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The *mtrAB* Operon of *Bacillus subtilis* Encodes GTP Cyclohydrolase I (MtrA), an Enzyme Involved in Folic Acid Biosynthesis, and MtrB, a Regulator of Tryptophan Biosynthesis

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***mtrA* of *Bacillus subtilis* was shown to be the structural gene for GTP cyclohydrolase I, an enzyme essential for folic acid biosynthesis. *mtrA* is the first gene in a bicistronic operon that includes *mtrB*, a gene involved in transcriptional attenuation control of the *trp* genes. *mtrA* of *B. subtilis* encodes a 20-kDa polypeptide that is 50% identical to rat GTP cyclohydrolase I. Increased GTP cyclohydrolase I activity was readily detected in crude extracts of *B. subtilis* and *Escherichia coli* in which MtrA was overproduced. Biochemical evidence indicating that MtrA catalyzes dihydroneopterin triphosphate and formic acid formation from guanosine triphosphate is presented. It was also shown that *mtrB* of *B. subtilis* encodes a 6-kDa polypeptide. Expression of *mtrB* is sufficient for transcriptional attenuation control of the *B. subtilis trp* gene cluster in *Escherichia coli*. Known interrelationships between genes involved in folic acid and aromatic amino acid biosynthesis in *B. subtilis* are described.**

GTP cyclohydrolase I from a variety of organisms catalyzes dihydroneopterin triphosphate and formic acid formation from GTP. This reaction is the first step in the biosynthetic pathway leading to synthesis of the pteridine portion of folic acid in prokaryotes (4, 40), tetrahydrobiopterin in mammals (5, 12, 32), and pteridine-containing pigments in insects (29, 39). GTP cyclohydrolase I has been identified in several bacterial species, including *Escherichia coli* (40), *Salmonella typhimurium* (7), *Serratia indica* (22), *Lactobacillus plantarum* (19), *Pseudomonas cocovenenans* (27), *Pseudomonas* sp. (6), and *Bacillus stearothermophilus* (36). Despite the numerous prokaryotic and eukaryotic species in which GTP cyclohydrolase I has been identified, the gene that putatively encodes this enzyme has been isolated only from *E. coli* (21), a rat (13), and *Drosophila melanogaster* (29).

The *mtr* (methyltryptophan resistance) locus of *Bacillus subtilis* was identified initially as the regulatory locus for the *trp* gene cluster of this organism (16). The term gene cluster is used in place of the term operon when referring to *trpEDCFBA* of *B. subtilis* because these genes are known to reside within a supraoperon concerned with aromatic amino acid and histidine biosynthesis (14, 15). The product of *mtr* is thought to be a *trans*-acting RNA-binding regulatory protein that is activated by tryptophan (34). Transcription termination at the attenuator preceding the *trp* gene cluster presumably occurs as a consequence of binding of the activated *mtr*-encoded protein to the nascent transcript (23, 34). The *mtr* locus has been cloned and sequenced and found to be a two-gene operon with the capacity to encode polypeptides of 22 (MtrA) and 8 (MtrB) kDa (8). MtrB has some sequence similarity to RegA, an RNA-binding regulatory protein of bacteriophage T4 (38). Previous searches of the available data bases did not reveal any proteins with significant homology to MtrA (8). However, J. O'Donnell, University of Alabama, recently informed us that *B. subtilis* MtrA is homologous to the recently published GTP cyclohydrolase I sequence from a rat (13) and to GTP cyclohydrolase I from *D. melanogaster* (31). Comparison of the predicted amino acid sequence of MtrA with that of rat GTP cyclohydrolase I revealed that they were 50% identical. On the basis of this homology, we performed experiments to determine whether MtrA of *B. subtilis* is GTP cyclohydrolase I. Here we describe results which established that MtrA is GTP cyclohydrolase I. We also demonstrate that MtrB is sufficient for regulation of expression of the *trp* gene cluster of *B. subtilis* in *E. coli*.

