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Biochemistry

Structure and regulation of the gene for dGTP triphosphohydrolase from *Escherichia coli*

(*dgt/optA*/bacteriophage T7 gene *1.2* protein/promoter)

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ABSTRACT ***Escherichia coli* encodes an enzyme, deoxyguanosine triphosphate triphosphohydrolase (dGTPase, EC 3.1.5.1), that cleaves dGTP into deoxyguanosine and tripolyphosphate. An *E. coli* mutant, *optA1*, contains a 50-fold increased level of dGTPase and cannot support the growth of phage T7 defective gene *1.2*, whose product is an inhibitor of dGTPase. The *optA1* mutation maps to 3.6 min on the *E. coli* chromosome and is closely linked to *dapD*. We have isolated the gene encoding dGTPase (*dgt*) from wild-type *E. coli* and determined its nucleotide sequence. The *dgt* gene lies immediately upstream of *htrA* and 6 kilobases from *dapD*, in the same region as the *optA1* mutation. The *dgt* structural gene is 1515 base pairs, encoding a protein of 59,315 daltons, in agreement with the size and N-terminal amino acid sequence of the purified protein. An *E. coli* strain containing a null allele has no detectable phenotype when grown at 30–42°C in rich medium. A transition of C to T in a potential promoter of *dgt* is required for expression of the *optA1* phenotype.**

Extracts of *Escherichia coli* contain a dGTPase (EC 3.1.5.1) that catalyzes the hydrolysis of dGTP to deoxyguanosine and tripolyphosphate (1, 2). *E. coli optA1* cells contain a 50-fold higher level of dGTPase than do *E. coli optA*$^+$ cells as determined by activity (2, 3), protein yield during purification (3), and Western blots (4). Biochemical analysis of the *optA1* mutant has shown that the intracellular dGTP pool is 5-fold lower than that in wild-type cells (5), presumably due to the increased level of dGTPase. In addition, the replication of some bacteriophages is restricted in *optA1* cells. In *optA1* cells infected with phage T7 defective in gene *1.2*, T7 DNA replication ceases prematurely and no viable phage are produced (6). Gene *1.2* encodes an inhibitor of dGTPase, enabling wild-type T7 phage to grow in *E. coli optA1* (7). The reduction in the dGTP pool can also explain the abortive infection of two mutants of bacteriophage T4, T4 *dexA* and T4 CB120 (8, 9), in *E. coli optA1*.

Biochemical characterization of the dGTPase has defined several interesting properties, but the physiological role of this enzyme is not known. For example, (*i*) dGTPase preferentially hydrolyzes dGTP over the other canonical NTPs (1–3); (*ii*) the association of dGTPase and its inhibitor, the gene *1.2* protein, is modulated by a complex mechanism (4, 7); (*iii*) dGTPase has a high affinity for DNA (2, 3); and (*iv*) the expression of the dGTPase gene, as evidenced by the *optA1* mutation, can vary 50-fold. To further our understanding of the function of dGTPase *in vivo*, and to determine the basis of the increase in dGTPase in *optA1* cells, we have isolated and characterized the dGTPase gene* from both *E. coli optA*$^+$ and *E. coli optA1*. Quirk *et al.* (10) have localized the dGTPase gene from wild-type *E. coli* on a phage λ clone containing an insert from the 3.6-min region of the *E. coli* chromosome.

