Nucleotide Sequence of the Genome of the Filamentous Bacteriophage I2-2: Module Evolution of the Filamentous Phage Genome

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Summary. The nucleotide sequence of the circular single-stranded genome of the filamentous Escherichia coli phage I2-2 has been determined and compared with those of the filamentous E. coli phages Ff (M13, f1, or fd) and IKe. The I2-2 DNA sequence comprises 6744 nucleotides; 139 nucleotides less than that of the N- and I2-plasmid-specific phage IKe, and 337 (336) nucleotides more than that of the F-plasmid-specific phage Ff. Nucleotide sequence comparisons have indicated that I2-2, IKe, and Ff have a similar genetic organization, and that the genomes of 12-2 and IKe are evolutionarily more closely related than those of I2-2 and Ff. The studies have further demonstrated that the I2-2 genome is a composite replicon, composed of only two-thirds of the ancestral genome of IKe. Only a contiguous I2-2 DNA sequence of 4615 nucleotides encompassing not only the coat protein and phage assembly genes, but also the signal required for efficient phage morphogenesis, was found to be significantly homologous to sequences in the genomes of IKe and Ff. No homology was observed between the consecutive DNA sequence that contains the origins for viral and complementary strand replication and the replication genes. Although other explanations cannot be ruled out, our data strongly suggest that the ancestor filamentous phage genome of phages I2-2 and IKe has exchanged its replication module during evolution with that of another replicon, e.g., a plasmid that also replicates via the so-called rolling circle mechanism.

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Introduction

A major characteristic of filamentous bacteriophages is that infection of their host cell occurs via attachment of a specific phage end, i.e., the one at which the coat proteins encoded by genes III and VI are located, to the tip of a conjugative pilus present at the surface of the host cell (Caro and Schnös 1966). This pilus is composed of filamentous polymers of identical protein subunits (pilin), generally encoded by conjugative plasmids (Marvin and Folkhard 1986). On the basis of their plasmid (pilus) specificity, filamentous phages have been subdivided into several groups that differ both in phage morphology and protein composition. The best-studied filamentous phages are the IncF-plasmid-specific phage Ff [M13, f1, and fd; for a recent review see (Model and Russel 1988)] and the IncI2- and IncNplasmid-specific phage IKe (Khatoon et al. 1972; Bradley et al. 1983; Peeters et al. 1985). They both have Escherichia coli as a host. Filamentous phages with other pilus- and host-specificity are Pf3, a Pseudomonas phage, and C-2, a Salmonella phage. They are specific for IncP-1- and IncC-plasmids, respectively (Stanisich 1974; Bradley et al. 1982).

The filamentous *E. coli* phage I2-2, which has been isolated from Pretoria sewage, is specific for pili encoded by conjugative plasmids of the IncI2-, IncN-, and IncP-incompatibility groups (Coetzee et al. 1982; Bradley et al. 1983). Its serological relationship with phage IKeh, a host range mutant of IKe that also infects *E. coli* cells containing plasmids of the IncP-incompatibility group, is indicative of an evolutionary relationship between these two phages (Grant et al. 1978; Bradley et al. 1983). Based upon this relationship it has been suggested that I2-2 is a naturally occurring host range mutant of IKe (Bradley et al. 1983).

Because we are interested in the evolutionary relationships of filamentous phages and because comparison of sequences of evolutionarily related genes or genomes might provide important clues about the significance of particular nucleotide and/or amino acid sequences with respect to their function, the nucleotide sequence of the circular single-stranded genome of I2-2 has been determined and compared with those of Ff and IKe. From these comparisons it became apparent that I2-2, IKe, and Ff have a similar genetic organization and that the genomes of I2-2 and IKe are evolutionarily more closely related than those of I2-2 and Ff. The homology between the nucleotide sequences of the genomes of I2-2 and IKe or Ff is, however, only partial and restricted to the *cis*-acting nucleotide sequence required for phage assembly and the genes coding for the coat and phage assembly proteins.

Materials and Methods

Bacteria, Bacteriophages, and Plasmids. Bacteriophage I2-2 and its host E. coli JE2571[N3] [leu, thr, str, fla, pil (N3, tet, strep, sulf)] were kindly provided by Dr. D. E. Bradley, St. John's, Newfoundland (Watanabe et al. 1964; Bradley et al. 1982). For propagation of recombinant pKUN19 phagemids (Konings et al. 1987), E. coli JM83 [ara, del(lac-proAB), rpsL, phi80, lac-ZdelM15] (Yanisch-Perron et al. 1985) was used. For the production of single-stranded phagemid DNA by recombinant pKUN19, phagemid-harboring cells [E. coli JM101 (supE, thi, del(lac-proAB) (F', traD36, proAB, lacIqZdelM15))], the helper phages IR1 (Enea and Zinder 1982), Mike (Konings et al. 1986), or Mike-delta (Konings et al. 1987) were used. The single-stranded bacteriophage vectors M13mp18 and M13mp19, which were propagated on E. coli JM101, have been described (Yanisch-Perron 1985).

Enzymes and Chemicals. Restriction endonucleases were purchased from Boehringer-Mannheim (Germany), New England Biolabs (Beverly, MA, USA), and Bethesda Research Laboratories (Gaithersburg, MD, USA). T4 DNA ligase, *E. coli* DNA polymerase I (Klenow fragment), and calf intestine alkaline phosphatase were purchased from Boehringer-Mannheim. All enzymes were used according to the manufacturers' instructions.

