Functional Analysis of the Adsorption Protein of Two Filamentous Phages with Different Host Specificities

By PETER BROSS, KLAUS BUßMANN, WALTRAUD KEPPLER
AND IHAB RASCHED*

Faculty of Biology, University of Konstanz, Postfach 5560, D-7750 Konstanz 1, FRG

(Received 9 June 1987; revised 25 September 1987)

The gene 3 coding for one minor coat protein (adsorption protein) of phage IK€ was cloned into an expression plasmid and overproduced. The presence of a promoter for this gene could be demonstrated as well as the incorporation of the IK€ gene 3 protein (g3p) into the cytoplasmic membrane of host cells. When 110 carboxy-terminal amino acids were deleted, the truncated protein was translocated across the cytoplasmic membrane into the periplasm. Thus the deleted amino acids bear a membrane anchor domain. In contrast to the partly homologous g3p of the Ff phages, IK€ g3p did not alter the membrane properties of its host. IK€ g3p was not incorporated into Ff phage particles in amounts detectable by our assays although the presence of IK€ g3p may affect the efficiency of Ff phage production. The existence of a structural feature necessary for the specific recognition of the respective g3p during phage assembly is deduced.

INTRODUCTION

The filamentous N-specific phage IK€ and the filamentous F-specific phages fd, fl and M13 (Ff phages; for a recent review see Rasched & Oberer, 1986) have probably evolved from a common ancestor. IK€ and Ff phages have an identical gene number and gene order and their genomes display 55% homology (Peeters et al., 1985). IK€ infects Escherichia coli cells carrying a plasmid of the N incompatibility group while Ff phages specifically infect E. coli cells harbouring F factor. For both phages the tip of the pilus (N- or F-pilus respectively) serves as the receptor on the surface of the host cell (Bradley, 1979; Jacobson, 1972).

The gene 3 proteins of Ff and IK€ are minor constituents of the rod-shaped phage particle. Ff gene 3 has been thoroughly investigated: its transcription starts from a weak promoter which directly precedes the gene (Smits et al., 1984) and partly overlaps with the rho-independent terminator for the preceding gene (gene 8). Ff gene 3 protein (g3p) is synthesized as a 424 amino acid precursor. Upon incorporation into the host cytoplasmic membrane the 'leader' peptide is cleaved off (Konings et al., 1975; Armstrong et al., 1981; Beck & Zink, 1981). The mature protein remains anchored in the membrane by a carboxy-terminal anchor sequence until it is incorporated into nascent phage particles (Boeke & Model, 1982a; Davis et al., 1985). Ff g3p is necessary for adsorption to the tip of the F-pilus on infection (Pratt et al., 1969). The receptor recognition site probably resides within a sequence of 169 amino acid residues in the amino-terminal half of Ff g3p (Nelson et al., 1981). As has been established by Boeke et al. (1982), Ff g3p induces pleiotropic effects in the host cell: (a) superinfection with other F-pilus specific (DNA- and RNA-) phages is blocked; (b) F-conjugation is strongly inhibited; (c) the cells show a higher sensitivity to detergents such as deoxycholate; and (d) the cells have a higher tolerance to certain colicins (Zinder, 1973; Smilowitz, 1974). A truncated g3p consisting of only the 115 amino terminal amino acid residues is sufficient to cause most of the effects. Within this amino

Abbreviations: RF, replicative form; TCA, trichloroacetic acid; X-Gal, 5-chloro-4-bromo-3-indolyI β-D-galactoside.

0001-4228 © 1988 SGM
terminal part of the protein is one of two glycine-rich domains occurring in g3p. Boeke et al. (1982) presented evidence that the amino-terminal glycine-rich domain represents the structural information responsible for these effects.

By comparing the amino acid sequences of the IKe gene products derived from the DNA sequence with the respective counterparts of the Ff phages, Peeters et al. (1985) found that there is considerable difference in the extent of homology from one gene to another. In general, genes which code for capsid proteins are less homologous (15.2-39.4%) than the other genes (44.3-58.7%). The gene products with least homology are gdp (20.7%) and g3p (15.2%), both of which are present at that end of the phage particle that forms the adsorption site. In g3p the homologous segments are occasionally found at different positions within the primary sequence of the gene 3 proteins indicating that rearrangements may have taken place (Peeters et al., 1985).

