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MINIREVIEW

Mutualistic Associations of Aphids and Prokaryotes: Biology of the Genus *Buchnera*

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INTRODUCTION

Aphids (class, Insecta; order, Homoptera; superfamily, Aphidoidea) are major pests of plants, including important agricultural crops (17, 44). Upon landing on a plant an aphid injects a flexible tube, which usually penetrates between plant cells until it reaches the phloem tissue (17, 44). The aphid sucks plant sap, a diet rich in carbohydrates but deficient in amino acids and other nitrogenous compounds (17, 44). This mode of feeding is conducive to the transmission of viruses, and the major economic impact of aphids arises from their role as vectors of viral plant pathogens (65). In addition, large populations of aphids may build up on plants and cause debilitation by depleting plant nutrients (17, 44). Many of the general properties of aphids are shared by whiteflies (Aleyrodoidea) and mealybugs (Pseudococcidae), which are related insects within the order Homoptera. All of these insects have symbiotic associations with intracellular prokaryotes (7, 19, 27, 30). In the case of aphids, the endosymbionts have been assigned to the genus *Buchnera*, which has one species, *Buchnera aphidicola* (53). There has been considerable new work on the endosymbionts of aphids, and in this review we will summarize the recent findings.

ULTRASTRUCTURE AND GROWTH

Within the body cavity of aphids is a bilobed structure called a bacteriosome which consists of an aggregate of 60 to 90 cells called bacteriocytes (22, 42, 60). The bacteriocytes are filled with vesicles whose membranes are derived from the host plasma membrane and which are called symbiosomes. Symbiosomes enclose *B. aphidicola*, an oval bacterium with a gram-negative cell wall (Fig. 1). *B. aphidicola* is maternally transmitted to its progeny by mechanisms which involve complex adaptations of the host (5–7). Aphids treated with antibiotics lose their endosymbionts and fail to reproduce (19, 21, 27, 30). The association between *B. aphidicola* and aphids is an example of mutualism, since neither partner is able to survive in the absence of the other.

During their most active reproductive stage, aphids are parthenogenetic females which contain embryos and give birth to live young (17, 44). In the case of the aphid *Schizaphis graminum*, a major pest of cereals, the newborn has an average wet weight of 24 μg and contains 0.2×10^6 endosymbionts (4). The increase in the numbers of *B. aphidicola* organisms approximately parallels the increase in the weight of the aphid. Maximal weight is achieved in 10 to 11 days, at which time the aphid has an average weight of 540 μg and contains 5×10^6 endosymbionts (4). Reproduction begins 8 days after birth, and each aphid can produce 50 to 60 live young during its lifetime.

EVOLUTIONARY RELATIONSHIPS AND rRNA GENE ORGANIZATION

Figure 2 presents a summary of the phylogenetic relationships of bacteriocyte-contained insect endosymbionts based on sequence comparisons of 16S DNA encoding rRNA (rDNA) (2, 11, 46, 47, 52, 54, 67). The results indicate that aphids, whiteflies, and mealybugs each contain a different group of bacterial endosymbionts. The closest relatives of aphid endosymbionts are members of the family *Enterobacteriaceae* and the recently characterized endosymbionts of tsetse flies (2). The phylogenetic tree for the endosymbionts of 12 aphid species was consistent with the phylogenetic tree for the aphids themselves, as deduced from aphid morphology (47). The similarity of the two trees is strong evidence that an ancestor of *B. aphidicola* infected an ancestor of modern aphids and that bacteria and hosts underwent subsequent cospeciation. Similar results involving fewer species have been obtained for endosymbionts of whiteflies, mealybugs, and tsetse flies (2, 12, 54). On the basis of the fossil record for aphids, dates could be assigned to various branch points of the *B. aphidicola* phylogeny, allowing the calibration of the rate of change of 16S rRNA sequences and providing one of the few cases where such an estimate could be obtained for a prokaryotic gene (47). The *B. aphidicola*-aphid association appears to have become established 200 to 250 million years ago (47).

