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Organization and Functions of Genes in the Upstream Region of *tyrT* of *Escherichia coli*: Phenotypes of Mutants with Partial Deletion of a New Gene (*tgs*)

MICHAEL BÖSL† AND HELGA KERSTEN*

Institut für Biochemie, Universität Erlangen-Nürnberg, Fahrstrasse 17, D-91054 Erlangen, Federal Republic of Germany

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A Δ*tyrT*::*kan* mutant from *Escherichia coli* K-12 (DTK-12) shows a transient growth lag that is caused by glycine starvation (U. Michelsen, M. Bösl, T. Dingermann, and H. Kersten, J. Bacteriol. 171:5987–5994, 1989). The same deletion, transduced into the *relA1 spoT1* mutant CA274 to construct strain DTC274, caused complete growth inhibition in glucose minimal medium. Here, we show that the *tyrT* 5′ region contains three new open reading frames in the order ORF37→ORF34→ORF32→*tyrT* and that the Δ*tyrT*::*kan* allele used previously deletes tyrT as well as a carboxy-terminal portion of ORF32. A plasmid encoding ORF32 totally complemented the inability of strain DTC274 to grow on glucose minimal medium as well as the transient glycine starvation phenomenon in DTK-12, and ORF32 was designated *tgs*. Partial deletion of *tgs*, cotransduced with the marker Δ*tyrT*::*kan*, was responsible for the completely different phenotypes of the deletion mutants DTK-12 and DTC274. The deduced Tgs protein sequence showed significant homology to the PurN protein of *E. coli* and to enzymes with glycinamide ribonucleotide transformylase activity. We discuss whether growth inhibition in strain DTC274 may be caused by synergistic effects with the preexisting mutations *relA1* and *spoT1*. The deduced protein sequence of ORF37 showed striking similarity to regulator response proteins and is probably a new member of this family. A spontaneous mutation in ORF37, caused by the integration of an insertion element, *IS1*, exhibited no phenotype.

Tyrosine-accepting tRNA is encoded by two genes in *Escherichia coli*, *tyrT* and *tyrU*. The *tyrT* operon and its regulation have been well investigated: it is under stringent control (17) and under growth rate control (40). An upstream activation sequence, including a binding site for the transactivator protein Fis (25), permits an optimal rate of transcription (16, 39).

The most striking feature of the *tyrT* operon is its structural arrangement (9, 20, 29, 30). The *tyrT* locus consists of two tandemly repeated $tRNA_1^{Tyr}$ genes separated by a 208-bp spacer segment including a sequence motif that is repeated three times downstream of the second $tRNA_1^{Tyr}$ gene. Each repeat, consisting of 178 bp, terminates with 19 nucleotides, identical with the 3′ end of the mature tRNA. The complex structure apparently confers some selective advantage for the cell and is therefore maintained (31). A very similar arrangement of terminal repeats has been found in four other tRNA genes of *E. coli* (for a comparison, see reference 3).

In order to investigate the biological significance of these repeated structures, a Δ*tyrT*::*kan* mutant has been constructed (22). Although the *tyrT* operon is not essential, the wild-type-derived deletion mutant DTK-12 shows a characteristic phenotype when shifted from a rich to an amino acid-limited medium: a transient glycine starvation phenomenon induces the regulatory network of stringent response and gives rise to a lag phase during mid-logarithmic growth (22). On the basis of complementation studies with plasmid-borne *tyrT* fragments, an RNA product of the repeated structures of the *tyrT* operon has been postulated to be the complementing unit (22).

In a further study, this RNA, designated rtT RNA, has been identified and characterized (3). We have made two unexpected observations. (i) Plasmid-expressed rtT RNA is not only involved in complementation of the glycine starvation in the Δ*tyrT*::*kan* strain DTK-12 but also influences growth arrest induced by isoleucine deprivation. Therefore, a general modulatory role of rtT RNA upon stringent control that is not restricted to glycine starvation in strain DTK-12 has been suggested. (ii) The extent of growth delay in the Δ*tyrT*::*kan* strain DTK-12 is increased under more-limiting conditions with glycerol as carbon source. Under these conditions, the characteristic lag phase in strain DTK-12 has been only partially overcome by the *tyrT* operon, probably because of the modulatory effect of rtT RNA upon stringent control, indicating that deletion of the *tyrT* operon is not or at least is not the only genetic cause for the phenomenon of transient glycine starvation, designated the *tyrT* deletion phenotype (22).

In the study presented here, the hypothesis that a second site mutation is involved in generating the *tyrT* deletion phenotype was further supported when the same Δ*tyrT*::*kan* marker was transduced into the *relA1 spoT1* mutant CA274 to construct strain DTC274. This strain showed a dramatic phenotype: when shifted to a minimal medium, growth was fully arrested, and, in contrast to that of strain DTK-12, the phenotype was not complemented or weakened by transformation with a plasmid containing exclusively the *tyrT* operon. This phenotype was completely unexpected, because the original *tyrT* deletion phenotype depends upon a functional stringent control system (22).

To identify the genetic basis of the *tyrT* deletion phenotype in strain DTC274, the 5′ region of *tyrT* was analyzed and revealed several new open reading frames. The mutation responsible for the phenotype of the *relA spoT*-derived strain was localized immediately upstream of the *tyrT* promoter in a new gene designated *tgs*. It was further shown that the *tgs*

* Corresponding author. Mailing address: Institut für Biochemie, Med. Fak., Universität Erlangen-Nürnberg, Fahrstrasse 17, D-91054 Erlangen, Germany. Phone: 49-9131-854188 or -854191. Fax: 49-9131-854605.

† Present address: Banyu Tsukuba Research Institute (Merck), Okubo 3, Tsukuba, 300-33, Japan.

TABLE 1. *E. coli* strains and plasmids

Strain or plasmid	Genotype or relevant characteristics	Source or reference[a]
Strains		
K-12	Wild type	DSM strain no. 498
$K\text{-}12_{BM}$	ORF37::*IS1*; wild type	Our laboratory stock K-12
W3110	Wild type	15
DTK-12	Δ*tyrT*::*kan*	22
CA274	*trp-49*(am) *lacZ125*(am) λ^- *spoT1 relA1*	B. J. Bachmann (CGSC)
DTC274	*trp-49*(am) *lacZ125*(am) λ^- *spoT1 relA1* Δ*tyrT*::*kan*	This work
CA275	*trp-49*(am) *lacZ125*(am) λ^- *spoT1 relA1 supF66*	B. J. Bachmann (CGSC)
MC4100	*thi araD139* Δ(*argF-lac*)*U169 ptsF2 deoC relA1 flbB5301 rpsL150* λ^-	5
JC9387	*hsdR thr-1 ara-14 leuB6* Δ(*gpt-proA*)*62 lacY1 sbcC201 tsx-33 supE44 galK2* λ^- *rac sbcB15 hisG4 rfbD1 recB21 recC22 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*	B. J. Bachmann (CGSC)
DH5α	*endA1 hsdR17 supE44 thi-1 recA1 gyrA96 relA1* Δ(*argF-lac*)*U169* φ80d*lacZ* ΔM15 λ^-	10
Plasmids		
pUC18, pUC19	Replicon ColE1; Ap^r, *lac*α peptide multiple cloning site	44
pACYC184	Replicon P15A; Cm^r Tc^r	6
pUM-E	pACYC184 [*supF66* containing a 5.7-kb *Eco*RI fragment] Tc^r	22
pUM101	pACYC184 [*tyrT* containing a 6.6-kb *Sal*I-*Bam*HI fragment] Cm^r	22
pUM302	pACYC184 [*tyrT* containing a *Pvu*II-*Eco*RI fragment] Tc^r	22
pUT-A1	pUC19 [*tyrT'* containing a 3.2-kb *Ava*I fragment] Ap^r	This work
pTT-EVA22	pACYC184 [*tyrT'* containing a 1.7-kb *Eco*RV-*Ava*I fragment] Cm^r	This work
pTT-3403A	pACYC184 [subfragment of pUT-A1 (pos. 2161-*Ava*I)[b]] Cm^r	This work
pTT-3513A	pACYC184 [*tgs* containing subfragment of pUT-A1 (pos. 2506-*Ava*I)[b]] Cm^r	This work
pTT-3513Bst	pACYC184 [*tgs* containing subfragment of pUT-A1 (pos. 2506-*Bst*EII)[b]] Cm^r	This work
pTT-3513Sc	pACYC184 [*tgs'* containing subfragment of pUT-A1 (pos. 2506-*Sca*I)[b]] Cm^r	This work

[a] CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.; DSM, Deutsche Sammlung von Mikroorganismen, Göttingen, Germany.
[b] 5′ end generated by exonuclease III treatment; pos., position in the sequence shown in Fig. 3.

mutation and not the *tyrT* mutation itself was responsible for the already described deletion phenotype of the wild-type-derived strain DTK-12.

