Cloning, Sequencing, and Overexpression of Gene 16 of Salmonella Bacteriophage P22

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It has been suggested that gene product 16 of bacteriophage P22 forms a pore for DNA transfer and/or that it functions as a pilot protein guiding the DNA across the membrane. We have cloned gene 16 and determined the nucleotide sequence. Within the sequenced region there is an open reading frame that could encode a protein of 609 amino acids having a molecular weight of 64,366. The hydropathic plot of this protein does not reveal putative membrane-spanning regions as expected for a protein forming a membrane pore. Overproduction of gene product 16 in *Escherichia coli* was successful only in a mutant in which the La protease was inactivated. Gene 16 mutants of phage P22 were not able to infect *rec*BCD mutants of *Salmonella typhimurium* nor was protein 16, synthesized in *E. coli* from a plasmid, able to substitute for the pilot protein of phage T4. It seems that gene product 16 is not a pilot protein in the meaning of binding to the ends of linear DNA, thus protecting it from degradation by nucleases.   © 1992 Academic Press, Inc.

INTRODUCTION

There are several biological processes that involve DNA transfer across a bacterial membrane, such as infection by bacteriophages, conjugation, and genetic transformation. Although there exist many studies dealing with the identification of genes involved in DNA transport, the mechanism of this process is poorly understood. Infection, conjugation, and transformation share at least the following general features: (i) For the initiation of the DNA transport a receptor is postulated on the surface of the recipient cell, (ii) the DNA crosses the membrane in the form of a complex consisting of DNA and pilot proteins and/or DNA-binding proteins, and (iii) a preexisting or newly formed pore in the membrane is needed for the uptake of the DNA–protein complex.

Bacteriophage P22 encodes at least three proteins that are essential for the DNA ejection/injection process (Israel, 1977). These three proteins, the products of genes 7, 16, and 20, are therefore good candidates for proteins involved in DNA transfer. It could be shown by photoinactivation experiments that in the phage particle the proteins of genes 7, 16, and 20 are closely associated with the phage DNA (Bryant and King, 1984). Phage mutants with a defect in gene 16 have been well characterized (Hoffman and Levine, 1975a,b). Gene product 16 is required at an early stage of the infection process and also at a late stage as it is incorporated in progeny phage particles. Infection and transduction require gp16 but induction of the prophage does not. Protein 16 can act *in trans* since

phage 16 mutants can be complemented in mixed infections by uv-inactivated wildtype phages. Gene 16 mutants normally adsorb to the cells and release at least the greater part of their DNA from the capsid; however, there is no expression of phage-specific genes. Thus it was postulated that gp16 may be involved either in the complete release of the phage DNA from the capsid or in guiding the DNA to the appropriate compartment of the host cell (pilot function). There also exists the hypothesis that gp16 could act as a hexamer forming a pore for the transfer of P22 DNA across the cytoplasmic membrane (Hoffman and Levine, 1975b; Labedan and Goldberg, 1981).

In order to investigate these different hypotheses it would be helpful to isolate and characterize the protein. We have therefore cloned the gene and successfully overproduced the gene product as a first step in analyzing its function in DNA uptake.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids

The pUC13 (Vieira and Messing, 1982) derivatives were propagated in *Escherichia coli* JM101 Δ*lac pro, thi, sup*E44 [F' *tra*D36, *pro*AB, *lac*I^{q}Z ΔM15]. The expression of genes from plasmid pJF118HE (Fürste *et al.,* 1986) was done in *E. coli* C600 F⁻, *thi*-1, *leu*B6, *lac*Y1, *sup*E44, *ton*A21 or in LC137 *htp*R165*am*, *lon*R9ts, *lac, trp, pho, rps*L, *sup*C$_{ts}$, *mal, tsx::Tn10*, which was a gift from J. Collins. *E. coli* E835 *rec*A56, *rec*B21, *su*⁻, *str*A was used for propagation of phage T4 2⁻. *Salmonella typhimurium* LT2 prototroph *su*⁻ (DB21, Botstein *et al.,* 1973) and *su*⁺, *cys*$_{am}$ were from H. Schmieger. *S.*

typhimurium *rec*BC531::*Tn10* was obtained from the Salmonella Genetic Stock Centre (SGSC595). Bacteriophages P22 16*am*34, 16*ts*, and 12*am*H80 were provided by the phage collection of H. H. Prell (Bode *et al.*, 1973).