In most prokaryotes the genes coding for rRNA are arranged as a single transcription unit in the order *16S-23S-5S* (40). *B. aphidicola* differs from most organisms in having these genes arranged as two transcription units (Fig. 3), each present as a single copy in the endosymbiont genome (50, 61). This feature, as well as the proximity of the same upstream structural genes (Fig. 3), was used to develop a PCR-based method for the identification of *B. aphidicola* (62). With oligonucleotide probes to *argS* and *16S* or *aroE* and *23S*, DNA fragments of the expected size were amplified from the DNAs of aphid endosymbionts (Fig. 3) but not from the DNAs of whitefly, mealybug, or tsetse fly endosymbionts or from members of the family *Enterobacteriaceae* (1, 2, 62). These results allow the differentiation of *B. aphidicola* from related organisms on the basis of linkage relationships and the unusual arrangement of

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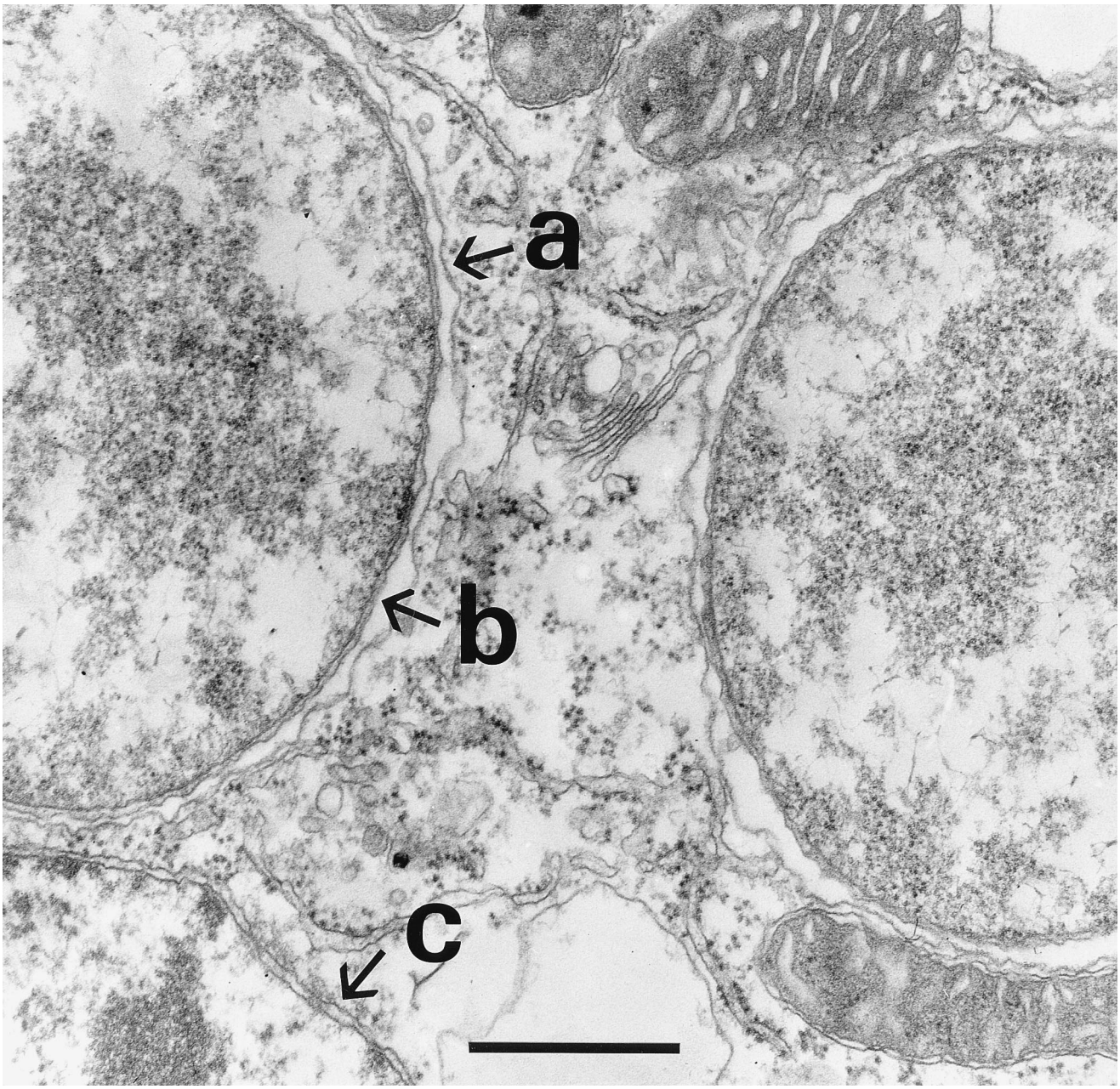

