Bot. Bull. Acad. Sin. (1994) 35:1– 8

(Invited review paper)

Xanthomonas campestris pv. *citri* infected with filamentous phage Cf1: Plaque turbidity, viral DNA integration, and immunity[1]

Tsong-Teh Kuo[2,3,4], Gow-Jen Shieh[2], Jung-Lieh Kuo[2], Wen-Ping Chen[2], and Huey-Jiunn Bau[2]

Institute of Botany[2] and Institute of Molecular Biology[3], Academia Sinica, Taipei, Taiwan 115, Republic of China

Abstract. Filamentous phage Cf1 isolated from *Xanthomonas campestris* pv. *citri* normally forms turbid plaques, and contains the integration life cycle. Genomic maps of Cf1, however, are still poorly defined. We show the localization of the plaque turbidity (pt) gene and of the integration-required region in the Cf1 phage genome. The involvement of pt gene in the regulation of Cf1 phage DNA synthesis was identified by substituting alternate DNA fragments between wild type and clear-plaque forming mutants. The functionally required region for integration of Cf1t were identified by analyzing the site-specific integration of plasmids containing different phage DNA fragments in the absence of any functional origin of replication. In the present study, a new phage mutant producing smaller plaques was obtained. This phage can infect Cf1 lysogens, and hence was named Cf1tv. Host cells infected by Cf1tv can release large numbers of phage particles. Furthermore, the ability to integrate Cf1tv DNA into the host chromosome is lost. Sequence data revealed that an alteration of one base pair occurred in the Cf1tv genome.

Keywords: Filamentous phage; Immunity; Plaque turbidity; Site-specific recombination.

Contents

Introduction

Filamentous bacteriophages, 1– 2 μm long and 6 – 7 nm wide, are hosted by gram-negative bacteria. Upon infection, the phages are absorbed at the tips of the threadlike host-bacteria appendages known as sex pili (Marvin and Hohn, 1969). Filamentous phages contain single-stranded DNA; their length is determined by the size of the DNA they encapsulate. Unlike other bacteriophages, filamentous phages are released from growing and dividing cells without marked harm to the cells. These carrier-strains of bacteria can be subcultured and will continue to release virus particles (Marvin and Hohn, 1969). Many filamentous phages have been identified in various bacteria. These include: (1) F-specific phage of *Escherichia coli*: f1, M13, ZJ/2, Ec9, AE2 and HR (Russel and Model, 1988); (2) N pili-specific phage of *E. coli*: IKe (Peeters et al., 1967); (3) *Salmonella* phage: If1 and If2 (Lawn et al., 1967); (4) *Pseudomonas* phage: Pf2 and Pf3 (Minamishima et al.,1986); (5) *Vibrio* phage: v6 (Marvin and Hohn, 1969); (6) *X. campestris* pv. *citri* phage: øLf (Lu, 1985); (7) *X. campestris* pv. *oryzae* phage: Xf (Kuo et al., 1969); (8) *X. campestris* pv. *citri* phage: Cf1 (Kuo et al., 1987a,b).

[1] This paper was presented at the Symposium on Botany (December 7–22, 1992) in commemoration of the 30th anniversary of the re-establishment in Taipei of the Institute of Botany, Academia Sinica. The abstract of the paper was also published in the Institute of Botany, Academia Sinica Monograph Series No. 13 (1993).

[4] Corresponding author.

The well-known bacteriophages f1, fd, and M13 are similar to one another, differing only in a few nucleotide positions in the whole genome. The genome of f1 phage contains 6.4 kilobases (kb) of single-stranded DNA encoding ten gene products and 0.5 kb of noncoding intergenic region. Three of the phage-encoded proteins (gIIp, gVp and gXp) participate in phage replication. When a phage enters, the parental "plus" single-stranded DNA is converted into a supercoiled, double-stranded molecule (RFI). Multiplication of the f1 genome proceeds according to a rolling-circle type of replication (Geider and Kornberg, 1974). Each replication round is initiated by gIIp, which creates a DNA replication site by introducing a nick in the viral strand of RFI DNA (Meyer et al., 1979). gXp, identical to the C-terminal one-third of the gIIp, inhibits at least some of the activities of the gIIp (Fulford and Model, 1988b). gVp of f1, a 9.8-kDa single-stranded DNA binding protein, has two known functions in the late stage of f1 replication. First, it sequesters the viral strands displaced by the rolling-circle replication of the plus-strand DNA to prevent their reconversion to double strands. Second, any gVp not bound to DNA can repress the translation of gene II (Fulford and Model, 1988a). Regulation of gIIp activity is crucial to the unique life cycle of f1 phage.

A number of clear-plaque mutants of f1 have been characterized. Some contain base alterations in the leader sequence of gIIp and others contain alterations in the coding region of gIIp or gVp (Michel and Zinder, 1989 a,b; Dotto and Zinder, 1984 a,b; Faber and Ray, 1980). Furthermore, the clear plaque mutants of f1 produce a lower phage yield than do the wild type (Marvin and Hohn, 1969).

