Synthesis of a *trans*-Acting Inhibitor of DNA Maturation by Prohead Mutants of Phage λ

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ABSTRACT

Bacteriophage λ with mutations in genes that control prohead assembly and other head precursors cannot mature their DNA. In this paper we present evidence that the failure of these phage mutants to mature DNA is a reflection of a mechanism that modulates terminase nicking activity during normal phage development. We have constructed plasmids that contain the λ-cohesive end site (*cos*) and the genes that code for DNA terminase, the enzyme that matures DNA by cutting at *cos*. The DNA terminase genes are under control of a thermosensitive *cI* repressor. These plasmids lack most of the genes involved in prohead morphogenesis and other head precursors. However, when repression is lifted by destruction of the thermosensitive repressor, the terminase synthesized is able to cut almost 100% of the plasmids. Therefore, these plasmids can mature in the absence of proheads and other head gene products. The plasmids are also able to complement mutants of λ deficient in terminase and DNA maturation. However, in these complementation experiments, if the phage carry mutations in prohead genes *E* or *B*, not only is phage DNA maturation blocked, but the plasmid also fails to mature. These experiments show that, in the absence of proheads, phage λ produces a *trans*-acting inhibitor of maturation. The genetic determinant of this inhibitor maps in a region extending from the middle of gene *B* to the end of gene *C*. A model is proposed in which the nicking activity of DNA-bound terminase is inhibited by the *trans*-acting inhibitor. Prohead (and other factors) binding to this complex would release the block to allow DNA cleavage and packaging.

THE normal DNA substrate used for packaging into virions are the concatemers synthesized late after infection (STAHL *et al.* 1972; MCCLURE and GOLD 1973; ENQUIST and SKALKA 1973; TAKAHASHI 1974, 1975; BASTIA, SUEOKA and COX 1975). The transformation of concatemers into monomeric virion chromosomes by the introduction of two staggered nicks 12 nucleotides apart on opposite strands of the duplex DNA is called DNA maturation (MOUSSET and THOMAS 1969). The nicks are introduced at a specific sequence called *cos* (FEISS and MARGULIES 1973; EMMONS 1974) by the enzyme λ-DNA terminase. Two λ head genes, *Nu1* and *A* code for the subunits of the terminase (SUMNER-SMITH, BECKER and GOLD 1982; GOLD and BECKER 1983). Of the other eight λ head genes, four, *B*, *C*, *Nu3* and *E* are involved in the morphogenesis of the prohead, an empty head precursor into which DNA is encapsulated (HOHN and HOHN 1974; HOHN *et al.* 1974; KAISER, SYVANEN and MASUDA 1974, 1975; HENDRIX and CASJENS 1975; HOHN, FLICK and HOHN 1975; BECKER, MURIALDO and GOLD 1977; MURIALDO and BECKER 1977, 1978a). Gene *D* codes for a protein that is added to the exterior of the prohead during DNA packaging (HOHN and HOHN 1973; KAISER, SYVANEN and MASUDA 1975; STERNBERG and WEISBERG 1977; IMBER *et al.* 1980), and gene *FI* product is also involved in DNA packaging in an, as yet, unclear manner (see FEISS and BECKER 1983). The product of the *W* and *FII* genes function after DNA packaging and cutting is complete (CASJENS, HOHN and KAISER 1972; KAISER, SYVANEN and MASUDA 1975).

Cos cleavage is intimately coupled to the packaging of the DNA into proheads. Thus, although DNA replication is normal, DNA maturation does not occur in cells infected with phage mutants in genes involved in prohead morphogenesis, mutants in genes *D* and *FI*, and in host mutants that block prohead assembly (DOVE 1966; MACKINLEY and KAISER 1969; WAKE, KAISER and INMAN 1972; SKALKA, POONIAN and BARTL 1972; GEORGOPOULOS *et al.* 1973; MCCLURE, MACHATTIE and GOLD 1973; LASKI and JACKSON 1982).

