An unexpected absence of queuosine modification in the tRNAs of an *Escherichia coli* B strain

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The post-transcriptional processing of tRNAs decorates them with a number of modified bases important for their biological functions. Queuosine, found in the tRNAs with GUN anticodons (Asp, Asn, His, Tyr), is an extensively modified base whose biosynthetic pathway is still unclear. In this study, it was observed that the tRNA^{Tyr} from *Escherichia coli* B105 (a B strain) migrated faster than that from *E. coli* CA274 (a K-12 strain) on acid urea gels. The organization of tRNA^{Tyr} genes in *E. coli* B105 was found to be typical of the B strains. Subsequent analysis of tRNA^{Tyr} and tRNA^{His} from several strains of *E. coli* on acid urea gels, and modified base analysis of tRNA preparations enriched for tRNA^{Tyr}, showed that *E. coli* B105 lacked queuosine in its tRNAs. However, the lack of queuosine in tRNAs was not a common feature of all *E. coli* B strains. The *tgt* and *queA* genes in B105 were shown to be functional by their ability to complement *tgt* and *queA* mutant strains. These observations suggested a block at the step of the biosynthesis of preQ₁ (or preQ₀) in the B105 strain. Interestingly, a multicopy vector harbouring a functional *tgt* gene was toxic to *E. coli* B105 but not to CA274. Also, in mixed cultures, *E. coli* B105 was readily competed out by the CA274 strain. The importance of these observations and this novel strain (*E. coli* B105) in unravelling the mechanism of preQ₁ or preQ₀ biosynthesis is discussed.

Keywords: *tyrT-tyrV*, *tyrU*, preQ₁, preQ₀, acid urea gels

INTRODUCTION

Transfer RNAs contain a variety of modified nucleosides, of which at least 80 have been catalogued so far (McCloskey *et al.*, 1998; Rozenski *et al.*, 1999). One of the most elaborately modified nucleosides among these is queuosine (7-{5-[(4S,5R-dihydroxy-2-cyclopenten-1S-yl)amino]methyl}-7-deazaguanosine). Introduction of queuosine into tRNAs is initiated by a transglycosylation reaction wherein a transglycosylase (Tgt) exchanges the guanine base at the first position of the GUN anticodon in tRNAs (Asp, Asn, His, Tyr) with 7-aminomethyl-7-deazaguanine (preQ₁), a precursor of the base (queuine) of queuosine (Q). Subsequently a substituted cyclopentenyl group is attached to preQ₁ in tRNA by *S*-adenosylmethionine-tRNA ribosyltransferase/isomerase (QueA). The resultant epoxy-

queuosine (oQ), 7-{5-[(2,3-epoxy-4,5-dihydroxycyclopent-1-yl)amino]methyl}-7-deazaguanosine, is ultimately converted into queuosine by an as yet unrevealed enzyme that requires vitamin B₁₂. Besides preQ₁, Tgt can also use 7-cyano-7-deazaguanine (preQ₀), another deazaguanine compound, as substrate. In *Escherichia coli*, both QueA and Tgt are monomers, of 39 and 46 kDa, respectively, and are encoded from a single operon (Slany & Kersten, 1994).

The queuosine in tRNAs has been proposed to modulate the interaction between the anticodon and the degenerate codons (Meier *et al.*, 1985), and shown to be important for survival of *E. coli* in the natural environment (Noguchi *et al.*, 1982). Interestingly, in *Shigella flexneri*, *vacC*, a virulence-associated chromosomal locus, is homologous to *tgt* of *E. coli* and a *vacC* mutant is complemented by *tgt* from *E. coli* (Durand *et al.*, 1994). The mutations in *vacC* in *S. flexneri* result in the loss of pathogenicity, suggesting *tgt* as a possible drug target for shigellosis (Gradler *et al.*, 2001). On the

Abbreviations: preQ₀, 7-cyano-7-deazaguanine; preQ₁, 7-aminomethyl-7-deazaguanine.

other hand, many tumours and neoplastic cell lines are deficient in queuosine modification (Aytac & Gunduz, 1994; Harada & Nishimura, 1972; Okada *et al.*, 1979; Randerath *et al.*, 1984). It has been suggested that queuosine modification in tRNA regulates protein synthesis and influences cellular growth and differentiation in tumour cells (Morris *et al.*, 1999). The eukaryotes are unable to carry out *de novo* synthesis of queuine, and as a nutritional factor, queuine has also been suggested to modulate receptor tyrosine kinases (Langgut, 1995). As the eukaryotes obtain queuine through diet or the gut microflora (Slany & Kersten, 1994), its analogues could serve as important therapeutic agents to treat cancer or related ailments (Nishimura, 1972; Nishimura *et al.*, 1983).

