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Characterization of the Helper Proteins for the Assembly of Tail Fibers of Coliphages T4 and λ

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Assembly of tail fibers of coliphage T4 requires the action of helper proteins. In the absence of one of these, protein 38 (p38), p37, constituting the distal part of the long tail fiber, fails to oligomerize. In the absence of the other, p57, p34 (another component of the long tail fiber), p37, and p12 (the subunit of the short tail fiber) remain unassembled. p38 can be replaced by the Tfa (tail fiber assembly) protein (pTfa) of phage λ, which has the advantage of remaining soluble even when produced in massive amounts. The mechanisms of action of the helpers are unknown. As a first step towards elucidation of these mechanisms, p57 and pTfa have been purified to homogeneity and have been crystallized. The identity of gene 57 (g57), not known with certainty previously, has been established. The 79-residue protein p57 represents a very exotic polypeptide. It is oligomeric and acidic (an excess of nine negative charges). It does not contain Phe, Trp, Tyr, His, Pro, and Cys. Only 25 N-terminal residues were still able to complement a g57 amber mutant, although with a reduced efficiency. In cells overproducing the protein, it assumed a quasi-crystalline structure in the form of highly ordered fibers. They traversed the cells longitudinally (and thus blocked cell division) with a diameter approaching that of the cell and with a hexagonal appearance. The 194-residue pTfa is also acidic (an excess of 13 negative charges) and is likely to be dimeric.

Receptors at the bacterial cell surface are recognized by phage T4 with the free ends of its long tail fibers (1, 26, 51). These fibers consist of four proteins: p34, p35, p36, and p37. p37 constitutes the distal part of the fiber and mediates receptor recognition (2, 51) with an area near its C terminus (17, 32). p34, p36, and p37 in these tail fibers are oligomers. It had earlier been assumed that they form dimers (50, 53); most likely, however, they are trimers. There is experimental evidence for the trimeric state of p34 and p37 (8), and regarding p37, strong indirect evidence stems from the fact that the tail fibers of phage T7 consist of trimers of this virion's p17 (49). Tail fiber genes are of a mosaic structure (summarized in reference 16), very strongly indicating horizontal transfer of gene fragments. Corresponding protein fragments, partially identical to sequences of p17, exist in p37 of phages K3 and T2 (16), which are very closely related to T4. There is little doubt, therefore that all p37 proteins are trimeric. Assembly of these fibers requires helpers (for a recent review, see reference 54), which are absent from the mature virion. In the absence of one of these, p38, p37 fails to oligomerize. A gene 38 (g38) partial bypass mutant has been isolated (3), and it has been shown to consist of a duplication of residues 797 to 805 (17) of the 1,026-residue p37 (36). This is the area where p38 is believed to act on p37 (48). Infection with the mutant phage leads to about 30% assembled distal half-fibers (compared with the amount produced in wild-type phage infection); hence, the bypass strongly suggests that p38 does not catalyze a covalent modification of p37 which would be required for assembly. p38 most likely acts as a specific chaperone (for reviews, see references 12 and 13), because, e.g., PapD is acting only in the bacterial pilus assembly (23). In line with this view is the recent demonstration that, depending on the type of p38 present, the folding pathway and the receptor specificity of p37 can differ, and we have suggested that this chaperone acts instructively (17). The thus rather interesting mechanism of action of p38 has remained unknown. The protein has not been purified, and the instability of its activity in extracts has hampered further study (53). The role of the other helper, p57, has remained entirely mysterious. Absence of p57 had pleiotropic effects: p34, p37, and p12, the subunit of the short tail fibers, remained unassembled (27, 50). p57 bypass mutants have also been isolated; they were located in the chromosome of the host (39), but their nature is not known. Oddly enough, even the identity of g57 has not been established beyond doubt.

In order to eventually understand the mechanism of action of the two helpers, it is our aim to develop an in vitro system consisting of the helpers and monomeric p37. This is not hopeless because it is possible in vivo. It has been shown that upon infection with a mutant phage carrying, among others, defective genes g34 and g36, p37 half-fibers were produced which were able to bind to a receptor for T4, the outer membrane's lipopolysaccharide (52).

Here we report the isolation and some properties of a homolog of p38, pTfa (tail fiber assembly protein) of phage λ, which can functionally replace p38 of T4 (33) and which, as we show here, can be replaced by p38. pTfa was chosen because, in contrast to p38, it remains soluble even when overproduced in massive quantities. g57 has been identified, and the rather unusual p57 has been isolated. Some of p57's properties are described.

MATERIALS AND METHODS

Bacterial strains, phages, plasmids, and growth conditions. As an indicator for all phage titrations, the K-12 strain MC4100 was used (F^- *araD134* Δ*lacU169 rpsL relA thi* [7]). UH300 (*recA56 proA* or *-B* F′ $lacI^q$ $lacZ\Delta M15$ $proA^+B^+$ in addition to the markers of MC4100 [30]) served as a host for most plasmids. *Escherichia coli* B (sup^+) and B/40 (*supD*) were from E. B. Goldberg. The indicator strain for cells transformed with phagemid Bluescript was XL-1-Blue

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(Stratagene [6]). The strain not expressing the OmpC porin was P400.6 (45). Phages T2 and T4D were from E. Kellenberger; TuIb (10), the T4-λ hybrid, T4-λ1 (35), and K3 (47) have been described previously. The T4g57 amber mutant amE198 (11) was given by W. B. Wood. The plasmids were pCG57-7 (carries g57 [22]), pTUλ-6 (carries *gtfa* [33]), pTUT4-4.9i (expressing g38 of T4 [34]), pHP45Ω-km (source of the interposon [14]; a gift from E. Bremer), pACI (carries *lamB* [4]; donated by M. Hofnung), and pUC19 (56). For DNA sequencing (44), the Bluescript phagemid SK was employed (Stratagene); the helper phage for the production of single-stranded DNA was R408 (40). Cells were grown at 37°C in L broth or M9 minimal medium (31), supplemented, when required, with glucose or glycerol (both 0.5%), ampicillin (100 μg/ml), kanamycin (30 μg/ml), IPTG (isopropyl-β-D-thiogalactopyranoside [1 mM]), or 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal [80 μg/ml]).

