taking the last encoded residue of a tRNA (the 3' A or discriminator) as position 0, any AUE starting from –1 to 8 or stopping before 12 is counted in region 1–10; an AUE starting from 9 to 18 or stopping before 22 is counted in region 11–20; and so on.

Note that the lengths of 3' trailers vary. It is necessary to calculate AUE abundance in each region according to the number of 3' trailer sequences present in that region. The number of 3' trailers in a particular region is determined by the presence of at least 3 nt of the trailer sequence in that region. For each bacterial genome, AUE abundance in each region (denoted as $R_1, R_2, \dots, R_{10}$) is calculated as the number of AUEs divided by the number of 3' trailers in that region. The results are presented in Table 2.

Interestingly, AUE abundance varies greatly among genomes (Table 2). AUE abundance across the 10 regions is high in some genomes but low in some others. This may reflect low or high GC percentages in those genome sequences (see below). Many genomes have a higher AUE abundance in the first region than in other regions of the 3' trailers. The AUEs, especially those selectively conserved close to the 3' end of tRNAs, probably are related to tRNA 3' processing. This point is further analyzed in the following sections.

Distribution of tRNA 3'-processing ribonucleases in 51 bacterial genomes

The differences in AUE abundance in the bacterial genomes may be associated with differences in tRNA 3' processing activities. We carried out a search for the activities potentially involved in the 3' processing of tRNA in all the genomes described above. In addition to RNase E/G and Z, we also searched for the exoribonucleases specific for tRNA 3' maturation (namely, RNase T, PH, and D). RNase BN was not included in this search because of the uncertainty in the identification of the gene encoding this enzyme (M.P. Deutscher, pers. comm.). Searches for the nonspecific exoribonucleases RNase II and PNPase were carried out to compare with the distribution of tRNA-specific enzymes. RNase R, a nonspecific exoribonuclease whose function has not yet been related to tRNA maturation, is also included in this

TABLE 1. AU-rich elements in the 3' trailer sequences of *E. coli* tRNAs

tRNA	Downstream gene	Length of 3' trailer	No. of AUE
ilev	alaV	42	3
alaV	rrlH	183	5
aspU	Ter	40	2
aspV	Ter	60	3
thrW	Ter	?	5
argU	Ter	?	7
glnX	Ter	37	1
glnV	glnX	37	2
metU	glnV	47	4
glnW	metU	15	1
glnU	metU	34	3
leuW	glnW	23	3
metT	leuW	9	1
lysT	valT	135	2
valT	lysW	2	0
lysW	valZ	149	3
valZ	lysY	3	0
lysY	lysZ	146	3
lysZ	lysQ	132	3
lysQ	Ter	31	2
serW	Ter	41	2
serT	Ter	43	2
serX	Ter	41	2
tyrV	tpr	55	3
tyrT	tyrV	209	4
valV	valW	4	0
valW	b1667	107	7
leuZ	Ter	67	3
cysT	leuZ	12	1
glyW	cysT	54	3
serU	Ter	?	6
asnT	Ter	?	4
asnW	Ter	97	3
asnU	Ter	36	3
asnV	Ter	39	2
proL	Ter	41	2
argW	Ter	?	4
alaX	Ter	35	1
alaW	alaX	39	3
valU	valX	44	2
valX	valY	46	2
valY	lysV	4	0
lysV	Ter	31	2
gltW	rrlG	184	5
ileY	Ter	?	6
argQ	Ter	?	5
argZ	argQ	198	6
argY	argZ	62	4
argV	argY	198	6
serV	argV	3	1
metZ	metW	33	1
metW	metV	33	1
metV	Ter	30	1
glyU	b2863	113	7
pheV	Ter	42	1
ileX	Ter	44	2
metY	yhbC	200	5
leuU	Ter	42	3
thrV	rrfF	37	3

(continued)

TABLE 1. Continued

tRNA	Downstream gene	Length of 3' trailer	No. of AUE
alaU	rrlD	174	5
ileU	alaU	42	3
proK	Ter	35	2
selC	Ter	?	7
gltU	rrlC	193	5
aspT	trpT	8	1
trpT	Ter	35	1
argX	hisR	57	3
hisR	leuT	20	2
leuT	proM	42	2
proM	Ter	30	1
ileT	alaT	42	3
alaT	rrlA	174	5
gltT	rrlB	193	6
thrU	tyrU	8	1
tyrU	glyT	116	5
glyT	thrT	6	1
thrT	tufB	114	8
gltV	rrlE	194	6
pheU	Ter	42	1
glyV	glyX	36	1
glyX	glyY	35	2
glyY	Ter	?	2
leuX	Ter	37	1
leuV	Ter	?	6
leuP	leuV	34	1
leuQ	leuP	28	2