Despite such crucial biological implications of queuosine modification in the tRNAs, the details of the biosynthesis of preQ₁ (or preQ₀) base are largely unknown. Availability of well-characterized mutants is an important tool in exploring the complex biochemical steps in the biosynthetic pathways. During the course of our studies on structure–function relationship of initiator tRNA, we discovered that an isolate of *E. coli* (*E. coli* B105) lacked queuosine modification. Further characterization of this strain suggests that the lack of queuosine in tRNAs in this strain is due to some defect(s) at the step(s) prior to the transglycosylation step. Using this novel strain, it should now become possible to gain insights into the steps involved in the *de novo* biosynthesis of the precursors of the base of queuosine.

METHODS

Bacterial strains and growth media. The strains (Table 1) were cultured in Luria–Bertani (LB) broth (1% tryptone, 0.5% yeast extract and 1% sodium chloride, w/v) or in solid LB (containing 1.5% agar). When needed, antibiotics were used at the following concentrations: ampicillin, 100 µg ml⁻¹; and kanamycin, 25 µg ml⁻¹ (Sambrook *et al.*, 1989).

Isolation of tRNAs and Northern blot analysis. The tRNAs were isolated under cold and acidic conditions to preserve the ester linkage between the amino acid and tRNA, electrophoresed on 6.5% polyacrylamide gels containing 8 M urea under acidic conditions (Varshney *et al.*, 1991), electroblotted onto nylon membranes and hybridized (Thanedar *et al.*, 2000) with 5′-³²P-end-labelled oligonucleotide. The probes were complementary to positions 29–47 of tRNA^{Met(CUA)}, 2–37 of tRNA^{Tyr} or 30–49 of tRNA^{His} (Sprinzl *et al.*, 1989). When needed, aminoacylated tRNAs were deacylated in 100 mM Tris/HCl (pH 9.0) at 37 °C for 15 min, recovered by ethanol precipitation and analysed on either acid urea or standard sequencing gels (Maxam & Gilbert, 1980; Varshney *et al.*, 1991).

Southern blot analysis. Genomic DNA (5 µg) from *E. coli* B105 (a B-strain) and *E. coli* CA274 (a K-strain) were digested with *Bst*EII or *Bst*NI following the supplier's instructions, resolved on agarose gel (0.9%), transferred onto nylon membrane under vacuum and hybridized with radiolabelled probe against tRNA^{Tyr} (Reed & Mann, 1985).

Cloning of the *tyrU* gene. The *tyrU* locus was PCR amplified from both the *E. coli* strains, CA274 and B105. Genomic DNA (200 ng) and 40 pmol each of the forward (5′-GGTCACGC-GTTCGATTCCGGTAG-3′) and reverse (5′-ACGGATCC-ATCGGTGATATCACC-3′) primers were used in PCR with Vent DNA polymerase (New England Biolabs). The reactions were subjected to initial incubation at 94 °C for 5 min followed by 30 cycles each consisting of 1 min at 94 °C, 1 min at 55 °C and 1.5 min at 68 °C. The PCR products were used for cycle sequencing; the one from B105 was cloned into pTrc99C and referred to as pTrc_{tyrU}.

Cloning of *queA* and *tgt* genes and generation of an internal deletion in *tgt* (Δ *tgt*). The *queA* and *tgt* cistrons from *E. coli* CA274 and *E. coli* B105 were PCR amplified using 200 ng genomic DNA, 40 pmol each of forward (5′-AGAATTCA-TCGATTATATTCTATCC-3′) and reverse (5′-CGAATTC-AAAGACATCGGGCTA-3′) primers and *Pfu* DNA polymerase (Promega). After initial denaturation at 94 °C for 5 min, DNAs were amplified in 30 cycles each consisting of 1 min at 94 °C, 1 min at 45 °C and 5 min at 68 °C. The 2.5 kb PCR products from B105 and CA274 were eluted from agarose gel, purified, digested with *Eco*RI and cloned into pTZN (a pTZ19R-derived vector) linearized with *Eco*RI, and referred

