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A Minor Arginine tRNA Mutant Limits Translation Preferentially of a Protein Dependent on the Cognate Codon

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The *Escherichia coli argU* gene encodes a rare arginine tRNA (anticodon UCU) that translates the similarly rare AGA codon. The *argU10*(Ts) mutation is a transition that changes the first nucleotide of the mature tRNA from G to A, presumably destabilizing the acceptor stem. This mutation, when present in haploid condition in the chromosome, reduces the growth rate at 30°C and results in cessation of growth after 60 to 90 min at 43°C. The mutation also preferentially limits (compared with total protein synthesis) translation of an induced gene that depends on five AGA codons, i.e., the λ *cI* repressor gene. Translation of another inducible protein, β-galactosidase, which does not involve AGA codons, was inhibited to a much lesser extent. The chromosomal *argU*(Ts) mutation also confers the Pin phenotype, that is, loss of ability of the host, as a P2 lysogen, to inhibit growth of bacteriophage λ, probably the result of reduced translation of the P2 *old* gene, which contains five AGA codons (E. Haggård-Ljungquist, V. Barreiro, R. Calendar, D. M. Kurnit, and H. Cheng, Gene 85:25–33, 1989).

The *argU* gene of *Escherichia coli* encodes a rare $tRNA^{Arg}_{UCU}$, which decodes the rare AGA arginine codon (8; R. A. Spanjaard, K.-S. Chen, J. R. Walker, and J. van Duin, personal communication). *argU* is an unusual tRNA gene for several reasons: (i) it is transcribed as a single unit rather than as the more common component of an operon (7), (ii) the stringency control sequence (20, 48, 49) is weak or missing, (iii) the *argU* promoter should be strong on the basis of sequence and spacing (13) but the product is one of the rarest tRNAs (5, 8, 17), (iv) a 31-base-pair (bp) region of dyad symmetry overlaps the promoter region and could be a regulatory site (8), and (v) immediately downstream of *argU* is a defective lambdoid prophage (DLP12), the integrase gene of which is transcribed toward and overlaps the *argU* primary transcript and the attachment site homolog of which is located within the *argU* mature tRNA sequence (26). Moreover, the *argU10*(Ts) mutation was originally recovered from a temperature-sensitive mutant that stopped growth at 43°C, although DNA replication was more sensitive to high temperature than were protein and RNA synthesis. It was proposed, therefore, that replication in the *argU*(Ts) mutant could be indirectly limited by the temperature-sensitive mutation (8), perhaps by limiting translation of an essential replication protein.

To determine whether a mutant tRNA could limit translation of a specific protein(s), we measured synthesis of induced λ *cI* repressor synthesis, which depends on AGA translation, in the $argU^+$ and *argU*(Ts) strains at 30 and 43°C. Translation of this protein was preferentially inhibited at both temperatures. Moreover, we report here that the *argU*(Ts) mutant has the Pin phenotype (which depends on limitation of the P2 prophage *old* gene, which in turn depends on translation of five AGA codons) (9, 11).

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids. *E. coli* K-12 $argU^+$ and *argU10*(Ts) strains GM241 and GM10, respectively, have been described (15). Strains C600 and λ *cI*857 were laboratory strains. Phage P2 was obtained from Ian Molineux. λD69 (30) was obtained from Sankar Adhya. Plasmid pEA305 (from Mark Ptashne) carries the λ *cI* gene under ptac control and ampicillin resistance on the pBR322 vector (1); pI^Q carries the $lacI^q$ allele and tetracycline resistance on the vector pACYC184 (24), which is compatible with ColE1 replicons. pMC1871 was obtained from Pharmacia, Inc.

pTZ2 consists of a 3.8-kilobase-pair (kb) *Sma*I-*Hin*dIII fragment from pMC1871 (41) cloned into the *Sma*I-*Hin*dIII sites of the pBR322 derivative pMB211 (Michael Bröker), which places *lacZ* under ptac control (simultaneously replacing the *Sma*I-*Hin*dIII *lacZ* fragment of pMB211). pBR1824 consists of a 118-bp $argU^+$ fragment cloned into pBR322 (31).

Phage λ growth. λ was normally grown in strain C600 on yeast extract-Tryptone plates and soft agar (16). When strains GM10 and GM241 and their derivatives were infected with λ, they had been grown in yeast extract-Tryptone broth without added NaCl and were plated in soft agar of the same composition.

