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A Component of the Side Tail Fiber of *Escherichia coli* Bacteriophage λ Can Functionally Replace the Receptor-Recognizing Part of a Long Tail Fiber Protein of the Unrelated Bacteriophage T4

DIRK MONTAG,†* HEINZ SCHWARZ, AND ULF HENNING

Max-Planck-Institut für Biologie, D-7400 Tübingen, Federal Republic of Germany

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The distal part of the long tail fiber of *Escherichia coli* bacteriophage T4 consists of a dimer of protein 37. Dimerization requires the catalytic action of protein 38, which is encoded by T4 and is not present in the virion. It had previously been shown that gene *tfa* of the otherwise entirely unrelated phage λ can functionally replace gene 38. Open reading frame (ORF) 314, which encodes a protein that exhibits homology to a COOH-terminal area of protein 37, is located immediately upstream of *tfa*. The gene was cloned and expressed in *E. coli*. An antiserum against the corresponding polypeptide showed that it was present in phage λ. The serum also reacted with the long tail fibers of phage T4 near their free ends. An area of the gene encoding a COOH-terminal region of ORF 314 was recombined, together with *tfa*, into the genome of T4, thus replacing gene 38 and a part of gene 37 that codes for a COOH-terminal part of protein 37. Such T4-λ hybrids, unlike T4, required the presence of outer membrane protein OmpC for infection of *E. coli* B. An *ompC* missense mutant of *E. coli* K-12, which was still sensitive to T4, was resistant to these hybrids. We conclude that the ORF 314 protein represents a subunit of the side tail fibers of phage λ which probably recognize the OmpC protein. ORF 314 was designated *stf* (side tail fiber). The results also offer an explanation for the very unusual fact that, despite identical genomic organizations, T4 and T2 produce totally different proteins 38. An ancestor of T4 from the T2 lineage may have picked up *tfa* and *stf* from a lambdoid phage, thus possibly demonstrating horizontal gene transfer between unrelated phage species.

The distal half of the long tail fibers of the classical *Escherichia coli* bacteriophage T4 consists of a dimer of the 1,026-residue (26) protein 37; the polypeptide chains are arranged in parallel, and the free end of the fiber corresponds to the COOH-terminal ends of the dimer (2, 3). Dimerization of protein 37 requires the catalytic action of protein 38 (39), which is not present in the mature virion (9, 24, 42). Computer analysis revealed that the hypothetical products of two adjacent open reading frames (ORFs), ORFs 314 and 194, in the genome of phage λ (35) exhibit homology to the COOH-terminal area of protein 37 and all of protein 38, respectively, of phage T4 (11, 20). We have cloned ORF 194 and have shown that the corresponding protein could functionally replace protein 38 of T4 (23); the gene was therefore designated *tfa* (tail fiber assembly). On the basis of DNA sequence homology, it had been speculated (20) that the ORF 314 protein is a subunit of the side tail fibers of phage λ (16). Here we show that this most likely is so. The *tfa* gene and part of ORF 314 were recombined into the genome of T4. The resulting hybrid phage had a host range different from that of T4 and, of course, from that of λ; the main receptor for the latter phage is the LamB protein (28).

MATERIALS AND METHODS

Bacterial strains, phages, plasmids, and media. The bacterial strains used are listed in Table 1. Strain 72.6 *ompC169* was selected for resistance to phage TuIb; the resulting mutant OmpC protein carries a single amino acid substitution which will be described elsewhere. λ *c*I857 *S*am7 was from Boehringer, Mannheim, Federal Republic of Germany, λ p*lac*5 (15) was from N. E. Murray. Phages TuIa and TuIb (7) belong to the T4 family of T-even-type phages (24). T4 amB262 (amber in gene 38) was provided by W. B. Wood. The pUC plasmids (43) were used for cloning, and the M13mp18 and pUC19 phages (44), together with strain JM103, were used for DNA sequencing (36). Plasmids pTUλ-6 (23) and pTUT4-4.9i (24) have already been described. pTUompC-65 was derived from pMY111 (22), which carries the *ompC* gene under the control of its own promoter. In pTUompC-65, the gene was placed under the control of the *lac* regulatory elements (unpublished data; the plasmid will be described elsewhere). pDMIb-17 (derived from pUC8) carries a 1.5-kilobase insert from the genome of phage TuIb. The cloned fragment codes for 267 COOH-terminal residues of gene 37, a complete gene 38, and 51 NH_2-terminal residues of gene *t* (unpublished data; the plasmid will be described elsewhere). Cells were grown in L broth (21) supplemented, when required, with ampicillin (20 μg/ml).

