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Pilin Gene Phase Variation of *Moraxella bovis* Is Caused by an Inversion of the Pilin Genes

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Moraxella bovis **Epp63 can express either of two different pilin proteins, called α and β. We have previously cloned and sequenced the β-pilin gene and now report that DNAs isolated from bacteria expressing α pilin have hybridization patterns consistently different from those of bacteria expressing β pilin. The phase variation between α- and β-pilin gene expression appears to be associated with an inversion of about 2 kilobases of DNA, whose endpoints occur within the coding region of the expressed pilin gene. Comparisons of the β-pilin gene sequence with those of well-studied bacterial inversion systems revealed a stretch of 58% sequence similarity (21 of 36 base pairs) between the left inverted repeat of the *Salmonella typhimurium* flagellar *hin* control region and the amino-terminal portion of the β-pilin gene.**

Moraxella bovis is the primary cause of infectious bovine keratoconjunctivitis, a widespread, highly contagious ocular disease of cattle that causes temporary and, occasionally, permanent blindness (16, 22, 28). Two factors, pili and hemolysin, have been established as being involved in the pathogenesis of *M. bovis* infection. The pili are probably required for the attachment of *M. bovis* to the corneal epithelium, with only piliated (P^+) strains able to infect experimentally exposed cattle (48). A wide variety of phenotypes are associated with piliation, including colony morphology and agar corrosion (4, 48), twitching motility (20, 21), and competence for DNA transformation (5). Furthermore, P^+ bacteria mediate the agglutination of bovine erythrocytes and form a pellicle on the surfaces of broth cultures (54). Piliated-to-nonpiliated (P^-) transitions occur spontaneously in vitro, with 1 in 10,000 colonies changing from a P^+ to P^- colony morphology when *M. bovis* is grown on agar (4). The reverse transition, from P^- to P^+, has been seen at various frequencies, from none to about 1 altered colony observed per 10,000 (4).

M. bovis produces serologically different pilus types (54), and a pilus vaccine induces protective immunity only against challenge with a homologous strain (52). Different strains make pilin (the repeating polypeptide subunit which makes up pili) of various molecular weights, and a single strain is capable of producing more than one type of pilin protein (41). *M. bovis* Epp63 produces two pilins, α (about 18,000 daltons) and β (about 16,000 daltons) (41; W. Ruehl, C. Marrs, R. Fernandez, S. Falkow, and G. Schoolnik, submitted for publication). Transitions from production of α pilin to production of β pilin and vice versa occur directly without the strain necessarily going through a P^- state (Ruehl et al., submitted).

The *M. bovis* pilin proteins are part of a conserved family that share extensive amino-terminal amino acid sequence homology and contain the modified amino acid *N*-methylphenylalanine (MePhe) as the first residue (23, 42). This family includes the pilins of *Moraxella nonliquefaciens* (15), *Neisseria gonorrhoeae* (23, 53, 57), *Bacteroides nodosus* (42), *Pseudomonas aeruginosa* (56), and *Vibrio cholerae* (70).

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We have previously cloned and sequenced the *M. bovis* β-pilin gene (41). To determine the number and arrangement of pilin genes present in *M. bovis* Epp63, we used our β-pilin gene as a probe in genomic Southern hybridization experiments. In this paper, we provide evidence that the switch in expression between α and β pilin of Epp63 is due to an inversion of a 2-kilobase (kb) region of DNA.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and media. *M. bovis* Epp63 and the recombinant plasmids pMxB7 and pMxB12 were described previously (41). *M. bovis* Mac74 and IBH64 were generously provided by G. W. Pugh, Jr., Agricultural Research Service, Ames, Iowa. *M. bovis* was grown on GC agar base (Difco Laboratories, Detroit, Mich.) with 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.). *Escherichia coli* strains containing drug-resistant plasmids were grown on L agar containing 100 μg of carbenicillin per ml (Sigma Chemical Co., St. Louis, Mo.).

DNA isolation and manipulation and Western blotting (immunoblotting). Total DNA was prepared by the method of Hull et al. (29). Plasmid DNA was isolated by the polyethylene glycol method of Humphreys et al. (30) followed by exhaustive dialysis in buffer (10 mM Tris [pH 7.9], 10 mM NaCl, 1 mM EDTA). Restriction endonucleases were purchased from New England BioLabs, Inc., Beverly, Mass.; Bethesda Research Laboratories, Inc., Gaithersburg, Md.; Boehringer Mannheim Biochemicals, Indianapolis, Ind.; or Promega Biotec, Madison, Wis. Restriction enzyme digests, agarose gel electrophoresis, and isolation of restriction enzyme-generated DNA fragments were carried out as described previously (39). Transfer of DNA fragments from agarose gels onto nitrocellulose paper was done by the method of Southern (64), and hybridizations were done by the method of Blattner et al. (3), except that hybridizations and washes were carried out at 56 instead of 68°C. DNA fragment probes were labeled with [α-^{32}P]dCTP (New England Nuclear Research Products, Boston, Mass.) by use of a nick translation kit (Bethesda Research Laboratories).

Western blotting of proteins from whole-cell extracts

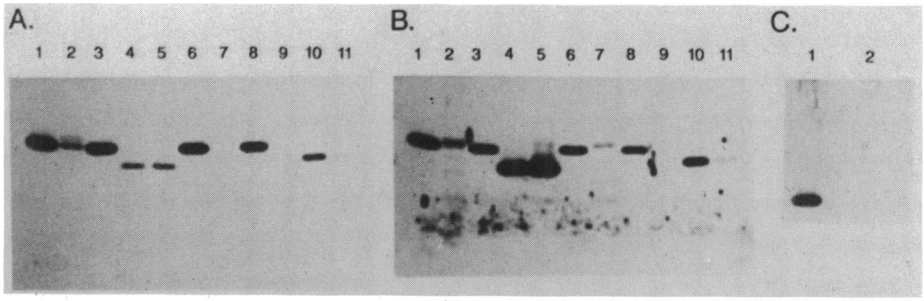

FIG. 1. Western transfer analysis of *M. bovis* bacteria lysed and electrophoresed on 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose paper. The resulting filters were incubated with antisera and ^{125}I-labeled protein A as described previously (8). In all cases, four times as much protein of the P^- samples as of the P^+ samples was loaded. P^- bacteria were defined by colony morphology and by the absence of pili on negatively stained bacteria observed with transmission electron microscopy. (A and B) Filter was reacted with antisera against α pili (A) and β pili (B). Lanes: 1 and 3, Epp63, α P^+; 2, Epp63 P^- derived from an α P^+ parent; 4, Epp63, β P^+; 5, Epp63 P^- derived from a β P^+ parent; 6 and 8, Mac74 P^+; 7 and 9, Mac74 P^-; 10, IBH64 P^+; 11, IBH64 P^-. (C) Filter was reacted with antiserum against β pili. Lanes: 1, Epp63 β P^+; 2, Epp63 P^- derived from a β P^+ parent.

separated according to molecular weight by sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as described previously (8), except that 1 mM EDTA was used in place of 0.15% sodium azide.