Transformation

The transformation of *E. coli* Ca^{2+} cells was performed as described (Maniatis *et al.*, 1982). Transformation of *S. typhimurium* was done by electroporation using a Bio-Rad Gene Pulser.

DNA sequencing

DNA sequencing was performed by the chain-termination method of Sanger (Sanger *et al.*, 1977) of deletion derivatives of hybrid pUC13 plasmids created by use of the "nested deletion kit" from Pharmacia. For DNA synthesis the T7 sequenase kit from U.S. Biochemical Corp. was used (Tabor and Richardson, 1987).

SDS–PAGE of *E. coli* proteins

Total protein for SDS–PAGE was prepared as described (Lanka and Barth, 1981). Samples of 10 μl were applied to polyacrylamide slab gels (17.5% polyacrylamide, 1 mm thick, 13 cm long) prepared as described (Laemmli, 1970). Electrophoresis was performed at 50 mA for about 3 hr. Gels were stained with Coomassie brilliant blue G-250. The protein marker mix from Pharmacia contained phosphorylase *b* (mol wt 94,000), BSA (mol wt 67,000), ovalbumin (mol wt 43,000), carboxyanhydrase (mol wt 30,000), trypsin inhibitor (mol wt 20,100), and α-lactalbumin (mol wt 14,400).