Cloning of ORF 314. The ORF 314 gene is located on a 2.296-kilobase *Pvu*II fragment of the λ genome (35). The fragment was isolated from λ cI857 Sam7 by agarose gel electrophoresis and digested with *Pst*I. The resulting 1.73-kilobase *Pvu*II-*Pst*I fragment was ligated into pUC8, linearized with *Sma*I and *Pst*I, to yield pDMλPP3 (Fig. 1).

Construction of plasmid pDMIbλT4. The operations for the construction of plasmid pDM1bλT4 are illustrated in Fig. 1. In plasmid pDMIb-17 (cloned from phage TuIb [see above]), the *Bam*HI site was converted to an *Eco*RI site by cutting with *Bam*HI, filling in with the Klenow fragment of DNA polymerase I, and religation in the presence of the *Eco*RI linker CCGAATTCGG. In the same way, the *Nco*I site was converted to an *Eco*RI site but with the linker CCGGAATTCCGG. The *Pst*I fragment was isolated from

* Corresponding author.
† Present address: Research Institute of Scripps Clinic, La Jolla, CA 92037.

TABLE 1. Bacterial strains

Strain	Genotype[a]	Source and/or reference
B	Prototroph	K. Jann; 27
B/40	Permissive for T4 amber mutants (*supF*?)	E. Goldberg; 33
HfrG6	*his*	W. Boos; 14
JM103	*recA endA gyrA thi hsdR supE relA rpsL sbcB* Δ(*lac-pro*) F′ *traD proAB*$^+$ *lacI*^{q} *Z ΔM15*	J. Messing; 19
F-Z-ΔM15	*thi rpsL recA* Δ(*lac-pro*) φ80 *lac Z ΔM15*	U. Rüther; 34
72.6	*fhuA phoA fadL relA pit spoT rpsE ompF glpT* HfrPO2A	K. Hantke; 5
72.6 *ompC169*	*ompC* (missense) derivative of 72.6	This study
P400.6	*thi argE proA thr leu rpsL non ompC supE*	37
C600	*thi thr leu lac supE*	1

[a] Fermentation markers other than *lac* are not given. Strain F-Z-ΔM15 is abbreviated M15 in this communication.

the plasmid containing these two *Eco*RI sites (Fig. 1, in parentheses on Ib-17) and digested with *Eco*RI. The small *Eco*RI fragment was ligated into pDMλPP3, from which the area delineated by the two *Eco*RI sites had been removed. This operation yielded pDMIbλ (step 1). An *Eco*RI fragment was removed from pTUT4-4.9i, and religation yielded PTUT4-4.9ΔE (step 2). The *Pst*I fragment from pTUλ-6 was cloned into pUC8, resulting in pDMtfaP (step 3). The *Hin*dII-*Hin*dIII fragment was isolated from this plasmid and restricted with *Alu*I, and the *Alu*I-*Hin*dII fragment was ligated into pTUT4-4.9ΔE, which had been linearized with *Sna*BI, resulting in pDMtfaT4t (step 4). Finally, the *Pst*I fragment from pDMtfaT4t was ligated into the *Pst*I site of pDMIbλ, resulting in pDMIbλT4 (step 5). In this plasmid, then, 76 base pairs (bp) from gene 37 of phage TuIb are followed sequentially by 3 bp from the linker, 747 bp of ORF 314, a complete *tfa* gene, 95 bp of a noncoding region, and 556 bp of gene *t* from phage T4. The nucleotide sequence of the beginning of the hybrid gene is shown in Fig. 1. Each construct was verified by DNA sequencing. All operations were executed essentially as already described (18).

DNA-DNA hybridization (40). Detection of the *tfa* gene and gene 38 was performed with probes (see Results) radiolabeled by nick translation (32) as detailed earlier (38). Hybridization was at 42°C in the presence of 50% formamide.

Antiserum. For reasons detailed in Results, the LacZ-ORF 314 fusion protein encoded by pDMλPP3ΔE was used as an antigen. Cells harboring this plasmid were grown in the presence of isopropyl-β-D-thiogalactopyranoside (1 mM) and broken by sonication. About 60% of the protein was found in the supernatant after centrifugation. The protein (largest polypeptide; see Fig. 4, lane 3) was isolated from the supernatant by electrophoresis in a Laemmli-type (17) sodium dodecyl sulfate-12.5% polyacrylamide gel, followed by electroelution. Antiserum was obtained from a rabbit treated with the protein as previously described (10).