RESULTS

M. bovis Epp63 can produce two different types of pili, which have pilin subunits α or β; these subunits differ serologically and in migration pattern in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 1). One of the derivatives of an α-piliated culture which appeared nonpiliated by colony morphology still produced reduced amounts of α pilin (Fig. 1A and B, lane 2). Similarly, production of β pilin by a phenotypically nonpiliated variant was observed (Fig. 1A and B, lane 5). In contrast, a second nonpiliated variant failed to produce any pilin (Fig. 1C). Thus, there appear to be at least two different types of nonpiliated variants, those which still produce pilin and those in which no detectable pilin can be visualized by Western analysis.

Pilin proteins of two other independently isolated *M. bovis* strains, Mac74 and IBH64, were also examined. The Mac74 pilin was similar to the α pilin of Epp63 both in size and in apparent reactivity to the α- and β-specific antisera. The pilin from the IBH64 isolate, on the other hand, migrated at an intermediate size between α and β pilins and seemed about equally cross-reactive to antiserum produced against either α or β pili.

Previously we cloned and sequenced the β-pilin gene of Epp63 (41). We used different regions of the cloned β-pilin gene (Fig. 2) as probes in genomic Southern hybridization analysis of DNA isolated either from a culture expressing β pilin or from a culture expressing α pilin as determined by Western blot analysis. Representative Southern hybridization data are illustrated in Fig. 3. Probe 1 (Fig. 2) consisted of the entire *M. bovis* insert region of pMxB12. Southern hybridizations of probe 1 to genomic DNA which was digested with some enzymes that did not have cleavage sites within probe 1 produced hybridization patterns, which were identical for α and β DNAs, consisting of one strong and one weak band of hybridization. Examples are *Ava*I and *Cfo*I (Fig. 3) and *Bcl*I, *Bgl*II, *Eco*RI, *Hae*II, *Hin*dIII, *Msp*I, *Nde*I, *Pst*I, *Pvu*I, and *Pvu*II (data not shown). A second class of enzymes, which also lacked sites within the pMxB12 insert, produced hybridization patterns that consisted of two strong bands of hybridization, which differed in α and β DNAs, and

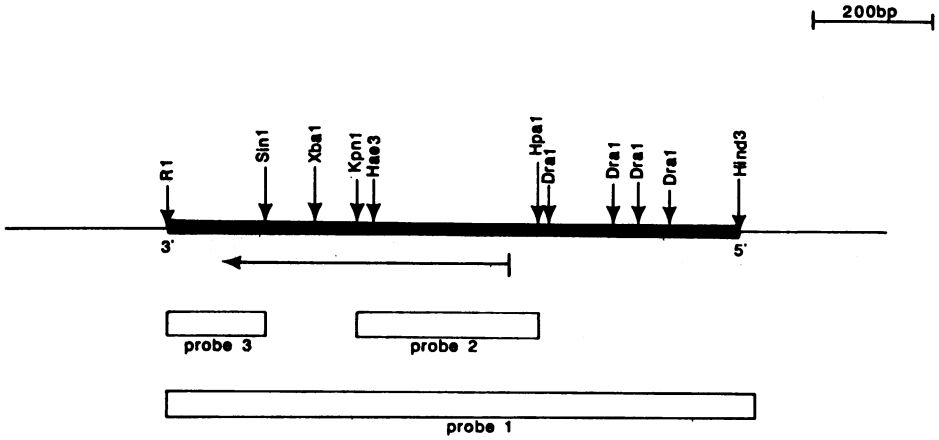

FIG. 2. Restriction map of the insert region of pMxB12, listing pertinent sites. Restriction sites for *Dra*I, *Eco*RI (R1), *Hae*III, *Hin*dIII, *Hpa*I, *Kpn*I, *Sin*I, and *Xba*I are shown. The thick line represents the region of *M. bovis* insert DNA, and the thin lines represent parts of pBR322. The arrow beneath the map represents the coding region of the β-pilin gene, with the arrowhead as the carboxy-terminal end.