In the present study we have tried to correlate several regions in IKe gene 3 proteins with functions of distinct domains within both proteins and amino acid homology is discussed.

METHODS

Bacteria, phages and plasmids. The genotype of the bacterial strains used is given in parentheses: K38 (HfrC su-; Lyons & Zinder, 1972); M5219 (M72lacZam_trp_amSm8 β bio252 c1857 ΔH1; Remaut et al., 1981); JE2571/N3 (leu thr str fli pil; Bradley, 1980); TL45 [araD139 ΔlacU169 rpsL relA thiA gpr β (ΔgprT-gprA)593; Larson et al., 1982]; JM103 [Δlac-pro] thi strA supE endA shcB15 hsdR4 F'(traD36 proAB lac) lacZ AM15]; Messing et al., 1981], MBM7014[F- araC arD MARGF-lac]U169 trp_am_malB_am rpsL rrelA thi.supF; Silhavy et al., 1984]; MC1061 [araD139 Δara-leu]7697 lac74 galU hsdR rpsL; Minton, 1984]. Phage IKe was a generous gift of Dr R. N. H. Konings (University of Nijmegen, Netherlands); phage fd was from our own collection. M13mp9 (Messing & Vieira, 1982) was obtained from BRL. M13mp18 was described by Yanisch-Perron et al. (1985). The vector plasmids were described by the following authors: pBR322 (Bolivar et al., 1977); pUR222 (Rüther et al., 1981); pPla2311 (Remaut et al., 1981); pNM480-482 (Minton, 1984).

Media, enzymes and chemicals. Bacteria were grown in LB-medium (Miller, 1972), which in the case of recombinant plasmid-carrying strains was supplemented with the appropriate antibiotic. Restriction enzymes and T4 ligase were purchased from Boehringer. DNA polymerase I (Klenow fragment) was from Pharmacia. BAL-31 nuclease was from BRL. Radiochemicals were purchased from Amersham. Alkaline-phosphatase-conjugated goat anti-rabbit antibodies and low-melting-point agarose were obtained from Sigma.

Isolation of replicative form DNA. Replicative form (RF) DNA was isolated from infected or transformed cells by the alkaline lysis method as described by Maniatis et al. (1982). For larger preparations the DNA was further purified by ethidium-bromide/CsCl density gradient centrifugation.

Recombinant DNA techniques and DNA sequencing. Buffers for restriction enzyme digests and ligation were prepared as recommended by the supplier. For cloning, restriction fragment separations were by agarose gel electrophoresis using low gelling type agarose. The gel was stained with ethidium bromide and the desired bands were excised with a scalpel and stored at 4 °C. For ligation excised bands (about 0.5 pmol in 20 μl) were melted at 65 °C and 2 μl of each of the fragments to be ligated was pipetted into an Eppendorf tube containing 11 μl H2O at 65 °C. This tube was then cooled to room temperature, and 2 μl 10 x ligation buffer (100 mM-Tris/Cl, pH 7-5; 12 mM-MgCl2; 10 mM-DTT), 2 μl 10 μM-ATP and 1 μl (1 unit) T4 ligase were added. Incubation was at room-temperature overnight. Transfection and transformation were done as described by Maniatis et al. (1982).

For BAL-31 digestion, 3 μg of plW1 were linearized with EcoRI. After phenol extraction and ethanol precipitation the DNA was resuspended in 50 μl BAL-31 buffer (12 mM-CaCl2; 12 mM-MgCl2; 0.2 mM-NaCl; 20 mM-Tris/Cl, pH 8.0; 1 mM-EDTA; 0.25 mg BSA ml-1) and incubated for 3 min at 28 °C. BAL-31 [1 μl (1 unit)] was added and after 1, 2 and 3 min samples (17 μl) were taken and diluted into 200 μl 'stop mixture' containing EDTA to give a final concentration of 0.2 mM-EDTA. After phenol extraction and ethanol precipitation the DNA was ligated overnight at 4 °C and subsequently used to transform E. coli M5219.