Isolation of pTfa and p57. Cells of strain UH300 carrying the appropriate plasmid were grown at 37°C aerobically in a fermentor in the presence of IPTG. Five grams of cell paste was suspended in 200 ml of 20 mM potassium phosphate buffer (pH 7.4), and cells were broken by sonication. Insoluble material was removed by centrifugation for 2 h at 38,000 × *g*. pTfa was precipitated at room temperature with ammonium sulfate (35% saturation), and the precipitate was dissolved in 20 ml of the potassium phosphate buffer described above. It was kept frozen (−24°C) overnight. A precipitate developed upon thawing, and it was removed by centrifugation for 40 min at 38,000 × *g*. The supernatant was brought to 35% saturation of ammonium sulfate at room temperature, and the precipitate was dissolved with 10 ml of 20 mM sodium *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonate (HEPES [pH 7.4]). Five milliliters of this solution was applied to a column (115 by 2.5 cm) of Sephadex G-150; the flow rate was 12 ml/h. Finally, the protein was precipitated again with ammonium sulfate. The precipitate was dissolved with 50 ml of 25 mM Tris-HCl (pH 7.5). The solution was applied to a column (12 by 2.5 cm) of DEAE cellulose equilibrated with the same buffer. The column was developed at room temperature with a gradient of NaCl (700 ml of the Tris buffer described above in one chamber and 300 ml of 0.3 M NaCl in this buffer in the other chamber of the gradient mixer at a flow rate of 23 ml/h). The protein eluted at about 240 mM NaCl and was concentrated with ammonium sulfate. This step separated pTfa from another protein which purified together with the former on the molecular sieve column and which migrated electrophoretically only very slightly faster than pTfa. pTfa was precipitated with ammonium sulfate; the yield was 150 to 160 mg/liter of culture. p57 was present in an ammonium sulfate fraction of the crude extract between 55 and 65% saturation. It was carried through the Sephadex step as described for pTfa. The fractions containing p57 were brought to 65% saturation of ammonium sulfate, and the precipitate was dissolved with 50 ml of 25 mM Tris-HCl (pH 7.5). This solution was chromatographed on DEAE cellulose as described for pTfa, except that another gradient of NaCl was used (300 ml of the Tris buffer versus 300 ml of 1 M NaCl in this buffer). The protein eluted at about 260 mM NaCl and was concentrated with ammonium sulfate. The yield was about 40 mg/liter of culture.

Inactivation of *gtfa* in Ur-λ. Plasmid pTUλ-6 (33) carries the complete *gtfa*. One site for *Bam*HI is located within this gene, and another is present in the vector (pUC8). The corresponding *Bam*HI fragment was removed and replaced with such a fragment from pHP45Ω-Km (14), which determines resistance to kanamycin. The resulting plasmid was opened with *Sal*I and made blunt ended with the Klenow fragment of T4 DNA polymerase. The *Bam*HI fragment described above was also made blunt ended and was ligated into the polished *Sal*I site. Plasmids with identical orientation of the two *tfa* fragments were identified by digestion with appropriate restriction enzymes. The insert in this plasmid then consists of *gtfa′* (0.43 kb)-Kan interposon (1.8 kb)-*g′tfa* (0.67 kb). Strain UH300 carrying this plasmid was lysogenized with Ur-λ, and phage replication was induced with UV irradiation. Strain UH300 was infected with this lysate (about two phage per cell), and selection was performed on plates containing kanamycin. Small and large colonies appeared; the small ones were resistant to kanamycin plus ampicillin (i.e., in the corresponding prophages, the replicating λ had probably swallowed the whole plasmid). Phage from one of the faster growing cells was called Ur-λ-kan15.

Structure of the T4-λ hybrid T4-λ1. From the construction of this phage (35), it should possess a complete *gtfa* (instead of g38) followed by *gt* of T4. g37 of T4 should switch to *gstf* of λ at about position 21227 of the sequence of this phage (9). (In the study by Montag et al. [35], it was still believed that an open reading frame [ORF] in the λ genome, ORF314, represented *gstf*. It is now known that this is not so [16, 18] and that ORF314 consists of a part of *gstf* encoding 314 C-terminal residues of the 774-residue pStf. The codon for residue 67 of the ORF314 protein mentioned by Montag et al. is in fact the codon for residue 527 of pStf—which corresponds to position 21227 of the λ sequence presented above.) The fragment described in the legend to Fig. 6 was obtained by PCR (42) with Pfu DNA polymerase (Stratagene) and the primers indicated. It was ligated into the Bluescript phagemid SK opened with *Sma*I. The sequences of the wild-type hybrid and of the two host range mutants were determined with the sequencing and reverse sequencing primers (New England Biolabs) and primers spaced about 200 nucleotides apart along the whole fragment; DNA from the helper phage was purified by the protocol supplied by the company (Stratagene).

