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Buchnera Plasmid-Associated *trpEG* Probably Originated from a Chromosomal Location Between *hsIU* and *fpr*

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Abstract. *Buchnera* are prokaryotic endosymbionts found in most aphids. One of their functions is the synthesis of the essential amino acid tryptophan for the aphid host. In *Buchnera* from some aphids that have a long development time, *trpEG*, which encodes the first enzyme of the tryptophan biosynthetic pathway (anthranilate synthase), is found as one copy on the endosymbiont chromosome and is located between *hsIU* and *fpr*. In *Buchnera* from *Schizaphis graminum*, which has a short development time, *trpEG* is amplified on plasmids. We have cloned and sequenced a 4.1-kb DNA fragment from *Buchnera* of *S. graminum* and have found the gene order *hsIU-ibp-fpr-yjeA-kdtB*. The proximity of *hsIU* and *fpr* is consistent with the excision, in an endosymbiont ancestor, of *trpEG* from a location between these two genes, with the excision either followed or preceded by acquisition of *ibp*.

Survival of most aphids is dependent on mutualistic associations with prokaryotic endosymbionts assigned to the genus *Buchnera* [2]. These organisms are found within specialized cells called bacteriocytes located within the body cavity of aphids. One of the functions of the endosymbionts is the synthesis of the essential amino acid tryptophan for the aphid host [2, 5]. In almost all organisms the tryptophan biosynthetic pathway is regulated by feedback inhibition of anthranilate synthase (*trpEG*) by tryptophan [4]. In aphids with a short development time, such as *Schizaphis graminum* (Sg), *trpEG* is present as four tandem repeats on plasmids and is amplified approximately 16-fold over the remaining chromosomal genes [*trpDC(F)BA*] of the tryptophan biosynthetic pathway [8, 12]. This amplification, which is widespread in aphids with a short development time, is interpreted as an adaptation to an endosymbiotic association in which the endosymbiont makes excess tryptophan for the aphid host [1, 8, 10, 13, 14]. In the aphid *Schlechtendalia chinensis* (Sc), which has a long development time, *Buchnera trpEG* has a chromosomal location and is present as only one copy [9]. This finding is consistent with a lower demand for tryptophan by this aphid.

Our initial cloning of a fragment of DNA containing *trpEG* from *Buchnera* (Sc) was performed in 1995 [9]. At this time the sequence of the complete genome of *Escherichia coli* had not yet been determined, and we failed to detect homologs for some of the open reading frames. Subsequently, upon completion of the *E. coli* genome, we were able to find additional homologs to *E. coli* genes (Fig. 1). Recently, we have also sequenced a

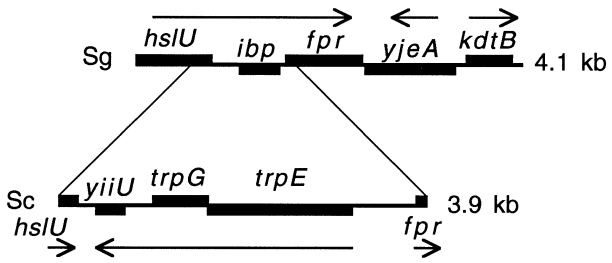

Fig. 1. Genetic map of DNA fragments from *Buchnera* (Sg) and (Sc). Thin line, DNA; thick line structural genes; arrows, direction of transcription. Data for *Buchnera* (Sc) from [9].