Immunoblotting and enzyme-linked immunosorbent assay. Western blotting (immunoblotting) was performed as already described (41). The antiserum and peroxidase-coupled goat antirabbit immunoglobulin G (Dianova, Hamburg, Federal Republic of Germany) were used essentially as already

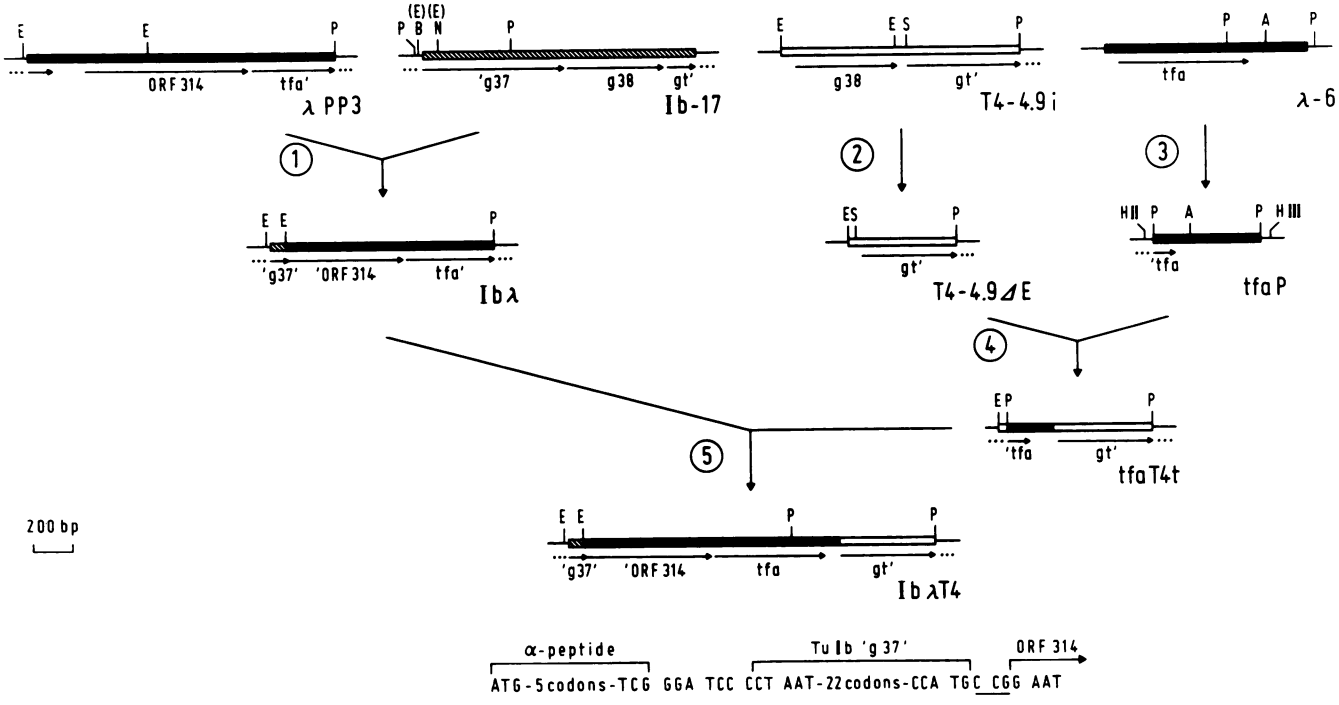

FIG. 1. Construction of pDMIbλT4. Restriction sites: A, *Alu*I; B, *Bam*HI; E, *Eco*RI; HII, *Hin*dII; HIII, *Hin*dIII; N, *Nco*I; P, *Pst*I; S, *Sna*BI. Thin lines, vector DNA (in all cases, pUC8); the *lac* regulatory elements are to the left of each insert. Boxes: open, T4 DNA; closed, λ DNA; hatched, TuIb DNA. Dots indicate that a gene continues in this direction. The DNA sequence shown is that of the beginning of the hybrid gene in pDMIbλT4; the underlined nucleotides are those from the linker (see the text).

