Bot. Bull. Acad. Sin. (1995) **36:** 207-214

# Molecular cloning and expression of the coat protein genes of Cf, a filamentous bacteriophage of *Xanthomonas campestris* pv. *citri*

Mei-Kwei Yang[1,4], Huei-Mei Huang[2], Yen-Chun Yang[1], and Wei-Chih Su[3]

[1]*The Graduate School of Biology, Fu-Jen University, Taipei, Taiwan, Republic of China*

[2]*Institute of Life Science, National Defense Medical Center, Taipei, Taiwan, Republic of China*

[3]*Graduate Institute of Botany, National Taiwan University, Taipei, Taiwan, Republic of China*

(Received April 11, 1995; Accepted August 18, 1995)

**Abstract.** Particles of the filamentous bacteriophage Cf contain a major coat protein, the B protein, with a molecular weight of approximately 6,000. In addition, a minor coat protein, the A protein, with a molecular weight of about 50,000, was also identified on sodium dodecyl sulfate-containing polyacrylamide gels. A 3.3 kbp *Hin*cII fragment derived from Cf genome was cloned into the expression plasmid pG308N, an *E. coli* plasmid which carries pL promoter. The recombinant plasmid pG33 and a series of deletion derivatives of pG33 were constructed and transformed into *E. coli* DG116 for expression of phage Cf genes. The genes coding for A and B proteins of Cf were found on the 2.0 kbp *Eco*RI-*Hin*cII fragment. The complete nucleotide sequences of the 2.0 kbp *Eco*RI-*Hin*cII insert were determined. The deduced amino acid sequence corresponds to a 62-amino acid-residue polypeptide that has a calculated Mr of 6070 was identified as the B protein by SDS/PAGE and immunoblotting. Another open reading frame (ORF419) downstream of the B protein gene (ORF62) was found, and was shown to code for a polypeptide of 419 amino acids with a calculated Mr of 44,676 that exhibits considerable identity to the A protein.

**Keywords:** Bacteriophage Cf; Coat protein genes; Gene expression; Host specificity; Nucleotide sequences.

## Introduction

Cf, Xf, and ΦLf are filamentous bacteriophages that infect different pathovars of *Xanthomonas campestris*. All three phages contain a single-stranded circular form DNA surrounded by a long protein coat (Dai et al., 1980; Kuo et al., 1967, 1969; Tseng et al., 1990). Similar to other filamentous phages isolated in *Escherichia coli*, the infection of these phages results in the releasing of progeny phages into the medium without cell lysis (Hofschneider and Preuss, 1963; Marvin and Hohn, 1969). Although the phages are of comparable genomic size, studies of their host range have revealed a significant difference. Phage Cf, isolated from *X. campestris* pv. *citri*, can infect neither *X. campestris* pv. *oryzae* nor *X. campestris* pv. *campestris*. Another filamentous phage (Xf) isolated from *X. campestris* pv. *oryzae*, cannot infect *X. campestris* pv. *citri* and *X. campestris* pv. *campestris.* We have recently reported, however, that DNAs of Cf and Xf can be transformed into non-host *X. campestris* strains by electroporation and propagated to accumulate intracellular phage DNA. They then export phage particles at a constant rate (Yang et al., 1991). This demonstrates that adsorption to host cell is responsible for the infectivity of the phage particles. Our interest in the detail of host specificity of phage infection has lead us to examine how Cf adsorbs to *X. campestris* pv. *citri.*

[4]Corresponding author.

The mechanism of adsorption in filamentous phages of *E. coli* has been inferred from genetic, electron microscopic, and molecular biological studies (Gray et al., 1981; Jacobson, 1972; Rasched and Oberer, 1986; Segawa et al., 1975). It was found that the A protein, specified by gene III of M13, has been implicated in the attachment of the phage to the host receptor (Henry and Pratt, 1969; Pratt et al., 1969), and it may also function in penetration (Goldsmith and Konigsberg, 1977). This adsorption protein comprises a minor fraction of the phage coat and is located only at one end of the phage (Lopez and Webster, 1982; Grant et al., 1981). After infection, the A protein remains with the phage, acting as a pilot protein to guide its DNA into the cell, and converts the single-stranded DNA to the double-stranded replicative form (RF) (Jazwinski et al., 1973; Lin and Bendet, 1976). The A protein also functions as a cut-off agent in the final stage of phage assembly (Crissman and Smith, 1984; Dotto and Zinder, 1983). The adsorption protein of the filamentous phage was found to have both very early and late functions.

Data concerning the structure and function of other coat proteins also exists (Rasched and Oberer, 1986; Simons et al., 1981). B protein encoded by gene VIII is the major protein constituent of the mature phage particles (Henry and Pratt, 1969; Luiten et al., 1983). Within the infected *Escherichia coli* cell, a large quantities of B protein is synthesized as precursor molecules with amino-terminal signal peptides of 18 to 23 amino acid residues. After insertion of the precursor coat protein into the membrane,

the amino-terminal peptides are removed by signal peptidase (Ohkawa and Webster, 1981). The mature B protein remains as an internal constituent of the cytoplasmic membrane until it is assembled into a phage particle. Besides these two proteins, two additional minor coat proteins, C protein and D protein, encoded by genes VI, VII, and IX of phage M13 have also been reported (Simons et al., 1981). Although data indicated that these minor coat proteins are located at one or both ends of the phage filament, their biological function is still unclear.