MATERIALS AND METHODS

Chemicals and enzymes. Neopterin and rifampin were purchased from Sigma Chemical Co. GTP was obtained from Pharmacia. *Taq* polymerase was purchased from Perkin-Elmer/Cetus and used in accordance with the manufacturer's specifications. Restriction enzymes and T4 DNA ligase were from Bethesda Research Laboratories. Mung bean nuclease was obtained from New England BioLabs. Prestained low-molecular-weight protein standards were from GIBCO-BRL.

Bacterial strains and plasmids. All of the bacterial strains used in this study are described in Table 1. *B. subtilis* CYBS13 was constructed by integration of a segment of ptrpBG1 that contains a translational fusion of *trpE* of *B. subtilis* and *lacZ* of *E. coli* (33) into the *amyE* locus of strain 1A62 by selecting for chloramphenicol resistance. Strain CYBS17 was constructed by transforming CYBS13 with chromosomal DNA prepared from strain JH584 (*aroB*). *trp*$^+$ transformants were screened for *aroB* (shikimic acid auxotrophy).

Plasmids pTZ18R (United States Biochemical Co.), pGP1-2 (37), pHY300PLK (18), pSI45 (8), pTTmtrAB (8), pTTmtrB (8), and ptrpE-lacZ (8) have already been described. Plasmid ptrpE-lacZ2 was constructed by linearization of ptrpE-lacZ at the *Pst*I site in the ampicillin resistance gene, removal of the sticky ends with mung bean nuclease,

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TABLE 1. Bacterial strains

Strain	Genotype	Source or reference
B. subtilis 1A62	*trpA5*	BGSC[a]
B. subtilis CYBS13	*trpA5 amyE*::[*trpp*-(*trpE'*-'*lacZ*)] Cm^r	This study
B. subtilis CYBS17	*aroB amyE*::[*trpp*-(*trpE'*-'*lacZ*)] Cm^r	This study
B. subtilis CYBS200	CYBS17/pHY300PLK (Tc^r)	This study
B. subtilis CYBS201	CYBS17/pSI45 (*mtrAB* Tc^r)	This study
B. subtilis JH584	*aroB*	J. A. Hoch[b]
E. coli JM109(DE3)	*endA1 recA1 gyrA96 thi hsdR17* (r_K^- m_K^+) *relA1 supE44* Δ(*lac-proAB*) [F' *traD36 proAB lacI*^{q} ZΔM15] λ(DE3)	Promega
E. coli K38/pGP1-2	HfrC (Km^r)	37
E. coli CY15250	(K38/pGP1-2)/pTZ18R (Ap^r)	This study
E. coli CY15251	(K38/pGP1-2)pTZmtrAB (Ap^r)	This study
E. coli CY15252	(K38/pGP1-2)/pTZmtrA (Ap^r)	This study

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and religation. pTZmtrAB was constructed by subcloning the 1,150-bp *Eco*RI-*Hin*dIII fragment containing *mtrAB* from pTTmtrAB (8) into the *Eco*RI-*Hin*dIII sites of pTZ18R. pTZmtrB was constructed by subcloning the 580-bp *Bam*HI-*Hin*dIII fragment containing *mtrB* from pTTmtrB (8) into the *Bam*HI-*Hin*dIII sites of pTZ18R. pTZmtrA was constructed by using the polymerase chain reaction to amplify the *mtrA* region from genomic DNA. The oligonucleotides used as primers in the reaction were designed such that an *Eco*RI site was created upstream of *mtrA* at position 371 (8) and a *Sal*I site was created downstream of *mtrA* at position 1022 (8). The 655-bp fragment that was produced was digested with *Eco*RI and *Sal*I and ligated into the *Eco*RI-*Sal*I sites of pTZ18R. The polymerase chain reaction was carried out by using an Ericomp Thermocycler. Oligonucleotides were synthesized by using an Applied Biosystems 380B DNA synthesizer. Plasmids pTZmtrAB, pTZmtrA, and pTZmtrB were constructed such that they retained the natural *mtrA* and/or *mtrB* ribosome-binding sites but not the endogenous *mtr* promoter.