FIG. 1. Electron micrograph of *B. aphidicola* within symbiosomes. a, symbiosome membrane; b, gram-negative cell wall; c, bacteriocyte membrane. Bar, 1 μm. Photograph courtesy of D. McLean and M. Kinsey.

their rRNA genes. Comparisons of the intergenic regions between *argS* and *16S* and *aroE* and *23S* (Fig. 3) from the endosymbionts of different species of aphids indicated the conservation of nucleotide sequences corresponding to putative −35 and −10 regions of the rRNA promoters (52, 61). The consensus sequences were TTGAC(A/T) and TGTA(A/T)T for the −35 and −10 regions, respectively (50, 61). DNAs containing the *argS-16S* intergenic region had promoter activity in *Escherichia coli* (50). Other conserved sequences following the −35 and −10 regions but preceding the rRNA genes were boxA and boxC, which have a role in antitermination. At the ends of both *16S* and *5S* (Fig. 3) of *B. aphidicola* from *S. graminum*, sequences resembling *rho*-independent terminators were detected, and in the case of *16S* this sequence functioned as a terminator in *E. coli* (50). Most prokaryotes appear to have multiple copies of the rRNA operon (68). The presence of one copy, as is the case in *B. aphidicola*, is rare and appears to be restricted to organisms which have a slow growth rate (68).

GENETIC AND PHYSIOLOGICAL PROPERTIES

The guanine-plus-cytosine content of *B. aphidicola* DNA is 28 to 30 mol%, similar to that of the aphid host (29, 66). Its genome size has been reported as 1.4×10^{10} Da, approximately five times that of *E. coli* (29). To date, over 60 kb of

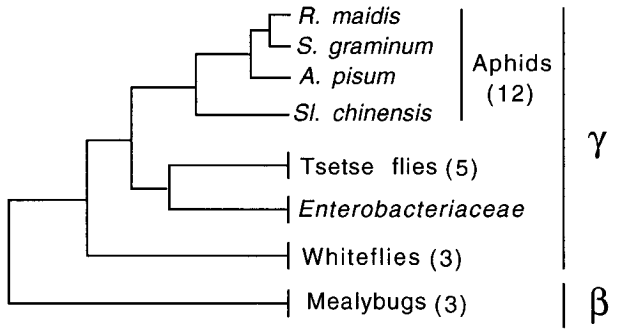

FIG. 2. Evolutionary relationships of the bacteriocyte-contained insect endosymbionts based on sequence comparisons of 16S rDNA. With the exception of the family *Enterobacteriaceae*, all of the designations are those of the insect hosts. The numbers in parentheses indicate the number of host species tested. γ and β refer to subdivisions of the class *Proteobacteria*. This information was compiled from references 2, 11, 47, 52, 54, and 67.

DNA from *B. aphidicola* has been cloned and sequenced. A list of most of the detected genes is presented in Table 1. This includes genes coding for proteins involved in DNA synthesis, transcription, and translation; the common portion of the aromatic amino acid biosynthetic pathway; and the tryptophan biosynthetic pathway; genes coding for chaperonins; and genes coding for proteins involved in other functions. It has been previously shown that isolated endosymbionts are able to synthesize DNA, rRNA, and over 210 different proteins (28, 29). As in the case of other intracellular bacteria, *B. aphidicola* has elevated levels of GroEL, and isolated endosymbionts exhibit the characteristics of a stress response (1, 9, 24, 25, 48, 72). The totality of these observations indicate that *B. aphidicola* has many of the genetic and physiological properties of free-living bacteria.