On the other hand, we have shown that, under certain conditions, *cos*-cutting *in vivo* in the absence of proheads can be extremely efficient (MURIALDO and FIFE 1984). In these experiments, a pBR322-derived plasmid, pFM3, that contains the terminase genes under control of the *cI* repressor was used. The plasmid also contains *cos* (for structure see Figure 1). When repression was lifted by destruction of the thermosensitive repressor, the terminase synthesized was able to cut essentially 100% of the intracellular plasmid. Since this plasmid lacks *C*, *Nu3* and *E* (as well as

genes *D* and *FI*), *cos* cutting took place in the absence of proheads. Several possible explanations were offered to reconcile the lack of prohead-dependence of *cos* cutting in this plasmid with the absolute requirement for proheads for λ-DNA *cos* cutting (MURIALDO and FIFE 1984). These include (1) failure to detect λ-DNA maturation due to exonucleolytic degradation of the unprotected ends. Indeed, in the biological assay for DNA maturation only DNA containing intact mature ends is infectious, and the removal of a few bases from the protruding ends destroys infectivity (MILLER and FEISS 1981; STRACK and KAISER 1965). In addition, if exonucleolytic attack extends about 200 base pairs (bp) or more from the ends, then it is quite possible that LASKI and JACKSON (1982), who used a radioactive probe containing only about 200 bp on either side of *cos*, might have missed *cos* cleavage. Moreover, if we assume that the concentration of terminase in λ-infected cells is limiting, then most concatemers would escape extensive cutting due to the lack of processivity in the absence of proheads. This would result in the production of minimal (undetectable) amounts of mature molecules and a very small decrease in concatemeric size. Sedimentation analysis of concatemeric DNA (MCCLURE, MACHATTIE and GOLD 1973) is not sensitive enough to detect a difference of this sort; (2) a different DNA topology between phage DNA and plasmid; (3) a different enzyme-substrate ratio; and (4) terminase inhibition in the absence of proheads by the product of another λ gene. Since the plasmid contains only genes *Nu1* to *B*, the hypothetical λ gene would have to map to the right of gene *B* (Figure 1). In this communication we present evidence that the latter possibility may well be correct.

MATERIALS AND METHODS

Bacterial strains: The *E. coli* K12 derivatives which are pertinent to the reported results are listed in Table 1. OR1265 (and OR1265[pFM3]) has been described (MURIALDO and FIFE 1984). It is a *sup*^{0} strain carrying a cryptic prophage; its relevant property for the present study is that it provides the cell with a thermosensitive λ repressor (*cI857*). Strain 594 is *sup*^{0}. The lysogens not carrying a plasmid and that are listed in the table, have been previously described (MURIALDO and SIMINOVITCH 1972). The lysogens carrying pFM3 (MURIALDO and FIFE 1984) and pFM4 (RACKWITZ *et al.* 1985) were constructed by transfection according to DAGERT and EHRLICH (1979) selecting for ampicillin resistance. The lysogens with λ-defective *gal* transducing prophages (λdg*al*, or λdg for short) are also listed in Table 1. The extent of the prophage deletions is shown in Figure 4. It was checked by marker rescue using a series of morphogenetic amber mutants as described before (MURIALDO *et al.* 1981). λdg*al580* and λdg*al616* were isolated by CAMPBELL (1961), and the *cI857* allele was crossed in by P. RAY (personal communication). The other λdg*als* were isolated by C. FUERST. The host of the λdg*al* prophages are W3350 which is *sup*^{0}, *galT1, galK2* (CAMPBELL 1961) and W3805 which is *sup*^{0}, *galE22* (ADLER and TEMPLETON 1963). We also used a series of lysogen derivatives of 594 which are not shown in Table 1. Their prophages carried, in addition to the *cI857* and *Sam7* mutations, amber mutations in two head genes. The head gene mutants combinations used were *A11E4, B1E4, C42E815, Nu3a8E815, D15E815, E815FI471, A11B10, W403B10, B1C42, B10Nu3a8, B1D15* and *B1FII423*. With the exception of *D15E815* and *E815FI471*, all these strains have been described in previous publications (BECKER, MURIALDO and GOLD 1977; MURIALDO and SIMINOVITCH 1972; MURIALDO and BECKER 1978a). λ*Dam15Eam815cI857Sam7* was constructed as follows: λ*Dam15cI857Sam7* (MURIALDO and SIMINOVITCH 1972) was crossed with λ*Uts08cI857* (MURIALDO *et al.* 1981) to obtain λ*Dam15Uts08cI857*. This latter phage was crossed with λ*Ats014Eam815cI857* (MURIALDO *et al.* 1981) to prepare λ*Dam15Eam815cI857*. Finally λ*Dam15Eam815cI857* was crossed with λ*Cts03cI857Sam7* (MCCLURE and GOLD 1973) to obtain λ*Dam15Eam815cI857Sam7*. The construction of λ*Eam815FIam471cI857Sam7* involved the following steps. First, λ*Eam815cI857Sam7* (MURIALDO and SIMINOVITCH 1972) was crossed with λ*Jts021cI857* (FUERST and BINGHAM 1978) to generate λ*Eam815Jts021cI857*. The latter phage was crossed with λ*Ets09FIam471cIt1* (MURIALDO *et al.* 1981) to obtain λ*Eam815FIam471cIt1*, which was in turn crossed with λ*Cts03cI857Sam7* (MCCLURE and GOLD 1973) to produce λ*Eam815FIam471cI857Sam7*. The methods used for the phage crosses and for the selection or screening of recombinants followed the principles previously described (MURIALDO *et al.* 1981).

