P. Baumann

Biology of Aphid Endosymbionts (Genus *Buchnera*)

Abstract

Most aphids are dependent on an association with an intracellular prokaryotic endosymbiont (*Buchnera*). Evolutionary studies are consistent with an infection of an aphid ancestor with *Buchnera* and subsequent cospeciation of the host and the bacterium. Genetic studies show that *Buchnera* resembles free-living bacteria in having genes coding for proteins involved in DNA replication, transcription, translation and a variety of other functions. Studies of the genetics of the tryptophan biosynthetic pathway indicate that *trpEG* genes of *Buchnera* are on plasmids and are amplified, relative to the remaining chromosomal genes. The *trpEG* genes encode anthranilate synthase, the first enzyme of the pathway, which is feedback-inhibited by tryptophan. Amplification of *trpEG* is consistent with past evidence suggesting that one of the functions of *Buchnera* is the overproduction of tryptophan for the aphid host.

Introduction

Aphids (class, Insecta; order, Homoptera; superfamily, Aphidoidea) are insects that feed on plant sap (Buchner, 1965; Douglas, 1989; Hauk and Griffiths, 1980). Aphids penetrate plant tissue by means of flexible stylets which probe until they reach the sieve tubes in the phloem tissue. This mode of feeding is conducive to the transmission of disease. Aphids are important vectors of plant viruses and cause major economic losses in agriculture. 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 a mutualistic association with intracellular prokaryotes (Douglas, 1989; Houk and Griffiths, 1980). The endosymbionts of aphids have been studied most extensively and have been assigned to the genus *Buchnera*, which currently has one species, *B. aphidicola*. (Recent reviews on endosymbionts are those of Douglas, 1989; Ishikawa, 1989; and Baumann *et al.*, 1995ab. Due to space limitations, many relevant references could not be cited in this discussion and are included in Baumann *et al.*, 1995ab).

Morphology, growth and transmission

Within the body cavity of most aphids is a bilobed structure (bacteriome) consisting of 60 to 90 cells called bacteriocytes. Within these cells are host-derived vesicles (symbiosomes) which contain *Buchnera* (Figure 1). This organism is oval-shaped and has a gram-negative cell wall. *Buchnera* and the aphid are dependent on each other. The endosymbionts have not been cultivated outside the host. Elimination of the endosymbionts by antibiotics or other treatments causes decreased growth of the aphid, sterility and eventual death. Some species of aphids have additional intracellular bacteria designated as secondary endosymbionts, which are absent from bacteriocytes and which do not appear to play an essential role in the life of the aphid.

Aphids vary greatly in their annual life cycles and host plant preferences. During their most active reproductive stage, aphids are parthenogenetic females which contain embryos and give birth to live young. Under laboratory conditions a typical aphid, *Schizaphis graminum*, which is a major pest of cereals, gives birth to young having an average weight of 24 μg and containing 0.2×10^6 endosymbionts. The increase in the number of *Buchnera* cells during aphid growth approximately parallels the increase in the weight and protein and total