General Characteristics of Cf1

Filamentous phage Cf1 was isolated from *Xanthomonas campestris* pv. *citri*, which is a pathogenic bacterium of citrus canker. The original filamentous phage Cf and Cf16 were isolated from the same host bacterium (Dai et al., 1980, 1987). The general properties and the morphology of these three isolates are the same, but they are believed to differ somewhat in DNA sequence (Kuo et al., 1987a). The genome size of Cf1 is 7308 bp (Kuo et al., 1991). The length of Cf1 is about 1,157 nm, and its diameter is 6 nm (Kuo et al., 1987a, b). DNA isolated from Cf1 phage particles is in the single-stranded form, but after infection the phage DNA is converted into the double-stranded (RF) form in infected cells (Kuo et al., 1987a, b). The restriction map of Cf1 is shown in Fig. 1.

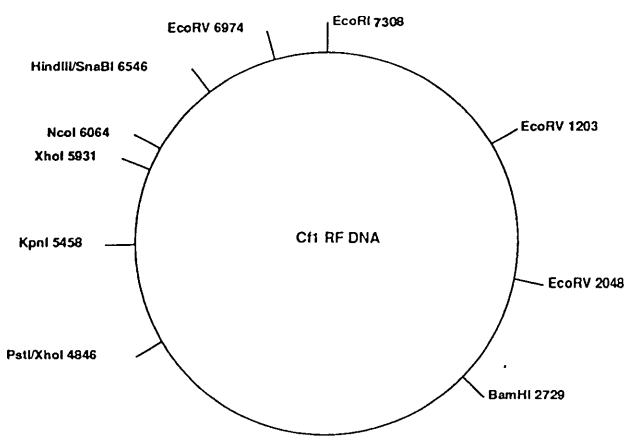

Fig. 1. The restriction map of Cf1 RF DNA.

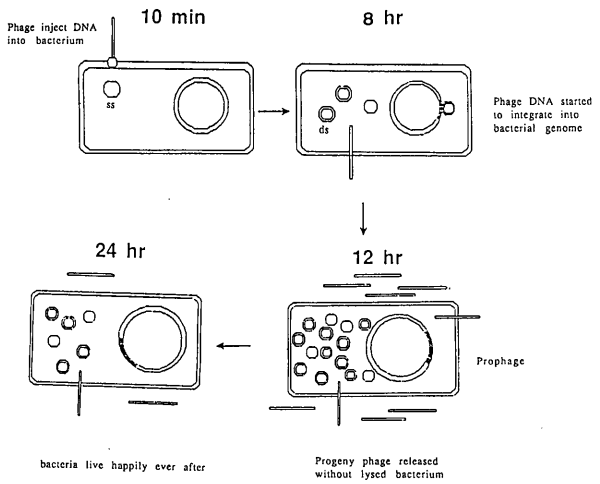

Fig. 2. Schematic diagram of the lysogenic cycle of Cf1 and phage production in infected cells. **ss**, single-stranded phage DNA; **ds**, double-stranded phage DNA.

During infection, Cf1 phage can undergo a lysogenic cycle and does not greatly affect the growth of cells (Kuo et al., 1987a). Our experiments show that the integration of phage DNA into the host chromosome can be detected at 8 h after infection, and that both single-stranded and double-stranded phage DNA could be detected at 12 h after infection. In addition, the phage titer of Cf1-infected cells can reach 2×10^{10} pfu/ml (Shieh et al., 1991) after 12 h of infection. It is suggested that Cf1 phage are produced progressively during the lysogenic life cycle. A schematic diagram of the lysogenic cycle of Cf1 phage is shown as in Fig. 2.

The Plaque Turbidity Gene of Cf1

Under normal cultivation conditions, Cf1 forms turbid plaque, but clear-plaque mutants derived from Cf1 phage are observed at a frequency of approximately

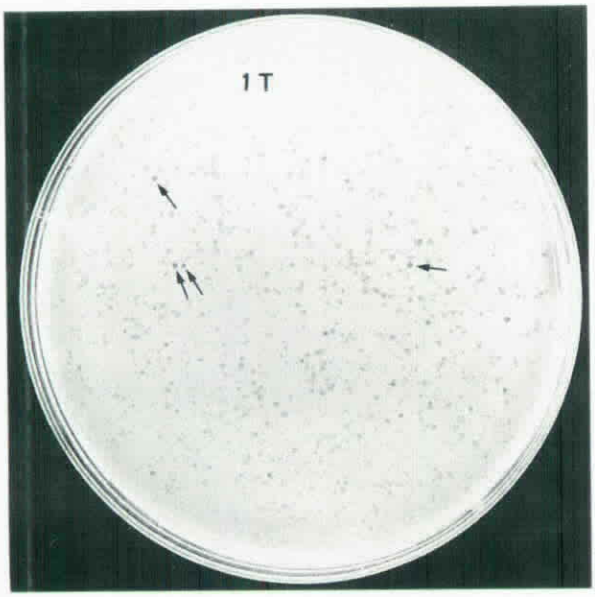

Fig. 3. A plate showing clear-plaque mutants (marked by arrows) among the turbid plaques of Cf1.