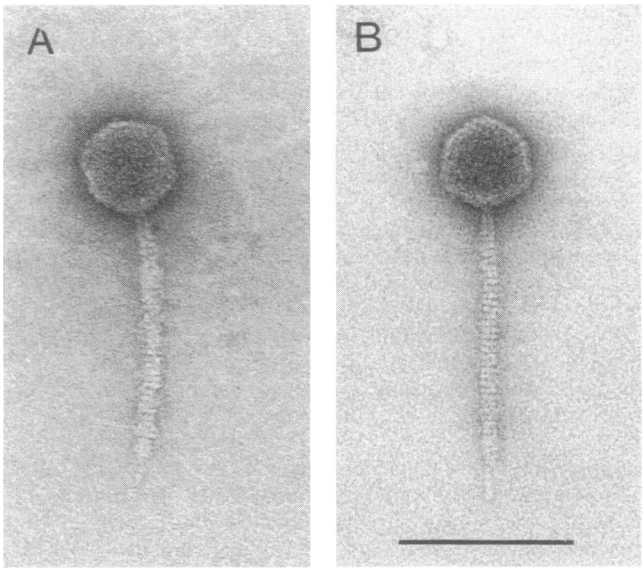

FIG. 2. Phage λ. A, λ *c*I857 *S*am7; B, λ p*lac*5. Bar, 100 nm.

described (12). An enzyme-linked immunosorbent assay was used to detect the ORF 314 protein in phage λ *c*I857 *S*am7. The phage was suspended in 34 mM Na_2CO_3–8 mM $NaHCO_3$, and 100 μl (~10^{10} PFU) was placed into wells of a microtiter plate and left for 12 h at 4°C. The wells were then incubated for 1 h with 250 μl of 3% bovine serum albumin (in phosphate-buffered saline) per well and subsequently washed three times with phosphate-buffered saline containing 0.3% serum albumin and 0.05% Tween 20 (wash buffer). Rabbit antiserum (250 μl; 10^{-3} dilution) was added, the plate was incubated for 1 h, the wells were washed four times with wash buffer, biotinylated goat anti-rabbit immunoglobulin G (250 μl; 10^{-3} dilution; Dianova) was added, and after 1 h they were washed as before. They were then rinsed twice with bicarbonate buffer containing 2 mM $MgCl_2$. Finally, wells were incubated for a further hour with alkaline phosphatase coupled to avidin (Sigma Chemical Co., St. Louis, Mo.), after which bicarbonate-$MgCl_2$ buffer containing dinitrophenylphosphate (1 mg/ml) was added. The dinitrophenol concentration was measured by A_{405} with a microtiter plate scanner (Titertec Multiscan; Flow Laboratories, McLean, Va.).

Electron microscopy. Visualization of antibodies bound to phages has already been described (38). To obtain better contrast concerning the side tail fibers of phage λ, the phages were first suspended in 10 mM Tris hydrochloride (pH 7.5) containing 10 mM sodium azide, 1 mM $MgCl_2$, and 0.1% bacitracin. The phages (mounted on grids) were stained with 2% phosphotungstate (pH 7.3), supplemented with 0.1% bacitracin, washed with Tris buffer, and negatively stained with 1% uranyl acetate.

RESULTS

Expression of ORF 314 in *E. coli* and presence of the corresponding protein in phage λ. ORF 314 is located within the *b* region of the λ genome (35), which is partially missing in phage λ p*lac*5 (6). The missing area includes only three ORFs, 401, 314, and 194 (*tfa*). Electron microscopy showed that phage λ *c*I857 *S*am7, which contains this region, possessed side fibers while the former phage did not (Fig. 2). Hence, a gene(s) required for establishment of these fibers is located within the region missing in λ p*lac*5, a fact lending much support to the notion that ORF 314 codes for a subunit in the side tail fiber of this phage. The presence of side fibers was strain dependent; in lysates from strain C600, the fibers were always present, while phages propagated on HfrG6 never possessed fibers.

	1	2	3	4	5	6	7	8	9
A	blank	-0.019	-0.047	-0.058	-0.085	-0.081	-0.079	-0.079	-0.083
B	blank	0.556	0.549	0.513	0.469	0.510	0.448	0.203	0.150
C	blank	0.950	0.786	0.587	0.729	0.663	0.635	0.331	0.209

FIG. 3. Presence of an antigen that reacts with anti-ORF 314 serum in phages λ and T4 (enzyme-linked immunosorbent assay). A printout of a microtiter plate scanner is shown; the instrument measured A_{405} (concentration of dinitrophenol; see Materials and Methods). Blank, phage λ p*lac*5 (7.2×10^9 PFU per well) was subjected to the procedure, and the absorptions of these wells were averaged and taken as 0. A2, 7.2×10^9 PFU of λ p*lac*5; A3 to A9, dilutions of this phage at 5×10^{-1}, 2×10^{-1}, 10^{-1}, 6.6×10^{-2}, 5×10^{-2}, 2×10^{-2}, and 10^{-3}, respectively. B2, 7.1×10^9 PFU of λ *c*I857 *S*am7; B3 to B9, dilutions of the phage as in line A. C2, 3×10^9 PFU of T4; C3 to C9, dilutions of this phage as in line A. Preimmune serum instead of anti-ORF 314 serum or omission of antibody 1 or 2 led to the same color development as the blank.