The plasmid pFM140 was constructed by cutting λDNA

TABLE 1

Terminase and prohead donor ability of bacterial strains

Strain	Ability to provide: Terminase: Prophage	Terminase: Plasmid	Prohead: Prophage	Prohead: Plasmid
1. OR1265 [pFM3]		+		−
2. 594 (λ*cI857 Sam7*)	+		+	
3. 594 (λ*Aam32 cI857 Sam7*)	−		+	
4. 594 (λ*Eam4 cI857 Sam7*)	+		−	
5. 594 (λ*Bam10 cI857 Sam7*)	+		−	
6. 594 (λ*cI857 Sam7*) [pFM3]	+	+	+	−
7. 594 (λ*Aam32 cI857 Sam7*) [pFM3]	−	+	+	−
8. 594 (λ*Eam4 cI857 Sam7*) [pFM3]	+	+	−	−
9. 594 (λ*Aam11 Bam10 cI857 Sam7*)	−		−	
10. 594 (λ*Aam11 Bam10 cI857 Sam7*) [pFM3]	−	+	−	−
11. 594 (λ*Aam11 Bam10 cI857 Sam7*) [pFM4]	−	+	−	−
12. 594 (λ*Aam32 cI857 Sam7*) [pFM4]	−	+	+	−
13. W3805 (λdg*al580 cI857*)	+		+	
14. W3350 (λdg*al101 cI857 Sam7*)	+		−	
15. W3805 (λdg*al616 cI857*)	+		−	
16. W3350 (λdg*al102 cI857 Sam7*)	+		−	
17. W3350 (λdg*al805 cI857 Sam7*)	+		−	

with *Cla*I. The DNA was then mixed with *Cla*I-cut pBR322, heated at 70°, cooled down slowly over a period of 3 hr and ligated using T4 DNA ligase. After transformation into OR1265, a transformant carrying a plasmid containing the *cos*-spanning *Cla*I fragment was identified by marker rescue using λ*Aam1 cI857* and λ*Bam1 cI857* as test lysates. The structure of the plasmid was confirmed by digestion with a series of restriction enzymes. The portion of λDNA inserted in pBR322 is shown in Figure 4.

General procedures: All the procedures used have already been described (MURIALDO and FIFE 1984). The experimental protocol to detect DNA maturation was as follows. Cells grown in LB medium to a concentration of 2 × 10^8 at 31° were thermoinduced by transferring the flasks to a 44° shaker water bath and then shaken for 15 min. At this time they were transferred to a 39° shaker water bath, and after a period of incubation, 10 ml of culture was pipetted into a centrifuge tube containing 5 ml of frozen U1 buffer (50 mM NaCl, 0.1 mM Na_2 EDTA, 40 mM Tris-HCl, pH 7.6). Immediately after the ice thawed, the suspensions were centrifuged at 6000 rpm at 2° for 10 min. The pellet was resuspended in 3 ml of ice-cold U1 buffer and centrifuged again in the same way. The pellet was then resuspended in 0.5 ml of ice-cold U1 buffer, and the nucleic acids were extracted with 0.5 ml of phenol:$CHCl_3$ (1:1). This was followed by three further 0.5-ml phenol extractions. The aqueous phase was treated with diethyl ether to remove residual phenol; the solutions brought to 0.3 M NaCl; and the DNA (and RNA) precipitated with 95% ethanol, washed three times with 75% ethanol, vacuum-dried and then dissolved in 50 μl of U2 buffer (10 mM NaCl, 0.1 mM Na_2 EDTA, 8 mM Tris-HCl, pH 7.6). One microliter of these preparations was diluted in restriction buffer, digested with the appropriate restriction enzymes and then electrophoresed in 0.5% agarose gels.

