Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 3819–3823, April 1994
Microbiology

Amplification of *trpEG*: Adaptation of *Buchnera aphidicola* to an endosymbiotic association with aphids

(*Schizaphis graminum*/tryptophan biosynthesis/anthranilate synthase)

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Communicated by Sydney Kustu, January 4, 1994 (received for review September 15, 1993)

ABSTRACT **Survival of aphids is dependent on an association with a prokaryotic endosymbiont (*Buchnera aphidicola*) found in specialized cells within the aphid body cavity. Recent nutritional and physiological studies have indicated that one of the functions of the endosymbionts is the synthesis of tryptophan [Douglas, A. E. & Prosser, W. A. (1992) *J. Insect Physiol.* 38, 565–568]. *B. aphidicola* resembles in many of its properties free-living prokaryotes. An adaptation to an endosymbiosis involving the overproduction of tryptophan would necessitate alterations that modify the effect of regulatory systems that in free-living organisms function to reduce enzyme activity under conditions of excess tryptophan. We have cloned and sequenced the genes for *B. aphidicola trpEG* encoding anthranilate synthase, the first enzyme of the tryptophan biosynthetic pathway, which in free-living bacteria is feedback-inhibited by tryptophan. Amino acid sequence comparisons indicate that the *B. aphidicola* enzyme has all of the key residues involved in allosteric feedback inhibition. Evidence is presented indicating that *trpEG* is present as four tandem repeats on a circular plasmid. Relative to *B. aphidicola trpDC(F)BA* (the chromosomal genes coding for the remaining enzymes of the tryptophan biosynthetic pathway) *trpEG* is amplified 14- to 15-fold. These findings suggest that the effect of inhibition by accumulated tryptophan may be overcome by overproduction of anthranilate synthase. Our results demonstrate the acquisition of a new property (gene amplification) as an adaptation to an endosymbiotic association in which *B. aphidicola* overproduces tryptophan for the aphid host.**

Aphids are major pests of agriculturally important plants (1). Their survival is dependent on an association with eubacterial endosymbionts [assigned to the genus *Buchnera* (2)], which are housed in host-derived vesicles within specialized cells called mycetocytes (3–5). The mycetocytes form a loose aggregate in the body cavity of the aphid known as the mycetome. The endosymbionts have not been cultured outside the aphid host. To gain understanding as to the nature of the endosymbionts and their essential contribution(s) to the aphid, we have initiated studies on *Buchnera aphidicola*, the endosymbiont from the aphid *Schizaphis graminum*. The results of these as well as other studies (6) indicate that *B. aphidicola* has many of the properties characteristic of free-living bacteria. These include genes for DNA replication, transcription, translation, biosynthesis, energy metabolism, chaperonins, and protein secretion (6–11). Evolutionary studies involving comparisons of genes coding for 16S rRNAs have indicated that *Buchnera* is a distinct lineage within the class *Proteobacteria*, with *Escherichia coli* as the nearest known relative (12). The endosymbiosis appears to have originated 200–250 million years ago (13).

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Insects are unable to synthesize 10 amino acids, which consequently are required in their diet (14). Aphids are plant sap suckers consuming a diet rich in carbohydrates but low in amino acids, and it is thought that *Buchnera* provides the aphid with these essential amino acids (3–5). Evidence has been presented indicating that the endosymbionts are able to reduce sulfate to sulfide and synthesize methionine, cysteine, and tryptophan (10, 15–17). Tryptophan synthase, the last enzyme in the tryptophan biosynthetic pathway, has been detected in the endosymbionts (17). Experiments in which aphids were fed on synthetic diets have shown that the addition of chlortetracycline leads to elimination of the endosymbionts and of tryptophan synthase activity and a concomitant dependence on exogenously added tryptophan (17). Recently it has been shown that *B. aphidicola* contains the genes coding for enzymes that catalyze the conversion of anthranilate to tryptophan [*trpD(C)FBA*]; these genes are probably part of a single transcription unit (18). The genes (*trpEG*) for anthranilate synthase (AS), the first enzyme of the tryptophan biosynthetic pathway which converts chorismate to anthranilate, were not found on the 2.3-kilobase (kb) DNA fragment upstream of *trpD(C)FBA* or the 1.5-kb fragment downstream of this gene cluster (18).