10^{-3}. The phages that form turbid plaque are named Cf1t and those forming clear plaque are named Cf1c (Fig. 3). In contrast to clear-plaque mutants from f1, Cf1c possesses higher phage-exporting ability and DNA replication rate than does the wild type. Analysis of DNA sequence data shows base alterations or deletions in the region upstream of the pt gene in Cf1 (Shieh et al.,1991).

Three characteristic differences between Cf1t and Cf1c phages have been observed: 1) The growth of Cf1c-infected cells is reduced compared with that of uninfected cells, while growth of Cf1t-infected cells remains normal; 2) The number of phages released from Cf1c-infected cells is 20-fold greater than that from Cf1t-infected cells; 3) Spot complementation tests indicate that the phenotype of turbid plaque is dominant and that of clear plaque is recessive (Shieh, 1992). It seems that a gene linked to this plaque turbidity may act as a genetic marker.

```
GCAACGCACAAACCGCCTGGGAAAAAGTAGGCAGTGGTGTCGCCACAGAACGAGCAGGTG      60
CCATCAGCCACGATAGAACTCCATGGCAGCGTCACGTGCATCAGCAGCATCGGTGCGCGA     120
TGCAAAATAGCTCTGTTCAACAGCGTTGCCGTTGACGACGATGGTCAAGACGTAAATGCG     180
GGGGCCGCCCTGCACTCGAGCTGGCGCAATCATCCATGCAACAGATCGAGACTCAGCCAC     240
GACGCACCTCGCGATATGCGATAGCGACCAATGCGGCTTGCTCAATTTGAGCAACCATCG     300
CGTCGTGCTTGCGATCAAGACACCAACAGATCACAGCGACGATTGCGTACCCGGTGAGCA     360
CGCAGATCGACACGAGAATGTGCAGCGCCAAAAGCTGGCCCAGGATCGAATATGGATGCA     420
                        G
CGGTGTAGCCCCCTCCCCTGCCCCTTGACGCGGACCCCGGAGGGGAGCCGGGGGTGCGCG     480
  C                     T
GTGCTTAGGGTTGCCTAAGCACGGGAGCATGTATAGTTTTCACTGTCCTCTCTGTCAAGA     540
                        M  Y  S  F  H  C  P  L  C  Q  E       11

AAAACCTGTCATGGACCATGTAAATAATTTGCTTGACACCGTGCGCAAGTCGTGCGCCAT     600
 K  P  V  M  D  H  V  N  N  L  L  D  T  V  R  K  S  C  A  I    31

ACCGTCAGACAACATGTTGAGCAAAAAAATTGGAGTGACGCGGGCGCTGATAAGCGGCTG     660
 P  S  D  N  M  L  S  K  K  I  G  V  T  R  A  L  I  S  G  W    51
          KpnI
GCGCGTTGGCCGGTACCCGGTTCCTGACGCACGAATTGCAGAGCTATACGCTATGGCACA     720
 R  V  G  R  Y  P  V  P  D  A  R  I  A  E  L  Y  A  M  A  H    71

TCTGGATGGCGGCGAATGGATGGCGAAAATTCACGCCGAAGCAGCGGCATCGCCGGCTGA     780
 L  D  G  G  E  W  M  A  K  I  H  A  E  A  A  A  S  P  A  E    91

AAAAGCGCTATGGCGATCAGTGTTGGACAGGCTAAGCGCGGCCGCCGCGGTGGTCGCGCT     840
 K  A  L  W  R  S  V  L  D  R  L  S  A  A  A  A  V  V  A  L   111

GCTGGTCCTGGCGGTACACACAGGGGCGCATGAGGCGCTGCTAGCGGCCCTCTCGCCGGT     900
 L  V  L  A  V  H  T  G  A  H  E  A  L  L  A  A  L  S  P  V   131

AGCCGTTACCCACCCTCTATACATTATGCGAAGTGTGCTGTTGACGCTGCTGTGCGCCCT     960
 A  V  T  H  P  L  Y  I  M  R  S  V  L  L  T  L  L  C  A  L   151

GGCGGCCTATCACTGCTGGTCCCTCCACAGGAAGCGGACTGGACAATGACGCTAGACACC    1020
 A  A  Y  H  C  W  S  L  H  R  K  R  T  G  Q  *                166

TACGATCGCGTAGACCTGACCGGCCCTTGGGCCGGTTTTGGTTTTCAGGGGCACCGGTTC    1080

TTCACCCCGGAGAATTACGACATCGAACCGTGCGATATGCGGTATTGGGCGCTGACATGC    1140

GCGATCGCACGGGAATGGTCACTGTTGATGTCAGAAGAACGCAATGCGCGATCGGCGAAT    1200

CCTCGAAAGCCTACTGCCACAAGATCTCCAGGGTCTCGTTTGTCTCGAGGCGCAGACGTG    1260

ATCTATCTGCGGGACGTGCTGCTGCAG
```