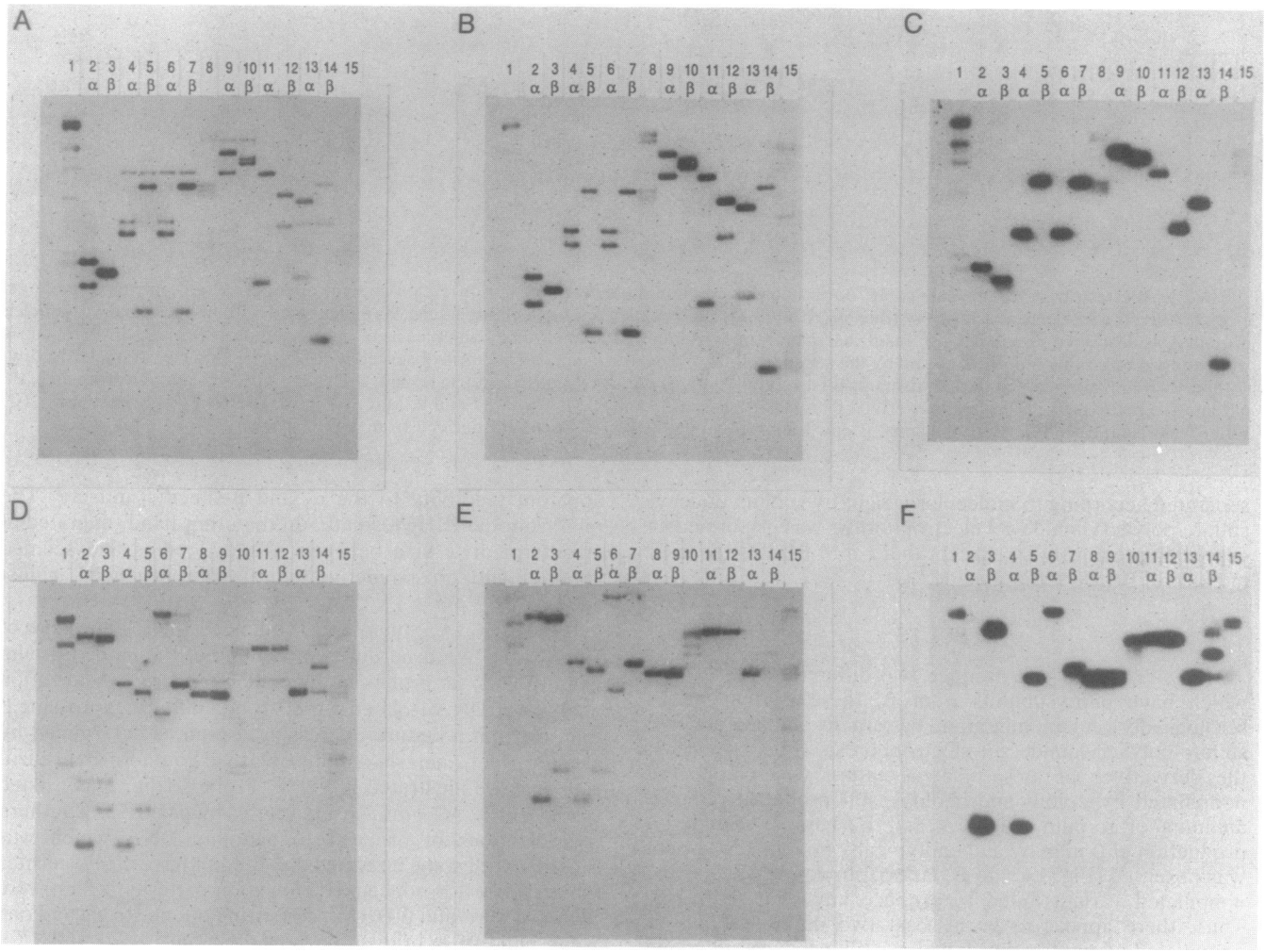

FIG. 3. Genomic Southern hybridizations with different β-pilin gene portions as probes (plus small amounts of labeled λ DNA to hybridize to the λ size standards). Panels A, B, and C contain the same patterns of DNA digests hybridized to probe 1 (A), probe 2 (B), and probe 3 (C). α and β DNAs were used as indicated; where neither α nor β is indicated, λ DNA was used. Lanes: 1, *Hin*dIII; 2 and 3, *Hin*fI; 4 through 7, *Hae*III; 8, *Pst*I; 9 and 10, *Sin*I; 11 and 12, *Sin*I plus *Hin*dIII; 13 through 15, *Dra*I. Panels D, E, and F contain identical patterns of DNA digests hybridized to probe 1 (D), probe 2 (E), and probe 3 (F). Lanes: 1, *Hin*dIII; 2 and 3, *Eco*RV; 4 and 5, *Eco*RV plus *Hin*dIII; 6 and 7, *Cla*I; 8 and 9, *Ava*I; 10, *Dra*I; 11 and 12, *Cfo*I; 13 and 14, *Sca*I; 15, *Pst*I.

one weaker band each, which was identical in both α and β DNAs (e.g., *Cla*I and *Eco*RV). *Hin*fI was unique in having a single strongly hybridizing band with β DNA but two different strongly hybridizing bands with α DNA; both DNA types showed an additional identical, weakly hybridizing band (Fig. 3).

Enzymes that had sites within the insert sequence of pMxB12, including *Dra*I, *Hae*III, and *Sin*I (Fig. 3) and *Xba*I (data not shown), also each produced two strong bands of hybridization that differed in α and β DNAs and a weaker band that was identical. The presence of two strong bands of hybridization with some enzymes which do not cleave within the probe used is evidence for the presence of at least two genes. However, the fact that digestion with any of a large number of enzymes resulted in only one strongly hybridizing band implies that the separate sequences that hybridize strongly are molecular neighbors. Since the *Ava*I band, which is the smallest unchanged band, is only 4.7 kb, the strongly hybridizing genes must all be present within that nucleic acid region.

The weak bands of hybridization seen with probe 1 which remain constant for α and β DNAs could be due to the presence elsewhere in the genome of less-homologous pilin gene sequences that do not differ between α and β DNAs. However, two observations lead us to believe that the weak bands of hybridization seen with probe 1 are not pilin gene sequences. The first observation was that the *Hin*dIII site present in pMxB12 did not have a corresponding site in genomic DNA at the predicted distance from the other known enzyme sites within the β-pilin gene. In addition, when the region further 5′ of the β-pilin gene, which was deleted when the plasmid pMxB12 was constructed from the plasmid pMxB7 (41), was used as a probe, it hybridized strongly to those weak bands of hybridization observed when probe 1 was used (data not shown). We feel that the most likely explanation for these data is that as an artifact of the original cloning, pMxB7 is made up of two separate, noncontiguous pieces of *M. bovis* DNA. Fortunately, only a small region past the *Hin*dIII site into pMxb12 appears to contain DNA that is not normally next to the pilin genes in the chromosome. Thus, the weakly hybridizing bands corresponding to this noncontiguous region show a relatively

TABLE 1. Sizes and sums of sizes of fragments hybridizing to probes 1 and 2

Enzyme(s)	DNA	No. of lanes	Band sizes[a]	Sum of band sizes
*Cla*I	α	2	~**25**, 3.9	~29
	β	2	~24, **5.3**	~29
*Sin*I[b]	α	9	**8.4**, 6.0	14.4
	β	6	7.5, 6.8	14.3
*Sin*I + *Hin*dIII[b]	α	3	**5.9**, 1.5	7.4
	β	3	4.5, **3.1**	7.6
*Eco*RV	α	5	12, **0.97**	13
	β	3	**11**, 1.4	12.4
*Eco*RV + *Hin*dIII	α	3	5.3, **0.95**	6.3
	β	3	**4.9**, 1.4	6.3
*Xba*I[b]	α	4	4.5, 3.9	8.4
	β	2	6.2, 2.2	8.4
*Hae*III[b]	α	6	3.3, **2.8**	6.1
	β	5	**5.0**, 1.1	6.1
*Dra*I[b]	α	3	**4.1**, 1.6	5.7
	β	3	5.0, **0.69**	5.7
*Hin*fI	α	10	**2.0**, 1.5	3.5
	β	8	**1.7** (×2)	3.4

[a] Fragments with size (in kilobases) in boldface also hybridize to probe 3.
[b] Sites which cut within pMxB12 insert.

fainter signal because they hybridize to less than 10% of probe 1 rather than because of low homology.

To obtain further information on the structure of the pilin gene region and to test the above-described theory, two additional probes which each contained only a portion of the β-pilin gene were used. Probe 2 was an *Hpa*I-*Kpn*I fragment of pMxB12 containing a small amount of the 5′ region plus the conserved amino-terminal region of the β-pilin gene, while probe 3 was a *Sin*I-*Eco*RI fragment of pMxB12 containing the carboxy-terminal region of the β-pilin gene (which by amino acid sequence differs significantly from the α-pilin gene [41]) plus a short region 3′ of the gene (Fig. 2). Examples of the hybridization patterns of these two probes compared with those of probe 1 are shown in Fig. 3. Hybridization patterns obtained with probe 2 or probe 3 instead of probe 1 resulted in the loss of the more weakly hybridizing bands that were invariant between α and β DNAs, a result that supported our hypothesis. Probe 2 often hybridized to two bands, while probe 3 hybridized to only one of those two bands. This implies that while probe 2 contains a region of the β-pilin gene that cross-hybridizes to the α-pilin gene sequences, probe 3 is β-pilin specific and does not cross-hybridize with the α-pilin gene sequences.