One of the most extensively studied biosynthetic pathways is that for the synthesis of tryptophan (19–22). In almost all organisms, tryptophan biosynthesis is regulated by feedback inhibition of AS by tryptophan (23) and by repression of enzyme synthesis (20–22). In *E. coli*, tryptophan mediates the repression of an operon containing genes coding for all of the enzymes of tryptophan biosynthesis [*trpEG(D)C(F)BA*] (21). In this and some other organisms, levels of tryptophan biosynthetic enzymes are also regulated by attenuation (transcription termination) based on the availability of charged $tRNA^{Trp}$ (21, 24, 25). A property of this attenuation mechanism is the presence of DNA coding for a short tryptophan-containing leader peptide that precedes the structural genes for AS (20, 21). Some species of *Bacillus* have a totally different mechanism of attenuation, which does not involve a leader peptide but does involve a tryptophan-dependent binding of a regulatory protein to trp mRNA (20, 26). All of these regulatory mechanisms, characteristic of free-living bacteria, respond to accumulation of tryptophan within the cell. If the endosymbiont overproduces tryptophan, modifications would have to occur to permit tryptophan synthesis in the presence of elevated levels of this amino acid. Among the possible adaptations are constitutive synthesis of the tryptophan biosynthetic enzymes and modifications of AS so as to overcome the effects of feedback inhibition by tryptophan (27). In studies of directed evolution, mutations to constitutivity are common (28); in addition, in some species the tryptophan biosynthetic pathway appears to be constitutive (20, 29). Alternatively, since feedback inhibition may not eliminate all enzyme activity, it is potentially possible to

Abbreviations: AS, anthranilate synthase; ORF, open reading frame.
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compensate for the reduction of AS activity in the presence of the feedback inhibitor by overproduction of the enzyme (30, 31). Microorganisms subjected to conditions in which growth is limited by an enzyme activity frequently produce tandem gene duplications that result in the production of more enzyme protein (30, 32). Such gene duplications arise at a frequency of 10^4 to 10^5, considerably above that of mutations in structural genes (30). These duplications are usually unstable and are eliminated once the selective pressure is removed. They can be present in the chromosome or on plasmids and appear to be a rapid response of the organism to environmental changes. In the present communication we present evidence indicating amplification of *B. aphidicola trpEG*,† a result consistent with the overproduction of tryptophan by the endosymbiont and its utilization by the host.

MATERIALS AND METHODS

General Methods. The conditions used for the cultivation of aphids, methods of purification of endosymbiont-aphid DNA, restriction enzyme analysis, Southern blot hybridization, and DNA sequencing have been described (9, 12, 33). The sole difference is the use of the Boehringer Mannheim Genius System (digoxigenin) for the labeling of hybridization probes. The DNA sequence was determined in both orientations.

Cloning of the *trpEG* Genes. The procedures used are the same as have been applied previously to the cloning of a variety of genes from *B. aphidicola* (9, 18). Only an outline of these methods will be given here. From the compilations of TrpE sequences (kindly provided by the late I. P. Crawford), several stretches of conserved amino acids were apparent. On the basis of two such regions, oligonucleotide primers were designed that took into account the codon usage of *B. aphidicola* (8) [Fig. 1, nucleotides 827–846, *Bam*HI–*Pst* I sites: 5′-CGG ATC CTG CAG TAT CC(T or A) AT(T or A) GC(T or A) GG(T or A) AC(T or A) (C or A)G-3′; and nucleotides 1298–1317, *Bam*HI–*Sal* I: 5′-CGG ATC CGT CGA CTC (A or T)AC (A or T)CC (A or T)GC (A or T)CC (A or T)GC TTG-3′]. By using these primers and the polymerase chain reaction, a 0.52-kb fragment was amplified, digested with *Pst* I and *Sal* I, cloned, and sequenced. An open reading frame (ORF) was detected, similar in its deduced amino acid sequence to the corresponding portion of *E. coli* TrpE. After restriction enzyme analysis and Southern blot hybridization of the endosymbiont-aphid DNA using as a probe the 0.52-kb fragment, a 3.6-kb *Xba* I fragment was detected. Subsequently this fragment was ligated to *Xba* I–*Eco*RI adaptors and cloned into *Eco*RI-cut and phosphatase-treated λZAP. The excised plasmid was designated pBS2AS9.

Time Course of Digestion of DNA Containing *trpEG*. The reaction mixture for each time point consisted of 5 μg of DNA with 1–2.5 units of the restriction enzyme. Electrophoresis was performed with 0.6% agarose gels followed by Southern blotting. The probe used consisted of nucleotides 827–1317 of Fig. 1.