Fig. 4. The nucleotide sequence of the Cf1t pt gene. The predicted amino acid sequence is shown below the Cf1t DNA sequence. The base alterations in Cf1c (nucleotide 442 and 491) and in Cf1tv (nucleotide 509) mutants are shown with capital letters above the Cf1t DNA sequence. The deletion site is marked by an arrow. The potential ribosome binding site and terminator signal are underlined.

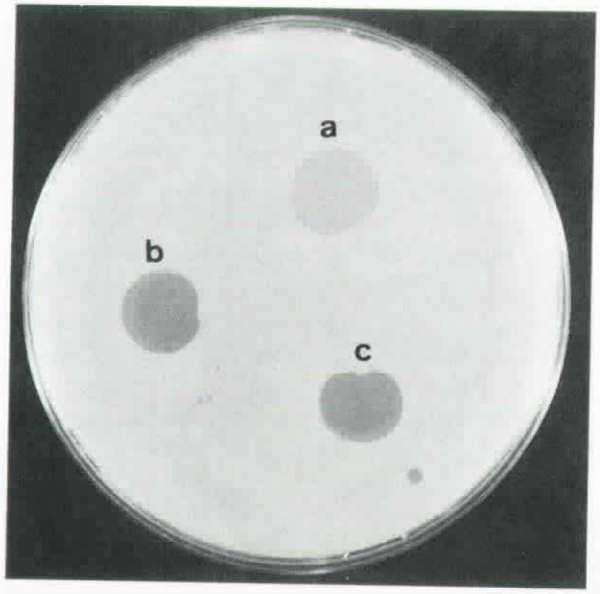

Fig. 5. Spots formed by **a**) Cf1t, **b**) Cf1c-1, and **c**) Cf1tdK.

In an attempt to identify the genomic differences between Cf1t and Cf1c, DNA fragment substitution studies were performed. They indicated that a NcoI/KpnI fragment of 591 bp was responsible for the determination of plaque turbidity. The analysis of sequence data for four Cf1c isolates revealed base pair alterations (Cf1c-1, Cf1c-2, and Cf1c-3) and a deletion (Cf1c-4) located in the upstream region of an open reading frame II (ORFII) which might encode an 18.2 kDa protein (Fig. 4).

KpnI cuts exclusively in the ORFII codon region between codons 55 and 56, without interrupting other ORFs (Fig. 4). KpnI digestion of Cf1t RF DNA followed by Klenow enzyme treatment generated a -4 frameshift mutant, which has a new stop codon TGA between codon 69 and 70 of ORFII. The resulting recombinant phage was named Cf1tdK, and forms clear plaque (Fig. 5) (Shieh,1991). These observations suggest that ORFII participates in the conversion of turbid plaque into clear plaque, and hence this gene was named the plaque turbidity (pt) gene.

The 5' upstream sequence has two unique aspects: the start codon of ORFII is preceded by a sequence which shows good base pairing with the 3' end of 16 S rRNA (Fig. 4), and the 70-nucleotide sequence in the upstream region of ORFII is able to form stem-loop structures. It has been shown that specific single-strand nucleotides in terminal loops or bulges are required in translational operator sequences (Romaniuk et al., 1987). The remaining nucleotides must be specified so that RNA can fold into its characteristic secondary hairpin loop structures (Romaniuk at al., 1987; Peattie et al., 1981). In the clear-plaque mutants of Cf1c, nucleotides 442 and 491 are located in the potential stem-loop structures upstream of the pt gene (Fig. 4). The deletion or the C to G transitional mutation at nucleotide 442 will create single-base bulges in addition to the original bulge in the structures, whereas the T to C substitution at nucleotide 491 in clear plaque mutants will alter one of the four bases in the terminal loop of the structures. Based on these observations we believe that base alterations or deletion in clear-plaque mutants may influence the expression of the pt gene, and that this in turn affects plaque turbidity (Shieh, 1991).