We measured the sizes of all the hybridizing bands shown in Fig. 3 and a large number of additional hybridization patterns not shown. Table 1 lists the sizes of the bands observed for a variety of different genomic digests hybridized to probe 2 or of the strong bands of hybridization seen when probe 1 was used. Although the sizes of the two hybridizing bands for a given enzyme used on α DNA generally differ from the size of the bands seen with that same enzyme used on β DNA, the sum of the sizes of the two bands observed for α DNA always equaled the sum of the sizes of the two bands observed for β DNA (Table 1). The one apparent exception was *Hin*fI, which gave only a single band of hybridization to β DNA but two different bands of hybridization to α DNA. This can be explained if the single β band of hybridization is actually a doublet made up of two identically sized DNA fragments. The conservation of sums seen in Table 1 is a pattern characteristic of DNA inversions.

The hybridization patterns observed with probes 1 and 2 and the identification of the β-pilin gene by the use of probe 3 allowed the construction of the maps of the pilin gene region for both α and β DNAs (Fig. 4). The maps are identical except for a 2-kb region which is inverted in α DNA relative to β DNA. It is important to keep in mind that since this map was generated solely from hybridization data, additional sites for the enzymes shown could occur within the region depicted if the additional fragments they produce do not contain any of the sequences that hybridize to the probes used. Also, very small fragments would have run off the agarose gels, and thus, closely spaced sites could not be seen. An example of this are the closely spaced *Dra*I sites 5′ of the coding region of the β-pilin gene, which are predicted from the sequence of pMxB12 (Fig. 2) (41).

A comparison of the *M. bovis* β-pilin sequences with the sequences of the recombination sites of some known bacterial inversion systems revealed a stretch of 58% homology (21 of 36 base pairs [bp]) between the left inverted repeat of the *Salmonella typhimurium* inversion region (*hixL*), which controls flagellar expression, and the amino-terminal portion (corresponding to amino acids 9 to 21 of the mature protein) of the β-pilin gene (Fig. 5). This 36-bp region of 58% sequence similarity falls entirely within a previously defined 40-bp region of homology between *hixL* and the other inversion recombination sites of the *hin* family of inversion sequences (24, 51). Figure 5 shows the homology between *hixL* and the other sites known to be recombined by the Hin invertase. Including the entire 40-bp region, there was 52.5% sequence similarity between *hixL* and the β-pilin gene, which is equal to the 52.5% similarity between *hixL* and the left bacteriophage P1 C region inversion recombination site (*cixL*) and is greater than the 45% similarity between *hixL* and the right inverted repeat of the *hin* inversion region (*hixR*). However, there was a qualitative difference in which bases are conserved throughout the *hin* family and those bases that are identical between *hixL* and the β pilin gene. Also, with the exception of *hixR*, the other recombination site regions of the *hin* family shown in Fig. 5 contained imperfect 9- to 14-bp inverted repeats with nonpalindromic dinucleotides at the center of symmetry. In *hixR* there was instead a small (5 of 6 bp) imperfect inverted repeat which was offset by 8 bp compared with the others. No imperfect inverted repeat was present in the β-pilin gene sequence shown in Fig. 5. The specific site at which recombination occurs in *hixL* would, in the β-pilin gene, correspond to the DNA codon for amino acid 13 of the mature β-pilin protein.

DISCUSSION

The data presented here provide evidence that a 2-kb region of DNA containig portions of the α- and β-pilin genes is inverted in α DNA relative to β DNA of strain Epp63. Figure 4 shows the maps derived from our hybridization data. In our model, an inversion event occurs in β-pilin-specific DNA, which changes it to the α-pilin DNA pattern (or vice versa), and this inversion event determines whether α- or β-pilin is expressed. The inversion appears to occur within the amino-terminal region of the pilin genes, and only the pilin sequences that have the promoter and beginning portion of the gene produce pilin protein. Thus, in one orientation of the 2-kb DNA region, the β-pilin-specific sequences are in the expression locus adjacent to the promoter and the initial pilin gene sequences which code for the leader sequence and the amino-terminal amino acids, while the α-pilin-specific sequences are in the inverted, nonex-

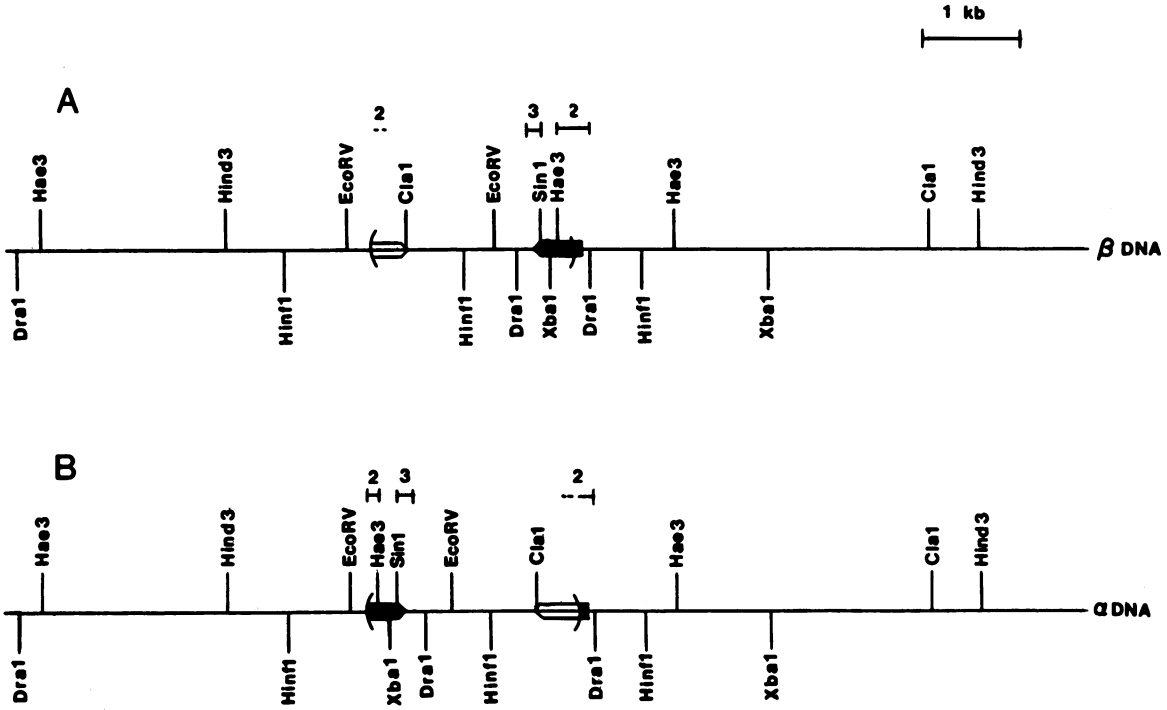

FIG. 4. Restriction maps of the chromosomal region containing the pilin genes as determined by genomic Southern hybridization analysis. (A), β-pilin-expressing DNA; (B), α-pilin-expressing DNA. ➡, Coding region of the β-pilin gene; ⊃, partial, nonexpressed α-pilin gene sequences; the arrowheads of both are the carboxy-terminal ends. In the α-pilin-expressing DNA map, the α-pilin-coding sequences are recombined next to the constant amino terminus, and the remainder of the β-pilin-coding sequences are present in the nonexpressed location. For each map, the bars above the map show the regions which hybridize to either probe 2 or probe 3. Some sites whose positions are known (e.g., the third *Eco*RV and *Cla*I sites) (Table 1) are not shown because they are off the region of the map being displayed.

pressed position. In the other orientation the reverse is true; that is, the α-pilin-specific sequences are in the expression locus and the β-pilin-specific sequences are in the inverted, nonexpressed position.

