The EMBO Journal vol.7 no.12 pp.4005-4010, 1988

# A novel replicon occurring naturally in *Escherichia coli* is a phage-plasmid hybrid

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Communicated by T.A.Trautner

**A novel DNA replicon in *Escherichia coli* was identified. It is the smallest natural isolate (1282 bp) found so far. In the presence of phage M13 it grows as a filamentous single-stranded DNA phage. Contrary to previously identified mini-phages this replicon displays sequence homology only to parts of the M13 viral and complementary strand origin. In the absence of M13 this DNA replicates autonomously. The only gene (*arp*) of the replicon encodes a 32-kd protein, which is essential for autonomous replication. The host *rep* gene required for replication of single-stranded DNA phages is dispensable. Distinct replication mechanisms are thus involved during growth as defective phage or as autonomous plasmid.**

*Key words:* DNA replication/phasyl/single-stranded DNA phage

## Introduction

Numerous extrachromosomal replicons have been identified in *Escherichia coli*. These are plasmids or phages with double-stranded DNA or phages containing single-stranded DNA. From the study of these replicons a general concept for the mechanism of DNA replication has emerged (Kornberg, 1980, 1982). In all replicons, the initiation of replication involves origin recognition, assembly of a protein complex and primer synthesis. With double-stranded DNA this is followed in *E.coli* by DNA unwinding and the coordinate replication of both strands. In the case of single-stranded DNA phages replication of both strands occurs in separate steps. Complementary strand synthesis converts the viral strand into the double-stranded replicative form (RF) DNA. From the RF molecule the viral strand is synthesized in a rolling circle mechanism. Phage- and plasmid-encoded replication proteins, in addition to proteins specified by the host, participate in these processes. Plasmids containing origins of single-stranded DNA phages were constructed *in vitro* to facilitate the isolation of cloned single-stranded DNA (Pouwels *et al.*, 1985).

In this work we describe the identification and characterization of a novel replicon in *E.coli*. This is the first natural isolate which is both a defective single-stranded mini-phage and an autonomous plasmid. Distinct replication mechanisms are involved during phage-like or plasmid-like growth of this replicon.

## Results

### *Detection of a defective mini-phage*

During cloning experiments employing phage-M13-derived vectors and appropriate *E.coli* host strains (Yanisch-Perron *et al.*, 1985), we observed in several cases, after agarose gel electrophoresis, a small DNA species in addition to the expected bands. This small DNA molecule, which we termed phasyl, was present not only in preparations of RF DNA from infected cells (Figure 1, lane D), but also in preparations of single-stranded DNA from phage in the culture supernatant (Figure 1, lane B).

Analysis of these DNA preparations by electron microscopy (Figure 2A and B) revealed that phasyl is a circular molecule of ~1.3 kb in size, it is single stranded when isolated from phage (Figure 2A), but double stranded when isolated from cells (Figure 2B). These data correspond to the observed electrophoretic mobility of phasyl DNA (Figure 1). Analysing phage preparations with phasyl in the electron microscope we observed in addition to M13 particles many small filaments of 1/5 M13 length (Figure 2C and D) obviously reflecting the DNA size ratio of phasyl versus M13. Apparently, phasyl is a filamentous mini-phage containing single-stranded DNA.

To analyse whether phasyl is an autonomous phage,

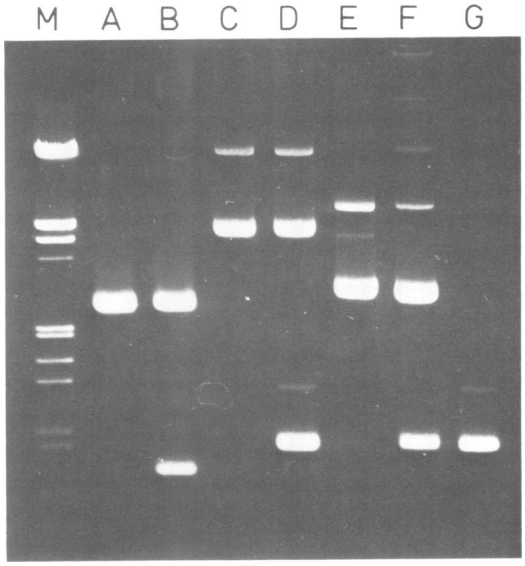


**Fig. 1.** Phasyl DNA from various sources. An ethidium-bromide-stained agarose gel (1%) is shown with λ DNA *Eco*RI-*Hin*dIII digested as marker (**M**), DNA prepared from M13 wild-type phages (**A**), from M13/phasyl phages (**B**), from strain JM103 infected with M13 (**C**) or infected with M13/phasyl phages (**D**), from strain WM1202 carrying plasmid pBR322 (**E**) or plasmid pBR322 and phasyl (**F**) and from strain H411 carrying phasyl (**G**).