Transformation procedures. *E. coli* cells were transformed by the method of Kushner (25). *B. subtilis* was transformed by using natural competence (1). Appropriate antibiotics were added, as needed, to the following concentrations: kanamycin, 50 μg/ml; ampicillin, 100 μg/ml; chloramphenicol, 20 μg/ml; tetracycline, 20 μg/ml.

DNA isolation. Plasmid DNA was isolated by the alkaline-lysis procedure of Birnboim and Doly (3) for CsCl-purified preparations and by a modified form of the method of Ish-Horowicz and Burke (17) for miniscreens. Chromosomal DNA was prepared from *B. subtilis* as previously described (28).

Cell growth and preparation of crude extracts. Growth of *E. coli* cells used in the preparation of crude extracts was done as previously described (37). The cells from each 200-ml culture were harvested, washed, and suspended in 5 ml of lysis buffer (50 mM Tris-HCl [pH 8.5], 1 mM dithiothreitol). Cells were lysed by sonication at 30 W with a Heat Systems-Ultrasonics W-225R sonicator. Cell debris was removed by centrifugation for 15 min in a microcentrifuge. Protein concentrations were determined by the Bio-Rad protein assay.

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MtrA   2 KEVNKEQIEQAVRQILEAIGEDPNREGLLDTPKRVAKMYAEVFSGLNEDP 51
         |:|   :  |   ||  :|||| |:||| || | |        | |
Rat   55 NELNLPNLAAAYSSILRSLGEDPQRQGLLKTPWRAATAMQFFTKGYQETI 104

      52 KEHF-QTIFGENHEELVLVKDIAFHSMCEHHLVPFYGKAHVAYIPRGGKV 100
         : : : ||:|:|:|:|:|||| : |||||||||| |: |::|:|    |
     105 SDVLNDAIFDEDHDEMVIVKDIDMFSMCEHHLVPFVGRVHIGYLP-NKQV 153

     101 TGLSKLARAVEAVAKRPQLQERITSTIAESIVETLDPHGVMVVVEAEHMC 150
         ||||||| ||   :| |:|||:|  ||  | | |:| || ||:|| |||
     154 LGLSKLARIVEIYSRRLQVQERLTKQIAVAITEALQPAGVGVVIEATHMC 203

     151 MTMRGVRKPGAKTVTSAVRGVFKDDAAARAEVLEHIKR 188
         | |||| |   ||||| : |||::|:  | | |  |:
     204 MVMRGVQKMNSKTVTSTMLGVFREDPKTREEFLTLIRS 241
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FIG. 1. Alignment of the predicted amino acid sequences of MtrA and rat GTP cyclohydrolase I. The numbers refer to the amino acid positions within each polypeptide. Identical residues are indicated by vertical lines; conservative substitutions are indicated by double dots.

B. subtilis cells were grown in Luria broth to the late log phase (Klett 110, green filter no. 54), at which time a 200-ml culture volume was harvested, washed, and suspended in 1.5 ml of lysis buffer. Cell lysis and protein determinations were done as described above.

Enzyme assays. GTP cyclohydrolase I activity was determined by the fluoresence method, essentially as described by Yim and Brown (40). This assay measures dihydroneopterin triphosphate formation from GTP. Standard 0.5-ml reaction mixtures contained 100 mM Tris-HCl (pH 8.5), 2.5 mM EDTA (adjusted to pH 8.0 with KOH), 1 mM dithiothreitol, 1 mM GTP, and protein extract. Incubation was at 42°C. At 30 min after the start of the reaction, an equal volume of activated charcoal (40 mg/ml) was added to adsorb both dihydroneopterin triphosphate and unreacted GTP. The mixture was filtered through a Millipore filter (type HA, 0.45 μm). The filter was then washed with 5 ml of water, 5 ml of 5% ethanol, and 5 ml of 50% ethanol that also contained 3.1% NaOH. During the adsorption and elution from the charcoal, dihydroneopterin triphosphate is quantitatively oxidized to neopterin triphosphate (40). The final wash was collected and analyzed for neopterin triphosphate by fluorescence (265-nm excitation, 450-nm emission) with a Gilford Fluoro IV spectrofluorometer. Neopterin at 25 μM was used as the standard.

