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Inhibition of Initiation of Bacteriophage T4 DNA Replication by Perturbation of *Escherichia coli* Host Membrane Composition

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3-Decynoyl-*N*-acetylcysteamine (3-decynoyl-NAC) is an analog which specifically causes the immediate cessation of the biosynthesis of unsaturated fatty acids in *Escherichia coli*, whereas the synthesis of saturated fatty acids is actually stimulated. As a result, the cell membrane accumulates saturated fatty acids in its phospholipid. Addition of the inhibitor at the time of infection of *E. coli* by T4 phage had no effect on normal phage replication and development, implying that the synthesis of unsaturated fatty acids per se has little effect on T4 DNA replication. However, if the integrity and composition of the bacterial membrane was grossly perturbed by first treating the cells with the inhibitor for 60 min before infection, the proper initiation and the attainment of a rapid rate of T4 DNA synthesis were not observed. Under these conditions, a full complement of T4 early proteins was synthesized. The membrane associability of the known DNA delay proteins induced by wild-type T4 phage in the treated cells resembled that expected of a culture of untreated cells infected with a DNA delay mutant. When any one of three DNA delay mutants was used to infect 3-decynoyl-NAC-treated cells, T4 DNA replication was aborted. These findings suggest that some kind of specific interactions among the initiation proteins defined by the DNA delay mutants and the bacterial membrane may be necessary to facilitate the normal initiation and rapid rate of T4 DNA replication. A model for the involvement of the three different initiation proteins and the subsequent attainment of rapid DNA synthesis is discussed.

The bacterial membrane has been implicated in T4 DNA replication based on the observation that parental and newly synthesized DNA were found associated with the membrane or a large particulate fraction of the cell lysate (18). We have studied the requirement for membrane participation in a more direct approach. Since phospholipid is a major component of the membrane, does perturbation of its biosynthesis affect the cell's ability to support T4 DNA replication? In the biosynthetic pathway of fatty acids in *Escherichia coli*, β-hydroxydecanoyl thioester dehydrase is an indispensable enzyme, responsible for the introduction of the double bond into the unsaturated fatty acids (12). The acetylenic analog of its substrate, 3-decynoyl-*N*-acetylcysteamine (3-decynoyl-NAC), is an inhibitor of the dehydrase in vitro and in vivo (11, 12). The addition of 3-decynoyl-NAC to an *E. coli* culture causes the immediate cessation of unsaturated fatty acid synthesis; however, other cellular functions appear to deteriorate at a much slower rate (11). The inhibition of the biosynthesis of unsaturated fatty acids by the analog is specific; the synthesis of saturated fatty acids is actually stimulated by the addition of the analog (12). As a result of treatment with the inhibitor, the cell membrane accumulates saturated fatty acids in its phospholipid. Results reported here show that the synthesis of fatty acids per se has no effect on T4 DNA replication. This observation is in agreement with an earlier report by Nunn and Cronan (16). However, the structural integrity of the bacterial membrane, specifically with regard to the appropriate composition of the saturated and unsaturated fatty acid moiety in the phospholipid, appears to be necessary for the proper initiation and rapid rate of T4 DNA synthesis. We have previously shown that the DNA initiation proteins of T4, defined by the DNA delay genes 39 and 52, are membrane associated and T4 DNA binding (7, 9). These findings, together with the observations reported in this communication, further suggest that the DNA initiation proteins, T4 DNA, and the bacterial membrane must interact in some yet un-

defined manner to facilitate initiation and rapid rate of T4 DNA replication.

MATERIALS AND METHODS

Bacterial and phage strains. *E. coli* B was used as nonpermissive host for amber mutants of T4D. *E. coli* B-40 (SuI) or CR63 (SuI) served as the permisssive host for phage propagation. All amber mutants of T4D were from the Caltech collection. For propagating phage, the media described by Edgar and Lielausis were used (4). Minimal medium, which was used for all experiments, contained the following: Na_2HPO_4, 0.02 M; KH_2PO_4, 0.02 M; NH_4Cl, 0.019 M; NaCl, 0.017 M; Trizma base, 0.05 M; $FeCl_3$, 3×10^{-6} M; $MgCl_2$, 0.003 M; $CaCl_2$, 0.1 mM; Na_2SO_4, 2 mM; Casamino Acids, 0.5%; and glucose, 0.5%. The pH of the medium was adjusted to pH 7.4 with HCl. For labeling proteins with radioactive ^{35}S, Casamino Acids was deleted from the medium. All cultures were grown at 37°C unless otherwise indicated.