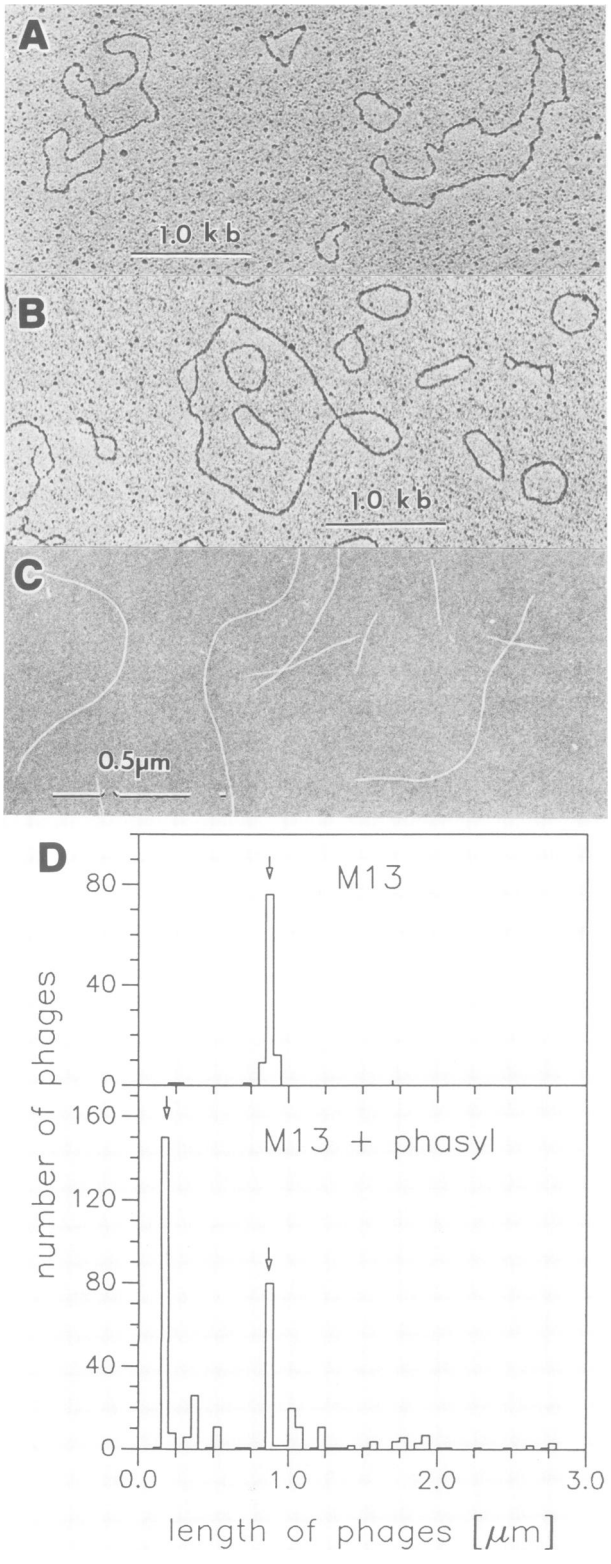


**Fig. 2.** Electron microscopic analysis of phasyl DNA and phages. Electron micrographs (magnification × 50 000) of DNA prepared from M13/phasyl phages (**A**) or from cells infected with this phage mixture (**B**) are shown (M13, large circles; phasyl, small circles). The phage mixture was visualized in the electron microscope by negative staining (**C**) and the length of particles in a pure M13 or an M13/phasyl phage preparation was quantified (**D**). The arrows indicate the mean values of the lengths of phasyl (0.186 µm) or M13 (0.87 µm) phage particles. Only one unit length was found in pure M13 phage preparations. In M13/phasyl preparations particles of mixed oligomeric length beside the expected monomers were present.

purified phasyl RF DNA was transfected into an $F^+$ strain. Even with large amounts of DNA (1 µg) no, or only very few, plaques were obtained, which in each case contained M13 in addition to phasyl DNA. Lack of plaque formation characterizes phasyl as a defective mini-phage.