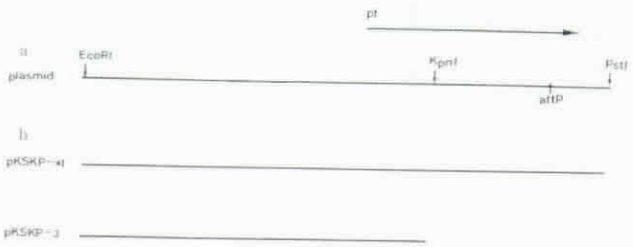

Fig. 6. Schematic diagram of pt gene carried by plasmids pKSKP-3 and pKSKP-4. **a**) localization of pt gene in EcoRI/PstI (2.5 kb) DNA fragment of Cf1 phage. **b**) EcoRI/PstI and EcoRI/KpnIDNA of Cf1 in plasmid pKSKP-4t and pKSKP-3.

Function of pt Gene

Synthesis of phage DNA, both single-stranded and double-stranded, and production of phages were lower in Cf1t-infected cells than in Cf1c- and Cf1tdK-infected cells. The transformants of host cells carrying the integration plasmid pKSKP-4t, which contains the intact pt gene, showed lower phage yield than did transformants carrying pKSKP-3 plasmid, which contains the disrupted pt gene (Fig. 6). This suggest that the function of pt gene is related to the inhibition of phage DNA synthesis and hence contributes to low phage yield (Shieh,1992). In f1 phage of *E. coli*, clear-plaque mutants are attributed to the mutation of gIIp and gVp, both of which are required for phage DNA replication. Based on these observations, we have compared the peptide sequence of Cf1 pt gene with those of gIIp and gVp of f1, but we could not find significant homology between them. Although pt gene protein shares up to 59.6% amino acid homology with gIIp and up to 15.4% homology with gVp, they share only a few identical amino acids.

In addition, clear-plaque mutants have also been observed in other phages. For example, øC31 phages of *Streptomycete* possess c gene mutations and produces clear plaque. It is well known that c gene (repressor gene) is essential for the maintenance of lysogeny (Harris et al., 1980; Sinclair and Bibb, 1988). In lambda phages of *E. coli.*, mutations that occur in the cI repressor cause clear plaque phenotype and concomitantly

destroy the ability of lambda to integrate by site-specific recombination (Arber, 1983). Both the cI gene protein of lambda phages and the c gene protein of øC31 phages contain the potential helix-turn-helix motif, a characteristic of DNA binding proteins (Pabo and Sauer, 1984). In light of this we have searched for potential DNA binding motifs in the pt gene protein. Residues 34 – 53 of pt gene protein appear to be likely candidates for this function (Shieh, 1992).

The Integration of Cf1t and Cf1c DNA into Host Chromosome

It is well known that some bacteriophages can integrate their DNA into host chromosomes as part of their reproduction cycle. This has been shown for restricted double-stranded DNA phages (Barksdale and Arden, 1974). Phage Cf1 undergoes a lysogenic cycle, which does not gravely affect the growth of cells, has a low phage yield, and renders its host immune to superinfection. The prophage is not cured by agents such as acridine orange, antiphage serum, or ethidium bromide (Russel and Model, 1988). The integration of Cf1 DNA into host DNA has been shown by Southern blot analysis (Kuo et al., 1987a). Furthermore, since the integrated DNA fragment patterns were always identical in different infected cells, one copy of Cf1 DNA is integrated at the same site on host chromosomes (Kuo et al., 1987a,b).

There are many well-characterized examples of site-specific recombination among Gram-negative bacteria. The integration and excision of bacteriophage lambda is the most thoroughly studied system, but others include resolution of Tn_3 and gamma-delta transposition (Kitt et al., 1983), bacteriophage P1 integration (Hoess et al., 1980), and site-specific inversion (Plasterk et al., 1983, 1984; Kahmann et al., 1985). Lambda is a temperate bacteriophage that can establish stable lysogeny by turning off its lytic functions and integrating its DNA into the host chromosome following the Campbell model. Integration occurs by a single reciprocal site-specific recombination event between the phage attachment site (*attP*) and the *attB* site on the host chromosome (Nash, 1977). As a result of integration, two additional attachment sites, *attL* and *attR*, are generated on the left and the right site of the prophage genome, respectively. The recombination takes place within a 15-base-pair sequence that is common to both attachment sites (Nash, 1977; Landy and Ross, 1977), and requires the phage protein Int (Gingery and Echols, 1967; Zissler, 1967; Gottesman and Yarmolinsky, 1968) and several host proteins (Arber, 1983). Excision of the prophage, a reversal of the integration process, requires an additional phage protein, named Xis, which works in concert with Int (Kaiser and Masuda, 1970; Guarneos and Echol, 1970). Control of site-specific recombination and direction (integration vs. excision) depend upon the regulatory mechanisms governing production of the two phage proteins : Int and Xis. The xis gene, int gene, and phage *att* site map adjacent to one another and thus form a compact 1.5-kilobase cluster of functions devoted to site-specific recombination (Enquist and Weisberg, 1977).

