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The Genetic Determinant of Adhesive Function in Type 1 Fimbriae of *Escherichia coli* Is Distinct from the Gene Encoding the Fimbrial Subunit

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The role of type 1 fimbriae in the mannose-sensitive attachment of *Escherichia coli* to eucaryotic cells was investigated by deletion mutation analysis of a recombinant plasmid, pSH2, carrying the genetic information for the synthesis and expression of functional type 1 fimbriae. A mutant, pUT2002, containing a deletion remote from the structural gene encoding the 17-kilodalton subunit protein of type 1 fimbriae failed to agglutinate guinea pig erythrocytes even though the bacteria expressed fimbriae morphologically and antigenically indistinguishable from those produced by the intact recombinant plasmid. Fimbriae isolated from pUT2002 failed to agglutinate guinea pig erythrocytes, but reacted with a monoclonal antibody specific for quaternary structural determinants of type 1 fimbriae. Moreover, the dissociated fimbrial subunits from this mutant were indistinguishable from normal fimbriae by their migration during electrophoresis in sodium dodecyl sulfate-polyacrylamide gels, by their reactivity with a monoclonal antibody directed against a subunit-specific epitope, and in enzyme-linked immunosorbent assays with monospecific antisera. These results indicate that the adhesive functions in type 1 fimbriae are dependent on a factor(s) encoded by a gene other than those required for synthesis, assembly, and expression of the structural 17-kilodalton subunit.

The determinants required for the synthesis of morphologically recognizable P and X fimbriae in *Escherichia coli* have been shown to be distinct from, but closely linked to, determinants which are required for expression of the adhesive functions of these organelles (5, 5a). The similarity between the genetic organization of the P and X determinants and the determinant encoding the type 1 fimbriae associated with mannose-sensitive hemagglutination by *E. coli* (8) suggested that adhesive function also may be genetically separable from the synthesis and expression of type 1 fimbriae. To test this hypothesis, we constructed and analyzed a series of deletion mutants of the recombinant plasmid pSH2; this plasmid carries the genetic information required for the synthesis of functional type 1 fimbriae (8).

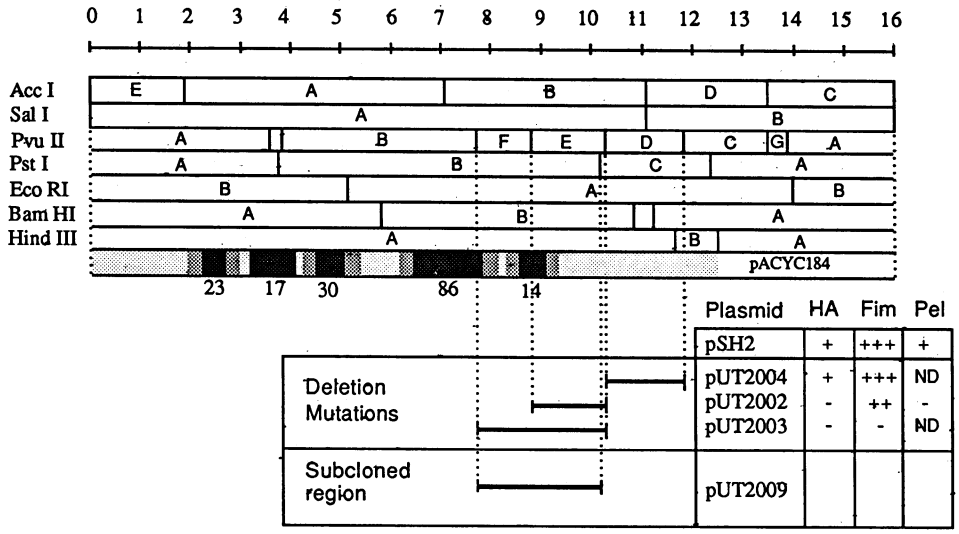

FIG. 1. Restriction map of pSH2 and phenotypes of pSH2 derivatives. Shaded areas show the approximate locations of the regions encoding the 23-, 17-, 30-, 86-, and 14-kDa peptides described and mapped by Orndorff and Falkow (8). Abbreviations: HA, hemagglutination of guinea pig erythrocytes; Fim, production of morphologically normal fimbriae as determined by electron microscopy; Pel, pellicle formation.