Table 1. Strains used in the study and their genotypes

<i>E. coli</i> strain	Genotype	Reference
CA274	Hfr, <i>lacZ</i> 125(Am) <i>trpA</i> 49(Am) <i>relA</i> 1 <i>spoT</i>	Brenner & Beckwith (1965)
CA275	Hfr, <i>lacZ</i> 125(Am) <i>trpA</i> 49(Am) <i>relA</i> 1 <i>spoT</i> 1 <i>tyrT</i> 66(As)	Brenner & Beckwith (1965)
B105	An <i>E. coli</i> B strain r _B ⁻ m _B ⁻ Gal ⁻ RecA ⁺	Mandal & RajBhandary (1992)*
MRE600	F ⁻ , <i>rna</i>	Gay (1984)
MG1665 (= DSM 3901)	A wild-type K-12 strain	Cayley <i>et al.</i> (1989)
BL21(DE3)	F ⁻ , <i>hsdS</i> _B (r _B ⁻ m _B ⁻) <i>gal</i> (<i>dcm</i>) <i>ompT</i> [<i>lon</i>]	Studier & Moffatt (1986)
KL16	<i>relA</i> 1 <i>spoT</i> 1 <i>thi</i> 1	Low (1968)
HB101	F ⁻ Δ (<i>gpt-proA</i>)62 <i>leuB</i> 6 <i>supE</i> 44 <i>ara</i> -14 Δ (<i>mcrC-mrr</i>) <i>rpsL</i> 20 <i>xyl</i> -5 <i>mtl</i> -1 <i>recA</i> 13 <i>lacY</i> 1 <i>galK</i> 2	Messing (1981)
JE7334	<i>lacY</i> <i>thi</i> <i>xyl</i> <i>metE</i> <i>ara</i>	Noguchi <i>et al.</i> (1982)
JE7336	<i>tgt</i> <i>lacZ</i> <i>xyl</i> <i>thi</i> <i>trp</i> <i>leu</i>	Noguchi <i>et al.</i> (1982)
JE10651	<i>queA</i>	Reuter (1991)

* First cited reference of the *E. coli* B105 strain.

to as pTZ*queA-tgt*(B105) and pTZ*queA-tgt*(CA274), respectively. Another construct, pTZ*queA-tgt1*(CA274), was similar to pTZ*queA-tgt*(CA274), except that it contained the *queA-tgt* insert in the opposite orientation. pTZ*queA-tgt*(CA274) was digested with *EcoRV* to excise an internal segment (~200 bp) of *tgt* and religated to generate pTZ*queA-Δtgt*(CA274).

Modified base analysis. Exponentially growing cells of *E. coli* KL16 or B105 harbouring pTrc*tyrU*(B105) were harvested and metabolically labelled with [³²P]orthophosphate in low-phosphate medium (Seong & RajBhandary, 1987; Thanedar *et al.*, 2001). The total tRNAs were fractionated on 15% polyacrylamide gels under non-denaturing conditions. The region in the gel corresponding to tRNA^{Tyr} was localized with the help of ethidium bromide staining of the gel and the marker tRNA^{Tyr} (a gift from Dr U. L. RajBhandary, Biology Department, Massachusetts Institute of Technology, Cambridge, USA), cut out and eluted in 5 × TE containing 5% phenol and 1 M LiCl. The tRNA preparation (enriched for tRNA^{Tyr}) was further purified by three steps of phenol extraction.

The labelled tRNA^{Tyr} (25000 c.p.m.) was mixed with 1 μg tRNA^{Tyr} and digested with 1 unit RNase T2 in 20 mM ammonium acetate buffer (pH 5.0) at 37 °C for 5 h, then subjected to repeated drying under vacuum to remove traces of ammonium acetate. The dried sample was resuspended in 3–4 μl water and spotted on cellulose sheets for two-dimensional chromatography (Noguchi *et al.*, 1982). The thin-layer chromatography was carried out using isobutyric acid/0.5 M ammonia 5:3 (v/v) solvent for the first dimension for 15–18 h until the solvent reached the top of the plate. The plates were dried for 12 h and then run in the perpendicular direction in the second solvent system (2-propanol/water/HCl, 14:3:3, by vol.) (Nishimura, 1972), air-dried and subjected to autoradiography using a BioImage analyser (Fuji Film BAS1800).

Complementation studies. The constructs (pTZ*queA-tgt* of CA274 or B105 origin) or the vector control (pTZN) were transformed into *tgt* mutant (JE7336), *tgt*⁺ (JE7334) or *queA* mutant (JE10651) strains. The tRNA preparations were analysed by Northern blotting.

