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Genes Coding for Integration Host Factor Are Conserved in Gram-Negative Bacteria

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A genetic system for the selection of clones coding for integration host factor and HU homologs is described. We demonstrate that the *himA* and *hip* genes of *Serratia marcescens* and *Aeromonas proteolytica* can substitute for the *Escherichia coli* genes in a variety of biological assays. We find that the sequence and genetic organization of the *himA* and *hip* genes of *S. marcescens* are highly conserved.

Integration host factor (IHF) and HU are similar, small, heterodimeric DNA binding proteins that have an important role in the formation of higher-order DNA structures and in gene regulation (2, 5, 19, 21). IHF has been studied extensively in *Escherichia coli*, and the genes *himA* and *hip*, coding for IHF, were isolated in this organism (4, 14, 16). Mutations in either of these genes can render the bacteria IHF$^-$. Recent reports have implicated IHF in the positive control of *E. coli* phage promoters (6, 12, 20), of the *nif* genes of *Klebsiella pneumoniae*, and of the flagellar genes in *Caulobacter crescentus* (7, 10). However, there is no direct proof for the presence of IHF genes in other organisms except for *E. coli*, *Salmonella typhimurium*, and *Haemophilus influenzae* (11, 13).

Here, we describe an experimental system for the isolation and characterization of genes coding for IHF-like proteins from different organisms. The method is based on our finding that phage λ is unable to grow on strains deficient in both HU (defective in *hupA* and *hupB* genes) and IHF (15). Our studies have suggested that either of these proteins is required to establish the higher-order DNA-protein structure, at the phage *cos* site, that is required for packaging. HU or IHF subunits supplied by a transducing λ phage at the time of infection permit phage development in mutant cells that are unable to synthesize that subunit. In the present work we used this system to isolate the *himA* and *hip* equivalent genes from two gram-negative organisms: *Serratia marcescens*, an opportunistic pathogen, and *Aeromonas proteolytica*, found in seawater. We demonstrate that these genes can substitute for the IHF of *E. coli* in a variety of biological assays.

λ DNA libraries of *S. marcescens* and *A. proteolytica* in the λD69 vector (17) were plated on lawns of *E. coli hupA hupB himA* or *hupA hupB hip* strains. Plaque-forming phage clones were purified and tested for their ability to grow on various tester strains. The results (Table 1) show that phages AO1086 and AO1085 provide a *himA*-like function whereas clones AO1060 and AO1084 express a *hip*-like function. We have also encountered phage clones that were able to grow on all three strains; these presumably express the HU function and were not further investigated.

The four IHF-complementing genes were then tested for their ability to support phage λ mutants and phage Mu that require IHF for growth. As seen in Table 2, the *S. marcescens himA*- and *hip*-like clones were able to complement the corresponding missing *E. coli* IHF subunits. Similar results were obtained with the *A. proteolytica* cloned genes (not shown).

We also showed that the cloned *himA* and *hip* genes complemented the *E. coli himA* and *hip* mutants in a test that measures λ prophage excision (3). In this assay a defective λ prophage is excised from within the *galT* gene to regenerate a functional *gal* operon. We used strain A6284, which was derived from strain RW842 (3) and carries the *gal-842* insert, the *hip*::Cm^r mutation, and a plasmid supplying the λ Int function (it was necessary to supply Int since the *Bam*HI cloning site of λD69 lies within the phage *int* gene) (9). We found that clones AO1060 and AO1084, carrying the *hip*-like genes (Table 1), were able to support λ site specific recombination (not shown). In a similar way we used strains A6969 and A6961, both *himA82* derivatives of RW842, that were made lysogenic for phages λ AO1086 and λ AO1085 carrying the *himA*-like genes (Table 1). We found that these strains were able to support site-specific excision following infection by wild-type λ phage.