Construction of plasmid pBU516

The 5' end of gene 16 was amplified by PCR using the following primers:

```
          HindIII      380         390
Primer 1  5'  GCGAAGCTTATGÀAAGTTACCGĊTAAT 3'
                      startcodon

          SacII    870        860
Primer 2  5'  TCACCGCGGCÀTTCTTCGĊAACGT 3'.
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Numbers above the sequence correspond to the nucleotide position in Fig. 3.

RESULTS

Mapping of gene 16 by subcloning experiments

On the physical map of phage P22, gene 16 is located on a *Sal*I–*Eco*RI DNA restriction fragment of

about 6 kb (Chisholm *et al.*, 1980). Since gene 16 is part of a large operon with the promoter located outside of the *Sal*I–*Eco*RI DNA fragment (Casjens and Adams, 1985), this fragment was inserted into the multicopy vector pUC13 to allow expression from the *lac*-UV5 promoter. The hybrid plasmid, termed pBU1, was used for further subcloning. A 2.5-kb *Sal*I–*Nru*I fragment was deleted between the *lac*UV5 promoter and the startpoint of gene 16. The resulting plasmid pBU2 carried an insert of 3.5 kb. Plasmid pBU2 was used for the production of a series of deletion derivatives by partial degradation of the insert with exonuclease III and S1 nuclease in the 5' region of gene 16 (Fig. 1).

Hoffman and Levine (1975a) have shown that a P22 mutant defective in gene 16 can be complemented by a functional gp16 from a second phage in mixed infections. That means the early function of gp16 can be complemented at the membrane. We utilized a trans-complementation assay to select for clones producing an active gp16. This complementation assay, however, is due to the late function of gp16. The gp16 synthesized from a plasmid should be incorporated into particles of gene 16 mutants, allowing them to infect their host in the following infection cycle. A suppressor-negative strain of *S. typhimurium* was transformed by electroporation with the hybrid plasmids previously established in *E. coli*. The plasmid-bearing Salmonella strains were infected by P22 16*am*34 phages which had been propagated in a suppressor strain. These phages are able to infect a suppressor-negative strain but they can produce infectious progeny phages only if a functional gp16 is provided by a plasmid. The plating efficiencies on the different plasmid-carrying suppressor-negative strains were compared to those on a suppressing host (Fig. 1). The progeny phages were plated again under permissive and nonpermissive conditions to distinguish between progeny phages resulting from complementation or recombination. About 30% of the progeny phages were recombinants. With the complementation assay the gene 16 was limited to a region of about 2.0 kb (pBU5). Plasmids with inserts smaller than pBU5 were no longer able to complement.

Nucleotide sequence of the gene 16 region

For the determination of the nucleotide sequence of gene 16, pBU3 (2.2-kb insert) was used. The sequencing strategy is summarized in Fig. 2. From analysis of proteins from P22 phages it is known that gp16 has a molecular weight in the range of 67,000–69,000 (Youderian and Susskind, 1980). Within the sequenced region there is only one open reading frame that could
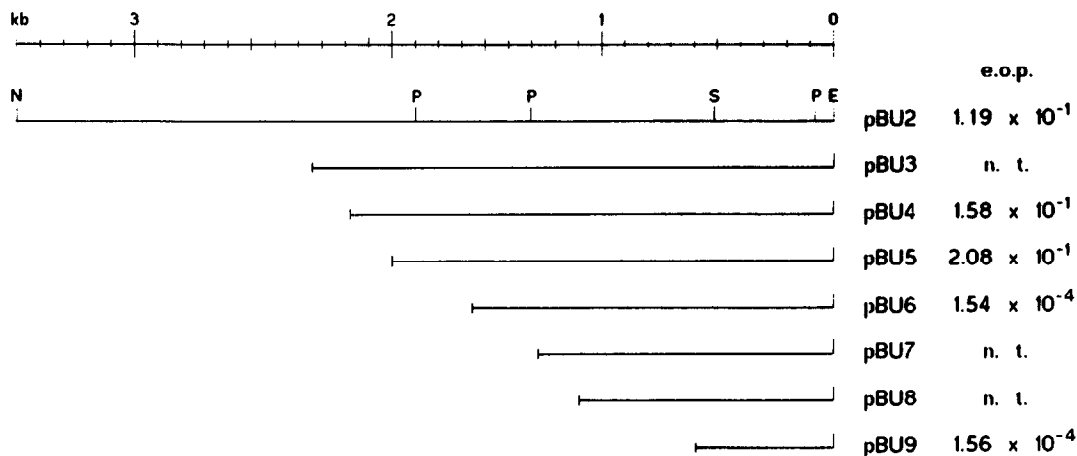
FIG. 1. Deletion derivatives of plasmid pBU1 and their effect on the efficiencies of plating (e.o.p.) of P22 gene 16 mutants. The size of the insert DNA was determined from the mobility of the DNA fragment during electrophoresis on a 1.2% agarose gel. The e.o.p. of phage P22 16 am34 on a suppressing host was taken as 1 (titer about 8×10^9). Under nonpermissive conditions the phage plated with a relative e.o.p. of 1.5×10^{-4} due to revertants in the lysate. (N, *Nru*I; P, *Pst*I; S, *Sac*I; E, *Eco*RI).