Recombinant DNA and DNA sequencing technology. Standard techniques were used for plasmid and phage DNA isolation, restriction endonuclease digestions, ligation, transformation, and gel electrophoresis (28). Nucleotide sequencing was performed on fragments cloned into M13mp8 by the dideoxy-chain termination method (37).

Isolation of chromosomal DNA. Chromosomal DNA was extracted from late-log-phase cells by a method similar to that of Blum et al. (4) except that proteinase K (100 μg/ml) was used. Phenol-extracted lysate was then extracted with ether, precipitated with ethanol, and dissolved in and dialyzed against TE buffer (26).

Cloning of the *argU*(Ts) allele. *Hin*dIII-digested DNA was fractionated on a 0.7% agarose gel, and the 4.4- to 4.6-kb fragments were electroeluted and ligated into *Hin*dIII-digested λD69. The ligated mixture was packaged in vitro (Promega Biotec packaging extract) and plated on strain C600. λD69 derivatives containing the 4.5-kb *argU*(Ts) *Hin*dIII fragment were identified by plaque hybridization (12), using a ^{32}P-labeled 118-bp $argU^+$ probe from pBR1824 (31). This fragment was purified from a 1.8% agarose gel by

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electroelution onto an NA-45 DEAE membrane (Schleicher & Schull, Inc.). The fragment was released from the membrane by a high-salt wash, precipitated, and end labeled with [α-^{32}P]dATP by the Klenow fragment of DNA polymerase I (28).

Pulse-labeling. Strains GM10 and GM241, each containing both pEA305 and pI^{Q}, were grown in minimal salts medium (16) plus carbenicillin, tetracycline, glucose, adenine, all of the common 19 L amino acids except methionine, and thiamine hydrochloride. All additions were at 50 μg/ml except glucose (10 mg/ml), thiamine hydrochloride (5 μg/ml), carbenicillin (500 μg/ml), and tetracycline (25 μg/ml). At A_{600} of 0.2, 10 mM isopropylthio-β-D-galactoside (IPTG) was added and a portion of each culture was shifted to 43°C. Samples of 1 ml were added to 20 μCi of [^{35}S]methionine (1,072 Ci/mmol) for 2 min, followed by a 2-min chase of 2.6 mM unlabeled methionine. Labeled cell pellets were washed with 1 ml of cold 10 mM Tris hydrochloride (pH 7.5), suspended in 90 μl of lysis solution (1% sodium dodecyl sulfate [SDS], 1 mM EDTA, 10 mM pH 7.5 Tris hydrochloride), and boiled for 3 min.

Radioimmunoprecipitation and quantitation of λ *c*I repressor. Pulse-labeled λ repressor was precipitated from 10 μl of boiled extracts by excess immune serum by the method of Lee et al. (23) except that the incubation period for immune complexes and protein A-Sepharose 4B was 2 h. Washed immune complexes were suspended in 50 μl of Laemmli SDS sample buffer and boiled for 2 min. A 20-μl sample was used for SDS-polyacrylamide gel electrophoresis (PAGE). Electrophoresis gels were autoradiographed by using Kodak XRP-1 film, and the repressor was measured by densitometry. Peak areas were proportional to amount of repressor, as shown by varying the amount of antigen in a control experiment.

β-Galactosidase assay. Strains GM10 and GM241, each containing both pTZ2 and pI^{Q}, were grown in the same medium as for pulse-labeling and induced by IPTG; a portion of each culture was shifted to 43°C and assayed by the procedure of Miller (29) except that the assays were conducted at 22°C. The units reported in Table 2 are similar to Miller units except that the Miller procedure conducts the assay at 37°C.

RESULTS

Nucleotide sequence of the *argU*(Ts) mutation. The *argU*(Ts) mutant allele was cloned on a 4.5-kb *Hin*dIII fragment from chromosomal DNA of strain GM10 into the vector λD69 and then into the *Hin*dIII site of phage M13mp8 for sequence analysis. The wild-type allele was cloned on a 4.5-kb *Hin*dIII fragment from pDM1 (31) into M13mp8. The region from +170 to −35 (Fig. 1) was sequenced along one strand, with the result that one point mutation change from C to T was found. This mutation results in changing the 5′ G of the mature tRNA to an A, thereby abolishing the normal G · C pair at the 5′ terminus of the acceptor stem.