Purification, digestion, mapping, ligation, tranformation, and electrophoretic analysis of plasmid DNA were performed by standard methods (6). Methods for agglutination

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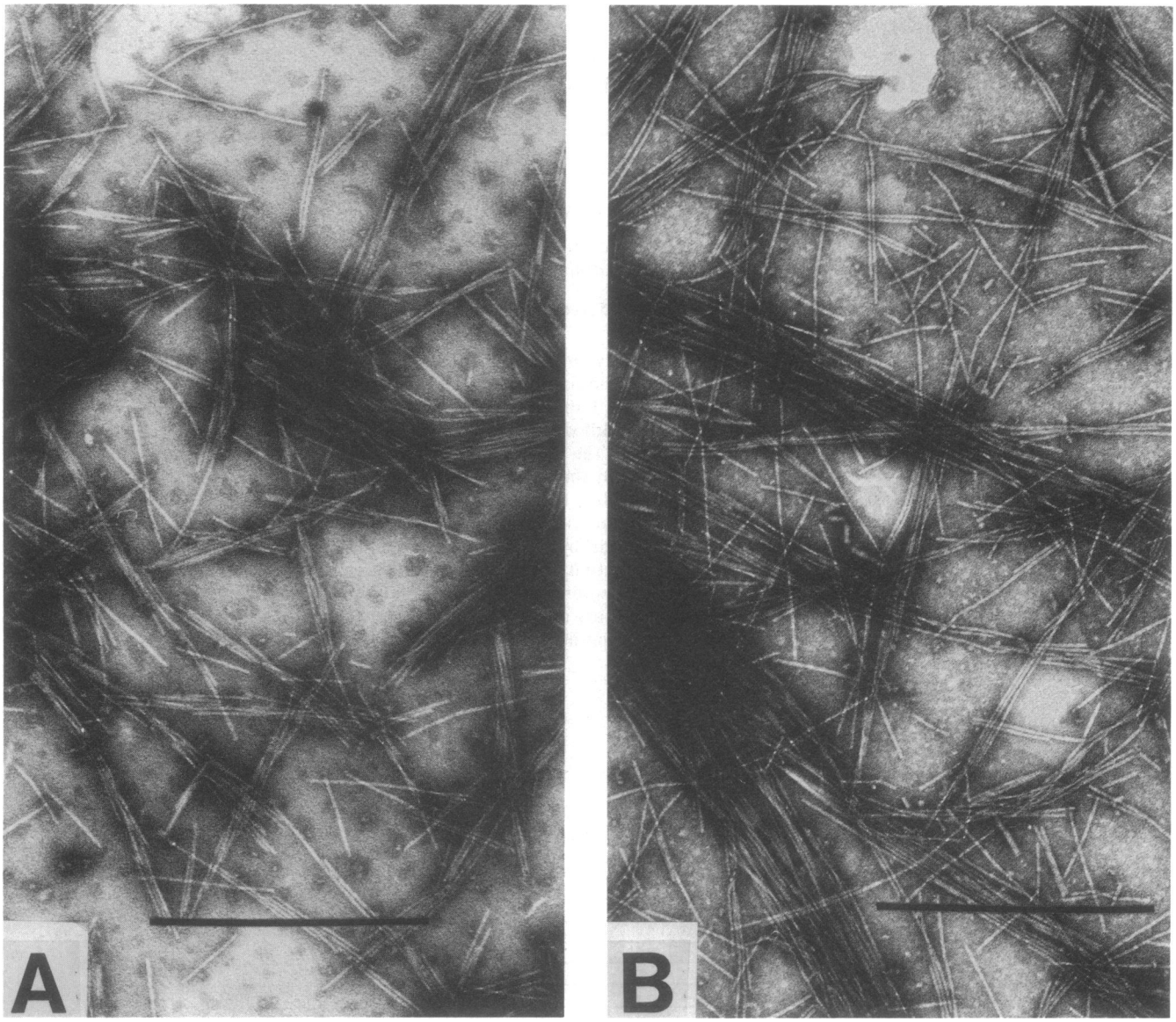