"Ter" stands for terminator. "?" represents unknown length of 3' trailer because of the uncertainty of the downstream gene.

analysis because of its similarity to RNase II. In fact, RNase II, R, and proteins with similar sequences are grouped into the RNR family (Zuo and Deutscher 2001).

The presence of the aforementioned ribonucleases in each organism was determined primarily by genomic BLAST searches using the *E. coli* proteins as query sequences (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi). Sequences of ElaC from *E. coli* and YqjK from *B. subtilis* were both used as queries in the RNase Z search. Membership of clusters of orthologous groups is also used in the search for RNase E/G (COG1530). We found that the BLAST result is inclusive of all COG members of RNase E/G, which indicates that genomic BLAST is a good tool for finding ribonucleases in bacteria. Since remote homologs of ribonucleases may or may not actually function as RNases, we did not include BLAST hits that have *E*-values >e-40. For the same reason, more sophisticated searches such as *psi*BLAST were not used. The RNases found in our search are essentially the ones that have already been annotated as the same enzymes in GenBank records. Ribonucleases found in various bacterial genomes are listed in Table 3. A similar search for most of the above enzymes was reported previously for some of the bacteria in our list (Minagawa et al. 2004). Our results agree well with the results of that search.

Based on the presence of RNase E or G, the bacteria can

TABLE 2. AUE abundance in tRNA 3' trailer sequences in 51 bacteria

Organism	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}
<i>Neisseria meningitidis</i> MC58	1.14	0.82	0.88	0.79	0.83	0.72	0.59	0.91	0.89	0.39
<i>Rickettsia conorii</i> str. Malish 7	1.09	1.12	1.03	0.93	0.79	1.11	0.93	0.96	1.00	0.54
<i>Lactococcus lactis</i> subsp. <i>lactis</i> II1403	1.08	0.72	0.84	0.79	0.97	0.78	0.84	0.75	0.75	0.61
<i>Brucella melitensis</i> 16M	1.05	0.83	0.92	0.84	0.76	0.54	0.54	0.40	0.50	0.38
<i>Xanthomonas campestris</i> pv. <i>campestris</i> str. ATCC 33913	1.04	0.47	0.33	0.60	0.38	0.43	0.40	0.41	0.26	0.33
<i>Chlamydia muridarum</i>	1.03	0.88	0.75	1.04	1.00	1.00	0.75	0.71	0.81	0.68
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> Mu50	1.03	0.92	0.91	1.24	1.07	1.21	1.15	1.23	1.00	0.62
<i>Yersinia pestis</i> KIM	1.03	0.58	0.71	0.72	0.95	0.67	0.69	0.54	0.58	0.20
<i>Brucella suis</i> 1330	1.02	0.85	0.92	0.71	0.61	0.50	0.46	0.37	0.46	0.42
<i>Xylella fastidiosa</i> 9a5c	1.00	0.73	0.78	0.87	0.62	0.43	0.60	0.73	0.52	0.42
<i>Escherichia coli</i> O157:H7	0.99	0.75	0.59	0.86	0.66	0.65	0.70	0.64	0.60	0.46
<i>Vibrio cholerae</i> O1 biovar <i>eltor</i> N16961	0.98	0.71	0.84	1.03	1.02	1.10	0.68	0.79	0.55	0.66
<i>Helicobacter pylori</i> 26695	0.97	1.03	0.79	0.90	0.76	0.73	0.54	0.82	0.67	0.50
<i>Escherichia coli</i> O157:H7 EDL933	0.96	0.78	0.61	0.86	0.73	0.68	0.77	0.68	0.69	0.51
<i>Clostridium perfringens</i> str. 13	0.96	1.00	1.06	1.07	0.98	0.98	0.93	1.16	1.14	0.86
<i>Streptococcus pyogenes</i> MGAS315	0.96	0.96	1.00	0.97	0.85	0.69	1.00	0.96	0.74	0.57