MATERIALS AND METHODS

Bacterial Strains. *E. coli* HR42 (*optA*$^+$) and HR44 (*optA1*) are isogenic except for the *optA1* mutation (6). *E. coli* MV1190 (11) and *E. coli* HMS254 (*hsdR thr*$^-$ *leu*$^-$ *lac*$^-$ *thi*$^-$ *supE*$^-$ *dapD4 optA1 tonA*$^-$) were from S. Tabor (Harvard Medical School). *E. coli* DH1 (12) was from M. Connor (University of California, Irvine). *E. coli* CG1090 (W3110 *galE*$^-$ with a kanamycin-resistance minitransposon insertion 92% linked to *optA1* by P1 transduction) was from C. Georgopoulus (University of Utah). *E. coli* HS40 was constructed by H. Nakai (this laboratory) by transducing HR42 to kanamycin resistance with a lysate of P1vir grown on *E. coli* CG1090. *E. coli* MC48 (CP366 *thr*$^-$ *leu*$^-$ *his*$^-$ *rpsL*$^-$ *lac*$^-$ *xyl*$^-$ *ara*$^-$ *tonA*$^-$ *tsx*$^-$ *thi*$^-$ *rha*$^-$ *zig*::Tn*10 polAts12*) was from M. Carson (Harvard Medical School). *E. coli* SW8 and SW9 were constructed by transducing HR42 and HR44, respectively, to tetracycline resistance with a lysate of P1vir grown on *E. coli* MC48.

Bacteriophages and Plasmids. λ EMBL4 clones (15A7, 9H2, 4E4, 23G6, 12D5) containing DNA inserts from the 3.6-min region of the *E. coli* chromosome were the gift of Yuji Kohara (Nagoya University). Plasmid pCD5, from C. Richaud (Institut de Microbiologie, Universite Paris-Sud), is a pBR322 plasmid with a 14-kilobase (kb) *Bam*HI–*Hin*dIII fragment of *E. coli* chromosomal DNA that contains *dapD*.

DNA Methods. *E. coli* HR42 and HR44 chromosomal DNA was isolated as described (13) except that the CsCl step was omitted and the DNA was treated with RNase A at 50 μg/ml and then with proteinase K at 100 μg/ml in 0.5% $NaDodSO_4$. Phage λ DNA iaolation (14) and Southern blot transfers (13) were carried out as described. Hybridizations with the degenerate 17-mer oligodeoxynucleotide (5′-TGCCARTTDATYTTYTT-3′) were as described (14) with incubations at room temperature. Nitrocellulose filters were washed to a stringency of 0.3 M NaCl/30 mM sodium citrate with 0.05% sodium pyrophosphate. Radioactively labeled nucleotides were from New England Nuclear.

DNA Sequence Analysis. The nucleotide sequence was determined by the dideoxy chain-termination method as described in the Sequenase kit from United State Biochemical. The M13 universal primer (−20) was from New England Biolabs. Other primers were provided by A. Nussbaum (Harvard Medical School). pSW1 DNA was prepared (15) and used to determine the sequence of the 3′ end of the dGTPase gene on one strand. Double-stranded DNA was alkali-denatured. Template and primer were annealed in 75 mM Tris·HCl, pH 7.5/50 mM $MgCl_2$ for 15 min at 37°C.

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Abbreviation: PCR, polymerase chain reaction.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M31772).

Polymerase Chain Reaction (PCR). Reactions were carried out according to Perkin–Elmer/Cetus with 3–50 μg of DNA and *Thermus aquaticus* (*Taq*) DNA polymerase (Perkin–Elmer/Cetus). The dGTPase structural gene from *E. coli* HR44 (*optA1*) was amplified as a 1606-base-pair (bp) fragment by using primers derived from the sequence of the *E. coli optA*$^+$ dGTPase gene. The primers specific for the 5′ end (5′-CCGGATCCGGGGAAGCGTATTTCTCACGC-3′) and for the 3′ end (5′-CCGAATTCCGAACTAAAGTTTCTGC-3′) each contained eight additional bases to include *Bam*HI and *Eco*RI restriction sites (underlined), respectively. The amplified DNA was digested with *Bam*HI and *Eco*RI. The 1600-bp fragment was cloned into M13mp18 and M13mp19. To sequence the region upstream of the dGTPase gene in *E. coli optA1*, two primers (5′-TGTTCGGTTTTAACGCCCTG-3′ and 5′-CAGGCTGGACTGTTTAGCGG-3′) were used to amplify an 819-base sequence. The double-stranded 819-bp fragment was isolated, digested with *Taq* I to generate a 563-bp fragment, and cloned into M13mp19. For direct sequencing, single-stranded DNA of the same region was prepared by asymmetric PCR amplification (16) with the primers present in a ratio of 20:1 with respect to one another.