Mini-M13 phages arising spontaneously upon repeated high multiplicity infections have been described (Griffith and Kornberg, 1974; Enea *et al.*, 1977; Ravetch *et al.*, 1979). These mini-phages retain the M13 intergenic region, where replication and packaging signals are specified (Zinder and Horiuchi, 1985) and depend on an M13 helper phage supplying gene products *in trans*. Contrary to these observations the pattern of restriction sites of phasyl DNA was completely different from the pattern of the M13 intergenic region. To clarify the structural relationship of phasyl and M13 DNA we performed hybridization experiments using either DNA as a probe. There was no cross-hybridization of phasyl and M13 DNA under standard stringency conditions (data not shown). Despite its characteristics of an M13-like phage, phasyl thus differs in its sequence from M13 DNA. Moreover, phasyl did not show any homology to λ, ΦX174, pBR322 or chromosomal DNA of the host strain (JM103) (data not shown).

### *Phasyl DNA sequence*

To characterize this novel type of mini-phage the nucleotide sequence of phasyl DNA was determined. The sequencing strategy is outlined in Materials and methods. The complete phasyl genome (Figure 3) encompasses 1282 bp. A non-coding region (position 1−424) is followed by an open reading frame (position 425−1282) potentially encoding a protein with a mol. wt of 32 kd. To identify the viral strand, purified single-stranded phasyl DNA was directly sequenced using small phasyl restriction fragments (*Hae*III−*Hae*II, position, 666−736; *Alu*I, position 514−595) as primers. A definite sequence was obtained characterizing the unique polarity of the template viral strand, which is shown with 5′ to 3′ polarity from left to right in Figure 3.

Comparison of the phasyl nucleotide sequence to those of filamentous phages revealed that two stretches from the non-coding region of phasyl are homologous to sequences in the intergenic region of filamentous phages (Figure 4). Multiple insertions, deletions and base substitutions explain the lack of M13/phasyl cross-hybridization under stringent conditions. We did not detect any further significant homology of the phasyl genome to sequences in GenBank or EMBL databases. Homology element I specifying two inverted repeat structures (loops D and E, Figure 5) constitutes the core region (domain A) of the viral strand origin, absolutely required for viral strand replication (Dotto *et al.*, 1984). Phage-encoded geneII protein produces a single-strand cut there (fd position 5781/5782; Meyer *et al.*, 1979), which is used for the rolling-circle-type replication of the viral strand.

Homology element II potentially forms two extensive hairpin loops (loops B and C, Figure 5). In single-stranded DNA these loops were found to be protected by RNA polymerase against nuclease attack (Gray *et al.*, 1978) and an RNA primer for complementary strand replication is synthesized there (fd position 5756−5728; Geider *et al.*, 1978). Interestingly, the first six nucleotides of the primer are not included in the homology element (Figures 4 and 5). Another difference to the filamentous phages is

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ACATCGAGTAGTCCACGCTGTTTAATAGTGGACTCTTGTTCCAATATGGAACACTTCCGCCTCACGCAGCCAGATTGTAAATCTGTCAGCGTTCACCGTT   100

GCTGAAATCGTCAAATGCTAAATCGGGGGTCCCTTTAGGGTTCCGATTTAGTGTTTGTACGACACCCCGACTAAAGACACTTCTTGCTGGTGTGTGTTAC   200

GTGCGCACGAGCGTGACACACACCTGTAAGACGGGTTTTAAGGGGTTCCGACCACTGACTAATCCGAAACAGCATGAGTGACCCCCGTTTGACCATGTTG   300

CCGAATCGCTGTAACGTTGTCCCGATGACCAAAAAGCAAAACCCCCGATGCGCAAACATCAGGGGTTTATGGTCAGTAAGCATTTCTAGGGCTCATTCTG   400

TACTTTACTTAACTGGACAACTCGATGTTACTCAATAAAGACCGCTTTGGCAAACTCACGTCAAAATCCCTCGATTCGATTGCCGCTCTTGGACTCCGCG   500
                        MetLeuLeuAsnLysAspArgPheGlyLysLeuThrSerLysSerLeuAspSerIleAlaAlaLeuGlyLeuArgG

AGGCGAATGCCGAGCTTCCTTGTCTATTTAGAAACAACTCAATCGAGAGTTCTTCAAAAACACCTCCAGAACCCTTATCACCTCTGGCTTTTGAGCTTTC   600
luAlaAsnAlaGluLeuProCysLeuPheArgAsnAsnSerIleGluSerSerSerLysThrProProGluProLeuSerProLeuAlaPheGluLeuSe