In Cf1, the phage attachment site has been localized to a unique KpnI/PstI fragment approximately 600 bp long (Kuo et al., 1987a). Furthermore, the integration region in the phage and in the host chromosome, and the two junctions in the lysogen chromosome have been isolated and sequenced. The phage and host attachment sites share an identical 15-bp core, 5'-TATACATTATG CGAA-3' (Fann, 1988).

It is suspected that a Cf1 phage-encoded protein exists which participates in integration and in retaining *attP* site binding ability. Hence, we are also interested in characterizing the minimal information necessary for Cf1-directed site-specific recombination in the Cf1 genome. We investigated whether integration required any region in addition to the *attP* site of Cf1 by analyzing the site-specific integration of plasmids containing phage *attP* sites and different Cf1 DNA fragments in the absence of any functional origin of replication. Our results suggest that besides *attP*, 2.0 kb of EcoRI/KpnI DNA fragments of Cf1 are also required for the integration function (Fig. 7). The participation of these DNA fragments in integration was severely impaired by adding amber stop linkers at NcoI, SnaBI, and EcoRV sites. This implies that EcoRI/KpNI DNA fragments might encode protein(s) (Shieh, 1992). Sequencing data analysis will be performed in the near future to identify an open reading frame involved in this DNA region.

The integration abilities of Cf1t and Cf1c have also been analyzed by using site-specific integration of plasmids in the absence of any functional origin of replication in *X. campestris* pv. *citri*. Results suggested that both Cf1t and Cf1c have the integration function (Shieh, 1992).

Virulence and Immunity Gene

A new phage mutant was isolated through enrichment of Cf1t lysogen cultures. This phage, designated Cf1tv, could superinfect Cf1t lysogens and cause them to lose their immunity. When the host bacteria were infected with Cf1tv, all infected cells released large quantities of phage particles. There was no latent period or burst found in the phage's one-step growth curve. Instead, the phage were continuously released soon after infection.

Cf1tv mutants can spontaneously arise from Cf1t with a high frequency. Therefore, we determined the gene responsible for immunity. DNA fragment substitution studies showed that a 590 – bp NcoI/KpnI fragment was responsible for the determination of phage immunity.

Sequence data revealed that only one base pair differed between Cf1t and Cf1tv (Fig. 4). When T was changed to A at nucleotide 509, located in the region required for integration, Cf1t phage lost its immunity and concomitantly lost its integration ability . It seems that Cf1tv is a virulent derivative form of Cf1t. The exact open reading frame(s) involved in phage immunity will be identified in the near future.

Conclusion

In filamentous phage systems, the infected cells are not killed or lysed. On the contrary, infection can persist for an indefinite number of cell divisions. To establish and maintain such a persistent infection, the phage must be able to adapt its replication cycle to the metabolic growth and division requirements of the host. In f1, gVp mediated translational repression of the synthesis of gIIp, which is the phage-specific DNA replication, has been envisioned as one of the mechanisms by which the phage exerts this control.

In Cf1, the infected cells containing intact pt gene have a lower phage yield than do those infected with phage containing a disrupted pt gene (Shieh, 1992). This implies that the pt gene participates in negative regulation of phage DNA production. Moreover, pt gene protein of Cf1 is not directly related to the phage integration function, but it contains the potential DNA-binding domain as observed in cI repressor of lambda and other phage DNA-binding proteins. Further studies are in progress to identify the target binding site of the pt gene protein.

It has never been reported that f1 possess a lysogenic life cycle. Cf1, however, retains the lysogenic life cycle (Kuo, 1987a,b). The participation of the integration function is impaired by adding amber stop linkers at the restriction enzyme sites in 2.0 kb of EcoRI/KpnI DNA fragments of Cf1. This implies that the phage-encoded protein(s) participate in integration (Shieh, 1992). Development of an in vitro assay system and identification of proteins participating in phage integration are in progress.

In lambda phage, immunity is determined by the cI gene, the cro gene, and the operator sites where they act. Both the cI gene and the cro gene of lambda determine the entry into the lysogenic or lytic life cycle (Arber, 1983). In Cf1, our results show that Cf1tv phage may lose the lysogenic life cycle due to a one-base pair alteration. We are now looking for evidence of regulation of the lysogenic life cycles in Cf1.

Literature Cited

Arber, W. 1983. A beginner's guide to Lambda biology. *In* R.W. Hendrix, F.W. Stahl, J.W. Roberts, and R. A. Weisberg (eds.), Lambda II. pp. 390 – 391. Cold Spring Harbor, NY.

Barksdale. L. and S. B. Arden. 1974. Persisting bacteriophage infections, lysogeny, and phage conversions. Annu. Rev. Microbiol. **28:** 265 – 295.

Chang, R.Y. 1989. Integration of cloned DNA into chromosome of *Xanthomonas campestris* mediated by filamentous phage DNA. Master's Thesis, Institute of Botany, National Chung Hsing University, Taiwan, R.O.C.