In *E. coli*, many of the genes homologous to those of *B. aphidicola* have upstream and downstream DNA sequences which are involved in the regulation of gene expression. Attempts to detect such sequences in *B. aphidicola* have for the most part been unsuccessful. There are a few differences which may be of interest, in that they could represent modifications which are an adaptation to the endosymbiotic association. DnaA is a protein which initiates chromosome replication by binding to a DNA segment known as the origin of replication (26, 71, 74). The characteristic features of the origin of replication are several 9-nucleotide sequences known as DnaA boxes and adenine- and thymine-rich direct repeats. The linkage relationship of genes around *dnaA* is highly conserved in bacteria: the order in most cases is *rnpA-rpmH-dnaA-dnaN-recF-gyrB* (57, 71). In most organisms the origin of replication is between *rpmH* and *dnaA*, but in *E. coli* it is approximately 40 kb away. In this species, a DnaA box is present upstream of *dnaA* and is involved in the autoregulation of the DnaA protein (57, 71). The *dnaA* region of *B. aphidicola* differs from that of most other organisms in two ways (36). The first is the absence of DnaA boxes and other properties characteristic of an origin of replication between *rpmH* and *dnaA*, suggesting that as in *E. coli*, the origin of replication lies elsewhere. Since the parallel growth of the aphid and *B. aphidicola* implies integration of endosymbiont growth with that of the host (4), the absence of a DnaA box upstream of *B. aphidicola dnaA* may be an indication of a different mechanism of regulation of DnaA synthesis and hence of initiation of chromosome replication. This regulation could be under the control of host signals. The absence of DnaA boxes upstream of *dnaA* has also been noted in *Borrelia burgdorferi*, an organism very distantly related to the lineage which includes *B. aphidicola* (63). A second difference in the *B. aphidicola dnaA* region is the absence of *recF* between *dnaN* and *gyrB*. This gene could be located elsewhere or alternatively, since RecF is involved in the repair of UV-damaged DNA (43), it is possible that because of the intracellular location of *B. aphidicola*, this function is no longer necessary. Curiously, the same absence of *recF* was also recently noted in *Spiroplasma citri*, another organism which leads a sheltered existence within plant tissue (70). In *E. coli* and a variety of other organisms, *rpoD* (σ^{70}) is followed by one or two inverted repeats characteristic of *rho*-independent terminators (37). Inverted repeats are not found downstream of *B. aphidicola rpoD* (37). In contrast to these differences, *B. aphidicola* from two aphid species resembles other bacteria in having *groEL* preceded by −35 and −10 promoter sequences characteristic of σ^{32}, which is involved in the stress response (58, 59, 73).

Previously, aphid endosymbionts were thought to resemble organelles, such as mitochondria and chloroplasts, and this has had considerable influence on the conceptual framework within which the endosymbionts were viewed. Characteristic of these organelles are the major reduction in the genome size and coding capacity and the transfer of some of the genes to the host nucleus (40). The genome size of *B. aphidicola* (29), as well as the recent information on its genetic properties (Table 1), suggests that comparisons to free-living bacteria are more meaningful. An unanswered question is that of why organelles such as mitochondria and chloroplasts differ so much from insect endosymbionts. One possible explanation is the age of the association. Both chloroplast and mitochondrial associations are over 1 billion years old (56), while the association between aphids and *B. aphidicola* is only 200 to 250 million years old (47). Consequently it is possible that insufficient time has elapsed for many of the alterations to occur. Perhaps a better explanation is that, unlike the cases for mitochondria and chloroplasts, the ancestor of *B. aphidicola* originally infected a complex multicellular host in which it was sequestered within cells which were separate from those of the germ line. This may have limited the opportunities for the transfer of genes from the prokaryotic chromosome to the host nucleus and for their incorporation into the aphid germ line.

