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# Chromosomal Map Position of Genes Encoding P Adhesins in Uropathogenic *Escherichia coli*

R. HULL,[1]* S. BIELER,[1] S. FALKOW,[2] AND S. HULL[1]

*Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030,[1] and Department of Medical Microbiology, Stanford University School of Medicine, Stanford, California 94305[2]*

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***Escherichia coli* isolates from upper urinary tract infections frequently express adherence to human uroepithelium and D-mannose-resistant hemagglutination of human erythrocytes. Such adherence is usually associated with P pili encoded by the *pap* operon(s). In this paper, we report approximate chromosomal map positions for two copies of the *pap* operon. Only one copy expressed an adhesin capable of D-mannose-resistant hemagglutination, although both expressed P-related antigen.**

The essential nature of bacterial adherence in the infection process was first clearly demonstrated by H. W. Smith (17). Since then, considerable progress has been made toward understanding the genetic and biochemical basis for specific adherence of bacteria to mammalian epithelial cells (2, 7).

Adherence to human uroepithelial cells and D-mannose-resistant hemagglutination (MRHA) of human erythrocytes are common features among *Escherichia coli* from upper urinary tract infections (13, 20). In a majority of *E. coli* urinary isolates, both of these adherence properties are mediated by bacterial cell surface antigens called P-type pili which bind to gal-gal-containing epithelial cell receptors and are genetically related to the *pap* operon (4, 8, 10). Uroepithelial cell adherence is required for colonization of the mouse urinary tract by *E. coli*, as demonstrated by using a BALB/c mouse model for ascending pyelonephritis, and is complemented in most strains by a second adherence antigen encoded by the *pil* operon (3, 9). Urinary colonization is blocked when receptor analogs or homologous antiadhesin antisera are used to prevent specific adherence (14, 19; J. Roberts, S. B. Svenson, G. Kallenius, T. Korhonen, B. Kaack, and J. Winberg, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, B5, p. 24).

In addition to their role in adherence, the gal-gal-adherent surface peptides may also have a role in evading the host immune system. Svanborg Edén et al. (18) have shown that bacteria bearing the P-type adhesin are less efficiently phagocytized than are homogenic nonadherent derivatives.

Genes encoding P adherence reside on the chromosome and may be present in more than one copy. In a recent study, Hull et al. (S. Bieler, S. Hull, C. Svanborg-Edén, and R. Hull, manuscript in preparation) found that 25% of *E. coli* strains from urinary tract infections possessed multiple copies of the *pap* operon. We describe here a genetic analysis of *pap* in a urinary *E. coli* strain containing two copies of the operon. The approximate position of each operon copy was determined, and the ability of each to independently express a hemagglutinin or cross-reactive antigen was examined.

**Genetic linkage of MRHA to auxotrophic loci in *E. coli* SH14.** *E. coli* SH14 is a multiply auxotrophic derivative of the urinary strain SH1. It is hemolytic (Hly$^+$) and expresses both MRHA and D-mannose-sensitive hemagglutination. Relevant properties of *E. coli* SH1 and various derivatives are listed in Table 1. The following experiments were done to determine whether genes required for MRHA were located near any of the auxotrophic mutations present as genetic markers in *E. coli* SH14. SH14 was used as a recipient in conjugational crosses with several *E. coli* K-12 Hfr strains. The origin and direction of transfer of the Hfr donors used are depicted in Fig. 1. Recombinants were selected for inheritance of genes required for leucine (*leu*), histidine (*his*), thymidine (*thy*), or isoleucine-valine (*ilv*) metabolism and tested for coinheritance of the donor hemolysin (Hly$^-$) or hemagglutination (MRHA$^-$) phenotype. The procedures used for detecting hemolysin expression and hemagglutination are described elsewhere (4, 6). The results are shown in Table 2. Two Hfr donors, PK191 and KL228, transferred the donor adherence phenotype, MRHA$^-$, to the recipient. For matings with Hfr PK191, MRHA$^-$ was inherited at low frequency when *ilv*$^+$ was selected but at slightly higher frequency when *thy* was selected. These data suggested that the genes for MRHA were near *thy*. For matings with Hfr KL228, half of the *thy*$^+$ recombinants inherited the donor MRHA phenotype. These data are consistent with a map position for MRHA near *thy* and between *thy* and the KL228 origin of transfer.

To confirm this position, a *serA*$^-$ derivative (HU752) of *E. coli* SH1 was prepared and used as a recipient in a mating with Hfr KL228. The donor MRHA phenotype was inherited by 76% of the *serA*$^+$ recombinants, suggesting that MRHA is linked to *serA* in *E. coli* SH1.

Hfr strains KL14 and KL16, which transfer the *serA* region only as a terminal marker, did not transfer the MRHA region.