Other members of the MePhe class of pilin genes have been cloned and sequenced from *N. gonorrhoeae* (43, 44), *B. nodosus* (2, 11–13), and *P. aeruginosa* (47, 66). *P. aeruginosa* strains have only one copy of the pilin gene in each

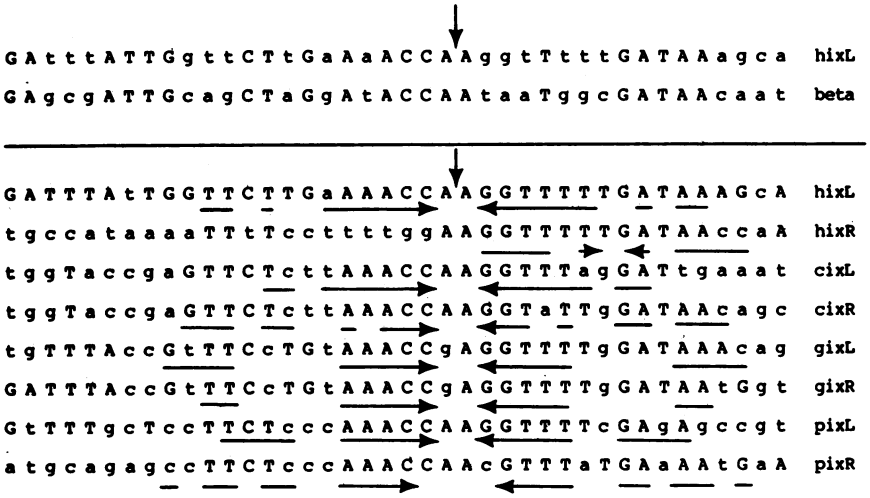

FIG. 5. Sequence comparison between the site-specific recombination sequence *hixL* of *S. typhimurium* and part of the *M. bovis* β-pilin gene sequence. The top line of both sections lists the 40-bp region of *hixL* containing the recombination site of the inversion which is homologous among the other recombination sites from phage P1 (C region; *cix* sites [24]), phage Mu (G region; *gix* sites [24, 49]), and the P region of *E. coli* (51). In the top comparison, the β-pilin gene sequence listed in line 2 represents the inverse-complementary sequence compared with the coding sequence of the β-pilin gene. Capitalized bases are identical. The arrow shows the site of recombination in *hixL*. The bottom section shows the homologies between the different members of the *hin* family of inversion sequences which are all capable of cross-complementation. In the *hixL* sequence, bases in lower case are those which are not present in any other member of the family. For the remaining sequences, capitalized bases are those identical to the *hixL* sequence. Underlined bases show the imperfect inverted repeat structures.

genome (47, 56), and so do most *B. nodosus* strains (called class I), although a few (class II) contain a second pilin gene sequence within 3 kb of the first (J. S. Mattick, personal communication). In contrast, every strain of *N. gonorrhoeae* possesses multiple pilin gene loci, and transitions from P^+ to P^- and between different P^+ pilin types are often accompanied by chromosomal DNA rearrangements (19, 43, 44, 58). Phase variation from one P^+ pilin type to a different P^+ pilin type is probably due to a gene conversion event between an expression site locus and one of the silent variant pilin sequences (18, 19, 59). Thus, despite the similarities found between the MePhe pilins of *M. bovis* and *N. gonorrhoeae*, it is clear that they differ in mechanisms of antigenic variation.

Another mechanism of phase variation seen in several systems involves a genetic inversion event which results in the reversible expression between just two alternative states (62). Examples of phase variation mediated by genetic inversion events include the *H1* and *H2* flagellar genes of *S. typhimurium* (31, 62), the tail fiber genes of bacteriophages Mu (17, 25, 49) and P1 (6, 7, 35, 71), and type 1 pili of *E. coli* (1, 9, 10, 14). It is interesting to compare the inversion system described here for *M. bovis* pilin genes to these other well-studied bacterial inversion systems. The *M. bovis* inversion system most closely resembles the inversion event seen in bacteriophage Mu, in which the promoter and amino-terminal portion of the *S* tail fiber gene are outside the 3-kb invertible G segment, and recombination occurs within the *S* gene to produce a switch to *S'* and also leads to the expression of *U'* in place of *U*, both of which are entirely within the G segment (26, 49, 72). In this case, the *gin* gene (located next to the G segment on the side opposite the *S* gene) encodes the recombinase that mediates the inversion event. For the *H1* and *H2* flagellar genes of *S. typhimurium*, the inversion involves a region of about 1 kb that contains the promoter controlling the expression of the *H2* gene as well as the *hin* gene, which makes the recombinase responsible for the inversion event (62). Expression of the *H1* gene is indirectly controlled by this switch, since the promoter which turns on the *H2* gene also turns on a gene which codes for a repressor of the *H1* gene. The inversion event mediating flagellar phase variation requires two other proteins in addition to Hin, a 12-kilodalton protein designated factor II and HU, one of the major histonelike proteins of *E. coli* (31). The recombinase activities of *hin*, *gin*, *cin* (of bacteriophage P1), and *pin* (an *E. coli* gene) are all capable of complementing each other for their respective inversion events (6, 34, 38, 49, 62, 69). The *hin* family of inversion systems also uses a recombinational enhancer site to greatly increase the inversion frequency (27, 32, 33).

The only other characterized inversion system that occurs in pilin gene regions is that of *E. coli* type 1 pili (fimbriae). A 314-bp invertible region containing a promoter controls the expression of the type 1 pilin gene (called *fimA* or *pilA*) (1, 36, 37). At least two genes, *fimB* and *fimE* (or *hyp*) are involved in the inversion event and in the general expression of *fimA*, and they are located immediately adjacent to the 314-bp invertible segment, on the side opposite the *fimA* gene (36, 45, 46). When the promoter is in the correct orientation to express the pilin gene, the bacteria have type 1 pili on their surfaces, but when it is inverted they lack type 1 pili. The *fimB* gene of the type 1 inversion system is not homologous with and does not complement the *hin* family of inversion systems (14).