<i>Chlamydia trachomatis</i> D/UW-3/CX	0.95	1.09	0.66	0.90	0.73	0.68	0.52	0.71	0.67	0.47
<i>Streptococcus pyogenes</i> M1 GAS	0.95	0.95	1.00	0.93	0.74	0.64	1.32	0.68	0.65	0.65
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> N315	0.95	0.97	1.10	1.33	1.00	1.13	1.14	1.29	1.07	0.79
<i>Clostridium acetobutylicum</i> ATCC824	0.95	0.90	0.89	0.97	1.07	0.93	1.04	0.96	0.88	0.70
<i>Fusobacterium nucleatum</i> subsp. <i>nucleatum</i> ATCC 25586	0.94	1.20	0.94	1.07	1.15	1.00	1.08	1.18	1.00	1.09
<i>Haemophilus influenzae</i> Rd KW20	0.91	1.02	0.90	0.91	1.05	1.16	1.06	0.91	0.80	1.06
<i>Escherichia coli</i> K12	0.91	0.77	0.64	0.78	0.68	0.62	0.68	0.77	0.55	0.46
<i>Bacillus subtilis</i> subsp. <i>subtilis</i> str. 168	0.91	0.92	0.66	0.88	1.04	0.93	0.96	0.68	0.90	0.75
<i>Streptococcus pneumoniae</i> TIGR4	0.91	0.76	1.11	1.12	0.96	0.91	0.87	0.74	0.76	0.85
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Typhi</i> str. CT18	0.91	0.82	0.70	0.86	0.77	0.71	0.57	0.70	0.74	0.55
<i>Ralstonia solanacearum</i> GMI1000	0.91	0.26	0.43	0.53	0.46	0.29	0.39	0.28	0.35	0.22
<i>Xanthomonas axonopodis</i> pv. <i>citri</i> str. 306	0.91	0.41	0.40	0.63	0.45	0.45	0.43	0.15	0.28	0.29
<i>Mycoplasma pulmonis</i> UAB CTIP	0.90	1.29	1.13	1.20	1.00	0.92	1.00	1.00	1.11	0.67
<i>Streptococcus pneumoniae</i> R6	0.90	0.76	1.00	1.00	1.04	0.83	0.78	0.70	0.74	0.86
<i>Chlamydophila pneumoniae</i> AR39	0.89	0.91	1.00	0.94	0.76	0.77	0.96	0.74	0.91	0.47
<i>Pseudomonas aeruginosa</i> PA01	0.89	0.30	0.30	0.50	0.33	0.40	0.35	0.27	0.13	0.36
<i>Streptococcus agalactiae</i> 2603V/R	0.86	1.17	1.06	0.93	1.32	0.96	0.91	1.26	0.96	0.78
<i>Chlamydophila pneumoniae</i> CWL029	0.84	1.03	1.12	0.79	1.03	0.91	1.00	0.93	0.86	0.67
<i>Chlamydophila pneumoniae</i> J138	0.84	1.06	1.12	0.79	0.97	0.88	1.00	0.90	0.86	0.68
<i>Oceanobacillus ihayensis</i> HTE831	0.81	0.86	0.88	1.15	1.00	1.05	1.00	1.00	0.85	0.75
<i>Agrobacterium tumefaciens</i> str. C58	0.78	0.75	0.54	0.51	0.45	0.35	0.49	0.40	0.50	0.36
<i>Aquifex aeolicus</i> VF5	0.77	0.50	0.45	0.48	0.62	0.45	0.45	0.26	0.32	0.11
<i>Mycoplasma pneumoniae</i> M129	0.76	0.71	0.70	0.55	0.68	0.59	0.76	0.69	0.53	0.43
<i>Thermoanaerobacter tengcongensis</i> str. MB4T	0.75	0.97	0.70	0.86	0.89	0.70	0.67	0.62	0.65	0.44
<i>Corynebacterium glutamicum</i> ATCC 13032	0.70	0.79	0.95	0.80	0.89	0.77	0.60	0.64	0.62	0.46
<i>Chlorobium tepidum</i> TLS	0.64	0.73	0.66	0.83	0.76	0.47	0.47	0.52	0.31	0.46
<i>Thermotoga maritima</i> MSB8	0.63	0.41	0.64	0.81	0.50	0.39	0.52	0.65	0.57	0.33
<i>Deinococcus radiodurans</i> R1	0.63	0.24	0.50	0.80	0.59	0.59	0.22	0.51	0.38	0.09
<i>Bifidobacterium longum</i> NCC2705	0.56	0.51	0.57	0.75	0.62	0.54	0.38	0.33	0.34	0.31
<i>Caulobacter crescentus</i> CB15	0.52	0.57	0.37	0.31	0.24	0.28	0.25	0.27	0.20	0.15
<i>Mycobacterium leprae</i> TN	0.44	0.44	0.53	0.54	0.49	0.32	0.32	0.41	0.25	0.29
<i>Treponema pallidum</i> subsp. <i>pallidum</i> str. Nichols	0.36	0.39	0.42	0.30	0.30	0.28	0.42	0.30	0.35	0.16
<i>Mycobacterium tuberculosis</i> H37Rv	0.33	0.30	0.23	0.20	0.16	0.19	0.25	0.31	0.17	0.11
<i>Mycobacterium tuberculosis</i> CDC1551	0.31	0.33	0.15	0.11	0.21	0.13	0.29	0.21	0.21	0.07
<i>Streptomyces coelicolor</i> A3(2)	0.23	0.11	0.17	0.28	0.19	0.14	0.12	0.04	0.13	0.13