Determination of the Ratio of *trpE*/*trpB*. The number of copies of *trpE* (on plasmid) and *trpB* (on chromosome) was determined by densitometric scans of Southern blots containing known amounts of pBS2AS9 (*trpE*), pBS2T-1 (*trpB*) (18), and endosymbiont-aphid DNA. In the case of *trpE*, the DNA was cut with *Eco*RV to give a 1.9-kb fragment; in the case of *trpB*, the DNA was cut with *Cla* I to give a 2.0-kb fragment. A standard curve was constructed by using digested plasmids at concentrations in which there was linearity between the number of molecules and the peak height of the densitometric scan (four replicates at four concentrations). Endosymbiont-aphid DNA was tested at two concentrations (four replicates).

†The sequence reported in this paper has been deposited in the GenBank data base (accession no. Z21938).

RESULTS

Identification of ORFs. The sequence of the *B. aphidicola* 3.6-kb *Xba* I DNA fragment is presented in Fig. 1. Four ORFs were detected; for three, the DNA sequence given in Fig. 1 is the coding strand. Comparisons with *E. coli* indicate that these three ORFs correspond to the major portion of TrpE, TrpG, and the beginning of TrpE (Fig. 1). The deduced amino acid sequence of the assembled *B. aphidicola* TrpE had a 59% sequence identity to the *E. coli* TrpE. The latter protein differs from *B. aphidicola* TrpE in having six additional amino acids at the C terminus. The amino acid sequence of *B. aphidicola* TrpG had a 61% identity to the *E. coli* protein. *B. aphidicola* TrpE and TrpG also have significant amino acid identity to *E. coli* PabB and PabA (*p*-aminobenzoate synthase; 30% and 42%, respectively) (35, 36). The complementary DNA strand had an ORF coding for a putative protein of 8.5 kDa that has at each end two potentially membrane-spanning hydrophobic regions separated by a hydrophilic stretch. A search in GenBank did not detect a protein with significant sequence similarity. The guanine + cytosine content of the DNA of the 3.6-kb fragment (Fig. 1) was 26 mol %; the guanine plus cytosine content of *Buchnera* was previously found to be 30 mol % (37).

Evidence for Tandem Repeats of *trpEG* on a Circular Structure. The presence of ORFs coding for parts of TrpE at both ends of the 3.6-kb *Xba* I fragment suggested either a 3.6-kb plasmid or tandem repeats of this gene. Restriction enzyme and Southern blot analysis of *B. aphidicola* DNA with enzymes that cut once in the 3.6-kb repeating unit (*Xba* I, *Pvu* II, *Hin*cII, *Sca* I, *Acc* I, *Bcl* I, *Hpa* I) resulted in the detection of only a single 3.6-kb band (results not shown). Digestion with *Eco*RV or with *Bgl* II (enzymes that cut twice) gave a single smaller fragment, its size consistent with the position of the hybridization probe. The absence of any DNA fragments other than those expected of a 3.6-kb repeating unit suggested a circular structure. Purified DNA preparations obtained with or without CsCl centrifugation gave the same pattern in Southern blots consisting of a major 19.0-kb band and a minor 14.3-kb band (Fig. 2 *A*, lane 1; and *B*, lane 2). Digestion of the DNA with DNase I, under conditions in which a control supercoiled plasmid was converted to an open circle (32), did not change the amounts or the electrophoretic properties of the 14.3- and 19.0-kb bands (results not shown). These findings indicate that neither of these two bands corresponds to a supercoiled plasmid. Digestion with *Xba* I (Fig. 2*A*) resulted in the eventual conversion of the 19.0-kb band to a 3.6-kb band via intermediates of 14.3, 10.8, and 7.2 kb, corresponding to 4, 3, and 2 tandem repeats of the 3.6-kb unit. Similar results were obtained with *Hin*cII, which cuts only once in the 3.6-kb unit (results not shown).

The major 19-kb band is probably an open circle containing four 3.6-kb repeats that is retarded in its migration because of its circular structure, while the minor 14.3-kb band is its linear degradation product. Evidence consistent with this interpretation is provided by experiments in which the kinetics of DNA digestion were examined by using *Eco*RV, an enzyme that cuts twice in the 3.6-kb unit, resulting in 1.7- and 1.9-kb DNA fragments. If the 19-kb band is a circular plasmid containing four 3.6-kb repeats, then the initial cut will give a 14.3-kb band, and all subsequent bands will be below this size. If the 19-kb band contains five 3.6-kb repeats, then cuts that excise a 1.7- or 1.9-kb *Eco*RV fragment will give bands above 14.3 kb. The results of such an experiment (Fig. 2*B*) indicated the presence of all of the bands expected of a circular molecule containing four 3.6-kb repeats. A similar