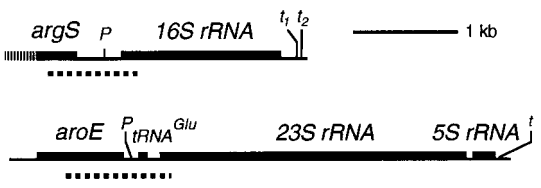

FIG. 3. Genetic maps of the two single-copy *B. aphidicola* transcription units which contain the genes for rRNA. The striped line indicates a partial sequence. The dashed lines indicate the positions of the oligonucleotide probes useful for the identification of linkage relationships by PCR and the size of the amplified fragment. *P*, putative promoter; *t*, putative terminator. This information was compiled from references 50, 61, and 62.

ROLE OF *B. APHIDICOLA*

Nutritional studies. It is generally thought that insects are unable to synthesize 10 essential amino acids and require them preformed in their diet (15, 45, 64). Some species of aphids are able to grow on synthetic diets lacking the essential amino acids, and it has been suggested that they are synthesized by *B. aphidicola* (15, 45, 64). Some evidence, consistent with this interpretation, has been obtained from experiments in which

TABLE 1. Partial list of detected genes and deduced gene products[a]

Protein type	Protein (gene) designations
Proteins involved in DNA synthesis	DnaA (*dnaA*), primase (*dnaG*), β and ε subunits of DNA polymerase III (*dnaN* and *dnaQ*), subunit B of gyrase (*gyrB*), RNase H (*rnh*)
RNA polymerase	α (*rpoA*), β (*rpoB*), β′ (*rpoC*), σ (*rpoD*) subunits
rRNAs	16S (*rrs*), 23S (*rrl*), 5S (*rrf*)
Ribosomal proteins	S1 (*rpsA*), S4 (*rpsD*), S11 (*rpsK*), L7/L12 (*rplL*), L20 (*rplT*), L34 (*rpmH*), L35 (*rpmI*)
tRNA synthases	*argS*, *cysS*, *thrS*
Chaperonins	SecB (*secB*), GroEL (*groEL*), GroES (*groES*)
Proteins involved in amino acid biosynthesis[b]	Serine acetyltransferase (*cysE*), 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (*aroH*), shikimate dehydrogenase (*aroE*)
Miscellaneous proteins	Initiation factor-3 (*infC*), RNase P (*rnpA*), β subunit of ATP synthase (*atpD*), glyceraldehyde-3-phosphate dehydrogenase (*gapA*), triose phosphate isomerase (*tpiA*)

[a] This information was compiled from references 10, 12, 32, 33, 35–38, 49–51, 58, 59, and 61.
[b] For genes and proteins involved in the tryptophan biosynthetic pathway, see Fig. 4.

the effect on nutritional requirements of the reduction or elimination of endosymbionts by antibiotics has been examined. Ideally, it would be expected that upon antibiotic treatment of the aphid there would be a requirement for the essential amino acids and a sparing effect upon their addition to the diet. Most of the nutritional studies involving antibiotics have not given such clear-cut results and are somewhat difficult to interpret. Nevertheless, on the basis of the results of these experiments, it is the general opinion that *B. aphidicola* synthesizes all or some of the essential amino acids required by the aphid host (15, 45, 64). Recently, by using modifications of this approach and radioactive tracer methods, evidence which implicates the endosymbionts in the reduction of sulfate to sulfite and in the synthesis of cysteine and methionine has been obtained (18, 20).