Growth competition experiments. The cultures of *E. coli* CA274 and B105 were started with 0.25% inoculum from overnight cultures in 25 ml LB broth and grown at 37 °C with shaking. Samples were taken periodically to read OD₆₀₀. In the growth competition assays, overnight cultures of *E. coli* CA274 and B105 were mixed in equal volumes (zero subculture) for use as inoculum and grown to saturation (first subculture). Inoculum from the first subculture was used to obtain the second subculture; and an inoculum from the second subculture yielded the third subculture. The abundance of CA274 and B105 in the cultures was determined by dilution plating on MacConkey's agar. *E. coli* CA274 with an amber mutation in the *lacZ* gene does not ferment lactose in the medium and produces white colonies, whereas *E. coli* B105, which utilizes lactose, grows as pink colonies.

RESULTS

The tRNA^{Tyr} from *E. coli* B105 migrates faster than that from *E. coli* CA274 on high-resolution polyacrylamide urea gels

Use of acid urea gels (Varshney *et al.*, 1991) in our studies on the structure–function relationship of initiator tRNAs revealed that the Tyr-tRNA^{Tyr} from *E. coli* B105, a B strain, migrated faster than that from *E.*

coli CA274, a K strain (Fig. 1a). In contrast, the aminoacyl- and formylaminoacyl- forms of the initiator tRNA from the two strains co-migrated (compare the fGln- and Gln-tRNA₂^{fMet(CUA)} bands in lanes 1–4 with lanes 5–8). Although a detailed description of the various forms of tRNA₂^{fMet(CUA)} mutants is not justified here, they serve as a control to rule out deacylation of the tRNA preparations from the B105 strain. To further examine this mobility difference, the total tRNAs (deacylated) from *E. coli* CA274 and B105 were analysed by urea-PAGE under completely denaturing conditions (Fig. 1b: compare lanes 1 and 2 with lanes 3 and 4). Consistent with the observation in Fig. 1(a), the tRNA^{Tyr} from strain B105 migrated faster than that from CA274. We reasoned that this mobility difference could be due either to a very small insertion/deletion in tRNA^{Tyr}, or to differential post-transcriptional processing in the two strains.

Organization of tRNA^{Tyr} genes, and sequence analysis of *tyrU* from *E. coli* B105 and CA274

In *E. coli*, tRNA^{Tyr} is encoded by the *tyrT-tyrV* (27 min) and *tyrU* (79 min) loci (Fig. 2a). The *tyrT-tyrV* locus is transcribed from a single promoter, and in *E. coli* K strains this locus contains a 3.14 × repeat of a 178 bp element, downstream of the *tyrV* gene (panel i). Interestingly, in the B strains, there is a deletion of 357 bp in the repeat region (panel ii) (Timms & Bridges, 1996). These differences in the K and B strains warranted a study of the organization of tyrosine tRNA genes in the CA274 and B105 strains by Southern blot analysis using two diagnostic restriction endonucleases, *Bst*EII and *Bst*NI (Fig. 2b). The bands of 2.2 kb in the *Bst*EII digest (lanes 1 and 2) and 0.5 kb in the *Bst*NI digest (lanes 3 and 4) show that the position of the *tyrU* locus is conserved in both the strains (Fig. 2a, panel iii). The bands of ~1.4 and 1 kb seen in the *Bst*EII digest (lanes 1 and 2) are also consistent with the expected pattern for the *tyrT-tyrV* genes of the K and B strains, respectively. The bands of ~0.9, 0.68 and 0.5 kb in the *Bst*NI digest (lane 3) agree with the sizes of the *tyrV*, *tyrT* and *tyrU* genes in the K strain. Interestingly, for the B105 strain, while the sizes of the 0.68 and 0.5 kb bands for the *tyrT* and *tyrU*, respectively, were conserved, the *tyrV* (~0.56 kb, lane 4) showed a deletion of ~0.35 kb from the 0.9 kb band (compare *tyrV* bands in lanes 3 and 4). Taken together, these observations reveal that the overall organization of tRNA^{Tyr} genes in *E. coli* CA274 and B105 is typical of the K and B strains, respectively.