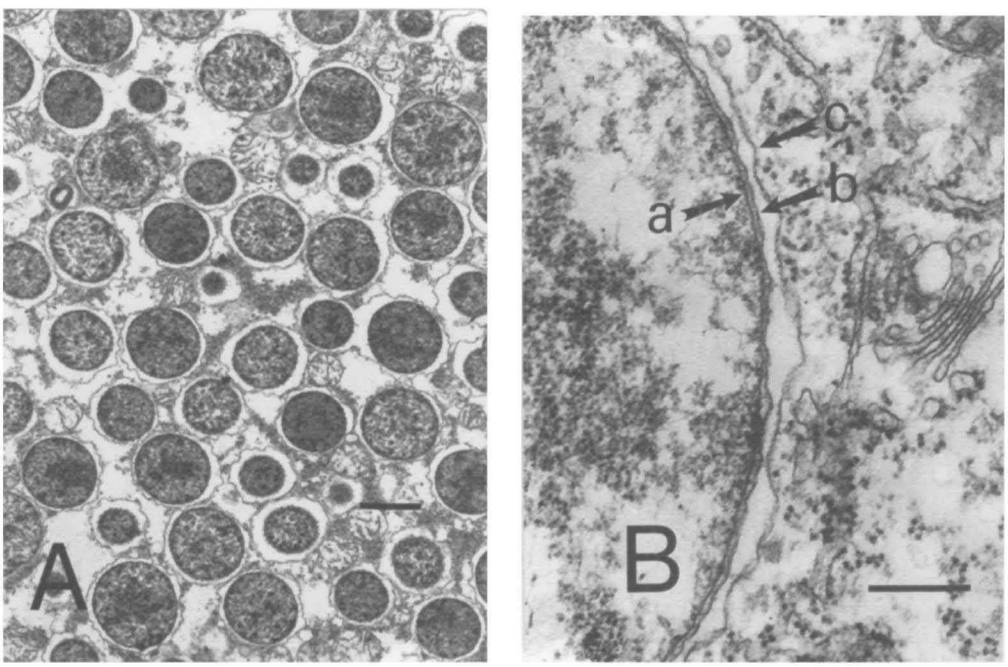

Fig. 1. Electron micrographs of *Buchnera* (A) within a bacteriocyte and (B) within a symbiosome showing the (a) cell membrane and (b) outer membrane of the endosymbiont and (c) the symbiosome membrane. Bar in (A) and (B), represents 2 μm and 0.5 μm, respectively. Photograph courtesy of D. McLean and M. Kinsey.

DNA content of the aphid, indicating a close integration of growth of the endosymbiont with that of the host. The aphid reaches a maximal weight of 540 ug in 10 to 11 days and contains $5x10^6$ endosymbionts, an approximately 28-fold increase in the *Buchnera* population. During this time there is an increase in the bacteriocyte volume, but not bacteriocyte number (Douglas and Dixon, 1987). New aphids are born about 8 days after the birth of the mother, and each aphid can produce 50 to 60 live young. *Buchnera* is transmitted maternally to the offspring by complex mechanisms which have not been extensively studied.

Evolutionary relationships and rRNA gene organization

Sequence comparisons of endosymbiont genes coding for 16S rRNA (rDNA) were used to establish evolutionary relationships. The results for *Buchnera* are presented in Figure 2. The nearest relatives were members of the *Enterobacteriaceae* (which includes *Escherichia coli*) and the newly characterized bacteriocyte endosymbionts of the tsetse fly (Aksoy *et al.*, 1994) (results not included). The latter were more closely related to *E. coli* than to *Buchnera* (Aksoy, 1994; Aksoy *et al.*, 1994). All of these organisms are in the gamma-3