MATERIALS AND METHODS

Materials. Restriction endonucleases, avian myeloblastosis virus reverse transcriptase, and DNA-modifying enzymes were purchased from Boehringer, Mannheim, Germany. Sequenase was from U.S. Biochemical Corp., Cleveland, Ohio. [α-^{32}P]dATP, [γ-^{32}P]ATP, [^{35}S]Met, and the prokaryotic DNA-directed translation kit were obtained from Amersham Corp., Little Chalfont, United Kingdom. Hybridization transfer membranes were from Schleicher and Schüll, Dassel, Germany, or from Du Pont de Nemours, Boston, Mass. (Gene Screen Plus). Culture media and Casamino Acids were purchased from Difco Laboratories, Detroit, Mich. Antibiotics, amino acids, and other chemicals were from Sigma Chemical Co., St. Louis, Mo. Qiagen plasmid preparation columns were obtained from Diagen, Düsseldorf, Germany. The nested deletion kit was from Pharmacia, Uppsala, Sweden.

Bacterial strains and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. Selection markers were transduced by generalized transduction with the phage P1 *kc* (23). Plasmids were introduced into bacteria by the standard transformation protocol (32).

Bacteria were cultured under aerobic conditions at 37°C in rich medium (YT) or in minimal medium (M9) (23) with 30 mM mannitol and either 0.4% glycerol or 0.4% glucose as carbon source. Cultures (10 ml) were grown in 100-ml Erlenmeyer flasks modified with sidearm cuvettes. Growth was monitored by measuring the A_{578}. Cultures were inoculated 1:100 with an overnight culture. The minimal medium for auxotrophic strains was supplemented with the corresponding amino acid at a final concentration of 50 μg/ml. Growth experiments were reproduced at least three times. If required, antibiotics were added in the following concentrations: kanamycin, 50 μg/ml; tetracycline, 20 μg/ml; chloramphenicol, 10 μg/ml; and ampicillin, 100 μg/ml. The vitamin mixture contained (per liter) biotin, 0.25 mg; pantothenic acid, 7.5 mg; folic acid, 1.25 mg; pyridoxine, 1.25 mg; nicotinic acid, 7.5 mg; thiamine, 12.5 mg; riboflavin, 2 mg; DL-6,8-lipoic acid, 2 mg; and cyanocobalamin, 2 mg.

Cloning procedures. Cloning was performed by established methods (32). For cloning purposes, plasmid DNA was purified with Qiagen pack 100 columns according to the manufacturer's protocol.

The plasmid pTT-3513Bst was constructed to obtain a *tgs*-containing plasmid without any adjacent *tyrT* sequences in the following way. An internal *Bst*EII site within the *tgs* gene required the construction of the helper plasmid pUT-Bst12 by subcloning the 0.5-kb *Bst*EII fragment of plasmid pTT-EVA22, which was blunt ended by Klenow polymerase, in the *Hin*cII site of the vector pUC19. The plasmid pUT-A3513 was a subclone of plasmid pUT-A1 with a 5′ deletion generated by exonuclease III treatment. The 0.5-kb *Hin*dIII*-*Hpa*I fragment of pUT-A3513 was replaced by the 0.33-kb *Hin*dIII*-*Hpa*I fragment of plasmid pUT-Bst12. The resulting insert terminating with the *Bst*EII site (inactivated by the fill-in procedure)

was cloned in the Tc^r gene of the vector pACYC184 as a 1.05-kb *Bam*HI-*Hin*dIII* fragment to obtain the plasmid pTT-3513Bst. The restriction sites marked by an asterisk are derived from the polylinker of pUC19. The *Bam*HI site was generated by cutting plasmid pUT-A3513 with *Eco*RI*, filling in the 5′ ends with Klenow polymerase, and performing a linker ligation with *Bam*HI linkers.

Nucleic acid analyses. Total RNA was prepared as already described (3). *tgs* mRNA was identified by Northern (RNA) blot analysis, performed as described elsewhere (3), with a specific nick-translated DNA fragment.

The transcription start of *tgs* mRNA was determined by primer extension analysis by using the conditions previously described (3) with a synthetic primer (23-mer) complementary to nucleotides 2618 to 2640. The extension products were analyzed on an 8% polyacrylamide sequencing gel together with a dideoxy-sequencing reaction of plasmid pUT-A1 as the size standard, performed with the 5′-phosphorylated extension primer.

Chromosomal DNA was isolated as described by Shapiro and Higgins (35), but before digestion with proteinase K, RNA was hydrolyzed with DNase-free RNase A for 30 min at 37°C at a final concentration of 100 μg/ml.

For Southern blot analysis, restricted (genomic) DNA was size fractionated in 0.6% agarose gels and transferred onto GeneScreen Plus membranes by an alkaline capillary blot with 0.5 M NaOH–1.5 M NaCl for 3 h. DNA-DNA hybridizations with nick-translated DNA fragments were performed at 42°C in 50% formamide–2 × SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.4% sodium dodecyl sulfate (SDS)–2× Denhardt's solution–2 mM EDTA–1% sarcosyl–0.1 M phosphate buffer (pH 7.0)–20 μg of denatured salmon sperm DNA per ml. Filters were washed twice for 10 min in 2× SSC at room temperature, twice for 30 min in 2× SSC–0.5% SDS at 65°C, and once for 15 min in 0.1× SSC at room temperature.

DNA sequence determination. Sequence analysis was performed by the dideoxy-chain termination method (33). The respective DNA fragments were cloned in pUC18 or pUC19. Nested deletions of the cloned inserts were constructed by partial, unidirectional hydrolysis with exonuclease III and S1 nuclease (11). Plasmid DNA for sequencing reactions was prepared according to Saunders and Burke (34). The sequence was determined with double-stranded DNA as a template by the modified T7 polymerase (Sequenase) reaction using both the universal M13 and the reverse M13 sequencing primer. Occasional band compression effects were resolved by the use of dITP instead of dGTP in the Sequenase reaction. The nucleotide sequence was determined from both directions.

Computer analysis. The sequence analysis was performed with the Genetics Computer Group Sequence Analysis Software package, version 7.1 (7).

Coupled in vitro transcription-translation assay. The expression of the plasmid-encoded open reading frames, determined by sequence analysis, was investigated by a coupled transcription-translation assay in vitro (45). The respective recombinant plasmids were used as DNA templates, and the translation products were labelled with [^{35}S]Met, separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (12% polyacrylamide) together with a molecular weight standard as a size reference, and identified by autoradiography.

Nucleotide sequence accession number. For the nucleotide sequence data given in Fig. 3, the GenBank accession number M64675 was assigned.

RESULTS

Growth inhibition in a *relA spoT* Δ*tyrT*::*kan* strain. The wild-type-derived Δ*tyrT*::*kan* strain DTK-12 shows a transient lag phase caused by glycine starvation when shifted from a rich to a minimal medium (Fig. 1C and D) that strictly depends on a functional stringent control system and is only detectable in a *relA*$^+$ background, whereas the Δ*tyrT*::*kan* derivative of the mutant *relA* strain MC4100 does not show this phenotype (22). The growth lag of DTK-12 is observed with mannitol as carbon source and to a greater extent with glycerol as carbon source (Fig. 1C and D), whereas with glucose as carbon source only a subtle lag phase was observed (data not shown).