The deletion in the 3.14 × repeat downstream of *tyrV* in *E. coli* B has been proposed to be responsible for the instability of the nascent transcripts arising from this locus (Timms & Bridges, 1996). Hence, in *E. coli* B, most of the tRNA^{Tyr} originates from the *tyrU* gene. Therefore, for a detailed analysis, we PCR amplified the *tyrU* genes from both the strains and sequenced them. The sequences of *tyrU* from both the strains were identical to each other and to the reported sequence of *tyrU* (NCBI accession number X04181; GenBank accession number gi:43228), ruling out mutations in the tDNA as a

Fig. 1. Differential migration of Tyr-tRNA^{Tyr} from *E. coli* B105 and CA274 strains on high-resolution gels. Total tRNA was prepared from *E. coli* B105 and CA274 harbouring mutant initiator tRNA (U35A36) genes under cold and acidic conditions and analysed on 6.5% acid urea gel (a) or a standard sequencing gel after deacylation (b), transferred to nytran membranes and hybridized with 5'-³²P-end-labelled oligonucleotides specific for tRNA^{Tyr} and tRNA₂^{fMet(CUA)} (a) or tRNA^{Tyr} alone (b). Autoradiograms are shown. Lanes 1–4 in (a), and 1 and 2 in (b), represent samples from *E. coli* B105; lanes 5–8 in (a), and 3 and 4 in (b), represent samples from *E. coli* CA274. (Note: because of the U35A36 mutation, the initiator tRNAs are aminoacylated with glutamine.)

Fig. 2. (a) A schematic representation of the tyrosine tRNA genes in *E. coli*. The *tyrT* locus contains tandemly duplicated genes *tyrT*-*tyrV* followed by a 3.14 × repeat element of 178 bp in the K strains (panel i). In *E. coli* B strains (panel ii) this repeat element suffers a deletion of 357 bp (Timms & Bridges, 1996). The *tyrU* locus (panel iii) is conserved in both the strains. Sizes of the expected fragments upon digestion with *BstEII* and *BstNI* are as shown. (b) Southern blot analysis: Genomic DNA from *E. coli* B105 and CA274 was digested with *BstEII* and *BstNI*, resolved on 0.9% agarose gel, transferred to nytran membrane and hybridized to ³²P-labelled probe against tRNA^{Tyr}. An autoradiogram is shown. Sizes and the identities of various hybridizing DNA fragments are indicated on the right. (c) Overexpression of *tyrU* in *E. coli* B105 and CA274. Total tRNA from the transformants of *E. coli* CA274 (lanes 1–3) or B105 (lanes 4–6) harbouring either pTrc99C vector alone or pTrctyrU as indicated was fractionated on a sequencing gel and processed for Northern blot analysis using a ³²P-labelled tRNA^{Tyr}-specific probe. Lanes 2 and 3, and lanes 5 and 6, represent independent clones transformed with pTrctyrU.

Fig. 3. Northern analysis for tRNA^{His} and tRNA^{Tyr}. Total tRNA from various *E. coli* strains was prepared under cold and acidic conditions, fractionated on acid urea gels, transferred to nytran membranes and hybridized to ³²P-labelled probes against tRNA^{His} (a) or tRNA^{Tyr} (b).

possible reason for the mobility difference in tRNA^{Tyr} of the two strains.

Expression of the cloned *tyrU* gene in *E. coli* CA274 and B105

To gain further insights, we transformed pTrctyrU into *E. coli* CA274 and B105. The analysis of tRNAs showed that the introduction of *tyrU* gene on a plasmid resulted in overproduction of tRNA^{Tyr} in both the strains (Fig. 2c: compare lane 1 with 2 and 3, and lane 4 with 5 and 6). Interestingly, the bulk of the tRNA^{Tyr} from the CA274 strain still migrated slower than that from the B105 strain (compare lanes 2 and 3 with 5 and 6). Also, overproduction of tRNA^{Tyr} in CA274 resulted in a faint band just below the major band (lanes 2 and 3), which co-migrated with the tRNA^{Tyr} of the B105 strain. This is most likely due to lack of modification in a minor population of tRNA^{Tyr} under these conditions. Taken together, these observations strongly suggest that the mobility differences in tRNA^{Tyr} are not caused by mutation, and in all probability arise from strain-specific modifications.

Lack of queuosine modification in *E. coli* B105

Since the initiator tRNAs from the two strains co-migrated on the same gel (Fig. 1a), differential biosynthesis of 2'-O-methylcytidine (Cm), pseudouridine

Fig. 4. Modified base analysis of tRNA^{Tyr}. The *in vivo* ³²P-body-labelled tRNA from *E. coli* B105 (a) or KL12 (CA274) (b) strains was enriched for tRNA^{Tyr}, digested with RNase T2, analysed by two-dimensional chromatography and autoradiographed. The presence or absence of a spot corresponding to queuosine (Qp) is indicated by an arrow.