As mentioned above, the mechanism used by coliphage to infect *E. coli* cell has been well-studied. It appears that F pili of male cells play a major role in the process of filamentous phage infection, but only limited data exists concerning the process of phage adsorption in *Xanthomonas campestris*. Whether Cf uses F pili or another portion of the cell surface as a receptor has not yet been studied. Unlike other filamentous phages, it was demonstrated that Cf can incorporate its DNA into the genome of its host (Kuo et al., 1987b). The life cycle of filamentous phages isolated from *X. campestris* may depart significantly from that of the coliphages (Kuo et al., 1987a). To further characterize Cf, and to investigate the mechanism by which it infects host cells, we need to identify genes coding for coat proteins with regard to their biological function. In this communication, we report on polyacrylamide gel studies of Cf, with particular emphasis on the cloning and expression of coat protein genes in *E. coli.*

## Materials and Methods

### *Bacterial Strains, Bacteriophages and Plasmids*

The bacterial strains, bacteriophages, and all plasmids used in this study are described in Table 1. *Escherichia coli* and *Xanthomonas campestris* strains were routinely grown at 37°C and 28°C respectively. For selection of *E. coli* transformants, LB was supplemented with ampicillin (100 $\mu$g/ml) or tetracycline (10 $\mu$g/ml).

### *DNA Manipulation and Cloning*

Plasmid DNA and replicative form DNA of the phage was isolated from the infected culture by the alkaline lysis procedure (Birnboim and Doly, 1979) and further purified by ethidium bromide/cesium chloride buoyant density gradient centrifugation. Restriction enzymes obtained from Bethesda Research Laboratories were used according to the manufacture's specifications. Restriction fragments were separated by agarose gel electrophoresis and then recovered from the gel by electroelution. The DNA cloning was performed in *E. coli*, and appropriate clones were selected as described by Sambrook et al. (1989).

### *Determination of Nucleotide Sequences*

The 2.0 kbp *Eco*RI-*Hin*cII fragment of cf RF DNA was subcloned into M13 mp18 and mp19. DNA from both strands was sequenced by the modified dideoxy chain termination method (Hattori and Sakaki, 1986) by following the instructions of the supplier (U.S. Biochemical Corp.)

### *Polyacrylamide Gel Electrophoresis and Immunoblotting*

Purified phage particles or soluble protein fraction of plasmid-containing strains were separated by electrophoresis through 15% sodium dodecyl sulfate-polyacrylamide gel as described (Weber and Osborn, 1969). Gels were either stained with Coomassie brilliant blue R250 or used for electrotransfer to a nitrocellulose membrane. Antibodies were raised against purified Cf particles in male New Zealand white rabbits by administering four injections, one month apart, of $1\times10^{11}$ pfu/ml phage plus Freund complete adjuvant into the backs of the rabbits. Approximately one

**Table 1.** Bacteria and plasmids.

| Strain and plasmid | Relevant characterizations | Source or reference |
|---|---|---|
| *Xanthomonas campestris* | | |
| *X. campestris* pv. *citri* | Host of phage Cf. | Our collection |
| *X. campestris* pv. *oryzae* | Host of phage Xf. | Our collection |
| *Escherichia coli* | | |
| DH5α | F'*lac*Z M15*rec*A1 (*lac*ZYA-*arg*F) | Sambrook et al., 1989 |
| DG116 | $F^-$ *end*A1 *thi*-1 *hsd*R17 *sup*E44 $\lambda cI_{857}$ | Huang et al., 1990 |
| JM103 | h*sd*R4Δ(*lac pro*)recA$^+$/F'*tra* D36 *pro*AB*lac*I$^q$M15 | Messing et al., 1981 |
| Plasmids | | |
| pG308N | An expression vector, containing a pL promoter and the upstream untranslated region of the N gene of phage lambda; 3.2 kbp. | Huang et al., 1990 |
| pUC18 | Ap$^r$ *lac*Z, 2.7 kbp. | Norrander et al., 1983 |
| M13mp18 | M13 derivative contains multiple cloning sites. | Yanisch et al., 1985 |
| pG33 | A 3.3 kbp H*inc*II fragment of phage Cf inserted in the *Pvu*II site of pG308N. | This study |
| pG13 | A 1.3 kbp *Hin*cII-*Eco*RI fragment of phage Cf inserted in the *Pvu*II site of pG308N. | This study |
| pG20 | A 2.0 kbp *Eco*RI-*Hin*cII fragment of phage Cf inserted in the *Pvu*II site of pG308N. | This study |
| pG04 | A 0.4 kbp *Eco*RI-*Bgl*I fragment of pG33 inserted in the *Pvu*II site of pG308N. | This study |
| pG06 | A 0.6 kbp *Bgl*I-*Hin*dIII fragment of pG20 inserted in the *Pvu*II site of pG308N. | This study |
| pG10 | Deletion of the 0.96 kbp *Hin*dIII fragment of pG20. | This study |
| pG16 | A 1.6kbp *Bgl*I-*Bam*HI fragment of pG20 inserted in the *Pvu*II site of pG308N. | This study |

month after the last injection, the rabbits were bled (50 to 60 ml) and antisera were prepared. The anti-cf serum was diluted 100- to 500-fold and used for Western blotting. Immunological detection was performed according to the method of Burnett (1981).

## Results

### *Proteins Present in Virions of the Filamentous Bacteriophage Cf and Xf*

To obtain information about the protein composition of the filamentous phages isolated from *Xanthomonas campestris*, Cf and Xf virions were propagated in *X. campestris* pv *citri* and *X. campestris* pv. *oryzae*, respectively. The purified virions were analyzed and size-fractionated on sodium dodecyl sulfate-polyacrylamide gels. As shown in Figure 1, it was found that in both cases the phage particles were composed of two different polypeptide chains. The faster-migrating major coat protein that has a molecular weight of about 6,000 was found in Cf and Xf. In addition, a faint band with a molecular weight of about 46,000-50,000 was assigned as a minor coat protein component of the virions. In accordance with the nomenclature of other filamentous phages, the major and minor proteins have been denoted as B protein and A protein, and are encoded by gene VIII and gene III, respectively.

