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Gene Organization of the *dnaA* Region of *Wolbachia*

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The *dnaA* region of *Wolbachia*, an intracellular bacterial parasite of insects, is unique. A *glnA* cognate was found upstream of the *dnaA* gene, while neither of the two open reading frames detected downstream of *dnaA* has any homologue in the database. This unusual gene arrangement may reflect requirements associated with the unique ecological niche this agent occupies.

Wolbachia is an obligatory intracellular and maternally inherited bacterium. It is widespread in insects (23) and is also known to infect other invertebrates (13). It is responsible for various reproductive alterations in the hosts it infects, such as cytoplasmic incompatibility, feminization of genetic males, and parthenogenesis (4, 13, 22). Although there is an appreciable and increasing amount of knowledge about the distribution, phylogeny, and population genetics of *Wolbachia*, little is known about its genomic organization.

In *Escherichia coli*, the DnaA protein is essential for initiation of bidirectional replication at the chromosomal origin of replication (8, 10, 21). The *dnaA* gene has been cloned from a number of eubacterial species, and the sequences are all highly conserved (12). Moreover, the gene arrangement of the *dnaA* region is also conserved, including (together with the *dnaA* gene) the *rpmH*, *rnpA*, *dnaN*, *recF*, and *gyrB* genes or a subset of these genes in close proximity (24). In this paper, we report the cloning and characterization of the *dnaA* chromosomal region of *Wolbachia* and show that this bacterium has a unique gene arrangement in this region.

The *Wolbachia*-infected *Drosophila simulans* Riverside (DSR) strain was used as a source of *Wolbachia* (9). A tetracycline-treated derivative strain of DSR was used as the *Wolbachia*-free control strain. Both strains were routinely grown on cornflour-sugar-yeast medium at 25°C. Unless otherwise mentioned, standard molecular methods were used (17). In previous work we have shown that at least part of the *Wolbachia dnaA* gene is located in a 3.3-kb *Eco*RI genomic fragment (3). After digestion with *Eco*RI, DNA from the DSR strain was size fractionated by electrophoresis, and DNA fragments of 3 to 4 kb in size were recovered and cloned into *Eco*RI-cut λZAP (Stratagene, La Jolla, Calif.). The previously described PCR-derived *dnaA* fragment (2, 3) was used as a probe for the detection of recombinants by plaque hybridization. The insert containing the partial *dnaA* fragment was excised from λZAP phagemids and was subcloned into a pBluescript vector. Both strands of the insert (3,324 bp) were sequenced by using the γδ transposon-facilitated DNA sequencing method (19). This *Eco*RI fragment did not contain the 5′ coding region of the *dnaA* gene which was cloned by the following method. Total DNA of DSR strain flies was digested with *Xba*I and ligated to similarly digested pBluescript. PCR was performed with the primer *dnaA* p1 (5′-GCT ATA GCA TGC ATT AGA TGT G-3′) and either the T3 or T7 primer, both of which recognize pBluescript. This was followed by nested PCR with the internal primer *dnaA* p2 (5′-GAA CCT TGG ATC CAG CGG CG-3′). The resulting PCR product of about 1.6 kb was cloned into pBluescript. *Pfu Taq* polymerase (Stratagene, Inc.) was used in all PCRs to maximize the sequence fidelity of the PCR product. Sequencing of both strands of this fragment was done at the Keck Sequencing Facility, Yale University. DNA and protein sequences were analyzed with the University of Wisconsin Genetics Computer Group programs (7).

The coding map of the *Wolbachia dnaA* region, which is present as a single copy in the genome (data not shown), is presented in Fig. 1. Three complete open reading frames (ORFs) and the start of a fourth one were found and analyzed by the BLAST program. The first ORF (Fig. 1) was identified as the *Wolbachia glnA* cognate, a gene which encodes the enzyme glutamine synthetase (GS), an enzyme responsible for ammonia assimilation and glutamine biosynthesis. There are two major families of GS: GS I (440 to 470 amino acids), which is present in most prokaryotes; and GS II (340 to 370 amino acids), which is present in eukaryotes and some prokaryotes (14). The *Wolbachia glnA* cognate is only 735 bp long. The deduced protein is much shorter than most of its homologues,