Translation of a protein dependent on AGA codons is preferentially inhibited in an *argU*(Ts) mutant. To determine whether the *argU*(Ts) mutation limits expression of a protein translated from AGA-containing messenger, the amount of induced λ *c*I repressor synthesis was compared with the amount of total protein synthesis at 30 and 43°C in *argU*$^{+}$ and *argU*(Ts) strains. The λ *c*I gene reading frame contains five AGA codons (39).

The wild-type and *argU10*(Ts) strains GM241 and GM10 were transformed with both pEA305, a pBR322 derivative containing the λ *c*I gene under ptac promoter control (1), and pI^{Q}, a compatible pACYC184 derivative containing the *lacI*^{q} gene (24). These strains were grown at 30°C and induced to synthesize *c*I repressor by addition of IPTG, and half of each culture was shifted to 43°C. The rate of protein synthesis was measured by 2-min pulses of [^{35}S]methionine. Rate of total protein synthesis was measured by counting trichloroacetic acid (TCA)-precipitable material; in addition, labeled proteins were separated by SDS-PAGE and visualized by autoradiography. λ *c*I protein was measured specifically by immunoprecipitation, SDS-PAGE, and densitometry.

At 30°C, the wild-type and mutant strains grew with doubling times of 1.5 and 1.6 h, respectively (Fig. 2). At 43°C, the wild-type strain continued exponential growth; the *argU*(Ts) strain continued growth for one mass doubling but stopped growth after 60 to 90 min. Labeled methionine continued to be incorporated but at a reduced rate during the 43°C incubation (Fig. 3A). The rate of [^{35}S]methionine incorporation into TCA-precipitable material was measured by 2-min pulses in mutant and wild-type strains at 30 and 43°C (Table 1). At 30°C, the mutant and wild type incorporated label at very similar rates. In the wild type at 43 and 30°C, rates of incorporation again were very similar. In the mutant at 43°C, pulse-label was incorporated at about two-thirds the level at 30°C over the entire 2-h incubation period. This was somewhat surprising because total mass increase stopped by 90 min at 43°C. Presumably, growth was limited by lack of complete or fully active proteins. Pulse-label incorporation and presumably turnover continued for at least 2 h at 43°C.

The rate of synthesis of the AGA-dependent *c*I repressor was compared with the rate of total [^{35}S]methionine incorporation. Repressor was precipitated from each pulse-labeled sample of Fig. 3A, electrophoresed by SDS-PAGE, and quantitated by densitometry of an autoradiogram (Fig. 3B). Comparison of rates of synthesis in the mutant and wild type at 30°C shows that repressor synthesis was reduced to about one-fifth the wild-type level even at the permissive temperature (Table 1). Presumably the *argU*(Ts) product is not fully active even at this temperature, which is permissive for growth.

In the mutant at 43°C, the repressor synthesis rate was reduced to one-third to one-fifth the 30°C rate (Table 1); in the wild-type at 43°C, the rate increased over the 30°C rate. At 43°C, the mutant synthesized repressor at about 1/50 the wild-type rate.

β-Galactosidase synthesis in *argU* and *argU*(Ts) strains. In the preceding experiment, the λ repressor rate was measured after induction from the ptac promoter by IPTG. To determine whether induction was somehow inhibited in the *argU*(Ts) mutant, β-galactosidase was assayed after induction from the same promoter by the same inducer. The *lacZ* gene contains no AGA codons (19). A pBR322 derivative containing the *lacZ* gene under ptac control and pI^{Q} were transformed into wild-type and *argU*(Ts) strains. These strains were grown at 30°C and induced with IPTG, half of each culture was shifted to 43°C, and total β-galactosidase was assayed at intervals (Table 2). At 30°C, the *argU*(Ts) strain synthesized β-galactosidase at 85% the wild-type rate (versus 20% for repressor). As 43°C, the β-galactosidase level in the mutant accumulated at about one-third the rate of the mutant grown at 30°C.

At 43°C in the wild type, β-galactosidase was synthesized at two-thirds the 30°C rate. At 43°C, the mutant supported 45% as much synthesis as did the wild type (versus 2% for repressor synthesis).