Construction of double amber mutants in T4. Plasmid pCG57-7 (Fig. 1) and an asymmetric PCR (37) were used for the generation of all mutants. The primers (Table 1) T4-g571 and r flank the insert of pCG57-7. To generate the two TAG codons with T4-g1mm1031, this primer was used together with T4-57r for PCR, and the product together with T4-571 was used for the next step. The same protocol was followed to obtain the other amber codons. In each case, 30 cycles were run with Pfu polymerase (1 min at 94°C, 1 min at 55°C, and 3 min at 65°C). The final products were digested with *Hin*dIII and *Pst*I and then ligated into pUC19. The presence of the stop codons in each cloned fragment was confirmed by DNA sequencing, which in these cases was performed commercially (GATC GmbH, Konstanz, Germany). Phage T4 was propagated on strain B/40 carrying one or the other recombinant plasmid. T4 amber mutants were identified essentially as described previously (41). In brief, B/40 was infected with a multiplicity of infection of 10^{-6}, plated with 2 ml of soft agar, and overlaid with 2 ml of soft agar containing about 1×10^6 to 5×10^6 cells of *E. coli* B. In contrast to wild-type plaques, plaques of an amber mutant are overgrown with microcolonies of the nonsuppressing *E. coli* B.

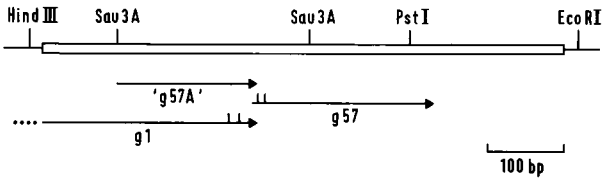

FIG. 1. Structure of the cloned fragment carrying g57. The *lac* promoter-operator region is to the left of the *Hin*dIII site in pUC19. The positions of the four double amber mutants in g1 and g57 are indicated by vertical lines; the dots at g1 indicate that the gene continues in this direction.

Electron microscopy. For conventional resin embedding (see Fig. 3b and c) cells were fixed with 2.5% glutaraldehyde (1 h), embedded in agarose, and postfixed with 1% OsO_4 (1 h) and 1% uranyl acetate (1 h). After dehydration in ethanol, specimens were embedded in Epon 812. For improved ultrastructural preservation (Fig. 3f and g), cells were cryoimmobilized with a Balzers JFD 030 propane jet, freeze-substituted in acetone (−90°C, 8 h; −60°C, 8 h; −40°C, 8 h) containing 1% OsO_4, and finally infiltrated with Epon 812 at room temperature. For immunolabeling of ultrathin cryosections (Fig. 3d) or Lowicryl HM20 sections (Fig. 3e), cells were processed as described earlier (15, 25).

Crystallization of proteins. The hanging drop vapor diffusion method was used. Sparse matrix sampling (24) was performed with all 98 commercially available Crystal Screen Reagents (Hampton Research, Laguna Hills, Calif.). With pTfa (11 mg/ml in 25 mM Tris-HCl [pH 7.5]), to date the best results were obtained by mixing 5 μl of this solution with 5 μl of 10% (wt/vol) polyethylene glycol in 2 M NaCl. Crystals appeared after 4 days (4°C) of vapor diffusion against the same precipitating solution. With p57, the largest crystals were obtained by mixing 5 μl of protein solution (5 mg/ml in 25 mM Tris [pH 7.5]) with 5 μl of a solution containing 0.2 M magnesium acetate, 0.1 M sodium cacodylate (pH 6.5), and 30% (vol/vol) 2-methyl-2,4-pentanediol. Crystals appeared after ~12 h (room temperature) of vapor diffusion against the same precipitating buffer.

Other techniques. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with Laemmli-type gels (28). Staining was with Coomassie brilliant blue or "Stains-All" (Serva, Heidelberg, Germany). For determination of such protein profiles, cells were broken by sonication; soluble and insoluble materials were separated by centrifugation for 30 min at 47,000 × *g*. Immunoblotting of electrophoretograms was performed as described previously (17). For amino acid sequencing, proteins from electrophoretograms such as that shown in Fig. 2, lane 4, were transferred electrophoretically with a dry blot apparatus (Pharmacia-LKB) onto a polyvinylidene difluoride membrane (Immobilon-P; Millipore). The membrane was stained with Coomassie brilliant blue, and the band consisting of p57 was cut out and subjected to automated Edman degradation. Phage from lysates was precipitated with polyethylene glycol (55) followed by centrifugation through a CsCl step gradient (43). For cross-linking in vivo with formaldehyde, cells harboring pTUT4g57 were pregrown in the presence of glucose. The sugar was removed by centrifugation, and the cells were suspended (dilution 1:20) in L broth containing IPTG and incubated for 2 h at 37°C. Treatment with formaldehyde was performed as described previously (38, 46). Cross-linked and control cells (carried through the same procedure without the aldehyde) were washed twice with 10 mM potassium phosphate buffer (pH 7.4). For lysis, they were resuspended in this buffer, and hen egg white lysozyme (100 μg/ml) was added, followed by sodium EDTA (1.25 mM). Protein concentrations were determined according to the method of Lowry et al. (29). This method is thought to be based mainly on the reaction of the phosphomolybdate-phosphotungstic acid in the Folin reagent with tyrosine and tryptophan residues in a protein and therefore should not work too well with p57. The dry weight of a sample of the isolated protein and the data obtained with the Folin reaction (bovine serum albumin was the standard), however, were in excellent agreement. Antiserum against p57 and pTfa was raised in rabbits as described previously (20).