For DNA transfer, the method of SOUTHERN (1975) was used as described by MANIATIS, FRITSCH and SAMBROOK (1982), with minor modifications. The probes were λ-DNA and pFM140. They were labeled by nick-translation according to the procedure described by MANIATIS, FRITSCH and SAMBROOK (1982), or by DNA polymerase (Klenow fragment) catalyzed extension of random primed DNA (FEINBERG and VOGELSTEIN 1983). Both the prehybridization and hybridization solutions contained 50 μg/ml of *E. coli* DNA (Sigma type VIII).

RESULTS

One of the hypotheses put forward to explain the prohead-dependence of λ*cos* cutting and the prohead-independence of plasmid pFM3 *cos* cutting was that the product of a λ gene inhibited *cos* cutting in the absence of proheads. Since this gene is absent in pFM3, it would have to map to the right of *B* (Figure 1) (MURIALDO and FIFE 1984). If the product of this putative gene(s) were able to act in *trans*, it should also be able to block plasmid DNA maturation. To check this model, we constructed a series of lysogens carrying pFM3. To detect DNA maturation, the prophage and plasmid were induced, and after an incubation period of 30 min. at 39°, the DNA was extracted and digested with the restriction enzymes *Apa*I and *Eco*RI. These enzymes cleave the DNAs as shown in Figure 1. Immature λ-DNA is characterized by the presence of a large fragment (labeled FA) of 13,616 bp spanning *cos*. This fragment generates, upon maturation, fragments of 3530 and 10,086 bp called F and A, respectively. Immature pFM3 produces a 11,570-bp fragment labeled L. Upon *cos* cutting, pFM3 digested with the above restriction enzymes gives rise to fragments of about 3900 and 7660 bp called a and b, respectively.

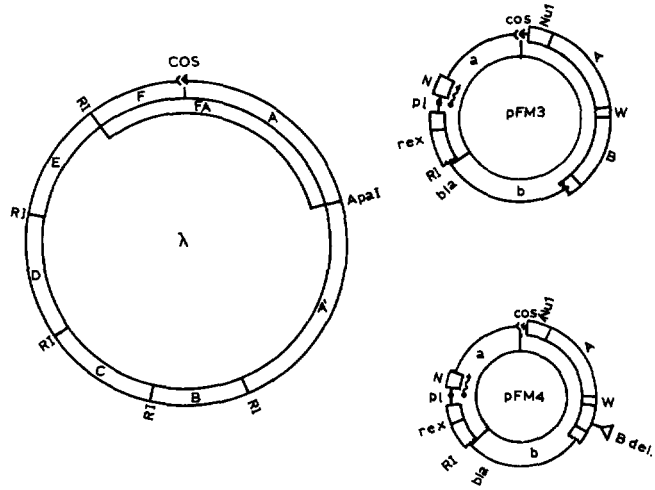

FIGURE 1.—Physical and genetic map of phage λ and plasmids pFM3 and pFM4. Restriction sites are shown only for *Apa*I and *Eco*RI. The restriction fragments are designated by upper-case letters for phage λ and by lower-case letters for the plasmids. The phage λ elements in the plasmids are indicated and are shown as boxes. The region between *N* and *cos* is from phage φ80 (MURIALDO and FIFE 1984), and its sequence is not known. pl, left promoter of λ; bla, β-lactamase gene of pBR322. The wavy arrows indicate the origin and direction of transcription of the fusion transcript encompassing *N* and the morphogenetic genes which are synthesized following derepression. The arrowhead indicates the left end and the arrowtail the right end of matured molecules. The plasmids are drawn at a scale one-half that of λ. The size of the elements are λ, 48,502 bp; pFM3 about 11,570 bp; and pFM4 about 10,636 bp. pFM4 has a 933-bp deletion in gene *B*, denoted *Bdel*.

The results of the experiments are shown in Figure 2. It can be observed that induction of cells carrying the plasmid alone ([pFM3]) resulted in the formation of three bands, labeled L, b and a. b and a are DNA maturation products, and we have previously shown that the molecules in the L band result from cleavage of one of the two *cos* present in dimer plasmids (MURIALDO and FIFE 1984). Induction of a prophage lacking any morphogenetic mutation (WT) resulted in extensive DNA maturation, as detected by the presence of large amounts of bands A and F. As expected, prophage maturation was blocked by a mutation in the terminase *A* gene (A^-) and by a mutation in *E* (E^-), the prohead major coat gene. Maturation of prophage and plasmid DNA proceeded normally when both shared the same cell (WT[pFM3]). Complementation took place when a terminase-deficient prophage DNA was matured by the terminase provided by the plasmid (A^-[pFM3]). Finally, if the prophage is deficient in prohead synthesis, not only did the prophage DNA fail to mature but plasmid DNA