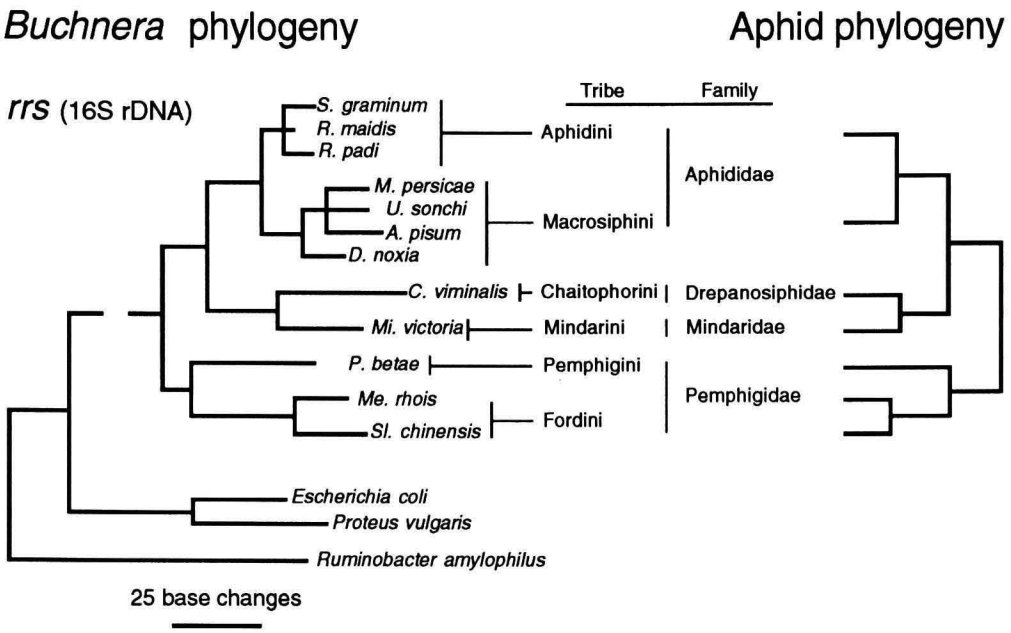

Fig. 2. *Buchnera* phylogeny based on 16S rDNA sequences and comparison with a proposed phylogeny of the aphid hosts. Names designate the aphid host. The following abbreviations designate aphid genera: *S, Schizaphis*; *R, Rhopalosiphum*; *M, Myzus*; *U, Uroleucon*; *A, Acyrthosiphon*; *D, Diuraphis*; *C. Chaitophorus*; *Mi, Mindarus*; *P, Pemphigus*; *Me, Melaphis*; *Sl, Schlechtendalia.* Redrawn from Baumann *et al.* (1995a) and Moran *et al.* (1993).

subdivision of the *Proteobacteria*. An inspection of Figure 2 indicates that the groupings of *Buchnera* agree with those of classical aphid taxonomy based on morphology and that the branching order within the *Buchnera* phylogenetic tree is identical to the proposed phylogeny of aphids (Moran and Baumann, 1993; Moran *et al.*, 1993). On the basis of the fossil record for aphids, dates could be assigned to the branch points of the *Buchnera* phylogenetic tree, allowing an estimation of the rate of change of 16S rRNA sequences (Moran *et al.*, 1993). The totality of these results is consistent with the following interpretation. Approximately 200 to 250 million years ago an ancestor of present-day aphids was infected with a free-living bacterium and an endosymbiotic association became established. Subsequent parallel divergence led to cospeciation of *Buchnera* and host, resulting in the present species of aphids and strains of *Buchnera*. A more limited analysis of the endosymbionts of whiteflies and mealybugs indicated that they constitute two different lineages distinct from *Buchnera*.

In most bacteria the genes for rRNA are arranged as a single transcription unit consisting of *16S-23S-5S*. *Buchnera* differs from these organisms in having the rRNA genes on two transcription units containing (i) *16S* and (ii) *23S-5S* (Figure 3) (Munson *et al.*, 1993; Rouhbakhsh and Baumann, 1995). Based on the linkage arrangements illustrated in Figure 3, pairs of oligonucleotide primers (*argS-16S*) and (*aroE-23S*) were designed and used in conjunction with the polymerase chain reaction to allow the identification of *Buchnera* (Rouhbakhsh *et al.*, 1995). Both *16S* and *23S* were preceded by conserved DNA sequences corresponding to putative -35 (TTGAC^{A}/T) and -10 (TGTA^{A}/T^{T}) promoter regions. A DNA fragment upstream of *16S* (Fig. 3) functioned as a promoter in *E. coli* and a DNA fragment downstream of *16S* functioned as a terminator (Munson *et al.*, 1993). As is characteristic of other slow-growing bacteria, *Buchnera* contains only one copy of the rRNA genes in its genome (Baumann and Baumann, 1994; Wolfe and Haygood, 1993).

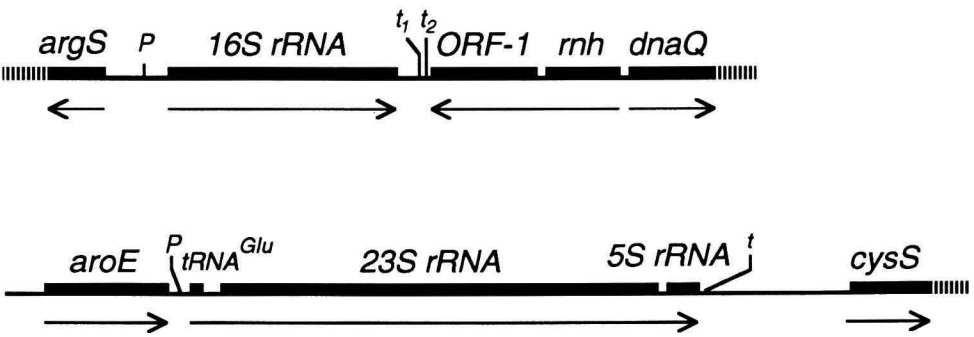

Fig. 3. Organization of rRNA operons in *Buchnera*. *P*, promoter; *t*, terminator; *ORF*, open reading frame; striped line, partial sequence of gene; arrow, direction of transcription. For explanation of gene designations see Tables 1. Redrawn from Munson *et al.* (1993) and Rouhbakhsh and Baumann (1995).