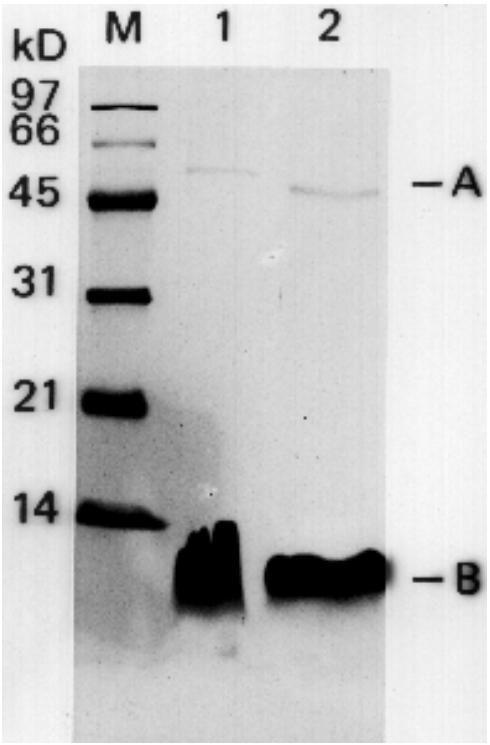


**Figure 1.** Polyacrylamide gel electrophoretic analysis of the coat proteins present in filamentous phages. Purified Cf (lane 1) and Xf (lane 2) phages were subjected to 15% SDS-polyacrylamide gel electrophoresis followed by staining with Coomassie brilliant blue.

### *Cloning of Bacteriophage Cf Coat Protein Genes*

Previous studies on the location of coat protein genes in Cf indicated that the 3.3 kbp *Hinc*II fragment contains the information for Cf A and B proteins (Kao, 1991). We have chosen a cloning strategy that should result in determining the entire nucleotide sequences for the coat protein genes. The 3.3 kbp *Hinc*II fragment derived from Cf DNA was cloned into the expression vector pG308N, an *E. coli* plasmid that carries the controllable pL promoter. The construction of the hybrid plasmid, pG33, is outlined in Figure 2. Cells transformed by this hybrid DNA were selected for growth in the presence of ampicillin. As shown in Figure 3, a series of deletion derivatives of pG33 was obtained by digestion with restriction enzymes and religation.

### *Expression of Cloned Cf Coat Protein Genes in E. coli*

To test the expression of the cloned bacteriophage Cf coat protein genes in *E. coli*, a culture of *E. coli* DG116 transformed with plasmid pG33 was grown at 37°C in LB medium and induced by heating at 42°C for 15 min. After induction, cells were collected, washed, and analyzed by SDS-polyacrylamide gel electrophoresis. As shown in Figure 4A, the major products specified by pG33 plasmid DNA comigrated with that specified by intact Cf phage particle. From its electrophoretic mobility, it was identi-

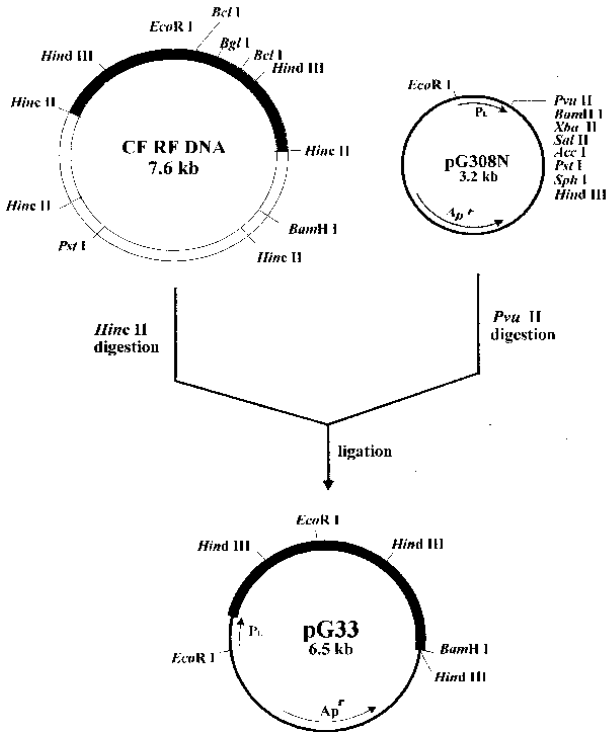


**Figure 2.** Construction of the expression plasmid pG33. The 3.3 kilo base-pair (kbp) *Hinc*II fragment of Cf RF DNA was cloned into the *Pvu*II site of pG308N, forming plasmid pG33.