encode a protein of such a molecular weight. The open reading frame starts at nucleotide 377 and ends at nucleotide 2203 (Fig. 3). The possible protein of 609 amino acids would have a molecular weight of 64,366. As expected there is no promoter in the 5′ region; a sequence resembling an ideal Shine–Dalgarno sequence is not present either.

If gp16 forms a pore for the phage DNA transport across the host membrane as proposed (Goldberg, 1980; Labedan and Goldberg, 1981), then the protein should have some hydrophobic regions long enough to span the membrane. The hydropathic plot of the hypothetical protein indicates that there are no hydrophobic regions (Fig. 4). This is also supported by calculations of the hydropathic index according to Kyte and Doolittle (1982) which is always below +1.6 at any part of the protein (window of 19 amino acids).

Overproduction of gene product 16

In order to initiate studies on the biological function of gp16 during P22 infection, the protein has to be
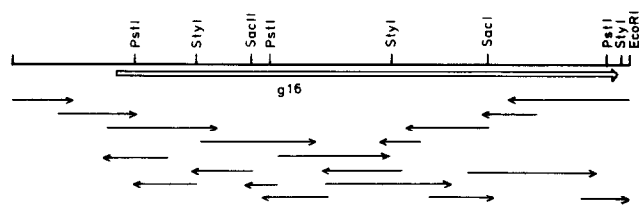

FIG. 2. Strategy for the nucleotide sequence determination of a 2.2-kb DNA fragment carrying gene 16. The sequence of the upper strand was mainly determined with the aid of deletion derivatives (see Fig. 1) and the universal primer. The lower strand was mainly determined using synthetic oligonucleotide primers complementary to sequences of the upper strand.

purified in sufficient quantities. For this purpose the 2-kb insert of pBU5 (beginning with nucleotide 343, Fig. 3) was inserted into different expression vectors with the p_L promoter of phage lambda, a promoter of phage T7, or the *tac* promoter (pBU216). None of the hybrid plasmids resulted in overproduction of a 64-kDa protein when the total protein of induced cells was analyzed by SDS–PAGE.

As there is no good Shine–Dalgarno sequence in front of gene 16 and the Shine–Dalgarno sequence of pJF118 is 63 bp from the start codon in plasmid pBU216, the *Hind*III–*Sac*II fragment of plasmid pBU5 was replaced by a *Hind*III–*Sac*II PCR product representing the 5′ end of gene 16 (Fig. 3). A new *Hind*III site, directly in front of the start codon, was constructed by the primer; the *Sac*II recognition site is located at position 875 (Fig. 3). PCR-amplified DNA was hydrolyzed with restriction endonucleases *Hind*III and *Sac*II and inserted into pBU5 digested with the same enzyme (pBU516). To ensure that gene 16 was not mutated by PCR amplification, we measured its biological activity by the complementation assay. The plating efficiency of gene 16 mutants of P22 on a strain with pBU516 was almost as high as that found on a suppressing host (relative e.o.p. 0.8 and 1, respectively). The total insert of pBU516 (*Hind*III–*Eco*RI) was then integrated into pJF118HE. In the resulting plasmid pBU316, the start codon of gene 16 is located 15 bp from the Shine–Dalgarno consensus sequence of vector pJF118HE. Although an active gene product could be identified by the biological test, overproduction of the protein in *E. coli* C600 extracts was not observed. In order to test for the function of the promoter and Shine–Dalgarno sequence, the *lacZ* gene was fused to

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        10        20        30        40        50        60
ATCCGGCAATTGGTGCAGATGTTCGCAGCCGACTCACAGGCAAAGAGCAACGCCAGTTAT

        70        80        90       100       110       120
ATAACTCCGCACAACGTATTCAGGGAAGAATGCAGAATCAGGGCGTGGCAGCAGCAAGAG

       130       140       150       160       170       180
ATATGGGCGCTAGGCGTATCAACACCATTGCAGAAGCGAAGATGTATTTTCAGGGGATGC

       190       200       210       220       230       240
CGCAGTTGACTACTCAAGCCCGGAGCTATGCAGCAGTCTATTCGTGAGATTCAGGAATAC

       250       260       270       280       290       300
