

Isolation and characterization of the *hupA* gene coding for HU of *Aeromonas proteolytica*

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Submitted July 2, 1992 GenBank accession no. M96574

Hu and integration host factor (IHF) are related, small, dimeric DNA-binding proteins, that play an important role in the formation of higher-order DNA-protein structures. Hu is found in bacteria in great abundance as a homodimeric protein, and is highly conserved in prokaryotes. However, in *Escerichia coli* and *Salmonella typhymurium*, HU consists of two subunits, HU-1 and HU-2, encoded by the *hupA* and *hupB* genes respectively. The main role of HU is believed to be in the condensation of the bacterial chromosome into its tightly compact conformation (see review by Drlica and Rouviere-Yaniv (1)).

IHF is conserved among gram negative bacteria (2) and is composed of two subunits encoded by two separate genes, *himA* and *hip* (see review by Friedman (3)). Mutation in one subunit of IHF is sufficient to render the cell IHF$^-$ whereas, for an HU$^-$ phenotype, the genes coding for both subunits have to be mutated.

We report here the isolation and characterization of the gene coding for HU of the gram negative bacterium *Aeromonas proteolytica*. The gene was isolated according to the system previously described (2), which exploits the finding that phage λ is unable to grow on *E.coli* strains deficient in both HU and IHF (4). An HU subunit supplied by the incoming phage, permits phage development.

A DNA library of *A.proteolytica* was constructed in the phage vector λD69 and screened on the *E.coli hupAhupBhimA* strain. Plaque-forming phage clones were isolated and tested further for their ability to grow on the isogenic *E.coli hupAhupBhip*, and *E.coli hupAhupBhimAhip* strains. Phage clones able to grow on all three mutant strains were considered as candidates for carrying the *A.proteolytica* HU-like gene. The DNA from one clone, AO1089, was subcloned into the plasmid vector pEMBL18. The plasmid clones were then tested for their ability to complement the growth of phage Mu in an *E.coli hupAhupB* strain. Plasmid clone pW4H, containing a 2.7 kb insert, was positive for Mu growth in the tester strain. In addition, at 30°C this clone imparted a mucoid appearance to these HU$^-$ cells, as did a control plasmid carrying the *hupA* gene of *E.coli*. Both, the control plasmid and pW4H, were able to complement *E.coli hupAhupBhimA* for growth at 42°C (4). These results indicate that the *A.proteolytica* HU can complement for HU of *E.coli*.

The DNA sequence reported here showed 78% and 67% homology to the *hupA* and *hupB* genes of *E.coli*, respectively. Sequences outside the region of the structural gene showed no significant homology to any *E.coli* sequences or to any published sequence. On the protein level, the *A.proteolytica* HU exhibited 82% homology to HU-2 and only 67% homology to HU-1 (see Figure).

Southern blot analysis of *A.proteolytica* DNA, probed with a 250 bp fragment containing the sequence coding for the first 55 amino acids and 31 bp upstream (isolated from pW4H), revealed only one band, suggesting that in *A.proteolytica*, HU is encoded by a single gene.

REFERENCES

1. Drlica,K., and Rouviere-Yaniv,J. (1987) *Microbiol. Rev.* **51**, 301-319.
2. Haluzi,H., Goitein,D., Koby,S., Mendelson,I., Teff,D., Mengeritsky,G., Giladi,H. and Oppenheim,A.B. (1991) *J. Bacteriol.* **173**, 6297-6299.
3. Friedman,D.I. (1988) *Cell* **55**, 545-554.
4. Mendelson,I., Gottesman,M. and Oppenheim,A.B. (1991) *J. Bacteriol.* **173**, 1670-1676.

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A.p.HU     MNKTQLIDFIAEKADLSKAQAKAALEATLDGVTDALKEGDQVQLIGF
E.c.HU-2   --------V-----E---T-------S--AAI-ES-----A---V--
E.c.HU-1   V--S----K--AG--I---A-GR--D-IIAS--ES-----D-A-V--

A.p.HU     GTFKVNHRAARTGRNPKTGAEIQIAAANVPAFVAGKALKDAVK*
E.c.HU-2   ---------E------Q--K--K----------S---------*
E.c.HU-1   ---A-KE---------Q--K--T----K--S-R---------N*
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Figure 1. Comparison of *A.proteolytica* HU protein sequence (A.p.HU) with the *E.coli* subunits of HU (E.c.HU-1; E.c.HU-2).