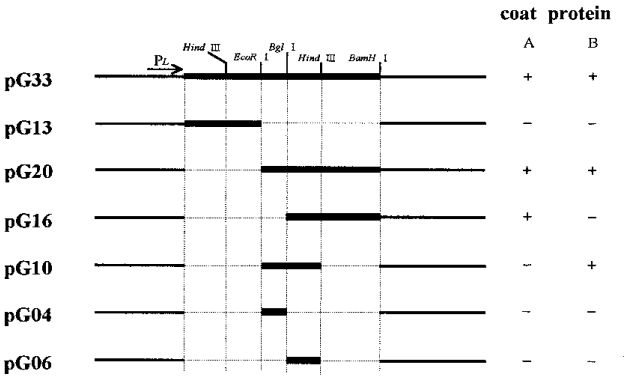


**Figure 3.** Structure and expression of pG33 and its derivatives. The 3.3 kbp *Hin*cII fragment of Cf RF DNA was inserted into pG308N and introduced into *E. coli* DG116. We isolated coat protein-expressing, ampicillin-resistant clones that carried pG33. Plasmid pG20, pG16, and pG13 were obtained from pG33 by digesting with *Hin*cII and *Eco*RI, *Hin*cII and *Bgl*II, and *Eco*RI and *Bam*HI, respectively, followed by religation. Plasmid pG10, pG04, and pG06 were constructed by ligation of fragments digested by *Eco*RI and *Hin*dIII, *Eco*RI and *Bgl*I, and *Bgl*I and *Hin*dIII, respectively, with P*vu*II-cleaved pG308N DNA. The regions derived from Cf RF DNA and pG308N are indicated by darkened lines and open boxes, respectively. Dashed lines represent deletions. The expression of coat proteins was detected by SDS-polyacrylamide gel electrophoresis.

fied as Cf A and B proteins, whereas products specified by vector pG308N did not correspond with those of phage Cf. The identity of the supposed Cf coat proteins was further confirmed by means of an immunoblot employing antiserum raised against bacteriophage Cf (Figure 4B).

Among deletion derivatives of pG33, only pG20 gave the same result as pG33. In plasmid pG13, which contains a 1.3 kbp *Hin*cII-*Eco*RI fragment of Cf RF DNA, no protein band was detected. Genes coding for the A and B proteins of Cf were found to be located on the 2.0 kbp *Eco*RI-*Hin*cII fragment (Figure 3). Further deletion was carried out by digesting this DNA fragment with *Bgl*I and religating to produce plasmid pG16. Only A protein with Mr of 50,000 was detected. In contrast to this, a protein with Mr of 6,000 was made in cells carrying pG10 (Figure 4C). In cells carrying the deletion plasmids pG04 and pG06, which contain the 400 bp *Eco*RI-*Bgl*I fragment and 600 bp *Bgl*I-*Hin*dIII fragment, respectively, the synthesis of both A and B proteins can no longer be detected.

## *Nucleotide Sequence Analysis of the Cloned Coat Protein Genes*

The complete nucleotide sequence of the 2.0 kbp *Eco*RI-*Hin*cII insert was determined and is shown in Fig-

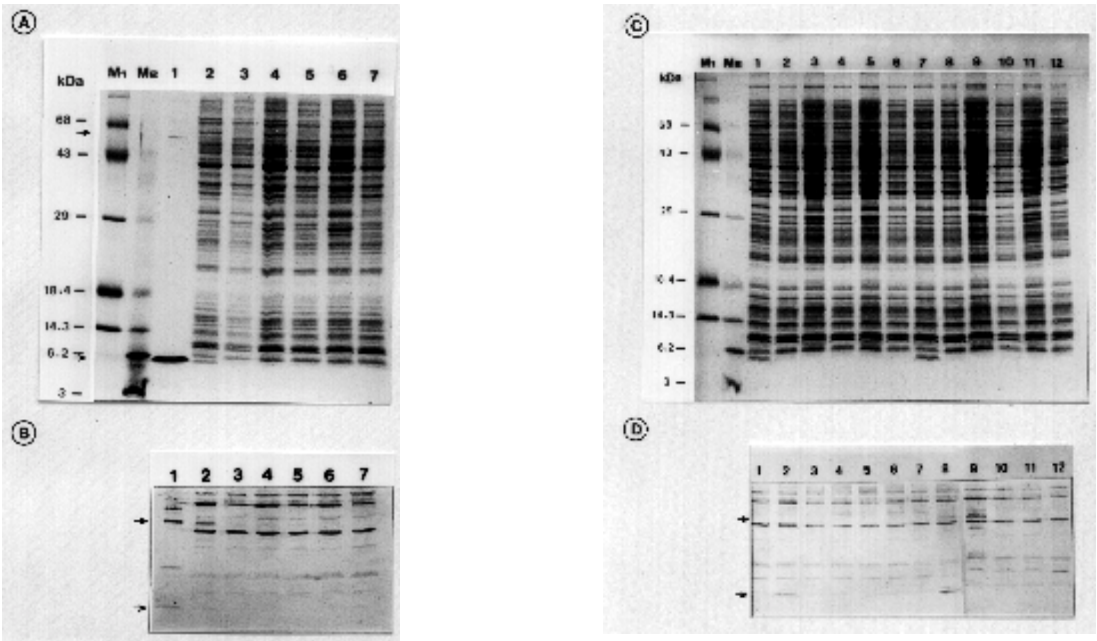


**Figure 4.** Expression of bacteriophage cf coat protein gene in *E. coli* cells transformed with recombinant plasmids. **A**, Samples of whole cells were subjected to SDS-polyacrylamide gel electrophoresis and the products were visualized by Coomassie brilliant blue staining. Lane 1, Cf particle; lane 2, pG33-transformed cells, induced at 42°C for 15 min; lane 3, pG33-transformed cells, not induced with heating; lane 4, *E. coli* DG116 induced at 42°C for 15 min; lane 5, *E. coli* DG116 not induced with heating; lane 6, pG308N-transformed cells, induced at 42°C for 15 min; lane 7, pG308N-transformed cells, not induced with heating; M1, high-molecular weight protein-size marker; M2, low-molecular weight protein-size marker. **B**, Immunoblot of a SDS-polyacrylamide gel employing antiserum raised against bacteriophage Cf. The lanes are as described for A. **C**, Cells transformed with various pG33 derivatives were also subjected to SDS-polyacrylamide gel electrophoresis and the proteins were stained with Coomassie Brilliant Blue. Lane 1, pG20, induced; lane 2, pG20, not induced; lane 3, pG04, induced; lane 4, pG04, not induced; lane 5, pG06, induced; lane 6, pG06, not induced; lane 7, pG10, induced; lane 8, pG10, not induced; lane 9, pG16, induced; lane 10, pG16, not induced; lane 11, pG308N, induced; lane 12, pG308N, not induced. M1 and M2 are high- and low-molecular weight protein-size markers, respectively. **D**, Immunoblot of a SDS-polyacrylamide gel employing antiserum raised against bacteriophage Cf. The lanes are as described for C.