Since IHF is a heterodimeric protein, the cloned gene product must specifically interact with the second subunit supplied by the host to produce chimeric IHF molecules. IHF acts by binding to specific DNA sites which share a consensus sequence (1, 8). It is possible that in the chimeric

TABLE 1. Identification of phage clones carrying the putative *himA* and *hip* genes from *S. marcescens* and *A. proteolytica*

Phage clone	Efficiency of plating with bacterial host[a]			Putative cloned gene
	himA	*hip*	*himA hip*	
S. marcescens				
AO1086	0.4	$<10^{-4}$	$<10^{-4}$	*himA*-like
AO1060	$<10^{-4}$	0.3	$<10^{-4}$	*hip*-like
A. proteolytica				
AO1085	0.3	$<10^{-4}$	$<10^{-4}$	*himA*-like
AO1084	$<10^{-4}$	1	$<10^{-4}$	*hip*-like

[a] All strains are derivatives of N99 and carry mutations in the *hupA* and *hupB* genes. The strains used as bacterial lawns were A5179, A5427, and A5477 (15). The values represent efficiencies of plating of phage clones relative to their plating on the parental N99 strain on tryptone agar plates at 30°C.

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TABLE 2. Function of *S. marcescens himA*- and *hip*-like genes as IHF in *E. coli*

Bacterial strain	Relevant host genotype		Complementary cloned genes		Growth of test phages[a]			
	himA	*hip*	*himA*	*hip*	λ	λc17	λcos154	Mu
A6614	−	+			+	−	−	−
A6617	−	+	+[b]		+	+	+	+
A6351	−	+	+[c]		+	+	+	+
A6284	+	−			+	−	−	−
A6324	+	−		+[b]	+	+	+	+
A6246	+	−		+[c]	+	+	+	+
A5445	−	−			+	−	−	−
A6694	−	−	+[c]		+	−	−	−
A6690	−	−		+[b]	+	−	−	−
A6691	−	−	+[c]	+[b]	+	+	+	+

[a] Serial dilutions of the test phages were spotted on the bacterial lawns and incubated at 37°C. +, plaque formation; −, restricted phage growth (efficiency of plating of less than 10^{-3}).

[b] The complementing *S. marcescens himA* on *hip* genes were introduced into the *E. coli* strains by lysogenization with phages AO1086 and AO1060, respectively.

[c] The complementing *S. marcescens* genes were introduced by transfection with plasmids pShimΔV and pShip derived from AO1086 and AO1060, respectively (see legends to Fig. 1 and 2).