The DNA of *Buchnera* has a guanine + cytosine content of 28 to 30 mol %, a value similar to that of the aphid host. Its genome size (1.4 x 10^{10} daltons) is approximately five times that of *E. coli* (Ishikawa, 1989). Over 60 kilobase pairs (kbp) of *Buchnera* DNA have been cloned and sequenced. Genes coding for proteins involved in DNA synthesis, transcription and translation, enzymes of the common portion of the aromatic amino acid biosynthetic pathways, enzymes of the tryptophan biosynthetic pathway, chaperonins, as well as genes for other functions have been detected in the endosymbiont (Table 1, Figure 4). Consistent with these results, it has been shown that isolated *Buchnera* are able to synthesize DNA, rRNA, and over 210 different proteins (Ishikawa, 1989). As in the case of other intracellular bacteria, *Buchnera* has elevated levels of GroEL (Aksoy, 1994; Ohtaka *et al.*, 1992). The totality of these observations indicates that *Buchnera* has many of the genetic and physiological properties of free-living bacteria.

In *E. coli*, many of the genes homologous to those of *Buchnera* have upstream and downstream DNA sequences involved in regulation of gene expression. In *Buchnera* such sequences have for the most part not been detected. 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 (Yoshikawa and Ogasawara, 1991). The characteristic features of the origin of replication are several nine-nucleotide

Table 1. Genes detected in *Buchnera* and the designations of the deduced products.[a]

Proteins involved in DNA synthesis: DnaA (*dnaA*), primase (*dnaG*), β- and ε-subunit of DNA polymerase III (*dnaN*, *dnaQ*), subunit B of gyrase (*gyrB*), RNase H (*rnh*).

RNA polymerase: α-(*rpoA*), β-(*rpoB*), β'-(*rpoC*), σ-(*rpoD*) subunits.

Ribosomal RNAs: 16S-(*rrs*), 23S-(*rrl*), 5S-(*rrf*) rRNA.

Ribosomal proteins: 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*).

Amino acid biosynthesis: Tryptophan biosynthetic pathway, serine acetyltransferase (*cysE*), 3-deoxy-*D*-*arabino*-heptulosonate 7-phosphate synthase (*aroH*), shikimate dehydrogenase (*aroE*).

Miscellaneous: Initiation factor-3 (*infC*), RNase P (*rnpA*), β-subunit of ATP synthase (*atpD*), β-subunit of integration host factor (*himD*), glyceraldehyde-3-phosphate dehydrogenase (*gapA*), triose phosphate isomerase (*tpiA*).

[a]Protein designation followed by gene designation in parentheses. Data from Lai and Baumann (1992ab), Lai *et al.* (1994), Munson and Baumann (1993), Munson *et al.* (1993) Ohtaka *et al.* (1992), Rouhbakhsh and Baumann (1995), and additional references given in Baumann *et al.* (1995a).

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* (Ogasawara and Yoshikawa, 1992). In most organisms the origin of replication is between *rpmH* and *dnaA*, but in *E. coli* it is approximately 40 kilobases away. In this species, a DnaA box is present upstream of *dnaA* and is involved in the autoregulation of DnaA protein. The *dnaA* region of *Buchnera* differs from that of most other organisms in two features (Baumann *et al.*, 1995a; Lai and Baumann, 1992a). 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. The absence of a DnaA box upstream of *Buchnera dnaA* may be an indication of a different mechanism of regulation of DnaA synthesis and, hence, initiation of chromosome replication. This regulation could be under the control of host signals. A second difference in the *Buchnera 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, it is possible that due to the intracellular location of *Buchnera*, this function is no longer necessary. The same absence of *recF* was also recently noted in *Spiroplasma citri*, another organism which leads a sheltered existence within plant tissue (Ye *et al.*, 1994). 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; inverted repeats are not found downstream of *Buchnera rpoD* (Lai and Baumann, 1992b).