ure 5. The deduced amino acid sequences of two open reading frames contained within this sequence are presented below the DNA sequences. Open reading frame 62, starting at position 312 and ending at 498, encodes a polypeptide of 62 amino acids with a calculated Mr of 6,070. This is in good agreement with the Mr of the B protein of Cf, determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and identified by immunoblotting techniques. A putative Shine-Dalgarno ribosome-binding site is located 9 base pairs upstream of the open reading frame 62 (Figure 5). In addition, analysis of the downstream region of ORF 62 showed the presence of putative rho-independent transcription termination signal, which is identical to that of the Cf1c (Kuo et al., 1991). The transcript of this sequence may be characterized by a high potential for forming base-paired stem and loop structure similar to Ff (van Wezenbeek et al., 1980). From these analyses, we conclude that ORF 62 is the real coding sequence of the B protein of phage Cf.

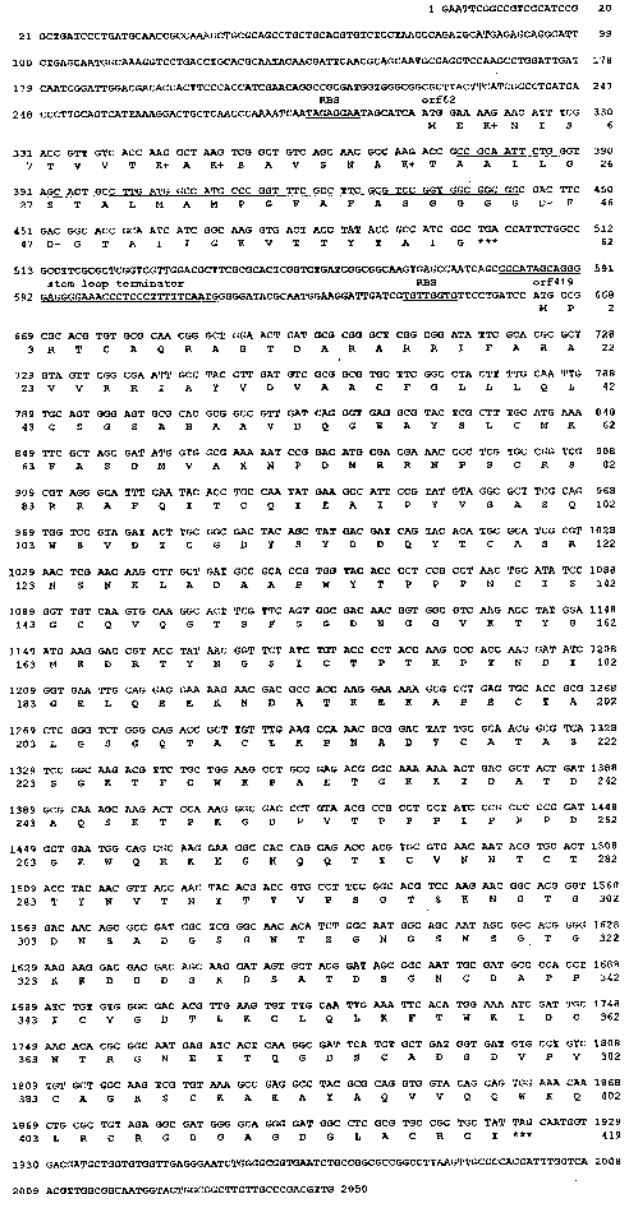

**Figure 5.** Nucleotide sequence and derived amino acid sequence of the phage Cf coat protein genes. The nucleotides are numbered above the sequence starting from the ATG initiation codon of the A and B protein. The deduced amino acid sequence is shown below the nucleotide sequence. The putative Shine-Dalgarno sequences and stem-loop terminators are underlined. The amino acids are represented by one-letter symbols. Negatively charged residues are labeled '-', positively charged residues are labeled '+', and the hydrophobic regions are overlined.

Another open reading frame (ORF419) starts at base pair +663, ten nucleotides downstream from a putative Shine-Dalgarno ribosome binding sequence, TGGTG (Figure 5). The structural part of this gene is 1,257 nucleotides long and encodes a polypeptide of 419 amino acids with a calculated Mr of 44,676. This Mr coincides with the value for the minor protein (the A protein) estimated from SDS-polyacrylamide gel electrophoresis and identified by immunoblot analysis. These findings indicated that ORF419 contains the whole coding sequence of the A protein of phage Cf.

## Discussion

In SDS-polyacrylamide gel electrophoresis of proteins from two filamentous phages isolated from *Xanthomonas campestris*, we found not only the major coat protein but also a minor coat protein. The rapidly moving major virion protein, B protein, was present in greater abundance than was the slower migrating minor coat protein, A protein. According to the reports (Goldsmith and Konigsberg, 1977; Woolford et al., 1977) on other known filamentous bacteriophages, a value of about 2,700 to 3,000 copies of B protein and approximately 5 copies of A protein per f1 and fd virion was estimated. Although the copy number of the A and B protein subunits of Cf had not been determined in the present study, our data presented here shows that B protein is the major constituents of the phage coat. But only one minor coat (A) protein associated with the Cf viral particle was identified in SDS-polyacrylamide gel. On the basis of composition of minor coat proteins in Ff coliphages, two additional capsid proteins were reported, but their presence was not revealed. Since they most probably existed in very small amounts, it was not possible to characterize them as phage-specific proteins. Until the application of radiochemical techniques to identify the appearance of protein, the actual composition of the coat protein in Cf will not be established with certainty.