Single-stranded DNA was isolated from recombinant plasmid plaques as described by Sanger et al. (1980). For DNA sequencing the chain-termination method was used (Sanger et al., 1977).

Infection tests and determination of detergent sensitivity. For infection tests 100 μl of serial dilutions of phage were plated with 200 μl of overnight cultures of the respective bacteria. The strains carrying one of the recombinant plasmids were grown and plated in the presence of the appropriate antibiotic.

Detergent sensitivity was determined by plating on plates containing 1.5% (w/v) deoxycholate (Boeke et al., 1982).
Adsorption proteins of filamentous phages

Fig. 1. Homology between the gene 3 proteins of IKe and fd. Homologous stretches were localized by alignment of the amino acid sequences of both proteins as described in the text. Homologous regions are joined by the bars emphasized with squares. Since the glycine-rich domains in fd g3p show high homology to several stretches within the corresponding domain in IKe g3p the entire domains of fd g3p were aligned with the IKe g3p sequence and those alignments displaying highest homology are shown. Under these conditions the two segments in IKe g3p with highest homology to the two domains in fd g3p overlap between amino acids 236 and 246. The scale gives the amino acid position. Defined domains in both proteins are emphasized: ■, 'leader' sequence; □, glycine-rich region; ⊙, membrane anchor sequence.

Cell fractionation procedures and detection of phage proteins by Western blotting. M5219 cells transformed with one of the plasmids were grown at 28 °C until a OD578 of 0.2 was reached; the cells were then shifted to 42 °C and grown for 1 h at this temperature. For analysis of unfractionated proteins 1 ml of cell culture was precipitated with 125 µl ice-cold 100% trichloroacetic acid (TCA) (30 min at 0 °C.) After centrifugation the pellet was washed twice with acetone, dried and resuspended in 200 µl of sample buffer per 1 OD578 unit of the culture.

Alkali fractionation was done as described by Russel & Model (1982). Cell culture (5 ml) was mixed with 25 ml ice-cold 0.1 M-NaOH, vortexed vigorously and centrifuged for 20 min at 27 000 g. The supernatant fraction was precipitated by addition of 2.75 ml 100% TCA. After 30 min at 0 °C, precipitated proteins were spun down as above. Both pellets were washed with 1 ml 10% (w/v) TCA and finally dissolved in 100 µl sample buffer per 1 OD578 unit of cell culture.

Osmotic shock treatment was as described by Neu & Heppel (1965).

Electrophoresis of protein samples through SDS-polyacrylamide gels (12.5%, w/v, acrylamide) was done using the discontinuous buffer system described by Laemmli (1970). The immunoblotting technique was as described by Towbin et al. (1979). Electrophoretic transfer was at 250 mA for 3 h. Bound antibodies were stained following the protocol of Blake et al. (1984).