Chemicals. Crystalline 3-decynoyl-NAC was a generous gift of K. G. Lark and was synthesized according to the published procedure (2, 5). It was dissolved in absolute ethanol and stored at −20°C at a concentration of 10^{-2} M. [*methyl*-^{3}H]thymidine and carrier-free ^{35}S (H_2SO_4) were purchased from New England Nuclear Corp. BrIJ 58 was obtained from Atlas Chemical Industries. Sodium dodecyl sulfate (SDS) was purchased from BDH Chemical, Ltd. Salt-free lysozyme and pancreatic DNase were obtained from Worthington Biochemicals Corp.

Measurement of cell number. *E. coli* cell density was determined two different ways. The culture was diluted in physiological saline (Abbott Laboratories) and the cell number was directly counted with a Coulter Counter. Alternatively, the growth of the culture was monitored by a Zeiss spectrophotometer at 595 nm, taking an absorbance of 0.3 as equivalent to 2×10^8 cells per ml.

Determination of the rate of T4 DNA synthesis. *E. coli* cells were grown in minimal medium to a cell density of 2×10^8 cells per ml. They were then infected with phage T4 at a multiplicity of infection of 8. At various times after infection, small samples (0.5 ml) were removed and mixed with 1 μCi of thymidine, and the incubation was continued for 2 min at 37°C. Then 1 ml of 10% trichloroacetic acid was added to stop further DNA synthesis. The amount of [^{3}H]thymidine incorporated was determined as acid-insoluble material collected on a GF/C filter (Whatman) and counted with a Beckman liquid scintillation system.

Preparation of ^{35}S-labeled lysate. A culture of *E. coli* B was grown in minimal medium, without Casamino Acids, to a cell density of 5×10^8 cells per ml. The cells were then collected by centrifugation, suspended in 1/20 the original volume, and infected with T4 phage at a multiplicity of 10 at 0°C. After adsorption for 15 min, the culture was diluted to the original volume with prewarmed (37°C) fresh medium to initiate phage development. ^{35}S as sulfate was added at the time of infection. At the end of the labeling period, the infected culture was quickly chilled and collected by centrifugation.

Preparation of membrane fractions. The membrane fraction was obtained as the pellet fraction of a gently disrupted BrIJ lysate of the phage-infected culture. Centrifugation was at 6,000 rpm for 15 min. The culture was usually harvested at 8 min postinfection at 37°C. During that time, all T4 early proteins have been induced and late protein synthesis has not yet begun (17). The detailed procedure for preparing the BrIJ lysate has been described (9). Briefly, the procedure involves treating a concentrated infected culture with lysozyme and EDTA, followed by addition of the neutral detergent BrIJ 58 to complete the lysis. Such a preparation consists mainly of disrupted membrane vesicles characterized by the bilayer structure as revealed by electron microscopy. This fraction is specifically enriched for a subset of phage proteins (7).

Gel electrophoresis. Analysis of protein content by electrophoresis on slabs of 10% polyacrylamide gel containing SDS has been described (17). All samples were heated in boiling water for 1 to 2 min before being applied to the slab gel. At the end of the electrophoresis (about 4 h), the gel was dried and subjected to autoradiography. To facilitate accurate comparison, samples from each figure shown were analyzed in the same slab. The assignment of protein bands in SDS-polyacrylamide gels as products of specific T4 phage genes has also been reported (17).

RESULTS

Effect of 3-decynoyl-NAC on *E. coli* B. The analog 3-decynoyl-NAC has been shown to inhibit bacterial cell growth by causing rapid and specific inhibition of the biosynthesis of the unsaturated fatty acids, whereas the synthesis of saturated fatty acids is actually stimulated (12). The inhibition of unsaturated fatty acid synthesis and cell growth can both be reversed by addition of the unsaturated fatty acid oleic acid. These effects have been shown in *E. coli* derivatives K-12 (11) and 15T$^-$ (5). Similarly, the analog can also be shown to effectively inhibit the growth of *E. coli* B, which is a natural host for phage T4 (Fig. 1). When 3-decynoyl-NAC was added to an exponentially growing culture at a concentration of the 10^{-4} M at 37°C, the culture of *E. coli* B continued to grow for about one doubling, and then growth ceased as determined by cell count with a Coulter Counter. This pattern of growth inhibition is characteristic of 3-decynoyl-NAC and was observed with other *E. coli* strains. The cessation of growth could also be restored by addition of oleic acid (data not shown). Therefore, a 2×10^{-4} M concentration of the analog was chosen for subsequent studies.