Enzymes. Most restriction enzymes were from New England Biolabs. T4 polynucleotide kinase and *Sma* I were from United States Biochemical. *E. coli* DNA polymerase I was from Pharmacia. Calf-intestine alkaline phosphatase was from Boehringer Mannheim.

Genetic Techniques. To replace the mutation in *E. coli* HR44 (*optA1*) with the analogous region from *E. coli* HR42 (*optA*$^+$), an approach similar to that of Lee *et al.* (17) was used. A *Hin*dIII–*Eco*RI fragment containing the *optA*$^+$ 1.5-kb *Stu* I–*Kpn* I segment flanked by M13mp18 polylinker sequences was cloned into plasmid pBR322 to generate pSK. *E. coli* SW9 (*polA*^{ts} *optA1*) was transformed with pSK or pBR322 and transformants resistant to ampicillin (25 μg/ml) at 30°C were isolated. Transformants were plated at 42°C in the presence of ampicillin (25 μg/ml) to isolate plasmid integrates. pSK yielded ampicillin-resistant colonies at 42°C at a frequency of 1/2500, whereas the frequency of integration of pBR322 was $<1 \times 10^{-6}$. To obtain ampicillin-sensitive segregates, plasmid integrates were grown without ampicillin at 30°C and plated at 42°C. Fourteen ampicillin-sensitive colonies were identified among the 12,000 screened (Bio-Rad AmpScreen kit). The *optA1* phenotype was scored with wild-type phage T7 or T7 ST16 (deleted for gene *1.2*).

Assay of dGTPase. Cells from 25 ml of culture were resuspended in 0.8 ml of cold 50 mM Tris·HCl, pH 7.5/10% sucrose. Samples were treated with lysozyme (1 mg/ml) for 15 min on ice and centrifuged (16,000 × *g*) for 15 min at 4°C. The supernatant was assayed for dGTPase activity as described (3).

RESULTS

Identification and Cloning of the dGTPase Structural Gene. Our approach to identify the dGTPase gene was based on the assumption that it contained or lay in close proximity to the *optA1* mutation. Since the concentration of dGTPase protein is increased 50-fold as a consequence of the *optA1* mutation (3, 4), we considered it likely that the mutation affected a regulatory region, leading to increased transcription of the dGTPase gene. Since *optA1* maps to the 3.6-min region on the *E. coli* linkage map (6), the dGTPase gene should be located near *dapD*; *dapD* is 92% cotransducible with *optA1* (6). On this assumption, a series of λ clones containing inserts of DNA from the 3.6-min region of the *E. coli* chromosome (18) were screened for the presence of the dGTPase gene. pCD5, a pBR322 plasmid with a 14-kb *Bam*HI–*Hin*dIII insert containing *dapD*, was included in this analysis. The N-terminal amino acid sequence of the dGTPase protein (2, 3) was used to synthesize a degenerate 17-base oligonucleotide, which, in turn, was used in Southern blots to probe restriction digests of five λ clones and pCD5. Three of the λ clones (9H2, 23G6, and 4E4) and pCD5 hybridized to the probe (Fig. 1). Hybridization to pCD5 localized the dGTPase gene to within 8 kb of *dapD*.