ACCAACAATTATAACCAGCAGTACAACGTTAATGTTGGTAATGGTGGGCTGAAATCACCA

       310       320       330       340       350       360
AGGCAGCAGCCAGATACTCAGCAATCAGCCGGAGAAGTTACACGTCTAAATCTGGCATTA

       370       380       390       400       410       420
AATTCACGGTGGAATAATGAAAGTTACCGCTAATGGCAAGACATTCAACTTCCCTGATGG
                   M  K  V  T  A  N  G  K  T  F  N  F  P  D  G

       430       440    PstI    460       470       480
AACCAGCACAGAAGACATCGGCGCTGCAGTTGATGAGTATTTTGCAGGGCAGGCATCAGC
 T  S  T  E  D  I  G  A  A  V  D  E  Y  F  A  G  Q  A  S  A

       490       500       510       520       530       540
AGCAGAAACACAACCAGCAGAACAGCAGGAAGAACCACAGCAGCCTGAACAATCCCTGAT
 A  E  T  Q  P  A  E  Q  Q  E  E  P  Q  Q  P  E  Q  S  L  M

       550       560       570       580       590       600
GCAACGGGCTGGTGACTTACTCACTGGCGGCCAGTCAGCAGGCCAGATTGCAGAGCAGGC
 Q  R  A  G  D  L  L  T  G  G  Q  S  A  G  Q  I  A  E  Q  A

       610       620       630       640       650       660
TGGGCGTGGGCTCGTAAATATCCCGTTTGATGTATTGCAGGGCGGCGCGAGTCTCATTAA
 G  R  G  L  V  N  I  P  F  D  V  L  Q  G  G  A  S  L  I  N

       670       680       690       700       710       720
CGCAATCAGCCAAGGGTTAGGCGGCCCGAAAGTGCTGGATGACGTGTATCGCCCGGTAGA
 A  I  S  Q  G  L  G  G  P  K  V  L  D  D  V  Y  R  P  V  D

       730       740       750       760       770       780
TCGCCCGACTGACCCATATGCGCAGGCAGGAGAATCCATTGGCGGGTATCTAATTCCAGG
 R  P  T  D  P  Y  A  Q  A  G  E  S  I  G  G  Y  L  I  P  G

       790       800       810       820       830       840
CGCTGGAGTCGCTGGCAACATGGCGATCGGCTCAGTGGCTGAGGCAGCCAATCAGCAGGG
 A  G  V  A  G  N  M  A  I  G  S  V  A  E  A  A  N  Q  Q  G

       850       860       SacII  880       890       900
AGATTTTGCTGGCAACGTTGCGAAGAATGCCGCGGTAAACCTCGGCGCTCAGGGGCTACT
 D  F  A  G  N  V  A  K  N  A  A  V  N  L  G  A  Q  G  L  L

       910       920       930    PstI    950       960
TTCTGGCGCAGCTAAATTAGTCGGGCGTGGCATTACTGCAGCAAGAGGTGAGATTGCACC
 S  G  A  A  K  L  V  G  R  G  I  T  A  A  R  G  E  I  A  P
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       970       980       990      1000      1010      1020
AGAGGCCAGACAACTGATTGATACCGCTGAGAGTATGGGTGTTAAGCCCATGACGTCAGA
 E  A  R  Q  L  I  D  T  A  E  S  M  G  V  K  P  M  T  S  D

      1030      1040      1050      1060      1070      1080
TATGATCAAGCCTGGCAATGCCTTTACTCGCAGCTTAATGCAAGGTGGTGAAGGTGCGTT
 M  I  K  P  G  N  A  F  T  R  S  L  M  Q  G  G  E  G  A  L

      1090      1100      1110      1120      1130      1140
GCTTGGAACGGGAGGAAAAAGAGCAGAACAGTACGCTATCCGCAGCAAACTTCTAGGCGA
 L  G  T  G  G  K  R  A  E  Q  Y  A  I  R  S  K  L  L  G  D

      1150      1160      1170      1180      1190      1200
CTATTTCGACCGAGTGGGAGGATACAATCCTGATGATATCGTTAAGTCAATGACCAGTAC
 Y  F  D  R  V  G  G  Y  N  P  D  D  I  V  K  S  M  T  S  T

      1210      1220      1230      1240      1250      1260
AGTAGGAGGGCGTAAAAATGCGGCAGGAGCAGTAAGAGATGAAATAGTAAATAGAATGGG
 V  G  G  R  K  N  A  A  G  A  V  R  D  E  I  V  N  R  M  G

      1270      1280      1290      1300      1310      1320
AAGCGCTCCAGTGGGAACCACCAATTCAATTAATGCAATTGATACAAATATTGCAAGACT
 S  A  P  V  G  T  T  N  S  I  N  A  I  D  T  N  I  A  R  L

      1330      1340      1350      1360      1370      1380
TGAGAAGCTCGGCACATCAGCGGACCAGAGGCTTTTGACAGCGCTTAAAAATCTAAAGGG
 E  K  L  G  T  S  A  D  Q  R  L  L  T  A  L  K  N  L  K  G

      1390      1400      1410      1420      1430      1440
GGAATTGAATAGCGGGAATGTTGATTTTGATCTCCTGCAACAGCATCGCACTGCATTCCG
 E  L  N  S  G  N  V  D  F  D  L  L  Q  Q  H  R  T  A  F  R

      1450      1460      1470      1480      1490      1500
CACCAATGTTCAGGGTGATGCGATGGTATTCCCAAATCAGGCCAAGGCTGCAACTAACAT
 T  N  V  Q  G  D  A  M  V  F  P  N  Q  A  K  A  A  T  N  M

      1510      1520      1530      1540      1550      1560
GGTTGAAAATGCAATGACTCGTGATTTGCGCAATGCTGTCGGTAAATCACTAGGGCCACA