ORF 314 was placed under the control of the *lac* regulatory elements in pUC8, resulting in plasmid pDMλPP3 (Fig. 1). Growth of cells harboring this plasmid in the presence of isopropyl-β-D-thiogalactopyranoside led, despite the strong *lac* promoter, to production of only very low amounts of the protein. ORF 401 precedes ORF 314 and is separated from ORF 314 by a fairly long (178-bp) intercistronic region (35). Since this area was probably responsible for the poor expression of ORF 314, plasmid pDMλPPΔE was constructed by eliminating the area flanked by *Eco*RI sites from plasmid pDMλPP3 (Fig. 1). In this plasmid, a protein is encoded where five NH_2-terminal residues of β-galactosidase are fused to residue 67 of the ORF 314 protein. Induction with isopropyl-β-D-thiogalactopyranoside resulted in massive accumulation of this fusion protein (see Fig. 4), which then was used to obtain an antiserum.

An enzyme-linked immunosorbent assay demonstrated that phage λ *c*I857 *S*am7, which had served as a source for ORF 314, contained a protein that reacted with the antiserum, while λ p*lac*5, lacking ORF 314, did not (Fig. 3). The assay also showed that phage T4 possesses such determinants (Fig. 3; for T4, see also below). An immunoblot (Fig. 4) provided additional evidence for the presence of an ORF 314-related product in λ *c*I857 *S*am7 and its absence in λ p*lac*5. Interestingly, the largest protein from phage λ *c*I857

TABLE 2. Reaction of phages with antiserum to the ORF 314 protein

Phage	Titer (% of input) after incubation with antiserum for:	
	10 min	60 min
T4	1	0.5
TuIa	20	2
TuIb	4	1
T2	100	100

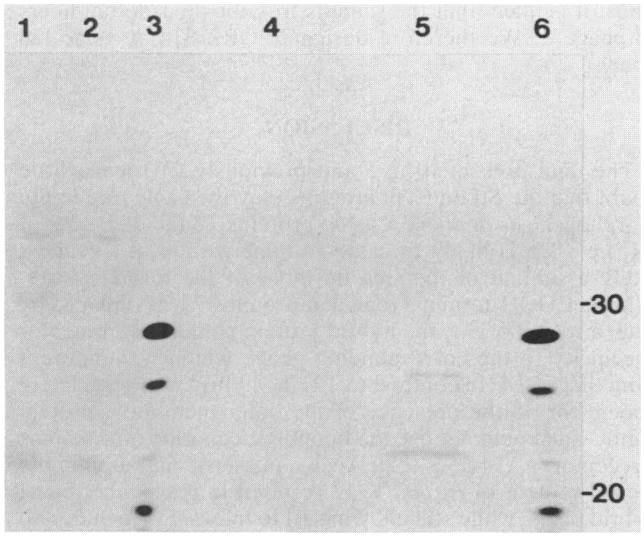

FIG. 4. Immunoblot of a sodium dodecyl sulfate-polyacrylamide gel electrophoretogram. Lanes: 1, cells of strain C600 (host for λ *plac*5 and λ *c*I857 *S*am7); 2, cells of strain M15; 3, cells of strain M15 carrying pDMλPPΔE; 4, phage λ *plac*5; 5, phage λ *c*I857 *S*am7 (for lanes 4 and 5, about 10^{11} PFU was placed onto the gel); 6, as lane 3. All strains were grown for 7 h in the presence of isopropyl-β-D-thiogalactopyranoside. For lanes 3 and 6, about 10^6 cells, and for lanes 1 and 2, about 10^7 cells were boiled in sample buffer and subjected to electrophoresis. The numbers on the right indicate molecular sizes in kilodaltons.