RESULTS AND DISCUSSION

Amino acid homology between g3p of IKe and fd

The gene 3 proteins of the related phages IKe and Ff present an example of two polypeptides with analogous functions but little homology (Peeters et al., 1985). We determined the presence of homology by searching for segments of 10 amino acids ('marker' segments) in fd g3p which could be aligned with a correspondence of at least five with 10 amino acids in a continuous segment in IKe g3p. Fig. 1 shows homologous regions that were localized using this alignment technique. Only larger homologous sections comprising one or several of the above 'marker' segments plus adjacent homologous sequences, allowing for short insertions and deletions, are included. Characteristic sequences such as the glycine-rich, and the leader- and anchor-type sequences occurring in both gene 3 proteins are emphasized. One larger section in both proteins - amino acids 11 to 109 in IKe g3p and amino acids 106 to 195 in fd g3p - does not have any homology satisfying these criteria. The regions with clearly identifiable homology occur occasionally at the same site within the primary sequence of both proteins (e.g. carboxy-terminal sequences) and sometimes at different sites (e.g. amino acids 137–193 in IKe are homologous to amino acids 23–79 in fd) (Peeters et al., 1985). The two glycine-rich sequences in fd g3p appear to occur in one domain in the IKe g3p. Whether distinct stretches in the IKe g3p fulfil the same functions as their homologous counterparts in Ff g3p was investigated in this study using genetic cloning techniques.
Fig. 2. Strategy for cloning of IKe gene 3. IKe RF DNA was digested with \textit{TaqI} and the fragments were shotgun-cloned into the \textit{AccI} site of vector pUR222. That of the resulting population of plasmids which contained the \textit{TaqI} A fragment with gene 3 in opposite orientation to \textit{lacZ} (pRW1) was identified by restriction analysis of a number of clones. The insert was subcloned into pPla2311 to give pLW1. Subsequently, the ribosome-binding site and part of the coding sequence of gene 8 were removed by BAL-31 digestion starting from the \textit{EcoRI} site. After recircularization and restriction analysis of several clones, one (pLW16) with a suitable deletion was chosen and the \textit{XhoI}/\textit{PstI} fragment containing the phage sequence was reintroduced into pPla2311 via an intermediate (pRW2). In order to exactly determine the extent of the deletion, the \textit{EcoRI}/\textit{PstI} B fragment of pLW17 was cloned between the respective sites in M13mp9 and the sequence proximal to the \textit{EcoRI} site was determined by dideoxy sequencing. By comparison of the sequence obtained with those published for IKe and the Km\textsuperscript{R} gene (Peeters et al., 1985; Oka et al., 1981) it turned out that altogether 676 bp had been removed: 169 bp between the \textit{EcoRI} site and gene 3 and 507 bp between the \textit{EcoRI} site and the Km\textsuperscript{R} gene.

\textbf{Construction of gene 3 plasmids}

Our aim was to construct plasmids containing either IKe gene 3 or fd gene 3. The strategy used to clone IKe gene 3 is represented in Fig. 2 and explained in the legend. The final plasmid, pLW17, contains nucleotides 1894–3580 of IKe RF (Peeters et al., 1985) comprising the coding sequence for the carboxy-terminal half of gene 8, the whole gene 3 and about two-thirds of gene 6. The orientation of the phage genes is the same as that of the lambda P\textsubscript{1}-promoter.
Adsorption proteins of filamentous phages

Fig. 3. Identification of IKe and fd g3p by Western blotting. M5219 cells transformed with the plasmids indicated were grown and induced as described in Methods. Cells were prepared for electrophoresis and loaded on a SDS-polyacrylamide gel (12.5%, w/v, acrylamide). Purified phage (IKe and fd) was dissociated in sample buffer and run in the same gel. After electrophoresis, proteins were transferred to a nitrocellulose sheet. The sheet was incubated with anti-IKe antibodies (a) or anti-fd antibodies (b) respectively and bound antibodies were visualized as described in Methods. The two sheets shown originate from one gel on which the samples were loaded twice. The molecular masses and positions of the coelectrophoresed molecular mass standard proteins are indicated on the right.

For cloning of fd gene 3 the Alul-A fragment of fd RF (nucleotides 1519–2964; Beck & Zink, 1981) was first introduced into the HindII site of vector pUR222. That of the resulting plasmids in which the EcoRI site of the linker was upstream of gene 3 was digested with EcoRI and PstI and the fragment containing the phage DNA was introduced between the respective sites of vector p PLA2311. The plasmid obtained, pLK15, contains the intergenic region between gene 8 and gene 3, the whole of gene 3 and about one-third of gene 6. The phage genes have the same orientation as the PL-p promoter.

Overexpression and detection of cloned gene 3

For overproduction of the cloned gene 3 proteins E. coli strain M5219 was chosen as host. This strain harbours a defective lambda lysogen comprising a temperature-sensitive cl-repressor and the lambda N-protein (Remaut et al., 1981). At low temperature (28 °C) the P1-p promoter will be repressed. With a temperature shift to 42 °C the cl-repressor is inactivated and P1-directed transcription is induced. The rho-independent terminator contained within the intergenic region between gene 8 and gene 3 of fd can be overriden by the action of lambda N-protein (Remaut et al., 1981). An equivalent sequence which presumably acts as transcription terminator is also present between gene 8 and gene 3 of IKe (Peeters et al., 1985). The use of M5219 as host therefore prevents premature termination of P1-driven transcription at the termination sequences preceding the respective gene 3 in plasmids pLW17 and pLK15.