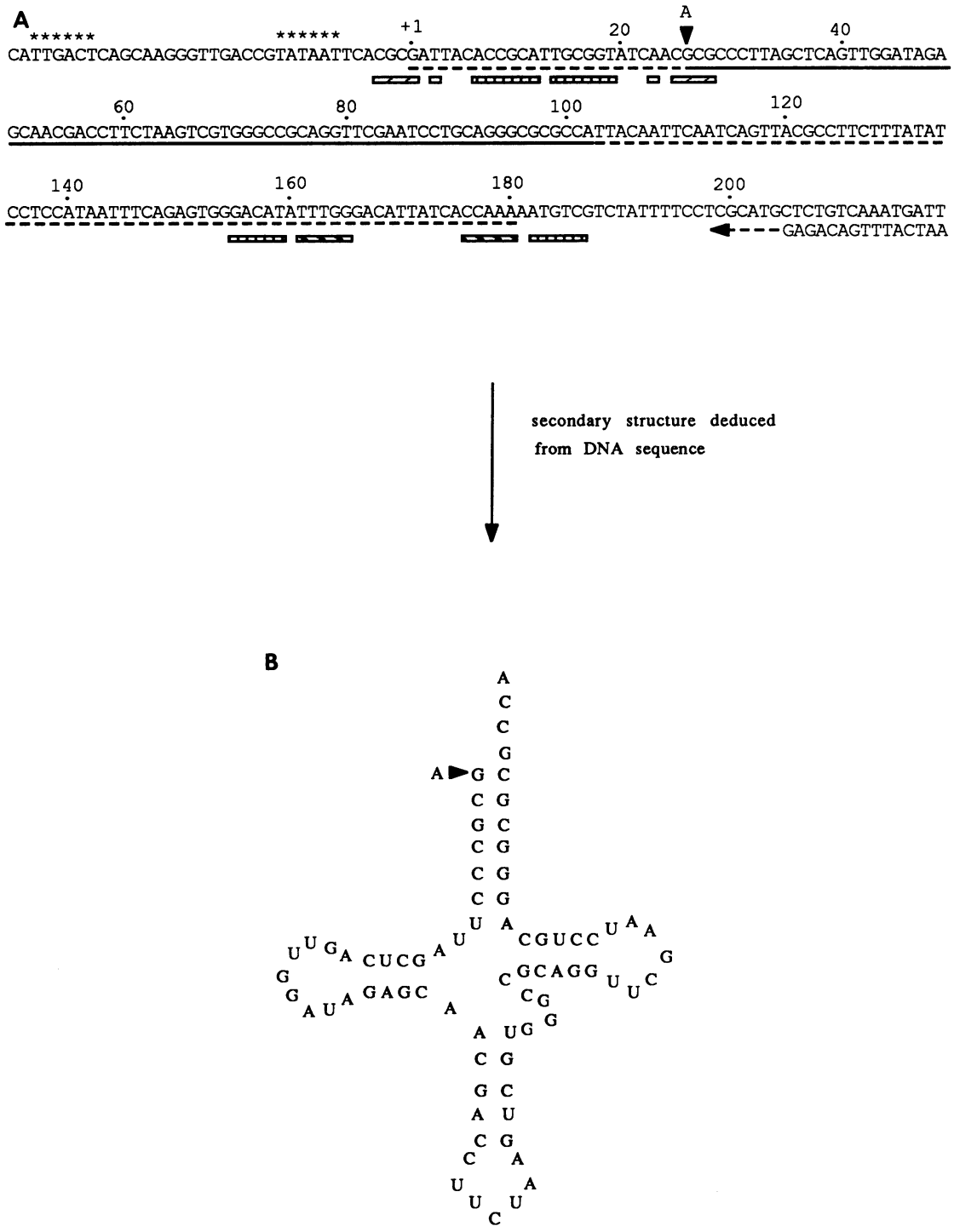

FIG. 1. *argU* sequences. (A) DNA sequence from Garcia et al. (8); (B) tRNA cloverleaf. The *argU*(Ts) G1-to-A1 transition is shown by the arrowhead. Transcription starts at the +1 nucleotide and forms precursors of 180 and 190 nucleotides (dashed underline), which are processed to form the mature form (solid underline). Promoter −35 and −10 sequences are indicated by asterisks. Dyad symmetry sequences in the promoter and termination regions are indicated by hatched bars.