The 2',3'-dideoxyribonucleosidetriphosphates and 2'-deoxyribonucleosidetriphosphates were purchased from Pharmacia (Uppsala, Sweden) and Boehringer-Mannheim, respectively. α -³²Plabeled deoxyadenosine triphosphate and α -³⁵S-labeled thiodeoxyadenosine triphosphate were obtained from Amersham (Buckinghamshire, UK).

The oligonucleotides, used for nucleotide sequence analysis of the viral strand, are listed in Table 1. They were synthesized

Table 1. Synthetic oligonucleotides and *Hae*III fragments of I2-2 RF that have been used as nucleotide sequence analysis primers for the viral strand of phage I2-2

Primer	Nucleotide positions in the I2-2 genome
5'-dCCCAGCCTAATTTACGGGC-3'	226–244
5'-dGTGAACTTTCGGTAATTG-3'	394-411
5'-dGCGCACAGGAGAATTG-3'	1070-1085
5'-dACTGTTATTGCCTGGGTA-3'	1485-1502
5'-dTTAACGCTTGCAGCC-3'	1895-1909
5'-dGGCAATGATAGCGTG-3'	3020-3033
5'-dGACGATACGATTCTAG-3'	3406-3421
5'-dGGCACAGACAGTTCC-3'	5515-5529
5'-dACATCTTCCTTTGTC-3'	5757-5771
5'-dCGCTATCTGGTAGTC-3'	6558-6572
HaeIII fragments	4490-4708
	4709-4832
	4833-5229
	5230-91

either by a Cyclone Plus DNA synthesizer (MilliGen/Biosearch) or by an Applied Biosystems DNA synthesizer.

I2-2 replicative form DNA and single-stranded DNA. Replicative form DNA (RF) was isolated from I2-2-infected *E. coli* JE2571[N3] cells, using the alkaline lysis method of Birnboim and Doly (1979) as described by Konings et al. (1987). The procedures for the preparation of a high-titer I2-2 phage stock and the isolation of viral single-stranded DNA have been described previously (Konings et al. 1987).

Cloning of I2-2 DNA and nucleotide sequence analysis. Basic DNA manipulations, which will not be elaborated here, were mainly performed according to Sambrook et al. (1989).

A library of the I2-2 genome was constructed by random cloning of *Hae*III restriction fragments of I2-2 RF in the *Sma*I site of either pKUN19, M13mp18, or M13mp19. After digestion with *Sau*3AI, the *Hae*III restriction fragments were subcloned in the *Bam*HI restriction site of pKUN19.

The nucleotide sequence of the cloned *Hae*III and *Sau*3AI fragments was determined by the chain termination method developed by Sanger et al. (1977). For the analyses also the single-stranded I2-2 genome was used as a template. As sequencing primers, synthetic oligonucleotides and *Hae*III fragments of I2-2 RF (Table 1) were used. To prevent band compression with dGTP analog 7-deaza-dGTP, instead of dGTP, was used.

The nucleotide sequence was analyzed by computer programs developed by Staden (1980, 1982) and Wilbur and Lipman (1983), and the computer program package IntelliGenetics Suite (release 5.37) of IntelliGenetics, Inc. (Mountain View, California, USA). To search for similar nucleotide sequences, the EMBL (release 23) and Genbank (release 60) Nucleic Acid Databases were scanned. The sequence data are stored in the EMBL, Genbank, and DDBJ Nucleotide Sequence Databases under the accession number X14336.

To estimate the evolutionary relationship between the homologous genes (I, III, IV, VI, VII, VIII, and IX) of I2-2, IKe (Peeters et al. 1985), and Ff (Van Wezenbeek et al. 1980), the extent of synonymous (Ks = synonymous substitutions/synonymous sites) and nonsynonymous amino acid substitutions (Ka = amino acid substitutions/amino acid sites) was calculated according to Miyata and Yasunaga (1980) and corrected for back mutations by the method of Jukes and Cantor (1969). Triplet



Fig. 1. Positions of individual gel readings in the final nucleotide sequence of the I2-2 genome. The axis represents the nucleotide map of the I2-2 genome, starting at the unique *Hind*III recognition site, numbered in the 5'-3' direction of the viral strand. Arrows pointing to the right and left refer to nucleotide sequences established for the viral and complementary strand, respectively. The length of the sequence derived is represented by the length of the arrow. Nucleotide sequences indicated by \rightarrow or \leftarrow are obtained from cloned DNA, whereas those indicated

insertions/deletions were excluded from the comparisons. The Ks and Kas calculated are thus overestimates.

Results and Discussion

Nucleotide Sequence of I2-2 DNA

The sequence analysis of the I2-2 genome was initiated by sequencing of I2-2 HaeIII fragments randomly cloned into phagemid pKUN19. Because three HaeIII fragments were longer than 1000 bp, and due to subcloning difficulties, several gaps remained. These gaps were filled in by using the viral genome as a template with synthetic oligonucleotides or purified HaeIII fragments as sequencing primers (vide supra). Furthermore, the nucleotide sequence of several Sau3AI subclones of HaeIII fragments was determined. The sequencing strategy followed is outlined in Fig. 1.