For further analysis of this phenotype, the Δ*tyrT*::*kan* marker was transferred to the *relA1 spoT1* mutant CA274 by generalized transduction to construct the Δ*tyrT*::*kan* variant DTC274 that is affected in ppGpp synthetase I (*relA*) and II (*spoT*) and ppGpp turnover (*spoT*) (14, 43). A phenotype different from that in DTK-12 was observed in strain DTC274. When the cells were precultured in rich medium and then shifted to M9 minimal medium supplemented with tryptophane and with glucose as carbon source, they were unable to grow, in contrast to the parent strain CA274 (Fig. 1A). Glucose was chosen as carbon source because with mannitol or glycerol as carbon source, the growth rate of the parent strain CA274 was markedly reduced. Under anaerobic conditions (M9 minimal medium with tryptophane and supplemented with 0.8% glucose, or with 0.8% glycerol and 50 mM nitrate, or with 0.8% glycerol and 50 mM fumarate), the growth of strains CA274 and DTC274 was very poor compared with that of wild-type strain K-12, but was identical between them. The colony-forming ability of strain DTC274 on minimal glucose plates with tryptophane was very low compared with that of the parent strain CA274 but was not completely abolished, indicating that strain DTC274 is not a classical auxotroph and may be favored by the microaerobic conditions on plates. Attempts to complement the growth arrest of DTC274 after shift down to minimal glucose medium by the addition of a vitamin mixture (see Materials and Methods), by the addition of nucleosides (inosine, uridine, and cytidine; 20 mg/liter), or by the addition of common trace elements did not show any effects. Increasing the amount of Casamino Acids from 0.03% to 0.5% shifted the growth arrest from 0.1 to 0.3 A_{578}. Only the addition of yeast extract and/or tryptone (0.5% to 1%) restored growth of strain DTC274 (data not shown). After prolonged incubation of strain DTC274 in minimal glucose medium for 36 to 48 h, the outgrowth of bacteria to stationary phase was observed in some cases. The outgrowing bacteria still showed the marker Kan^r Lac$^-$ Trp$^-$. The phenomenon was interpreted as the outgrowth of very rare spontaneous mutants rather than an adaptation to the minimal glucose medium, because it was not observed reproducibly and demanded prolonged incubation periods.

In contrast to strain DTK-12, the phenotype of DTC274 was restored neither by the addition of glycine (data not shown) nor by transformation of DTC274 with plasmid pUM302, which contains solely the *tyrT* operon (Fig. 1A). The failure to complement the phenotype of strain DTC274 by the *tyrT* operon suggested a synergistic effect of the newly introduced Δ*tyrT*::*kan* marker with preexisting mutations and/or an additional unknown mutation within the *tyrT* region that was cotransduced and raised the question whether the different phenotypes of the two Δ*tyrT*::*kan* strains DTK-12 and DTC274 had a common genetic origin.

Restriction analysis of the *tyrT* region. The *tyrT* region has been isolated from two different *E. coli* strains: the *supF* allele

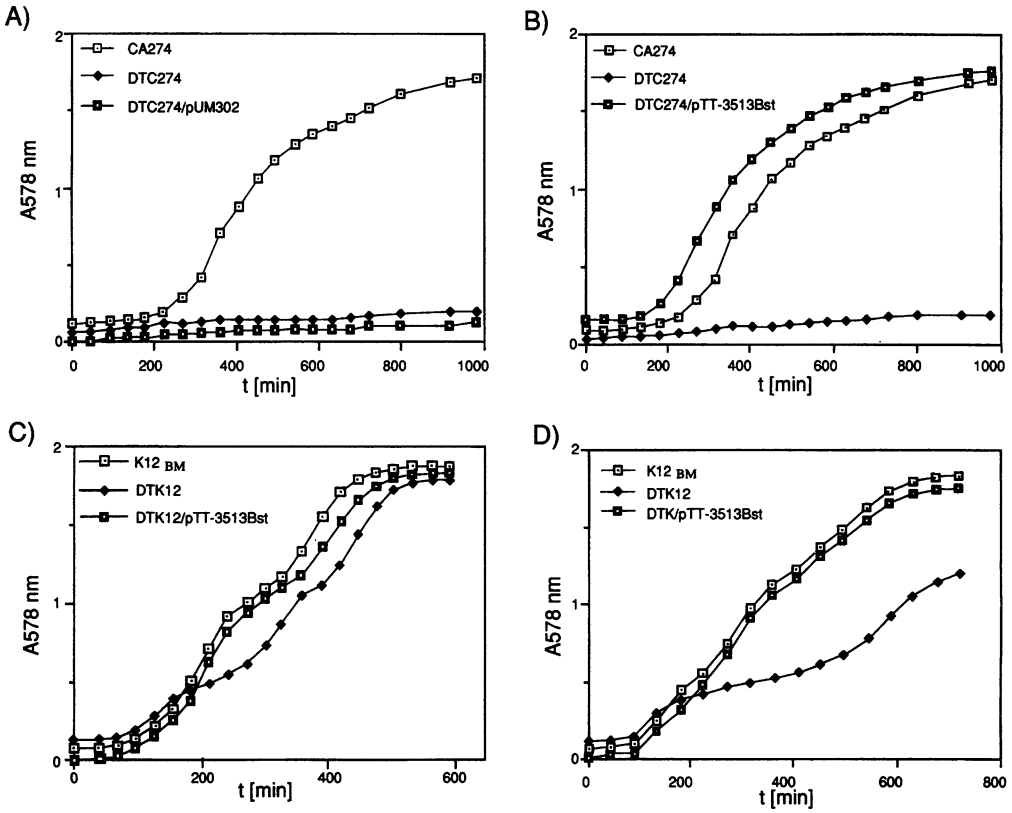

FIG. 1. Growth phenotype of *E. coli* Δ*tyrT*::*kan* strains and its genetic complementation. Symbols representing the strains are indicated on panels A to D. Strains were precultured in rich medium and then transferred to a minimal medium, supplemented with 0.03% Casamino Acids and with (A and B) tryptophane (50 μg/ml) and 0.4% glucose, (C) 0.25% mannitol, and (D) 0.4% glycerol. For strains CA274 and DTC274, glucose was used as carbon source instead of mannitol or glycerol to improve the growth rate. (A) Growth of the Δ*tyrT*::*kan* strain DTC274 and its transformant DTC274/pUM302 compared with that of the parent strain CA274. (B) Growth of strain DTC274 and its transformant DTC274/pTT-3513Bst compared with that of CA274. (C) Growth of the Δ*tyrT*::*kan* strain DTK-12 and its transformant DTK12/pTT-3513Bst compared with that of the parent strain $K\text{-}12_{BM}$ with mannitol as carbon source. (D) The same as in panel C but with glycerol as carbon source. For strain DTK-12 (shown in panels C and D) but not for strain DTC274 (panel A, B), the parental growth pattern is also restored by the addition of glycine.

is from strain CA275, the daughter strain of strain CA274 used in this study (see Table 1), and the *tyrT* allele is from our laboratory strain $K\text{-}12_{BM}$ (22). Both genomic regions were available for this study (plasmids pUM-E containing the *supF* region as a 5.7-kb *Eco*RI fragment and pUM101 containing *tyrT* as a 6.6-kb *Sal*I-*Bam*HI fragment).

A detailed restriction fragment analysis of both plasmids was performed to elucidate putative differences between the two genomic clones within the *tyrT* region. The *tyrT* allele contained an 800-bp insertion within a *Sal*I-*Eco*RV fragment located about 3 kb upstream of *tyrT* that was marked by an additional *Pst*I site (data not shown). This region was characterized in detail because it was supposed that the genomic differences between the *tyrT* and *supF* clones were responsible for the phenotypic differences between the two Δ*tyrT*::*kan* strains DTC274 and DTK-12. The *Sal*I-*Eco*RV fragment was subcloned from both plasmids, and their nucleotide sequences were determined (positions 81 to 818; see Fig. 3).

The restriction length polymorphism resulted from the integration of an insertion element, *IS1* (27), in the *tyrT* allele of the laboratory strain $K\text{-}12_{BM}$ at position 430 (the integration site is marked *IS1*/*DR* in Fig. 3) flanked by the 9-bp direct repeat sequence GGCAGCAAG that was duplicated during the integration event.

The integration site was found within a continuous reading frame extending beyond the sequenced *Sal*I-*Eco*RV fragment. To complete the sequence of the reading frame, the 1.5-kb *Pst*I fragment and the 0.56-kb *Stu*I-*Sal*I fragment of plasmid pUM-E were subcloned and their sequences were determined.

A new gene coding for a regulator response protein. The overlapping sequences resulted in an open reading frame (ORF37) that was oriented opposite to *tyrT* and consisted of 337 codons from nucleotides 1069 to 59 (see Fig. 3) coding for a protein with a calculated molecular mass of 37.3 kDa, a net charge of −7 at physiological pH, and a pI of 5.7. Its expression was shown by a coupled transcription-translation assay in vitro using plasmid pUM-E as the template (see Fig. 4). A putative ribosome binding site (GGAG) was located 5 bp upstream of the start codon. As putative regulatory sequences, a −10 box from positions 1159 to 1154 (**CAGAAT**) and, in a 16-bp distance, a −35 region from positions 1181 to 1176 (**TTGGTA**) were found.