The *argU10*(Ts) mutation confers the Pin phenotype. *E. coli* C *pin* mutations restore the ability of P2 lysogens to plate superinfecting λ phages and map at min 12.2, near *argU* (9). To test the possibility that *pin* mutations are in *argU*, the ability of *argU*(Ts) and wild-type P2 lysogens to support λ growth was tested by superinfecting P2 lysogens at 30°C. The *argU*(Ts) mutation, even at 30°C, allowed λ to grow, corroborating the conclusion that even 30°C is not fully permissive for the *argU*(Ts) strain. The *argU10*(Ts) mutation is responsible for the Pin phenotype because the 118-bp *argU*$^+$ fragment of pBR1824 (31) complemented *argU*(Ts) and restored the ability to restrict λ (Table 3).

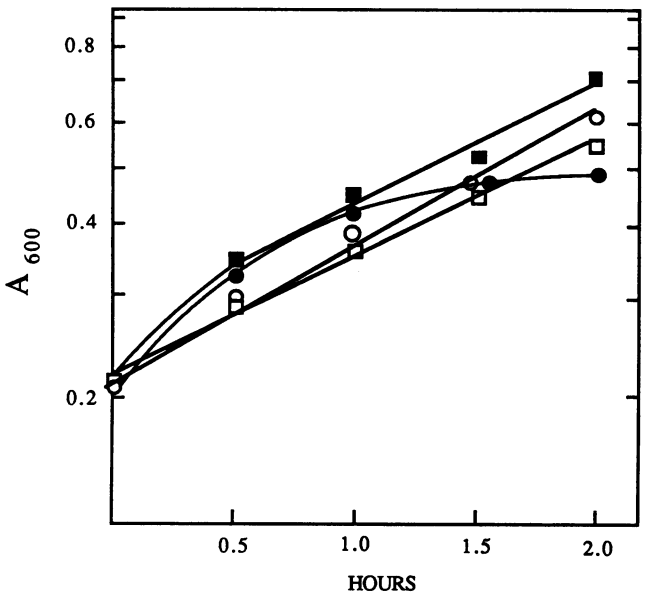

FIG. 2. Growth of *argU*$^+$ strain GM241 (□, ■) and *argU*(Ts) strain GM10 (○, ●) at 30 and 43°C. Cultures growing at 30°C (○, □) were divided at 0 h, and a portion of each was shifted to 43°C (●, ■).

TABLE 1. Rate of [^{35}S]methionine pulse-label incorporation into TCA-precipitable material and into induced λ *c*I repressor synthesis

Strain	Genotype	Rate of incorporation[b]			
		TCA insoluble		*c*I repressor	
		30°C	43°C	30°C	43°C
GM241	*argU*$^+$	1.0		1.0	
		1.1	0.8	1.8	2.1
		1.3	1.3	2.0	3.1
		1.7	1.6	2.1	4.6
		1.7	1.3	2.6	4.9
GM10	*argU*(Ts)	0.97		0.08	
		1.05	0.6	.28	0.07
		1.43	1.0	.42	0.16
		1.4	0.9	.27	0.08
		1.5	0.9	.55	0.1

[a] Both strains contained pEA305 and pI^Q.
[b] Results are normalized to the values for strain GM241 at 30°C. For TCA-insoluble material, 1.0 represents 34,400 cpm/275 μl of culture. For *c*I repressor, 1.0 represents the area of the peak measured by densitometry of Fig. 3B, lane C0 (strain GM241 at zero time).

DISCUSSION

The *argU10*(Ts) tRNA product has an alteration of the first nucleotide of the acceptor stem from G to A, which is expected to reduce the stem stability, possibly reducing its affinity for elongation factor (EF) Tu-GTP complex (27, 36, 40). Translation of AGA-containing messenger might be restricted because stable cognate tenary complex of arginyl $tRNA^{Arg}_{UCU}$ bound to elongation factor Tu-GTP complex is not available. Two approaches indicate that in the *argU* mutant, translation of specific proteins can be preferentially inhibited even at the 30°C temperature normally used as the permissive condition. First, direct measurement of *c*I repressor synthesis demonstrated preferential inhibition compared with total [^{35}S]methionine incorporation into protein. *c*I repressor synthesis depends on translation of five AGA codons (39). This inhibition was exacerbated by increasing the temperature to 43°C.