*S*am7 exhibited an apparent molecular weight (27,000) considerably smaller than that expected on the basis of the DNA sequence (34,500), indicating proteolytic processing or degradation (see Discussion). The antiserum did not inactivate phage λ, nor could antibodies be visualized at the side fibers of the phage with the electron microscope. However, the antiserum did inactivate all three phages of the T4 group (T4, TuIa, and TuIb), i.e., the phages possessing similar genes 38 and similar COOH-terminal areas of gene 37 (24; Table 2). Immunoelectron microscopy revealed that the antibodies bound to the long tail fibers of these phages and did so, interestingly, 15 to 18 nm away from the tip of the fiber (Fig. 5). Phage T2 was used as a negative control. In T2 and several of its relatives, the organization of this tip is completely different from that of the T4 family; protein 38 is present at the free end of the fiber and acts as an adhesin (31). As expected, the antiserum did not react with this phage.

These results strongly suggested but did not prove that ORF 314 codes for a component of the side fiber of λ. Because the antibodies could not be localized on the phage, the protein could have been present somewhere else in the phage and, although unlikely, the cross-reaction with T4 could have been fortuitous. Therefore, assuming that the Tfa protein acts in the assembly of the side fibers of λ as it did for protein 37 of T4, we placed part of ORF 314, together with *tfa*, in the genome of T4.

Part of ORF 314 plus *tfa* can functionally replace a COOH-terminal area of gene 37 and all of gene 38, respectively, of phage T4. The homology between ORF 314 and the COOH-terminal part of gene 37 is by far not perfect, and it was doubtful whether this ORF could be fused to gene 37 by recombination. Even if so, it was unpredictable whether or not a resulting hybrid protein would be able to form a functional fiber. For this reason and those detailed below, plasmid pDMIbλT4 (Fig. 1) was constructed. In this plasmid, the codon for residue 67 of the ORF 314 protein is preceded by 24 codons for a part of gene 37 and two residues (Cys and Arg) introduced by the fill-in reaction and the linker, relatively near the 3′ end of the gene, from phage TuIb. This TuIb sequence is nearly identical to that of gene 37 of phage T4. The plasmid harbors a complete *tfa* gene which is followed by part of gene *t* from T4. Hence, recombination should at least be possible within the regions flanking the λ genes. Furthermore, the host range of T4 is determined by an area of protein 37 near the COOH terminus, and protein 38 also acts at this area (3). If a COOH-terminal region of the ORF 314 protein can be fused to gene 37 and if the Tfa protein can catalyze dimerization of this hybrid polypeptide, formation of a functional tail fiber could ensue. This fiber should determine the receptor specificity of the side tail fiber of λ, which may differ from that of T4.

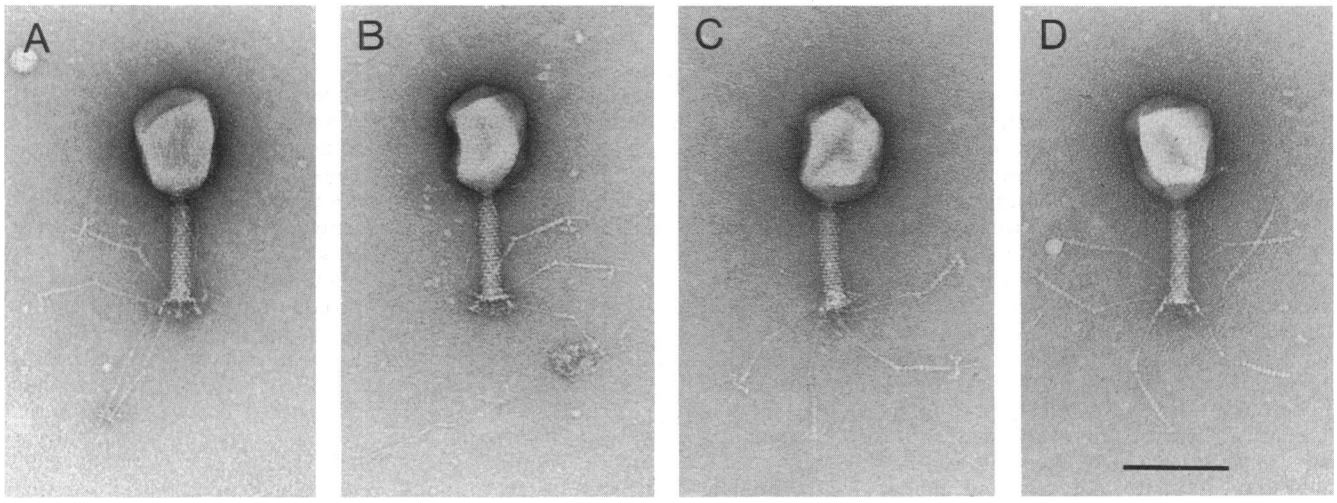

FIG. 5. Phages after treatment with antiserum to the Stf protein. A, T4; B, TuIa; C, TuIb; D, T2. Note that because of the dimeric nature of the distal part of the tail fiber, two immunoglobulin G molecules are frequently bound to the fiber. Bar, 100 nm.