M5219 cells transformed with pLW17 or pLK15 were grown at 28 °C, shifted to 42 °C and incubated for 1 h at this temperature. The proteins synthesized were separated by SDS-PAGE and the phage coat proteins were identified by immunoblotting. The primary antibodies used were prepared against purified (CsCl density gradient) IKe or fd phage respectively. Fig. 3 shows blots of samples run in the same gel after reaction with anti-IKe or anti-fd antibodies. G8p binds only weakly to the nitrocellulose membrane using our procedure and is therefore not visualized, although this protein is present in much higher quantities in purified phage than g3p. Anti-IKe antibodies effectively bind to IKe g3p and anti-fd antibodies to fd g3p. However, there is no cross-reaction between anti-IKe antibodies and fd g3p or between anti-fd antibodies and IKe g3p.
In M5219 cells transformed with pLW17 a band comigrating with IKe g3p from purified phage can be visualized with anti-IKe antibodies. This band is not present in cells transformed with pLK15. If anti-fd antibodies are used a band comigrating with fd g3p is visualized only in cells carrying pLK15. The faintly visible extraneous bands may partially be due to contaminating activity against *E. coli* proteins present in the antibody preparation or to degradation products of the respective gene 3 proteins. The band visible in B in the track with pLW17 which migrates slightly faster than g3p is not a degradation product of IKe g3p since this band is not visible if the anti-IKe antibodies are used. These results show that the respective gene 3 proteins are synthesized in detectable amounts using our constructed plasmids. The molecular mass of IKe g3p deduced from its electrophoretic behaviour is much higher (about 72 kDa) than that deduced from the DNA sequence (42 kDa). In this respect IKe 3p resembles the Ff protein, which is known to migrate much slower in SDS-polyacrylamide gels than would be expected from its actual molecular mass.

Localization of IKe g3p and a carboxy-terminally truncated g3p* in the host cell

Localization of Ff g3p in the host cell is determined by an amino-terminal leader sequence and a carboxy-terminal anchor sequence. IKe g3p also possesses an amino-terminal leader sequence which fulfils the requirements of standard leader sequences (Perlman & Halvorson, 1983) but is only homologous to Ff g3p within the first 10 amino acids. The anchor sequence of Ff g3p consists of a stretch of 23 uncharged, mostly hydrophobic, amino acid residues flanked by positively charged residues. Deletion of this part of the protein results in the loss of the anchoring capacity (Davis et al., 1985). In Ike g3p, there exists an equivalent stretch of amino acids surrounded by positively charged residues at the carboxy terminus. The questions we addressed were (a) is IKe g3p incorporated into the membrane and (b) is IKe g3p anchored by the homologous carboxy-terminal domain? We constructed a plasmid which contains an inducible IKe g3p* in which the codons for the 110 carboxy-terminal amino acid residues are deleted. For this purpose pLW17 was digested with *EcoRI* and *HindII* and the appropriate fragment (see Fig. 2) was cloned between the *EcoRI* and *SmaI* sites of mp18. By sequencing the *SmaII/HindII* junction it was determined that during cloning a G at the junction had been lost. As a consequence of this deletion the translational reading frame of gene 3 is interrupted at a TAG nonsense codon contained in the linker sequence three codons downstream from codon 324 of IKe gene 3. The cloned fragment was reexcised with *EcoRI* and *PstI* and introduced between the respective sites of pPla2311 downstream of the P1 promoter so producing plasmid pLW18.

M5219 cells transformed with pLW17, pLW18 or pPla2311 were grown at 28 °C and shifted to 42 °C to induce the P1 promoter. After incubation at this temperature for 1 h, samples were either precipitated with TCA or subjected to alkali fractionation in order to separate integral membrane proteins from non-integral membrane-associated and soluble proteins. Fig. 4(a) shows an immunoblot of a representative experiment. The overproduced IKe g3p segregates with the membrane fraction while the carboxy-terminally truncated IKe g3p* is in the soluble fraction. Thus removal of the 110 carboxy-terminal amino acid residues results in loss of the anchoring capacity.