Effect of 3-decynoyl-NAC on T4 DNA replication. The addition of 3-decynoyl-NAC to an *E. coli* B culture at the time of infection has little effect on T4 DNA replication as determined by [^{3}H]thymidine incorporation and has no significant effect on the eventual phage yield.

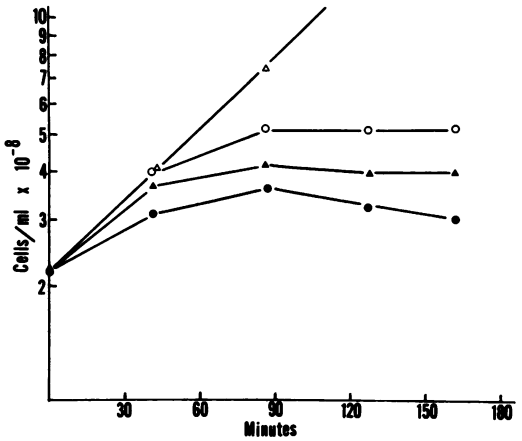

FIG. 1. *Effect of 3-decynoyl-NAC on the growth of E. coli B at 37°C. The culture was grown in minimal medium, and the analog was added at the indicated concentration at zero time. Cell density was monitored by direct counting of the cell numbers with a Coulter Counter. The concentrations of 3-decynoyl-NAC used were* (△) *none,* (○) 10^{-4} *M,* (▲) 2×10^{-4} *M, and* (●) 3×10^{-4} *M.*

As shown by Kass et al. (12), 3-decynoyl-NAC is readily taken up by *E. coli* cells and causes immediate and complete cessation of unsaturated fatty acid synthesis. Our results, therefore, suggest that the active synthesis of the unsaturated fatty acid moiety of the phospholipid of the bacterial membrane is not required for T4 phage development. However, it has been suggested that T4 DNA replication occurs on the bacterial membrane (18). Therefore, it is important to investigate whether the integrity of the bacterial membrane specifically with regard to the ratio of saturated to unsaturated fatty acids is necessary for T4 DNA replication. Figure 2 shows the rate of T4 DNA synthesis in a culture of *E. coli* B pretreated with the inhibitor for various lengths of time before infection with wild-type T4 phage. When the culture was pretreated for 60 min or longer, the profile of T4 DNA synthesis showed a delay in the onset of DNA replication and a reduced rate of DNA synthesis at later times. This level of DNA synthesis corresponded to a phage yield of about 10% of the normal burst of T4 phage in untreated cells (Table 1). Since the inhibition of the growth of the host cell by 3-decynoyl-NAC at 37°C was not complete until about 1 h after the addition of the inhibitor at 2×10^{-4} M (Fig. 1), we chose to pretreat *E. coli* B cells for 75 min for Table 1 in order to allow significant accumulation of saturated fatty acids in the phospholipid at the expense of the unsaturated fatty acids. Under these conditions, the rates of *E. coli* DNA and protein synthesis were about 30 and 50%, respectively, of the level of the untreated cells.

DNA replication in 3-decynoyl-NAC-treated cells infected with T4 mutants defective in T4 DNA initiation. The profile of