A computer search in the GenBank data base with the amino acid sequence of ORF37 showed striking similarities to a couple of proteins belonging to the regulator response family (for a review, see reference 38), with complete conservation of invariant amino acids (D-15, D-58, and K-108) and of highly conserved amino acids (data not shown). These proteins are

part of a signal transduction pathway in bacteria. The similarity was only found with the N-terminal part known to carry the regulatory domain that is modified by a corresponding histidine kinase at an aspartyl residue. The C-terminal part of ORF37 did not show any similarity, so that conclusions with respect to the catalytic function of the protein were not possible.

The ORF37 gene that was isolated from our laboratory strain K-12_{BM} carried an insertion element, *IS1*, at position 430. The open reading frame was interrupted at the 197th amino acid within the functional (catalytic) domain (part) of the protein. This integration event had occurred in the laboratory strain K-12_{BM}, whereas the other strains investigated (W3110, DTK-12, JC9387, MC4100, and CA275) did not contain an *IS1* element in the corresponding restriction fragment (Fig. 2A and B). Although strain DTK-12 is derived from strain K-12_{BM}, that *IS1* element was missing in DTK-12 demonstrating that the intact ORF37 was cotransduced from strain JC9387 together with the marker Δ*tyrT*::*kan* during construction of the *tyrT* deletion strain (22). Therefore, it was ruled out that the phenotypic differences of strains DTK-12 and DTC274 were caused by a mutation in ORF37. The *IS1* pattern of the laboratory strain K-12_{BM} was compared with that of strain K-12 obtained from the Deutsche Sammlung von Mikroorganismen (DSM) strain (number 498; see Table 1). In the DSM strain, the specific *IS1* element was also missing (Fig. 1C). The laboratory strain was designated with the preliminary appendix BM (K-12_{BM}; see Table 1).

The spontaneous mutation ORF37::*IS1* is a distinctive genetic marker of strain K-12_{BM}, but it is not yet possible to assign any phenotype to it. Strain K-12_{BM} did not show any auxotrophy or any alterations in growth behavior, so ORF37 is not essential under normal growth conditions. Anaerobic growth (under glycolytic conditions or under nitrate- or fumarate-respirative conditions) was not impaired. The genetic markers *galU* and *drs* were also tested, because both have been mapped at 27 min (18, 19), but growth on minimal medium with galactose as the carbon source (*galU*) or with glucose, both supplemented with leucine and serine (*drs*), was not affected.

Nucleotide sequence of the 3.6-kb 5′ region of *tyrT*. During analysis of ORF37, complementation studies in strain DTC274 were performed with the plasmid pUM-E, containing the intact ORF37, and plasmid pUM101, containing most of ORF37::*IS1*. Both plasmids restored wild-type behavior, confirming that ORF37 was not involved in complementation, whereas the plasmid pUM302 containing only the *tyrT* operon did not complement the phenotype. Therefore, the complementing region was mapped between ORF37 and *tyrT*.

The complementing region was subcloned as a 3.2-kb *Ava*I fragment from plasmid pUM101 in pUC19, resulting in plasmid pUT-A1. The sequence of pUT-A1 was determined for both strands from overlapping deletion subclones and linked to the sequence of ORF37. The complete sequence is presented in Fig. 3. In addition to ORF37, three further reading frames were found.

Upstream of ORF37, in the same orientation, an open reading frame (ORF34) from positions 2105 to 1164, starting with a GTG codon, which might encode a protein consisting of 314 amino acids with a molecular mass of 34.4 kDa and a calculated pI of 6.7, was found. A possible ribosome binding site (GAG) was located 10 bp upstream of the start codon (positions 2118 to 2116). A putative −10 region was from positions 2218 to 2213 (**TATACT**); however, a suitable −35 region was not found.

In the opposite direction, a small reading frame (URF13)

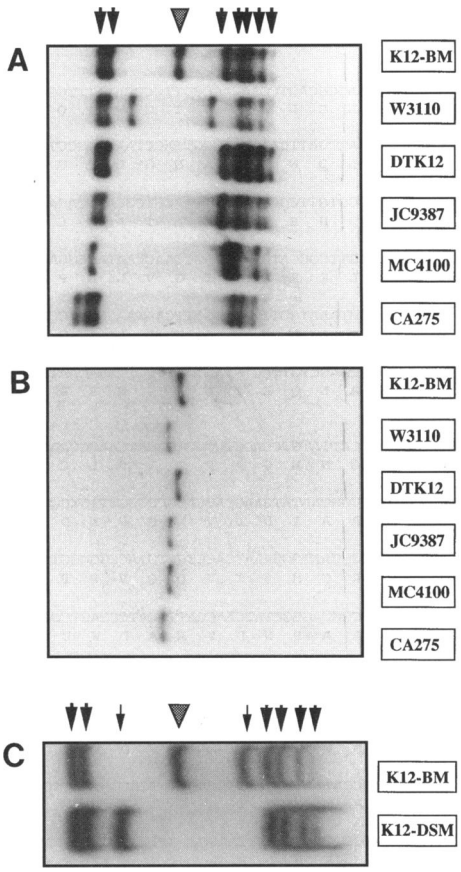

FIG. 2. Distribution patterns of insertion elements *IS1* in different *E. coli* strains. Genomic DNA was restricted with *Eco*RI, separated by electrophoresis on a 0.6% agarose gel, and blotted as described in Materials and Methods. The strains are indicated to the right of the corresponding lanes. (A) Hybridization with an *IS1*-specific nick-translated restriction fragment (0.3-kb *Bst*EII-*Ava*I fragment). (B) The same blot as in panel A, but hybridized against an ORF37-specific probe (0.46-kb nick-translated *Ava*I-*Eco*RV fragment) to identify specifically that *IS1* element that had integrated into ORF37. (C) Comparison of the *IS1* pattern of our laboratory strain K-12_{BM} with the K-12 strain obtained by the DSM (in this figure indicated as K12-DSM). Note that Fig. 2C is not in scale with Fig. 2A and B. *IS1* elements are marked by arrows. One element differing between strains K-12_{BM} and K-12-DSM (Fig. 2C) because of a restriction length polymorphism is marked by two thin arrows. The *IS1* element specific for strain K-12_{BM} that had integrated into ORF37 is indicated by a dotted triangle.

from positions 2278 to 2634, consisting of 113 amino acids with the coding capacity for a 13.5-kDa peptide with a pI of 8.1, was found. A putative ribosome binding site or promoter region was not detected; furthermore a gene product corresponding to this reading frame was not identified. Therefore, its amino acid sequence was omitted from Fig. 3.

Finally, an open reading frame (*tgs*) from positions 2687 to 3529 with the coding capacity for a 280-amino-acid peptide with a molecular weight of 31.9 kDa and a theoretical pI value of 7.0 was found. A putative ribosome binding site (AAGG) 11 bp upstream of the start codon, a possible −10 region (**TATATT**) from positions 2548 to 2553, and, in an 18-bp distance, a −35 region (**TTGAAA**) were located. The *tgs* reading frame terminated adjacent to the promoter and upstream activating sequence of *tyrT*, which is also indicated in Fig. 3.