Overproduction of biosynthetic end products. The presumed ancestor of *B. aphidicola* was a free-living bacterium which consequently had a variety of regulatory features designed to respond to concentrations of essential nutrients either in the cell or in the environment (55). In the case of biosynthetic pathways this usually involves two levels of regulation: (i) allosteric end product inhibition of the activity of the enzyme which initiates the pathway and (ii) repression of enzyme synthesis by the end product (55). In order to change from a free-living bacterium, with a mechanism for integrating the availability of an end product with its demand for growth and efficient energy utilization, to an endosymbiont which overproduces an end product, two regulatory changes are necessary. These changes are potentially similar to those required in industrial fermentations where the goal is the development of strains for the overproduction of biosynthetic end products (16, 31). The first change is the constitutive production of the enzymes; such changes are frequent and well documented in experiments on directed evolution of metabolic pathways (13). The second change is the retention of or increase in the activity of the first enzyme of the pathway in the presence of the end product. Two strategies for accomplishing this change have been observed. The first is a structural gene mutation which desensitizes the enzyme to its allosteric inhibitor (16). The second is gene amplification, either on the chromosome or on a plasmid, which allows the synthesis of more enzyme protein (3). Since allosteric enzymes are generally not totally inhibited by their effectors, overproduction of the enzyme will allow the retention or increase of activity even in the presence of elevated levels of the end product. Gene amplification may be the mechanism of choice, since it is known in some cases to occur at a frequency of 10^{-4} to 10^{-5}, which is above the mutation rate for changes in the structural genes which would lead to allosteric desensitization (3).

An example of a potential desensitization is provided by *B. aphidicola* serine acetyltransferase (*cysE*). In *E. coli* this enzyme is constitutive, and its action is the sole regulated activity in the pathway of cysteine biosynthesis (34). The portion of the enzyme involved in allosteric regulation is located on the C terminus of the protein (16). *B. aphidicola cysE* differs from that of *E. coli* in having a totally different sequence at the C terminus, suggesting that this enzyme is not allosterically inhibited by cysteine (37). This lack of inhibition would lead to overproduction of this amino acid and its availability to the aphid host. An example of gene amplification within *B. aphidicola* is observed in tryptophan biosynthesis, which is considered in the next section.

Tryptophan biosynthesis. By far the most clear-cut results of nutritional studies implicate *B. aphidicola* in the biosynthesis of tryptophan (23). Tryptophan synthase has been detected in the endosymbiont and is eliminated from aphids treated with antibiotics (23). The genes of the tryptophan biosynthetic pathway of aphids are arranged in two separate, unlinked transcription units consisting of *trpEG* and *trpDC*(*F*)*BA* (Fig. 4) (35, 38, 51). *trpEG* codes for anthranilate synthase, the first enzyme of the tryptophan biosynthetic pathway, which is allosterically inhibited by tryptophan (14, 69). The sequence of this enzyme from *B. aphidicola* has all of the residues which have been shown to be required for feedback inhibition (8, 41). *trpBA* codes for tryptophan synthase, the last enzyme of the pathway. The aphids *Rhopalosiphum maidis*, *S. graminum*, and *Acyrthosiphon pisum* are all members of the family Aphididae and have a rapid growth rate (38). *Schlechtendalia chinensis* is a member of the family Pemphigidae and has a slow growth rate (38). In the case of endosymbionts of the three aphid species from the family Aphididae, *trpEG* is amplified by being on a plasmid (Fig. 4) (35, 39). *R. maidis* has endosymbionts with plasmids consisting of a single 3.7-kb unit (39). *S. graminum* endosymbionts contain a plasmid consisting of four tandem

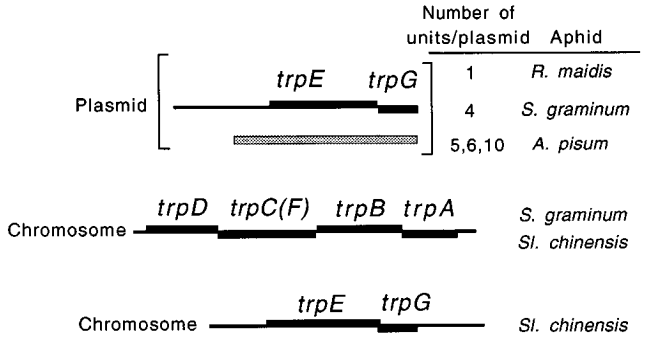

FIG. 4. Arrangements of genes of the tryptophan biosynthetic pathway in *B. aphidicola* from different aphid hosts. Brackets indicate genes on plasmids, which in endosymbionts from different hosts are present as tandem repeats of a single unit. The stippled line indicates conserved DNA sequences in the plasmid units. This information was compiled from references 35, 38, 39, and 51.