Second was the observation that the *argU*(Ts) strain has the Pin phenotype even at 30°C. *E. coli* lysogenic for phage P2 do not support growth of phage λ because of expression of the P2 *old* gene, which interferes with λ late gene expression (25, 42). Action of the P2 *old* gene is inhibited in *E. coli pin* mutants, which allow λ growth (9). Haggård-Ljungquist et al. (11) recently sequenced the P2 *old* gene, which contains five AGA codons, two of them in tandem. On the basis of their observation that *E. coli* C *pin* mutants were complemented by a 118-bp *argU*$^+$ plasmid, they concluded that hosts defective in *argU* could not support P2 *old* protein synthesis at the level necessary to inhibit λ growth. Inasmuch as the *E. coli* C *pin* mutants used by Haggård-Ljungquist et al. were not temperature sensitive for growth, it seems likely that these *pin* mutations had partially active $tRNA^{Arg}_{UCU}$. These observations are consistent with a recent observation that the *argU*(Ts) mutant is defective in translating two AGA codons in tandem (Spanjaard et al., personal communication). The observation that the wild-type P2 lysogen [strain GM241(P2); Table 3] allowed λ to plate with an efficiency of about 1 at 37 but not 30°C suggests that the pool of $tRNA^{Arg}_{UCU}$ is minimal even in this strain and that incubation at the higher temperature further restricts the pool or increases demands on it.

Rare codons are avoided in highly expressed genes such as those for protein synthesis factors, ribosomal proteins, and outer membrane components. On the other hand, repressor and DNA replication genes do not discriminate against rare codons (10). This nonrandom distribution of codons could result in preferential inhibition of proteins involved in replication, thus explaining the preferential inhibition of replication observed in this *argU*(Ts) strain (15).

The facts that *argU* is adjacent to the defective prophage DLP12 *int* gene, that *int* contains the unusually high number of eight AGA codons, and that the *argU* and DLP12 *int* transcripts overlap (8, 26) suggest a relationship between expression of these genes in wild-type cells. One model (Fig. 4) predicts that when the $tRNA^{Arg}_{UCU}$ level is high, Int protein is synthesized and the Int messenger interacts with the *argU* transcript to limit tRNA availability, perhaps by affecting its synthesis, processing, or folding. When the $tRNA^{Arg}_{UCU}$ level decreases, Int translation decreases and its messenger is degraded (11), thus allowing the tRNA level to increase.

Although it is assumed that the G1→A1 mutant tRNA is defective in translation, it is not excluded that mischarging of the mutant tRNA could occur also. Smith and Celis (43) reported that a G1-to-A1 mutation in a tyrosine tRNA led to mischarging with glutamine. Mischarging would not inhibit translation but could result in reduced activity of specific proteins. Other possible effects of the *argU10*(Ts) mutation include inhibition of precursor transcript processing to form the mature tRNA and increased ribosomal frameshifting, especially at tandem AGA codons (45). Nonprogrammed frameshifts could result in reduced activity of many proteins. This latter point seems relevant because a *Salmonella typhimurium* mutant $tRNA^{Gly}_2$ with a mutation changing G1 to A1 [disrupting the potential for normal base pairing in the acceptor stem and identical to the *argU10*(Ts) mutation] promotes frameshifting (34, 35).

Perhaps differential inhibition of translation explains pleiotropic effects of other tRNA mutants. The *E. coli divE42*(Ts) mutation alters G10 to A10 in serine $tRNA_1$, disrupting the