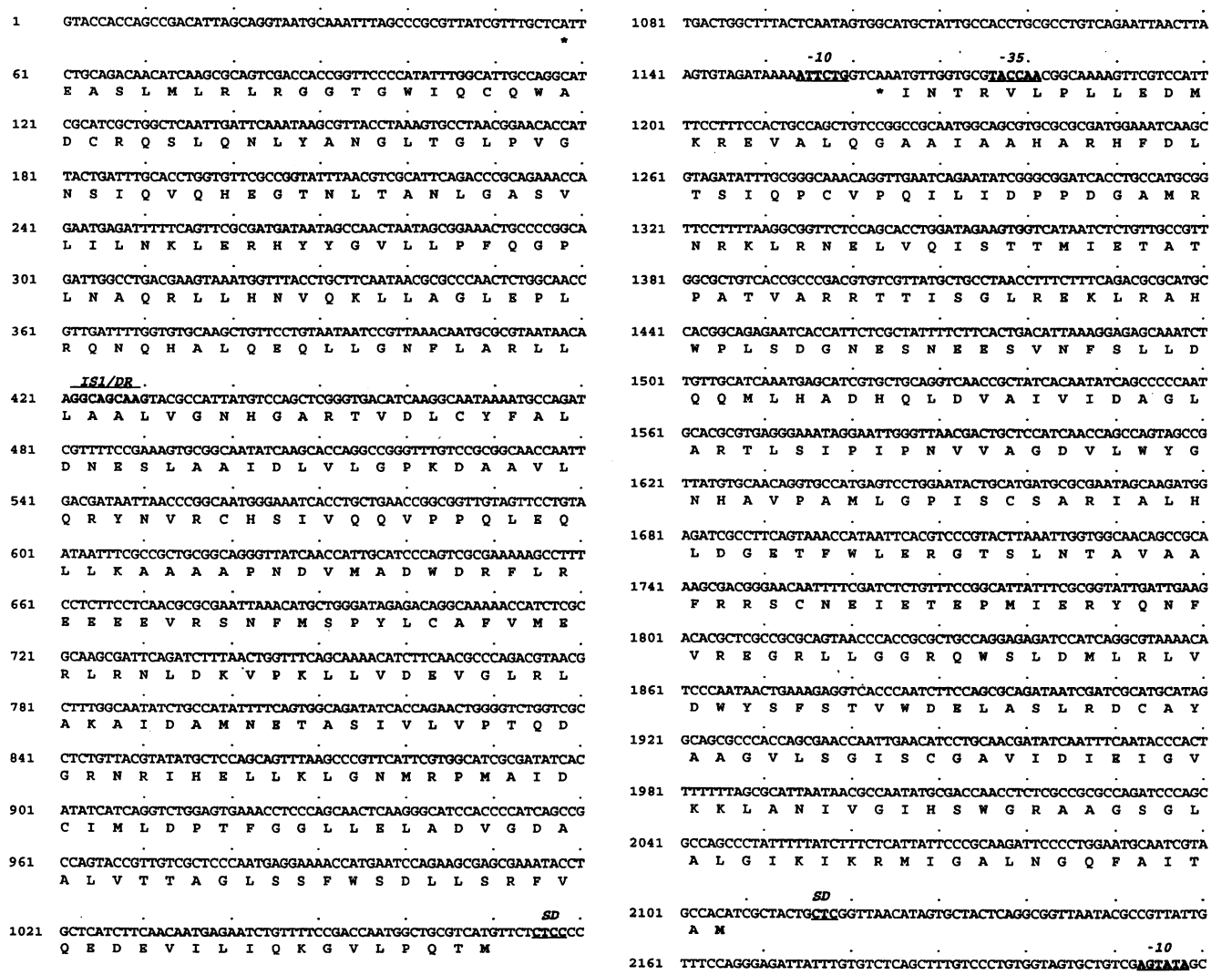

FIG. 3. Nucleotide sequence of the *tyrT* 5′ region. The deduced open reading frames are presented in the standard one-letter code. Asterisks denote stop codons. Potential ribosome binding sites (*SD*) and putative promoter regions (−10 and −35) are underlined. The transcriptional start sites for the *tgs* gene and the *tyrT* operon are indicated by arrowheads (>). The putative PurR binding site in front of the *tgs* gene and the binding site for the transactivator protein Fis in the upstream activating sequence of the *tyrT* operon are also indicated. The integration site of an insertion element, *IS1*, identified in the corresponding DNA sequence derived from strain K-12$_{BM}$ (see the text), is marked *IS1/DR*. The GenBank accession number M64675 was assigned to the sequence described in this figure.

By the method of Brendel and Trifonov (4), a factor-independent RNA polymerase terminator was not found between *tgs* and *tyrT*, between ORF34 and ORF37, or downstream of ORF37, suggesting that the latter two reading frames are organized in an operon that extends beyond the sequenced region.

A coupled transcription-translation assay was performed to determine whether the open reading frames were actively transcribed and to identify their gene products (Fig. 4). The proteins were synthesized in vitro from plasmid templates and were labelled with [^{35}S]methionine. They were separated by SDS-PAGE and analyzed by autoradiography. Plasmid pTT-EVA22 (containing the insert from positions 1962 to 3703) expressed, in addition to vector-encoded proteins, a peptide with a relative molecular mass of 32 kDa that agreed well with the predicted molecular mass of *tgs*. Plasmid pUT-A1 expressed *tgs* and ORF34, which migrated slightly differently, as expected at 36 kDa. Plasmid pUM-E expressed three further genes: ORF37, which migrated according to the calculated molecular mass, and two peptides with relative molecular masses of 34 and 16 kDa. The latter two are encoded downstream of ORF37 and are presently under investigation.

Complementation of growth inhibition in the *relA spoT* Δ*tyrT*::*kan* mutant DTC274. The 3.2-kb *Ava*I insert of plasmid pUT-A1 was further subcloned in the vector pACYC184 as shown in Fig. 5. The resulting plasmids were transformed in strain DTC274 and tested for their ability to complement the *tyrT* deletion phenotype. The 5′ end of plasmid pTT-3513Bst was generated by exonuclease III treatment; its 3′ end was marked by the restriction site *Bst*EII and is located upstream of any known *tyrT* regulatory sequences. The plasmid exclusively contained the *tgs* gene. Growth inhibition in strain DTC274 was completely overcome in the transformant DTC274/pTT-3513Bst (Fig. 1B). Plasmid pTT-3513Bst also fully restored

```
2221  CTATGTTGCCACCCTTATGTGTCTGGTGAAAAGGTTGCACCTGATCCAGAACATCTCATG
2281  CGTTCGCGTTACTGCGCTTTTGTGATGCAAGACGCAGATTATTTAATAAAGACCTGGCAT
2341  CCCTCTTGTGGAGCAGCGGCGTTACGTGCCGAATTGATGGCCGGATTTGCGCATACTGAA
2401  TGGTTGGGATTGACCGTATTTGAACATTGTTGGCAGGACGCCGACAATATCGGATTTGTC
                                                        PurR
2461  AGTTTTGTTGCTCGATTTACCGAAGGTGGTAAAACAGGTGCAATAATTGAACGTTCGCGA
        -35                 -10      >> >
2521  TTTTTGAAAGAAAACGGTCAATGGTACTATATTGACGGTACACGTCCGCAGTTTGGTCGC
                                          --->tgs
2581  AACGATCCCTGCCCTTGTGGTTCAGGTAAAAAATTTAAAAAGTGCTGCGGCCAATAATGG
                                  SD
2641  TTGACGGTACGGTTTAGCAAACACTCTCAACAAGGTTTTTCCAGCAATGCATTCACTCCA
                                                    M  H  S  L  Q
2701  ACGTAAAGTTCTGCGTACTATTTGTCCGGACCAAAAAGGTCTGATCGCACGTATTACCAA
       R  K  V  L  R  T  I  C  P  D  Q  K  G  L  I  A  R  I  T  N
2761  TATTTGCTACAAGCACGAGTTAAATATCGTACAGAACAATGAATTTGTTGATCACCGTAC
       I  C  Y  K  H  E  L  N  I  V  Q  N  N  E  F  V  D  H  R  T
2821  CGGGCGCTTTTTTATGCGCACGGAACTGGAAGGGATTTTTAATGATTCCACCCTGCTGGC
       G  R  F  F  M  R  T  E  L  E  G  I  F  N  D  S  T  L  L  A
2881  GGATCTCGATAGCGCATTGCCAGAAGGCTCCGTGCGTGAGCTGAATCCTGCCGGTCGTCG
       D  L  D  S  A  L  P  E  G  S  V  R  E  L  N  P  A  G  R  R
2941  CCGGATAGTGATTCTGGTCACTAAAGAAGCGCATTGCCTTGGCGATTTGTTGATGAAAGC
       R  I  V  I  L  V  T  K  E  A  H  C  L  G  D  L  L  M  K  A
3001  CAATTACGGCGGCCTGGATGTCGAAATCGCGGCAGTTATTGGTAACCACGATACTTTACG
       N  Y  G  G  L  D  V  E  I  A  A  V  I  G  N  H  D  T  L  R
3061  TTCTCTGGTTGAGCGTTTTGATATTCCGTTTGAGCTGGTAAGCCATGAAGGGTTAACCCG
       S  L  V  E  R  F  D  I  P  F  E  L  V  S  H  E  G  L  T  R
3121  CAACGAGCACGATCAAAAGATGGCGGATGCCATTGATGCTTATCAACCTGACTACGTGGT
       N  E  H  D  Q  K  M  A  D  A  I  D  A  Y  Q  P  D  Y  V  V
3181  GCTGGCGAAGTATATGCGGGTATTAACGCCGGAATTTGTGGCACGCTTCCCGAATAAGAT
       L  A  K  Y  M  R  V  L  T  P  E  F  V  A  R  F  P  N  K  I
3241  CATCAATATTCACCATTCATTCCTGCCAGCGTTTATTGGCGCACGTCCTTATCACCAGGC
       I  N  I  H  H  S  F  L  P  A  F  I  G  A  R  P  Y  H  Q  A
3301  CTATGAACGTGGTGTGAAGATTATTGGCGCAACCGCTCACTATGTGAATGACAATCTGGA
       Y  E  R  G  V  K  I  I  G  A  T  A  H  Y  V  N  D  N  L  D
3361  CGAAGGCCCAATCATCATGCAGGACGTTATTCATGTCGATCATACCTACACAGCTGAAGA
       E  G  P  I  I  M  Q  D  V  I  H  V  D  H  T  Y  T  A  E  D
3421  TATGATGCGCGCAGGTCGTGACGTCGAGAAAAACGTCTTAAGTCGTGCACTATACAAAGT
       M  M  R  A  G  R  D  V  E  K  N  V  L  S  R  A  L  Y  K  V
3481  ACTGGCACAGCGCGTCTTTGTTTACGGTAATCGAACGATTATTCTTTAATCGCCAGCAAA
       L  A  Q  R  V  F  V  Y  G  N  R  T  I  I  L  *
                                  Fis
3541  AATAACTGGTTACCTTTAATCCGTTACGGATGAAAATTACGCAACCAGTTCATTTTTCTC
            -35                 -10      >
3601  AACGTAACACTTTACAGCGGCGCGTCATTTGATATGATGCGCCCCGCTTCCCGATAAGGG
                                                    --->tyrT
3661  AGCAGGCCAGTAAAAAGCATTACCCCGTGGTGGGGTTCCCGAG
```