β-Galactosidase assays were performed as described by Miller (30). Cells used in the assay were grown in minimal medium supplemented with 1% casein hydrolysate in the absence or presence of tryptophan (100 μg/ml).

Analysis of protein extracts. Protein extracts were fractionated on a 15% sodium dodecyl sulfate-polyacrylamide gel as described by Laemmli (26) and stained with Coomassie blue.

RESULTS

Homology between MtrA and rat GTP cyclohydrolase I. As reported previously (8), the *mtrA* nucleotide sequence from *B. subtilis* contains an open reading frame that can encode a 190-residue polypeptide. Initial searches of the available protein data bases did not disclose any protein with significant homology to MtrA (8). A subsequent search of the updated GenBank/EMBL data bank performed by J. O'Donnell, University of Alabama (31), revealed extensive homology between a predicted *Drosophila* protein, MtrA, and rat GTP cyclohydrolase I (13). It can be seen in Fig. 1 that MtrA

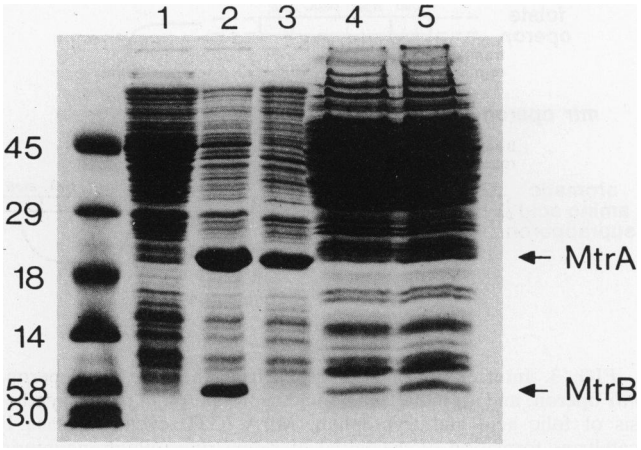

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of cell extracts from the following strains: lane 1, CY15250(pTZ18R); lane 2, CY15251(pTZmtrAB); lane 3, CY15252 (pTZmtrA); lane 4, CYBS200(pHY300PLK); lane 5, CYBS201 (pSI45) *mtrAB*. A 25-μg protein sample was loaded in lanes 1 to 3, and a 100-μg sample was used in lanes 4 and 5. Locations of prestained low-molecular-weight protein standards are indicated in kilodaltons.

and the rat protein are 50% identical. When the comparison is extended to include conservative amino acid differences, the proteins are 65% similar. These comparisons suggested that MtrA was GTP cyclohydrolase I of *B. subtilis*.

Identification of MtrA as GTP cyclohydrolase I. Our initial strategy to determine whether MtrA has GTP cyclohydrolase I activity was to overexpress the *B. subtilis mtr* operon in *E. coli*. We used the T7 expression system described by Tabor and Richardson (37). Recombinant plasmid pTZ mtrAB and control plasmid pTZ18R were transformed into *E. coli* K38/pGP1-2. By using this system, we produced MtrA and MtrB to approximately 30% of total *E. coli* protein (Fig. 2). The 20- and 6-kDa polypeptides that were observed are in excellent agreement with the predicted molecular masses of MtrA (22 kDa) and MtrB (8 kDa), respectively (Fig. 2, lane 2). Neither of these polypeptides was present in the extract from the control strain (Fig. 2, lane 1). These extracts were then assayed for GTP cyclohydrolase I activity by using the procedure of Yim and Brown (40). Overproduction of MtrA and MtrB resulted in a 66-fold increase in the level of GTP cyclohydrolase I activity (Table 2).