(ψ), dihydrouridine (D), thiouridine (S) and ribothymidine (rT), which are common to the tRNA^{Tyr} and the initiators (as well as other tRNAs) was unlikely to be responsible for the faster mobility of tRNA^{Tyr} from *E. coli* B105. We therefore focused our attention on queuosine modification, which is present in a set of tRNAs containing the GUN anticodon (encoding Tyr, His, Asp and Asn). The total tRNAs from several *E. coli* strains were fractionated on acid urea gels and probed for tRNA^{His} and tRNA^{Tyr} (Fig. 3a and b, respectively). Importantly, in this analysis we included *E. coli* CA275, which differs from the CA274 strain in that its *tyrT* locus

Fig. 5. (a) Complementation analysis using *E. coli* JE7334 (*tgt*⁺) or JE7336 (*tgt*) strains. The vector pTZN (lanes 1 and 2) or the constructs pTZqueA-*tgt*(B105) (lane 3) or pTZqueA-*tgt*(CA274) (lane 4) were transformed into *E. coli* JE7334 (lane 2) or JE7336 (lanes 1, 3 and 4). Total tRNA was prepared under cold and acidic conditions, fractionated on acid urea gel, transferred to nylon membranes and hybridized to ³²P-labelled probe against tRNA^{Tyr}. (b) Complementation analysis using *E. coli* JE10651 (*queA*). The deacylated (lanes 1–6) or acylated (lanes 7–12) total tRNA samples from various transformants were fractionated on acid urea gel and processed as in (a). An autoradiogram is shown. Lanes: 1 and 7, total tRNA from *E. coli* CA274 harbouring pTZN vector; 2 and 8, total tRNA from *E. coli* B105 harbouring pTZN vector; 3–6 and 9–12, total tRNA from *E. coli* JE10651 harbouring plasmids as indicated on top of the lanes.

Fig. 6. Biosynthetic pathway of queuosine modification in tRNAs in *E. coli*: Various known and unknown steps leading to queuosine incorporation into tRNAs with GUN anticodons are shown. Abbreviations are as follows: preQ₁, 7-aminomethyl-7-deazaguanosine; preQ₀, 7-cyano-7-deazaguanine; Tgt, tRNA guanine transglycosylase; QueA, S-adenosylmethionine-tRNA ribosyltransferase/isomerase; SAM, S-adenosylmethionine; oQ, epoxyqueuosine (7-[5-[(2,3-epoxy-4,5-dihydroxycyclopent-1-yl) amino] methyl]-7-deazaguanosine). PreQ₀ could be a precursor of preQ₁. While preQ₁ is the likely physiological substrate of Tgt, this enzyme also utilizes preQ₀ as substrate (reviewed by Slany & Kersten, 1994).

encodes a tRNA with CUA anticodon (an amber suppressor tRNA, *supF*).

Consistent with the prediction of the lack of queuosine modification, even the tRNA^{His} from *E. coli* B105 (Fig. 3a, lane 3) showed faster mobility. All other strains, including CA275, showed a single tRNA^{His} band comigrating with that from the CA274 strain. Remarkably, when the blot was probed for tRNA^{Tyr}, the *E. coli* strain CA275 (Fig. 3b, lane 2) showed two bands, the lower of which co-migrated with the band from *E. coli* B105. As in the CA275 strain, *tyrT* encodes tRNA^{Tyr} (*supF*), which cannot be modified at position 34 with queuosine (CUA anticodon); this observation was quite revealing and conveniently limited our investigation to the lack of queuosine modification in the B105 strain. Interestingly, it may be noted that the faster mobility of the tRNAs for His and Tyr was not seen for other *E. coli*

B strains such as BL21 and HB101, suggesting that the lack of the suspected (queuosine) modification was restricted to the B105 strain.

Modified base analysis

To confirm that the faster mobility of the His- and Tyr-specific tRNAs in the B105 strain was due to the lack/deficiency of queuosine modification, we carried out a modified base analysis (Noguchi *et al.*, 1982) of tRNA preparations enriched for tRNA^{Tyr} (Fig. 4). While a spot corresponding to queuosine (Qp) was seen in the control K strain (Fig. 4b), it was absent from the B105 strain even upon overexposure of the autoradiogram (Fig. 4a), confirming that the B105 strain lacked queuosine modification. However, other identifiable modified nucleosides were present commonly in both the strains.

