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

HADARA HALUZI, DAVID GOITEIN, SIMI KOBY, ITAI MENDELSON, DINAH TEFF, GALINA MENGERITSKY, HILLA GILADI, AND AMOS B. OPPENHEIM

Department of Molecular Genetics, The Hebrew University-Hadassah
Medical School, Jerusalem 91010, Israel

Received 11 April 1991/Accepted 20 July 1991

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 λD9 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' mutation, and a plasmid supplying the λ Int function (it was necessary to supply Int since the BamHI 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

<table>
<thead>
<tr>
<th>Phage clone</th>
<th>Efficiency of plating with bacterial host</th>
<th>Putative cloned gene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>himA</td>
<td>hip</td>
</tr>
<tr>
<td>S. marcescens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AO1086</td>
<td>0.4</td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>AO1060</td>
<td>&lt;10^-4</td>
<td>0.3</td>
</tr>
<tr>
<td>A. proteolytica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AO1085</td>
<td>0.3</td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>AO1084</td>
<td>&lt;10^-4</td>
<td>1</td>
</tr>
</tbody>
</table>

* 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.

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

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Relevant host genotype</th>
<th>Complementary cloned genes</th>
<th>Growth of test phages$^a$</th>
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<tbody>
<tr>
<td></td>
<td>himA</td>
<td>hip</td>
<td>himA</td>
</tr>
<tr>
<td>A6614</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>A6617</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A6351</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>A6264</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>A6324</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>A6264</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>A5445</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A6604</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A6609</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$ 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$^{-3}$).

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

The complementing *S. marcescens* genes were introduced by transfection with plasmids pShim$^\Delta$ and pShip derived from AO1086 and AO1060, respectively (see legends to Fig. 1 and 2).

![Fig. 1](http://jb.asm.org/) Comparison of the himA nucleotide sequences of *S. marcescens* and *E. coli*. A 3-kbp BamHI fragment of phage AO1086 was cloned into the BamHI site of pBR322, yielding plasmid pShim. Two subclones of pShim were prepared: pShim$^\Delta$, containing a 700-bp EcoRV-BamHI insert, able to complement *himA* deficiency (Table 2); and pShip$m^\Delta$, containing a 500-bp HindIII-BamHI 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](http://jb.asm.org/) FIG. 2. Comparison of the *hip* nucleotide sequences of *S. marcescens* and *E. coli*. A 3.3-kbp HindIII fragment of phage AO1060 was cloned into the HindIII site of pGem3Zf(+) (Promega), producing plasmid pShip. The HindIII 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 39-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.

IFH heterodimers, only the *E. coli* subunit is providing the DNA binding specificity. To test whether the putative IFH of *S. marcescens* can substitute for both *E. coli* IFH subunits, we constructed an *E. coli* strain (A6691) in which both IFH genes were replaced by the *S. marcescens* IFH-like genes. Table 2 shows that the *S. marcescens* IFH can function effectively in *E. coli*. The DNA segments coding for the himA and *hip* genes of *S. marcescens* were subcloned and sequenced. The *hip* gene and the upstream sequence show high similarity to the
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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