The majority of the I2-2 sequence was established via sequence analysis of both the viral and complementary strand. On the average each nucleotide is supported by at least three separate gel readings. We therefore feel confident that the nucleotide sequence as presented in Fig. 2 is correct. As nucleotide number 1 we have chosen the first nucleotide (A) of the unique *Hin*dIII restriction endonuclease cleavage site. The circular single-stranded genome of I2-2 is 6744 nucleotides long. This is 139 nucleotides less than that of IKe (Peeters et al. 1985), and 337 (336) nucleotides more than that of Ff (Beck et al. 1978; Van Wezenbeek et al. 1980; Beck and Zink 1981; Hill and Petersen 1982).

Gene Identification

The five coat protein genes (i.e., genes III, VI, VII, VIII, and IX), as well as the two phage assembly

by $\leftarrow x$ and $\leftarrow o$ are obtained from nucleotide analysis of viral DNA primed with synthetic oligonucleotides or *Hae*III restriction fragments, respectively. The linear genetic map of the I2-2 genome is given in the lower portion of the diagram. Genes are denoted by Roman numerals; IR refers to the major intergenic region in which the origins for viral and complementary strand synthesis and the signal required for efficient phage assembly are located.

genes (I and IV) and the phage morphogenetic signal located immediately distal to gene IV, have been identified by alignment of the I2-2 sequence with that of phage IKe. The respective gene products are not only highly homologous but also of similar lengths (Fig. 2). Although no significant homology was observed between the open reading frames II, X, and V of I2-2 and the DNA replication genes II, X, and V of IKe, as demonstrated here, we feel confident in concluding that the proteins encoded by these open reading frames fulfill an identical function in the DNA replication process. This conclusion is based partly on the observation that the biochemical and physical properties (DNA-binding properties, amino acid composition, and molecular weight) of the I2-2 encoded single-stranded DNAbinding protein are in accordance with those of the protein encoded by open reading frame V (data not shown). Furthermore, genetic and biochemical studies have unambiguously demonstrated that the filamentous phage genome codes for three different functions that are indispensable for the phage (DNA) replication process. One of these functions is encoded by gene II and indispensable for initiation and termination of viral strand replication. The second function is encoded by gene X, a gene that completely overlaps with the 3' terminal region of gene II, and is somehow involved in the regulation of the synthesis of the complementary strand. The third function is the single-stranded DNA-binding protein encoded by gene V that, at a late stage of the infection cycle, is indispensable for the sequestration of the newly synthesized viral strand from the DNA replication cycle. Because the only DNA region that is left in the I2-2 genome that might code for these proteins is located between the major intergenic region and gene VII (Fig. 3), and on the basis of the strong resemblance with the genetic or144

ganization of IKe and Ff, we conclude that the open reading frames indicated code for these indispensable DNA replication functions. As in the phages IKe and Ff, in I2-2 the 3' terminal part of gene II overlaps gene X completely.

The proposed genetic organization is further substantiated by the observation that all initiation codons of the proposed genes and/or open reading frames are preceded by a sequence that is significantly homologous to the consensus sequence for ribosome binding (Table 2) (Shine and Dalgarno 1974). All I2-2 genes start with an ATG codon, except gene III, which starts with the codon GTG. Similar observations have been made during our studies of the genomes of Ff and IKe (Van Wezenbeek et al. 1980; Peeters et al. 1985).

Genetic Organization

The genetic maps of the genomes of I2-2, IKe, and Ff (M13) are depicted in Fig. 3 (Van Wezenbeek et al. 1980; Peeters et al. 1985). The three genomes consist of an equal number of genes, which are arranged in an identical order (vide supra). Comparison of the genomes of I2-2 and Ff reveals that, with the exception of genes II and VII, all I2-2 genes are larger than their Ff counterparts. The larger length of I2-2 compared with Ff is mainly the result of length differences between their respective major intergenic regions (Fig. 3).

Apart from the major intergenic region that is located between genes IV and II, a much smaller one is located between genes VIII and III. It contains a rho-independent transcription termination signal, identical in sequence to that of IKe and very similar to that of M13 (Fig. 4) (Van Wezenbeek et al. 1980; Peeters et al. 1985). In Ff and IKe the DNA region encompassing genes II through VIII is expressed in a nested set of mRNAs whose synthesis is initiated at different promoters but terminated at the aforementioned rho-independent transcription termination signal (Edens et al. 1978). Because in the DNA region of I2-2 encompassing the major intergenic region and genes II-VIII, multiple nucleotide sequences are found that resemble the consensus sequence of E. coli promoters and which have an almost identical map position as the promoters found in IKe and Ff (data not shown), we suggest that this part of the I2-2 genome is expressed via a similar transcription mechanism.

Besides the complete overlap of gene X with the 3' terminal region of gene II, in I2-2 the reading frames of genes V and VII, VII, and IX, and of III and VI overlap each other with a few nucleotides. A similar compact genetic organization has been found to be present in the genomes of IKe (Peeters et al. 1985) and Ff (Van Wezenbeek et al. 1980),

but with the exception that in Ff genes IX and VIII and genes I and IV overlap, rather than genes V and VII and genes III and VI (Figs. 2 and 3).