A comparison of the *M. bovis* β-pilin DNA sequence and the recombination sites of the above-listed inversion systems showed no sequence similarities between the *M. bovis* β-pilin DNA sequence and the type 1 pili system. However, we discovered a stretch of 58% sequence similarity over a 36-bp region between the left inverted repeat of the *hin* segment (*hixL*) (32, 49) and the amino-terminal portion of the β-pilin gene at a place consistent with our data for the position of the recombination site for the pilin gene inversion (Fig. 5). This 36-bp section is part of the previously defined 40-bp region of homology between the members of the *hin* family of invertible sequences (24, 50). Counting the entire 40-bp region, DNA sequence similarity between the β-pilin gene and *hixL* is 52.5%. By comparison, homology within the *hin* family ranges from 75%, between *hixL* and *gixR*, to 45%, between *hixL* and *hixR*. It will be instructive to test for complementation of the *M. bovis* pilin gene inversion system by the *hin* gene system. That this region of DNA similarity to the *hin* recombination sequence should occur in the region of the gene that codes for the highly conserved amino acid sequence of the MePhe class of pilins is somewhat surprising, since no evidence exists for such an inversion recombination site in any of the other MePhe pilin gene sequences. Comparisons of the *hixL* sequence with the published sequences of the other MePhe pilin genes show DNA sequence similarities ranging from 25 to 35% for the same 40-bp region.

Our model explains how the switch between α- and β-pilin production occurs, and it also offers mechanisms for formation of some of the variants which lack pili on their cell surfaces. At least two different types of P^- variants appear to exist, (i) phenotypically P^- bacteria that still produce the original pilin protein and (ii) a second class of P^- isolate that produces no detectable pilin proteins. To date, all the P^- variants that we have examined by genomic Southern hybridization analysis are indistinguishable from either an α or a β pattern. No third pattern characteristic of a deletion or major chromosomal rearrangement has been observed, other than the inversion already described. Our model for *M. bovis* pilin gene switching would predict that an imperfect recombinational inversion event might lead either to a frameshift mutation or to an assembly missense pilin polypeptide. A frameshift mutation would most likely result in a short, rapidly degraded polypeptide, which might be undetectable in our assay system, appearing as the above-mentioned P^- variant which made no pilin protein. An assembly missense pilin polypeptide would probably be indistinguishable from normal pilins by Western analysis, producing the other observed class of P^- variants that produce pilin protein but are phenotypically P^- because they cannot assemble pili on the cell surface. Since P^- variants which still express pilin generally have substantially lower amounts of pilin, it may be that missense pilin proteins would be more rapidly degraded. Other mechanisms for producing P^- variants which are not related to the inversion event might also exist.

Transitions from P^+ to P^- in *N. gonorrhoeae* often involve deletions or recombination events at the expression loci (19, 58, 63), although some P^- variants still contain intact expression sites. *E. coli* that contains clones of these intact *N. gonorrhoeae* expression sites makes pilin transcripts, suggesting that pilus assembly or expression control involves some additional regulatory factor (19, 58, 63, 65). Swanson et al. have shown that only some *N. gonorrhoeae* P^- strains are capable of reverting to P^+ and that one class of revertible P^- strains produce an assembly missense pilin polypeptide which reverts to P^+ by gene conversion events similar to those that cause pilus antigenic variation (68).

The following possible roles that phase variation of pili

(between P^+ and P^- states) or antigenic variation between different P^+ types may play in the disease process have been postulated: (i) the variation may assist in avoidance of the host immune defense mechanisms, especially for *N. gonorrhoeae* (67); (ii) the presence of pili may help in adherence to host cells but may also make the bacteria more susceptible to ingestion by phagocytes (10, 60, 61); and (iii) expression of different pili types may allow the bacteria to bind to different host cell tissue types (67). We have evidence that for *M. bovis* the different pili types may play different roles in the pathogenic process. In a study on the relative infectivity and pathogenicity in calves of α-piliated, β-piliated, and P^- isolates of strain Epp63, the β-pilin phenotype was statistically correlated with enhanced infectivity and pathogenicity compared with the other types (Ruehl et al., submitted). Thus, the ability to switch between α- and β-pilus types may have important consequences in the disease process.