Table 2. Number of transformants obtained upon introduction of various plasmids into *E. coli* CA274 and B105

Construct	No. of transformants obtained	
	<i>E. coli</i> CA274	<i>E. coli</i> B105
Vector alone (pTZN)	1116	796
pTZ <i>queA-tgt</i> (CA274)	792	0
pTZ <i>queA-tgt1</i> (CA274)	576	0
pTZ <i>queA-Δtgt</i> (CA274)	848	564
pTZ <i>queA-tgt</i> (B105)	476	0

The *queA* and *tgt* genes from *E. coli* B105 code for functional proteins

To investigate the reasons for the lack of queuosine modification in the B105 strain, we cloned the *tgt* and *queA* genes from both the B105 and CA274 strains. The two constructs and the vector alone were transformed into JE7336 (*tgt*), JE7334 (*tgt*⁺) and JE10651 (*queA*) strains. As expected, the Northern analysis showed that the Tyr-tRNA^{Tyr} from JE7336 (*tgt*) harbouring vector alone migrated faster than that from JE7334 (*tgt*⁺) (Fig. 5a: compare lane 1 with lane 2). However, introduction of pTZ*queA-tgt* constructs (of either B105 or CA274 origin) into JE7336 restored the slow mobility to the Tyr-tRNA^{Tyr} (compare lane 1 with lanes 3 and 4), suggesting that the *tgt* genes of the B105 and the CA274 strains encoded functional proteins. Similarly, while the difference in the mobility of the tRNA^{Tyr} prepared from the *queA* mutant (JE10651) and the wild-type strain (JE7334) is not as prominent as for the *tgt*⁺ versus *tgt* mutant strains (JE7334 vs JE7336), there is a small mobility difference, irrespective of the acylation status of the samples (Fig. 5b: compare lanes 1 and 2 with 6, and 7 and 8 with 12). Interestingly, the mobility of tRNA^{Tyr} isolated from JE10651 harbouring pTZ*queA-tgt*(B105) was identical to that of the tRNA^{Tyr} from CA274 (compare lane 4 with lane 1, and lane 10 with 7) and from JE10651 harbouring either *queA-tgt* or *queA-Δtgt* from CA274 (compare lane 4 with 3 and 5, and lane 10 with 9 and 11), indicating that the *queA* gene of B105 also encodes a functional protein. Since for queuosine modification in tRNA, the QueA step occurs after the Tgt step (Fig. 6), these data suggest that the defect in queuosine biosynthesis in the B105 strain must precede the Tgt step. Thus, the defect in B105 is most likely in the biosynthesis of preQ₁ (or preQ₀).

Overproduction of Tgt in *E. coli* B105 is toxic

To further investigate the consequences of the lack of Q-base modification, we wished to introduce the pTZ*queA-tgt* constructs into the B105 strain. As is clear from the number of transformants obtained in various experiments (Table 2), introduction of plasmids carrying *queA-tgt* genes, irrespective of their source [pTZ*queA-*

Fig. 7. (a) Growth curves of *E. coli* CA274 and B105 in LB medium. (b) Growth competition assay using mixed inoculum of *E. coli* CA274 and B105. Total viable counts corresponding to *E. coli* B105 and CA274 were determined by dilution plating on MacConkey's agar. The viable counts of the strains individually and in total are shown in the histogram. For details see Methods.

tgt(B105) or pTZ*queA-tgt*(CA274)] or orientation [pTZ*queA-tgt*(CA274) or pTZ*queA-tgt1*(CA274)], into *E. coli* was toxic to the B105 strain, but not to the CA274 strain. However, when we used a construct (pTZ*queA-Δtgt*) containing an internal deletion in the *tgt* gene, the construct was no longer toxic to the B105 strain (Table 2), suggesting that expression of Tgt from a multicopy vector is toxic to the B105 strain.

Growth curve and competition studies

Since the lack of queuosine in tRNAs in strain B105 was unexpected, we wished to study the fate of *E. coli* B105 in mixed-culture growth experiments (see Methods). The growth curves of the individually grown strains were very similar (Fig. 7a). However, when we carried out sequential subculturing from a mixed inoculum, we noticed that even though the mixed cultures were started with a slight advantage to B105 (as seen from the higher total viable counts for B105 in the inoculum for the zero

subculture, Fig. 7b), this strain rapidly declined in favour of CA274 in the subsequent subcultures. While it remains to be established that the lack of queuosine modification in B105 contributes to its lesser fitness in this experiment, the observation is consistent with the reports that *E. coli* strains lacking queuosine in their tRNAs do not compete well with their counterparts containing this modification (Noguchi *et al.*, 1982; Frey *et al.*, 1989).