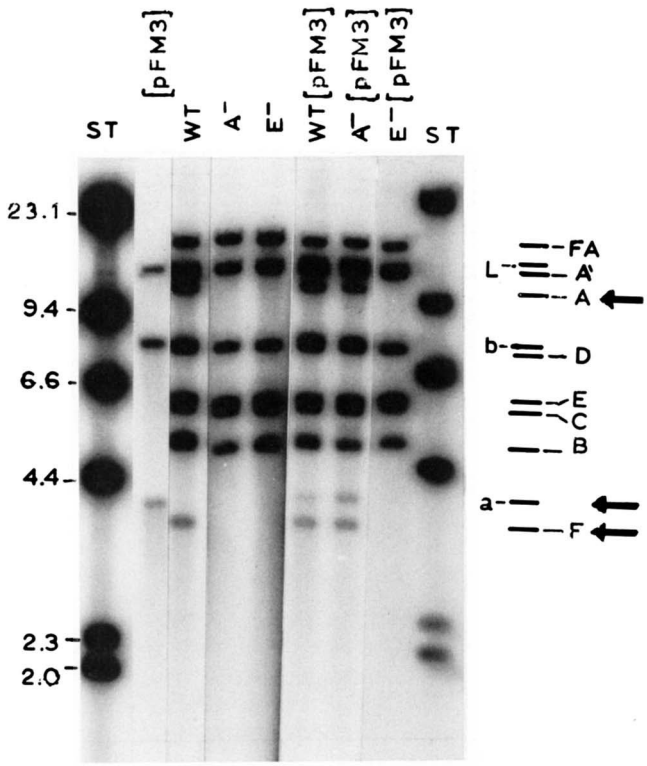

FIGURE 2.—A λE^- mutant inhibits plasmid DNA maturation. The cells carrying various prophages and/or plasmids were thermoinduced and incubated for 30 min at 39° to allow DNA replication and phage development. The DNA was extracted, digested with *Apa*I and *Eco*RI, electrophoresed in agarose gels, and transferred to nitrocellulose sheets. λ and plasmid DNA were visualized by probing with radioactive λ-DNA followed by autoradiography. ST, λ-DNA digested with *Hin*dIII. The presence of a plasmid is indicated within a square bracket. The presence of a prophage is specified as WT when no morphogenetic mutation is present, or by its morphogenetic mutation, *i.e.*, A^-. All of the prophages carry, in addition, the *cI857* mutation, which renders them thermoinducible (SUSSMAN and JACOB 1962) and the *Sam7* mutation which blocks cell lysis (GOLDBERG and HOWE 1969). The size, in kilobase pairs, of the marker bands are indicated. A scheme depicting all the possible bands expected, appropriately labeled (refer to Figure 1), is shown at the right. The thick arrows at the right indicate the maturation product fragments of λ, A and F; and of pFM3 (a). The other maturation fragment of pFM3 (b) is obscured by fragment D of λ.