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

Dai, H., S. H. Tsay, T. T. Kuo, Y. H. Lin, and W. C. Wu. 1987. Neolysogenization of *Xanthomonas campestris* pv. *citri* infected with filamentous phage Cf16. Virology **156:** 313 – 320.

Devereux, J. and P. Haeberli. 1987. Symbol comparison tables. *In* J. Devereux, P.Haeberli, and P. Marquess (eds.), The Program Manual for the Sequence Analysis Software Package of the Genetic Computer Group. Appendix II, pp. 13 – 14.

Dotto, G. P. and N. D. Zinder. 1984a. Increased intracellular concentration of an initiation of DNA synthesis. Proc. Natl. Acad. Sci. USA **81:** 1336 – 1340.

Dotto, G.P. and N. D. Zinder. 1984b. Reduction of the minor sequence for initiation of DNA synthesis by qualitative or quantitative change of an initiator protein. Nature **311:** 279 – 280.

Enquist, L.W. and R. A. Weisberg. 1977. A genetic analysis of the att-Int-Xis regulation of coliphage lambda. J. Mol. Biol. **111:** 97 – 120.

Fann, C. H. and T. T. Kuo. 1988. Studies on the structure of attachment site for filamentous phage Cf1t from *Xanthomonas campestris* pv. *citri*. *In* C.S. Chen and J. Tu (eds.), Plant Molecular Biology. pp. 1 – 7. Academia Sinica, R.O.C.

Farber, M. B. and D. S. Ray. 1980. A clear plaque mutation of bacteriophage M13 affects the regulation of viral DNA synthesis. J. Virol. **33:** 1106 – 1110.

Fulford, W. and P. Model. 1988a. Bacteriophage f1 DNA replication genes: The roles of gene V protein and gene II in complementary strand synthesis. J. Mol. Biol. **203:** 39 – 48.

Geider. K. and A. Kornberg. 1974. Conversion of M13 viral single stranded to double-stranded replicative forms by purified proteins. J. Biol. Chem. **249:** 3999 – 4005.

Gingery, R. and H. Echols. 1967. Mutants of bacteriophage unable to integrate into the host chromosome. Proc. Natl. Acad. USA **58:** 1507 – 1514.

Gottesman, M. E. and M. B. Yarmolinsky. 1968. Integration-negative mutants of bacteriophage lambda. J. Mol. Biol. **31:** 487 – 505.

Harris, J. E., K. F. Chater, C. J. Bruton, and J. M. Piret. 1983. The restriction mapping of c gene deletions in *Streptomyces* bacteriophage øC31 and their use in cloning vector development. Gene **22:** 167 – 174.

Hoess, R. H., C. Foeller, K. Bidwell, and A. Landy. 1980. Site-specific recombination functions of bacteriophage DNA

sequence of regulatory regions and overlapping structure genes for Int and Xis. Proc. Natl. Acad. Sci. **77:** 2482 – 2486.

Johnson, K. S., G. B. L. Harrion, M. W. Lightowlers, K. L. O'Hoy, W. G. Cougle, R. P. Dempster, S. B. Lawrence, J. G. Vintons, D. D. Heath, and M. D. Rickard. 1989. Vaccination against ovine cysticercosis using a defined recombinant antigen. Nature **338:** 585 – 587.

Kahmann, R., F, Rudt, C. Koch, and G. Mertens. 1985. Gene inversion in bacteriophage Mu DNA is stimulated by a site within the invertase genes and a host factor. Cell **41:** 771 – 780.

Kaiser, A. and T. Masuda. 1970. Evidence for prophage excision gene in lambda. J. Mol. Biol. **47:** 557 – 564.

Kitts, P. A., L. S. Symington, P. Dyson, and D. J. Sherratt. 1983. Transposon-encoded site-specific recombination: nature of the Tn3 DNA sequences which constitute the recombinant site *res*. EMBO. J. **2:** 1055 – 1060.

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

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

Kuo, T. T., M. S. Tan, M. T. Su, and M. K. Yang. 1991. Complete nucleotide sequence of filamentous phage Cf1c from *Xanthomonas campestris* pv. *citri*. Nucleic Acid Res. **19:** 2498.

Landy, A. and W. Ross. 1977. Viral Integration and excision: Structure of Lambda att sites. Science **197:** 1147 – 1160.

Lawn, A. M., E. Meynell, G. G. Meynell, and N. Datta. 1967. Sex pili and the classification of sex factors in the enterobacteria. Nature **216:** 343 – 346.

Lu, M. C. 1985. A filamentous bacteriophage LF of *Xanthomonas campestris* pv. *campestris*. Master Thesis, Institute of Botany, National Chung Hsing University, Taiwan, R.O.C.

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

Meyer, T. F. and K. Geider. 1979. bacteriophage fd gene II protein. II: specific cleavage and relaxation of supercoiled RF from filamentous phage. J. Biol. Chem. **254:** 12642 – 12646.

