Biochemistry. In the article “Structure and regulation of the gene for dGTP triphosphohydrolase from Escherichia coli” by Susannah M. Wurgler and Charles C. Richardson, which appeared in number 7, April 1990, of Proc. Natl. Acad. Sci. USA (87, 2740–2744), a printer’s error resulted in the omission of the word “in” from the second sentence of the Abstract. The sentence should read “An E. coli mutant, optA1, contains a 50-fold increased level of dGTPase and cannot support the growth of phage T7 defective in gene 1.2, whose product is an inhibitor of dGTPase.”

Genetics. In the article “Mitochondrial genotype of a unisexual salamander of hybrid origin is unrelated to either of its nuclear haplotypes,” by Fred Kraus and Michael M. Miyamoto, which appeared in number 6, March 1990, of Proc. Natl. Acad. Sci. USA (87, 2235–2238), the authors request that the following error be noted. In Fig. 4B, step III, the hexagon labeled A. laterale-texanum should be a square labeled A. laterale.

Medical Sciences. In the article “Human immunodeficiency virus type 1 envelope glycoprotein gp120 produces immune defects in CD4+ T lymphocytes by inhibiting interleukin 2 mRNA” by Naoki Oyaizu, Narendra Chirmule, Vaniambadi S. Kalyanaraman, William W. Hall, Robert A. Good, and Savita Pahwa, which appeared in number 6, March 1990, of Proc. Natl. Acad. Sci. USA (87, 2379–2383), the authors request that the following errors be noted. There should be two additional authors, with author 5 becoming Rajendra Pahwa of the Department of Pediatrics, Schneider Children’s Hospital, Long Island Jewish Medical Center, New Hyde Park, NY 11040, and author 6 becoming Michael Shuster of the Department of Medicine, North Shore University Hospital–Cornell University Medical College, Manhasset, NY 11030. The acknowledgment should read as follows:

“We are grateful to Dr. H. Slade for providing mannose-BSA. This work was supported in part by National Institutes of Health Grants AI26281 and CA40931.”
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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Contributed by Charles C. Richardson, January 18, 1990

ABSTRACT Escherichia coli encodes an enzyme, deoxyguanosine triphosphate triphosphohydrolase (dGTPase, EC 3.1.5.1), that catalyzes the hydrolysis of dGTP to deoxyguanosine and triphosphophate. An E. coli mutant, optA, 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 optA 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 optA 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 optA phenotype.

Extracts of Escherichia coli contain a dGTPase (EC 3.1.5.1) that catalyzes the hydrolysis of dGTP to deoxyguanosine and triphosphophate (1, 2). E. coli optA 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 optA 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 optA cells. In optA 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 codes for an inhibitor of dGTPase, enabling wild-type T7 phage to grow in E. coli optA (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 optA.

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 optA 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 optA cells, we have isolated and characterized the dGTPase gene* from both E. coli optA* and E. coli optA. 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 (optA) are isogenic except for the optA mutation (6). E. coli MV1190 (11) and E. coli HMS254 (hKdr thr leu lac thi supE dapD optA 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 optA by P1 transduction) was from C. Georgopoulos (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 z: Tn10 polA ts12) 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) BamHI–HindIII 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% NaDodSO4. Phage λ DNA isolation (14) and Southern blot transfers (15) were carried out as described. Hybridizations with the degenerate 17-mer oligodeoxynucleotide (5'-TGCCARTTDA-TYTTRTTT-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 States 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 MgCl2 for 15 min at 37°C.

Abbreviation: PCR, polymerase chain reaction.

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

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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 gene. The primers specific for the 5' end (5'-CCGATCCGGGGAACGTAATTCACCGC-3') and for the 3' end (5'-CCGAAATCCGAACTAAGTTTCTGC-3') each contained eight additional bases to include BamHI and EcoRI restriction sites (underlined), respectively. The amplified DNA was digested with BamHI and EcoRI. 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'-TGGTCTGTTTAAAGCCTG-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 SmaI 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 (optA1*), an approach similar to that of Lee et al. (17) was used. A HindIII-EcoRI fragment containing the optA1 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*) 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 x 10^-6. To obtain ampicillin-sensitive segregates, plasmid integrates were grown without ampicillin at 30°C and plated at 42°C. Fourteen ampicillin-sensitive clones 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 x 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 BamHI–HindIII 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.