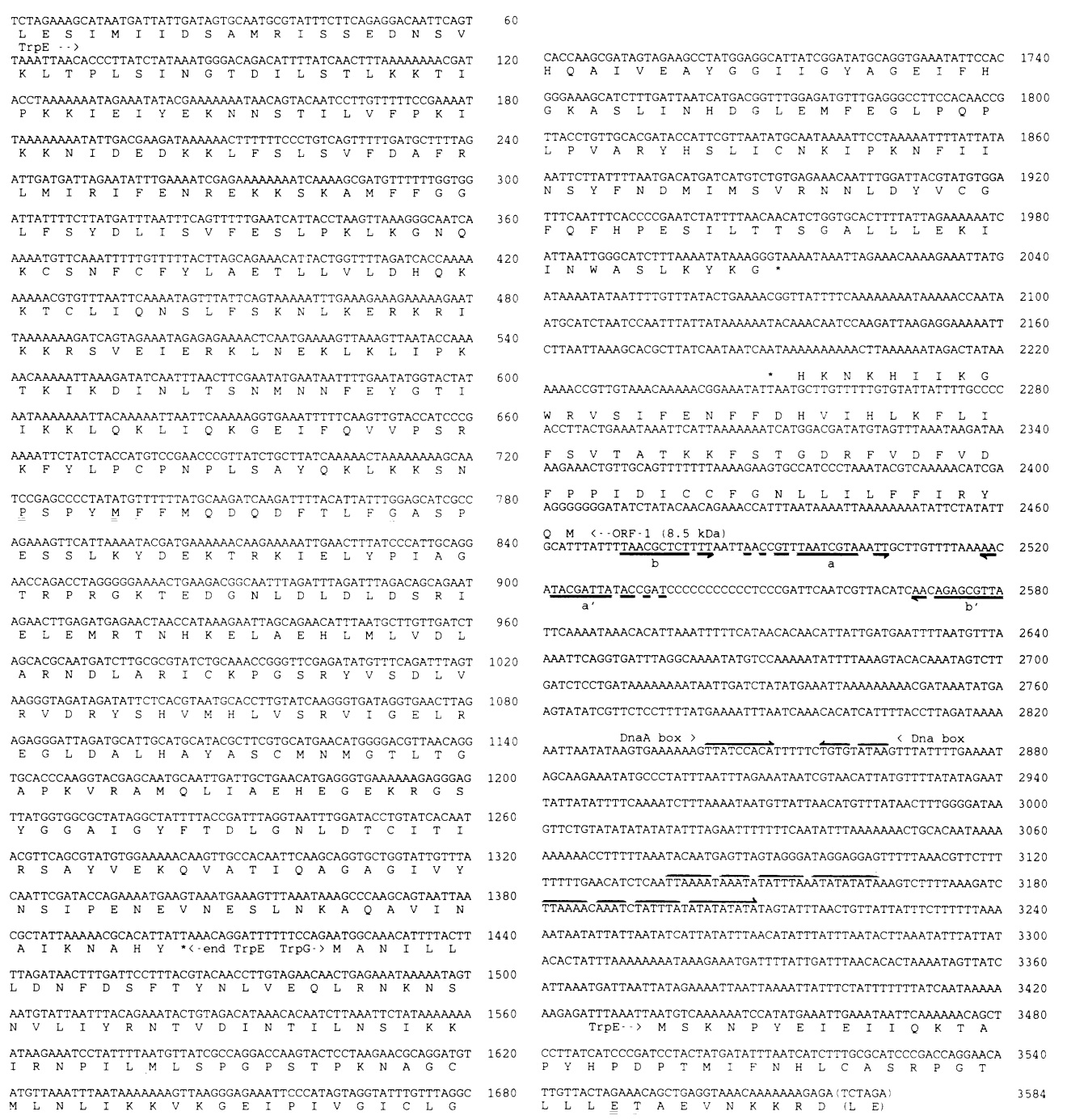

FIG. 1. Sequence of a 3584-nucleotide *B. aphidicola* DNA fragment containing *trpEG*. The upper line is the 5′ → 3′ nucleotide sequence; the lower line is the deduced amino acid sequence in single-letter code. The amino acid sequence above the nucleotide sequence between positions 2280 and 2520 is the ORF deduced from the complementary strand. *, Stop codon; arrows, direct or inverted repeats. The double-underlined amino acids in the AS sequence (below positions 719, 722, 734, 737, 770, 3550, 3553, and 3556) are essential conserved residues for allosteric inhibition by tryptophan (27, 34). The end of sequence in parentheses is a repeat of the beginning sequence.

pattern was obtained with *Bgl* II, an enzyme that also cuts twice in the 3.6-kb unit (results not shown). An interpretation of the above results is presented in Fig. 3.