TABLE 1. Sequences of the PCR primers used in this study[a]

Primer	Sequence
T4-571	C G C C *A A G C T T A T C G A* C C G C A T G
T4-57r	A G T C G A C C *C T G C A G* A T T T C A G C
T4-g1mm1031	A T T A C A G A A T A G G G A T A G C C A A T T C G T G A T
T4-g1mm1051	T G A T G G C G A T T A G G T A T A G A C A A A C G A T G G
T4-g57mm1182	T A C G A T T T C A G C A G C T A T T T C T A T T C A A C A G T T T G T T C A
T4-g57mm1208	G T A T C A A G A A T G C G A G A C T A C T A A G T T A C G A T T T C A G C A G

[a] The sites for *Hin*dIII, *Taq*I (in T4-571), and *Pst*I are in italics. The numbers 1031 to 1208 are positions according to reference 5, and the T in the *Taq*I site and the second G in the *Pst*I site are positions 852 and 1352 according to the same authors. The stop codons in the mismatch primers are underlined (for their locations, also see Fig. 1); they correspond to four to five mismatches per primer; all are written in the 5′→3′ direction.

RESULTS

g57. The most likely candidates for g57, then called g57A, were two mostly overlapping ORFs on a cloned fragment of T4 DNA coding for polypeptides with molecular weights of 6,459 and 5,843 (21). This conclusion was based on the facts that (i) a cloned fragment not harboring any other complete ORF complemented g57 amber mutants of T4, and (ii) the electrophoretically determined molecular weight of the protein made in vitro, directed by this fragment, was about 6,000 (22). Doubts about this identification arose when it was found that g57A would have to completely overlap g1, a gene coding for deoxynucleotide monophosphate kinase (5). The situation does not seem to have been investigated further.

We wished to isolate p57. Therefore, the *Hin*dIII-*Pst*I fragment (Fig. 1) was transferred to pUC19, thus presumably placing g57A under the control of the *lac* regulatory elements. This plasmid, pTUT4g57P, caused the synthesis of a protein with a size of about 5 kDa (Fig. 2, lane 2) and, as described previously (21, 22), complemented the g57 amber mutant amE198. This polypeptide was recovered from electrophoretograms and subjected to Edman degradation. The N-terminal sequence found was Ser-Glu-Gln-Thr-Ile-Glu-Gln-. This sequence does not exist in g57A, but the gene immediately downstream from it encodes a protein starting with Met-Ser-Glu-Gln-Thr-Val-Glu-Gln-. Consequently, the *Eco*RI-*Hin*dIII fragment was also transferred to pUC19, resulting in pTUT4g57. IPTG now caused the production of large quantities of an about 6-kDa polypeptide, which was soluble (Fig. 2, lane 4). To make the case airtight and to be able to eventually look for g57 bypass mutants, two double amber mutants in each of the genes g1 (g57A) and g57 were prepared (Fig. 1). Two double mutants in each gene were constructed in order to increase the probability that at least one of them would be suppressible in terms of function (all four were suppressible in this way by *supD*). pTUT4g57P did not complement those in g1 but did fully complement both mutants derived from g57, and the complemented efficiency of plating was identical to that found with the *supD* strain B/40. Clearly, g57A does not exist, and this sequence represents the C-terminal sequence of g1 only. The C-terminal sequence of the correct g57 is -Ser-Ala-Asp-Glu-Ala-Lys-Asp-Glu-Gln-CO_2H. The action of *Pst*I alters it to -Ser-Ala-Gly-Arg-Leu-CO_2H, and this does not cause any measurable loss of function. Amazingly, a much smaller frag-

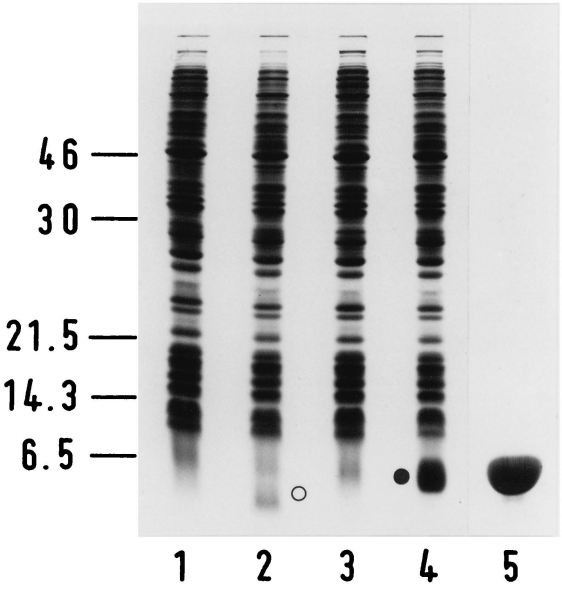

FIG. 2. p57. Lanes 1 to 4 show soluble proteins of cells carrying pTUT4g57P (lanes 1 and 2) or pTUT4g57. Cells had been grown in the presence of glucose (lanes 1 and 3) or of IPTG (lanes 2 and 4). Open circle, the C-terminally shortened p57; solid circle, complete p57. Lane 5, the isolated p57. Numbers to the left represent molecular mass standards in kilodaltons. A stained SDS-PAGE (16% polyacrylamide) electrophoretogram is shown.

TABLE 2. Amino acid compositions of pTfa and p57

Amino acid	Amino acid concn (mol/mol) in:	
	pTfa	p57[a]
Ala	21	10
Val	11	6
Leu	16	7
Ile	11	7
Pro	14	0
Phe	7	0
Trp	6	0
Met	4	2[a]
Gly	11	3
Ser	11	5
Thr	13	7
Cys	0	0
Tyr	5	0
Asn	7	1
Gln	4	5
Asp	12	5
Glu	20	13
Lys	10	6
Arg	7	3
His	4	0
No. of residues	194	79
M_r	23,600	8,600

[a] g57 codes for a polypeptide with two methionines. The mature protein apparently arises by cleavage of the initiator *N*-formylmethionine.