TABLE 3. Host ranges

Phage	Relative efficiency of plating with the following *E. coli* strain[a]:	
	B	72.6 *ompC169*
T4	1	0.3
T4-λ1	$<10^{-6}$	$<10^{-6}$
T4-λ2	1	0.3
T4-λ3	$<10^{-6}$	$<10^{-6}$
T4-λ4	1	NT[b]
T4-λ7	1	NT

[a] The plating efficiencies obtained with strain M15 were defined as 1 (reference). Those obtained with strains B *ompC*$^+$ (*E. coli* B carrying pTUompC65) and 72.6 were also 1, and those obtained with strain P400.6 were all $<10^{-6}$.

[b] NT, Not tested.

With an amber mutant in gene 38 of T4 (amB262) and a *sup*^{0} host (M15) carrying plasmid pDMIbλT4, selection for the desired recombinants was possible. A lysate of T4 amB262 was prepared on this host. Most of the progeny consisted, of course, of T4 amB262 complemented by the cloned *tfa* gene. Therefore, this progeny was plated on strain M15, allowing growth of revertants and any viable recombinants. Plaques appeared with a frequency of $\sim 10^{-5}$ compared with the titer on the amber-permissive host B/40. Among this progeny, two types of host ranges existed, one identical to and one different from T4. Five isolates were analyzed further; their host ranges are shown in Table 3. For infection of *E. coli* K-12 but not of *E. coli* B, T4 requires the outer membrane protein OmpC (13, 25). *E. coli* B was resistant to phages T4-λ1 and T4-λ3; thus, these two must be recombinants, probably requiring the OmpC protein, which is not present in *E. coli* B. That this indeed was so is evidenced by strains P400.6 (*ompC*, missing the OmpC protein) and 72.5 *ompC169* (*ompC169* encodes an OmpC protein which carries a missense mutation). Both strains were resistant to T4-λ1 and T4-λ3, while T4 could infect 72.5 *ompC169*, although with a somewhat reduced efficiency of plating. Also, introducing plasmid pTUompC65, which carries a wild-type *ompC* gene, into *E. coli* B conferred sensitivity to T4-λ1 and T4-λ3 to this strain. The host range of the other T4-λ phages was that of T4.

To prove that recombination between T4 and the plasmid had occurred, *Taq*I digests of the DNAs of the five T4-λ phages were subjected to agarose gel electrophoresis and probed with a radioactive fragment of gene 38 of T4 (fragment T4-2 [24]) or with such a *Pst*I fragment of *tfa* from pTUλ-6 (Fig. 1). DNAs from phages T4-λ1, T4-λ2, and T4-λ3 reacted with the *tfa* probe but not at all with the T4 probe, and the reverse was true for the other two phages which, therefore, most likely represented revertants of T4 amB262 (data not shown). T4-λ2, possessing the *tfa* gene or at least part of it, but not the area determining the host range of ORF 314, probably recombined within gene *t*, and ORF 314 of the plasmid probably recombined with gene *t* and gene 37 of T4, respectively; the DNA sequences encoding some 30 COOH-terminal residues of ORF 314 and gene 37 are almost identical. Agreeing with this interpretation was the result obtained by treating phages T4-λ1, T4-λ2, and T4-λ3 with antiserum against the ORF 314 protein and then examining them in the electron microscope. All three had the immunoglobulin bound in exactly the way shown in Fig. 5 for phages T4, TuIa, and TuIb. We conclude that a COOH-terminal area of the ORF 314 protein, upstream from this region of near identity, determines specificity for the OmpC protein. Thus, it is likely that the same is true for the side tail fibers of phage λ. We therefore designate ORF 314 *stf* (side tail fiber).