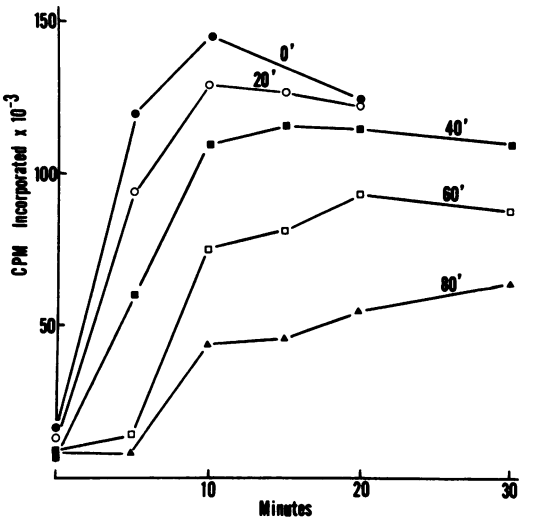

FIG. 2. *T4 DNA synthesis in E. coli cultures pretreated for various times with the inhibitor 3-decynoyl-NAC. E. coli B was grown to a cell density of 2* $\times 10^8$ *cells/ml in minimal medium, and 3-decynoyl-NAC was added at a concentration of* 2×10^{-4} *M. At various times after treatment with the inhibitor, a portion was removed and infected with wild-type phage T4 (multiplicity of infection = 10). Zero time denotes the time of phage infection. The rate of T4 DNA synthesis was determined as the amount of [³H]thymidine incorporation in the 2-min period beginning at the indicated time (see text for detail). The length of pretreatment (in minutes) of the E. coli cells with the inhibitor before T4 phage infection is indicated by the numbers listed next to each curve.*

TABLE 1. *Effect of 3-decynoyl-NAC on T4 phage development in E. coli B at 37°C*[a]

Phage	Relative phage yield	
	Untreated	Treated
T4D	100	8
39⁻ (*am*N116)	40	<0.1
52⁻ (*am*H17)	120	<0.1
58⁻ (*am*HL627)	93	<0.1
43⁻ (*am*E4314)	<0.1	

[a] *E. coli* B was grown to a cell density of 2×10^8 cells/ml in minimal medium. Treatment with the inhibitor was at 2×10^{-4} M for 75 min. The cells were then infected with T4 phages (multiplicity of infection = 10) for 90 min and lysed with chloroform; the phage yields were determined by using *E. coli* B-40 as the permissive host.

DNA replication in wild-type phage T4-infected cells which were pretreated with the inhibitor for 70 min showed the characteristics of a DNA delay mutant. Figure 3A shows the rate of DNA synthesis at various times after infection at 37°C of T4 DNA delay mutants in genes 39, 52, and 58. (T4 mutants defective in gene 60 were originally defined as another DNA delay mutant. However, it appears that these mutants are defective only when *E. coli* B is used as host and are not defective in *E. coli* K-12 strains with or without amber suppressors [19]; thus, they appear not to be nonsense mutants. Since it is not clear whether gene 60 defines a protein, we have not studied mutants in gene 60 in detail.) These mutants were defective mainly at the onset of DNA replication. After a delay of approximately 10 min, DNA synthesis resumed at a fast rate, accounting for the near-normal burst of phage at the end of the infectious cycle (Table 1). The phenotype of these mutants therefore suggests that the DNA delay proteins coded by these genes may function at the onset (or chromosomal initiation) of T4 DNA replication rather than at the actual synthetic process of DNA chain elongation. Recently, we have further shown that in phage T4, DNA replication is positively regulated by the DNA delay protein coded by gene 39 (8).

When mutants in DNA delay genes were used to infect 3-decynoyl-NAC-treated cells, after the expected delay in the onset of DNA synthesis, DNA replication was not resumed even at later times (Fig. 3B). The lack of significant DNA replication was also reflected by the low phage yield (Table 1). It is interesting to note that the level of late DNA synthesis in these cases was less than 1/50 of the normal DNA replication of wild-type T4; nonetheless, it was about 10 times higher than that of a DNA-negative mutant of T4, such as the mutant in T4 DNA polymerase (gene 43) used as a control in Fig. 3A.

T4 proteins synthesized in 3-decynoyl-NAC-treated cells. To determine whether the defect in T4 DNA replication in 3-decynoyl-NAC-treated cells was due to the failure to induce some essential phage replication proteins, infected cells were labeled with ^{35}S at various times afterwards, and the protein content was analyzed on SDS-polyacrylamide gels. Figure 4 shows the temporal pattern of T4 protein synthesis in *E. coli* cells pretreated with the inhib-