FIG. 3—*Continued.*

wild-type behavior in strain DTK-12, resulting in a short transient lag phase with mannitol as carbon source (Fig. 1C) or abolishing an extended lag phase with glycerol as carbon source (Fig. 1D). Because of the complementation of the transient glycine starvation phenomenon in strain DTK-12, the gene was designated *tgs*.

In plasmid pTT-3513Sc, a small deletion of 68 bp in *tgs* including the coding sequence for the 15 C-terminal amino acids completely abolished the complementing activity in strain DTK-12 as well as in strain CA274, confirming that an intact *tgs* gene is the complementing unit and suggesting that the C-terminal sequence of *tgs* is essential to express a functional

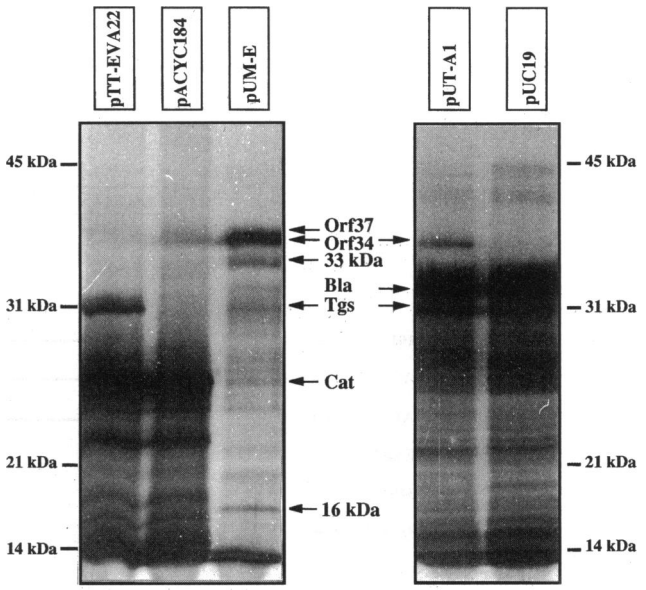

FIG. 4. Identification of plasmid-encoded proteins by a coupled transcription-translation assay in vitro. The plasmids used as templates are indicated above the lanes. The synthesized proteins were labelled with [^{35}S]methionine, separated on an SDS–12% PAGE gel, and detected by autoradiography. The migration of the molecular mass marker proteins is shown. The vectors pACYC184 and pUC19 were used as controls to determine vector-encoded proteins. Abbreviations: Bla, β-lactamase; Cat, chloramphenicol acetyltransferase. Plasmid pTT-EVA22 expressed Tgs, plasmid pUT-A1 expressed Tgs and ORF34, and plasmid pUM-E expressed Tgs, ORF34, ORF37, and two so-far uncharacterized proteins with molecular masses of 16 and 33 kDa.

product. The truncated *tgs* gene in plasmid pTT-3513Sc resembled the genomic situation in the Δ*tyrT*::*kan* strains. The *Sca*I restriction site at the end of *tgs* marks the start of the kanamycin resistance gene that replaces the *tyrT* operon (22). Therefore, the Δ*tyrT*::*kan* allele inactivates both the *tgs* gene and the *tyrT* operon.

Characterization of the new gene *tgs*. The mRNA of the *tgs* gene was identified by Northern blot analysis using a nick-translated 500-bp *Bst*EII fragment (Fig. 6). According to rRNA as a size marker the length was estimated to be 1,000 nucleotides. The *tgs* mRNA was identified in total RNA from strain K-12$_{BM}$ (Fig. 6, lane 1) but was completely absent in the Δ*tyrT*::*kan* strain DTK-12 (lane 2), confirming the results of the complementation experiments. Most probably, the truncated mRNA was rapidly degraded and was therefore not detected. In strain DTK-12 transformed with a *tgs*-containing plasmid (DTK12/pTT-3403A; Fig. 6, lane 3), the mRNA was strongly overexpressed.

The transcription start site of *tgs* was determined by primer extension analysis with a 23-mer oligonucleotide complementary to positions 2618 to 2640 (Fig. 7). Total RNA was isolated from strain DTK12/pTT-3403A (Fig. 7, lane 1) and from strain K-12$_{BM}$ (lane 2); crude tRNA from *E. coli* was used as a control (lane 3). A dideoxy-sequencing reaction performed with plasmid DNA (plasmid pUT-A1) with the 5′-phosphorylated extension primer served as the size marker. The identified cDNAs mapped at G-2557, G-2558, and to a minor extent at A-2560. The analysis confirmed the predicted promoter sequence **TTGAAA-N$_{18}$-TATATT** (positions 2524 to 2553), resembling a characteristic σ^{70} promoter.

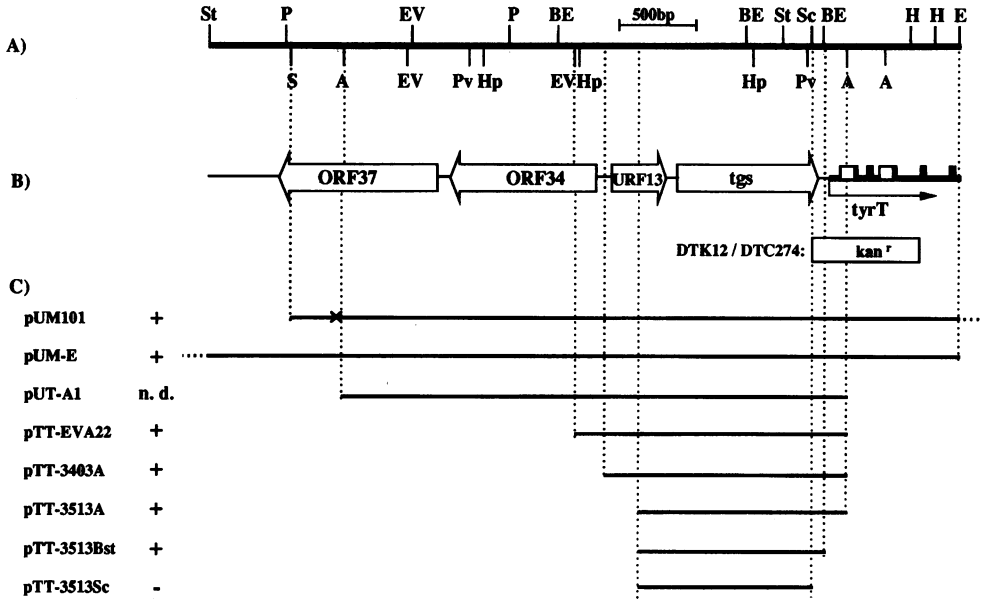

FIG. 5. Organization of the *tyrT* 5′ region and identification of the genetic unit complementing the *tyrT* deletion phenotypes in strains DTK-12 and DTC274. (A) Restriction map of the *tyrT* 5′ region. Abbreviations: St, *Stu*I; P, *Pst*I; S, *Sal*I; A, *Ava*I; EV, *Eco*RV; Pv, *Pvu*II; Hp, *Hpa*I; BE, *Bst*EII; Sc, *Sca*I; H, *Hin*dIII; E, *Eco*RI. (B) Genetic map of the *tyrT* 5′ region. Open reading frames (ORF37, ORF34, and *tgs*) and the unidentified reading frame URF13 are represented by open arrows. The *tyrT* operon is drawn as a thick line; the two $tRNA_1^{Tyr}$ genes are drawn as open boxes with the last 19 nucleotides of the tRNA sequence as dotted boxes (CCA motif). Below, the sequence replaced by a kanamycin cassette (kan^r) in the Δ*tyrT*::*kan* strains DTK12 and DTC274 is indicated as an open rectangle. (C) Genetic complementation of the *tyrT* deletion phenotype in strains DTK-12 and DTC274. The thick lines represent the DNA inserts of the corresponding plasmids, drawn in scale to the restriction map and the genetic map. The plasmids pUM101 and pUM-E exceed the map (indicated by the dotted lines). The cross in plasmid pUM101 symbolizes the integration site of an insertion element, *IS1*. The complementing activity of the plasmids is indicated by + or −, as appropriate.