Using a similar methodology, we have also cloned a 1.7-kb *B. aphidicola* DNA fragment containing a portion of *trpE* (Fig. 3). The nucleotide sequence of this fragment was with one exception identical to the corresponding sequence of the cloned 3.6-kb fragment. The sole difference was the number of consecutive C bases: the 1.7-kb fragment had 9 while the 3.6-kb fragment had 11 (Fig. 1, nucleotides 2537–2547). These differences are real in that the results of the sequencing were unambiguous and are consistent with multiple copies of the 3.6-kb unit.

Determination of the Ratio of *trpE* to *trpB*. Previously it has been shown that the *B. aphidicola* genome contains one copy of *trpB* (18). By using endosymbiont-aphid DNA preparations, it was found that 1 μg of DNA contains $5.1 \pm 1.0 \times 10^6$ copies of *trpB* and $7.4 \pm 1.2 \times 10^7$ copies of *trpE*. These results indicate a 14- to 15-fold amplification of *trpE* relative to *trpB* and suggest that each cell of *B. aphidicola* has three to four copies of the *trpE*-containing plasmid (Fig. 3).

Properties of the DNA Sequence Between ORF-1 and *trpE*. The DNA segment consisting of nucleotides 2465–3435 (Fig. 1) contains inverted and direct repeats. The inverted repeat (ba–a′b′, between nucleotides 2471 and 2582) has a calculated ΔG° of −19.7 kcal/mol (Genetics Computer Group Sequence

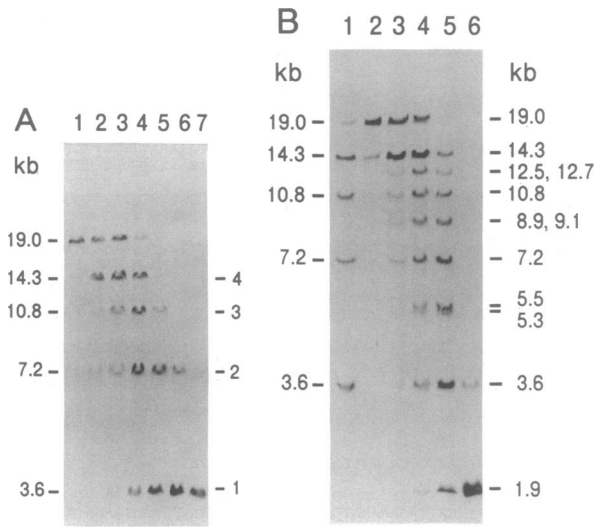

FIG. 2. Time course of restriction enzyme digestion of *B. aphidicola* DNA containing *trpEG* determined by means of Southern blots. (*A*) Digestion with *Xba* I. Lanes: 1, undigested DNA; 2, digestion for 5 min; 3, 10 min; 4, 20 min; 5, 30 min; 6, 45 min; 7, 1 hr. Numbers to the right refer to multiples (repeats) of 3.6 kb. (*B*) Digestion with *Eco*RV. Lanes: 1, digestion with *Xba* I for 20 min; 2, undigested DNA; 3–6, *Eco*RV; digestion for 30 sec (lane 3), 2 min (lane 4), 4 min (lane 5), and 15 min (lane 6).

Analysis Software, Madison, WI) and contains a loop with the sequence $C_{11}TC_3G$ (nucleotides 2537–2552). The high C content in this sequence is unusual since *B. aphidicola* has a low G+C content in its DNA (30 mol %) (36). Line 2880 (Fig. 1) contains two inverted repeats, followed by two direct, A+T-rich repeats having 25 identical bases out of 28 (lines 3180 and 3240). The DNA upstream of *trpE* does not code for a leader peptide, indicating the absence of control by an attenuation mechanism similar to that of *E. coli* (21, 22).