be divided into four groups (Table 3). Group I contains both RNase E and G. Interestingly, bacteria in this group also have the three tRNA-specific exoribonucleases RNase T, PH, and D, with a few exceptions in which one or two of the three enzymes exist. Note that RNase T is exclusively found in this group of organisms. Bacteria of this group

except *Caulobacter crescentus* belong to proteobacteria β - and γ -subdivisions. *C. crescentus* is the only member from the α -subdivision. Bacteria in Group II each have one RNase E/G-like enzyme that is close to RNase E in size and contains the RNase E/G domain. This domain contains an S1 RNA-binding domain, confers catalytic activity, and en-

TABLE 3. Ribonucleases present in the 51 bacteria

Organism	3'-processing endo			tRNA-specific exo			Nonspecific exo		
	E	G	Z	T	PH	D	II	PNP	R
Group I									
<i>H. influenzae</i>	+	+	–	+	+	+	+	+	+
<i>Y. pestis</i> KIM	+	+	–	+	+	+	+	+	+
<i>V. cholerae</i>	+	+	+	+	+	+	+	+	+
<i>E. coli</i> O157:H7 EDL933	+	+	+	+	+	+	+	+	+
<i>E. coli</i> O157:H7	+	+	+	+	+	+	+	+	+
<i>E. coli</i> K12	+	+	+	+	+	+	+	+	+
<i>N. meningitidis</i>	+	+	–	–	+	–	+	+	+
<i>S. enterica</i>	+	+	+	+	+	–	–	+	+
<i>X. fastidiosa</i>	+	+	–	+	+	+	–	+	+
<i>X. axonopodis</i>	+	+	+	+	+	+	–	+	+
<i>X. campestris</i>	+	+	+	+	+	+	–	+	+
<i>P. aeruginosa</i>	+	+	–	+	+	+	–	+	+
<i>R. solanacearum</i>	+	+	+	–	+	–	+	+	+
<i>C. crescentus</i>	+	+	+	+	–	+	–	+	^b
Group II									
<i>R. conorii</i>	^a	–	–	–	–	+	–	+	–
<i>C. glutamicum</i>	^a	–	+	–	+	–	–	+	–
<i>B. melitensis</i>	^a	–	–	–	+	+	–	+	+
<i>B. suis</i>	^a	–	–	–	+	–	–	+	+
<i>M. leprae</i>	^a	–	+	–	+	+	–	+	–
<i>A. tumefaciens</i>	^a	–	+	–	+	+	–	+	+
<i>B. longum</i>	^a	–	–	–	+	–	–	+	–
<i>M. tuberculosis</i> H37Rv	^a	–	+	–	+	+	–	+	–
Group III									
<i>F. nucleatum</i>	–	+	–	–	+	–	+	+	–
<i>C. perfringens</i>	–	+	+	–	+	–	–	+	+
<i>C. acetobutylicum</i>	–	+	+	–	–	+	–	+	+
<i>T. tengcongensis</i>	–	+	+	–	–	–	–	+	^b
<i>C. muridarum</i>	–	+	+	–	–	–	–	+	^b
<i>C. pneumoniae</i> AR39	–	+	+	–	–	–	–	+	+
<i>C. pneumoniae</i> CWL029	–	+	+	–	–	–	–	+	+
<i>C. pneumoniae</i> J138	–	+	+	–	–	–	–	+	+
<i>C. trachomatis</i>	–	+	+	–	–	–	+	+	–
<i>A. aeolicus</i>	–	+	–	–	+	–	–	+	+
<i>T. maritima</i>	–	+	+	–	–	–	–	+	+
<i>C. tepidum</i>	–	+	–	–	–	–	+	+	–
<i>M. tuberculosis</i> CDC1551	–	+	+	–	+	–	–	+	–
Group IV									
<i>M. pulmonis</i>	–	–	–	–	–	–	–	–	^b
<i>S. aureus</i> N315	–	–	+	–	–	–	–	+	+
<i>S. aureus</i> Mu50	–	–	+	–	–	–	+	+	–
<i>L. lactis</i>	–	–	+	–	+	–	–	+	+
<i>S. agalactiae</i>	–	–	+	–	–	–	–	+	^b
<i>O. ihayensis</i>	–	–	+	–	+	–	–	+	+
<i>S. pyogenes</i> M1GAS	–	–	+	–	–	–	–	+	+
<i>S. pyogenes</i> MGAS315	–	–	+	–	–	–	–	+	+
<i>H. pylori</i>	–	–	–	–	–	–	+	+	+
<i>S. pneumoniae</i> TIGR4	–	–	+	–	–	–	–	+	^b
<i>S. pneumoniae</i> R6	–	–	+	–	–	–	–	+	+
<i>M. pneumoniae</i>	–	–	–	–	–	–	–	–	+
<i>B. subtilis</i>	–	–	+	–	+	–	–	+	+
<i>T. pallidum</i>	–	–	+	–	–	–	+	+	–
<i>D. radiodurans</i>	–	–	+	–	+	–	+	+	+
<i>S. coelicolor</i>	–	–	+	–	+	+	–	+	–