At 7 bp upstream of the −35 region, an 8-bp palindromic sequence was found. Comparison with known operator sequences revealed some similarity with the *pur* operator, the binding site for the PurR repressor protein. Of 16 bp, 10 bp (written in bold and uppercase letters) of the sequence AattgAAC*GTTcGCGa matched the PurR binding consensus sequence A(c/g)G(c/a)AAAC*GTTT(t/g)CGT derived from previously published sources (28, 37, 42).

As determined by the program TFastA, the deduced protein sequence of Tgs showed striking similarity to that of the purine biosynthesis enzyme glycinamide ribonucleotide (GAR) transformylase of *Drosophila melanogaster* (13) and with the corresponding *purN*-encoded enzyme of *E. coli* (36) and *Bacillus subtilis* (8). The protein sequences of these enzymes were aligned with the Tgs sequence (Fig. 8), and common sequence motifs are indicated. Similar results were obtained with the GAR transformylase of *Drosophila pseudoobscura* (12) and with the corresponding human cDNA clone (1) (data not shown). An optimal alignment with the program Bestfit of the 200 C-terminal amino acids of Tgs showed 48% similarity and 27% identity of Tgs with PurN of *E. coli*, 54% similarity and 28% identity with PurN of *B. subtilis*, and 49% similarity and 24% identity with the GAR transformylase domain of *D. melanogaster*. The values for the homolog PurN proteins of *E. coli* and *B. subtilis* were only slightly higher, with 59% similarity and 30% identity.

The similarity of Tgs to a purine biosynthesis enzyme led us to investigate whether the *tyrT* deletion phenotypes were complemented by supplementing the growth medium with purines or purine derivatives. The minimal medium was supplemented either with guanine (100 μM), adenine (100 μM), hypoxanthine (100 μM), AICA [5(4)-aminoimidazol-4(5)-carboxamide] as the only precursor compound that can be added and used by a salvage pathway (100 μM), thiamine (20 mg/liter) that is synthesized from the purine precursor 5′-phosphoribosyl-5-aminoimidazole, or the pyrimidine uracil (100 μM) as a control. However, none of the supplements restored wild-type behavior, indicating that purine biosynthesis is not impaired in the Δ*tyrT*::*kan* strains.

The 60 N-terminal amino acids of Tgs did not show any similarity to GAR transformylase sequences. In this domain, the motif $H\text{-}X_{10}\text{-}C\text{-}X_{13}\text{-}C\text{-}X_2\text{-}H\text{-}X_{12}\text{-}H$ (corresponding to amino acids 2 to 43 of Tgs) is unusually rich in histidine and cytidine residues, which might complex divalent cations. A similar arrangement of amino acid residues has been found in aminoacyl-tRNA synthetases and has been called the Cys-His box (24).

DISCUSSION

Deletion of the *tyrT* operon in a wild-type strain of *E. coli* causes a transient glycine starvation phenomenon during midlogarithmic growth that strictly depends on a functional stringent control system (22). For further analysis of this phenotype, the Δ*tyrT*::*kan* mutation was transduced into the *relA1 spoT1* strain CA274 to construct strain DTC274. This strain showed a dramatic, completely unexpected phenotype: when it was shifted to a minimal medium, growth was fully arrested, and in contrast to that of strain DTK-12, the phenotype was not complemented or weakened by transformation with a plasmid containing solely the *tyrT* operon.

In the wild-type-derived strain DTK-12, expression of rtT

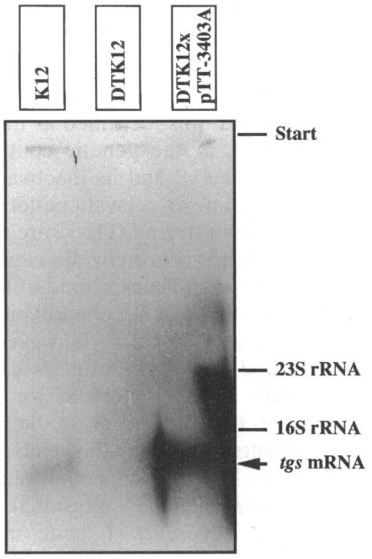

FIG. 6. Identification of *tgs* mRNA by Northern blot analysis. Total RNA was isolated from strains K-12$_{BM}$, DTK-12, and DTK-12/pTT-3403A grown in minimal medium supplemented with mannitol and 0.05% Casamino Acids and harvested at an A_{578} of 0.5. A 20-μg quantity of RNA was separated by formaldehyde gel electrophoresis on a 1% agarose gel, blotted onto a cellulose nitrate membrane, and hybridized against a *tgs*-specific probe (nick-translated 500-bp *Bst*EII fragment; see Fig. 5A). rRNA served as a size marker.

RNA, encoded by the repeated units of the *tyrT* operon, markedly curtails the characteristic lag phase in the Δ*tyrT*::*kan* mutant, but under more restricted growth conditions only a partial restoration of the wild-type behavior, instead of full complementation, has been obtained (3). Furthermore, a

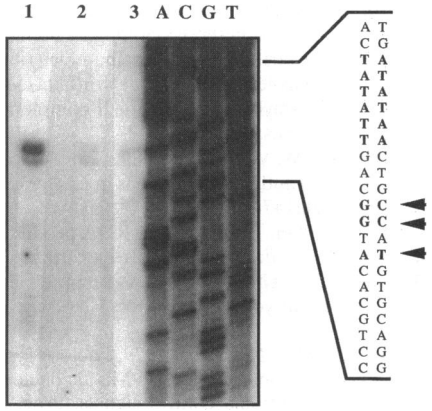

FIG. 7. Determination of the transcription start site of *tgs* mRNA by primer extension analysis. Total RNA was isolated as described for Fig. 6 from strains DTK-12/pTT-3403A (lane 1) and K-12$_{BM}$ (lane 2). A crude tRNA preparation served as a negative control (lane 3). A ^{32}P-end-labelled oligonucleotide, complementary to positions 2618 to 2640, was annealed to 20 μg of total RNA, extended by avian myeloblastosis virus reverse transcriptase, and analyzed on an 8% phosphonoacetic acid sequencing gel together with a dideoxy-sequencing reaction performed with the same 5′-phosphorylated oligonucleotide and plasmid pUT-A1 (A, C, G, and T). The relevant sequence is indicated. The −10 promoter region is written in bold, and the transcription start sites (G-2557, G-2558, and A-2560) are marked by arrows.

```
Drogart  ---RKDPKKS QVVVQNFEAS L..ARTQKML SQRRKRVAVL ISGTGSNLQA
Tgs      -TELEGIFND STLLADLDSA LPEGSVRELN PAGRRRIVIL VTKEAHCLGD
B.s.PurN ..........  ..........  ..........  ...MKKFAVF ASGNGSNFEA
E.c.PurN ..........  ..........  ..........  ....MNIVVL ISGNGSNLQA

Drogart  LIDATRDSAQ GIHADVVLVI SNKPGVLGLQ RATQAGIP..  SLVISHKDFA
Tgs      LL..MKANYG GLDVEIAAVI GN..HDTLRS LVERFDIP..  .FELVSHEGL
B.s.PurN IV..TRLKEE NWDASRALLV CDKPQAKVIE RAERFHIPSF  AFEPKSYEN.
E.c.PurN II..DACKTN KIKGTVRAVF SNKADAFGLE RARQAGIATH  TLIASAFDS.

Drogart  SREVYDAELT RNLKAARVDL ICLAGFMRVL SAPFVREWRG  RLVNIHPSLL
Tgs      TRNEHDQKMA DAIDAYQPDY VVLAKYMRVL TPEFVARFPN  KIINIHHSFL
B.s.PurN .KAAFEQAII EQLRLHEVEL IALAGYMRLI GDTLLQAYGG  KIINIHPSLL
E.c.PurN .REAYDRELI HEIDMYAPDV VVLAGFMRIL SPAFVSHYAG  RLLNIHPSLL

Drogart  PKYPGLHVQK QALEAGEKES GCTVHFVDEG VDTGAIIVQA  AVPILPDDDE
Tgs      PAFIGARPYH QAYERGVKII GATAHYVNDN LDEGPIIMQD  VIHVDHTYTA
B.s.PurN PAFPGIDAVG QAFRAGVKVA GITVHYVDEG MDTGPIIAQK  AIEIDEHDTL
E.c.PurN PKYPGLHTHR QALENGDEEH GTSVHFVTDE LDGGPVILQA  KVPVFAGDSE

Drogart  DSLTQRIHKA EHWAFPRALA MLVN..GTAL ISPEVSSQ..  ..........
Tgs      EDMMRAGRDV EKNVLSRALY KVLA..QRVF VYGNRTIIL.  ..........
B.s.PurN ETIEQRIHKL E....HKWYP SVIKQLLG..  LNNRGEKA..  ..........
E.c.PurN DDITARVQTQ E....HAIYP LVISWFADGR LKMHENAAWL  DGQRLPPQGY
```