Intergenic Region

The major intergenic region (IR; 508 nucleotides) of phage Ff is a noncoding sequence that encompasses all cis-acting elements required for phage assembly and DNA replication (Zinder and Horiuchi 1985). As one moves from the 5' to the 3' end, there are five consecutive regions (designated A-E) of dyad symmetry capable of forming stable stem-loop structures, followed by an AT-rich sequence without self-complementarity and that fulfills an enhancer function in the DNA replication process (Cleary and Ray 1980, 1981; Dotto et al. 1981, 1982). Region A functions both as a rho-dependent transcription termination signal (Moses and Model 1984; Smits et al. 1984) and as a morphogenetic signal for the proper packaging of the viral strand into filamentous particles (Dotto and Zinder 1983). Regions B and C function as origin for the synthesis of the complementary viral strand (Schaller et al. 1976; Gray et al. 1978), whereas the "rabbit ears structure" consisting of regions D and E fulfills an indispensable role in the initiation and termination processes of viral strand replication (Meyer et al. 1979).

The major intergenic region of the genome of IKe is 738 nucleotides long. Similar to Ff this intergenic region consists of five regions with dyad symmetry, which are homologous to those found in the intergenic region of Ff (Peeters et al. 1985). The homology does not hold, however, for the major intergenic region of I2-2, although in this region of 817 nucleotides five stretches with dyad symmetry are also located, which resemble in number, relative position, and structure those found in the intergenic regions of IKe and Ff (Fig. 5). Only stretch A has a homologous counterpart in the genomes of Ff and IKe (Fig. 5B). Because the phage-encoded proteins that cross-talk with this sequence (i.e., the coat proteins and probably also the phage assembly proteins encoded by genes I and IV) are highly conserved, we propose that this sequence functions in I2-2 also as a phage morphogenesis signal. The observation that the secondary structure characteristics of the intergenic regions of I2-2, IKe, and Ff are very much alike highly favors the hypothesis that, despite the lack of homology, in I2-2 the DNA stretches of dyad symmetry have the same function in the phage replication cycle as they do in the phages Ff and IKe.

Evolution of the Filamentous Phage Genome

Based upon the data presented it is clear that the bacteriophages I2-2, IKe, and Ff are evolutionarily

ĸ L H H F P Q V G R Y A K Q C R V M R I A D K P T L E D L E A 1 G R G N L S Y D 10 20 30 40 50 60 70 80 90 100 110 120 * * * * * TWFNSRNWSDKSROP ID¥ F F SKNG 1. I VLDECG v L н A R ĸ L G 160 200 220 230 240 130 140 150 170 180 190 210 D V I F I I Q D I S L M D K Q A R E A L A E H V V Y C R R L DKL NIP 1 1 G TGGGATGTAATTTTTATTATTCCCGATATTTCCCCTGATGGATAAGCAGGCACGCGAGGCTTTAGCCGAACATGTTGTTTATTGCCGTCGATTAGATAAATTAAATATACCTATCGT 280 250 260 270 290 300 310 320 330 340 350 360 G L I S V L S G G R L P I P K V H F G I V K Y G D N P Q S L T V D K W I Y T G Т GENE GGTTTAATTTCGGTTCTTTCAGGTGGTAGATTGCCATTACCGAAAGTTCACTTTGGTATTGTTAAATATGGTGATAATCCTCAGTCATTAACTGTAGATAAATGGATTTATACTGGTACA 370 380 390 400 410 420 430 440 450 460 470 480 κQ S P Ρ СР VSP G Y D Т 1 F Т s D RΕ L F Y н F v ĸ R D D L Y A A Y Т 1 A A 500 510 520 540 560 570 580 600 490 530 550 590 KSRQI YFKKM NR VWL SF 1 LG G V G F F YMR ΚI MA 4 A м AAATATTATATGCGTATGACAAAAATCTATTTTAAGAAGATGAATCGTGTTAGGTTTAATGGCTTCCTTTCTTGGTGCTGGCGTAGGGTTTTTTTATAAATCTCGCCAGATAAAT 670 **69**0 630 640 650 660 680 700 710 610 620 720 E Q L S N M P V A S A Q A N T T K T D H T I D E L P R L S I N S F A Q M G Y DV GAGCAACTATCAAATATGCCTGTAGCGTCTGCTCAGGCTAATACCACTAAAACAGATCACACTGATGAATTGCCACGATTATCGATCAACTCATTTGCACAAATGGGTTATGATGAT 740 760 790 820 830 730 750 770 780 800 810 840 v s FKDA KG KIYY SFDL MKSGYA DI ΚD S C н ΙT R KR I AATGTAAGTTTTAAAGATGCAAAGGGAAAAATCTATTACTCATTTGATTTAATGAAATCTGGTTATGCCTTAGATATTAAAGATTCCTGTCATATTACATTGCGTAAACGAAACTACATT 850 860 870 880 890 900 910 920 930 940 950 960 * * * QVTCEG* M K T F F A K F I V A L F A F T Y F S A F A E P V T ۵ INN S P 990 970 980 1000 1010 1020 1030 1040 1050 1060 1070 1080 * s F VOUY s **зк**т GKS v I V N P D v KGN T Т v F N D v Ν A N D D R ۵ N Ĩ TGCGCTCATTCGTTCAATGGTATTCATCCAAAACAGGAAAATCAGTTATTGTTAACCCTGATGTTAAGGGGAATATCACCGTATTTAATGCTGATGTTAACAACGCAAATATTGATGAC 1090 1100 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200 * FKSVLN Α NGLVVV Α GNP A V V ТР S L Т ĸ L. ASQP S N EET ۰¥ D D 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320 GENE Е S D G V A Y E A V P Q S A A P A V P A D L TVRNFNVTR ٧ RS S D V L Ρ L $\overline{}$ 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440 * * * * * * * * * * * K I F V D S N G G G N V V D Y P G N N S L V V S G S A Q V M P A L S D FI T S CTAAGATTTTCGTTGATTCAAATGGCGGCGGTAATGTCGTCGATTACCCAGGCAATAACAGTCTTGTTGTTTCCGGCTCAGGTTATGCCTGCTTTATCCGATTTCATTACGTCAA 1460 1470 1480 1450 1490 1500 1560 1510 1520 1530 1540 1550 VSN D V A R E Q V L I Q S L M F E T S GVDLSF LA A 5 6 G K V GG L Δ Δ. 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 * * T S A L G T A L S T A G G S F G VQSD м IFN G NTL A L S L Q A S N S κv TTAATACCTCAGCTCTCGGCACTGCTCTTTCTACTGCTGGCGGTAGCTTCGGTATCTTTAACGGTAACATTCTGGCGTTATCTCTCCAGGCTGTTCAGAGTGAT TCTAACTCTAAGGTTA 1690 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800