To determine whether MtrA was solely responsible for the observed increase in GTP cyclohydrolase I activity, plasmid pTZmtrA was introduced into strain K38/pGP1-2. This plasmid is essentially identical to pTZmtrAB, except that *mtrB* has been deleted. pTZmtrA clearly directed overexpression of only *mtrA* in K38/pGP1-2 (Fig. 2, lane 3). This extract was also assayed for GTP cyclohydrolase I activity. A 40-fold increase in enzyme activity was observed (Table 2), which was comparable to that obtained with the extract containing both MtrA and MtrB. Divalent cations were apparently not required for activity, as the enzyme was equally active in the presence of 25 mM EDTA (data not shown).

TABLE 2. Assay of extracts of various strains for GTP cyclohydrolase I activity

Strain	Plasmid	Activity (U)[a]	Sp act (U/mg of protein)
CY15250	pTZ18R	1	10
CY15251	pTZmtrAB	66	660
CY15252	pTZmtrA	40	400
CYBS200	pHY300PLK	8	8
CYBS201	pSI45 (*mtrAB*)	94	94

[a] One unit of activity is defined as the amount of enzyme needed to catalyze the formation of 1 nmol of dihydroneopterin triphosphate in 60 min.

TABLE 3. MtrB regulation of attenuation in the *B. subtilis trp* leader region

Plasmids present	Relative β-galactosidase activity[a]	
	No tryptophan	With tryptophan
ptrpE-lacZ2 + pTZ18R	1.00	0.77
ptrpE-lacZ2 + pTZmtrAB	0.60	0.10
ptrpE-lacZ2 + pTZmtrB	0.58	0.04
ptrpE-lacZ2 + pTZmtrA	0.75	0.50

[a] The values shown are averages of four experiments. The value obtained with ptrpE-lacZ2 plus pTZ18R without tryptophan was set at 1.00.

In addition to the experiments performed by using overexpressing strains of *E. coli*, it was also of interest to determine whether GTP cyclohydrolase I activity could be increased by overexpressing *mtrA* in *B. subtilis*. Strain CYBS17 was transformed with plasmid pSI45, which carries *mtrAB* under control of its natural promoter. The same strain was also transformed with vector pHY300PLK as a control. An increased level of MtrA was observed in the strain that contained pSI45 relative to the control strain, in which *mtrA* was present in a single copy (Fig. 2, lanes 4 and 5). These extracts were assayed for GTP cyclohydrolase I activity, and the extract from the strain harboring pSI45 showed a 12-fold increase in enzyme activity compared with the control strain (Table 2). Interestingly, a low but reproducible level of enzyme activity was detected in the control strain containing a single copy of *mtrA*. Taken together, these results demonstrate that *mtrA* encodes GTP cyclohydrolase I from *B. subtilis*.

MtrB is sufficient for regulation of expression of the *trp* genes of *B. subtilis* in *E. coli*. In a previous study, plasmids containing *mtrB*, or *mtrA* and *mtrB*, were introduced into an *E. coli* strain containing a plasmid having a fusion of the *B. subtilis trp* regulatory region to *E. coli lacZ* as a *trpE'-'lacZ* gene fusion. In the presence of tryptophan, the plasmid containing both *mtrA* and *mtrB* conferred regulation of β-galactosidase synthesis, whereas the plasmid containing *mtrB* did not (8). In view of the finding reported here, that *mtrA* encodes GTP cyclohydrolase I, the above-described experiment was repeated by using more efficient expression plasmids to test the possibility that the *mtrB*-expressing plasmid used previously did not direct the synthesis of sufficient MtrB protein to be effective. Accordingly, the above-described experiment was performed with plasmids in which *mtrB* (pTZmtrB), *mtrA* (pTZmtrA), or *mtrA* and *mtrB* (pTZmtrAB) were expressed from a T7 promoter in strain JM109(DE3), which produces T7 RNA polymerase. The presence of either plasmid containing *mtrB* permitted tryptophan regulation of expression of the *B. subtilis trpE'-'lacZ* fusion (Table 3). Expression of *mtrA* alone did not allow tryptophan regulation of β-galactosidase production from ptrpE-lacZ2 (Table 3). These findings demonstrate that *mtrB* is sufficient for regulation of transcription termination in the leader region preceding the *trp* genes of *B. subtilis*.