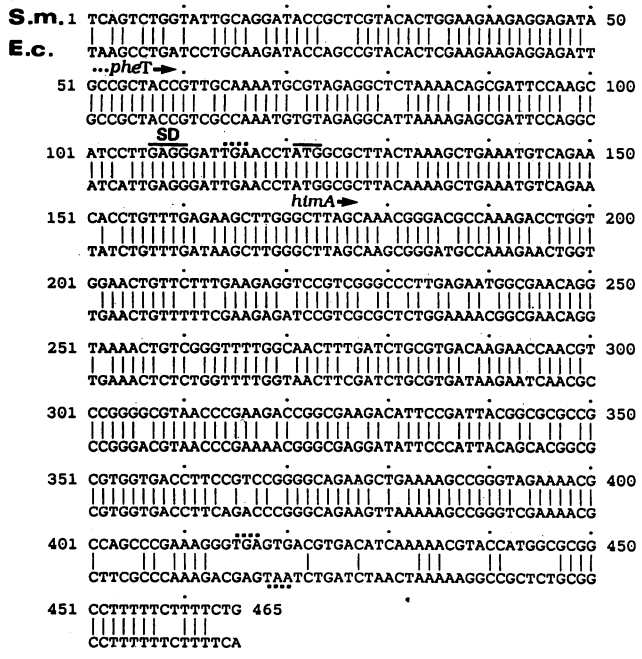

FIG. 1. Comparison of the *himA* nucleotide sequences of *S. marcescens* and *E. coli*. A 3-kbp *Bam*HI fragment of phage AO1086 was cloned into the *Bam*HI site of pBR322, yielding plasmid pShim. Two subclones of pShim were prepared: pShimΔV, containing a 700-bp *Eco*RV-*Bam*HI insert, able to complement for *himA* deficiency (Table 2); and pShimΔH, containing a 500-bp *Hin*dIII-*Bam*HI fragment with the same orientation, which has lost the ability to complement for *himA*. Sequencing was performed by the Sanger dideoxy chain termination method (18) directly on both plasmids. The top line (S.m.) shows the sequence of the *S. marcescens himA*-like clone, and the line below (E.c.) shows the sequence of the *E. coli himA* gene (16). The AUG initiation codon and the Shine-Dalgarno sequence (SD) of *himA* are shown by a solid bar; the stop codons of the two *himA* genes and of the upstream *pheT* gene are shown by a dotted line. The designated GenBank accession number of the *S. marcescens himA* gene is M62644.

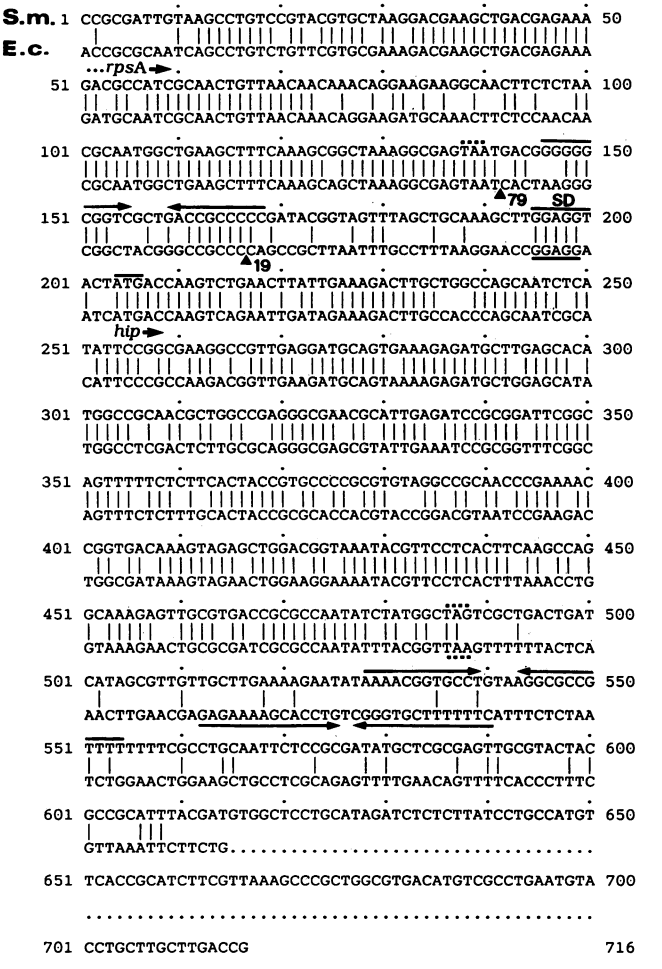

FIG. 2. Comparison of the *hip* nucleotide sequences of *S. marcescens* and *E. coli*. A 3.3-kbp *Hin*dIII fragment of phage AO1060 was cloned into the *Hin*dIII site of pGem3Zf(+) (Promega), producing plasmid pShip. The *Hin*dIII subcloned fragment contains only the putative *hip* structural gene, without the upstream sequences. To sequence the upstream region, we performed a polymerase chain reaction on phage AO1086 and used the polymerase chain reaction product for sequencing. The top line (S.m.) shows the sequence of the *S. marcescens hip*-like clone, and the line below (E.c.) shows the sequence of the *E. coli hip* gene (4). The AUG initiation codon and the Shine-Dalgarno sequence (SD) of *hip* are shown by a solid bar; the stop codons of the two *hip* genes and of the upstream *rpsA* gene are shown by a dotted line. Triangles denote a 79-bp region and a 19-bp region absent from the *S. marcescens* clone which were placed to achieve maximal alignment. The arrows represent inverted repeats. The GenBank accession number of *S. marcescens hip* is M62643.