TACAAACCTCAAAAAATCTGCCTCGGCTCTGGCATGGAATGTACAGTATTTTGTCGATACCTACGGCCTTTCAAACGTTGGATTTTTAACCCTTACCTTT   700
rThrAsnLeuLysLysSerAlaSerAlaLeuAlaTrpAsnValGlnTyrPheValAspThrTyrGlyLeuSerAsnValGlyPheLeuThrLeuThrPhe

CGTGACCATGTGACCGACCCAAAAGAGGCGCAGCGCCGTTTTAACTCCCTCAAAACAAATATCCTTGCCAAAAGATACCGCGCTTACATCCGTGTTATGG   800
ArgAspHisValThrAspProLysGluAlaGlnArgArgPheAsnSerLeuLysThrAsnIleLeuAlaLysArgTyrArgAlaTyrIleArgValMetG

AGCCTATGAAGTCAGGGCGCATCCACTATCACTTACTCGTTGCACTGCACTCCGACATTCGAACCGGATTCGATTTCCCCGCTGTATATCGCCAGGACTA   900
luProMetLysSerGlyArgIleHisTyrHisLeuLeuValAlaLeuHisSerAspIleArgThrGlyPheAspPheProAlaValTyrArgGlnAspTy

TTCCTCCGCAAACAAGGCTATCCGCTCTGAATGGTCATTCTGGCGCAAGACTGCGCCGAAATATGGCTTCGGTCGCACTGAATTGATGCCCGTTCGGTCA  1000
rSerSerAlaAsnLysAlaIleArgSerGluTrpSerPheTrpArgLysThrAlaProLysTyrGlyPheGlyArgThrGluLeuMetProValArgSer

AATTCTGAGGGGATAGGGCGCTATGTAGGCAAATACATTTCTAAGGGCATAGAATCGCGTACAGAGCAATTTAAGGGCGTTAGGCTGGTAGAGTATTCCC  1100
AsnSerGluGlyIleGlyArgTyrValGlyLysTyrIleSerLysGlyIleGluSerArgThrGluGlnPheLysGlyValArgLeuValGluTyrSerA

GCAAGGCTAAATCGCTTCTACGCGCTTCCAGTTCGTTTCTGACGGGTCTTATGAGTGGCGTCGCAAACTTTCGATATTCGTTCATTACATCGCGGACAAT  1200
rgLysAlaLysSerLeuLeuArgAlaSerSerSerPheLeuThrGlyLeuMetSerGlyValAlaAsnPheArgTyrSerPheIleThrSerArgThrIl

ATGGGCTGTGAACCAACTTTTGACGGCTTACGCCGTGTTCTCGGCTCTCGTTGGTCGTATCACTGGCGTGATTTTATTATGA
eTrpAlaValAsnGlnLeuLeuThrAlaTyrAlaValPheSerAlaLeuValGlyArgIleThrGlyValIleLeuLeuEnd
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**Fig. 3.** Complete nucleotide sequence of phasyl DNA. The sequence of the circular viral strand with 5′ to 3′ polarity from left to right is shown. Position 1 was defined as the first nucleotide following the stop codon of the open reading frame.

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                    ← homology element I →                                          ← homology element II →

phasyl      8 GTAGTCCACGCTGTTTAATAGTGGACTCTTGTTCCAATATGGAACA  53          82 TCTGTCAGGGTTCACCGTTGCTGAAATCGTCAAATGCTAAATC..GGGGG

M13  5765 GGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACA 5810      5600 TCTCGCCACGTTCGCCG..GCTTTCCCCGTCAAGCTCTAAATC..GGGGG
f1   5765 GGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACA 5810      5600 TCTCGCCACGTTCGCCG..GCTTTCCCCGTCAAGCTCTAAATC..GGGGG
fd   5766 GGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACA 5811      5601 TCTCGCCACGTTCTCCG..GCTTTCCCCGTCAAGCTCTAAATC..GGGGG
IKe  6553 TGAGTCCACTATGTTTAATAGTGGACTCTTGTTGCATTTTGCAACG 6598      6309 TCTTTCGGCGTTCTCCGGCGTCG.AATCGTCAAACGCTAAATCGTGGGGG

phasyl  .TCCCTTTAGGGTTCCGATTTAGTGTTTGTACGACACCCCGA.CTAAAGACACTTCTTGCTGGTG.TGTGTTACGTGCGCACGAGCGTGACACACACCTGTAAGACGGGTTTT  239