TABLE 1. Strains used

| Strain | Relevant properties | Reference |
|---|---|---|
| SH1 | *E. coli* J96[a] *lac* Hly$^+$ MRHA$^+$ | 6 |
| SH14 | SH1 *leu his thy rpsL ilv* Hly$^+$ MRHA$^+$ | 6 |
| HU752 | SH1 *serA* MRHA$^+$ | This paper |
| HU784 | HU752 *serA*$^+$ MRHA$^-$ | This paper |
| HU786 | HU752 *serA*$^+$ MRHA$^+$ | This paper |
| HU779 | SH14 *thy*$^+$ MRHA$^-$ | This paper |
| HU788 | SH14 *thy*$^+$ MRHA$^-$ | This paper |
| HU780 | SH14 Hly$^-$ MRHA$^-$ | This paper |
| HU781 | SH14 Hly$^-$ MRHA$^+$ | This paper |
| HU849 | *E. coli* K-12(pRHU845) | 4 |

[a] Isolated from an upper urinary tract infection.

* Corresponding author.

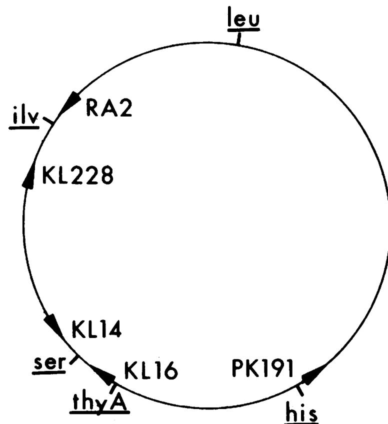


FIG. 1. Origin and direction of transfer of *E. coli* K-12 Hfr strains used (12).

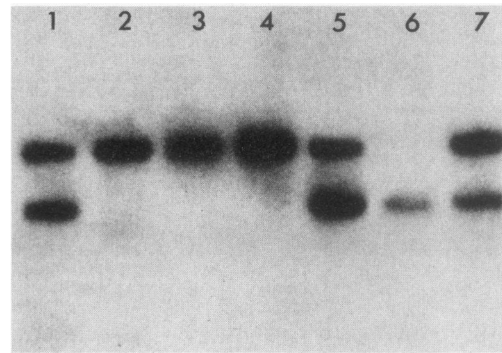


FIG. 2. Autoradiograph of blot of restriction endonuclease *Eco*RI-digested whole-cell DNA hybridized with the *pap*-specific pRHU845 *Hin*dIII C probe. Chromosome digests: 1, SH1; 2, HU779; 3, HU788; 4, HU784; 5, HU786; 6, HU780; 7, HU781.

**Association of MRHA and Hly.** In many of the Hfr crosses, the inheritance of Hly was also recorded. Previous studies have shown that the *hly* genes of *E. coli* SH1 are linked to *ilv* (6). In crosses where the MRHA phenotype was transferred at high frequency, Hly was transferred at reduced frequency or not at all. For example, none of the *E. coli* SH14 $thy^+$ recombinants from the Hfr KL228 cross received the donor $Hly^-$ phenotype. When cotransfer of Hly and MRHA could be measured, as among the $ilv^+$ recombinants from the Hfr PK191 cross with *E. coli* SH14, the two phenotypes were not linked; only 6 of 21 $ilv^+$ $Hly^-$ recombinants also inherited the donor MRHA region. These results show that genes necessary for expression of hemolysin and MRHA are not closely linked in *E. coli* SH14.

**Hybridization analysis.** Previous hybridization studies have shown that there are two copies of the *pap* operon in *E. coli* SH1 (5). The following experiments were done to determine if one or both copies had been deleted in the various recombinant classes described above. DNA was purified from representative isolates and digested to completion with endonuclease *Eco*RI. This enzyme does not cut within either *pap* operon copy. The DNA was transferred to nitrocellulose and hybridized with a $^{32}P$-radiolabeled pRHU845 *Hin*dIII C probe to detect fragments with *pap* homologous sequences as described previously (4). This probe consists of a 4-kilobase (kb) DNA fragment, derived from the recombinant cosmid pRHU807, which is contained entirely within the *pap* operon and is specific for *pap* (4). The results are shown in Fig. 2 and Table 3. Strains HU784 ($MRHA^-$) and HU786 ($MRHA^+$) are $serA^+$ recombinants from the mating of Hfr KL228 with HU752; the $MRHA^-$ recombinant has lost the 15.5-kb *pap* copy, while the $MRHA^+$ recombinant retained both copies. Strains HU779 and HU788 are *E. coli* SH14 $thy^+$ recombinants from matings with Hfr PK191 and Hfr KL228, respectively. These strains have also lost the 15.5-kb *pap* fragment and are $MRHA^-$. These results show that genes necessary for expression of a functional adhesin reside on a 15.5-kb *Eco*RI fragment and that this fragment is in the *serA* region of the chromosome.