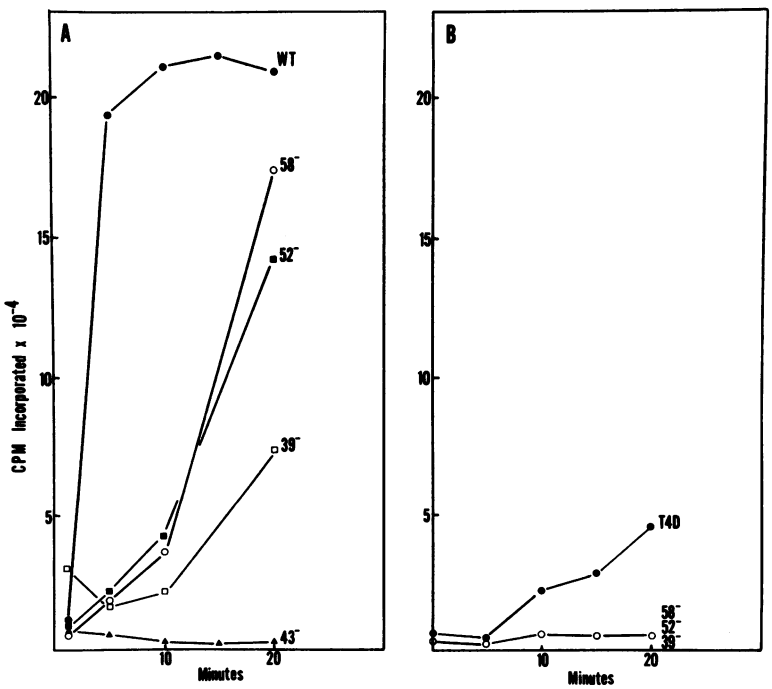

FIG. 3. *T4 DNA replication in cultures infected with T4 wild type or mutants defective in DNA delay genes. Cultures of E. coli B, untreated (A) or pretreated with 3-decynoyl-NAC for 75 min (B) were infected at 37°C with a multiplicity of infection of 10 at zero time. The mutants used were: amN116 (39⁻), amH17 (52⁻), amHL627 (58⁻), and amE4314 (43⁻). The rate of [³H]thymidine incorporation was determined as described in the text.*

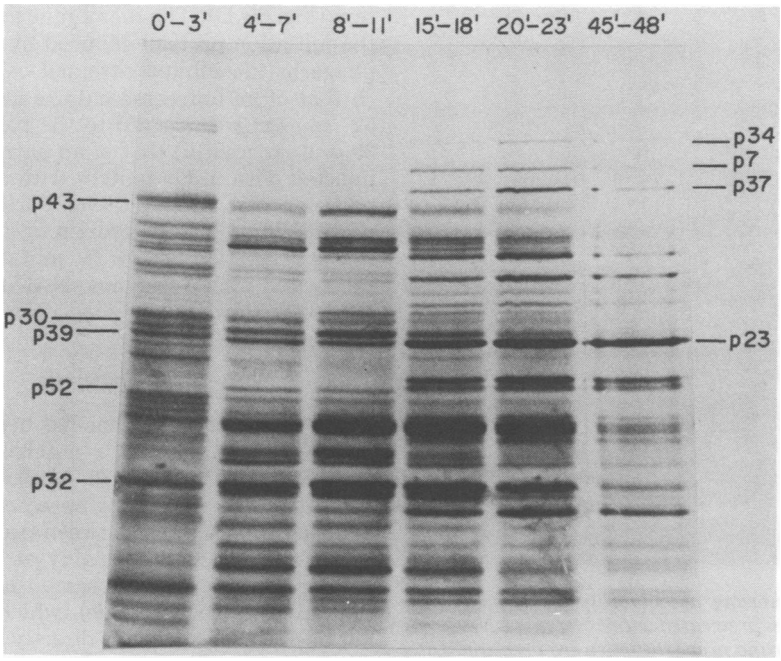

FIG. 4. *Time course of T4 protein synthesis in E. coli cells pretreated with 3-decynoyl-NAC. Cells were pretreated with the inhibitor for 75 min at 37°C and were infected with T4 phage (multiplicity of infection = 10). At various times afterwards, portions were removed and pulse-labeled with ^{35}S (H_2SO_4) for 3 min. The samples were analyzed on a 10% SDS-polyacrylamide slab gel (see text). Gene assignments for some of the protein bands are listed on both sides of the slab gel; for example, p43 denotes the band corresponding to gene 43.*

itor 3-decynoyl-NAC. A full complement of the essential T4 phage proteins was induced. Furthermore, the level of phage protein synthesis as determined by the ^{35}S incorporation was not grossly different from that of an untreated culture. It is important to note that the proteins that are required for the proper initiation of T4 DNA replication, as defined by the DNA delay genes 39 and 52, were present. Furthermore, they appeared to be synthesized in approximately the same amount, relative to the other early proteins, as in the untreated cells (see Fig. 6 for the protein patterns of T4 in untreated cells). Since we do not yet have a band assignment for the protein coded by gene 58, its fate in the inhibited cells remains unknown.