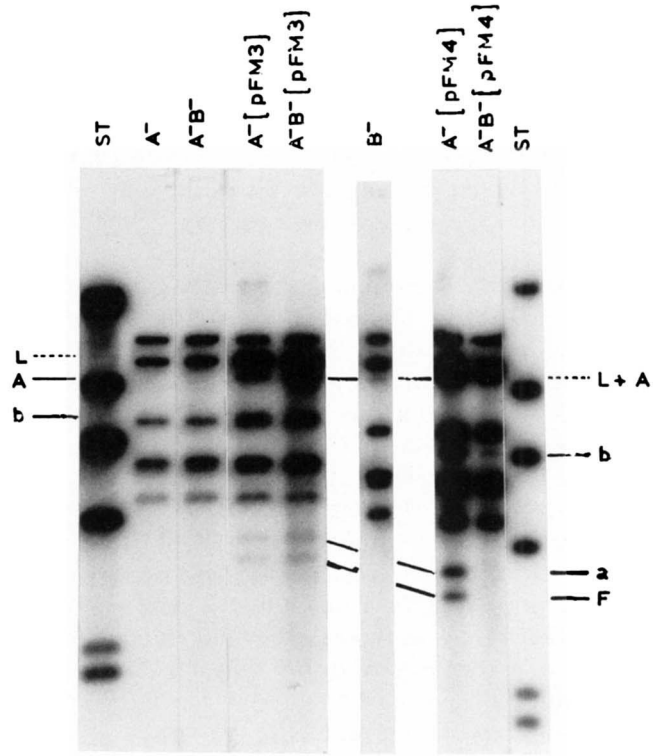

FIGURE 3.—Prohead complementation between phage and plasmid. See legend to Figure 2 for details and nomenclature. Linearized pFM4 is approximately the same size as λ fragment A, and they cannot be distinguished. L and b bands on the left margin refer to pFM3 only, whereas L and b on the right margin refer to pFM4 only.

maturation was also inhibited essentially 100% (E^-[pFM3]). This result shows that a prohead-deficient phage provides a function that blocks DNA maturation in *trans*. We call this function MIF, for maturation inhibition factor.

To check if there were something unusual about the particular isolate of the E^- lysogen carrying pFM3, the following experiment was done. Lysogens with mutations in the terminase gene *A* and the prohead gene *B* were transformed by pFM3 and pFM4. The first plasmid carries intact genes *A* and *B* and therefore should be able to complement the prophage deficiency leading to maturation of both phage and plasmid DNAs. pFM4, on the other hand, has a deletion in gene *B* (Figure 1) and therefore should fail to complement the prophage in prohead production. Furthermore, plasmid DNA maturation should be inhibited by the prophage-directed substance. Due to the deletion in *B*, *Eco*RI-linearized pFM4 is about 10,636 bp. The maturation product fragment a is the same size as in pFM3, about 3900 bp, and the b fragment is 6727 bp long (see Figure 1). The results of this experiment are shown in Figure 3.

It can be observed that the expectations were met. pFM3 complemented the A^-B^- lysogen allowing substantial DNA maturation. In lysogens carrying pFM4, however, cutting was very inefficient. In fact, only a small amount of the larger b fragment was observable in the autoradiogram, and no fragment a was detected. Therefore, although the block was not 100% efficient, plasmid DNA maturation was strongly inhibited. The incomplete block to DNA maturation is a property of *B* mutants, as can be observed by the formation of low levels of band A and undetectable levels of band F in induced B^- lysogens. In conclusion, this experiment shows that a block in prohead synthesis is specifically responsible for the inhibition, in *trans*, of DNA maturation.

To determine if any of the identified genes of the head genetic region of the λ chromosome were MIF, we attempted to find what mutation in combination with an *E* or a *B* mutation allowed phage DNA mat-

uration. However, none of the mutant combinations (the lysogens used for these experiments are listed in the MATERIALS AND METHODS section) allowed DNA maturation (results not shown). Therefore, we resorted to localizing the genetic determinant of MIF by deletion mapping using a series of defective lysogens containing *gal* transducing prophages. The deletions in the prophages extend from *att* to various points inside the head genetic region (Figure 4). In this experiment the incubation period at 39° after thermoinduction was only 20 min to prevent lysis of the cells with S^+ prophages, and the DNA was digested with *Bst*XI. As can be seen in Figure 4, a phage containing no deletion (WT) and a phage with all the tail genes and *FII* deleted, but with intact terminase and prohead genes (d*g580*), matured the DNA with the production of the two *Bst*XI restriction fragments labeled L-end and R-end. Phages in which the deletion extended into *E* (d*g101*) and into *C/Nu3* failed to mature their DNA, as it was expected. Longer deletions, such as the ones in d*g102* and d*g805* allowed DNA maturation as can be deduced by the appearance of the R-end restriction fragment. The reason for the failure to detect the L-end restriction fragment in these phages is not known (but see DISCUSSION). Despite this shortcoming, it can be concluded that the genetic determinant of MIF maps between the end points of the deletions in λd*g102* and λd*g616*; that is, a region comprising a portion of *B* and most of *C/Nu3*.