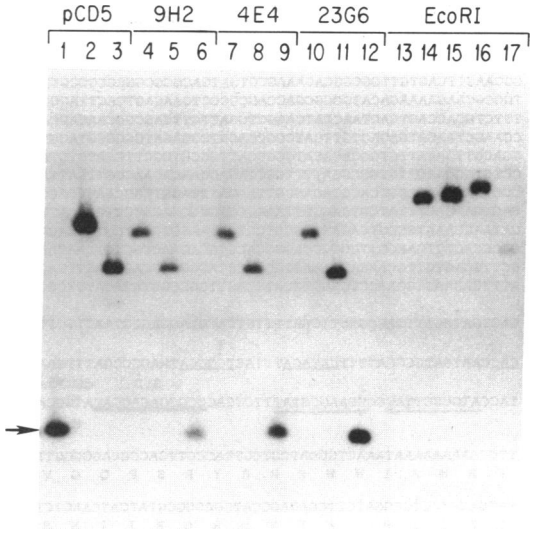


FIG. 1. Southern blot analysis of selected λ EMBL4 clones (15A7, 9H2, 4E4, 23G6, 12D5) and the plasmid pCD5 cleaved with various restriction enzymes and hybridized to a degenerate 17-mer. Lanes 1, 6, 9, and 12, *Pst* I and *Pvu* II; lanes 2, 4, 7, and 10, *Pst* I; lanes 3, 5, 8, and 11, *Pvu* II; lanes 13–17, *Eco*RI. The DNAs used in lanes 13–17 were λ clones 15A7 (lane 13), 9H2 (lane 14), 4E4 (lane 15), 23G6 (lane 16), and 12D5 (lane 17). Arrow indicates the 1.5-kb *Pvu* II–*Pst* I fragment that contains the N terminus of the dGTPase structural gene.

In order to clone the dGTPase gene and possibly the *optA1* mutation from the isogenic strains, *E. coli* HR42 (*optA*$^+$) and HR44 (*optA1*), we compared restriction digest maps of the 3.6-min region of the *E. coli* chromosome provided by Y. Kohara (18) and pCD5 from C. Richaud (personal communication). This analysis indicated that a 15-kb *Hin*dIII fragment contained both the dGTPase structural gene and *dapD*. *Hin*dIII fragments in this size range were isolated from *E. coli* HR42 and HR44 chromosomal digests and cloned into

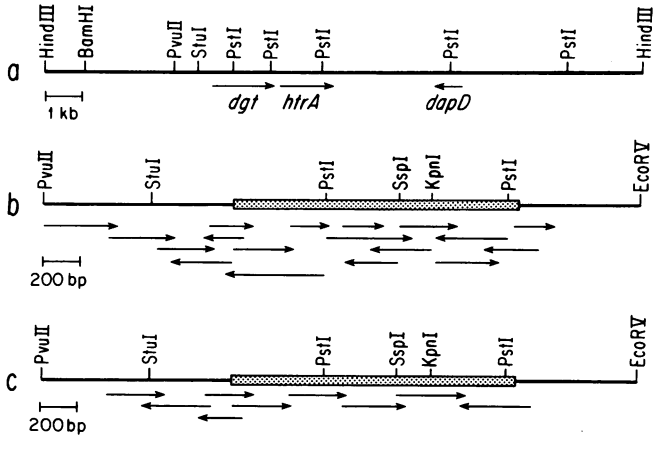
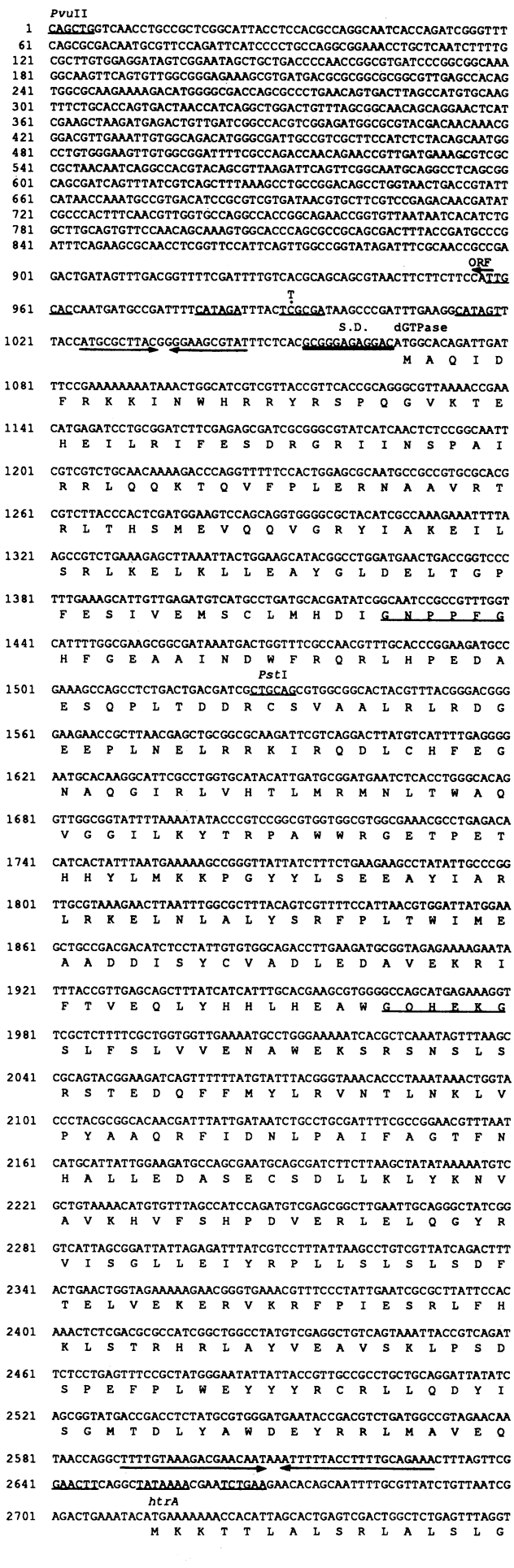