To gain a better understanding of the biological function of two proteins associated with the Cf particle, molecular cloning techniques were used to study these proteins in more detail. We have shown that coat protein genes (gene III and VIII) of bacteriophage Cf can be cloned and successfully expressed in *E coli* under the control of the pL promoter in the plasmid pG308N. We determined the nucleotide sequences of a DNA fragment containing two coat protein genes of Cf. Two open read-

ing frames, encoding certain proteins of 62 and 419 amino acid residues, respectively, were found. The estimated Mr of these two proteins were in excellent agreement with those of the predicted mature polypeptides B and A. In comparing the amino acid sequences of ORF62 with that of gene VIII of *E. coli* filamentous phages, no significant homology was found between the two coat proteins. The major coat protein of some other filamentous phages not belonging to the Ff group have been investigated in more detail (Peeters et al., 1985; Putterman et al., 1984; Thomas et al., 1983). The secondary structure of B protein is basically the same for all phages examined, but there are differences in amino acid sequences. As has been pointed out by Nakashima et al. (1974, 1981), the primary structure of major coat (B) protein was segregated into three domains: acidic N-terminus, hydrophobic central part, and basic C-terminal region. This amino acid distribution determines the orientation of the mature B protein in its host cell membrane and provides specific biological function. In phage Cf, similar functional side chains were also found in the major coat protein, the product of ORF 62. The involvement of B protein in phage morphogenesis needs to be investigated.

Study of the major coat protein of the filamentous phages of *E. coli* and *Pseudomonas* has shown that B protein is synthesized as a precursor form with an N-terminal signal peptide and is correctly inserted in the membrane (Boeke et al., 1980 ; Greenwood and Perham, 1989; Luiten et al., 1983; William, 1988). The recognition and cleavage of precoat by signal peptidase takes place in the membrane. After processing to mature coat protein, its central hydrophobic region is retained in the membrane bilayer, while the C-terminal part will interact via its basic residues with the negatively charged DNA when progeny phage is assembled (Andreans and Willian, 1986). The data presented in the present study demonstrated the existance of gene coded for B protein of phage Cf, but the primary sequence of B protein was only deduced from nucleotide sequence data. The processing of this protein, although not shown in the present study, will be identified by direct sequence analysis. In an attempt to examine the hydrophilicities of ORF62, we found a striking internal hydrophobic sequence that is preceded by a positively charged amino acid and followed by two negatively charged residues (Figure 5). We suggest that the charged residues halts the passage of the polypeptide through the cytoplasmic membrane. If this interpretation is correct, we propose a similar mechanism of the insertion of the major coat protein of Cf into the cell membrane as Ff phage (Andreas and Willian, 1986 ; Greenwood and Perham, 1989 ; Boeke and Model, 1982; Dotto and Zinder, 1983 ; Boeke et al., 1980).

The product of ORF419 appeared to be the only protein of phage Cf besides the major coat (B) protein. In comparing our results from the amino acid analysis with sequences in the A protein of Ff, no possible homologies were found. These differences may be a result of differences in the bacteriophages, since adsorption protein of the phage mediates recognition of and attachment to the cell surface of its specific host (Gray et al., 1981; Lopez and Webster, 1982; Segawa et al., 1975). Like the major coat protein, A protein is initially synthesized in a precursor molecules with 18 extra amino acods at its N-terminus (van Wezenbeek et al., 1980). The amino-terminal part of the protein has been shown to attach to the tip of F pilus on *E. coli* cell, whereas the C-terminal part anchors the protein to the host membrane (Boeke and Model, 1982). A central hydrophobic domain was also found, which may be essential to its aggregation to oligomers. Phage Cf, unlike Ff coliphages that require F pili as the host receptor, attach to certain receptor on the surface of *X. campestris* pv. *citri* by unknown mechanism. In analysis of the amino acid sequence that was deduced from the nucleotide sequence of ORF419, no distinct functional domains were found.

Although a substantial amount of information is now available concerning the morphogenesis of filamentous phage coat protein, relatively little is known about how Cf coat proteins are assembled at the molecular level. The data presented here shows that the products of ORF62 and ORF419 are major and minor constituents of the Cf coat. Examination of the sequences flanking these two ORFs reveals a potential regulatory signal for the genes. At 75 base pairs upstream of the start codon ATG of the ORF62 is a region of dyad symmetry capable of folding into a stem-and-loop structure followed by five U residues, if transcribed into RNA. This hairpin structure corresponds to a probable rho-independent transcription terminator. The existence of this terminator may halt the translation of the following gene and result in a very low level of A protein expression. It is of particular interest to investigate the coat protein synthesis in more detail. To this end, examination of the apparent promoter activity of sequences upstream of ORF62 and ORF419 will be carried out. DNA fragments, including upstream sequences of the B protein and A protein genes, will be placed on a promoter probe plasmid and their influence on the expression of reporter gene will be tested.

**Acknowledgments.** The authors thank Dr. Yu-Shen Chang for valuable advice, generous assistance, and helpful discussions. This research was supported by a grant from the National Science Council, Republic of China (NSC83-0211-B030-004).