Michel, B. and N. D. Zinder. 1989a. In vitro binding of bacteriophage f1 gene V protein to the gene II RNA operator and its DNA analog. Nucleic Acid Res. **17:** 7333 – 7340.

Michel, B. and N. D. Zinder. 1989b. Translational repression in bacteriophage f1 characterization of the gene V protein target on the gene II mRNA. Proc. Natl. Acad. Sci. USA **86:** 4002 – 4006.

Minamishima, Y., K. Takeya, Y. Ohnishi, and K. Amako. 1986. Physicochemical and biological properties of fibrous *Pseudomonas* bacteriophage. J. Virlo. **2:** 208 – 213.

Nash, H. A. and C. Robertson. 1981. Purification and properties of the *E. coli*. protein factor required for lambda integrative recombination. J. Biol. Chem. **256:** 9246 – 9253.

Pabo, C. O. and R. T. Sauer. 1984. Protein-DNA recognition. Annu. Rev. Biochem. **53:** 293 – 321.

Peattie, D. A., S. Douthwaite, R. A. Garrett, and H. F. Noller. 1981. A bulged helix in a RNA-protein contact site. Proc. Natl. Acad. Sci. USA **78:** 7331 – 7335.

Peeters, B. P., R. M. Peter, J. G. G. Schoenmarker, and R. N. H. Konings. 1967. Nucleotide sequence and genetic organization of the genome of N-specific filamentous bacteriophage Ike. J. Mol. Biol. **81:** 27 – 39.

Plasterk, R. H. A., A. Brinkaman, and P. Van de Putte. 1983. DNA inversions in the chromosome of *Escherichia coli* and in bacteriophage Mu: Relationship to other site-specific recombination systems. Proc. Natl. Acad. Sci. USA **80:** 5355 – 5358.

Plasterk, R. H. A., A. Brinkaman, and P. Van de Putte. 1984. Genetic switch by DNA inversions in prokaryotes. Biochem. Biophys. Acta. **782:** 111 – 119.

Romaniuk, P. J., P. Lowary, H. N. Wu, G. Stormo, and O. C. Uhilenbeck. 1987. RNA binding site of R17 coat protein. Biochemistry **26:** 1563 – 1568.

Russel, M. and P. M.odel. 1988. Filamentous bacteriophage. *In* R. Calendar (ed.), The bacteriophage. Vol. 2, pp. 375 – 456. Plenum, New York.

Shieh, G. J., Y. C. Chang, B. C. Yang, J. Tu, H. J. Bau, and T. T. Kuo. 1991. Identification and nucleotide sequence analysis of an open reading frame involved in high frequency conversion of turbid to clear plaque mutants of filamentous phage Cf1t. Virology **185:** 316 – 322.

Shieh, G. J. 1992. Studies on the plaque turbidity gene and the integration of phage DNA into host chromosome of filamentous phage Cf1 from *Xanthomonas campestris* pv. *citri*. Ph. D. Thesis. Graduate Institute of Botany, National Taiwan University.

Sinclair, R. B. and M. J. Bibb. 1988. The repressor gene (c) of the streptomyces temperate phage øC31: Nucleotide sequence analysis and functional cloning. Mol. Gen. Genet. **213:** 269 – 277.

Zissler, J. 1967. Integration-negative (int) mutants of phage lambda. Virology **31:** 189.

Botanical Bulletin of Academia Sinica, Vol. 35: 1, 1994

柑橘潰瘍病病原菌線狀噬菌體 Cf1 之溶菌斑混濁度，病毒 DNA 之插入及免疫性

郭宗德[1,2]　謝國珍[2]　郭榮烈[2]　陳文彬[2]　包慧俊[2]

[1]中央研究院植物研究所
[2]中央研究院分子生物所

線狀噬菌體 Cf1 的寄主爲柑橘潰瘍病病原菌，所呈現的溶菌斑爲混濁型（turbid），而且具有潛溶性的生活史。然而 Cf1 的基因組尚未被研究完全，本篇論文將溶菌斑混濁度基因及插入染色體所需的區域定出。關於溶菌斑混濁度基因是利用突變株及野生株之噬菌體 DNA 片段交換的方法定出的；而插入寄主染色體所需的區域乃是利用不具複製功能的質體來分析。目前本實驗室亦純化出一較小溶菌斑之突變株，此突變株會感染 Cf1 之 lysogen，被感染之寄主，會釋放出大量的噬菌體，並且失去將噬菌體 DNA 插入寄主染色體的現象，因此將此突變株命名爲 Cf1tv，經由 DNA 序列分析結果顯亦在 Cf1tv 之基因組中有一鹼基發生突變。

關鍵詞：線狀噬菌體；免疫性；溶菌斑；特定位置基因重組。