repeats of a 3.6-kb unit (35), while *A. pisum* contains endosymbionts with plasmids consisting of five, six, and ten tandem repeats of a 3.7-kb unit (39). For *A. pisum*, it is not known whether different aphids have endosymbionts containing plasmids of one or several sizes. In the case of *B. aphidicola* from *S. graminum*, it has been shown that the number of copies of the plasmid-borne *trpEG* genes is 14- to 15-fold greater than that of the chromosomal *trpB* gene (35). These results suggest that the overproduction of tryptophan is a consequence of the amplification of the genes for the allosterically inhibitable anthranilate synthase; activity in the presence of tryptophan is retained due to the increased amount of the enzyme protein. Thus, the amplification of *trpEG* and its location on a plasmid appear to be adaptations of the endosymbionts to the mutualistic association with aphids. These adaptations may have occurred in an ancestor of the family Aphididae after divergence from the family Pemphigidae. The latter possibility is suggested by the fact that *trpEG* is located on the chromosome as a single copy in the endosymbionts of the aphid *S. chinensis* (Fig. 4) (38). Possibly, the slow growth rate of this aphid obviates the need for an elevated rate of tryptophan biosynthesis.

Comparisons of *trpEG* and the deduced protein products from endosymbionts of several aphid species indicate that their sequences are highly conserved (Fig. 4) (38, 39). In the plasmids, an additional DNA region containing conserved sequences is present upstream of *trpE* (Fig. 4) (35, 39). This region has sequences resembling an origin of replication and presumably the promoter of *trpEG* (37, 39). Phylogenetic analyses of *trpE* and *trpG* and of the deduced protein products from the endosymbionts of the four aphid species listed in Fig. 4 yield a branching order that is the same as that based on 16S rDNA (Fig. 2) or on aphid morphology. This result indicates vertical evolution of the *trpEG* genes and 16S rDNA (52), that is, the absence of genetic exchange of endosymbiont plasmid or chromosomal DNA between aphid species. In the case of *B. aphidicola* from *S. graminum*, it has been shown that the sequences of the intergenic region within two 3.6-kb tandem repeats are virtually identical (35). The lack of sequence conservation in most of the intergenic region of plasmids from the endosymbionts of closely related aphid species (*R. maidis* and *S. graminum*) indicates that the sequence change in this region is relatively rapid. The virtual identity of the same intergenic region in at least two tandem repeats in the same plasmid (from *S. graminum* endosymbionts) and the presence of plasmids of five, six, and ten tandem repeats in the endosymbionts of a single aphid species (*A. pisum*) suggest that their number is subject to rapid variation.

CONCLUSIONS AND PERSPECTIVES

The results of these studies provide a beginning for our understanding of the nature of *B. aphidicola* and its function in the aphid host. In most respects *B. aphidicola* resembles free-living bacteria, and comparisons with near relatives may be fruitful for understanding some of the adaptations to an endosymbiont association. Currently, the best-documented function of *B. aphidicola* is the overproduction of tryptophan. Because of the complex nature of the system, none of the data can be considered definitive according to standards applicable to bacteria with well-developed genetic systems. However, the studies leading to this conclusion are quite extensive and all are consistent with this interpretation.

The data summarized in this review provide potentially useful information applicable to future aphid endosymbiont research. We now have several strong promoters and terminators which function both in *B. aphidicola* and in *E. coli*. In addition, we have a DNA region in endosymbiont plasmids which probably serves as an origin of replication. This may facilitate the construction of plasmids functional in *B. aphidicola*, although their delivery and maintenance in the endosymbiont may present formidable obstacles. It is the ultimate goal of this research to provide the necessary groundwork which might be useful for future approaches to the control of aphid populations. Since *B. aphidicola* is essential for the survival of the host, knowledge of its physiology and genetics may allow us to interfere with its growth, resulting in sterility and eventual death of the aphids. Alternatively, *B. aphidicola* may be used as a delivery system of components which restrict the ability of aphids to act as vectors of important plant viruses (2).

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