The time course of T4 protein synthesis in 3-decynoyl-NAC-treated cells also showed several other features. (i) The shutoff of host protein synthesis appeared to be somewhat slower than normal but adequate. Even at very early times after infection (0 to 3 min), the protein pattern of the infected culture consisted of mainly phage proteins and few *E. coli* host proteins. This implies that the treatment of *E. coli* B cells with the inhibitor 3-decynoyl-NAC did not significantly alter the susceptibility of *E. coli* cells to T4 phage infection in that the adsorption of the phage, the injection of phage DNA, and the induction of early phage protein synthesis all appeared to be normal. (ii) The synthesis of late proteins, however, did not occur until after 11 min postinfection. T4 late protein synthesis can easily be identified by the appearance of the protein of gene 23 (which is the major capsid protein) and the high-molecular-weight, tail fiber proteins which appear near the top of the gel. The delay in the onset of late protein synthesis was not surprising because viral DNA replication was also delayed in the inhibitor-treated cells (Fig. 2), and significant late protein synthesis in T4 is dependent on viral DNA replication (1).

Partition of DNA delay proteins in 3-decynoyl-NAC-treated cells. We have previously shown that the protein products of genes 39 and 52, which are required for normal T4 DNA initiation, are membrane associated (7). The membrane associability of T4 proteins synthesized in 3-decynoyl-NAC-treated cells was examined by analyzing the protein content of the membrane fraction, using SDS-polyacrylamide gels (Fig. 5). Our results show that a set of T4 proteins which we had previously reported

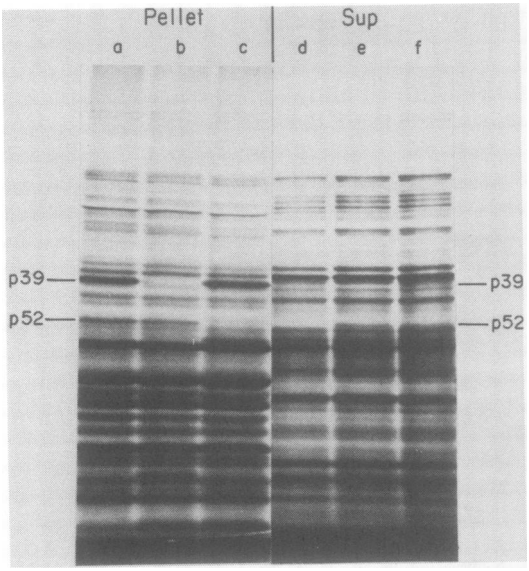

FIG. 5. *Membrane associability of T4 early proteins in cultures pretreated with 3-decynoyl-NAC. ^{35}S-labeled membrane preparations were prepared from T4 phage-infected cultures pretreated with the inhibitor at 2×10^{-4} M at 37°C for 75 min as described in the text. Both the membrane (pellet) and the soluble (cytoplasmic) fractions were analyzed on a 10% SDS-polyacrylamide slab gel. Columns a, b, and c represent the protein contents of the membrane fractions in cultures infected with T4D wild type, 39$^-$, and 52$^-$, respectively. Columns d, e, and f represent the protein contents of the soluble fractions of the 52$^-$, 39$^-$, and wild-type phage-infected cultures, respectively.*

to be enriched in the bacterial membrane (7) was also found in the membrane fraction of the treated cells (column a). The initiation proteins p39 and p52 were among them. Furthermore, it was noted that the 39 protein was partitioned exclusively in the membrane and was absent from the supernatant (cytoplasmic fraction), whereas the 52 protein was found in both fractions (columns a and f). The exclusive partition of the 39 protein in the (treated) membrane in the presence of a full complement of T4 early proteins (induced by wild-type T4) was not observed in cultures not pretreated with the inhibitor; however, it was observed in untreated culture if one of the DNA delay proteins was defective. In the absence of the 52 protein (as in an *am*52$^-$ mutant-infected culture not treated with inhibitor), all of the 39 protein was normally found partitioned into the membrane fraction (Fig. 6A,B). On the other hand, the absence of the 39 protein (as in an *am*39$^-$ mutant-infected culture) did not cause a change in the partitioning of the 52 protein; 52 protein was present in both the supernatant and the membrane fraction (Fig. 6C,D). The membrane associability of the initiation proteins induced by wild-type T4 phage in the inhibitor-treated cells resembled (i) that of an untreated culture infected with a 52$^-$ mutant with regard to the partition of the 39 protein, and (ii) that of an untreated culture infected with a 39$^-$ mutant with regard to the partition of the 52 protein. Furthermore, the partitioning of the 52 protein or the 39 protein in the respective 39$^-$ or 52$^-$ mutant in cultures pretreated with 3-decynoyl-NAC remained the same as in the corresponding, untreated cultures (Fig. 5C and 5D,B,E).