Molecular studies have shown that adherence genes may be adjacent to genes for hemolysin expression in certain *E. coli* strains (11). We therefore screened SH14 $Hly^-$ recombinants to determine whether one of the *pap*-hybridizing *Eco*RI fragments had also been deleted. Strains HU780 and HU781 are recombinants from a mating with Hfr KL14 which have received the donor $Hly^-$ region. Results from hybridization studies with these strains show that HU780 has lost the 19.5-kb *pap* fragment. Both strains are $MRHA^+$. These results indicate that the 19.5-kb copy of the *pap* operon is linked to the *hly* genes in *E. coli* SH1, but that the *pap* genes on this operon copy are not required for expression of a D-mannose-resistant hemagglutinin.

**Expression of *pap* antigen.** Each of the strains from Fig. 2

TABLE 2. Hfr matings with *E. coli* SH1 derivatives

| Donor | Selected marker[a] | No. tested | % of recombinants inheriting donor phenotype | | |
|---|---|---|---|---|---|
| | | | MRHA | Hly | MRHA and Hly |
| PK191 | *his* | 48 | 2 | 6 | 0 |
| | *thy* | 48 | 33 | 0 | 0 |
| | *ilv*[b] | 48 | 23 | 44 | 13 |
| KL16 | *his* | 48 | 0 | | |
| | *thy* | 48 | 0 | | |
| KL14 | *leu*[b] | 46 | 0 | 20 | 0 |
| | *ilv*[b] | 48 | 0 | 38 | 0 |
| KL228 | *his* | 48 | 10 | 2 | 2 |
| | *thy* | 144 | 53 | 0 | 0 |
| | *serA*[c] | 96 | 76 | 0 | 0 |
| RA2 | *leu* | 64 | 0 | 14 | 0 |

[a] Matings were conducted for 120 min at 37°C as described elsewhere (6). The recipient was SH14, and donors were counterselected with streptomycin except as noted.
[b] Data from Hull et al. (6).
[c] The recipient used was HU752. Donors were counterselected by growth in the absence of methionine.

TABLE 3. Properties of recombinant strains

| Strain | *pap* fragment size (kb)[a] | MRHA phenotype | *pap* antigen |
|---|---|---|---|
| SH1 | 19.5, 15.5 | + | + |
| HU779 | 19.5 | − | + |
| HU788 | 19.5 | − | + |
| HU784 | 19.5 | − | + |
| HU786 | 19.5, 15.5 | + | + |
| HU780 | 15.5 | + | + |
| HU781 | 19.5, 15.5 | + | + |

[a] *pap*-related restriction endonuclease *Eco*RI fragments were identified with the pRHU845 *Hin*dIII C probe (4).
[b] Isolates were tested by slide agglutination with antiserum specific for the adhesin encoded by pRHU845.

was tested for agglutination by antibody specific for the surface antigens encoded on the *pap* recombinant plasmid pRHU845. Antiserum specific for the P pili purified from strain HU849 was prepared as described previously (4). All of the strains tested agglutinated in the presence of *pap* antiserum (Table 3).

The following conclusions may be drawn from this study. Genes necessary for MRHA are linked to *serA* in *E. coli* SH1. These genes are located on a 15.5-kb *Eco*RI fragment and have been cloned into the recombinant plasmid pRHU845 (4). This *pap* operon copy when present alone in *E. coli* SH1 is sufficient for bacterial adherence. A second copy of *pap* is located near the *hly* operon of *E. coli* SH1. This operon is not required for expression of MRHA and in itself is insufficient for expression of a functional hemagglutinin. However, when this operon was cloned into the plasmid vector pHC79, it produced an adhesin which bound to gal-gal receptors (11). This copy of *pap* resides on a 19.5-kb *Eco*RI fragment. Although functional adherence could not be demonstrated in *E. coli* SH14 strains containing only the 19.5-kb *pap* copy, surface antigens related to P-type pili were detected. This indicates that the operon is not completely silent and that it expresses surface peptides similar to the adhesin encoded on the 15.5-kb copy. The expression of adherence by bacteria that harbor the cloned 19.5-kb copy may result from the increased gene dose of the high-copy-number recombinant molecule or from more efficient expression of the cloned operon, perhaps from a vector-encoded promotor. Molecular studies have shown that the 19.5-kb copy contains DNA sequences which hybridize with essentially all of the *pap* operon, suggesting that it has no major rearrangements or deletions (5).

We have also determined that the *pil* genes of *E. coli* SH14, which encode D-mannose-sensitive adherence, map near *leu* near the position reported for *pil* in *E. coli* K-12 (1; unpublished data). Thus, none of the three adherence gene clusters in *E. coli* SH1 seem to be genetically linked. However, both of the *pap* operons may be linked to other virulence determinants. As reported earlier, the 19.5-kb copy of *pap* is linked to genes for hemolysin expression, a property highly correlated with symptomatic urinary tract infection. The 15.5-kb *pap* copy is near *kpsA*, a genetic locus near *serA* that has been shown to encode various K antigens, including K1, K4, K10, and K54 (15, 16). Capsular antigens, and especially K1, contribute to the virulence of *E. coli* in extraintestinal infections.

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