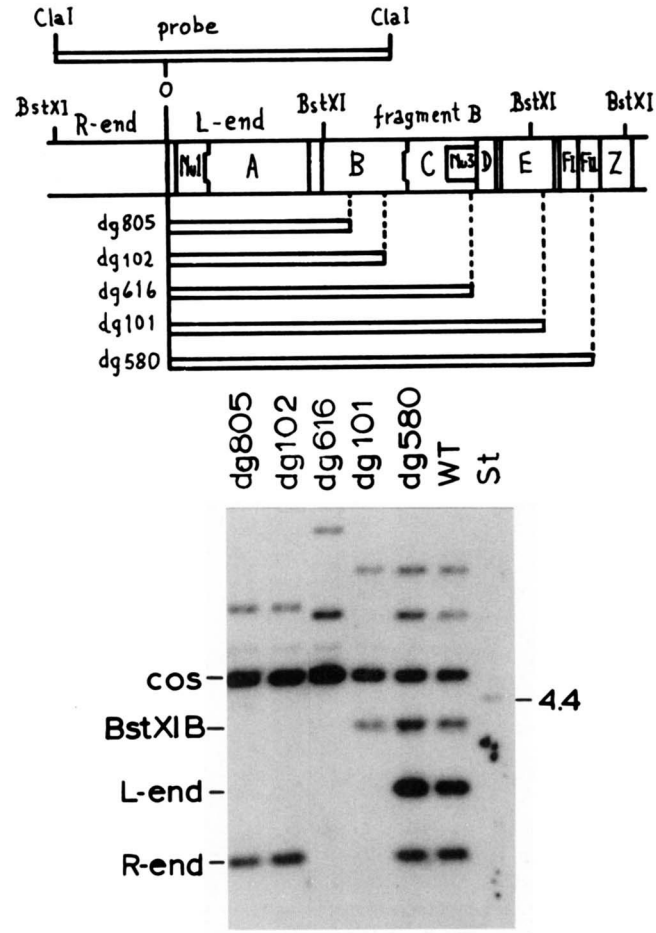

FIGURE 4.—DNA maturation in *gal*-transducing defective phages. *Top:* Genetic map of a region of λ surrounding *cos*. The *Cla*I fragment of λ-DNA inserted into pBR322 to generate pFM140 is shown as a bar and is labeled probe. A portion of the λ chromosome is represented from about 2100 bp to the left of *cos* to about 8700 bp to the right of *cos*. This section includes all the known head genes (*Nu1* to *FII*). The space between *A* and *B* corresponds to gene *W*. Spaces between other genes corresponds to short intergenic regions. Gene *Nu3* is encoded in the same translational reading frame within gene *C*. The position of *Bst*XI sites are indicated on top of the lines representing the λ chromosome. The extent of λ-DNA not deleted in the transducing phages listed is shown underneath as bars, the right ends of which are joined with dashed lines to the λ chromosome to indicate the beginning of the deletion. *Bottom:* The cells carrying various prophages were thermoinduced and incubated for 20 min at 39° to allow DNA replication and phage development. The DNA was extracted, digested with *Bst*XI, electrophoresed in an agarose gel, transferred to nitrocellulose sheets, and fragments were visualized by probing with radioactive pFM140, followed by autoradiography. St, λ-DNA digested with *Hin*dIII. WT, no morphogenetic mutation (λ*cI857 Sam7*). The precise genotype of the prophages in the other strains is described in Table 1. COS, *Bst*XI fragment spanning *cos*. BstXIB, *Bst*XI fragment of λ-DNA adjacent to the left end fragment. L-end and R-end, *Bst*XI fragments of λ-DNA containing the left end and right end of the mature molecule, respectively. Note that the probe is able to hybridize to end fragments only. Therefore, only the 23-kbp left end and the 4.4-kbp right end of *Hin*dIII-cut λ-DNA should appear in the autoradiogram. However, the 23-kbp band is not shown to save space. The bands above the COS band correspond (precisely) to incomplete digestion products in the case of d*g101*, d*g580* and WT. Presumably, the bands above the COS band for the other phages also correspond to incomplete restriction enzyme digestion, or to hybrid λ-*E. coli* DNA-containing fragments.

DISCUSSION

The experiments presented in this paper confirm the observation that mutations in prohead genes such as *B* and *E* block DNA maturation in phage λ (MACKINLEY and KAISER 1969; WAKE, KAISER and INMAN 1972; MCCLURE, MACHATTIE and GOLD 1973; LASKI and JACKSON 1982). Our results show that DNA restriction fragments corresponding to the ends of mature DNA are not detected in B^- and E^- (and A^-) mutants.

We have already demonstrated that plasmids containing *cos* and the terminase genes under control of a thermosensitive repressor can, upon induction, mature their DNA very efficiently in the absence of proheads (and gp*D* and gp*FI*) (MURIALDO and FIFE 1984). We have shown here that, in the absence of proheads (by maturation), a *trans*-acting inhibitor (MIF) coded for, or controlled by, phage λ blocks both phage DNA and plasmid maturation. The nature of MIF and its mechanism of action are unknown.