DISCUSSION

Folic acid biosynthesis in prokaryotes is a multistep process requiring formation of a pteridine ring and subsequent addition of ρ-aminobenzoic acid and glutamic acid residues. Folic acid, in turn, is involved in one carbon metabolism in the synthesis of methionine, glycine, thymine, purines, and pantothenic acid (11). We have identified *mtrA* of *B. subtilis* as the gene that encodes GTP cyclohydrolase I. This enzyme catalyzes formation of dihydroneopterin triphosphate by removal of carbon 8 from GTP as formic acid. This reaction is the first step in the synthesis of the pterin moeity that, upon further modification, is incorporated into folic acid (40).

We directed the synthesis of appreciable levels of GTP cyclohydrolase I in *E. coli* and *B. subtilis* by overexpressing the *B. subtilis mtrAB* operon. Overexpression of *mtrAB*, and *mtrA* alone, allowed us to establish that *mtrA* encodes a 20-kDa polypeptide (Fig. 2) that is sufficient for GTP cyclohydrolase I activity (Table 2). In addition, we found that the enzyme does not require divalent cations for activity. The absence of a divalent-cation requirement for GTP cyclohydrolase I activity has been observed in all of the organisms in which this enzyme has been characterized (4–7, 12, 19, 22, 27, 32, 36, 39, 40).

Comparison of the amino acid sequence of MtrA with that of the rat enzyme revealed striking homology. The two enzymes are 50% identical, and homology extends throughout MtrA (Fig. 1). This degree of homology between enzymes from organisms as unrelated as *B. subtilis* and rats suggests that strong selective pressure to maintain the overall structure of GTP cyclohydrolase I exists.

No prokaryotic strain deficient in GTP cyclohydrolase I has been described. In a previous study, Gollnick et al. (8) reported the inability to construct a strain in which *mtrA* was disrupted or deleted. However *mtrB* was dispensable. They reasoned that *mtrA* may serve some essential function in *B. subtilis*. The finding that *mtrA* of *B. subtilis* encodes GTP cyclohydrolase I is consistent with this explanation. The essential nature of MtrA may indicate that one or more of the one-carbon metabolites that are products of folate-dependent pathways are limiting in Luria agar. Alternatively, a branch point leading to the synthesis of some, as yet unidentified, essential pteridine derivative may exist. This second possibility is consistent with the isolation of several pteridine compounds from bacteria that are not involved in folic acid synthesis. Examples are the pteridine cofactors of alcohol dehydrogenase (2) and phenylalanine hydroxylase (10) of *Pseudomonas* species. In addition, the azapteridine antibiotic toxoflavin has been isolated from *P. cocovenenans* (27). The roles played by various pterins in *B. subtilis* have not been established.

The findings described in this report reveal an interesting interrelationship between folic acid and tryptophan biosynthesis in *B. subtilis* (Fig. 3). Transcription of the *trp* gene cluster (*trpEDCFBA*) is regulated in response to the availability of tryptophan (34). It is thought that a *trans*-acting regulatory factor encoded by the *mtr* locus recognizes a specific 10-base nucleotide sequence in the leader transcript, causing transcription termination (24). In addition, binding of this protein to nonterminated *trp* transcripts may inhibit translation by favoring a transcript secondary structure that sequesters the *trpE* ribosome-binding site (24). Inhibition of translation of the unlinked *trpG* coding region is also believed to be mediated by an *mtr* product (23). In a previous study, evidence was presented suggesting that both MtrA and MtrB are necessary for attenuation control of the *B. subtilis trp* gene cluster (8). The findings that *mtrA* encodes GTP cyclohydrolase I and that MtrB is sufficient for *trp* attenuation regulation indicate that MtrB is the *trans*-acting regulator of the *B. subtilis trp* gene cluster and that MtrA is not involved in this process.