DISCUSSION

Previous work on the genetics and physiology of aphid endosymbionts and the nutrition of aphids has provided evidence consistent with the synthesis of the essential amino acid tryptophan by the endosymbionts and its utilization by the aphid host (17, 18). These studies include the cloning and sequencing of genes converting anthranilate to tryptophan [*trpDC(F)BA*] (18), the detection of endosymbiont-associated tryptophan synthase activity (17), and the demonstration of a requirement for tryptophan and a decrease in tryptophan synthase upon treatment of the aphids with chlortetracycline (17). The present work completes these investigations by a genetic characterization of AS (*trpEG*), the first enzyme of the tryptophan biosynthetic pathway. Our results show that the deduced amino acid sequence of *B. aphidicola* TrpEG resembles the sequence of the enzyme from *E. coli*. There is no leader peptide preceding TrpE, which indicates that *B. aphidicola* AS, is not regulated by a transcription attenuation mechanism similar to that of *E. coli* and a variety of other organisms (20, 21). The conversion of chorismate to anthranilate and allosteric inhibition by tryptophan is associated with TrpE (23). It has been shown that in the case of the *Salmonella typhimurium* TrpE, eight amino acid residues are essential for allosteric inhibition (34). With one exception these amino acids are conserved in *B. aphidicola* TrpE (Fig. 1); the single difference was a change of serine to threonine (Fig. 1, nucleotide 3553) and is probably without significance. The conservation of these amino acids suggests that the *B. aphidicola* AS is subject to allosteric inhibition by tryptophan. We were unable to demonstrate AS activity in endosymbiont-aphid cell-free extracts by the fluorometric assay for anthranilate formation from chorismate, probably because of the low level of activity in such crude extracts and interference of other constituents with the enzyme assay (38).

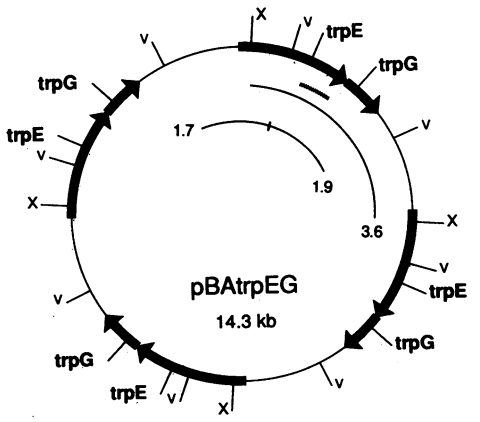

FIG. 3. Proposed structure of the *B. aphidicola* plasmid (pBAtrpEG) containing four tandem repeats of *trpEG*. Arrows show the coding region and direction of transcription, the broken line shows the location of the hybridization probe, and numbers show sizes in kb. V, *Eco*RI; X, *Xba* I; arcs indicate V and X repeating units.

In relation to *trpDC(F)BA*, *trpEG* is amplified 14- to 15-fold. The amplification is a consequence of four tandem repeats of *trpEG*, which constitute a circular plasmid (pBAtrpEG, Fig. 3). There appear to be three or four such plasmids per endosymbiont. The totality of these results suggests the following interpretation. One of the functions performed by *B. aphidicola* is the overproduction of tryptophan, and this adaptation to an endosymbiotic association necessitates a modification that would assure sufficient AS activity in the presence of the endproduct, tryptophan. The solution found in *B. aphidicola* is one observed in many other organisms subjected to growth limitation due to the presence of limiting amounts of an enzyme activity (30–32). It consists of an increase in the enzyme protein due to tandem gene duplication and further amplification by localization on a multicopy plasmid. Since the allosteric inhibition of AS (23, 39) is not complete even at high concentrations of tryptophan, gene amplification and consequent overproduction of the protein would allow for sufficient AS activity, even in the presence of inhibitory concentrations of tryptophan.

Suggestive evidence indicating a modification of an enzyme resulting in elimination of feedback inhibition has been found in *B. aphidicola cysE*. This gene codes for a key enzyme in cysteine biosynthesis (40), which in *E. coli* is synthesized constitutively and is feedback-inhibited by cysteine. Mutational analysis has indicated that the C terminus of CysE is involved in feedback inhibition (41). Comparison of the deduced *B. aphidicola* CysE amino acid sequence with that of *E. coli* indicated that these two proteins differ in that the *B. aphidicola* CysE has a truncation at the C terminus eliminating the region involved in allosteric inhibition (10). Unfortunately extensive attempts to overproduce *B. aphidicola* CysE in *E. coli* and examine its allosteric properties were unsuccessful because of the degradation of this enzyme (C.-Y.L., unpublished data).