IHF heterodimers, only the *E. coli* subunit is providing the DNA binding specificity. To test whether the putative IHF of *S. marcescens* can substitute for both *E. coli* IHF subunits, we constructed an *E. coli* strain (A6691) in which both IHF genes were replaced by the *S. marcescens* IHF-like genes. Table 2 shows that the *S. marcescens* IHF can function effectively in *E. coli*.

The DNA segments coding for the *himA* and *hip* genes of *S. marcescens* were subcloned and sequenced. The *himA* gene and the upstream sequence show high similarity to the

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HimA-E.c.  MALTKAEMSEYLFDKLG.LSKRDAKELVELFFEEIRRALENGEQVKLSGFGNFDLRDKNQRPGRNPKTGEDIPITARRVVTFRPGQKLKSRVENASPKDE
HimA-S.m.  ----------H--E---.-------D--------V-----------------------------------------------------------G

Hip-E.c.     MTKSELIERLATQQSHIPAKTVEDAVKEMLEHMASTLAQGERIEIRGFGSFSLHYRAPRTGRNPKTGDKVELEGKYVPHFKPGKELRDRANIYG
Hip-S.m.     ----------G--------A-------------A---E--------------------V-----------D---------------------
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FIG. 3. Comparison of the protein sequences, derived from the DNA sequences, of HimA and Hip of *S. marcescens* and *E. coli*. The amino acids that differ between the IHF subunits of *E. coli* (HimA-E.c., Hip-E.c.) and *S. marcescens* (HimA-S.m., Hip-S.m.) are shown. One amino acid interval (.) was inserted into HimA to achieve best alignment with Hip. Sequencing both strands of the *E. coli hip* gene, using dGTP or dITP, revealed unequivocally the presence of an additional GCC trinucleotide coding for alanine at position 90.

E. coli himA gene (Fig. 1). As in *E. coli*, the *himA* gene of *S. marcescens* is located downstream of the *pheT* gene, which terminates where *himA* translation is initiated. This arrangement may indicate that the expression of *himA* genes is coupled to the expression of *pheT*. The DNA sequences following the *himA* genes are highly divergent.

The *hip* gene of *S. marcescens* is also very similar in sequence to the *E. coli hip* gene and is similarly located downstream of the *rpsA* gene, although the distance between the *rpsA* and *hip* genes in *S. marcescens* is shorter by 98 bp (Fig. 2). This 98-bp deletion is not continuous; it is interrupted by a conserved inverted repeat sequence. The sequences downstream of the *hip* genes are also highly divergent; however, both contain a putative *rho* independent transcription terminator. Thus, in both organisms the genes coding for the IHF subunits are situated downstream of genes whose products play an important role in protein synthesis, suggesting the presence of a regulatory circuit controlling their expression.

The protein sequences of the two cloned genes are highly conserved (Fig. 3): the HimA protein of *S. marcescens* differs by five amino acids from and is shorter by one residue than its *E. coli* homolog; Hip differs by six amino acids. Eight of the eleven substitutions are conservative amino acid changes. Note that the Hip protein of *E. coli* contains an extra alanine residue (A90) that was previously overlooked (4).

The two bacteria studied here, *S. marcescens* and *A. proteolytica*, express a large number of genes that are not present in *E. coli*. For example, both bacteria are able to degrade chitin and secrete a number of hydrolytic enzymes to the surrounding medium. The results presented here suggest that IHF genes are probably widespread and are evolutionarily conserved in gram-negative bacteria. It would be interesting to find out whether IHF-like genes also play a role in lower and higher eukaryotes.

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