FIG. 2. (*a*) Restriction digest map of the *E. coli* HR42 15.8-kb *Hin*dIII fragment isolated from the *dapD* ampicillin-resistant transformant pSW1. (*b*) Sequencing strategy of the wild-type dGTPase gene and flanking sequences. The fragments from pSW1 used for sequencing the dGTPase gene were 1.5 kb *Pvu* II–*Pst* I, 0.98 kb *Pst* I, 1.5 kb *Stu* I–*Kpn* I, and 0.7 kb *Pst* I–*Eco*RV. (*c*) Sequencing strategy of the dGTPase gene from *E. coli* HR44 (*optA1*).



pBR322. Recombinant plasmids containing *dapD* were isolated by transforming *E. coli* HMS254 (*optA1 dapD4*) and selecting for *dapD* ampicillin-resistant transformants. *dapD4* mutants normally require exogenous diaminopimelic acid for growth (19). Six *dapD* ampicillin-resistant transformants were obtained from clones with HR42 DNA but none with HR44 DNA. Restriction digest and Southern blot analysis of the *dapD* ampicillin-resistant transformants with HR42 inserts showed that all six contained the same *Hin*dIII insert and the dGTPase structural gene (data not shown). One of these clones, pSW1, was used in all subsequent experiments. Using a similar approach, Quirk *et al.* (10) also identified a λ clone with an insert carrying the dGTPase gene. We have maintained the mnemonic *dgt* for the dGTPase structural gene.

An extract prepared from DH1 cells harboring pSW1 was analyzed by Western blot using polyclonal antibodies to purified dGTPase. These extracts contain a 5- to 10-fold higher level of a 59-kDa protein that comigrates with dGTPase from *E. coli* HR44 extracts in denaturing polyacrylamide gels.

When pSW1 is introduced into *E. coli* HR42, the level of dGTPase activity in extracts increases 5-fold. These cells, however, are not *optA1* as judged by their ability to support the growth of T7 gene *1.2* mutant phage. Quirk *et al.* (10), however, demonstrated that *E. coli* strains harboring a plasmid similar to pSW1 did not support the growth of phage T4 *dsd* mutants (8), indicating that the strain had become phenotypically *optA1*. We believe that this difference stems from a difference in plasmid copy number and/or from the difference in phage used to screen for the *optA1* phenotype. Surprisingly, we observed that *E. coli* HR42 is phenotypically *optA1* when the 4.1-kb *Hin*dIII–*Stu* I fragment (see Fig. 2*a*) is deleted from pSW1 (data not shown).

DNA Sequence Analysis of the dGTPase Gene. Southern blot analysis of restriction digests of pCD5 showed that the sequence encoding the N terminus of the dGTPase resided on a 1.5-kb *Pvu* II–*Pst* I fragment (Fig. 1). The corresponding fragment was isolated from the HR42 insert in pSW1 to carry out DNA sequence analysis as outlined in Fig. 2*b*.