DISCUSSION

The bacterial membrane has been implicated in DNA replication in a number of systems, including *E. coli* and T4 (3, 6, 18). Evidence has been obtained suggesting specific (though undefined) interactions between bacterial membrane and DNA, presumably at the origin of DNA replication. At the same time, it is likely that an initiation protein(s) (which may or may not be one of the proteins directly involved with

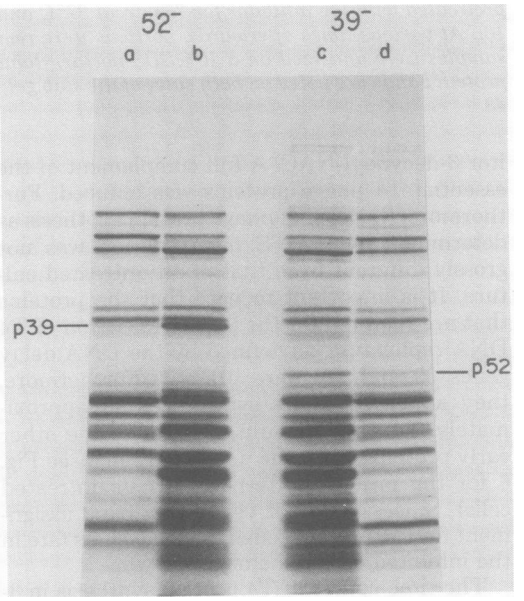

FIG. 6. *Membrane associability of T4 early proteins in cultures infected with 39$^-$ and 52$^-$ mutants. E. coli B cultures were infected with either amN116 (39$^-$) or amH17 (52$^-$) and labeled with ^{35}S for 8 min postinfection. Membrane preparations were prepared as described in the text, and both the membrane and the soluble fractions were analyzed on a 10% SDS-polyacrylamide slab gel. Columns a and d are the soluble fractions from 52$^-$- and 39$^-$-infected cultures; columns b and c represent the corresponding membrane fractions.*

the synthetic process of DNA chain elongation) will be needed to facilitate site-specific recognition of a DNA sequence at or near the origin as a means of regulating DNA replication (10). These components, namely bacterial membrane, DNA, and initiation protein(s), may have to interact with each other to facilitate initiation of the replicative process. To date, unequivocal biochemical evidence in support of this mechanism is lacking. In this communication, we have presented evidence suggesting that in the case of T4, such an interaction does occur although the detailed mechanism remains to be elucidated.

We have examined the requirement of membrane participation in T4 DNA replication by lowering the level of unsaturated fatty acids in the phospholipids. Since phospholipid is a major component of the membrane, does gross perturbation of the *E. coli* phospholipid composition affect its ability to support T4 DNA replication? Our results show that the integrity of the bacterial membrane, specifically with regard to the appropriate composition and possibly the distribution of the saturated and unsaturated fatty acid moiety in the phospholipids, is necessary for the proper initiaton and rapid rate of T4 DNA replication. If the inhibitor was added at the time of the phage infection, normal T4 DNA initiation and replication as well as subsequent phage yield were obtained, implying that active and continued unsaturated fatty acid synthesis per se is not necessary for T4 development. Similar conclusions were obtained by Nunn and Cronan, using mutants defective in *E. coli* membrane lipid biosynthesis (16). The inability of 3-decynoyl-NAC-treated cells to support normal initiation of T4 DNA synthesis occurred only after a long treatment (70 min) with the inhibitor. At the end of this period, significant amounts of saturated fatty acid had accumulated. The ratio of saturated to unsaturated fatty acid in the membrane in the treated cells increased to 11.6 from the level of 0.6 found in normal, untreated cultures of *E. coli* (11). Therefore, it appears that it is the integrity of the membrane's composition, as reflected by the distribution of the saturated and unsaturated fatty acids, which is required for successful T4 phage infection. The physiological effect of the accumulation of excess saturated fatty acid, and possibly alteration of other cellular processes due to the addition of the inhibitor, is not clear at present. Nonetheless, the antibacterial effect of the inhibitor is readily reversible by the simple addition of oleate to the culture, implying that few major cellular processes are permanently inactivated by the inhibitor. Furthermore, we have shown that the cumulative effect of the inhibitor does not appear to cause significant changes in the synthesis of T4 early proteins; therefore, it did not affect T4 early development such as penetration and the injection of viral DNA and the shutoff of host protein synthesis.