Refer to Table 2 for the full name of the bacteria in this table. endo, endoribonuclease; exo, exoribonuclease. Groups are divided based on the presence of different RNase E/G proteins.

^aProtein belongs to the RNase E/G family, more like RNase E.

^bExoribonuclease of the RNR family, more like RNase R.

compasses the N-terminal half of the *E. coli* RNase E and almost the full length of the *E. coli* RNase G (Taraseviciene et al 1995; McDowall and Cohen 1996; Bycroft et al. 1997). Most of these bacteria also have RNase PH and D. This group of bacteria belongs to the proteobacteria α -subdivision and actinobacteria. Group III bacteria clearly have RNase G but not E, since the enzymes are much closer to RNase G in size and sequence. Group II bacteria are present in a wider range of eubacterial families, including actinobacteria, chlamydiae, clostridia, aquificales, thermotogales, and the Chlorobium–Flavobacterium group. Group IV has neither RNase E nor G. Bacteria in Group IV are widely present in actinobacteria, the proteobacteria ϵ -subdivision, spirochaetales, firmicutes (bacillales mollicutes lactobacillales), and the Thermus–Deinococcus group. Very few bacteria in the third and fourth groups have RNase PH or D. In contrast to the tRNA-specific exoribonucleases, the nonspecific exoribonucleases RNase II, R, and PNPase are present indistinguishably among all groups. PNPase is present in all bacteria in this list except the two mycoplasmas. RNase R is present in most of the bacteria, and is much more frequently found than RNase II, in agreement with previous observations (Zuo and Deutscher 2001). RNase Z is present in most bacteria in Table 3, and its presence does not seem to be different among the four groups.

AUE abundance in tRNA 3' trailers among genomes with different percentages of GC

As described above, the abundance of AU-rich elements in tRNA 3' trailers varies among bacterial genomes. Analysis of nucleotide frequencies in *E. coli* tRNA 3' trailers suggested that adenine and uridine are most abundant within the first 10 nt downstream of tRNA. AUEs are present in the first 10 nt of the 3' trailers in most *E. coli* tRNA precursors. Therefore, we focused our analysis on the comparison of AUE abundance in the first 10-nt region (R_1) and the

average AUE abundance of the other nine regions ($\bar{R}_{2-10}$) in the 3' trailers.

