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REPORTS

A Small Microbial Genome: The End of a Long Symbiotic Relationship?

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Intracellular bacteria are characterized by genome reduction. The 422,434–base pair genome of *Buchnera aphidicola* BCc, primary endosymbiont of the aphid *Cinara cedri*, is ~200 kilobases smaller than the previously sequenced *B. aphidicola* genomes. *B. aphidicola* BCc has lost most metabolic functions, including the ability to synthesize the essential amino acid tryptophan and riboflavin. In addition, most retained genes are evolving rapidly. Possibly, *B. aphidicola* BCc is losing its symbiotic capacity and is being complemented (and might be replaced) by the highly abundant coexisting secondary symbiont.

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Genome reduction in endosymbiotic bacteria is a continuous process derived from their adaptation to intracellular life. One unsolved question is whether reduction reaches a threshold, or if it is an ongoing process that inevitably leads to bacterial extinction and replacement by a new symbiont. Current debate centers on whether genomic streamlining is a result of deletion bias or natural selection, and has implications for the theory of genome complexity evolution (*1*). The obligate association between aphids and their maternally transmitted intracellular symbiont *Buchnera aphidicola* offers a model system to analyze genome reduction and its consequences. Genome sizes ranging from 450 to 641 kb have been reported in *B. aphidicola* strains from different aphid subfamilies, with the genome of *B. aphidicola* from the aphid *Cinara cedri* (*B. aphidicola* BCc) being the most dramatically reduced (*2*). A particular feature of *C. cedri* is the presence of large numbers of a secondary symbiont (*3*), "*Candidatus* Serratia symbiotica" (*S. symbiotica*) (*4*).

The genome comparison of three previously sequenced *B. aphidicola* strains (*5*–*7*) showed almost total conservation of genome architecture since their last

common symbiotic ancestor. Selective gene losses in the extant lineages appear to be mainly related to host-specific properties (6, 7).

The *B. aphidicola* BCc genome is composed of a 416,380–base pair (bp) circular chromosome plus a 6045-bp plasmid for leucine biosynthesis (tables S1 to S3) (8, 9). Gene loss, scattered along the chromosome (fig. S1), is the main cause of genome shrinkage, because there is no reduction in the sizes of intergenic regions and open reading frames. With only 362 protein-coding genes, this genome represents the minimal known gene set able to support cellular life.

Gene loss affects all functional categories (figs. S1 and S2; table S4), although not evenly. Genes necessary for RNA metabolism (transcription and translation) are the most preserved, representing 35% of the genome's coding capacity. The DNA replication machinery is also complete, but the repair machinery is further reduced than in other strains. Chaperone systems and all essential components for protein translocation are also well preserved, ensuring proper folding and positioning of membrane protein components. These include a highly simplified flagellar apparatus, composed only of those elements homologous to the type III virulence secretion system required for the invasion of the host cells (10).

Gene losses affecting biosynthesis of nucleotides, cofactors, cell envelope, and transport are particularly acute. Hence, *B. aphidicola* BCc depends entirely on its host for nucleotide and cofactor provisioning. In addition, and in contrast to what has been described in other strains (11), *B. aphidicola* BCc is clearly unable to provide riboflavin to its host. Finally, it lacks most of the transporters encoded by other *B. aphidicola* genomes. Because it has also lost all the genes for aminosugar and peptidoglycan biosynthesis, it appears that *B. aphidicola* BCc must be close to a free-diffusing cell, in which most metabolites can be passively exchanged through a highly simplified cell envelope.

The putative *B. aphidicola* BCc metabolism inferred from the extant genes (fig. S3) is reduced simply to using glucose to obtain energy through substrate-level phosphorylation, plus the production of saturated fatty acids and all the essential amino acids, except tryptophan. All genes encoding the adenosine 5'-triphosphate synthase subunits have been lost, indicating that the retained components of the electron transport chain must be involved in the regeneration of nicotinamide adenine dinucleotide for glycolysis and acetyl–coenzyme A biosynthesis. In the absence of all genes necessary for the biosynthesis of phospholipids, the preservation of the complete saturated fatty acid pathway indicates that *B. aphidicola* BCc, and *B. aphidicola* in general, probably provide them to the host.