Tryptophan biosynthesis

Plant sap, the diet of aphids, is rich in carbohydrates but deficient in amino acids and other nitrogenous compounds. Insects are thought to require ten essential amino acids and one of the proposed functions of the endosymbionts is the synthesis of these amino acids for the aphid host. Some aphids are able to grow and reproduce on a synthetic diet. There is an extensive literature on the amino acid requirements of aphids. In principle, the demonstration of a requirement in the presence but not in the absence of an antibiotic is strong evidence that the biosynthetic activities of the endosymbionts are the source of the amino acid. In practice, such clear-cut results have rarely been obtained and the results are often difficult to interpret. Nevertheless, the consensus of opinion derived from such experiments is that endosymbionts produce essential amino acids for the aphid host (Baumann *et al.*, 1995ab; Douglas, 1989; Ishikawa, 1989). Using nutritional methods as well as radioactive tracers evidence has been obtained indicating that *Buchnera* is able to reduce sulfate to sulfide and synthesize methionine and cysteine (Douglas, 1990). By far, the best evidence has been obtained for the synthesis of tryptophan by the endosymbionts. Douglas and Prosser (1992) have found that most aphids survive to adulthood on a synthetic diet containing tryptophan and chlortetracycline, while the omission of tryp-

tophan leads to death of the aphids. In addition, these investigators have detected in *Buchnera*, tryptophan synthase, the last enzyme of the tryptophan biosynthetic pathway. The inclusion of chlortetracycline in the diet led to the elimination of tryptophan synthase activity. While this work was in progress we independently initiated studies on the genetics of the *Buchnera* tryptophan biosynthetic pathway (Lai *et al.*, 1994; Munson and Baumann, 1993).

The modifications which *Buchnera* potentially must undergo in order to overproduce tryptophan or any other biosynthetic endproduct involve a major change in the regulation of the biosynthetic pathway (Baumann *et al.*, 1995ab; Lai *et al.*, 1994). The ancestor of *Buchnera* probably resembled other free-living bacteria in that it had mechanisms which integrated the rate of tryptophan synthesis to the intracellular and extracellular availability of this amino acid. In almost all bacteria, the immediate effect of tryptophan accumulation is feedback inhibition of anthranilate synthase (AS, encoded by *trpEG*), the first enzyme of the pathway. The long-term effect of tryptophan accumulation is repression of synthesis of the enzymes of this pathway. The first step of endosymbiont adaptation could be a mutation to constitutivity, so that the enzymes of the pathway would be produced in the presence of tryptophan. Such mutations are common in studies of directed evolution. Even if tryptophan is exported from the cell, its overproduction could result in some accumulation which would be expected to reduce activity of AS by feedback inhibition. As in the case of many other allosteric enzymes, considerable activity of AS is present even at high levels of tryptophan. In order to augment AS activity, *Buchnera* could have a mutation in sites which would desensitize the enzyme to allosteric feedback inhibition. An alternative modification is gene amplification resulting in the production of more AS protein, which, even in the presence of tryptophan, would provide sufficient AS activity for the synthesis of this amino acid. Gene amplification is a common mechanism for the overproduction of enzyme protein under conditions where the growth rate is limited by a particular enzyme activity (Anderson and Roth, 1977). Its frequency can be as high as 10^{-4} to 10^{-5}, which is considerably higher then the frequency of mutations leading to changes in structural genes resulting in desensitization of feedback inhibition. Our studies indicate that gene amplification of *trpEG* is the alternative favored by several rapidly growing species of aphids.

Studies of *Buchnera* from the aphid *S. graminum* have indicated the presence of all of the genes of the tryptophan biosynthetic pathway (Fig. 4). The arrangement *trpDC(F)BA* suggests a single transcription unit and restriction enzyme analysis is consistent with a single copy of these genes on the endosymbiont chromosome. On the other hand, *trpEG* is amplified 14- to 15-fold, relative to the chromosomal genes and is found on plasmids consisting of four tandem repeats of a 3.6-kbp unit (Figure 4). Since *Buchnera* AS has all of the amino acid residues which are required for allosteric feedback inhibition of activity (Lai *et al.*, 1994), gene amplification would result in an increase in AS protein which could still provide sufficient activity for the overproduction of tryptophan, even in the presence of this end product.