The nucleotide sequence of the dGTPase structural gene and the upstream region is shown in Fig. 3. The open reading frame codes for a 505-amino acid protein whose deduced amino acid sequence is in agreement with the N-terminal sequence determined from the purified protein (2, 3). The calculated molecular weight of the dGTPase is 59,315, similar to that determined for the purified protein (2, 3). Searches of the Protein Identification Resource data bank of the National Biomedical Resource Foundation (June 1989) and the GenBank data base (release 61.0) did not identify any proteins or nucleotide sequences with significant homology to dGTPase.

FIG. 3. Nucleotide sequence and potential regulatory elements of the dGTPase gene (*dgt*). The nucleotide sequence shown begins at the *Pvu* II site 1066 nucleotides upstream of the dGTPase translation initiation site. The Shine–Dalgarno ribosome binding site (S.D.) is underscored with a heavy line. The region of dyad symmetry in the presumptive promoter region is indicated by the inverted arrows. The C → T transition identified in *E. coli* HR44 is indicated 74 nucleotides upstream of the dGTPase translation initiation codon. The −35 and −10 regions of the potential *E. coli* RNA polymerase promoters are underlined. The two Gly-Xaa-Xaa-Xaa-Xaa-Gly loops are underlined below the predicted amino acid sequence of dGTPase. Downstream of the dGTPase termination codon, the A+T-rich region is indicated by arrows, followed by *htrA*; the *htrA* σE promoter is underlined and the beginning of the *htrA* translation product is shown (20). Upstream of the dGTPase regulatory region, ORF indicates the initiation codon of the potential gene that is oriented in the direction opposite to that of *dgt*.

Immediately upstream of the translation initiation site is a Shine–Dalgarno ribosome binding site (21). Also present are two regions with homology to the consensus recognition sequence for the *E. coli* RNA polymerase holoenzyme, which consists of TTGACA as the −35 sequence and TATAAT as the −10 sequence (22). The region ≈50 bp upstream of the *dgt* translational initiation site (−35 TCGCGA, −10 CATAGT) has stronger homology than the region 80 bp upstream (−35 TTGCAC, −10 CATAGA). A short region of dyad symmetry lies between the −10 CATAGT sequence and the translational start codon. Immediately following the termination codon of *dgt* is an A+T-rich sequence containing dyad symmetry. One hundred and thirty base pairs downstream of *dgt* is *htrA* (*degP*), a gene encoding a periplasmic protease required for growth of *E. coli* at elevated temperatures (20, 23). *htrA* has a potential σE promoter (20) and does not appear to form an operon with *dgt*.

A second, nonoverlapping open reading frame with the coding potential for a protein of 24 kDa exists upstream of *dgt* (see Fig. 3, ORF). If expressed, this gene would be transcribed in the direction opposite to that of *dgt* transcription, and its promoter region would overlap that of *dgt*.

Characterization of the *optA1* Mutation. Attempts to clone the region analogous to pSW1 from *E. coli optA1* into a multicopy vector have been unsuccessful. We therefore took advantage of the known sequence of this region from wild-type *E. coli* to use PCR to determine the nucleotide sequence of the dGTPase gene and the upstream region in *E. coli optA1* DNA. The sequence of *dgt* from *optA1* cells is identical to that of the wild-type dGTPase gene. Sequencing of the presumptive promoter region revealed a single nucleotide change: a C → T transition 74 nucleotides upstream of the dGTPase translation initiation site (see Fig. 3). This C → T transition removes an *Nru* I site, providing a convenient screen for its presence.

The Promoter Mutation Is Necessary for Expression of the *optA1* Phenotype. In order to determine whether the C → T transition identified in *E. coli* HR44 was necessary for expression of the *optA1* phenotype, the region containing this mutation in *E. coli* HR44 was replaced by homologous recombination with that from *E. coli* HR42 (*optA*$^+$). pSK is a pBR322 plasmid with a 1.5-kb *Stu* I–*Kpn* I fragment (see Fig. 2*a*) from *E. coli* HR42. The only difference between the cloned sequence in pSK and the chromosomal sequence in *E. coli* HR44 is the C → T transition in a potential promoter region of *dgt*.