DISCUSSION

The data presented here and previously (23) leave little doubt that the Stf and Tfa proteins play the same role in the morphogenesis of phage λ as do proteins 37 and 38 in that of T4; i.e., Tfa is likely to catalyze dimerization of Stf, most likely a subunit of the side tail fiber of the former phage. When a COOH-terminal region of protein 37 was replaced by such a region of Stf, the hybrid protein conferred a receptor specificity to the corresponding phage which was different from that of T4. In contrast to T4, the hybrid was completely dependent on the presence of the outer membrane protein OmpC and could not use the lipopolysaccharide of *E. coli* as a receptor, as T4 does (27). Also, a missense mutation in the OmpC protein of *E. coli* K-12 resulted in resistance to the hybrid phage while still allowing T4 to infect. Since in *E. coli* K-12 the OmpC protein is necessary for infection with T4, the hybrid tail fiber apparently recognizes a region of the protein which is different from that required for T4. Although in the context of protein 37 of T4 the Stf moiety may differ in receptor specificity from the Stf protein alone, it is certainly likely that the side tail fiber of λ recognizes the OmpC protein. Both *stf* and *tfa* are nonessential genes. As discussed previously for *tfa* (23), preservation of the function of the Stf protein very strongly suggests that the two genes provide some selective advantage not clearly evident in infections of cells with λ under laboratory conditions. Obviously, this advantage may involve increased successful formation of λ-cell complexes. It has been observed that an *E. coli* strain completely missing the LamB protein can be transduced, albeit with low efficiency, with a λ *pgal* transducing phage (4). If this phage still possessed the *stf* and *tfa* genes, it may have used the small tail fibers for adsorption.

An antiserum against the Stf protein did not inactivate phage λ, but it did inactivate T4. The inability to neutralize λ was not unexpected, since the antibodies would probably not interfere with the interaction of the tail with the essential λ receptor, the LamB protein. However, the immunoglobulin also could not be visualized electron microscopically, while it was visible at the tail fibers of phages of the T4 family. It has remained unknown why the side fibers could not be coated with the antibodies. If they would recognize a site of the side fibers which is structurally homologous to the site to which they bind in T4, one would expect them to bind around the middle of the λ fibers. Treatment of λ with the antiserum resulted in neither loss of side fibers (the particle shown in Fig. 2 comes from a population which had been incubated with the antiserum) nor loss of the reactive proteins shown in Fig. 4. It appears, therefore, that in the context of the side fiber an antigenic epitope, exposed when present in the T4 fiber, is masked.

Why was the λ protein, which reacted on an immunoblot with antiserum to the Stf protein, smaller than expected? Evidently the polypeptide was processed, and this proteolysis was either physiological or pathological. Although protein 37 from phage T2 is processed at its COOH terminus (10), protein 37 from phage T4 is not. The primary structures of the COOH-terminal areas of the T2 precursor and those of protein 37 of T4 are completely different (29), while the latter is homologous to the Stf protein, indicating that processing does not occur at the COOH terminus of the Stf protein. Hence, processing could proceed at the NH_2 terminus of Stf.

However, the Stf protein, synthesized in the absence of other λ genes, was also partially degraded and one of the products was of the same size as one small polypeptide stemming from phage λ (Fig. 4). Side tail fibers were observed on phages propagated in strain C600 but not at all when lysates were obtained from strain HfrG6. This fact may indicate complete proteolytic loss of the fibers in the latter strain. It is, therefore, possible that the tail fibers on phages grown in C600 were partially degraded by a cellular protease. Alternatively, strain HfrG6 may lack a factor required for assembly of the fiber, such as a protease for hypothetical physiological processing of the Stf protein.

We have previously offered an explanation for the very strange fact that closely related phages, such as T2 and T4, harbor genes 38 which code for proteins with nothing in common either structurally or functionally; we speculated that perhaps gene 38 of T4 was derived from gene 37 of a T2-type phage (24). The very existence of the T4-λ recombinants described here strongly suggests a much simpler mechanism, namely, that an ancestor of T4 from the T2-type lineage picked up a *tfa* gene and an *stf* gene (or part of the latter) from a lambdoid phage or prophage. If so, such a horizontal gene transfer has most likely occurred more than once. We have cloned and sequenced a region of gene 37 of phage TuIb that codes for 250 COOH-terminal residues (manuscript in preparation). The amino acid sequences of the regions thought to determine receptor specificity are quite different in the proteins from T4 and TuIb and in the Stf polypeptide. Moreover, T4 was isolated in 1945 on Long Island, N.Y. (8), while TuIb was isolated from a sewage plant near Tübingen, Federal Republic of Germany, in 1976 (7). Since it appears that such interspecies recombination can occur naturally, the very high variability of the receptor specificity of phages—comparable in several aspects to the immune systems of animals (30, 38)—would increase even further and become nearly limitless.

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