M13  CTCCCTTTAGGGTTCCGATTTAGTGCTT.TACGGCACCTCGACCCCAAAAAACTTGATTTGGGTGATGGTTCACGT.......AGTGGGCCATCGCCCTGATAGACGGTTTTT 5749
f1   CTCCCTTTAGGGTTCCGATTTAGTGCTT.TACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGT.......AGTGGGCCATCGCCCTGATAGACGGTTTTT 5749
fd   ATCCCTTTAGGGTTCCGATTTAGTGCTT.TACGGCACCTCGACCTCCAAAAACTTGATTTGGGTGATGGTTCACGT.......AGTGGGCCATCGCCCTGATAGACGGTTTTT 5750
IKe  C.CCCTTT.GGG..CCGATTTAGTGATT.TACGACACTCC.A..TAAATATCCTTGTTATGGGTGATGGTTCACGT.......AGTGGGCCATCGCCTTGTTAGACGTTTTTT 6454
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**Fig. 4.** Comparison of the phasyl DNA sequence with those of filamentous phages. Stars below the nucleotide sequence of phasyl DNA indicate identical bases in the sequence of phages M13, f1, fd or IKe. The nucleotide positions limiting homology elements I and II are given. Sequence data refer to van Wezenbeek *et al.* (1980) for M13, Beck and Zink (1981) for f1 and fd, and Peeters *et al.* (1985) for IKe.

remarkable. The order of homology elements I and II in phasyl is reversed (Figure 5). The efficient production of phasyl phages in the presence of M13 implies that these homology elements specify functional complementary and viral strand origins of phasyl.

### *Phasyl encodes a 32-kd protein*

We used an *in vitro* transcription–translation assay to visualize proteins encoded by the phasyl genome. Proteins synthesized in an *E.coli* extract with phasyl RF DNA as template were labelled with [$^3$H]leucine and separated on an SDS–polyacrylamide gel. A single protein band was detected which was absent in a control without phasyl DNA (Figure 6). This band was also absent when the template was cleaved with an enzyme which cuts within the coding region, e.g. *Hae*III (data not shown). The apparent mol. wt of the protein agrees well with the size of 32 kd predicted from the phasyl DNA sequence.

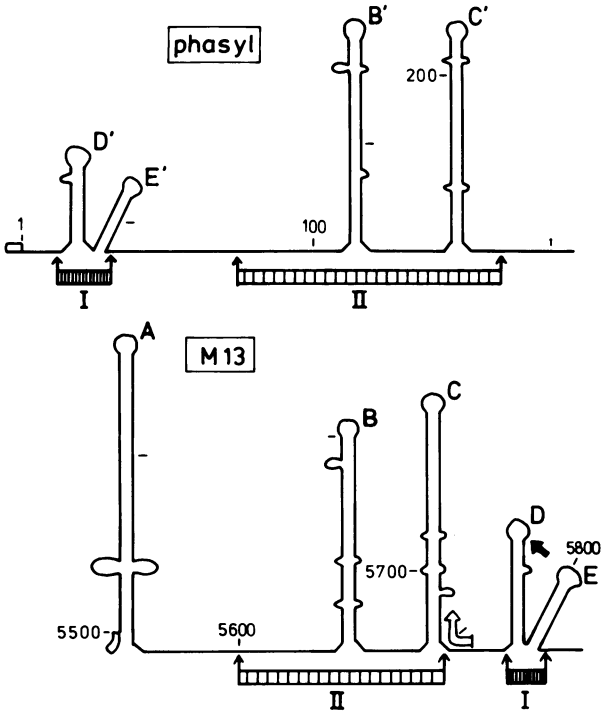


**Fig. 5.** Potential secondary structure in the intergenic region of phasyl and M13. M13 loop A specifies the morphogenetic signal (Dotto and Zinder, 1983), loops B and C the complementary strand origin (Gray *et al.*, 1978), loops D and E domain A of the viral strand origin (Dotto *et al.*, 1984). Start and 5′ to 3′ orientation of the complementary strand primer (Geider *et al.*, 1978) are indicated by an open arrow, the geneII protein nicking site (Meyer *et al.*, 1978) by a filled arrow. Homology elements I and II (Figure 4) are shown.