Fourteen colonies that had integrated and then resolved pSK from the chromosome were analyzed for their *optA1* phenotype. The region encompassing the mutation was PCR-amplified from chromosomal DNA to yield an 819-bp fragment, which was then digested with *Nru* I to test for the presence of the *optA1* mutation. Six of the 14 colonies had lost the mutation. These same 6 colonies also recovered the ability to support the growth of T7 phage deficient in gene *1.2*. Extracts prepared from 2 of the 6 colonies had levels of dGTPase activity comparable to that in *E. coli* SW8 (*optA*$^+$) (Table 1). Western blot analysis of extracts prepared from these two strains confirmed that they no longer overproduced dGTPase (data not shown). We conclude that the C → T transition present in *E. coli* HR44 is required for expression of the *optA1* phenotype.

Table 1. dGTPase activity in extracts of *E. coli* cells

Strain	Activity, pmol of dGTP hydrolyzed per 30 min per mg of protein
SW8 (*optA*$^+$)	4.7
SW9 (*optA1*)	120
SW9 *optA*$^+$ (isolate 1)	5.2
SW9 *optA*$^+$ (isolate 2)	4.8
HS40 (*dgt*::mini-Tn*10*)	2.1

Extracts (0.3–8.4 μg of protein) were tested for their ability to catalyze the hydrolysis of ^{32}P from [α-^{32}P]dGTP (3).

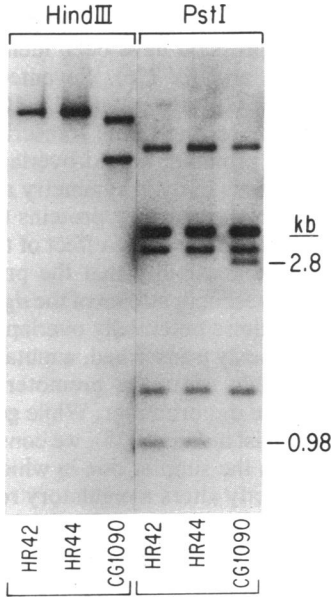


FIG. 4. Southern blot analysis to localize the kanamycin-resistance minitransposon insertion linked to *htrA* and *optA1* in *E. coli* CG1090. Bacterial chromosomal DNA was digested with *Hin*dIII or *Pst* I and probed with nick-translated pSW1. Lanes 1 and 4, *E. coli* HR42; lanes 2 and 5, *E. coli* HR44; lanes 3 and 6, *E. coli* CG1090. The *Hin*dIII digest of CG1090 contains an additional fragment due to the presence of a *Hin*dIII site within the minitransposon insertion (24). In the *Pst* I digest of CG1090, the 0.98-kb *Pst* I fragment containing the majority of the dGTPase coding region is replaced by a 2.8-kb fragment due to the minitransposon insertion.

The dGTPase Gene Is Not Essential. We have characterized a second *E. coli* dGTPase mutant. *E. coli* CG1090 and *E. coli* HS40 both contain a 1.8-kb kanamycin-resistance minitransposon insertion that disrupts the dGTPase coding (Fig. 4). No dGTPase mRNA transcripts were detected in a Northern blot analysis of RNA from *E. coli* CG1090 (data not shown). The level of dGTPase activity in extracts of *E. coli* HS40 (*dgt*::mini-Tn*10*) is less than half that in extracts of *E. coli* SW8 (*optA*$^+$) (Table 1). Presumably, the residual dGTPase activity present in *E. coli* HS40 extracts is due to phosphatases with other specificities. Thus, *E. coli* CG1090 and *E. coli* HS40 are null mutants of dGTPase. Since these strains have no detectable phenotype in LB medium (14) at 30–42°C, an intact *dgt* gene is not required for bacterial growth under these conditions.