In order to determine whether the g3p* is secreted into the periplasm or remains in the cytoplasm, we subjected cells induced for overproduction to osmotic shock treatment. Fig. 4(b) shows an immunoblot of shock-fractionated cells. The truncated g3p* is quantitatively released into the supernatant by this treatment. These results show that IKe g3p is integrally incorporated into the membrane while g3p* is secreted into the periplasm. This confirms that the leader- as well as the anchor-type sequence in IKe g3p fulfil the expected functions.

Does IKe g3p compete with fd g3p in fd phage assembly?

In order to test whether IKe g3p can be incorporated into an Ff phage in the presence of the Ff g3p, we subcloned IKe gene 3 into an M13 derivative. The *EcoRI/PstI* fragment of pLW17 containing IKe gene 3 was introduced between the respective sites of M13mp18. The resulting construct – mp18LW17 – harbours the IKe gene in the same orientation as the unidirectionally transcribed M13 genes.
**Adsorption proteins of filamentous phages**

Fig. 4. Localization of IKe g3p and g3p* in the cell. Cells transformed with the plasmids indicated were grown and induced as described in the legend to Fig. 3. Subsequently, cells were either prepared directly for electrophoresis (c), subjected to alkali in order to separate integral membrane proteins (p) from soluble or non-integral membrane proteins (s) or fractionated by osmotic shock treatment into periplasmic fraction (f) and the remainder of the cells (x). Samples were run on (12.5%, v/v, polyacrylamide) SDS-polyacrylamide gels and phage coat proteins were identified by immunoblotting using anti-IKe antibodies. IKe: purified phage dissociated in sample buffer. The molecular masses and positions of coelectrophoresed marker proteins are shown on the right.

Mpl8LW17 was propagated in E. coli F+ cells (JM103) and the phage particles produced were purified from the culture supernatant. The plaque-forming ability of this phage and of phage M13mp18 on E. coli cells carrying either an F or an N3 episome was assayed. mpl8LW17 as well as mp18 formed plaques only on F+ cells (data not shown).

In order to determine whether IKe g3p is incorporated into the M13 particles without conferring the ability to use N3 pili for infection, we analysed the proteins present in mp18LW17 phage by Western blotting with anti-fd and anti-IKe antibodies. A band with a molecular mass of about 70 kDa was observed only if anti-fd antibodies were used; anti-IKe antibodies were not effective even if high quantities of phage were loaded on the gel (data not shown). Since this assay gives positive results only if IKe g3p is incorporated in considerable amounts into M13 phage particles we investigated whether the presence of IKe g3p interferes with phage production. Cells were infected with mp18 or mp18LW17 and the time course of production of p.f.u. was monitored. As shown in Fig. 5, production of mp18LW17 phage was slightly slower than that of mp18. Furthermore, the culture infected with mp18LW17 reached a plateau at about $1 \times 10^{10}$ p.f.u. ml$^{-1}$ while mp18 phage were released beyond this level. It is possible, therefore, that the presence of IKe g3p weakly interferes with phage production; alternatively, the difference may be simply due to the presence of additional DNA in mp18LW17. Overall, these data suggest that despite the demonstrated functional and structural homology of several domains in the linear sequence of the gene 3 proteins of IKe and Ff, these two proteins are not complementary. Furthermore, the binding of g3p to the phage particle appears to be mediated by protein–protein interaction rather than direct binding to the single-stranded DNA. This means that the gene 3 proteins bear an as yet unidentified structural feature which signals their specific recognition by the respective progeny assembly apparatus. Non-complementarity of the highly homologous gene 2 proteins of IKe and Ff phages has also been
Fig. 5. Growth curves of phages mp18 (○) and mp18LW17 (○). JM103 cells were grown to a density of $5 \times 10^7$ cells ml$^{-1}$ and then infected with mp18 or mp18LW17 respectively at a multiplicity of infection of 1. The no. of p.f.u. in the supernatant of the cultures was monitored over time.

demonstrated (Peeters et al., 1986). The gene 5 proteins of both phages are interchangeable in their function to bind single-stranded DNA, however the cooperativity of binding resulting from protein–protein interactions is not observed between IKe g5p and M13 g5p (de Jong et al., 1987).