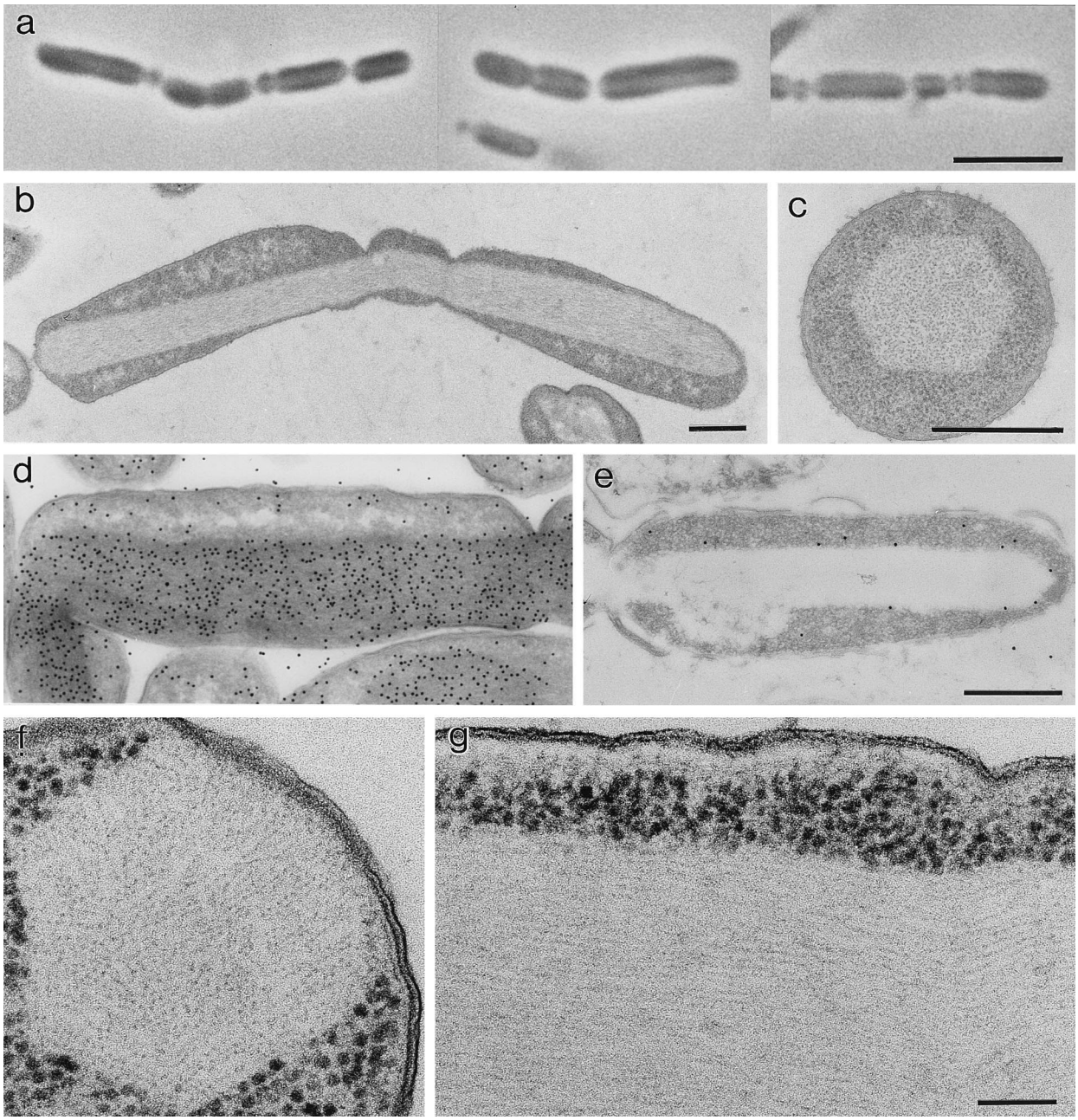

FIG. 3. p57 in cells. Phase-contrast (a) and electron (b to g) micrographs of cells overexpressing p57 are shown. (b and c) Ultrathin longitudinal and cross-sections. (d) Ultrathin frozen section, thawed and labeled with rabbit anti-p57 serum followed by treatment with protein A–15-nm-diameter gold particles. (e) Ultrathin Lowicryl HM20 section, same procedure as for panel d with cells cross-linked with formaldehyde and treated with lysozyme and EDTA. (f and g) Cross- and longitudinal sections of cryoimmobilized and freeze-substituted cells showing the fibers in greater detail. Bars: a, 5 μm; b to e, 0.5 μm; f and g, 0.1 μm.

ment of p57 had still not completely lost function. The 0.25-kb *Sau*3A fragment (Fig. 1) of g57 encodes only 25 N-terminal residues of the 79-residue protein. It was cloned into the *Bam*HI site of pUC19. Cells carrying the recombinant plasmid complemented mutant amE198 with an efficiency of plating of about 10 to 20% of that measured on the *supD* host; pUC19 alone had no such effect.

p57. The molecular weight of the monomer is 8,600; hence, the protein migrates electrophoretically rather aberrantly (see above). This fact, together with a truncated p57 being able to function, of course, explains why Herrmann (21) was misled regarding the identity of g57. The amino acid composition is shown in Table 2. With an excess of nine negative charges, the polypeptide is very acidic. No less than six amino acids are not present; in particular, the absence of all aromatic residues makes the protein probably fairly unique in the prokaryotic world. Therefore, the protein could not be recognized by conventional UV detectors of fraction collectors: manual shaking of fractions was used—foaming indicated p57.

p57 was recovered in pure form with only three steps of purification: ammonium sulfate fractionation, molecular sieve chromatography, and chromatography on DEAE-cellulose.