FIG. 2. Electron micrographs of negatively stained preparations of isolated fimbriae. (A) *E. coli* K-12 VL361(pSH2); (B) *E. coli* K-12 VL361(pUT2002). Bars represent 1 μm.

of guinea pig erythrocytes as a test of fimbrial function, purification of fimbriae, electron microscopy, treatment of intact bacteria with fimbria-specific antibodies, enzyme-linked immunosorbent assay of fimbriae, and detection of the 17-kilodalton (kDa) fimbrial subunit on immunoblots were described previously (1). Properties of all plasmids with respect to synthesis or function of fimbriae were assessed in *E. coli* K-12 VL361 (4). This strain does not produce either intact fimbriae (4) or the 17-kDa fimbrial subunit (unpublished observations) owing to the effects of a chromosomal Mu d1(Ap *lac*) insertion. The effect of this insertion on production of other proteins associated with synthesis of fimbriae has not been determined.

A restriction map of pSH2 and the structures of three deletion mutants (pUT2002, pUT2003, and pUT2004) constructed by partial digestion with *Pvu*II followed by religation are shown in Fig. 1. The structure of this plasmid differed somewhat from that reported by Orndorff and Falkow (8), although these differences occur largely outside the region required for the synthesis of functional fimbriae. The ability of the mutant plasmids to direct synthesis of functional fimbriae as determined by mannose-sensitive agglutination of guinea pig erythrocytes and to form pellicles is also indicated (Fig. 1). pUT2004 containing a deletion to the right of the *Pvu*II site at 10.3 kilobases (kb) directed the synthesis of functional fimbriae (Fig. 1). Neither pUT2002 nor pUT2003 was capable of conferring hemagglutinating or pellicle-forming activity (3) upon *E. coli* K-12 VL361 (Fig. 1). These results indicate that DNA sequences to the right of the *Pvu*II site near 10 kb are not required for formation of functional fimbriae in the VL361 background, whereas sequences within the 1.33-kb fragment to the left of this site are essential.

The mutant plasmids which failed to produce functional fimbriae differed with respect to the production of morphological fimbriae. VL361(pUT2003) lacked suface fimbriae,