Fig. 2. Nucleotide sequence of the single-stranded genome of the N/I2/P-specific filamentous *E. coli* phage I2-2. Numbering is in the 5'-3' direction of the viral strand and begins at the first nucleotide of the unique *Hind*III recognition site. The genes I-X are boxed and the amino acid sequences of their encoded proteins are shown. For clarity, gene VII is boxed with a broken line. The amino acid residues that have an identical position in

the corresponding gene products of I2-2 and IKe are indicated by asterisks. Termination codons are denoted by asterisks above the nucleotide sequence. IR refers to the major intergenic region in which the origins for viral and complementary strand synthesis and the signal required for efficient phage assembly are located. Continued on pp. 146–148.

related. Their genetic organization is almost identical and genes I, III, IV, VI, VII, VIII, and IX of I2-2 and IKe are highly homologous. Furthermore, there are no significant differences in codon usage and at the third codon position there is a strong preference for a T (46%). The evolutionary relationship between filamentous phages can be estimated by comparing the extent of nucleotide se-

* * * * * * * * * * I S T P R I L T Q TCTCTACCCCTCGCATCCTCACGCA 1810 1820	* * * * * * S G Q S G Y GTCCGGCCAGAGTGGTTATA 1830 1840	* * * * * * * * ISVGQNV TTTCAGTTGGCCAGAATGTT 1850 1860	PFVTGKV CCTTTTGTGACAGGTAAAGT 1870 1880	T G E A A S (Cactggcgaggctgcaago 1890 1900	* * * * * * V N N P F GTTAATAATCCTTTCC 1910 1920	
* * * * * * * * * * Q T I E R R D V G AGACAATCGAGCGCCGCGACGTAGG 1930 1940	* * * * * * V S Ł K V T CGTATCACTAAAGGTAACGC 1950 1960	* * * * * * * P V V M G N G CGGTTGTTATGGGAAATGGT 1970 1980	QLVLTIC CAGTTAGTICTCACCATCGA 1990 2000	TKADSL TKADSL TACTAAAGCCGACTCTCTC 2010 2020	* SNQAI CAGCAATCAGGCGATTG 2030 2040	
* * * * * * * * * * A S D I I T N Q R CCTCTGACATCATTACCAATCAGCGG 2050 2060	* * * * Q I Q T T V CCAGATACAAACCACCGTTC 2070 2080	* * * * * * * * Q I K D G Q T AGATTAAAGACGGTCAGACO 2090 2100	LLLGLI CTGCTTTTAGGCGGCCTGAT 21102120	* * * * S S N Q F D TAGCTCTAACCAGTTCGAC 2130 2140	* * * S D R S V CAGCGATCGTTCTGTGC 2150 2160	
* * * PFMSKIPLI CTTTTATGTCGAAGATTCCTTTAAT 21702180	G W L F R S CGGCTGGCTTTTCCGCAGCC 2190 2200	* * * * H S D S K D D ATTCAGACTCGAAAGATGAT 2210 2220	R T M F V L L CGCACTATGTTCGTTTTGCT 2230 2240	TAHVIR TACTGCTCACGTTATCAGO 2250 2260	A L * GGCGCTTTGAGGGTGCG 2270 2280	
GGGTAGGTGCGTTAGCCCTGCCCCG	TATCCTCACAGCGTCCCCTG	ACCCGTATCATTCGCTTAT(GCCGTATGTGCTTCCCTGTC	CTTGTCCCTAAGGCTACGTA	ATTGCCTCCCCGTTTAA	
2290 2300	2310 2320	2330 2340	2350 2360	2370 2380	2390 2400	
ATCGTCAAGCTATAAATCGTGGGGG	CCCCATCGCCCTGTTAAGCT	GCCTTTTTTCCTGCGATCC	CCCGCCTCTCTGTTGTCTGA	CGGTCGCAGACGAACAGGA	ACGTTACAGCCATTGTT	
2410 2420	2430 2440	2450 2460	2470 2480	2490 2500	2510 2520	
TTTCGTTCTGCTGGCTTCTTTTTT	GGGGGGTGTACTCTTGTTGC	AAAATCGCAACGCAGCGGGG	GTTAAATCATGCTGCACGAA	AGTCTCACTTTCGGGATTT	TGGTTTTTGGATTTCT	I R
2530 2540	2550 2560	2570 2580	2590 2600	2610 2620	2630 2640	
GCTGCGGAGTCAGGCGGGGCGGGGT	GAACGTGCAGTGTATGTAAT	ACTGCAACGTTTGTCTCAA	CTGATACCTCTCTACTAAAC	GCCTCCTTTCTTTTAAGC	CTCTTTAATCATTTTCT	
2650 2660	2670 2680	2690 2700	2710 2720	2730 2740	2750 2760	
TTTCTGCTAATTCTGTATAGTTTTC	AATGACATAGTTTAAGTGAA	TCTGTCCATGTGACCTGTAC	GCCCGTACTTGTACTAATCC	TGATCGCAGTATCACTCAC	GTTTTTCTTTTTCTCG	
2770 2780	2790 2800	2810 2820	2830 2840	2850 2860	2870 2880	