 V  E  N  A  M  T  R  D  L  R  N  A  V  G  K  S  L  G  P  Q

      1570      1580      1590      1600      1610      1620
AGCTGCATCAAAATATCTCAAATCCAACTCAGACTTCGCAAACATTTACAATAAGGTTCT
 A  A  S  K  Y  L  K  S  N  S  D  F  A  N  I  Y  N  K  V  L

      1630      1640      1650      1660      1670      1680
GAATAAGCGCATCTCTAATACGCTAAATAAAGCCAGAAGCGAATACACACCCGAGCTTAT
 N  K  R  I  S  N  T  L  N  K  A  R  S  E  Y  T  P  E  L  I

      1690      1700      1710      1720      1730      1740
TAACACCGTTGTTTTCAGTCGCAAACCGTCAGATATAAAGCGCATATGGAGCTCCCTGGA
 N  T  V  V  F  S  R  K  P  S  D  I  K  R  I  W  S  S  L  D

      1750      1760      1770      1780      1790      1800
TAACAAAGGAAAGGACGCAATGCGAGCTGCATACATCAGCAAGATTGCTGAAAAAACTGG
 N  K  G  K  D  A  M  R  A  A  Y  I  S  K  I  A  E  K  T  G

      1810      1820      1830      1840      1850      1860
TGATTCTCCAGCTAAGTTCATAACCGAAGTAAACAAATTAAAAGCGCAATCCGGAGGTGA
 D  S  P  A  K  F  I  T  E  V  N  K  L  K  A  Q  S  G  G  E

      1870      1880      1890      1900      1910      1920
GATTTACAACACCATTTTCAGCGGACGACACATGAAGGAACTTGATGCGCTTCATGATGT
 I  Y  N  T  I  F  S  G  R  H  M  K  E  L  D  A  L  H  D  V

      1930      1940      1950      1960      1970      1980
GCTGAGACAAACAGCTAGGTCTGATTCGGCAAATGTTGTCACACAGACGGGGCAGGCGCT
 L  R  Q  T  A  R  S  D  S  A  N  V  V  T  Q  T  G  Q  A  L

      1990      2000      2010      2020      2030      2040
GGCAAATCCGGTAAGGCTTGGCGCTGCAATTCCTACTTTAGGTAAGTCACTCGCAGCAGA
 A  N  P  V  R  L  G  A  A  I  P  T  L  G  K  S  L  A  A  E

      2050      2060      2070      2080      2090      2100
GGCCGGCTATGGCTTGGCAATGAGGGTGTATGAGAGCAAGCCAATAAGAAATATGCTACT
 A  G  Y  G  L  A  M  R  V  Y  E  S  K  P  I  R  N  M  L  L

      2110      2120      2130      2140      2150      2160
CAGGCTGGCTAACACCAAGCCGGGCACACCTGCATATGAGCGTGCGCTGAATCAGGCCGC
 R  L  A  N  T  K  P  G  T  P  A  Y  E  R  A  L  N  Q  A  A

    PstI          2180      2190      2200      2210      2220
TACTGCAGTGCGCCCTCTTTTAGCTAACGAAGCTACCCGGCAGTAGCGCTATAAGCCAAG
 T  A  V  R  P  L  L  A  N  E  A  T  R  Q

      2230      2240
GACGGCATTTATTTTATAGTTTTTAT
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FIG. 3. Nucleotide sequence of the 2.2-kb DNA fragment containing gene 16. The deduced amino acid sequence of gene product 16 is shown below the nucleotide sequence.
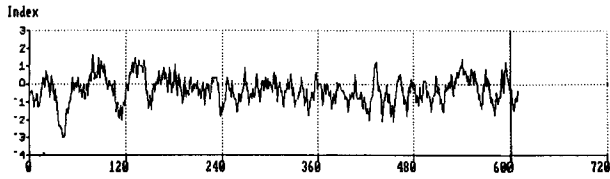
FIG. 4. Hydropathic plot of gene product 16 according to Kyte and Doolittle (1982). The hydropathic plot was performed at a span setting of 9.

FIG. 6. Dependence of gene product 16 abundance on La protease activity. SDS–PAGE (15%) of total protein from induced cells (*E. coli* LC137 pBU316). Cells were induced by the addition of 2 m*M* IPTG after inactivation of La protease by incubation at 42° for 20 min. Lane 1, 5 hr induction at 37°; lane 2, 3 hr at 37° followed by 2 hr at 28° to allow synthesis of La protease; lane 3, 3 hr at 37°; lane 4, uninduced cells; lane 5, marker proteins.

the 5′ part of gene 16 by exchanging a *Pst*I DNA fragment of gene 16 (position 444 to 2163, Fig. 3) for the *Pst*I *lacZ* cassette of plasmid pMC1871 (Pharmacia). The β-galactosidase activity of the fusion protein was determined and compared with the activity of β-galactosidase from a plasmid with an intact *lacZ* gene expressed from the *tac* promoter. For both plasmids a β-galactosidase activity of about 110–130 units was determined when cells were grown in the presence of IPTG, indicating that the promoter and the Shine–Dalgarno sequence of plasmid pBU316 allow high expression after induction. Without induction, the β-galactosidase activity was about 10 units. Therefore it is possible that although gene 16 is well expressed, protein 16 has only a short half-life and is rapidly degraded by proteases. To test this hypothesis, plasmids pBU216 and pBU316 were introduced into a *lon* mutant of *E. coli*. After thermic inactivation of the La protease and induction of the *tac* promoter by IPTG, overproduction of a 64-kDa protein was observed (Fig. 5). Thus it seems that gp16 indeed is quite rapidly degraded in *E. coli*. In a further experiment we wanted to test whether gp16 is sensitive to protease degradation only during its synthesis or also in its native form. Therefore, after 3 hr of induction at nonpermissive temperature, the tem-