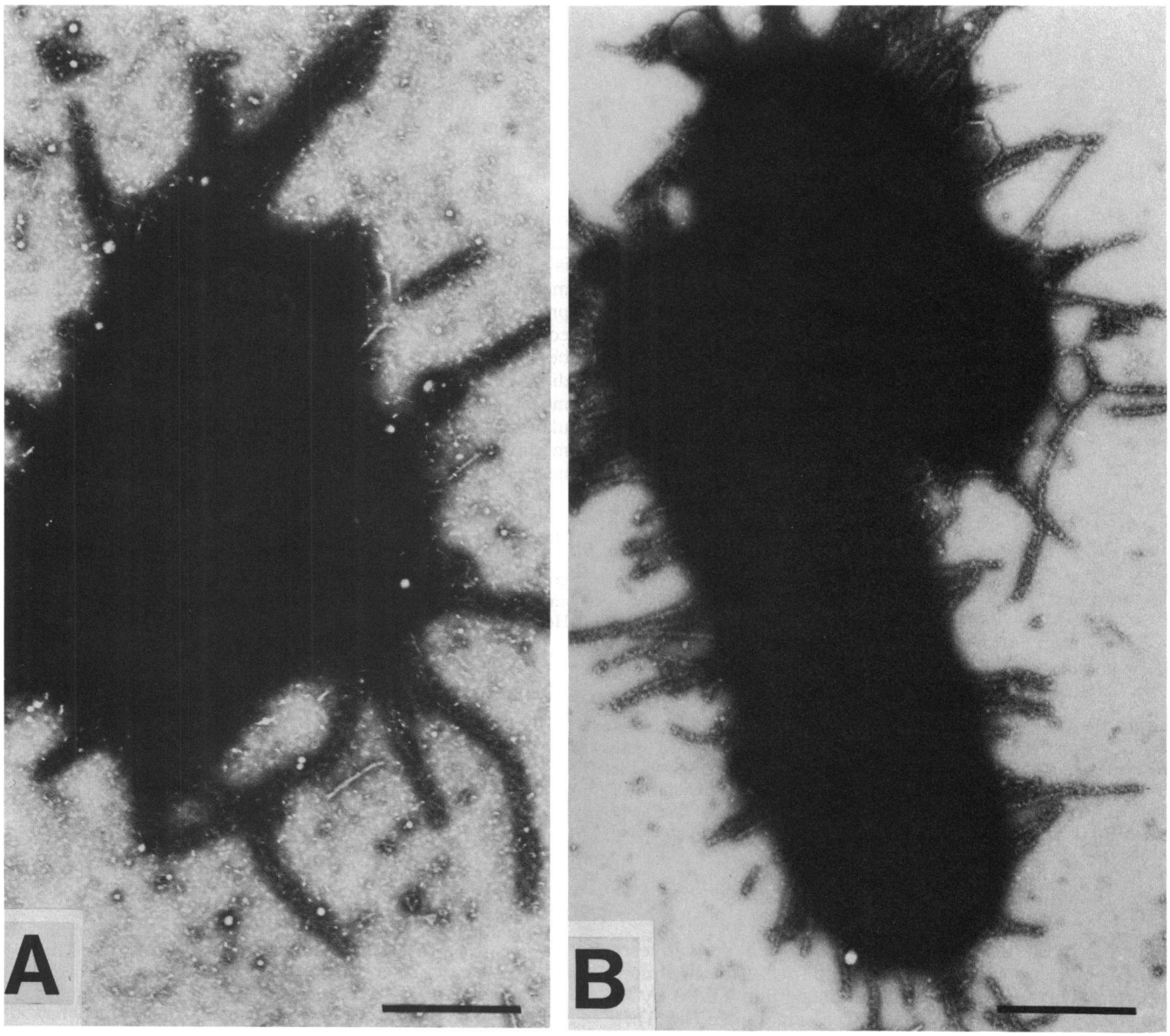

FIG. 3. Electron micrographs of negatively stained *E. coli* K-12 VL361(pSH2) (A) and *E. coli* K-12 VL361(pUT2002) (B) after incubation in a 1:1,000 dilution of a quaternary-structure-specific monoclonal antibody (G10A). Bars represent 1 μm.

suggesting that the deletion in this plasmid may extend into the structural gene for the 86-kDa protein known to be required for fimbria production (8). The precise limits of this gene are not known. In contrast, pUT2002 directed the synthesis of morphologically intact fimbriae; fimbriae produced by VL361(pUT2002) were indistinguishable by electron microscopy from those produced by VL361(pSH2) (Fig. 2). Consistent with the loss of hemagglutinating activity of the pUT2002-transformed strain VL361, isolated pUT2002 fimbriae failed to agglutinate guinea pig erythrocytes, whereas isolated pSH2 fimbriae agglutinated the erythrocytes in a D-mannose-sensitive manner (data not shown).

Despite their lack of adhesive activity, the mutant pUT2002 fimbriae were recognized by a monoclonal antibody specific for a quaternary structural determinant of adhesive type 1 fimbriae (Fig. 3). Furthermore, the subunits of both pSH2 and the mutant pUT2002 fimbriae reacted with each of three different monoclonal antibodies that recognize epitopes on the dissociated 17-kDa subunits of type 1 fimbriae of *E. coli* K-12. The reactivity of one of these antibodies with the subunits of the adhesive and nonadhesive fimbriae in a Western blot is shown in Fig. 4; the antibody reacted with a protein band of apparent molecular size 17 kDa in each of the dissociated fimbrial preparations. When samples of purified pSH2 and pUT2002 fimbriae containing equal concentrations of protein were titrated in a standard enzyme-linked immunosorbent assay by using monospecific polyclonal rabbit antiserum prepared against normal *E. coli* K-12 fimbriae from strain CSH50, equal titers were obtained. In conjunction with the observations from electron microscopy, these findings indicate that there are no gross structural differences between the pUT2002-encoded and wild-type fimbriae.