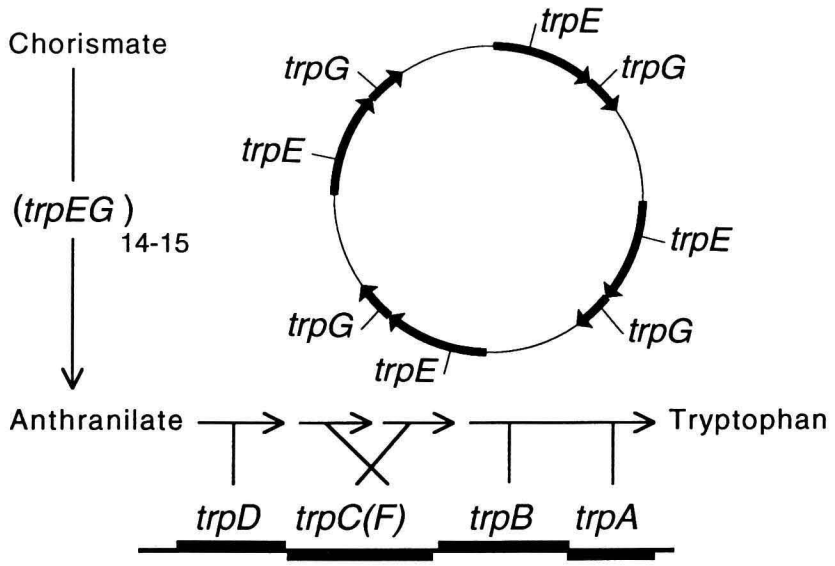

Fig. 4. Genetics of tryptophan biosynthesis in *Buchnera* from the aphid *S. graminum*. *trpEG* is on a plasmid (four tandem repeats) and is amplified 14 to 15-fold relative to the remaining genes of the pathway which are present as one copy on the chromosome. Redrawn from Lai *et al.* (1994) and Munson and Baumann (1993).

Gene amplification of plasmid-borne 3.6-kbp units containing *trpEG* has also been found in *Buchnera* from the aphids *R. maidis* and *A. pisum* (Figure 5). In *R. maidis* the 3.6-kbp unit constitutes a single plasmid, while in *A. pisum* plasmids containing 5, 6, and 10 tandem repeats of this unit have been found. In the latter aphid it is not known whether *Buchnera* from a single aphid has one or several different-sized plasmids. All of these aphids as well as *S. graminum* have a short development time and are in the family Aphididae (Lai *et al.*, 1995). In contrast, the aphid *Schlechtendalia chinensis*, which is in the family

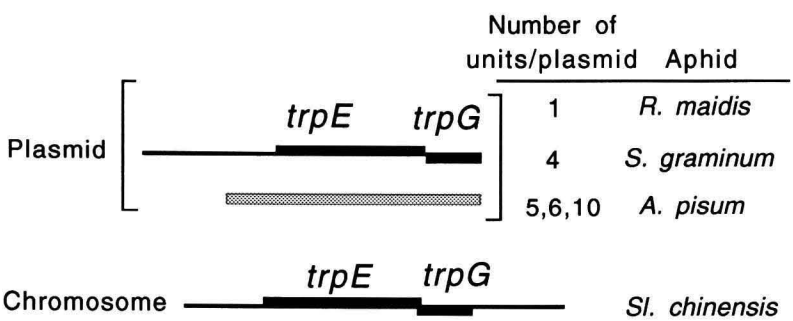

Fig. 5. Arrangements of *trpEG* in *Buchnera* from different aphid hosts. Brackets designate a *trpEG*-containing 3.6-kbp unit which, singly or as tandem repeats, constitutes a plasmid. Stippled line indicates conserved DNA sequences in the plasmid units. Redrawn from Baumann *et al.* (1995b).

Pemphigidae, has a long development time (Lai *et al.*, 1995). We have cloned and sequenced the *Buchnera* genes for the tryptophan biosynthetic pathway from this aphid. The results indicate that the genes are organized into two single chromosomal transcription units consisting of *trpEG* and *trpDC(F)BA*. The absence of amplification of *trpEG* in the endosymbiont of *Sl. chinensis* may be due to the fact that its development time is long and consequently the demand for tryptrophan is reduced.