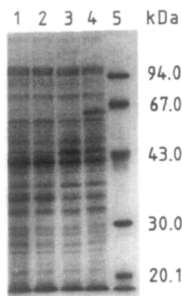
perature was shifted down to 28° for 2 hr to allow the synthesis of active La protease. There was no visible difference in the amount of gp16 between cells with inactivated and reactivated La protease (Fig. 6). It seems that once it has its native conformation, gp16 is no longer degraded by the La protease.

Some aspects of the biological function of gene product 16

The gene product 16 produced from a plasmid is able to complement gene 16 mutants. Plating of the progeny phages on suppressing and nonsuppressing hosts showed that the gp16 is incorporated into the phage particle of progeny phages. These progeny phages behave like mutant phages grown on a suppressing host.

It has been shown that 16⁻ particles can be complemented at the membrane, if gp16 is supplied from outside the cell by a helper phage (Hoffman and Levine, 1975a). To test whether gp16 can also complement 16⁻ particles from inside the cell, we infected *S. typhimurium su⁻* pBU516 with 16⁻ phage particles. The infective center assay was done as described (Hoffman and Levine, 1975a). The results of Table 1 show that complementation at the membrane is not possible from inside the cell. Complementation is possible from outside the cell by a helper phage (P22 12 *am*H80), indicating that the phage lysate contained 16⁻ particles.

It has been suggested that gp16 of P22 might be a pilot protein (Hoffman and Levine, 1975b; Bryant and King, 1984). Pilot proteins of phages with linear DNA are thought to protect the DNA against degradation by the RecBCD nuclease. A well-studied example for such a pilot protein is the gene 2 protein of bacteriophage T4 (Lipinska *et al.*, 1989). It has been shown that gene 2 mutants of T4 are able to infect *rec*BCD mu-

FIG. 5. Overproduction of gene product 16 in *E. coli*. SDS–PAGE (17.5%) of total protein from induced and uninduced cells. Induction was performed by adding 2 m*M* IPTG to the cells at a titer of 3 × 10⁸/ml followed by incubation at 37° for 3 hr. *E. coli lon* mutants were incubated for 20 min at 42° to inactivate the La protease prior to induction. Lane 1, LC137 pBU216 uninduced; lane 2, LC137 pBU216 induced; lane 3, LC137 pBU316 uninduced; lane 4, LC137 pBU316 induced; lane 5, marker proteins.
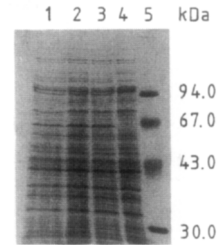
Plasmid	Phage	moi	Infective centers/ml infected culture
—	16⁻ts Defective particles	0.5	1.1×10^7
—	12 amH80	5	1.1×10^4
—	16⁻ts Defective particles + 12 amH80	0.5 5	2.7×10^8
pBU516	16⁻ts Defective particles	0.5	9.0×10^6

Note. Salmonella typhimurium su⁻ was infected at a titer of 1 × 10⁸/ml. Adsorption was done at 25° for 15 min. Unadsorbed phage were removed by centrifugation. Resuspended, infected cells were plated with indicator cells (S. typhimurium su⁻) and incubated at 28°.