GTGGTGATACTGGCTGTTGTCCTAA	CTGGTTTTTTCATTTTATGC	CTTTGAAAAATATAAAGTT	CTACCGAAAATAGGTTTTTA	AGCGTAATCTTATTGTCAA	ATAGATGTAAAAATTTT	
2890 2900	2910 2920	2930 2940	2950 2960	2970 2980	2990 3000	
TGTTGCGCACGTTATTTTTCACGCT 3010 3020	ATCATGCCTGCATTGGATTT 3030 3040	CATCTTGATACGTGTATAC/ 3050 3060	CGTATACATCACTAAGGGAA 3070 3080	M I D W V ITGCGTATGATTGATTGGGT 3090 3100	TAVLP TACAGCCGTCTTGCCT 3110 3120	
CLKVPVDA	G R V L S V A	P D G S V E N	ESVKFS	R V T G S F G	SSISV	GE
TGTCTGCATGTGCCTGTTGATGCTG	GTCGCGTCCTCTCTGTTGCG	CCGGATGGCTCTGTAGAAT	GGAATCAGTAAAGTTTTCCC	GCGTTACTGGCTCCTTTCA	AAGCTCCATCAGTGTA	
3130 3140	3150 3160	3170 3180	3190 3200	3210 3220	3230 3240	
R S Q G S D G N	G K A T H L Y	V D G N P S I	CWLQGHN	IVGSDDL	NGLMI	NE II
CGTTCGCAGGGTTCTGACGGTAATG	Ggaaagccactcatttatac	GTTGATGGTAACCCGTCAA	GTGGTTGCAGGGTCATAATA	Itcgttggaagcgacgatc1	CAACGGCCTTATGATT	
3250 3260	3270 3280	3290 3300	3310 3320	3330 3340	3350 3360	
A F Y A R M L S	L L N I P H H	LESYRQ	/LSGQYE	L K R V D I M	LYMFEL	
GCTTTTTACGCTCGTATGTTGTCAC	TACTCAATATACCTCACCAT	CTAGAATCGTATCGTCAGG	GCTTTCTGGTCAGTACGAGC	TAAAGCGCGTTGACATTAA	Actacatgtttgagctt	
3370 3380	3390 3400	3410 3420	3430 3440	3450 3460	3470 3480	
P T L I D V R S CCTACGCTCATTGATGTACGGTCATTGATGTACGGTCATTGATGTACGGTCATT 3490 3500	WLHAAEF GGTTACATGCTGCTGAGTTC 3510 3520	KAKTRH AAGGCTAAGACTCGTCACG 3530 3540	G R P A T A K GCGCCCGGCAACTGCAAAGG 3550 3560	G T L Y F G K GCACTCTGTACTTTGGTAA 3570 3580	KNSRRW MAAATTCTCGCCGTTGG 3590 3600	

Fig. 2. Continued

quence similarity of genes I, III, IV, VI, VII, VIII, and IX of I2-2, IKe, and Ff(M13), respectively. The extent of synonymous and nonsynonymous amino acid substitutions between the various genes was estimated according to Miyata and Yasunaga (1980) (Table 3). In virtually all comparisons the Ks value is larger than 1, indicating that the genes under consideration did not diverge recently. The reason why the nucleotide sequences of genes III and VIII of I2-2 and IKe are more strongly conserved than all others is unclear. The Ka values (Table 3), i.e., the values for the relative number of amino acid substitutions, clearly indicate that I2-2 is more closely related to IKe than to Ff(M13).

Genetic and cell biologic studies indicate that the protein encoded by gene III of Ff is specifically re-



Fig. 2. Continued

quired for attachment of the phage to the conjugative pilus of the host cell. The minor, randomly distributed differences between the gene III proteins of IKe and I2-2 (Fig. 2; 30 out of 434 amino acids) indicate that only a few amino acid substitutions are sufficient to alter/extend the plasmid specificity of the phage (vide supra). The observed serological relationship (Bradley et al. 1983) between IKe, IKeh (a host range mutant of IKe), and I2-2, is in accordance with the high amino acid sequence homology found between the coat proteins encoded by gene VIII of both I2-2 and IKe. However, our sequencing data prove that, contrary to earlier speculations (Bradley et al. 1983), I2-2 is not a host range mutant of IKe. This can also be concluded from dot-matrix comparisons of the genomic sequences of I2-2, IKe, and Ff (Fig. 6). In these analyses the homology between successive stretches of heptanucleotides of the genomes of either I2-2 and IKe (Fig. 6A), I2-2 and Ff(M13) (Fig. 6B), or IKe and Ff(M13) (Fig. 6C) are