In order to clone the dGTPase gene and possibly the optA1 mutation from the isogenic strains, E. coli HR42 (optA1*) 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 HindIII fragment contained both the dGTPase structural gene and dapD. HindIII fragments in this size range were isolated from E. coli HR42 and HR44 chromosomal digests and cloned into

![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.](image1)

![Fig. 2. (a) Restriction digest map of the E. coli HR42 15.8-kb HindIII 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–Kpn I, and 0.7 kb Pst I–EcoRV. (c) Sequencing strategy of the dGTPase gene from E. coli HR44 (optA1).](image2)
pBR322. Recombinant plasmids containing dapD were isolated by transforming E. coli HMS254 (optA1 dapDM) and selecting for dapD ampicillin-resistant transformants. dapD mutants normally require exogenous diaminopimelic acid for growth (19). Six dapD ampicillin-resistant transformants were obtained from clones with HR42 DNA but none with HR4 DNA. Restriction digest and Southern blot analysis of the dapD ampicillin-resistant transformants with HR42 inserts showed that all six contained the same HindIII 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 phase. Quirk et al. (10), however, demonstrated that E. coli strains harboring a plasmid similar to pSW1 did not support the growth of phase T4 dsd mutants (8), indicating that the strain had become phenotypically optA1. We believe that this difference stems from a difference in amino acid copy number and/or in the difference in phase used to screen for the optA1 phenotype. Surprisingly, we observed that E. coli HR42 is phenotypically optA1 when the 4.1-kb HindIII–StrI fragment (see Fig. 2a) 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 PvuII–Par 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. 2b.

The nucleotide sequence of the dGTPase structural gene and the upstream region shown is 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 Research 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 PvuII 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 by 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–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 oE promoter is underlined and the beginning of the htra aE 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 TTGCGA, −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 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 ω 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 dGTase 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 dGTase gene. Sequencing of the presumptive promoter region revealed a single nucleotide change: a C → T transition 74 nucleotides upstream of the dGTase translation initiation site (see Fig. 3). This C → T transition removes an NruI 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 StuI–KpnI fragment (see Fig. 2a) 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 NruI 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 dGTase 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 dGTase (data not shown). We conclude that the C → T transition present in E. coli HR44 is required for expression of the optA1 phenotype.

The dGTase Gene Is Not Essential. We have characterized a second E. coli dGTase mutant. E. coli CG1090 and E. coli HS40 both contain a 1.8-kb kanamycin-resistance minitransposon insertion that disrupts the dGTase coding region (Fig. 4). No dGTase mRNA transcripts were detected in a Northern blot analysis of RNA from E. coli CG1090 (data not shown). The level of dGTase activity in extracts of E. coli HS40 (dgt::mini-Tn10) is less than half that in extracts of E. coli SW8 (optA+) (Table 1). Presumably, the residual dGTase 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 dGTase. 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.

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Activity, pmol of dGT hydrolyzed per 30 min per mg of protein</th>
</tr>
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<tbody>
<tr>
<td>SW8 (optA+)</td>
<td>4.7</td>
</tr>
<tr>
<td>SW9 (optA)</td>
<td>120</td>
</tr>
<tr>
<td>SW9 optA* (isolate 1)</td>
<td>5.2</td>
</tr>
<tr>
<td>SW9 optA* (isolate 2)</td>
<td>4.8</td>
</tr>
<tr>
<td>HS40 (dgt::mini-Tn10)</td>
<td>2.1</td>
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Extracts (0.3–8.4 μg of protein) were tested for their ability to catalyze the hydrolysis of 32P from [α-32P]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 HindIII or PstI 1 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 HindIII digest of CG1090 contains an additional fragment due to the presence of a HindIII site within the minitransposon insertion (24). In the PstI 1 digest of CG1090, the 0.98-kb PstI fragment containing the majority of the dGTase coding region is replaced by a 2.8-kb fragment due to the minitransposon insertion.

**DISCUSSION**

We have characterized the gene encoding dGTase from *E. coli* HR42 (wild-type) and from *E. coli* HR44 (optA), a strain that overexpresses dGTase. Sequence analysis of the dGTase 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 dGTase 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 dGTase translation initiation site (Fig. 3). This mutation, which is required for overexpression of the dGTase, is in a position to alter a potential transcriptional promoter of dgt. Mutating TCGCGA to TTGGCGA increases the homology of this sequence to the −35 consensus recognition sequence, TTGACA, of the *E. coli* 70 RNA polymerase holoenzyme.
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 optAI strains directly alters a regulatory region of the dgt gene.

Although we have shown that the C → T transition is essential for the optAI phenotype, we do not know if it is sufficient. Since the original optAI strain (HR44) was isolated through nitrosoguanidine mutagenesis, it is possible that more than one mutation is required for expression of the optAI phenotype. Attempts to demonstrate that this single mutation is sufficient for expression of the optAI phenotype have thus far been inconclusive. The inability to clone the dGTPase gene from the optAI 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-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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