FIG. 8. Amino acid alignments of the GAR transformylase domain of *D. melanogaster* (Drogart [13]), of Tgs, and of the PurN proteins of *B. subtilis* (B.s. PurN [8]) and *E. coli* (E.c. PurN [36]). Identical or functionally homologous amino acids within the proteins are written in boldface and italic. Common sequence motifs are underlined. The standard one-letter amino acid nomenclature is used.

similar influence of rtT RNA has been observed under artificially induced growth arrest by isoleucine deprivation, suggesting a general modulatory effect of rtT RNA on stringent response. In addition to the incomplete complementation of the phenotype of strain DTK-12, the failure to influence the *tyrT* deletion phenotype of strain DTC274 by a plasmid expressing rtT RNA demonstrated that deletion of the *tyrT* operon was not or at least was not the only genetic cause of the observed phenotype.

The main intention of this study was therefore to find the second site mutation that is involved or responsible for the dramatic *tyrT* deletion phenotype in strain DTC274 and to investigate whether the completely different phenotypes in strains DTC274 and DTK-12 have a common genetic basis. A restriction length polymorphism in the *tyrT* 5′ region between a genomic *tyrT* clone isolated from our laboratory strain K-12$_{BM}$ and strain CA275 was found. Although not confirmed during the course of this study, we originally supposed that this genetic difference could account for the phenotypic differences between the K-12- and the CA274-derived *tyrT* deletion strains and that it characterized the respective DNA region in detail. It encoded ORF37, a new member of the regulator response family, which was identified by extensive primary and secondary structure homology to already-known members of this family of homologous proteins. They are characterized by a conserved N-terminal domain that is enzymatically converted by a cognate histidine kinase. Together with the cognate sensory kinase, the regulatory response protein defines an adaptive response system. In *E. coli*, members of these homologous families have been identified in more than 10 different adaptive response systems (for a review, see reference 38), including chemotaxis (CheA-CheY), nitrogen regulation (NtrB-NtrC), phosphate regulation (PhoR-PhoB), nitrate reductase regulation (NarX-NarL), oxygen regulation (ArcB-ArcA), and osmotic porin regulation (EnvZ-OmpR). The cognate sensory kinase of ORF37 is not known.

The restriction length polymorphism resulted from the integration of an insertion element, *IS1*, within the ORF37 coding region of the laboratory strain K-12$_{BM}$. It was shown by Southern blot analysis that the localization of the *IS1* element

in ORF37 is a unique feature of our laboratory strain. The transposition event probably happened within the last 15 years. In genetic maps describing the localization of insertion elements (2, 41), it is also not included. Although transposition of *IS* elements is a rare event, the distribution patterns of *IS* elements are of course strain specific but obviously also change with time. The laboratory strain K-12_{BM} with the marker ORF37::*IS1* showed wild-type behavior, so the ORF37 gene is not essential under standard growth conditions.

Complementation studies with the strain DTC274 mapped the complementing unit between ORF37 and *tyrT*. In this region, three reading frames were found: ORF34; URF13, which is not expressed according to our analysis; and ORF32, designated here as *tgs*. Although synergistic effects with the deletion of the *tyrT* operon itself cannot be completely excluded, the *tgs* gene was found to be the genetic basis of the phenotypes caused by the Δ*tyrT*::*kan* mutation for the following reasons. (i) A plasmid-borne *tgs* gene without any adjacent genetic regions fully complemented the growth inhibition in strain DTC274, which was not complemented by *tyrT*. (ii) The same plasmid complemented the *tyrT* deletion phenotype in strain DTK-12 when grown with glycerol or mannitol as carbon sources. (iii) The *tgs* gene was found to be deleted in its 3′ sequence coding for the 15 C-terminal amino acids of Tgs in all Δ*tyrT*::*kan* strains, because the kanamycin cassette that has been introduced to replace the *tyrT* operon (22) is cloned in a *Sca*I site that is located within the *tgs* coding sequence. A plasmid-borne *tgs* gene truncated at that *Sca*I site lost its ability to complement the deletion phenotype, indicating that the 3′ sequence of *tgs* is essential to code for a functional product. (iv) A recently constructed true *tyrT* mutant of strain CA274, including deletion of the terminal repeats but without an additional mutation in *tgs*, showed no corresponding phenotype (unpublished results). Therefore, the new data presented here define the *tgs* gene as the complementing unit of the *tyrT* deletion phenotype (as it was originally called) instead of the *tyrT* gene itself. The former conclusion that the *tyrT* gene is the complementing unit is based on the analysis of strain DTK-12 when shifted to a minimal medium supplemented with mannitol as carbon source (22). Under these conditions, the growth lag, 1 to 2 h, is quite short compared with 5 h when grown in minimal glycerol medium (3) and is no longer observed when strain DTK-12 is transformed with the *tyrT*-containing plasmid pUM302, which lacks the *tgs* gene. We now know that transformation with the *tyrT*-containing plasmid also reduces growth lags artificially induced by amino acid deprivation (3). This implies that overexpression of *tgs* alone or to a certain extent of *tyrT* alone can abolish the growth lag of strain DTK-12 by independent mechanisms.

The *tgs*-encoded protein showed significant similarity to the PurN protein of *E. coli* and *B. subtilis* and to the corresponding protein domains with GAR transformylase activity of higher eukaryotes. The PurN protein of *E. coli* is encoded at 54 min at the *purMN* operon (36). Therefore, the Δ*tyrT*::*kan* strains (which also carry the 3′ deletion of *tgs*) were not affected in purine metabolism, as verified by supplementing the growth medium with several purine compounds, because they have a functional *purN* gene. But on the basis of its similarity, the Tgs protein could have GAR transformylase activity. Smith and Daum have postulated that a second gene encoding a GAR transformylase should exist because a *purN* deletion mutant still exhibits purine prototrophy (36). During preparation of this paper, Nygaard and Smith reported a novel GAR transformylase (GAR transformylase T) that requires formate as C1 donor and has been mapped at 40 min on the *E. coli* chromosome (26). Therefore, *tgs* seems not to encode the second GAR transformylase activity despite the striking similarity on the molecular level.

Although the phenotypes of the two Δ*tyrT*::*kan* strains DTK-12 and DTC274 differ completely, the phenotypes have the same genetic basis, which was identified as the 3′ deletion of the *tgs* gene. Obviously, in the genetic context of strain DTC274 the *tgs* gene is essential and its inactivation leads to growth inhibition. The only known relevant genetic markers of strain DTC274 compared with strain DTK-12 are the *relA1* and *spoT1* mutations. Although not proven by direct genetic experiments, it is likely that the genetic background of the *relA1* and *spoT1* mutant is responsible for the phenotypic differences between strains DTK-12 and DTC274. However, additional unknown and unidentified genetic differences cannot be excluded, but they should be characteristic for CA strains, because transduction of the Δ*tyrT*::*kan* marker into strain CA275 (see Table 1) resulted in exactly the same phenotype as that observed with strain DTC274 (data not shown). It has been shown that the *relA1* gene has a residual ppGpp synthetase activity and that severe *spoT* gene mutants have extremely slow growth rates in *relA1* strains (21). If the *tgs* mutation disturbs the balance between residual *relA1* and *spoT1* activity, this could account for *relA1*-mediated ppGpp synthesis after nutritional shift down that is accumulated and inhibits growth. This would explain the growth inhibition in strain DTC274 and its need of a complex medium.

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