**Expression signals for IKe gene 3**

Further experiments were done to study whether the expression signals for IKe g3p are contained within the cloned sequence, in order to ensure that the plasmid coded IKe g3p is indeed synthesized even in the cases where $P_L$ induced overexpression is not effective. We constructed translational fusions of IKe gene 3 and lacZ. For this purpose we used the three pNM-vectors described by Minton (1984) which allow the construction of translational fusions in all three reading frames with respect to lacZ. The truncated gene 3 of pLW18 was excised with EcoRI/PstI and introducted into each of the three pNM-plasmids. The resulting constructs were called pNW18-0, pNW18-1 and pNW18-2. As deduced from the DNA sequences, the IKe gene 3 reading frame should fuse with that of lacZ only in pNW18-1. However, the TAG codon mentioned above, which is also present in this construct allows translation of the fusion protein only in strains carrying an amber suppressor. These plasmids were transformed into strains MC1061 (sur$^-$) and MBM7014 (supF) and plated on ampicillin/X-gal plates. Blue colonies were formed only if MBM7014 was transformed with pNW18-1. This indicates that as in Ff phages, a promoter is present upstream of gene 3 which promotes transcription of IKe gene 3. Therefore the transcriptional control elements, with partly overlapping weak promoter/strong terminator at the border between the frequently and the unfrequently transcribed regions of the IKe and Ff phage genomes, appear to be identical.

**Tests for pleiotropic effects**

The function of the glycine-rich regions in Ff g3p is still unclear. Boeke et al. (1982) presented evidence that the first (amino terminal) of these domains in Ff g3p may trigger the previously mentioned pleiotropic effects. Peeters et al. (1985) suggest that host cells infected with IKe do
Adsorption proteins of filamentous phages

Table 1. Test for pleiotropic effects of gene 3 of IKe and fd

The strains indicated were transformed with the respective plasmids. Phage and detergent sensitivity were analysed as described in Methods. R, resistant; S, sensitive.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid (gene 3 contained)</th>
<th>Sensitivity to filamentous phages:</th>
<th>Sensitivity to desoxycholate</th>
</tr>
</thead>
<tbody>
<tr>
<td>JE2571/N3</td>
<td>-</td>
<td>fd R</td>
<td>IKe S</td>
</tr>
<tr>
<td></td>
<td>pLa2311 (none)</td>
<td>fd R</td>
<td>IKe S</td>
</tr>
<tr>
<td></td>
<td>pLK15 (fd)</td>
<td>fd R</td>
<td>IKe S</td>
</tr>
<tr>
<td></td>
<td>pLW16 (IKe)</td>
<td>fd R</td>
<td>IKe S</td>
</tr>
<tr>
<td></td>
<td>pLW17 (IKe)</td>
<td>fd R</td>
<td>IKe S</td>
</tr>
<tr>
<td>K38 (Hfr)</td>
<td>-</td>
<td>fd S</td>
<td>IKe R</td>
</tr>
<tr>
<td></td>
<td>pLa2311 (none)</td>
<td>fd S</td>
<td>IKe R</td>
</tr>
<tr>
<td></td>
<td>pBK15 (fd)</td>
<td>fd S</td>
<td>IKe R</td>
</tr>
<tr>
<td></td>
<td>pLK15 (fd)</td>
<td>fd S</td>
<td>IKe R</td>
</tr>
<tr>
<td></td>
<td>PLW16 (IKe)</td>
<td>fd S</td>
<td>IKe S</td>
</tr>
<tr>
<td></td>
<td>pLW17 (IKe)</td>
<td>fd S</td>
<td>IKe S</td>
</tr>
<tr>
<td>TL45/N3-F1ac</td>
<td>-</td>
<td>fd S</td>
<td>IKe S</td>
</tr>
<tr>
<td></td>
<td>pLa2311 (none)</td>
<td>fd S</td>
<td>IKe S</td>
</tr>
<tr>
<td></td>
<td>pLK15 (fd)</td>
<td>fd S</td>
<td>IKe S</td>
</tr>
<tr>
<td></td>
<td>PLW16 (IKe)</td>
<td>fd S</td>
<td>IKe S</td>
</tr>
<tr>
<td></td>
<td>pLW17 (IKe)</td>
<td>fd S</td>
<td>IKe S</td>
</tr>
</tbody>
</table>