## Literature Cited

Andreas, K. and W. Willian. 1986. The cytoplasmic carboxyl terminus of M13 precoat is required for the membrane insertion of its central domain. Nature **322:** 335–339.

Boeke, J. D., M. Russel, and P. Model. 1980. Processing of filamentous phage pre-coat protein. Effect of sequence variations near the signal peptidase cleavage site. J. Mol. Biol. **144:** 103–116.

Boeke, J. D. and P. Model. 1982. A prokaryotic membrane anchor sequence: carboxyl terminus of bacteriophage f1 gene III protein retains it in the membrane. Proc. Natl. Acad. Sci. USA **79:** 5200–5204.

Birnboim, H. C. and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:** 1513–1523.

Burnett, W.N. 1981. Western blotting: electrophoretic transfer of unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112:** 195–203.

Crissman, J. and G. Smith. 1984. Gene 3 protein of filamentous phages: evidence for a carboxyl-terminal domain with a role in morphogenesis. Virology **132:** 445–455.

Dai, H., K. S. Chiang, and T.T. Kuo. 1980. Characterization of a new filamentous phage Cf from *Xanthomonas citri.* J. Gen. Virol. **46:** 277–289.

Dotto, G. and N. Zinder. 1983. The morphogenetic signal of bacteriophage f1. Virology **130:** 252–256.

Goldsmith, M. E. and W. H. Konigsberg. 1977. Adsorption protein of the bacteriophage fd: isolation, molecular properties, and location in the virus. Biochemistry **16:** 2686–2694.

Grant, R., T. Lin, W. Konigsberg, and R. Webster. 1981. Structure of the filamentous bacteriophage f1. Location of the A, C, and D minor coat proteins. J. Biol. Chem. **256:** 539–546.

Gray, C., R. Brown, and D. Marvin. 1981. Adsorption complex of filamentous fd virus. J. Mol. Biol. **146:** 621–627.

Greenwood, J. and R. N. Perham. 1989. Dual importance of positive charge in the C-terminal region of filamentous bacteriophage coat protein for membrane insertion and DNA-protein interaction in virus assembly. Virology **171:** 444–452.

Hattori, M. and Y. Sakaki. 1986. Dideoxyl sequencing method using denatured plasmid templates. Anal. Biochem. **152:** 232–238.

Henry, T. J. and D. Pratt. 1969. The proteins of Bacteriophage M13. Proc. Natl. Acad. Sci. USA **62:** 800–807.

Hofschneider, P. H. and A. Preuss. 1963. M13 bacteriophage liberation from intact bacteria as revealed by electron microscopy. J. Mol. Biol. **7:** 450–451.

Huang, L. H., H. F. Tsi, and S.T. Liu. 1990. High level expression of porcine growth hormone in *Escherichia coli* from an expression vector containing bacteriophage λ. $P_L$ and N gene untranslated region. Biochem. Biophys. Res. Commun. **173:** 711–717.

Jacobson, A. 1972. Role of F pili in the penetration of bacteriophage f1. J. Virol. **10:** 835–843.

Jazwinski, S. M., R. Marco, and A. Kornberg. 1973. A coat protein of the bacteriophage M13 virion participates in membrane-oriented synthesis of DNA. Proc. Natl. Acad. Sci. USA **70:** 205–209.

Kao, E.T. 1991. Cloning and expression of the filamentous bacteriophage Cf coat protein genes in *Escherichia coli.* Master thesis. Fu-Jen University.

Kuo, T.T., T. C. Huang, R.Y. Wu, and C. M. Yang. 1967. Characterization of three bacteriophages of *Xanthomonas oryzae* (Uyeda Et Ishiyama) Dowson. Bot. Bull. Acad. Sin. **8:** 246–254.

Kuo, T. T., T. C. Huang, and T. Y. Chow. 1969. A filamentous bacteriophage from *Xanthomonas oryzae*. Virology **39:** 548–555.

Kuo, T.T., Y.H. Lin, C.M. Hung, S.F. Dai, and T.Y. Feng. 1987a. The lysogenic cycle of the filamentous phage Cf1t from *Xanthomonas campestris* pv. *citri*. Virology **156:** 305–312.

Kuo, T. T., Y. S. Chao, H. Y. Lin, B. Y. Lin, C. F. Liu, and T. Y. Feng. 1987b. Integration of the DNA of filamentous bacteriophage Cf1t into the chomosomal DNA of its host. J. Virol. **61:** 60–65.

Kuo, T. T., M. S. Tan, and M. K. Yang. 1991. Complete nucleotide sequence of filamentous phage Cf1t from *Xanthomonas campestris* pv. *citri*. Nucleic Acids Res. **19:** 2498.

Lin, T. and I. Bendet. 1976. The A protein of bacteriophage fd: its interference with viral infection. Biochem. Biophys. Res. Commun. **72:** 369–372.

Lopez, J. and R. Webster. 1982. Minor coat protein composition and location of the A protein in bacteriophage f1 spheroids and I-forms. J. Virol. **42:** 1099–1107.

Luiten, R. G. M., J. G. G. Schoenmaker, and R. N. H. Konings. 1983. The major coat protein gene of filamentous *Pseudomonas aeruginosa* phage pf3: absence of an N-terminal leader signal sequence. Nucleic Acids Res. **11:** 8073–8085.

Marvin, D. and B. Hohn. 1969. Filamentous bacterial viruses. Bacteriol. Rev. **33:** 172–209.

Marvin, D. and E. Wachtel. 1975. Structure and assembly of filamentous bacterial viruses. Nature (London) **253:** 19–23.