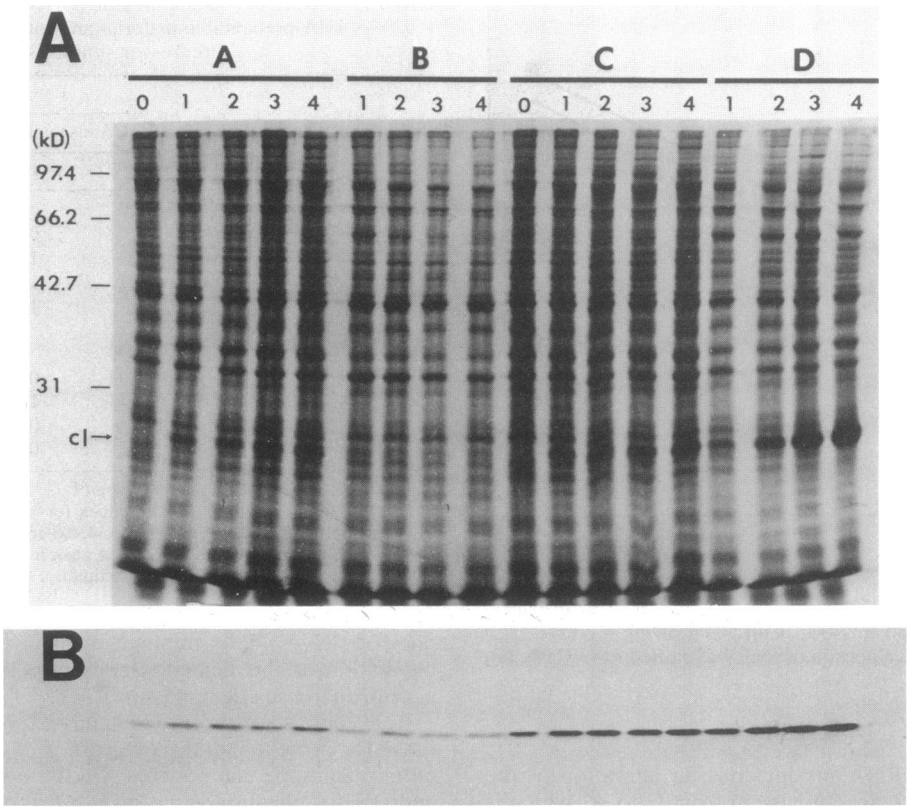

FIG. 3. Protein synthesis in *argU*$^+$ and *argU*(Ts) strains at 30 and 43°C. The *argU*$^+$ and *argU*(Ts) strains GM241 and GM10 containing pEA305 and pI^Q were grown to A_{600} of 0.2 and induced by addition of 10 mM IPTG, and a portion of each culture was shifted to 43°C. A 1-ml sample of each culture was pulsed with [^{35}S]methionine (20 μCi/ml) for 2 min at each time interval (0, 1, 2, 3, and 4 refer to 0, 30, 60, 90, and 120 min) and chased for 2 min by addition of 2.6 mM unlabeled methionine. Washed pellets were lysed by boiling in 90 μl of lysis buffer. (A) Total protein. A 10-μl sample of each lysate was loaded in each lane and electrophoresed on a 5 to 13% composite SDS-polyacrylamide gel, which was dried and autoradiographed. Lanes A and B contained extracts of strain GM10 at 30 and 43°C; lanes C and D contained extracts of strain GM241 at 30 and 43°C. Molecular size markers are shown on the left (kD, kilodaltons). *c*I refers to the λ repressor. (B) λ repressor, A 10-μl sample of each lysate was mixed with antibody to the *c*I repressor, which was precipitated and electrophoresed. The portion of the autoradiograph containing the repressor is shown. Lanes are indicated by the headings in panel A.

usual base pairing of the D stem. A *divE42*(Ts) mutant is temperature sensitive for growth. Upon a shift to 42°C, the mutant at first continued growth and then gradually stopped after a twofold mass increase. Upon reduction of the temperature, mutant cells sustained a burst of synthesis of certain membrane and cytoplasmic enzymes (e.g., succinate dehydrogenase) and then resumed growth and divided synchronously. Because the D-stem change did not affect synthesis of most cellular proteins, it was proposed that $tRNA_1^{Ser}$ is specifically involved in regulation of cell cycle-specific protein synthesis, with a resulting role in cell division (outside the general decoding function of tRNAs) or that

TABLE 2. β-Galactosidase synthesis in *argU*$^+$ and *argU*(Ts) strains

Strain	Genotype	Time	β-Galactosidase activity (U)[a]/μl of extract	
			30°C	43°C
GM241	*argU*$^+$	0	100 ± 10	
		0.5	7,200 ± 170	4,100 ± 100
		1.0	11,700 ± 340	7,400 ± 370
		1.5	15,800 ± 930	10,300 ± 710
		2.0	22,000 ± 1,530	15,800 ± 1,300
GM10	*argU*(Ts)	0	120 ± 10	
		0.5	6,900 ± 610	1,800 ± 80
		1.0	9,300 ± 400	3,300 ± 70
		1.5	15,600 ± 1,690	4,700 ± 110
		2.0	17,800 ± 1,760	7,700 ± 220

[a] Similar to Miller units except that the assays were incubated at 22 rather than 37°C. Activities ± 1 standard deviation are reported.