tants successfully (Silverstein and Goldberg, 1976). We have tested whether a gene 16 mutant of P22 can also grow on recBCD mutants. In such an experiment we observed no increase in the efficiency of plating of phage mutants on suppressor-negative S. typhimurium recB mutants compared to the e.o.p. on wildtype cells. In both cases the plaques obtained (2×10^4/ml) were due to wildtype revertants. Pilot proteins probably bind to the end of the linear phage DNA, but they do not require specific DNA sequences. Therefore the pilot proteins of phages T4 and Mu are interchangeable, and the pilot protein of phage Mu enables gene 2 mutants of T4 to grow on a suppressor-negative strain (Schaus and Wright, 1980). In order to determine if this was also accomplished by gp16 of P22, E. coli su⁻ carrying plasmid pBU5 was infected by a gene 2 mutant of phage T4 grown on a suppressor-free E. coli recBCD. The efficiency of plating of phage T4 did not increase in comparison to a plasmid-free strain. While the phage titer was about 1×10^{10} on a recBC host, the titer was about 3×10^7/ml on JM101 and JM101 pBU5.

DISCUSSION

We have cloned and overexpressed gene 16 of bacteriophage P22. The nucleotide sequence includes an open reading frame encoding a protein of 609 amino acids with a molecular weight of 64,366. Recently the eight aminoterminal amino acids of gp16 isolated from phage particles have been determined (Eppler et al., 1991). This amino acid sequence agrees with that deduced from the nucleotide sequence beginning at nucleotide 377 (Fig. 3). There are no membrane-spanning regions in either part of the protein arguing against the possibility that gp16 forms a membrane pore for DNA transport (Goldberg, 1980; Labedan and Goldberg, 1981). It could be shown, for example, that gp3 of

phage fd can oligomerize and form an aqueous pore (Glaser-Wuttke et al., 1989). The gp3, although it does not have many α-helical domains, exhibits at least several possible membrane-spanning regions. Gene product 16 itself is probably not a transmembrane protein, but the possibility remains that gp16 could modify a preexisting pore or form a pore in interaction with other proteins.

The protein 16 does not protect P22 DNA from degradation by the RecBCD enzyme. It also cannot substitute for the gene product 2, the pilot protein of phage T4. Therefore, it is not likely that gp16 is a pilot protein in the sense that it binds to the end of linear DNA protecting it from degradation by exonucleases. However, it cannot be ruled out that gp16 is a pilot protein acting by another mechanism than known for gp2 from phage T4. It has also been suggested that gp16 may interact with gp20 and/or gp7 in guiding the DNA across the membrane. Supporting this is a recent report that gp16, if purified from procapsids, is complexed with gp20 (Thomas and Prevelige, 1991).

Protein 16 is only overproduced when its gene is under the control of a strong promoter and a Shine–Dalgarno consensus sequence in a lon mutant of E. coli. At present, overproduction has only been shown in E. coli. It would be interesting to know if the half-life time is as short in Salmonella strains, the natural host, as in E. coli. Since protein 16 is probably needed only during the injection of DNA, it could be convenient that it is rapidly degraded when it has fullfilled its function during the infection process. When gp16 is produced during late stages of phage development, the protein may be incorporated very rapidly into the proheads (Thomas and Prevelige, 1991) and thus be protected from degradation by proteases. Protein 16 may also be protected from degradation by forming oligomers or a complex, for example, with gp20. Reactivation experiments of protease La showed that gp16 is indeed a substrate for the protease only during synthesis. The native protein is no longer degraded.

Overproduction of gp16 in E. coli renders it possible to purify the protein in sufficient amounts for further studies. It will be possible to test DNA binding, a prerequisite for a pilot protein. With the help of antibodies it may be possible to determine the location of the protein after infection.

ACKNOWLEDGMENTS

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