DISCUSSION

We have characterized the gene encoding dGTPase from *E. coli* HR42 (wild-type) and from *E. coli* HR44 (*optA1*), a strain that overexpresses dGTPase. Sequence analysis of the dGTPase gene (*dgt*) from *optA1* has ruled out the possibility that a mutation in the structural gene gives rise to the 50-fold higher levels of dGTPase activity. DNA sequence analysis of the *dgt* region from *E. coli optA1* identified only one nucleotide change, a C → T transition 74 nucleotides upstream of the dGTPase translation initiation site (Fig. 3). This mutation, which is required for overexpression of the dGTPase, is in a position to alter a potential transcriptional promoter of *dgt*. Mutating TCGCGA to TTGCGA increases the homology of this sequence to the −35 consensus recognition sequence, TTGACA, of the *E. coli* σ^{70} RNA polymerase holoenzyme

(22). Analogous mutations in the −35 region that result in large increases in expression have been identified in the *E. coli* genes *lacI* (25) and *fol* (26). Seventeen nucleotides downstream of TCGCGA is a potential −10 region of the promoter (Fig. 3). Interestingly, the transcription initiation site of this potential promoter would overlap the region of dyad symmetry. Regions of dyad symmetry are often recognition sites for bacterial regulatory proteins (27).

An alternative explanation of the effect of the mutation on *dgt* expression is the possibility that the promoter for the open reading frame present upstream of the *dgt* gene overlaps the *dgt* promoter. If these potentially overlapping promoters cannot be simultaneously transcribed, a mutation preventing transcription from this upstream promoter would permit transcription from the *dgt* promoter. While genes with overlapping promoters exist in *E. coli* (28), we consider this model to be less likely than the simpler one in which the mutation in *optA1* strains directly alters a regulatory region of the *dgt* gene.

Although we have shown that the C → T transition is essential for the *optA1* phenotype, we do not know if it is sufficient. Since the original *optA1* strain (HR44) was isolated through nitrosoguanidine mutagenesis, it is possible that more than one mutation is required for expression of the *optA1* phenotype. Attempts to demonstrate that this single mutation is sufficient for expression of the *optA1* phenotype have thus far been inconclusive. The inability to clone the dGTPase gene from the *optA1* strain onto a multicopy vector also indicates that the mutation may be lethal under certain conditions.

What is the role of dGTPase? Under the conditions tested, dGTPase is not essential for the growth of *E. coli*; strain CG1090, containing a minitransposon insertion that disrupts the coding region of *dgt*, has no detectable phenotype. It is, of course, possible that *E. coli* CG1090 contains a partially active truncated dGTPase or that *E. coli* contains one or more additional proteins with comparable function.

Among possible nonessential roles for the dGTPase, the regulation of intracellular dGTP pools would appear to be an energetically wasteful pathway. It seems more likely that, *in vivo*, dGTPase may be part of a larger enzyme complex. For example, dGTPase activity may be coupled to other enzymatic activities such that the products of dGTP hydrolysis are utilized in other metabolic pathways. Alternatively, dGTP may not be the preferred substrate for the enzyme. Seto *et al.* (2) have proposed a preferential hydrolysis of dGTP in the *syn* configuration, a reaction that would decrease the chance of an A·G mispair during DNA replication. Related to these points is the question of whether the multiple conformational states observed during the interaction with T7 gene *1.2* protein are fortuitous or represent a need for control of dGTPase activity (4, 7).

Unfortunately, the amino acid sequence of the dGTPase offers little insight into these potential roles. dGTPase has two Gly-Xaa-Xaa-Xaa-Xaa-Gly loops (Fig. 3), a motif found in other nucleotide-binding proteins (29). A computer generated Chou–Fasman (30) analysis predicted that the first of these loops is more likely to lie within a region of secondary structure that resembles mononucleotide-binding folds (29). However, since previous experiments indicate that there is one NTP binding site for every two dGTPase monomers (4), it is possible that the mononucleotide-binding domain is composed of residues from more than one monomer.

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