DISCUSSION

In this study, we show that *E. coli* B105 lacks queuosine modification in its tRNA. Our observation may appear in conflict with an earlier report on the presence of an intact pathway for queuosine modification in *E. coli* B (Harada & Nishimura, 1972). However, it may be noted that the lack of queuosine modification was restricted to the B105 strain, and not a common feature of all B strains (Fig. 3). Furthermore, as demonstrated by the various complementation analyses using the *tgt* and *queA* mutants of *E. coli*, both the *tgt* and *queA* genes in the B105 strain encode functional proteins. These analyses have also shown that the lack of queuosine modification is most likely due to some defect(s) at step(s) prior to the Tgt step. In fact, the steps leading to biosynthesis of the precursors of the base of queuosine utilized as substrate by Tgt are entirely unknown. We believe that the availability of the B105 strain provides an important genetic system towards identifying the enzymic activities involved in these steps. Interestingly, expression of Tgt in *E. coli* B105 from a multicopy vector was found to be toxic. A possible explanation for this observation could be that Tgt cleaves the N-glycosidic bond between the G34 and the ribose sugar of the tRNAs with GUN anticodons, leading to depurination. In the absence of preQ₁ or preQ₀, the covalent complex formed between the tRNA and Tgt (Romier *et al.*, 1996) may sequester a significant amount of the substrate tRNAs, especially the low-abundance ones, and make them unavailable for protein synthesis.

The biosynthesis of preQ₁ or preQ₀ in bacteria is thought to result from the action of GTP-cyclohydrolase-like enzyme on GTP (Fig. 6). However, the two enzymes presently known to possess such activity (cyclohydrolase I and cyclohydrolase II) in *E. coli* are involved in pteridine and riboflavin biosyntheses, respectively, and their role in the biosynthesis of preQ₁ or preQ₀ has been ruled out. Moreover, a mutant of *E. coli* (*ribA*) defective in cyclohydrolase II still had queuosine modification in its tRNA (Slany & Kersten, 1994). In fact, the only mutations that have been characterized so far in the pathway leading to queuosine modification in tRNA are at the steps of Tgt and QueA (Noguchi *et al.*, 1982; Reuter *et al.*, 1991). The *E. coli* B105 strain thus provides a tool to investigate the very early steps of biosynthesis of the precursor(s) of queuosine base. Importantly, since synthesis of the queuosine base is unique to prokaryotes (Slany & Kersten, 1994), detailed knowledge of the enzyme(s) involved at this step could make it a novel target to design a new class of antibacterials.

Tgt overproduction is tolerated in *E. coli* CA274 but not in *E. coli* B105. The lethal phenotype in B105 may allow one to isolate gene(s) involved in the synthesis of the precursors of the base of queuosine by suppression of the toxic phenotype of Tgt overexpression. The result of the growth competition experiment shown in Fig. 7(b), which was performed to illustrate the reduced fitness of strain B105 due to lack of queuosine modification, would be more valid if carried out with isogenic strains. Nevertheless, our observation that B105 (Q⁻) fails to compete with CA274 (Q⁺) suggests that introduction of gene(s) from a library (Q⁺) into B105, followed by multiple subculturing, may result in enrichment/selection of transformants wherein the resident plasmid confers a growth advantage to the host due to queuosine modification. Thus, characterization of the *E. coli* B105 strain in this study will be helpful in the elucidation of the queuosine biosynthetic pathway, which may even be a boon to the field of cancer biology (Nishimura *et al.*, 1983) and contribute to a better understanding of the virulence of *Shigella* (Gradler *et al.*, 2001).

Finally, the electrophoretic analysis of tRNAs on acid urea gels has provided a very useful and sensitive tool to separate the various forms (deacylated, aminoacylated or formylaminoacylated) of tRNA in a large number of studies. These gels have also provided crucial insights into the aminoacylation of tRNA with different amino acids (Li *et al.*, 1996) or their differential modification status for the 2-methylthio-N⁶-(Δ^2 -isopentenyl)-adenosine (ms²i⁶A) (Mangroo *et al.*, 1995). In this study, we provide yet another example of discrimination of tRNA species differentially modified for the queuosine base. In fact, detection of mobility differences even for the tRNAs isolated from the *queA* mutant background suggests that the acid-urea-gel-based separation system can be exploited for wide-ranging applications.

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