not show comparable pleiotropic effects. We used several plasmids carrying gene 3 of IKe to investigate whether IKe g3p affects detergent sensitivity and infectibility of different host bacteria. The IKe gene 3 plasmids used were pLW17 and pLW16. pLW16 is the intermediate in the construction of pLW17 after BAL-31 digestion. In this plasmid no P_L-promoter is present. For tests with fd gene 3 the plasmids pLK15 and pBK15 were used. pBK15 was constructed by introduction of the EcoRI/PstI fragment of pLK15 containing fd gene 3 between the respective sites of pBR322. In pLW16 and pBK15 transcription of the respective genes 3 is controlled only by the gene 3 promoters. This is important since the strains used for the tests carry no lambda cI-repressor and elevated expression levels resulting from initiation of transcription at P_L and eventual read-through over the terminator might influence the results.

We tested the strains K38 (Hfr), JE2571/N3 and TL45/F-N3. The latter strain was constructed by transferring an F-lac and an N3 episome in two consecutive matings into E. coli TL45. The F- and N-plasmids belong to different incompatibility groups and therefore can be maintained in the same strain. Table 1 summarizes the results. The presence of IKe gene 3 caused no changes in detergent sensitivity or infectibility with fd or IKe in the strains tested. fd gene 3, as expected, enhanced detergent sensitivity. Infection with fd but not with IKe is blocked by fd gene 3 in normally permissive cells. This indicates that fd gene 3 influences the function of F-pili but not that of N-pili. This is confirmed by the observation that N3-dependent-conjugation is not influenced by fd gene 3 while F-conjugation is shut off (data not shown). Therefore we can conclude that IKe g3p does not have the same effects on the host cell as Ff g3p. It is then also possible that the pleiotropic effects observed with Ff g3p are a byproduct of certain properties of the protein with no positive or negative consequences on the phage life cycle.

One of the glycine-rich domains in Ff g3p has been implicated in the pleiotropic effects (Boeke & Model, 1982). Although the Ff g3p mitigated pleiotropic effects are not observed with IKe g3p, the latter also contains a glycine-rich domain which is homologous to the Ff g3p domains. The domain in IKe g3p (D-G-D-T-G-G-T-G-E-G-G-S-D-T-G-G-D-T-G-G-S-T-G-G-G-S-S-G-G-G-S-S-G-G-G-S-S-G-G-G-S-S-G-G-G-S-S-G-G-G-S-T-G) does not show the same uniform order (repeats of E-G-G-G-S and G-G-G-S) as those in Ff. However, there is a striking similarity in the type of amino acids contained: glycine, amino acids with acidic side chains (E, D) and residues with polar side chains (S, T). In the IKe domain aspartic acid is preferred to
glutamic acid and threonine to serine. The use of an acidic amino acid with a side chain one carbon unit shorter seems to be balanced by use of the polar amino acid threonine (one carbon unit longer than serine) indicating that steric demands might be similar within the glycine-rich regions. This suggests that the glycine-rich domains may have a primarily structural role and that the implied involvement of one of the domains with the pleiotropic effects may be wrong.

Interest in glycine-rich proteins has increased recently. Such proteins are prominent in the cell wall of certain plant species and in spider web and cocoon silk (for references see Varner & Cassab, 1986). Condit & Meagher (1986) have tentatively proposed a beta-pleated sheet structure with eight anti-parallel strands for a glycine-rich protein of petunia. Our current knowledge suggests that repeating glycine-rich regions are present in proteins with structural function requiring flexibility as well as strength. The fact that sequences hybridizing with the genome (Vassart et al., 1987) underlines the importance of these relatively simple proteins.

We thank Dr B. Böhler-Kohler for her help in the preparation of the manuscript.

REFERENCES


Adsorption proteins of filamentous phages