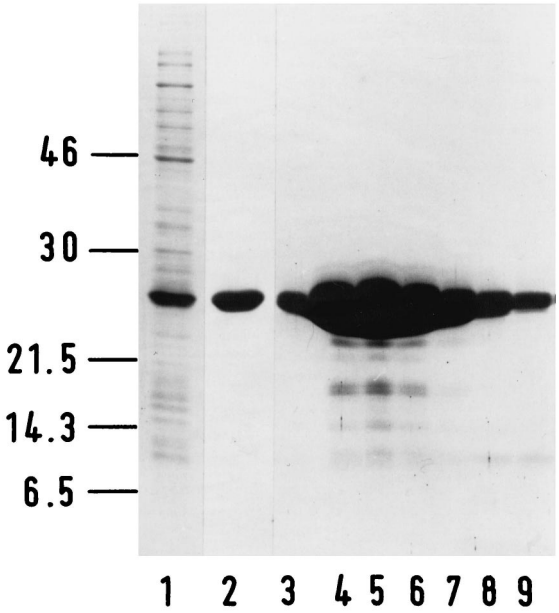

FIG. 4. pTfa. Lanes: 1, soluble proteins of cells harboring pTU-λ6 and grown in the presence of IPTG; 2, the isolated pTfa; 3 to 9, fractions (in this order) of the DEAE cellulose column. The proteins below pTfa in lanes 4 to 6 react with anti-pTfa serum; those in lanes 8 and 9 do not. A stained 12.5% polyacrylamide gel is shown; numbers to the left represent molecular mass standards in kilodaltons.

Figure 2 shows the crude bacterial extract (lane 4) and the purified protein (lane 5). On columns with Sephadex G-50 or G-75, the polypeptide eluted in the void volume. On Sephadex G-150, aldolase (M_r of 158,000) and bovine serum albumin (M_r of 68,000) were well separated, and p57 appeared between the two, partially overlapping both; hence, it appears to be oligomeric. The protein was crystallized as described in Materials and Methods (see Fig. 5).

Overexpression of g57 (in contrast to that of pTfa) was toxic. Cells growing in L broth or M9 minimal medium (both in the presence of IPTG and with 0.5% glycerol as a carbon source in M9 medium) exhibited inhibition of cell division (Fig. 3a); upon longer incubation, the cells lysed. The reason for this effect appears to be the presence of the crystal-like, fibrous structures shown in Fig. 3b, c, f, and g). The material was deposited in a highly ordered fashion; longitudinal sections always showed an apparent parallel arrangement of fibers, and cross-sections revealed hexagons. Most, and probably all, of this material consisted of p57. Its formation was specifically associated with the presence of IPTG in the growth medium. Comparison of electrophoretograms of cells carrying plasmid pTUT4g57 and grown in the presence of glucose or IPTG did not reveal other differences than the absence or presence, respectively, of p57 (by staining with Coomassie or "Stains-All"). Nothing stainable migrated towards the cathode, and there was no indication for the existence of a substance too large to enter the separating gel. Cells synthesizing g57 were then treated with formaldehyde, followed by incubation with lysozyme plus EDTA. This treatment without prior cross-linking caused complete lysis and complete release of p57 in soluble form, while cells pretreated with the aldehyde remained morphologically intact; however, they had lost the material in question (Fig. 3e). Electrophoretic analyses of such cells and of the supernatant revealed that all of p57 was in the supernatant, with about 20% in monomeric form; the missing 80% was recovered as monomers upon boiling of the supernatant (data not shown), a condition known to break the chemical cross-links (38, 46). Consistent with p57, cross-linked or not, being soluble upon cell lysis, the fibrous material seen intracellularly was not found in the supernatant fractions by electron microscopy. Finally, immunoelectron microscopy showed that the material reacted with antiserum against p57 (Fig. 3d). Hence, p57 aggregates intracellularly in an ordered, rather unique fashion.

pTfa. pTfa, encoded by phage λ, complements mutants of T4 not producing p38 (33). We had placed *gtfa* under *lac* control (on pTUλ-6); cells carrying this plasmid and growing in the presence of IPTG synthesized very large quantities of the protein, all of which remained soluble. Ammonium sulfate fractionation followed by molecular sieve chromatography and chromatography on DEAE cellulose yielded the polypeptide in an almost homogeneous form (Fig. 4). It has not been possible to remove by molecular sieve or ion-exchange chromatography the faster-migrating proteins shown in lanes 4 to 6 of Fig. 4. They reacted well with antiserum against pTfa, and their maximum coincided with that of pTfa. Most likely, therefore, they represent degradation products of pTfa that remain associated with the complete protein (see below). The protein was crystallized as described in Materials and Methods (Fig. 5).

The amino acid composition of pTfa is shown in Table 2. The polypeptide is also rather acidic: there is an excess of 13 negative charges (assuming that histidine is dissociated by only 50%). On Sephadex G-150, bovine serum albumin (M_r of 68,000), hen egg albumin (M_r of 45,000), and chymotrypsinogen A (M_r of 25,000) were fairly well separated. pTfa eluted together with hen egg albumin and thus appears to be a dimer. This would explain the coincidence of the peaks of the degra-