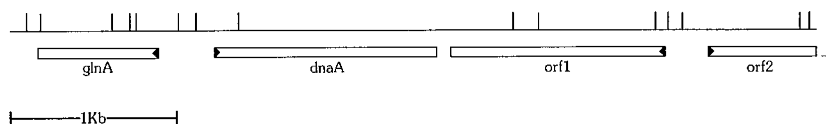

FIG. 1. Coding map of the *Wolbachia dnaA* region. The boxes represent ORFs. The thin vertical lines indicate the presence of putative DnaA boxes. The entire region (4,838 bp) was sequenced (for details see text).

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TABLE 1. The products of the *dnaA* region of *Wolbachia* and the identities to their closest relatives

Wolbachia gene	Product	Nearest relative(s)	Accession no.	% Identity
glnA	Putative GS	*R. prowazekii* hypothetical protein	AJ235272	38.2
		E. coli putative GS	AE000228	34.9
		E. coli putative GS	D90767	36.0
		E. coli GS (GlnA)	AE000462	26.9
dnaA	DnaA protein	*R. prowazekii* DnaA protein	Q59758	58.7
orf1		None		
orf2		None		

with a molecular weight (MW) of 30 and predicted isoelectric point of approximately 6.7. Sequence analysis indicated that the putative *Wolbachia* GlnA protein is a member of the GS I family (data not shown) and is closely related to a hypothetical protein of *Rickettsia prowazekii* and two *E. coli* GlnA cognates as determined based on analysis made by the MegAlign program of the DNASTAR software (Table 1).

The next ORF encodes the *Wolbachia dnaA* homologue, which contains 454 amino acids with an MW of 52 and a calculated isoelectric point of 8.6. It is very similar to other bacterial *dnaA* genes, being most closely related to the *R. prowazekii* homologue (Table 1). This similarity is most pronounced in the ATP-binding domain and the carboxy-terminal region, which includes the DNA binding domain (12). Neither of the two ORFs (*orf1* and *orf2*) found downstream of the *dnaA* gene has any obvious homologue in the databases. In addition, the entire *dnaA* region contains 15 potential DnaA boxes with 100% homology to the degenerate consensus sequence (YYHTMCRHM) (18), approximately three times the number of boxes expected by chance.

The gene arrangement in the *Wolbachia dnaA* region, *glnA-dnaA-orf1-orf2*, has not been observed in any other known bacterial genome (1, 6, 11, 15, 16, 20, 25). In most of the various bacteria studied so far, the *dnaA* gene is present in a quite conserved gene cluster while exceptions can be explained by small chromosomal rearrangements in this region. It has been suggested that this conserved gene arrangement is of ancestral origin and evolved more than one billion years ago (24). However, several diverse bacteria do not follow this rule. The exceptions identified so far are as follows: (i) the hyperthermophilic bacterium *Aquifex aeolicus*, which belongs to the family *Aquificaceae*, the most deeply branching family of bacteria (5); (ii) two marine cyanobacteria, *Prochlorococcus marinus* and *Synechocystis* sp., which have a light-dependent cell cycle (15, 16); (iii) the gastric pathogen *Helicobacter pylori* (20); and (iv) four members of the α subdivision of the *Proteobacteria*, namely, *Caulobacter crescentus*, which divides asymmetrically (25), *Rhizobium meliloti*, which can differentiate from a free-living to a symbiotic nitrogen-fixing bacterium within the root nodules of alfalfa, a change which is accompanied by a cessation of DNA replication and cell division (11), and *R. prowazekii* (1) and *Wolbachia*, both intracellular bacteria. It is likely that the unique arrangements in each of these bacteria may reflect adaptive changes associated with a unique mode of regulation of the *dnaA* gene and DNA replication, which in turn might reflect their unique physiology and the environmental niches they occupy. It is notable that the *dnaA* regions of *Wolbachia* and its closest known relative, *Rickettsia*, have different organizations, suggesting that such adaptive changes may have arisen independently in the corresponding lineages.

Nucleotide sequence accession number. The nucleotide sequence (4,838 bp) reported in the present study has been deposited in the EMBL database under the accession no. AJ012073.

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