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LITERATURE CITED

1. **Abraham, J. M., C. S. Freitag, J. R. Clements, and B. I. Eisenstein.** 1985. An invertible element of DNA controls phase variation of type 1 fimbriae of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82**:5724–5727.
2. **Anderson, B. J., M. M. Bills, J. R. Egerton, and J. S. Mattick.** 1984. Cloning and expression in *Escherichia coli* of the gene encoding the structural subunit of *Bacteroides nodosus* fimbriae. J. Bacteriol. **160**:748–754.
3. **Blattner, F. R., A. E. Blechl, K. Denniston-Thompson, H. E. Faber, J. E. Richards, J. L. Slightom, P. W. Tucker, and O. Smithies.** 1978. Cloning human fetal gamma globin and mouse alpha-type globin DNA: preparation and screening of shotgun collections. Science **202**:1279–1284.
4. **Bovre, K., and L. O. Froholm.** 1972. Variation of colony morphology reflecting fimbriation in *Moraxella bovis* and two reference strains of *M. nonliquefaciens*. Acta Pathol. Microbiol. Scand. Sect. B **80**:629–640.
5. **Bovre, K., and L. O. Froholm.** 1972. Competence in genetic transformation related to colony type and fimbriation in three species of *Moraxella*. Acta Pathol. Microbiol. Scand. Sect. B **80**:649–659.
6. **Chow, L. T., T. R. Broker, R. Kahmann, and D. Kamp.** 1978. Comparison of the G DNA inversion in bacteriophages Mu, P1, and P7, p. 55–56. *In* D. Schlessinger (ed.), Microbiology—1978. American Society for Microbiology, Washington, D.C.
7. **Chow, L. T., and A. I. Bukhari.** 1976. The invertible DNA segments of coliphages Mu and P1 are identical. Virology **74**: 242–248.
8. **Cohen, M. L., and S. Falkow.** 1980. Protein antigens from *Staphylococcus aureus* strains associated with toxic-shock syndrome. Science **211**:842–844.
9. **Eisenstein, B. I.** 1981. Phase variation of type 1 fimbriae in *Escherichia coli* is under transcriptional control. Science **214**: 337–339.
10. **Eisenstein, B. I.** 1982. Genetic control of type 1 fimbriae in *Escherichia coli*, p. 308–311. *In* D. Schlessinger (ed.), Microbiology—1982. American Society for Microbiology, Washington, D.C.
11. **Elleman, T. C., and P. A. Hoyne.** 1984. Nucleotide sequence of the gene encoding pilin of *Bacteroides nodosus*, the causal organism of ovine footrot. J. Bacteriol. **160**:1184–1187.
12. **Elleman, T. C., P. A. Hoyne, D. L. Emery, D. J. Stewart, and B. L. Clark.** 1984. Isolation of the gene encoding pilin of *Bacteroides nodosus* (strain 198), the causal organism of ovine footrot. FEBS Lett. **173**:103–107.
13. **Elleman, T. C., P. A. Hoyne, N. M. McKern, and D. J. Stewart.** 1986. Nucleotide sequence of the gene encoding the two-subunit pilin of *Bacteroides nodosus* 265. J. Bacteriol. **167**:243–250.
14. **Freitag, C. S., J. M. Abraham, J. R. Clements, and B. I. Eisenstein.** 1985. Genetic analysis of the phase variation control of expression of type 1 fimbriae in *Escherichia coli*. J. Bacteriol. **162**:668–675.
15. **Froholm, L. O., and K. Sletten.** 1977. Purification and N-terminal sequence of a fimbrial protein from *Moraxella nonliquefaciens*. FEBS Lett. **73**:29–32.
16. **Gallaghar, C. H.** 1954. Investigation of the etiology of infectious ophthalmia of cattle. Aust. Vet. J. **30**:61–68.
17. **Grundy, F. J., and M. M. Howe.** 1984. Involvement of the invertible G segment in bacteriophage Mu tail fiber biosynthesis. Virology **134**:296–317.
18. **Haas, R., and T. F. Meyer.** 1986. The repertoire of silent pilus genes in *Neisseria gonorrhoeae*: evidence for gene conversion. Cell **44**:107–115.
19. **Hagblom, P., E. Segal, E. Billyard, and M. So.** 1985. Intragenic recombination leads to pilus antigenic variation in *Neisseria gonorrhoeae*. Nature (London) **315**:156–158.
20. **Henrichsen, J.** 1983. Twitching motility. Annu. Rev. Microbiol. **37**:81–93.
21. **Henrichsen, J., L. O. Froholm, and K. Bovre.** 1972. Studies on bacterial surface translocation. Acta. Pathol. Microbiol. Scand. Sect. B **80**:445–452.
22. **Henson, J. B., and L. C. Grumbles.** 1960. Infectious bovine keratoconjunctivitis. I. Etiology. Am. J. Vet. Res. **21**:761–766.
23. **Hermodson, M. A., K. C. S. Chen, and T. M. Buchanan.** 1978. *Neisseria* pili proteins: amino-terminal amino acid sequences and identification of an unusual amino acid. Biochemistry **17**: 442–445.
24. **Hiestand-Nauer, R., and S. Iida.** 1983. Sequence of the site-specific recombinase gene *cin* and of its substrates serving in the inversion of the C segment of bacteriophage P1. EMBO J. **2**: 1733–1740.
25. **Howe, M. M.** 1980. The invertible G segment of phage Mu. Cell **21**:605–606.
26. **Howe, M. M., J. W. Schumm, and A. L. Taylor.** 1979. The S and U genes of bacteriophage Mu are located in the invertible G segment of Mu DNA. Virology **92**:108–124.
27. **Huber, H. E., S. Iida, W. Arber, and T. A. Bickle.** 1985. Site-specific DNA inversion is enhanced by a DNA sequence element in *cis*. Proc. Natl. Acad. Sci. USA **82**:3776–3780.
28. **Hughes, D. E., and G. W. Pugh.** 1970. A five-year study of infectious bovine keratoconjunctivitis in a beef herd. J. Am. Vet. Med. Assoc. **157**:443–451.
29. **Hull, R. A., R. E. Gill, P. Hsu, B. H. Minshew, and S. Falkow.** 1981. Construction and expression of recombinant plasmids encoding type 1 or D-mannose-resistant pili from a urinary tract infection *Escherichia coli* isolate. Infect. Immun. **33**:933–938.
30. **Humphreys, G. O., G. A. Willshaw, and E. S. Anderson.** 1975. A simple method for the preparation of large quantities of pure plasmid DNA. Biochim. Biophys. Acta **383**:457–463.
31. **Johnson, R. C., M. F. Bruist, and M. I. Simon.** 1986. Host protein requirements for in vitro site-specific DNA inversion. Cell **46**:531–539.
32. **Johnson, R. C., and M. I. Simon.** 1985. Hin-mediated site-specific recombination requires two 26 bp recombination sites and a 60 bp recombinational enhancer. Cell **41**:781–791.
33. **Kahmann, R., F. Rudt, C. Koch, and G. Mertens.** 1985. G inversion in bacteriophage Mu DNA is stimulated by a site within the invertase gene and a host factor. Cell **41**:771–780.
34. **Kamp, D., and R. Kahmann.** 1981. The relationship of two invertible segments in bacteriophage Mu and *Salmonella typhimurium* DNA. Mol. Gen. Genet. **184**:564–566.
35. **Kamp, D., E. Kardas, W. Ritthaler, R. Sandulache, R. Schmucker, and B. Stern.** 1984. Comparative analysis of invertible