Eubacterial origins of replication contain two or more copies of a nucleotide sequence known as a DnaA box (consensus TTATCCACA) followed by two direct A+T-rich repeats (42–44). Such an arrangement is found between ORF-1 and *trpE* (Fig. 1) (two DnaA boxes, two direct repeats), consistent with the presence of an origin of replication on each of the four 3.6-kb units that form pBAtrpEG. With the exception of *E. coli* and closely related species, all

of the organisms examined appear to have the origin of chromosomal replication immediately preceding *dnaA* (43). In *E. coli* the origin of replication is located 40 kb away from *dnaA* (43, 44). A single DnaA box is, however, present before *dnaA*, since it is required for autoregulation of DnaA expression. The absence of a DnaA box in front of *B. aphidicola dnaA* (9) was unusual and suggested a different mode of regulation of this protein. The presence of DnaA boxes on pBAtrpEG indicates that in *B. aphidicola* these structures may be involved in initiation of replication and that, as in the case of *E. coli*, the origin of replication is found in some region other than in front of *dnaA*.

An arrangement in which *trpEG* is unlinked to other *trp* genes is found in *Rhizobium meliloti* (24), *Thermus thermophilus* (25), and *Leptospira biflexa* (45). In *R. meliloti trpE(G)* genes are fused, and enzyme activity is regulated by attenuation as is indicated by DNA encoding a tryptophan-containing leader peptide preceding *trpE*(G) and by mutational analysis (24, 46). There is also evidence for regulation by attenuation in *T. thermophilus* (25). In *L. biflexa, trpEG* codes for separate proteins and, as in the case of *B. aphidicola*, there is no DNA encoding a leader peptide preceding *trpE*. *R. meliloti trpE(G)* is followed by inverted repeats suggestive of rho-independent terminators. The *B. aphidicola* inverted repeat, which follows *trpEG* and precedes ORF-1 (Fig. 2), contains a stretch of C bases, an unusual property due to the low G+C content of endosymbiont DNA. The significance of this putative structure is not known.

The present study demonstrates a positive attribute (gene amplification) that is a potential adaptation of *B. aphidicola* to an endosymbiotic association with the aphid host. Previous results indicated the absence of regulatory structures in the endosymbiont DNA that have been found in other organisms or the presence of one copy of the gene coding for 16S rRNA, a finding consistent with a slow growth rate of the endosymbionts (8–10, 12).

Note Added in Proof. Katsumata and Ikeda (47) have found that a modification of *Corynebacterium glutamicum* that primarily involves an increase in the allosterically inhibited AS protein results in a major accumulation of tryptophan in the culture medium.

This material is based upon work supported by Entotech, Inc. (Novo Nordisk) and the National Science Foundation Award IBN-9201285.