Through random variation, AUE is expected to exist at a higher abundance in genomes with a lower percentage of G and C (GC%). We plotted AUE abundance (R_1 and $\bar{R}_{2-10}$) in parallel to GC percentage in order to discriminate the effect of GC% and selective conservation on AUE abundance. In Figure 4, bacteria are presented on the X-axis with increasing GC%. R_1 and $\bar{R}_{2-10}$ were plotted separately. $\bar{R}_{2-10}$ decreases as GC% increases. Although the curve of $\bar{R}_{2-10}$ is not smooth, an inverse correlation is clearly present between $\bar{R}_{2-10}$ and GC percentage. The distribution of $\bar{R}_{2-10}$ is consistent with the notion that AUEs in the region 11–100 nt in the 3' trailers occur in a relatively random manner. This finding suggests that their relationship to tRNA maturation, if any, is weak.

In contrast, R_1 shows a very interesting pattern when GC% increases. At low GC percentages, R_1 is not distinguishable from $\bar{R}_{2-10}$. However, the two curves separate as GC% increases. At higher GC%, R_1 is lower in some genomes, but stays high in many others. In a few genomes with >60% GC, R_1 is as high as that in genomes with <40% GC. This indicates that AUE in the first 10 nt is strongly conserved in tRNA precursors in certain high-GC bacteria. This behavior is not related to transcription termination since no U residues in the first 10 nt are from the terminator. Instead, the conserved high abundance of AUE im-

mediately after tRNA is most likely related to tRNA processing.

AUEs are highly conserved immediately downstream of tRNA in organisms with RNase E and tRNA-maturation exoribonucleases

To further investigate the relationship between AUE abundance and tRNA processing, we have studied AUE abundance within the four groups of bacteria that have different RNase profiles (Table 3). AUE abundance in the first region (R_1) and the average of the next nine regions ($\bar{R}_{2-10}$) was plotted in the order of increasing GC percentages for each group of bacteria. As shown in Figure 5, the difference of R_1 and $\bar{R}_{2-10}$ now is shown almost exclusively in Group I and II bacteria having RNase E or E-like enzymes.

In Group I bacteria, R_1 stays high across the group, whereas $\bar{R}_{2-10}$ drops quickly as GC% increases. The high-GC bacterium *C. crescentus* CB15 is exceptional in that R_1 is lower but is still much higher than $\bar{R}_{2-10}$. The result indicates that AUEs are selectively conserved close to the 3' end of tRNA in all members of this group. This is consistent with the idea that RNase E may play a major role in tRNA 3' processing in these bacteria.

Group II bacteria show a mixed pattern. As GC% increases, $\bar{R}_{2-10}$ drops in the same way as in Group I. R_1 stays high in half of the bacteria. However, in other members of this group, R_1 decreases and is not different from $\bar{R}_{2-10}$. Therefore, it appears that only some members of Group II resemble Group I bacteria.

In order to understand the mixed pattern of R_1 in Group II bacteria, we have compared RNase E and E-like enzymes in Group I and II by sequence alignment (Fig. 6). Interestingly, RNase E proteins in Group I all have similar features, i.e., close in sizes and very similar in sequences to the N-terminal half of *E. coli* RNase E. The RNase E-like enzymes in Group II, however, are divided into two types. Type I has an insert of varying lengths within the N-terminal catalytic domain, interrupting the S1 RNA-binding domain. Type II has a long N-terminal sequence in front of the continuous catalytic domain. Remarkably, all the Group II bacteria with high R_1 have Type I enzymes, whereas the bacteria with low R_1 have Type II enzymes. This strongly suggests that Type I enzymes function like RNase E in tRNA processing, depending on the selectively conserved AUE immediately downstream of tRNA. The role of Type II en-

FIGURE 4. The abundance of AU-rich elements (AUEs) in 3' trailers of bacterial tRNA as an effect of GC percentage. AUE abundances in the first 10-nt region after tRNA (R_1) and the average of abundance in the nine regions downstream ($\bar{R}_{2-10}$) are plotted for 51 bacteria. The bacteria on the X-axis are arranged with increasing GC%.