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Table 1. Gene and protein designations as well as properties of the proteins

Gene[a]	First-last codon	Size of product (kDa/aa)	Description of product	% identity to *E. Coli* protein	*E. coli* homolog[b]
hsIU	-TAA	partial/277	Heat-shock protein	76	1790366
ibp	ATG-TAA	18.9/161	Heat-inducible protein HSP20 family	26	1790122
fpr	ATG-TAA	30.1/257	Ferredoxin $NADP^+$ reductase	52	1790359
yjeA	ATG-TAA	38.3/324	Hypothetical lysyl-tRNA synthase homolog	49	1790599
kdtB	ATG-TAA	19.0/165	Putative enzyme of lipopolysaccharide biosynthesis	48	466772

[a] Gene abbreviations are those used in *E. coli*.
[b] GenBank PID;g followed by number.

similar fragment from *Buchnera* of the related aphid *Melaphis rhois* (Mr) and have found the same gene order. The chromosomal location of *trpEG* in *Buchnera* from these aphids, which have relatively slow development times, was hypothesized to correspond to the ancestral state. Plasmid-associated amplification of *trpEG* may have involved the removal of these genes from a position between *hsIU* and *fpr*. In order to test this hypothesis we cloned and sequenced a 4.1-kb DNA fragment from *Buchnera* (Sg) that contained *hsIU*.

The methods used for obtaining endosymbiont DNA, restriction enzyme and Southern blot analyses and cloning into λZAP (Stratagene, LaJolla, CA) have been described in our past publications [8, 9]. Using the sequence of *hsIU* of *Buchnera* (Sc) (GenBank No. U09184), the following oligonucleotide primers were designed: *Bam*HI, 5′-GAC GGA TCC GCT ATC TAA ATG TTT ACT TAC ATA TTC TTC ATC G-3′ and *Eco*RI, 5′-GAC GAA TTC GTC ATA TTG CCG AAG CTG CTT GG-3′. Following amplification by the polymerase chain reaction a 0.19-kb DNA fragment was obtained, cloned into pBCKS (Stratagene), and used as a probe in restriction enzyme and Southern blot analyses. A 4.1-kb *Eco*RI DNA fragment was cloned into λZAP and its sequence determined (GenBank No. AF108665).

Figure 1 presents a genetic map of the 4.1-kb *Buchnera* (Sg) DNA fragment, and some of the properties of the genes are listed in Table 1. HsIU and Ibp are, in *E. coli*, heat-inducible proteins [6]. In this organism *ibp* is preceded by a σ^{32} promoter [6]. There is no evidence for a similar promoter upstream of *Buchnera* (Sg) *ibp*. This gene is, however, followed by inverted repeats (AATCAT-TGTACTT-10nt-AAGTACAATGATT) of unknown function. Fpr is a protein involved in catalyzing the reduction of ferredoxin and consequently has a role in anaerobic metabolism [3]. It is also a protein that is induced by superoxide-generating agents and may play a role in protection against oxidative stress [11]. KdtB is putative enzyme involved in the biosynthesis of lipopolysaccharide, which is a component of the outer membrane of *Buchnera*.

The position of *trpEG* in *Buchnera* (Sc), between *hsIU* and *fpr* and the proximity of these two genes in *Buchnera* (Sg) (Fig. 1) suggests that the origin of the *trpEG* plasmid involved the excision of these genes from their position between *hsIU* and *fpr* in an endosymbiont ancestor. The evolutionary divergence between the lineage leading to *Buchnera* (Sg) and that leading to *Buchnera* (Sc) and *Buchnera* (Mr) corresponds to the basal split in the phylogeny of aphids, about 100 to 200 million years ago [7]. The excision of *trpEG* from the ancestral location between *hsIU* and *fpr* must have occurred after this split, in an ancestor shared by *Buchnera* (Sg) and *Buchnera* of other members of the Aphididae, which also contain *trpEG* on a plasmid. [13, 14]. This interpretation is complicated by the presence of *ibp* between *hsIU* and *fpr* in *Buchnera* (Sg). This gene may be an addition following excision of *trpEG*, or it may have been inserted prior to or simultaneous with the excision. The possibility that *ibp* has some mobility is indicated by its presence on a *Buchnera* plasmid, totally different in organization from the *trp*-plasmids, which contains the genes for the biosynthesis of leucine [15].

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