Comparisons of the sequences of the 3.6-kbp units from *Buchnera* of three aphid species have indicated that the nucleotide sequence of the genes and the amino acid sequences of the proteins are highly conserved (Figure 5). A phylogenetic tree based on these sequences gives a branching order identical to that observed in the tree derived from 16S rDNA (Figure 2) which is consistent with the vertical evolution of the endosymbionts and the *trpEG* plasmids. A sequence approximately 600 base pairs upstream of *trpEG* is also highly conserved (Fig. 5). The conserved sequence proximal to *trpEG* is the putative ribosome binding site and the promoter. The more distal conserved upstream sequence has a number of properties, such as DnaA boxes and in some cases tandem high AT-containing repeats, which suggest that it functions as the plasmid origin of replication. There is no conservation of the remaining upstream sequence of approximately 900 base pairs. In the case of *Buchnera* from *S. graminum*, graminum, two different 3.6-kbp units have been sequenced and the intergenic region between *trpG* and *trpE* has been found to be virtually identical. The major difference in the intergenic sequence of plasmids from *Buchnera* of the three closely related aphid species and the virtual identity of the intergenic sequence in the units from the plasmid of *Buchnera* from the same aphid suggest that the number of tandem repeats is subject to relatively rapid variation. One possible mechanism of gene amplification is an increase in the number of tandem repeats which constitute a plasmid. Another is an increase in the copy number of a plasmid. These mechanisms may be a response to the nutritional status of the aphid: aphids provided with a good source of food and growing rapidly would have a greater *trpEG* amplification than aphids growing slowly on a poor nutrient source.

As in the case of the obligate intracellular pathogens, *Rickettsia* and *Chlamydia*, application of recombinant DNA methodology to aphid endosymbionts has resulted in considerable new information on the genetics, physiology and evolutionary relationships of these organisms. Much, however, remains to be learned concerning the nutritional contributions which *Buchnera* makes to the aphid host and nothing is known about the mechanisms which are involved in the transmission of *Buchnera* to aphid progeny.

References

Aksoy, S., 1995 - Molecular analysis of the endosymbionts of tsetse flie: 16S rDNA locus and over-expression of a chaperonin. *Insect Mol. Biol.* **4**, 23–29.

Aksoy, S., A.A. Pourhosseini and A. Chow, 1995 - Mycetome endosymbionts of tsetse flies constitute a distinct lineage related to *Enterobacteriaceae. Insect Mol. Biol.* **4**, 15–22.

Anderson, R.P. and J.R. Roth, 1977 - Tandem genetic duplications in phage and bacteria. *Annu. Rev. Microbiol.* **31**, 473–505.

Baumann, L. and P. Baumann, 1994 - Growth kinetics of the endosymbiont *Buchnera aphidicola* in the aphid *Schizaphis graminum. Appl. Environ. Microbiol.* **60**, 3440–3443.

Baumann, P., L. Baumann, C.Y. Lai, D. Rouhbakhsh, N.A. Moran and M.A. Clark, 1995a - Genetics, physiology and evolutionary relationships of the genus *Buchnera*: intracellular symbionts of aphids. *Annu. Rev. Microbiol.* **49**, 55–94.

Baumann, P., C.-Y. Lai, L. Baumann, D. Rouhbakhsh, N.A. Moran and M.A. Clark, 1995b - Mutualsitic associations of aphids and prokaryotes: biology of the genus *Buchnera. Appl. Environ. Microbiol.* **61**, 1–7.

Buchner, P., 1965 - Endosymbiosis of Animals with Plant Microorganisms, p. 210–338. Interscience Publishers, Inc., New York.

Douglas, A.E., 1989 - Mycetocyte symbiosis in insects. *Biol. Rev. Camb. Phil. Soc.* **64**, 409–434.

Douglas, A.E., 1990 - Nutritional interactions between *Myzus persicae* and its symbionts, p. 319–327. Aphid-plant genotype interactions, R.K. Campbell and R.D. Eikenbary (ed.). Elsevier Biomedical Press, Amsterdam.

Douglas, A.E. and A.F.G. Dixon, 1987 - The mycetocyte symbiosis of aphids: variation with age and morph in virginoparae of *Megoura viciae* and *Acyrthosiphon pisum. J. Insect Physiol.* **33**, 109–113.

Douglas, A.E. and W.A. Prosser, 1992 - Synthesis of the essential amino acid tryptophan in the pea aphid (*Acyrthosiphon pisum*) symbiosis. *J. Insect Physiol.* **38**, 565–568.

Houk, E.J. and G.W. Griffiths, 1980 - Intracellular symbiotes of the Homoptera. *Annu. Rev. Entomol* **25**, 161–187.