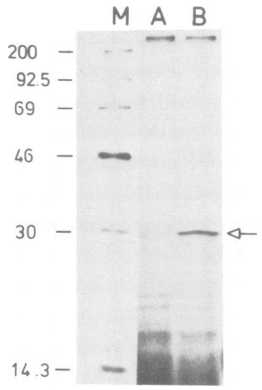


**Fig. 6.** *In vitro* transcription–translation of phasyl DNA. An autoradiograph of an SDS–polyacrylamide gel (12.5%) of $^{3}$H-labelled proteins synthesized in an *E.coli* extract without (**A**) or with (**B**) phasyl DNA as template is shown. The phasyl-encoded gene product is indicated by an arrow. Mol. wts of marker proteins (M) are given.

Immediately upstream of the single phasyl gene an RNA polymerase binding site was mapped by electron microscopy. This was the only RNA polymerase binding site detected on the phasyl genome (data not shown).

### *Phasyl is an autonomous replicon*

We carried out an analysis to find out if phasyl, which requires phage M13 for growth as a filamentous phage, can replicate its DNA autonomously. To avoid any alteration of the phasyl genome by insertion of a selectable marker

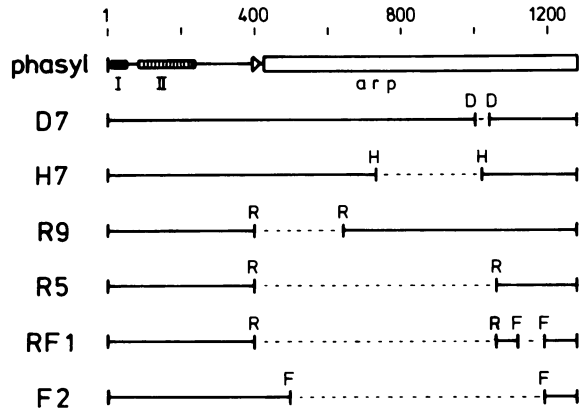


**Fig. 7.** Phasyl deletion derivatives. A map of the phasyl genome, linearized at position 1, with homology elements I and II, the *arp* gene (open box) and promoter (triangle) is shown. Single letters denote sites for restriction enzymes *Dde*I (D), *Hae*II (H), *Rsa*I (R) and *Fnu*DII (F). Deleted regions are indicated by a dashed line.

a co-transformation assay was used. Competent cells of various strains (JM103, C600 and WM1202) were transformed with saturating amounts (0.2 μg) of purified phasyl RF DNA. To select for transformed cells a small amount (0.2 ng) of a second selectable plasmid, e.g. pBR322, was added. Resistant colonies were grown, plasmid DNA was prepared and analysed by agarose gel electrophoresis. Typically 50–90% of the transformants contained phasyl DNA in addition to the resistance marker plasmid. A DNA preparation from strain WM1202 harbouring both phasyl and pBR322 DNA is shown in Figure 1, lane F. The absence of M13 DNA was verified by titrating the culture supernatant on an $F^{+}$ strain. Even after multiple passages no decrease in the amount of phasyl DNA was observed, which replicates at a copy number about equal to that of pBR322 (Figure 1, lane F). These data demonstrate that phasyl is a stable autonomous replicon with a high copy number.

### *Phasyl encodes an essential replication protein*

To define the sequences essential for autonomous replication we constructed deletion derivatives of phasyl (Figure 7). The DNA was digested partially or completely with enzymes *Dde*I, *Hae*II, *Rsa*I or *Fnu*DII, religated and co-transformed with M13 RF DNA into JM103. Cultures were grown from individual plaques, DNA was prepared and analysed. These experiments defined the phasyl region from restriction sites *Fnu*DII (position 1192) to *Rsa*I (position 401), where both segments homologous to parts of the M13 viral and complementary strand origin are located, to be sufficient for growth as a filamentous phage. An intact phasyl gene is obviously not required.

Phasyl deletion derivatives were tested for replication in the absence of M13 using the co-transformation assay described above. Any deletion in the open reading frame, even the removal of only 36 bp in the case of D7 (Figure 7), resulted in loss of the autonomous replication capacity of phasyl. The intact open reading frame is obviously essential for autonomous phasyl replication. We therefore termed the gene *arp* for autonomous replication of phasyl DNA.