FIGURE 5. tRNA 3'AUE abundance in bacterial groups harboring different RNases. Bacteria are divided into four groups based on the presence or absence of different RNase E/G proteins, as demonstrated in Table 3. AUE abundances are plotted the same way as in Figure 4, within each group of bacteria marked on top of each graph. RNase profiles for the groups are described in Table 3. (●) AUE abundance in the first region (R_1). (○) The average AUE abundance in the nine regions downstream (R_{2-10}). (Δ) GC percentage.

zymes in tRNA 3' maturation, if any, may not be related to AU-rich elements. It should be noted that all bacteria with Type I enzymes belong to the α -subdivision of proteobacteria, whereas those with Type II enzyme are members of the actinobacteria.

Group III and IV bacteria show essentially the same pattern: both R_1 and R_{2-10} decrease with increasing GC%. This indicates that AUE is generally not selectively conserved in Group III and IV bacteria. A Group III bacterium, *Aquifex*

aeolicus VF5, shows a much higher R_1 than R_{2-10} , suggesting a possible selective pressure for 3' proximal AUE in this organism. Alternatively, it is likely that its R_{2-10} is unusually lower than those with similar GC% (Fig. 5) for unknown reasons. Interestingly, an activity has been demonstrated in *A. aeolicus* that cleaves AU-rich sequences in tRNA 3' trailers in an in vitro processing reaction (Willkomm et al. 2002). It remains to be determined if RNase G confers this activity, and if it plays a role in tRNA 3' maturation in this bacterium.

It should be noted that GC percentages also segregate among the bacterial groups. The majority of high GC bacteria fall into Group I and II. Only one organism in each of Groups I and II has a GC percentage <40%. In contrast, most low-GC bacteria belong to Groups III and IV. For the few high GC% organisms in these groups, both R_1 and R_{2-10} drop sharply as GC increases. R_{2-10} drops steadily above ~45% GC (Fig. 5), indicating that selective pressure is required to keep high AUE abundance in bacteria with GC of >45%. R_1 is high in all the low-GC bacteria. It remains to be determined if the high R_1 in Groups III and IV is important for tRNA 3' processing by enzyme(s) other than RNase E.

DISCUSSION

Our analysis has shown that RNase E and the Type I E-like proteins are present in the proteobacteria α -, β -, and γ -subdivisions. These bacteria also have an abundant AU-rich element (AUE) identified immediately downstream of their tRNA-coding sequences. The abundance of AUE in the distal regions of 3' trailers becomes much lower when GC% increases. In contrast, AUE present within 10 nt downstream of tRNA is selectively conserved even in genomes of high GC%. Selective conservation of the proximal AUE is almost exclusively observed in the aforementioned bacteria. It has been previously shown that in *E. coli*, RNase E is responsible for the maturation of a number of tRNAs by cleaving at AU-rich sequences in the 3' trailers (Li and Deutscher 2002; Ow and Kushner 2002). RNase E cleavages within the first 10 nt (R_1) may be responsible for the processing of most tRNAs in *E. coli* since AUEs are highly present in this region. Cleavages also happen at downstream AUEs (Fig. 2), and can be important for tRNA processing if no cleavage occurs in R_1 for a pre-tRNA. The general importance of AUEs in R_1 for tRNA processing is further demonstrated by its selective conservation in bacteria having RNase E. Together, the results demonstrate that RNase E co-evolves with its potential cleavage sites, i.e., AUEs im-

FIGURE 6.—Alignment of RNase E and E-like proteins. The alignment was constructed based on BLAST pairwise alignments of each protein with the *E. coli* RNase E with E-values shown. Black bars represent highly homologous regions. Gray bars are regions showing no homology to *E. coli* RNase E by BLAST. Dashed lines show regions missing in those proteins. Hatched bars represent the conserved catalytic domain of RNase E/G enzymes with S1 domain represented in trellis pattern (Diwa et al. 2002). As a comparison, RNase G from *E. coli* K12 was included in the alignment.

mediately downstream of the tRNA. This work strongly suggests that RNase E is generally important for tRNA 3' processing in the above bacteria.