The region deleted in pUT2002 contains at least part of the structural gene for the 14-kDa protein identified by Orndorff and Falkow (8). To determine whether this gene could

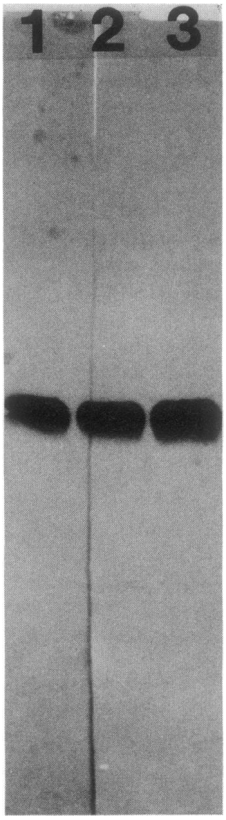

FIG. 4. Blots of dissociated fimbrial preparations on nitrocellulose. Lane A, *E. coli* K-12 VL361(pSH2) fimbriae stained with amido black. This blot shows a single band corresponding to the 17-kDa subunit of type 1 fimbriae. Lanes B and C, immunoblots of fimbrial preparations from the *E. coli* strains VL361(pSH2) (B) and VL361(pUT2002) (C), reacted with a subunit-specific monoclonal antibody (H3).

complement the lesion in pUT2002, a *recA* derivative of VL361 carrying both pUT2002 and pUT2009 was constructed. The *recA* allele was introduced into VL361 by P1 transduction of *recA56* and the closely linked *srl*::Tn*10* marker from strain JC10275 (2). pUT2009 consists of the region of pSH2 between the *Pvu*II site near 7.4 kb and the *Pst*I site near 9.4 kb inserted into the *Pst*I site of pBR322 with the aid of synthetic linkers. This region contains the fragment of pSH2 previously shown to express the 14-kDa protein (8). The strain containing both plasmids produced fimbriae, but they were hemagglutination negative. These results indicate that the 14-kDa protein is probably not sufficient to restore adhesive function. Either a *cis*-acting element within the deleted region of pUT2002 or a product encoded by sequences beyond the *Pst*I site at 9.6 kb may also be necessary.

The data presented here show that, as is true for P and X fimbriae, the DNA sequences required for the synthesis of apparently normal type 1 fimbriae as judged by morphological and immunological criteria are distinct from the sequences which confer the adhesive function associated with these structures. The region conferring adhesive function is separated from the gene encoding the major fimbrial subunit by the ancillary genes required for fimbrial assembly (8), a feature which resembles the organization of the P and X determinants (5). This region is less than 2.4 kb in size. It is known to encode a 14-kDa protein, but this protein apparently is not sufficient by itself to confer adhesive function.

The observation that the behavior of isolated fimbriae reflects the phenotype of the intact bacteria with respect to hemagglutination is strong evidence that the adhesive moiety is an integral part of the assembled fimbriae and suggests two models for the function of the region deleted in pUT2002; a product(s) of this region may modify the fimbrial subunit to confer adhesive function, or it may itself be the adhesin and be incorporated into the fimbriae. Our failure to detect differences between adhesive and nonadhesive fimbriae in morphology, reaction with monoclonal antibodies, or molecular mass of the fimbrial subunit suggests that possible modifications are subtle or that additional adhesive components incorporated into the assembled fimbriae are minor.

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ADDENDUM IN PROOF

Similar findings have recently been published by L. Maurer and P. E. Orndorff (FEMS Microbiol. Lett. **30**:59–66).

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