1. Minks, A. K. & Harrewijn, P. (1987) *Aphids: Their Biology, Natural Enemies, and Control* (Elsevier Biomedical, Amsterdam), Vol. 2A.
2. Munson, M. A., Baumann, P. & Kinsey, M. G. (1991) *Int. J. Syst. Bacteriol.* **41**, 566–568.
3. Douglas, A. E. (1989) *Biol. Rev. Cambridge Philos. Soc.* **64**, 409–434.
4. Houk, E. J. & Griffiths, G. W. (1980) *Annu. Rev. Entomol.* **25**, 161–187.
5. Ishikawa, H. (1989) *Int. Rev. Cytol.* **116**, 1–45.
6. Ohtaka, C., Nakamura, H. & Ishikawa, H. (1992) *J. Bacteriol.* **174**, 1869–1874.
7. Clark, M. A. & Baumann, P. (1993) *Curr. Microbiol.* **26**, 233–237.
8. Clark, M. A., Baumann, L. & Baumann, P. (1992) *Curr. Microbiol.* **25**, 283–290.
9. Lai, C.-Y. & Baumann, P. (1992) *Gene* **113**, 175–181.
10. Lai, C.-Y. & Baumann, P. (1992) *Gene* **119**, 113–118.
11. Munson, M. A., Baumann, L. & Baumann, P. (1992) *Curr. Microbiol.* **24**, 23–29.
12. Munson, M. A., Baumann, P., Clark, M. A., Baumann, L., Moran, N. A., Voegtlin, D. J. & Campbell, B. C. (1991) *J. Bacteriol.* **173**, 6321–6324.
13. Moran, N. A., Munson, M. A., Baumann, P. & Ishikawa, H. (1993) *Proc. R. Soc. London B* **253**, 167–171.
14. Dadd, R. H. (1985) in *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, eds. Kerkut, G. A. & Gilbert, L. I. (Pergamon, Elmsford, NY), Vol. 4, pp. 315–319.
15. Douglas, A. E. (1988) *Insect Biochem.* **18**, 599–605.
16. Douglas, A. E. (1990) in *Aphid–Plant Genotype Interactions*, eds. Campbell, R. K. & Eikenbary, R. D. (Elsevier Biomedical, Amsterdam), pp. 319–327.
17. Douglas, A. E. & Prosser, W. A. (1992) *J. Insect Physiol.* **38**, 565–568.
18. Munson, M. A. & Baumann, P. (1993) *J. Bacteriol.* **175**, 6426–6432.
19. Crawford, I. P. (1987) *Methods Enzymol.* **142**, 293–300.
20. Crawford, I. P. (1989) *Annu. Rev. Microbiol.* **43**, 567–600.
21. Yanofsky, C. & Crawford, I. P. (1987) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, eds. Neidhardt, F. C., Ingraham, J. E., Low, K. B., Magasanik, B., Schaechter, M. & Umbarger, H. E. (Am. Soc. Microbiol., Washington, DC), Vol. 2, pp. 1453–1472.
22. Yanofsky, C., Platt, T., Crawford, I. P., Nichols, B. P., Christie, G. E., Horowitz, H., van Cleemput, M. & Wu, A. M. (1981) *Nucleic Acids Res.* **9**, 6647–6668.
23. Zalkin, H. A. (1980) in *Multifunctional Proteins*, eds. Bisswanger, H. & Schmincke-Ott, E. (Wiley, New York), pp. 123–149.
24. Bae, Y. M. & Crawford, I. P. (1990) *J. Bacteriol.* **172**, 3318–3327.
25. Sato, S., Nakada, Y., Kanaya, S. & Tanaka, T. (1988) *Biochim. Biophys. Acta* **950**, 303–312.
26. Otridge, J. & Gollnick, P. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 128–132.
27. Matsui, K., Miwa, K. & Sano, K. (1987) *J. Bacteriol.* **169**, 5330–5332.
28. Clarke, P. H. (1978) in *The Bacteria*, eds. Ornston, L. N. & Sokatch, J. R. (Academic, New York), Vol. 6, pp. 137–218.
29. DaCosta e Silva, O. & Kosuge, T. (1991) *J. Bacteriol.* **173**, 463–471.
30. Anderson, R. P. & Roth, J. R. (1977) *Annu. Rev. Microbiol.* **31**, 473–505.
31. Stark, G. R. & Wahl, G. M. (1984) *Annu. Rev. Biochem.* **53**, 447–491.
32. Beverley, S. M., Coderre, J. A., Santi, D. V. & Schimke, R. T. (1984) *Cell* **38**, 431–439.
33. Unterman, B. M., Baumann, P. & McLean, D. L. (1989) *J. Bacteriol.* **171**, 2970–2974.
34. Caligiuri, M. G. & Bauerle, R. (1991) *J. Biol. Chem.* **266**, 8328–8335.
35. Goncharoff, P. & Nichols, B. P. (1984) *J. Bacteriol.* **159**, 57–62.
36. Kaplan, J. B., Merkel, W. K. & Nichols, B. P. (1985) *J. Mol. Biol.* **183**, 327–340.
37. Ishikawa, H. (1987) *J. Mol. Evol.* **24**, 205–211.
38. Bauerle, R., Hess, J. & French, S. (1987) *Methods Enzymol.* **142**, 366–386.
39. Ito, J., Cox, E. C. & Yanofsky, C. (1969) *J. Bacteriol.* **97**, 725–733.
40. Kreditch, N. M. (1987) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, eds. Neidhardt, F. C., Ingraham, J. E., Low, K. B., Magasanik, B., Schaechter, M. & Umbarger, H. E. (Am. Soc. Microbiol., Washington, DC), Vol. 2, pp. 419–428.
41. Denk, D. & Böck, A. (1987) *J. Gen. Microbiol.* **133**, 515–525.
42. Georgopoulos, C. (1989) *Trends Genet.* **5**, 319–321.
43. Yoshikawa, H. & Ogasawara, N. (1991) *Mol. Microbiol.* **5**, 2589–2597.
44. Zyskind, J. W. (1990) in *The Bacterial Chromosome*, eds. Drlica, K. & Riley, M. (Am. Soc. Microbiol., Washington, DC), pp. 269–278.
45. Yelton, D. B. & Peng, S. L. (1989) *J. Bacteriol.* **171**, 2083–2089.
46. Bae, Y. M. & Stauffer, G. V. (1991) *J. Bacteriol.* **173**, 3382–3388.
47. Katsumata, R. & Ikeda, M. (1993) *Biotechnology* **11**, 921–925.