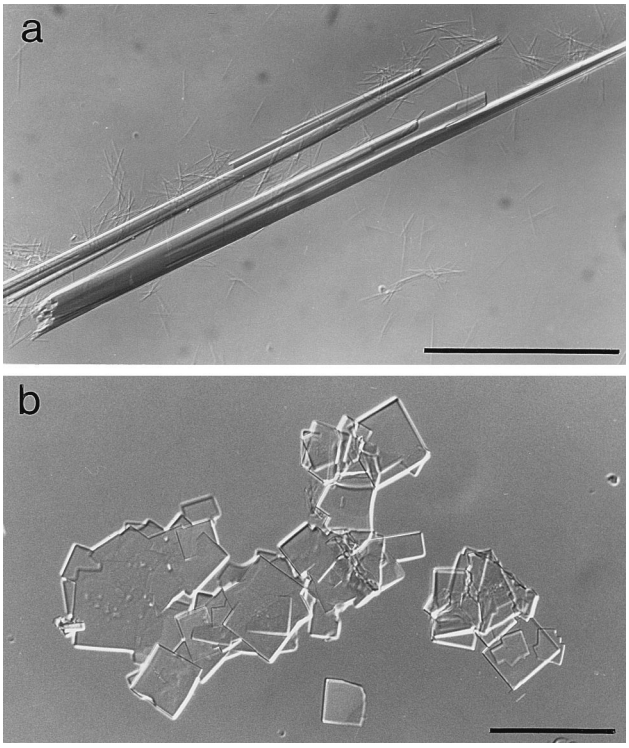

FIG. 5. Differential interference contrast images of crystallized proteins. (a) p57. (b) pTfa. Photograph a was taken after 5 days of vapor diffusion and shows that the protein can assume two crystal forms. Incubation overnight yielded only the large needles. Bar, 100 μm.

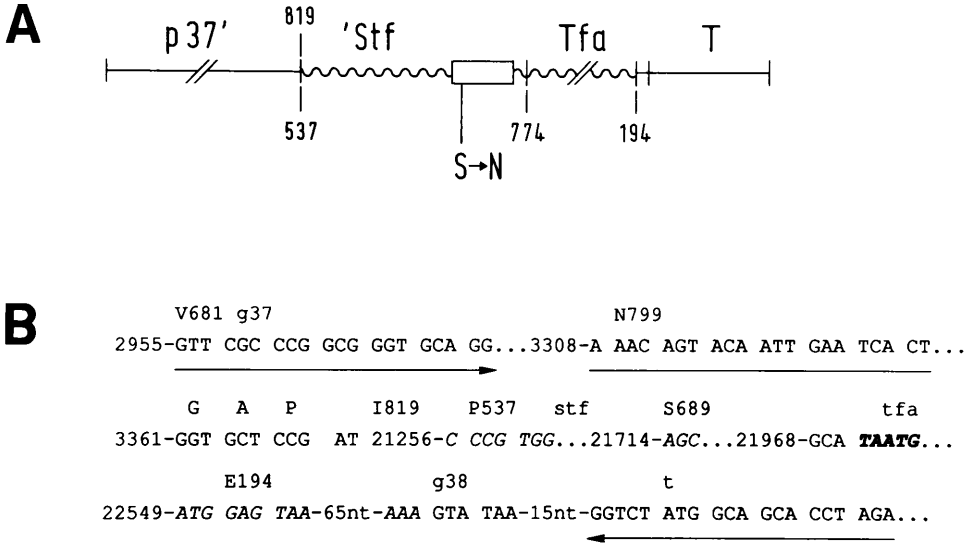

FIG. 6. (A) Schematic view of proteins encoded by the hybrid T4-λ1; the wavy line represents λ sequences. The 1,026-residue p37 of T4 was fused at position 819 to residue 537 of the 774-residue pStf of λ, followed by this phage's complete protein, pTfa. Lysis protein T is from T4. The boxed area represents the region of the protein which should serve in receptor recognition (see text); the amino acid substitution (Ser→Asn) in the host range mutants is indicated. (B) Details of the structure of the hybrid genes. Sequences of phage λ are in italics, and the overlapping stop codon for *gstf* and start codon for *gtfa* are in boldface. The positions of amino acid residues and nucleotides from T4 are according to reference 36, and those from λ are according to reference 9. t, start of lysis *gt*. The underlined sequence is duplicated in the p38 bypass mutant ts3813. Two codons of g38 are present (GTA TAA). In the two host range mutants, the AGC at position 21714 was changed to an AAG. Arrows, primers used for amplifying the fragment.

dation products matching those of the complete protein. A minority of dimers may exist that contain one or two of the shortened monomers.

p38 and the tail fiber. Two types of phage λ exist. One, called Ur-λ (18), possesses side tail fibers (see Fig. 7). The subunit of these fibers is a 774-residue protein, pStf. In the other, λPaPa, g*stf* is interrupted by a frameshift, and the phage lacks the fibers (16, 18). pTfa, as p38 in T4, serves in assembly of the fibers (described below). Unlike p38 in T4, however, pTfa was found to be a constituent of Ur-λ (18). It has not yet been possible to determine the protein's location at the virion, but most likely, of course, it is in the side tail fiber. The degree of identity with p38 is rather low (about 40% including conservative substitutions), and the possibility existed that pTfa represents a bifunctional protein, acting as a chaperone in the fiber assembly and serving in receptor recognition by the fiber (19). We had constructed a hybrid between T4 and λ (35). In this phage, g38 was replaced by g*tfa*, and a 3′ area of g37 was replaced by such an area of g*stf* (both corresponding to a C-terminal part of the proteins). The receptor for the phage was the outer membrane porin OmpC. The relevant sequence of the hybrid has now been determined (Fig. 6), and the question was if receptor recognition was mediated by the Stf moiety or pTfa of the hybrid. Host range mutants could be obtained on a host missing OmpC (frequency of $\sim 10^{-7}$). Selection for resistance of this *ompC* host against two independently isolated such host range mutants showed that the maltoporin LamB was missing in strains resistant to either of the mutants (data not shown). Sensitivity was restored by introduction of a plasmid harboring the *lamB* gene (pAC1); obviously, a receptor switch had occurred. DNA sequencing revealed that there were no substitutions in the pTfa proteins but that in both mutants the same substitution in the g*stf*-derived part of the tail fiber gene had caused an amino acid substitution (Fig. 6). This location is within an area of the protein which by an alignment with the p37 proteins of other phages of the T4 family (32) and analyses of host range mutants of such phages (17) had been identified as the receptor-binding region.