DNA in phage genomes. Cold Spring Harbor Symp. Quant. Biol. **49**:301–311.
36. **Klemm, P.** 1986. Two regulatory *fim* genes, *fim*B and *fim*E, control the phase variation of type 1 fimbriae in *Escherichia coli*. EMBO J. **5**:1389–1393.
37. **Klemm, P., B. J. Jorgensen, I. van Die, H. de Ree, and H. Bergmans.** 1985. The *fim* genes responsible for synthesis of type 1 fimbriae in *Escherichia coli*, cloning and genetic organization. Mol. Gen. Genet. **199**:410–414.
38. **Kutsukake, K., T. Nakao, and T. Iino.** 1985. A gene for DNA invertase and an invertible DNA in *Escherichia coli* K-12. Gene **34**:343–350.
39. **Labigne-Roussel, A. F., D. Lark, G. Schoolnik, and S. Falkow.** 1984. Cloning and expression of an afimbrial adhesin (AFA-I) responsible for P blood group-independent, mannose-resistant hemagglutination from a pyelonephritic *Escherichia coli* strain. Infect. Immun. **46**:251–259.
40. **Lund, B., F. P. Lindberg, M. Båga, and S. Normark.** 1985. Globoside-specific adhesins of uropathogenic *Escherichia coli* are encoded by similar *trans*-complementable gene clusters. J. Bacteriol. **162**:1293–1301.
41. **Marrs, C. F., G. Schoolnik, J. M. Koomey, J. Hardy, J. Rothbard, and S. Falkow.** 1985. Cloning and sequencing of a *Moraxella bovis* pilin gene. J. Bacteriol. **163**:132–139.
42. **McKern, N. M., I. J. O'Donnell, A. S. Inglis, D. J. Stewart, and B. L. Clark.** 1983. Amino acid sequence of pilin from *Bacteroides nodosus* (strain 198), the causative organism of ovine footrot. FEBS Lett. **164**:149–153.
43. **Meyer, T. F., E. Billyard, R. Haas, S. Storzbach, and M. So.** 1984. Pilus genes of *Neisseria gonorrhoeae*: chromosomal organization and DNA sequence. Proc. Natl. Acad. Sci. USA **81**: 6110–6114.
44. **Meyer, T. F., N. Mlawer, and M. So.** 1982. Pilus expression in *Neisseria gonorrhoeae* involves chromosomal rearrangement. Cell **30**:45–52.
45. **Orndorff, P. E., and S. Falkow.** 1984. Identification and characterization of a gene product that regulates type 1 piliation in *Escherichia coli*. J. Bacteriol. **160**:61–66.
46. **Orndorff, P. E., P. A. Spears, D. Schauer, and S. Falkow.** 1985. Two modes of control of *pilA*, the gene encoding type 1 pilin in *Escherichia coli*. J. Bacteriol. **164**:321–330.
47. **Pasloske, B. L., B. B. Finlay, and W. Paranchych.** 1985. Cloning and sequencing of the *Pseudomonas aeruginosa* PAK pilin gene. FEBS Lett. **183**:408–412.
48. **Pedersen, K. B., L. O. Froholm, and K. Bovre.** 1972. Fimbriation and colony type of *Moraxella bovis* in relation to conjunctival colonization, and development of keratoconjunctivitis in cattle. Acta Pathol. Microbiol. Scand. Sect. B **80**:911–918.
49. **Plasterk, R. H. A., A. Brinkman, and P. van de Putte.** 1983. DNA inversions in the chromosome of *Escherichia coli* and in bacteriophage Mu: relationship to other site-specific recombination systems. Proc. Natl. Acad. Sci. USA **80**:5355–5358.
50. **Plasterk, R. H. A., R. Kanaar, and P. van de Putte.** 1984. A genetic switch *in vitro*: DNA inversion by Gin protein of phage Mu. Proc. Natl. Acad. Sci. USA **81**:2689–2692.
51. **Plasterk, R. H. A., and P. van de Putte.** 1985. The invertible P-DNA segment in the chromosome of *Escherichia coli*. EMBO J. **4**:237–242.
52. **Pugh, G. W., D. E. Hughes, and G. D. Booth.** 1977. Experimentally induced infectious bovine keratoconjunctivitis: effectiveness of a pilus vaccine against exposure to homologous strains of *Moraxella bovis*. Am. J. Vet. Res. **38**:1519–1522.
53. **Rothbard, J. B., and G. K. Schoolnik.** 1985. The primary sequence and antigenic structure of gonococcal pilin: approaches towards a gonococcal vaccine Adv. Exp. Med. Biol. **185**:247–273.
54. **Sandhu, T. S., F. H. White, and C. F. Simpson.** 1974. Association of pili with rough colony type of *Moraxella bovis*. Am. J. Vet. Res. **35**:437–439.
55. **Sanger, F., A. R. Coulson, B. G. Barrell, A. J. H. Smith, and B. A. Roe.** 1980. Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. **143**:161–178.
56. **Sastry, P. A., B. B. Finlay, B. L. Pasloske, W. Paranchych, J. R. Pearlstone, and L. B. Smillie.** 1985. Comparative studies of the amino acid and nucleotide sequences of pilin derived from *Pseudomonas aeruginosa* PAK and PAO. J. Bacteriol. **164**:571–577.
57. **Schoolnik, G. K., R. Fernandez, Y. T. Tai, J. Rothbard, and E. C. Gothschlich.** 1984. Gonococcal pili: primary structure and receptor binding domain. J. Exp. Med. **159**:1351–1370.
58. **Segal, E., E. Billyard, M. So, S. Storzbach, and T. F. Meyer.** 1985. Role of chromosomal rearrangement in *N. gonorrhoeae* pilus phase variation. Cell **40**:293–300.
59. **Segal, E., P. Hagblom, H. S. Seifert, and M. So.** 1986. Antigenic variation of gonococcal pilus involves assembly of separated silent gene segments. Proc. Natl. Acad. Sci. USA **83**:2177–2181.
60. **Silverblatt, F. J.** 1974. Host-parasite interaction in the rat renal pelvis: a possible role for pili in the pathogenesis of pyelonephritis. J. Exp. Med. **140**:1696–1711.
61. **Silverblatt, R. J., and I. Ofek.** 1975. Effects of pili on susceptibility of *Proteus mirabilis* to phagocyosis and on adherence to bladder cells, p. 49–59. *In* E. H. Kass and W. Brumfitt (ed.), Infections of the urinary tract. University of Chicago Pres, Chicago.
62. **Silverman, M., and M. Simon.** 1983. Phase variation and related systems, p. 537–557 *In* J. Shapiro (ed.), Mobile genetics elements. Academic Press, Inc., New York.
63. **So, M., E. Billyard, T. F. Meyer, and E. Segal.** 1985. Regulation of pilus phase variation in *Neisseria gonorrhoeae*, p. 287–291. *In* L. Leive (ed.), Microbiology—1985. American Society for Microbiology, Washington, D.C.
64. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
65. **Sparling, P. F., J. G. Cannon, and M. So.** 1986. Phase and antigenic variation of pili and outer membrane protein II of *Neisseria gonorrhoeae*. J. Infect. Dis. **153**:196–201.
66. **Strom, M. S., and S. Lory.** 1986. Cloning and expression of the pilin gene of *Pseudomonas aeruginosa* PAK in *Escherichia coli*. J. Bacteriol. **165**:367–372.
67. **Swanson, J.** 1983. Gonococcal adherence: selected topics. Rev. Infect. Dis. **5**(Suppl. 4):S678–S684.
68. **Swanson, J., S. Bergstrom, K. Robbins, O. Barrera, D. Corwin, and J. M. Koomey.** 1986. Gene conversion involving the pilin structural gene correlates with pilus + pilus − changes in *Neisseria gonorrhoeae*. Cell **47**:267–276.
69. **Szekely, E., and M. Simon.** 1981. Homology between the invertible deoxyribonucleic acid sequence that controls flagellar-phase variation in *Salmonella* sp. and deoxyribonucleic acid sequences in other organisms. J. Bacteriol. **148**:829–836.
70. **Taylor, R. K., V. L. Miller, D. B. Furlong, and J. J. Mekalanos.** 1987. Use of *pho*A gene fusions to identify a pilus colonization factor coordinately regulated with cholera toxin. Proc. Natl. Acad. Sci. USA **84**:2833–2837.
71. **Toussaint, A., N. Lefebvre, J. R. Scott, J. A. Cowan, F. de Bruijn, and A. I. Bukhari.** 1978. Relationships between temperate phages Mu and P1. Virology **89**:146–163.
72. **van de Putte, P., S. Cramer, and M. Giphart-Gassler.** 1980. Invertible DNA determines host specificity of bacteriophage Mu. Nature (London) **286**:218–222.