It should be noted that the definition of AUE is quite arbitrary since RNase E cleavage sites do not have a known sequence consensus. However, we find that the AUE designation is useful in finding sequence features for RNase E-dependent tRNA processing. A closer examination of the content of AUEs may be helpful to better decipher RNase E cleavage sites. Furthermore, neighboring structural features have been shown to be important for RNase E cleavage (for review, see Li and Deutscher 2004). It remains to be elucidated if tRNA itself and any other sequence or structures surrounding AUEs play roles for determination of RNase E cleavage sites in the 3' trailers. The conservation of AUEs proximal to tRNA 3' ends adds another example to the list of conserved sequence elements important for RNA processing. Conservation of nucleotides in the 3' trailer that is important for tRNA processing was also described in mammalian systems (Nashimoto 1997).

Interestingly, as described above, the tRNA-specific exoribonucleases also co-evolve with RNase E. It is therefore likely that in bacteria having these enzymes, RNase E usually cleaves a few nucleotides downstream of tRNA, and that the extra residues are removed by exoribonucleases. Therefore, RNase E-containing bacteria may use a pathway for tRNA processing very similar to that in *E. coli* (Fig. 1A).

We are prompted to ask if the sequences downstream of tRNA also affect exonucleolytic trimming reactions. RNase T, one of the major tRNA maturation exoribonucleases, has been shown to preferentially remove 3' terminal residues in the order of $A \approx G > U \gg C$ (Zuo and Deutscher 2002). It has also been demonstrated that in vivo, RNase PH is the most efficient enzyme for removal of C at the 3' +2 position of pre-tRNA₁^{Tyr} (Li and Deutscher 1996). U is most abundant at positions +2 to +6 of *E. coli* tRNAs (Fig. 3), and can be removed efficiently by RNase T or PH under normal growth conditions (Li and Deutscher 1996). The base specificity of other tRNA-processing exoribonucleases has not yet been defined. It is likely that no individual exoribonuclease can trim all the extra residues efficiently enough to support optimal cell growth. On the other hand, RNase T's ability to efficiently remove A residues results in the loss of the 3' terminal A in a fraction of mature tRNAs, and the A is added back by tRNA nucleotidyltransferase (Deutscher 1990). Therefore, multiple exoribonucleases and tRNA nucleotidyltransferase act together for generating mature 3' ends of tRNA in *E. coli* (Reuven and Deutscher 1993; Li and Deutscher 1994, 1996), and they may also be required in other bacteria using this pathway.

RNase E-like enzymes were identified in our search. These enzymes resemble RNase E more than G. However, sequence alignments show two different types of arrangement of protein segments among the E-like enzymes: Type I with inserts in the catalytic domain and Type II with a

large domain at the N terminus (Fig. 6). Since AUEs are abundant immediately downstream of tRNA in bacteria having Type I but not Type II proteins, Type I proteins may function in tRNA processing similarly to RNase E. This finding also predicts that the two types of proteins may have different activities. Such an alignment provides a way of distinguishing RNase E/G-like proteins.

Despite the importance of RNase E in tRNA maturation in *E. coli*, this enzyme, and the Type I E-like proteins, are only present in three subdivisions of proteobacteria. RNase Z may function in tRNA processing in many of the other bacteria, such as *B. subtilis* and *T. maritima* (Fig. 1). However, the substrate specificity of RNase Z (3' tRNase) in *B. subtilis* has been shown to be different from that in *T. maritima* (Pellegrini et al. 2003; Minagawa et al. 2004). In addition, RNase Z does not seem to be important for tRNA maturation in *E. coli* (Minagawa et al. 2004; Schilling et al. 2004). Therefore, it is difficult to predict the role of RNase Z in tRNA processing in other bacteria. RNase G and Type II RNase E-like proteins are found in some bacteria without RNase E. It remains to be determined if these enzymes play roles in tRNA processing in these organisms. Furthermore, the Group IV bacteria *Mycoplasma pulmonis*, *Mycoplasma pneumoniae*, and *Helicobacter pylori* do not have any of the endoribonucleases discussed above. These bacteria also lack the tRNA-specific exoribonucleases. It is likely that tRNA 3' processing in these bacteria uses a mechanism yet different from any of the ones described in Figure 1. For instance, the 3' trailers may be removed solely by the previously known nonspecific exoribonucleases (RNase R, II, or PNPase). Finally, it will be interesting to learn if there are sequence features important for tRNA 3' processing by the other enzymes.

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