The profile of DNA synthesis in wild-type T4-infected, 3-decynoyl-NAC-treated cells showed a delay in the onset of DNA synthesis characteristic of a DNA delay mutant in untreated cells. Since the DNA delay proteins were synthesized in the inhibitor-treated cells, failure to induce proper initiation of T4 DNA synthesis may indicate unsuccessful interaction of the DNA initiation proteins with their intended target. This notion is further supported by the observation that the DNA initiation proteins (p39 and p52) synthesized in the inhibitor-treated cells in a wild-type T4 phage infection had a distribution in the soluble and the membrane fractions similar to that found in mutants in DNA delay genes. We have previously shown that the DNA initiation proteins bind T4 DNA tightly and are membrane associated by a number of criteria (7). With the observations reported in this communication, it may be proposed that the DNA delay proteins interact with the bacterial membrane, with DNA (presumably at the origin), and with each other to function as initiators of T4 DNA replication. Proper initiation at one specific DNA origin is probably not an absolute requirement for successful T4 DNA replication. The physical origin(s) of T4 DNA replication has not yet been unequivocally identified. Genetic evidence suggests that multiple origins may be involved in T4 DNA replication (13), implying that a number of DNA sites may be used as replicative origins.

The following model may be proposed to explain the initiation process of T4 DNA replication, which incorporates most of the observations pertaining to the early development of T4 phage. The initiation process (which may include the synthesis of a short stretch of DNA near the origin) might be a slower reaction than the subsequent reaction of fork movement and chain elongation catalyzed by the replication protein complex containing all the proteins of the DNA-negative genes (15). In a normal T4 phage infection, the initiation proteins presumably interact with the host membrane at the DNA origin(s), causing initiation at the correct or preferred site(s) on the parental T4 DNA molecule and, subsequently, rapid chain growth. Since not all the initiation proteins are absolutely required to start DNA synthesis (as in the case involving a single DNA delay mutant, where significant DNA synthesis does occur),

the absence of one of the initiation proteins does not abolish DNA initiation. The remaining proteins in the infected cell can apparently supply the initiation function. In this case, fewer or different origins may be used to start DNA synthesis. McCarthy et al., using emulsion autoradiography, have indeed shown that in a gene 52^- mutant-infected culture, the number of growing points per unit of template DNA is much reduced when compared with a wild-type T4 phage-infected culture (14). These initiated pieces in the DNA delay mutant-infected cells might accumulate and eventually lead to long DNA molecules either by chain elongation or by DNA recombination. Once long chains of DNA molecules are made, rapid DNA synthesis may be achieved as shown by the rapid rate of DNA replication at late times of infection even in cultures infected with T4 phage defective in one of the DNA delay genes (Fig. 3). It should be noted that in the simultaneous absence of the products of genes 39, 52, and 58, no significant DNA replication in vivo or in vitro was observed even at late times after infection (Huang, manuscript in preparation). When the *E. coli* membrane composition was grossly disturbed, as in the 3-decynoyl-NAC-treated cells, the interaction between all the initiation proteins and the bacterial membrane became aberrant, resulting in very poor or "incorrect" initiation. Since the initial accumulation of newly synthesized DNA in this case was low, no rapid DNA synthesis was observed even at late times after infection. At present, since we do not know the physiological effect of excess saturated fatty acids, other cellular parameters, which may in turn influence rapid DNA replication, cannot be eliminated. When a DNA delay mutant was used to infect the inhibitor-treated culture, the absence of a full complement of the initiation proteins together with the aberrant bacterial membrane would not allow even the incorrect initiation or subsequent rapid chain growth to occur. This resulted in no phage production. It should be noted that in these cases the T4 DNA synthetic machinery is present, and a low level of DNA synthesis is indeed detected. This lower level of DNA synthesis is not sufficient for subsequent phage development; nonetheless, the level of DNA synthesis is much higher than that obtainable when a DNA-negative mutant is used to infect the *E. coli* culture.

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