Aphids, like other animals, require adequate quantities of 10 essential amino acids that are lacking in their diet and must be provided by their endosymbionts. *B. aphidicola* BCc has retained the biosynthetic capacity for all essential amino acids except tryptophan. The importance of tryptophan production by the endosymbiont has been experimentally demonstrated (12), and the close relative *B. aphidicola* BCt, endosymbiont of the aphid *Cinara*

tujafilina, possesses *trpE and trpG* (the two regulatory genes of the tryptophan pathway) on a plasmid (*9*). *C. cedri* and *C. tujafilina* are almost identical (*13*), and their plant hosts are also very similar. Yet, *B. aphidicola* BCt contains the genes for tryptophan biosynthesis whereas *B. aphidicola* BCc has lost the complete pathway, which suggests that *B. aphidicola* BCc is not only unable to provide tryptophan to its host, but must obtain it from another source. Although secondary symbionts are considered facultative in other aphids, *S. symbiotica* is present in all the *C. cedri* clones we have worked with. They are always contained within well-defined bacteriocytes, are present at a density similar to that of *B. aphidicola* (Fig. 1; fig. S5), are located in the central part of the bacteriome, and are surrounded by primary bacteriocytes (*3*). The polymerase chain reaction amplification and sequencing of a *trpE* fragment from *S. symbiotica* (*8*) indicate that this symbiont synthesizes tryptophan and supplies it to the whole symbiotic system.

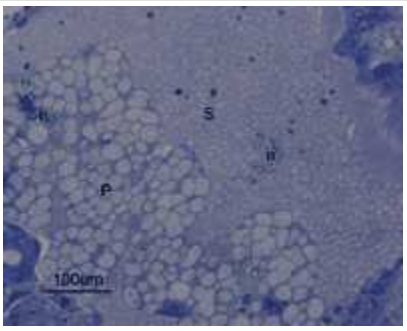

Fig. 1. Microscopic analysis of *C. cedri* bacteriocytes. Semi-thin section showing two types of bacteriocytes, identifiable by their different tonality with toluidin blue. P, primary symbiont (*B. aphidicola*); S, secondary symbiont (*S. symbiotica*); n, bacteriocyte nuclei. [View Larger Version of this Image (177K GIF file)]

The evolution of *B. aphidicola* BCc sequences appears to have been particularly rapid. In general, the ratio of synonymous to nonsynonymous substitutions, *dN*/*dS*, of *B. aphidicola* protein-coding genes is higher than those of free-living bacteria, owing to an accelerated rate of nonsynonymous substitution (*14*). This pattern is more marked in *B. aphidicola* BCc (*8*) (table S5). Tests of the relative accumulation of nucleotide substitutions performed for all possible *B. aphidicola* strain pairs (table S6) revealed that the *B. aphidicola* BCc branch accumulates a significantly higher number of substitutions in most of its genes (table S7). The genes with higher *dN*/*dS* ratios are not associated with any particular functional role (fig. S4). Finally, we analyzed the type of selection that operates on protein-coding genes in *B. aphidicola* (table S8). Most of the genes are under purifying selection (*dS* > *dN*), but about 12% of the genes are under neutral selection in *B. aphidicola* BCc (*dS* ≈ *dN*), as expected for pseudogenes.

Taking together all functional, evolutionary, and microscopic data, we postulate that *B. aphidicola* BCc is undergoing a process of genome degradation and functional replacement by the coexisting *S. symbiotica* (*3*). Natural symbiont replacement of *B. aphidicola* by a fungus was postulated to have occurred in aphids of the tribe Cerataphidini (*15*), whereas experimental evidence of secondary symbionts taking on the role of *B. aphidicola* has been

demonstrated in infection experiments of *B. aphidicola*–cured aphids (*16*). All the analyses performed point to a more extreme gene-degradation effect occurring in the *B. aphidicola* BCc genes than in other *B. aphidicola* lineages. Indeed, the loss of most DNA-protecting and DNA-repair mechanisms in *B. aphidicola* BCc, more so than in the other *B. aphidicola* lineages, would enhance the mutation rate. Further, *B. aphidicola* BCc has apparently lost its role as a tryptophan and riboflavin supplier to its host and indeed cannot even supply its own needs, which must be provided by *S. symbiotica*, not only to the host but also to *B. aphidicola* BCc. Thus, the mutualistic relationship between *B. aphidicola* and its aphid host seems to have taken on a new, more complex role that includes a second endosymbiont and might end up in a replacement.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/314/5797/312/DC1

Materials and Methods

SOM Text

Figs. S1 to S5

Tables S1 to S8

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