TABLE 3. Evidence that an *argU*(Ts) P2 lysogen does not restrict λ growth

Strain	Temp (°C)	Relevant genotype	λ titer	λ EOP[a]
GM241	30	*argU*$^+$	1.6×10^{10}	
	37		1.6×10^{10}	1.0
GM241(P2)	30	*argU*$^+$	0.04×10^{10}[b]	0.026
	37		1.8×10^{10}	1.1
GM241(P2) (pBR1824)	30	*argU*$^+$ *argU*$^+$	$<1 \times 10^3$	$<10^{-7}$
	37		$<1 \times 10^3$	$<10^{-7}$
GM10	30	*argU*(Ts)	1.4×10^{10}	
GM10(P2)	30	*argU*(Ts)	1.4×10^{10}	1.0
GM10(P2) (pBR1824)	30	*argU*(Ts) *argU*$^+$	$<1 \times 10^3$	$<10^{-7}$

[a] Efficiencies of λ plating (EOP) on derivatives of strains GM241 and GM10, using titers on the nonlysogens at 30°C as the standards.
[b] Plaques were about 1/10 the normal diameter.

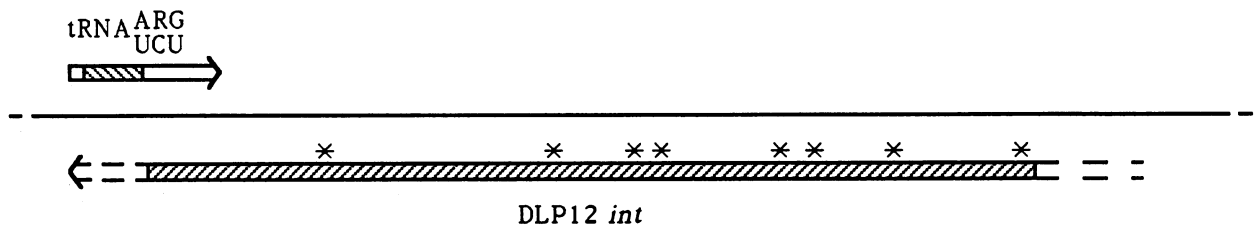

FIG. 4. Relation between *argU* and DLP12 *int* expression. The solid line is the chromosome. The rightward arrow is the *argU* transcript, with the mature tRNA region cross-hatched. The leftward arrow is the DLP12 *int* transcript, with the reading frame cross-hatched. Asterisks mark the locations of AGA codons. The dashed regions of the *int* transcript indicate uncertainty about the exact initiation and termination points.

this tRNA is specific for translation of specific proteins (32, 33, 46). However, two-dimensional gel electrophoresis of pulse-labeled proteins did not detect a specific defect in translation in the *divE*(Ts) mutant (38). Moreover, as pointed out by Tamura et al. (46), $tRNA_1^{Ser}$ is a major species of the serine isoacceptors and has a 5′ U*GA anticodon (U* is uridine-5-oxyacetic acid), which decodes UCA, UCG, and UCU, which are common serine codons (18). Thus, the specific defect of the mutant $tRNA_1^{Ser}$ in these pleiotropic phenomena is unclear, and it is possible that translation of one or a few proteins absolutely requires the native $tRNA_1^{Ser}$ at high temperature (46).

The *bldA* mutants of *Streptomyces coelicolor* are defective in antibiotic production and the development of aerial hyphae and spores but are not affected in vegetative growth (21). The *bldA* gene encodes a leucine tRNA that decodes UUA, a rare codon in this organism. Five *bldA* mutations were single-base changes in the tRNA, one of which altered the anticodon to decode serine. Since the wild-type *bldA* product accumulates late in the *Streptomyces* growth cycle, it is possible that this tRNA plays a role in translational control of *Streptomyces* development, although nontranslational functions were considered also (21).

A missense suppressor mutant tRNA preferentially affects cell division and DNA metabolism in *E. coli*. The cell division mutation *ftsM1* and the *supH120* suppressor mutation (6, 47) are likely to be identical alleles of *serU*, the gene for $tRNA_2^{Ser}$ (22). Thus, the *ftsM1* phenotype of temperature sensitivity, filamentous growth, and UV sensitivity is the result of the missense suppressor tRNA. *serU* encodes a minor serine tRNA (2, 44) of anticodon 5′ CGA; the *supH* allele contains an anticodon change to 5′ CAA, allowing the suppressor tRNA to insert serines at UUG leucine codons (47). Leclerc et al. (22) proposed that the minor, suppressor tRNA modifies a cell division factor or protein by inserting serine into a normal leucine position(s). UUG is a frequently used codon that is read by one major ($tRNA_{NAA}^{Leu}$) and one minor ($tRNA_{NAA}^{Leu}$) isoacceptor (3, 14, 17, 50).

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