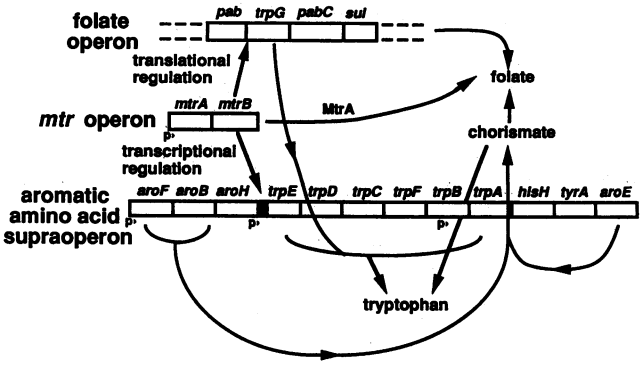

FIG. 3. Interrelationships of genes from the folic acid operon, *mtr* operon, and aromatic amino acid supraoperon in the biosynthesis of folic acid and tryptophan. MtrA (GTP cyclohydrolase I) catalyzes formation of the initial pteridine ring (dihydroneopterin triphosphate) of folic acid. AroF, AroB, and AroE are required for chorismate synthesis. Pab, TrpG, and PabC are involved in ρ-aminobenzoic acid synthesis from chorismate. Sul catalyzes condensation of ρ-aminobenzoic acid and the pteridine ring. MtrB is believed to bind to a target sequence that overlaps the *trpG* ribosome-binding site, resulting in translational regulation (35), and to the *trpE* leader transcript, resulting in transcriptional regulation of the *trp* gene cluster (24). The *trpEDCFBA* and *trpG* gene products are necessary for tryptophan biosynthesis from chorismate. Note that AroH is involved in phenylalanine and tyrosine biosynthesis from chorismate in some strains of *B. subtilis* (15). p> marks the positions of known promoters. ■ denotes the position of the *trp* attenuator. === indicates that open reading frames exist upstream of *pab* and downstream of *sul* (35).

Another gene cluster that links folic acid formation and tryptophan biosynthesis in *B. subtilis* is the folic acid operon described by Slock et al. (35). This operon includes *pab*, *trpG*, *pabC*, and *sul*, as well as two or more additional open reading frames. *pab* and *pabC* are involved in ρ-aminobenzoic acid synthesis, while *trpG* encodes an amphibolic polypeptide that is required for synthesis of both ρ-aminobenzoic acid and tryptophan (35). The *sul* gene encodes dihydropteroate synthase, the enzyme responsible for condensation of ρ-aminobenzoic acid and the pteridine ring (Fig. 3). In an analysis of the *trpG* nucleotide sequence (35), a nine-base sequence (AGATGAGGT) that overlaps the *trpG* ribosome-binding site was found to have eight nucleotides in common with the regulatory sequence (AGAATGAGTT) thought to be involved in *trp* attenuation and translational repression of the *trpEDCFBA* gene cluster (24, 34). Slock et al. (35) postulated that the product of the *mtr* locus may bind to this nine-base sequence, which overlaps the *trpG* ribosome-binding site, thereby blocking translation initiation (Fig. 3). This interpretation is supported by the observation that synthesis of TrpG is negatively regulated by tryptophan and *mtr* in vivo (20) and by analysis of *trpG* expression using a *trpG'-'lacZ* fusion (23).

It is important to note that the *aroF*, *aroB*, and *aroH* genes lie immediately upstream and the *hisH*, *tyrA*, and *aroE* genes lie immediately downstream of the *trpEDCFBA* gene cluster, in what has been termed a supraoperon involved in aromatic

amino acid and histidine biosynthesis (14, 15) (Fig. 3). Transcripts that presumably originate from the *aroFBH* gene cluster enter the *trp* leader region (34). Since the stop codon of *aroH* is located in the *trp* promoter (9), the *trp* attenuator is the initial transcription terminator for the *aroFBH* gene segment. Thus, the *mtr* and folate operons, as well as the aromatic amino acid supraoperon, contain genes involved in the biosynthesis of both folic acid and tryptophan. (Fig. 3). These gene arrangements appear to facilitate coordinate regulation of the biosynthesis of folic acid, the aromatic amino acids, and histidine in *B. subtilis*.

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