Ishikawa, H., 1989 - Biochemical and molecular aspects of endosymbiosis in insects. *Int. Rev. Cytol.* **116**, 1–45.

Lai, C.-Y. and P. Baumann, 1992a - Genetic analysis of an aphid endosymbiont DNA fragment homologous to the *rnpA-rpmH-dnaA-dnaN-gyrB* region of eubacteria. *Gene* **113**, 175–181.

Lai, C.-Y. and P. Baumann, 1992b - Sequence analysis of a DNA fragment from *Buchnera aphidicola* (an endosymbiont of aphids) containing genes homologous to *dnaG*, *rpoD*, *cysE*, and *secB. Gene* **119**, 113–118.

Lai, C.-Y., L. Baumann and P. Baumann, 1994 - Amplification of *trpEG*; adaptation of *Buchnera aphidicola* to an endosymbiotic association with aphids. *Proc. Natl. Acad. Sci. USA* **91**, 3819–3823.

Lai, C.-Y., P. Baumann and N.A. Moran, 1995 - Genetics of the tryptophan biosynthetic pathway of the prokaryotic endosymbiont (*Buchnera*) of the aphid *Schlechtendalia chinensis. Insect Mol. Biol.* **4**, 47–59.

Moran, N.A. and P. Baumann, 1993 - Phylogenetics of cytoplasmically inherited microorganisms of arthropods. *Trends Ecol. Evol.* **9**, 15–20.

Moran, N.A., M.A. Munson, P. Baumann and H. Ishikawa, 1993 - A molecular clock in endosymbiotic bacteria is calibrated using the insect hosts. *Proc. R. Soc. Lond. B* **253**, 167–171.

Munson, M.A. and P. Baumann, 1993 - Molecular cloning and nucleotide sequence of a putative *trpDC(F)BA* operon in *Buchnera aphidicola* (endosymbiont of the aphid *Schizaphis graminum*). *J. Bacteriol.* **175**, 6426–6432.

Munson, M.A., L. Baumann and P. Baumann, 1993 - *Buchnera aphidicola* (a prokaryotic endosymbiont of aphids) contains a putative 16S rRNA operon unlinked to the 23S rRNA-encoding gene: sequence determination, and promoter and terminator analysis. *Gene* **137**, 171–178.

Ogasawara, N. and Y. Yoshikawa, 1992 - Genes and their organization in the replication origin region of the bacterial chromosome. *Mol. Microbiol.* **6**, 629–634.

Ohtaka, C., H. Nakamura and H. Ishikawa, 1992 - Structures of chaperonins from an intracellular symbiont and their functional expression in *Escherichia coli groE* mutants. *J. Bacteriol.* **174**, 1869–1874.

Rouhbakhsh, D. and P. Baumann, 1995 - Characterization of a putative 23S-5S rRNA operon of *Buchnera aphidicola* (endosymbiont of aphids) unlinked to the 16S rRNA-encoding gene. *Gene* **155**, 107–112.

Rouhbakhsh, D., N.A. Moran, L. Baumann, D.J. Voegtlin and P. Baumann, 1994 - Detection of *Buchnera*, the primary prokaryotic endosymbiont of aphids, using the polymerase chain reaction. *Insect Mol. Biol.* **3**, 213–217.

Wolfe, C.J. and M.G. Haygood, 1993 - Bioluminescent symbionts of the Caribbean flashlight fish (*Kryptophanaron alfredi*) have a single rRNA operon. *Mol. Mar. Microbiol.* **2**, 189–197.

Ye, F., J. Renaudin, J.-M. Bové and F. Laigret, 1994 - Cloning and sequencing of the replication origin (*oriC*) of the *Spiroplasma citri* chromosome and construction of autonomously replicating artificial plasmids. *Curr. Microbiol.* **29**, 23–29.

Yoshikawa, H. and N. Ogasawara, 1991 - Structure and function of DnaA and the DnaA-box in eubacteria: evolutionary relationships of bacterial replication origins. *Mol. Microbiol.* **5**, 2589–2597.

Acknowledgments

Work from the author's laboratory was supported by the National Science Foundation IBN-9201285, MCB-9402813, DEB-9306495 (to Nancy A. Moran), Entotech Inc. (Novo Nordisk), and the University of California Experiment Station.

Microbiology Section, University of California, Davis, CA, USA 95616-8665