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s P 5 FKTESW S IRAQVEGD MO R S V P D Е Ρ s ۹ Е Ε т P Е Ε 1 C E TCGCCTAGTGAAAAAACTGAAAGTTGGTCTATTCGCGCTCAGGTTGAGGGTGATATGCAACGTTCTGTACCAGATGAAGAACCTTCTGAACAAACGCCAGAAGAAATCTGTGAAGAAAACTGAAGAAACTG 5290 5300 5310 5320 5330 5340 5350 5360 5370 5380 5390 5400 * I D G VE N N VSKGDEGGF Y INY NGCE ΕA TGV τν O N Y С D GΤ 5410 5420 5430 5440 5450 5460 5470 5480 5490 5500 5510 5520 * r S S A W K P T G Y V P E S G E S SSSPVKDG D TGGTGE GGS D v A TG 5530 5540 5550 5560 5570 5580 5590 5600 5610 5620 5630 5640 GENE TGGGDTGGGSTGGDTGGSTGGG STG GGSTG G D GST GKS L T Ξ 5650 5660 5670 5680 5690 5700 5710 5720 5730 5740 5750 5760 * * * * * * ٠ * * * D s **V К D** ε D V т A A I н A Ρ S I G D A s L т E D N D Q N D N QKK A D Ε 0 5 AAGGAAGATGTAACCGCAGCAATACATGATGCTTCCCCTTCTATTGGTGATGCTGTTAAAGATTCCCTTACAGAAGATAATGACCAGAACGACAATCAGAAGGAAAGCAGATGAACGATCA 5770 5780 5790 5800 5810 5820 5830 5840 5850 5860 5870 5880 * * * * * * * * * * * * * * ASVSDAI SDGM RGVGNFVDD GGESSQY KAS L GI GNSEM D GCTAAAGCCTCTGCTTCTGTATGCTATCTCTGATGGTATGCGTGGCGTTGGTAATTTCGTTGACGATCTTGGCGGTGAGTCGTCGCAATACGGAATCGGTAATTCTGAAATGGAT 5890 5900 5910 5920 5930 5940 5950 5960 5970 5980 5000 6000 * * * * * * * * * * * * * * * * * * * V S L A K G Q L G I D L E G H G S A W E S F L N D G SIPSG H G C S н CTCTCCGGTAGCCTGGCTAAAGGGCAACTTGGAATTGATCTTGAAGGTCATGGTTCAGCTTGGGAATCCTTTTTAAATGACGGTGCTTTACGTCCCTCCATTCCTTCTGGTCATGGCTGT 6010 6020 6030 6040 6050 6060 6070 6080 6090 6100 6110 6120 * * * * * * * * * * * * * * * * * * FVMFQGSVYQLD IGCDKLGD IΚ s v S W V M Y С F W ۷ т D L L Т Y F 6130 6140 6150 6160 6170 6180 6190 6200 6210 6220 6230 6240 × ATSL L RK GΕ Q * * * * * * * * * * * * * * * Ρ I P A L I R F I M G L V P M A LLG 1 A IG YF ĸ A CAGTCTGCTACTTCATTACTTCGTAAAGGTGAACAATAATGCCAGCTTTATTAGGTATTCCGGCTTTAATTCGTTTCATAATGGGGCTTGTGCCTCTTGCTATTGGTTATTTGCTAAGT 6360 6250 6260 6270 6280 6290 6300 6310 6320 6330 6340 6350 E * * * * * * * * * * NE TRNGLM A A A L IGA ILS FVT FA I 0 I L GD A L. S S м G GΚ II A L \leq 6370 6380 6390 6400 6410 6420 6430 6440 6450 6460 6470 6480 MSSVL P D τττς ΙΤΥ I D Y O v E v F D 1 KDR м Ρ ADFGN L G I 1 A GAATGCCTGCTGATTTTGGAAATTTAATGTCCTCTGTATTACCTGATGGTACAACAACTTGTATTACAGTAATTATAGACTACCAGATAGCGGTCTTTGTTTTCGATATTAAAGATCGCT 6520 6490 6500 6510 6530 6540 6550 6560 6570 6580 6590 6600 * * * * * * MAVY VVTGKLGAGK T L V A V S R I Q R T L A K G G 1 G TACTCGGCATTAGCTAATAAGGTAATTTGATATGGCTGTTTATGTTGTTACCGGAAAATTAGGCGCAAGACGCTTGTAGCTGTATCGCGTATACAAAGGACTTTAGCTAAAGGTGGT ENE 6660 6670 6680 6700 6710 6720 6650 6690 6610 6620 6630 6640 * * * * * * A T NL NL ATTGTTGCCACCAATCTAAATTTA 6730 6740



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compared. The genomes are aligned by using the initiation codon of gene VII as a reference point. From these plots it can be concluded that genes VII, IX, VIII, III, VI, I, and IV and the 5' end of the intergenic region (hairpin A) of I2-2 and IKe are highly similar. Immediately after the morphogenetic signal the sequence correspondence decreases abruptly. A similar observation has been made when the genomes of I2-2 and Ff(M13) were compared, although in this case the similarity between the DNA sequence located between the 5' end of gene VII and the 3' end of hairpin A is less pronounced (Fig. 6B).