Messing, J., R. Crea, and P. H. Seeburg. 1981. A system for shotgun DNA sequencing. Nucleic Acids Res. **9:** 309–321.

Nakashima, Y. and W. Koningsberg. 1974. Reinvestigation of a region of the fd bacteriophage coat protein sequence. J. Mol. Biol. **88:** 598–600.

Nakashima, Y., B. Frangione, R. Wiseman, and W. Konigsberg. 1981. Primary structure of the major coat protein of the filamentous bacterial viruses If1 and Ike. J. Biol. Chem. **256:** 5792–5797.

Norrander, J., T. Kempe, and J. Messing. 1983. Construction of improved M13 vectors using oligodeoxynucleotide-directed mutagenesis. Gene **26:** 101–106.

Ohkawa, I. and R. E. Webster. 1981. The orientation of the major coat protein of bacteriophage f1 in the cytoplasmic membrane of *Escherichia coli.* J. Biol. Chem. **256:** 9951–9958.

Peeters, B. P. H., R. M. Peters, J. G. G. Schoenmakers, and R. N. H. Konings. 1985. Nucleotide sequence and genetic organization of the genome of the N-specific filamentous bacteriophage Ike: Comparison with the genome of the F-specific filamentous phages M13, fd and f1. J. Mol. Biol. **181:** 27–39.

Pratt, D., H. Tzagoloff, and J. Beaudoin. 1969. Conditional lethal mutants of the small filamentous coliphage M13. II. Two genes for coat proteins. Virology **39:** 42–53.

Putterman, D. G., A. Casadevall, P. D. Boyle, H. L. Yang, B. Frangione, and L. A. Day. 1984. Major coat protein and single-stranded DNA binding protein of filamentous virus pf3. Proc. Natl. Acad. Sci. USA **81:** 699–703.

Rasched, I. and E. Oberer. 1986. Ff coliphages: structural and functional relationships. Microbiol. Rev. **50:** 401–427.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Segawa, K., K. Ikehara, and Y. Okada. 1975. Isolation and chemical properties of A-protein from filamentous phage fd. J. Biochem. **78:** 1–7.

Simons, G., R. Konings, and J. Schoenmakers. 1981. Gene VI, VII and IX of phage M13 code for minor capsid proteins of the virion. Proc. Natl. Acad. Sci. USA **78:** 4194–4198.

Thomas, G., B. Prescott, and L. Day. 1983. Structure similarity, difference and variability in the filamentous viruses fd, Ike, If1, pf1 and Xf. Investigation by Laser Raman Spectroscopy. J. Mol. Biol. **165:** 321–356.

Tseng, Y. H., M. C. Lo, K. C. Lin, C. C. Pan, and R. Y. Chang. 1990. Characterization of filamentous bacteriophage ΦLf from *Xanthomonas campestris* pv. *campestris*. J. Gen. Virol. **71:** 1881–1884.

Van Wezenbeek, P. M. G. F., T. J. M. Hulsebos, and J. G. G. Schoenmakers. 1980. Nucleotide sequence of the filamentous bacteriophage M13 DNA genome: comparison with phage fd. Gene **11:** 129–148.

Weber, K. and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. J. Biol. Chem. **244:** 4406–4412.

William, W. 1988. Mechanisms of membrane assembly: general lessons from the study of M13 coat protein and *Escherichia coli* leader peptidase. Biochemistry **27:** 1081–1086.

Woolford, J. L., H. M. Steinman, and R. E. Webster. 1977. Adsorption protein of bacteriophage f1 : solubilization in deoxycholate and localization in the f1 virion. Biochemistry **16:** 2694–2700.

Yang, M. K., W. C. Su, and T. T. Kuo. 1991. Highly efficient transfection of *Xanthomonas campestris* by electroporation. Bot. Bull. Acad. Sin. **32:** 197–203.

Yanisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:** 103–119.

# 線狀噬菌體 Cf 殼蛋白基因之選殖與表現

楊美桂 黃惠美 楊源昌 蘇偉誌

輔仁大學生物研究所

由柑橘潰瘍病菌 *Xanthomonas campestris* pv. *citri* 分離的線狀噬菌體 Cf，以 SDS-polyacrylamide 凝膠電泳分析而知是由 A 蛋白與 B 蛋白構成其蛋白外殼， A 蛋白之分子量爲 50,000 daltons，含量極少； B 蛋白分子量爲 6,000 daltons，含量極多，爲主要的殼蛋白。將 Cf 基因體以限制酶 *Hinc*II 作用，分離出 3.3 kbp 之 DNA 片段，插入 *E. coli* 之表現載體 pG308N，所形成之重組質體稱 pG33，當送入 *E. coli* DG116 後會產生 A 與 B 兩種蛋白，表示此 3.3 kbp DNA 片段含有 A 蛋白與 B 蛋白的基因，以不同限制酶作用，得到許多不同位置的缺失質體，再分別送入 *E. coli*，由 A 蛋白與 B 蛋白的是否形成，因而得知兩個基因是位於自 *Eco*RI 至 *Hinc* II 之 2.0 kbp 片段內。於是將 *Eco*RI 至 *Hinc*II 之 2.0 kbp DNA 片段的核甘酸序列訂出，由讀出之氨基酸序列，可知有兩個 ORFs 的存在，分列由 62 個與 419 個氨基酸所組成，其形成的蛋白質分子量約爲 6,070 daltons 與 44,676 daltons，此與 SDS-PAGE 所訂出之 B 蛋白與 A 蛋白大小很相近，利用抗 Cf 血清以免疫反應確定之。

**關鍵詞**：線狀噬菌體 Cf ； 殼蛋白基因；基因選殖；核甘酸序列；基因表現；寄主特異性。