Since plasmids able to mature their DNA contain genes *Nu1* to *B* only, MIF must map to the right of *B* (in the λ genetic map). In an attempt to map MIF, we used a series of λ d*gal*s in which the deletions encompassed the genetic material from *att* to various points within the head morphogenetic region. It was found

that phages in which the deletion ended within *E* or *C* did not mature their DNA, as expected. If the deletion extended further to the left, into *B*, cutting was observed. These results fully agree with those obtained with the plasmids and position the MIF genetic determinant (or one of them) in the region between the middle of *B* and the end of the *C* and *Nu3* overlapping genes. However, MIF does not seem to be gp*B*, gp*C* or gp*Nu3*, as deduced from the observation that the double λ mutants B^-E^-, C^-E^-, $Nu3^-E^-$ and B^-C^- and B^-Nu3^- failed to mature phage DNA. It is possible that MIF is coded for in a different reading frame to genes *B* and *C* and *Nu3*. An analysis of the λ-DNA sequence (SANGER *et al.* 1982) in the region where MIF maps showed the presence of several open reading frames that could potentially code for small proteins.

Although the results obtained with the λdg*al* series agreed with those obtained with the plasmids, the absence of the left end in the autoradiograms is disturbing. Two possible explanations for this result are, first, that terminase remains bound to the left end fragment blocking its migration in gel electrophoresis or, second, that the left end fragment is preferentially and efficiently degraded. We cannot distinguish between these alternatives at the moment, but it is pertinent to note, however, that both left and right end fragments are detected during plasmid maturation. Therefore, it appears that the activity of cell nucleases is minimal and that, if the left end fragment of λdg*al*s that mature DNA is indeed degraded, the enzyme(s) responsible must be induced by phage development. It may be possible to verify that the DNA has been cut at *cos* by isolating the right end fragment and determining its sequence at the terminus.

In order to explain the prohead dependence of DNA maturation, several models have been suggested. One model (the religation model) stated that *cos* cutting occurs, but that, in the absence of proheads, the sticky ends are immediately ligated by *E. coli* DNA ligase (WANG and KAISER 1973). A second model (the activation model) stated that terminase binds to *cos* but does not cut until proheads attach to the complex (KAISER and MASUDA 1973). Finally, a third and more popular model (the first-packaging-then-cutting model) suggested that *cos* cutting at the left and right ends of the chromosome takes place after packaging is completed, in a mechanism where prohead-bound terminase is actuated by head fullness (HOHN *et al.* 1974; HOHN 1975; KAISER, SYVANEN and MASUDA 1975; SYVANEN 1975; STERNBERG and WEISBERG 1977).

We have shown that the products of terminase action in the absence of proheads can be detected in normal *E. coli* cells (*lig*$^+$). Hence, the religation model as an explanation for the absence of mature DNA in prohead-deficient infections becomes extremely unlikely. Likewise, the fact that *cos* cutting can take place in the absence of proheads discards the activation model. Finally, the fact that *cos* cutting can be uncoupled from DNA packaging contradicts the first-packaging-then-cutting model.

We would like to present the following model. Although terminase is able to bind to *cos* in the absence of proheads (complex I) (HOHN 1975; BECKER, MARKO and GOLD 1977), its catalytic action is impaired by MIF. A prohead, binding to complex I would displace or inactivate MIF, allowing the proper terminase-*cos* configuration for cutting to occur. The putative existence of an inhibitor of terminase had been proposed to explain the absence of prohead dependence for *cos* cutting in an *in vitro* system (MURIALDO and BECKER 1978b; FEISS and BECKER 1983).

Whereas the "activation" model assumed that the prohead activates *cos*-bound terminase, our data suggests that the prohead enables terminase to function by displacing or inactivating the inhibitor. The two mechanisms can be distinguished, because a mutation in the gene coding for the inhibitor should allow DNA maturation in the absence of proheads. Experiments are in progress to try to localize the gene coding for the inhibitor.

The ability of terminase preparations to mature *cos*-containing DNA *in vitro* in the absence of proheads (WANG and KAISER 1973) suggests that MIF is dilution-sensitive or unstable *in vitro*. It is likely that MIF plays a role in modulating λ-DNA terminase in a wild-type situation, assuring that the DNA is not cut until a prohead (and other factors) is properly positioned to start the packaging process.

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