The observation that no significant homology ex-

ists between the DNA regions of I2-2 and IKe or Ff that encompass the major part of the intergenic region (vide supra), genes II, X, and V suggests that the genome of I2-2 is composed of DNA sequences derived from two different replicons. We propose that the I2-2 genome is the result of a recombination event in which the replication cassette, i.e., the DNA fragment containing the viral and complementary strand origins of DNA replication and the phageencoded replication genes of its filamentous phage ancestor genome, has been exchanged for the rolling circle replication cassette of another replicon. Whether the latter cassette originated from another

 Table 2.
 Shine–Dalgarno sequences for the assigned and proposed genes of the I2-2 genome

16S RNA	3' OH-AUUCCUCCACUAG			
Gene I	CAATAAGGTAATTTGAT.ATG			
Gene II	TAAGGGAATGCGT.ATG			
Gene III	TAAGGTGTAATT.GTG			
Gene IV	TGTGAAGGCTAATT.ATG			
Gene V	TAAGGGGTTTTAA.ATG			
Gene VI	CGTAAAGGTGAACAATA.ATG			
Gene VII	TGAAGAGCTGATTT.ATG			
Gene VIII	TAAGGAATACAC.ATG			
Gene IX	TGCTGGGGTACTCCG.ATG			
Gene X	TATGGGGCGTATCGAA.ATG			

Nucleotides that are complementary to the 3' terminal end of 16S ribosomal RNA are underlined (Shine and Dalgarno 1974). Initiation codons are indicated in bold



M13

Fig. 4. Nucleotide sequences and presumed secondary structures of the rho-independent transcription termination signals of phages I2-2, IKe, and Ff(M13), which are located in the intergenic region between genes VIII and III. Nucleotides in the Ff sequence, encompassing the region of dyad symmetry, that are different from those in I2-2 and IKe are indicated with asterisks.







Fig. 3. Comparison of the circular genetic maps of the genomes of the filamentous phages I2-2, IKe, and Ff(M13). Genes are indicated by Roman numerals. IR refers to the major intergenic region located between genes IV and II, in which both the origins for viral and complementary strand synthesis and the *cis*-acting element required for efficient phage assembly are located.



Fig. 5. Comparison of the nucleotide sequence and secondary structural configuration of the regions of dyad symmetry in the major intergenic regions of the phages I2-2, IKe, and Ff(M13). A Nucleotide sequence (5'-3') and potential secondary structures in the I2-2 major intergenic region. B Comparison of the nucleotide sequence of region A of the intergenic region of the phages I2-2 and IKe. Within this sequence both the phage morphogenetic signal and a rho-dependent transcription termination signal are

located. Sequences are aligned in such a way that maximum homology is achieved. Regions of dyad symmetry are indicated by brackets. The stop codons of genes IV of I2-2 and IKe are over- and underlined. C Comparison of morphology and relative map position of the potential stem-loop structures (hairpins A through E) in the major intergenic region of the phages I2-2, IKe, and Ff(M13). The configuration are drawn to scale.



Fig. 6. Dot-matrix analysis of the extent of homology between the genomes of the phages I2-2, IKe, and Ff(M13). Starting from the 5' terminal nucleotide of gene VII consecutive stretches of seven nucleotides are compared. Each dot represents a stretch of

seven identical nucleotides. Parallel to the axes the genetic maps are indicated. A Dot-matrix of I2-2 versus IKe. B Dot-matrix of I2-2 versus Ff(M13). C Dot-matrix of IKe versus Ff(M13).

Table 3. Extent of synonymous (Ks) and nonsynonymous (Ka) substitutions in genes I–IX of the phages I2-2, IKe, and Ff(M13)

			Non-
	Sites compared	Synonymous difference (Ks)	synonymous difference (Ka)
I2-2 vs IKe			
Gene I	365	>1	0.107
Gene III	432	0.115	0.032
Gene IV	427	>1	0.163
Gene VI	116	>1	0.062
Gene VII	29	>1	0.148
Gene VIII	79	0.563	0.158
Gene IX	33	>1	0.120
I2-2 vs M13			
Gene I	342	>1	0.395
Gene III	338	>1	0.709
Gene IV	408	>1	0.458
Gene VI	109	>1	0.737
Gene VII	28	>1	0.573
Gene VIII	63	>1	0.429
Gene IX	31	>1	0.786
IKe vs M13			
Gene I	342	>1	0.410
Gene III	337	>1	>1
Gene IV	409	>1	0.482
Gene VI	109	>1	0.729
Gene VII	31	>1	0.818
Gene VIII	64	>1	0.571
Gene IX	31	0.874	0.752

The extent of synonymous and nonsynonymous substitutions was calculated as indicated in Materials and Methods

filamentous phage or from a plasmid with a rolling circle replication mode is not yet clear, as computer searches in the EMBL (release 23) and Genbank (release 60) nucleic acids databases have not yet revealed the existence of nucleotide sequences that are evolutionarily related to the replication module of I2-2. Acknowledgments. We thank our colleagues, Lettie Lubsen and Graham Elliott, for reading the manuscript and making corrections and helpful suggestions. The work has been supported (Maoxiao-Yu) by a grant from the Dutch Ministry of Education and Science.

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