GeneII of phage fd has been described to be sufficient for

replication of plasmids carrying the fd intergenic region (Meyer and Geider, 1981). Similarly we analysed if phasyl derivatives, defective in the phasyl *arp* gene, replicate in strain H411 (F′ fd geneII$^+$) expressing the fd geneII product. By co-transformation we could establish all phasyl deletion derivatives in the fd geneII-expressing strain. The fd geneII product complements the replication defect of phasyl derivatives with deletions in the *arp* gene.

Consequently we carried out an analysis to find out if, in the reciprocal case, phasyl *arp* gene can substitute for fd geneII. Phage fdTn9 is defective in geneII, carrying a transposon insertion. Phage fdTn9 grows efficiently ($5 \times 10^8$ plaques/ml phage lysate) on strain H411 which supplies the fd geneII product *in trans*. But this lysate did not produce plaques on a standard F$^+$ strain (JM103) nor on JM103 harbouring phasyl ($< 10^2$ plaques/ml phage lysate). These data show that the phasyl *arp* gene cannot substitute for fd geneII, which, on the other hand, is sufficient for growth of *arp*-gene-defective phasyl derivatives.

### *Two distinct mechanisms for phasyl replication*

Lack of reciprocal complementation of the phasyl *arp* protein and the fd geneII product implies two different mechanisms of phasyl replication involving one or the other gene product. GeneII-dependent replication of filamentous phages requires the host *rep* (helicase) gene (Dumas, 1978). Using again the co-transformation assay phasyl could be efficiently established in a *rep*$^-$ host strain (H517).

In addition, strain H517 (F$^+$ *rep*$^-$) was infected with a mixture of phasyl and M13 phages. The culture was grown for 5 h, plated, fresh cultures were grown from individual colonies and DNA was prepared. Phasyl was found to replicate efficiently in a *rep*$^-$ strain (Figure 1, lane G). No M13 DNA or phage was detected. Since phasyl DNA replication, in contrast to single-stranded DNA phages, does not require the host *rep* gene, the mechanisms involved in *arp*-gene-dependent plasmid-type replication or in fd-geneII-dependent phage-type replication of phasyl are different.

## Discussion

We have detected and characterized a combined phage–plasmid replicon (termed phasyl) in *E.coli*. It contains two segments homologous in sequence and potential secondary structure to filamentous phages (M13, f1, fd and IKe). But the order of homology elements in phasyl is reversed (Figure 5) and there are a number of insertions and deletions in addition to base substitutions (Figure 4). Therefore, a direct derivation of these phasyl sequences from known filamentous phages can be ruled out. Rather, these sequence and secondary structure homologies seem to reflect conservation of origin function, since they lie within the minimal region sufficient for growth of phasyl DNA as defective phage (Figure 7).

Homology element I exactly corresponds to the core region of the viral strand origin defined by deletion analysis (Dotto *et al.*, 1982, 1984) and by geneII protein-binding studies (Greenstein and Horiuchi, 1987). Element II covers most of the complementary strand origin characterized by RNA polymerase protection (Gray *et al.*, 1978) and by deletion analysis (Kim *et al.*, 1981). From the comparison of phasyl DNA to filamentous phages (Figure 4) a consensus sequence of viral and complementary strand origins can be derived.

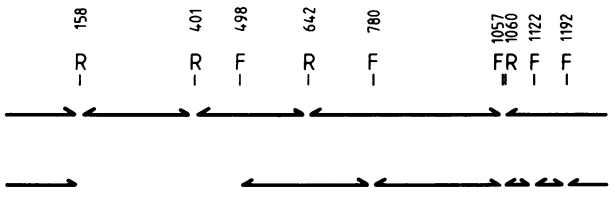


**Fig. 8.** Outline of the sequencing strategy. A physical map of the phasyl genome, linearized at position 1, is shown. Restriction sites for enzymes *Rsa*I (R) and *Fnu*DII (F) with their exact map positions are given. For sequencing, fragments generated by these enzymes were inserted into vectors M13mp18/19 or pUC18 in both orientations.

Phasyl does not contain homology to the morphogenetic signal of filamentous phages (loop A, Figure 5), which was reported to be required for efficient phage packaging (Dotto and Zinder, 1983) nor does phasyl DNA contain homology to the replication enhancer sequence (domain B of the viral origin; Dotto *et al.*, 1984). Phasyl obviously defines the minimal requirements for efficient phage growth.