Rather unexpectedly, it then turned out that these experiments were (almost) superfluous. Knowing the exchangeability of p38 and pTfa for T4, we have asked if this is also true for Ur-λ. Its g*tfa* was inactivated by interposon mutagenesis, and, expectedly, this phage no longer possessed side tail fibers. They reappeared quantitatively when the cloned g38 of T4 was present in the cells of the host (Fig. 7). For determination of the presence or absence of pTfa or p38 in mature phages, these were subjected to electrophoresis and subsequent immunoblotting with antiserum against pTfa or p38 (these experiments were performed exactly as described previously [17]). The outcome was unambiguous. pTfa was present only in Ur-λ and not in the T4-λ hybrid; p38 was also not detectable in the Ur-λ with the inactivated g*tfa* (data not shown). We conclude that (i) pTfa is not directly involved in receptor binding and that (ii) it is irrelevant for the function of the chaperones whether or not one of them remains bound to the mature phage.

DISCUSSION

The somewhat confusing history of various attempts to identify p57 has been discussed earlier (22) and does not need to be repeated here. One result adding to the confusion was that in an amber mutant of g57, two T4-specific polypeptides with molecular weights of 6,000 and 18,000 called p57A and p57B, respectively (21), were missing. It was suggested later (5) that g57A is the gene identified here as g57 and that p57B is encoded by a gene immediately downstream from g57A. We have shown that the *Hin*dIII-*Pst*I fragment, which does not carry any sequences of g57B, complements a g57 amber mutant. Hence, g57B is not required for phage maturation and should no longer be designated this way but, for the time being, should be designated ORF1 (5). The absence of the 18,000-kDa protein in the g57 amber mutant may simply be due to

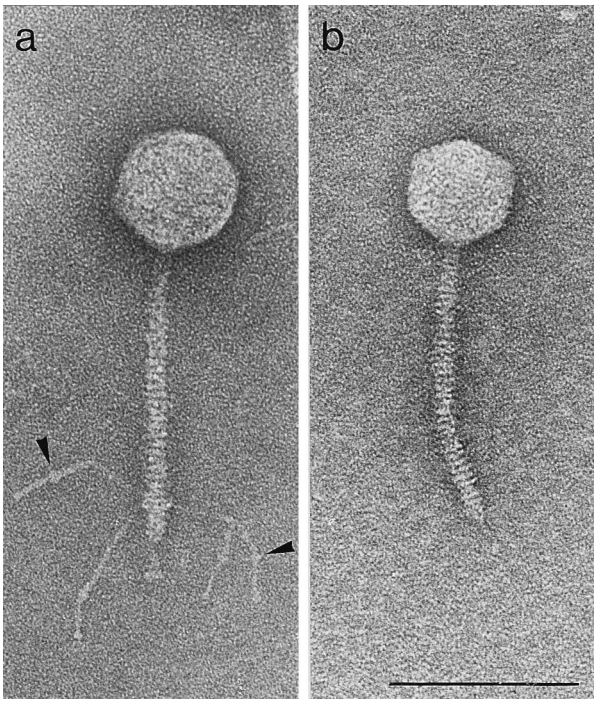

FIG. 7. Phage Ur-λ. (b) Phage Ur-λ-kan15 (*gtfa* inactivated). (a) Phage Ur-λ-kan15 grown in cells carrying pTUT4-4.9i (possessing g38 of T4; the bulges demonstrate that the architecture of the distal part of the fiber is not the same all along this structure). The phage on a and b are indistinguishable from Ur-λ and λPaPa, respectively (18). The virions were negatively stained with uranyl acetate. Bar, 100 nm.

polarity: g1 (the deoxynucleotide monophosphate kinase), g57, and ORF1 appear to belong, in this order, to the same transcriptional unit (5).

p57 represents a very remarkable polypeptide. The 79-residue acidic polypeptide misses no less than six amino acids. An N-terminal fragment of only 25 residues was still active in complementing a g57 amber mutant. The protein, when overproduced, assumed a quasi-crystalline structure intracellularly which was stable only in the cytosolic environment, and all of the polypeptide went into solution upon opening of the cells. Chromosomal mutants of the host have been isolated (39) which specifically allowed growth of g57 amber mutants, and some of these exhibited temperature sensitivity (poor growth at 42°C) or cold sensitivity (poor growth at <30°C). Unfortunately, these mutants apparently no longer exist. We have performed several searches for such mutants and have found them in *E. coli* K-12 and B strains; however, none of 12 independent isolates exhibited a phenotype other than the specific bypass of a g57 amber mutant. Therefore, we have so far not been able to elucidate the nature of these interesting mutants.

Finally, one point of interest concerning phage λ should be mentioned. It has been suggested that Ur-λ may not possess four side tail fibers but six side tail fibers (18). The authors argued that six fibers would match the rotational symmetry of the tail and that many other phages possess six but not four fibers. We have been looking at rather large numbers of this phage, decorated with λ-specific Fab fragments for enhanced visibility of tail fibers, and have never seen a single phage with more than four fibers; we therefore believe that there are only four fibers.

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ADDENDUM IN PROOF

The trimeric state of p34, p36, and p37 has now been proven beyond doubt (M. E. Ceritelli, J. S. Wall, M. N. Simon, J. F. Conway, and A. C. Steven, Stoichiometry and domainal organization of the long tail fiber of bacteriophage T4: a hinged viral adhesin. J. Mol. Biol. **260:**767–780, 1996).

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