In the absence of helper phage or helper replication protein (geneII product) phasyl DNA still replicates (Figure 1, lane F). The *arp* gene product encoded by the phasyl genome is essential for autonomous replication (Figure 7). Phasyl Arp protein cannot substitute for geneII product of filamentous phages. Most striking, autonomous phasyl replication is independent of the host *rep* function (Figure 1, lane G), a helicase required for rolling-circle replication of all single-stranded DNA phages in *E.coli* (Dumas, 1978). Obviously, replication as defective phage or as autonomous plasmid follows distinct mechanisms. Rolling-circle replication of plasmids in Gram-positive bacteria like *Bacillus subtilis* has been reported (Viret and Alonso, 1987; Gros *et al.*, 1987) but phasyl plasmid replication is apparently different.

Several common features of plasmid replication origins are absent from phasyl DNA. There are no obvious repeated sequences or AT-rich regions, no DnaA protein binding sites or recognition sites for Dam methylase. Analysis of the mechanism of autonomous phasyl replication will therefore contribute new aspects to our knowledge about replication.

The small size of phasyl DNA, its stable replication, high copy number, its novel sequence and the option of phage or plasmid replication all make phasyl DNA an attractive starting material for cloning vector development.

Phasyl is a natural isolate which can propagate as a plasmid or as a defective single-stranded DNA phage. It is this dual property which we find particularly interesting from an evolutionary point of view. Such a combination was so far only known from cloning vectors constructed in the laboratory. The evolutionary advantage for phasyl might be the potential of horizontal transmission in a culture.

## Materials and methods

### *Bacterial strains, plasmids and phages*

Strains used in this study were C600 (Maniatis *et al.*, 1982), JM103 (Messing *et al.*, 1981), WM1202 (*ara*, Δ(*lac–pro*), φ80 dΔ(*lac*)M15, *rec*A, *rps*L, *thi*), H517 (F$^+$, *end*A, *rep*) and H411 (=C600 F′phage fd geneII). Phage fdTn*9* carries a Tn*9* transposon insertion in geneII. Strain H411 and phage fdTn*9* were kindly provided by M.Metzger and K.Geider, Heidelberg, FRG.

### *General techniques*

Standard protocols (Maniatis *et al.*, 1982) and manufacturer's instructions were followed for preparation of DNA, restriction enzyme digestion, ligation,

transformation and agarose gel electrophoresis in TA-buffer. Phages were precipitated from the culture supernatant with polyethylene glycol 6000 (4%) and NaCl (0.5 M) for 30 min on ice. The phage pellet after centrifugation was suspended in TE-buffer. For preparation of single-stranded DNA, phages were phenolized twice followed by ethanol precipitation.

### *Sequencing*

Restriction fragments of the phasyl genome generated by enzymes *Rsa*I and *Fnu*DII (Figure 8) were inserted into M13mp18/19 vectors (Yanisch-Perron *et al.*, 1985) in both orientations. We did not succeed in inserting a 588-bp *Fnu*DII (position 1192–498) and a 380-bp *Rsa*I (position 1060–158) fragment into these vectors. Therefore, the latter fragment and a 248-bp *Fnu*DII–*Rsa*I fragment (position 1192–158) were cloned in plasmid pUC18 (Yanisch-Perron *et al.*, 1985), and the plasmid sequencing technique (Chen and Seeburg, 1985) was employed in this case. For each fragment both strands were sequenced by the chain-termination method (Sanger *et al.*, 1977). A sequencing kit from BioLabs (Beverly, MA) and conditions as specified by the supplier were used. Data were evaluated with the help of computer programs from the University of Wisconsin Genetics Computer Group (Devereux *et al.*, 1984).

### *In vitro transcription–translation*

For *in vitro* synthesis of proteins conditions are detailed in Kölling *et al.* (1988).

### *Electron microscopy*

Phages were prepared for electron microscopy by negative stain (Valentine *et al.*, 1968) using 2% sodium phosphotungstate pH 7.0. Single- and double-stranded DNA was prepared by cytochrome C spreading in 50% formamide and carbonate buffer on a water hypophase (Burkardt and Lurz, 1984). Phages and DNAs were traced on enlarged (×16) electron microscopic negatives (primary magnification ×5200). The data were evaluated with the aid of computer programs.

## Acknowledgements

We thank Beate Dobrinski for expert technical assistance in electron microscopy and Christina Lange for construction of some deletion derivatives. Strain H411 and phage fdTn9 were kindly provided by M.Metzger and K.Geider, Heidelberg. For this and previous work support by Sylvia Abel to W.S. was vital.

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*Received on June 28, 1988; revised on September 8, 1988*