Impaired Expression of Certain Prereplicative Bacteriophage T4 Genes Explains Impaired T4 DNA Synthesis in Escherichia coli rho (nusD) Mutants

BARBARA L. STITT† and GISELA MOSIG*
Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235

Received 20 September 1988/Accepted 1 April 1989

The Escherichia coli rho026 mutation that alters the transcription termination protein Rho prevents growth of wild-type bacteriophage T4. Among the consequences of this mutation are delayed and reduced T4 DNA replication. We show that these defects can be explained by defective synthesis of certain T4 replication-recombination proteins. Expression of T4 gene 41 (DNA helicase/primase) is drastically reduced, and expression of T4 genes 43 (DNA polymerase), 30 (DNA ligase), 46 (recombination nuclease), and probably 44 (DNA polymerase-associated ATPase) is reduced to a lesser extent. The compensating T4 mutation goF1 partially restores the synthesis of these proteins and, concomitantly, the synthesis of T4 DNA in the E. coli rho mutant. From analyzing DNA synthesis in wild-type and various multiply mutant T4 strains, we infer that defective or reduced synthesis of these proteins in rho026-infected cells has several major effects on DNA replication. It impairs lagging-strand synthesis during the primary mode of DNA replication; it delays and depresses recombination-dependent (secondary mode) initiation; and it inhibits the use of tertiary origins. All three T4 genes whose expression is reduced in rho026 cells and whose upstream sequences are known have a palindrome containing a CUUCGG sequence between the promoter(s) and ribosome-binding site. We speculate that these palindromes might be important for factor-dependent transcription termination-antitermination during normal T4 development. Our results are consistent with previous proposals that the altered Rho factor of rho026 may cause excessive termination because the transcription complex does not interact normally with a T4 antiterminator encoded by the wild-type goF gene and that the T4 goF1 mutation restores this interaction.

Transcription termination factor Rho plays an important role in regulating gene expression in Escherichia coli and its bacteriophages (for reviews, see references 7, 13, 52, and 69). Some E. coli mutants with defective Rho factors show reduced transcription termination at Rho-dependent termination sites and thus suppress transcriptional polar effects. These mutants can also partly overcome the effects of defective lambda gene N antiterminator function at the lambda Rho-dependent termination sites. In contrast, another class of rho mutants, sometimes called nusD, has a different phenotype. These mutants do not support the normal growth of wild-type bacteriophage lambda or T4, but their defects can be partially suppressed by mutations in phage antiterminator genes: N in lambda and probably goF, comCα, or go9H (which are probably the same genes [23, 64, 66]) in T4. It is thought that in these rho mutants, the altered protein is unable to respond to phage-encoded antitermination functions (10, 55, 64).

The T4 goF gene is dispensable in many hosts; it maps in a nonessential part of the T4 genome (64, 66). Presumably, in wild-type hosts T4 is far less dependent than phage lambda on antitermination functions because many of the T4 delayed-early genes have dual controls: they can be transcribed from (early) promoters far upstream, in this case requiring antitermination, but they can also be transcribed without antitermination from (middle) promoters located closer to the coding regions (35). In motA (39), motB, or motC mutants (54) in which T4 middle promoters function poorly (7, 18, 47), T4 antitermination functions are more essential.

T4 DNA replication is one of the processes which is aberrant in E. coli rho mutants of the NusD phenotype infected with wild-type T4. The onset of overall DNA synthesis appears to be delayed, and the rate is lower than in T4-infected rho" bacteria (8, 23, 64, 65, 73). In the work described in this paper, we have asked whether this defective DNA replication is a direct or indirect consequence of the altered Rho factor. Altered termination-antitermination patterns could affect the expression of genes whose products are necessary for DNA replication. Alternatively, they could interfere with DNA replication by affecting the formation or processing of primer RNAs or their precursors.

DNA replication in T4 infections is initiated in at least three modes (28, 30, 36, 43). It begins at specific primary or tertiary origins of replication (for reviews, see references 26, 27, 29, 43, and 72). For at least one primary origin, oriA, at 16.5 kilobases on the T4 map, it has been shown that E. coli RNA polymerase initiates primers for leading-strand DNA synthesis (37, 49; P. Macdonald, Ph.D. thesis, Vanderbilt University, 1983). Because the host RNA polymerase is modified by several proteins (including gp33 and gp55 [for reviews, see references 15, 47, and 56]) during the T4 infectious cycle and this modification directly or indirectly prevents the use of origin promoters, T4 thereafter initiates DNA replication differently: in its secondary mode it uses recombinational intermediates as primers. At tertiary origins of replication, T4 topoisomerase and T4 primase may be required for initiation (29, 30, 41). Altered E. coli Rho factors could interfere with primary-mode T4 DNA replication directly (for example, by affecting primer synthesis by RNA polymerase) or indirectly with any or all three initiation

* Corresponding author.
† Present address: Department of Biology, New York University, New York, NY 10003.
modes by exerting effects on the synthesis of certain proteins.

We have therefore investigated T4 DNA synthesis and early T4 protein synthesis in rho026 mutant bacteria (which are of the NusD phenotype [10]). We observed delayed and reduced synthesis of at least five T4 proteins in T4-infected rho026 cells. Four of these proteins are important in all modes of DNA replication mentioned above: gp43 (DNA polymerase), gp41 (DNA helicase and subunit of the T4 primase), gp44 (DNA polymerase-associated ATPase), and gp30 (DNA ligase). For primary-mode T4 DNA replication, host ligase can substitute for T4 DNA ligase under certain conditions (6, 11, 24, 25). gp46 (recombination nuclease) is important for initiation of replication from recombinational intermediates.

Because we found that the defective synthesis of these proteins can largely account for the partial defect in T4 wild-type DNA replication in NusD mutant hosts, we will describe the protein synthesis results before we discuss DNA replication.

**MATERIALS AND METHODS**

**Bacteria and phage strains.** The *E. coli* K-12 strain SKB178 (galE, McrA) (56a) is the wild-type parental bacterium; HDF026 is a rho mutant derived from it by nitrosoguanidine mutagenesis (64). It was isolated and described as HDF by Revel et al. (57). As originally isolated, HDF026 was temperature sensitive for bacterial growth; the strain derived from it and used here is thermoresistant. Its rho mutation has been called rho026 by Das et al. (10). Phage T4D was obtained from W. B. Wood. The spontaneous gof1 mutant, which grows in HDF026, was isolated by Revel et al. (57). T4 amber mutants were originally obtained from R. S. Edgar and backcrossed to the wild type by us: N130 (gene 46), A456 (gene 47), H39 (gene 30), A453 (gene 32), BL292 (gene 55), N134 (gene 33), N116 (gene 39), and N81 (gene 41). The double mutant N130-A456 and the quadruple mutant N134-130-A456, referred to below as 4×, were constructed and described by Luder and Mosig (36).

**Media.** Phosphate-buffered minimal medium (M9) (1) or low-phosphate Tris minimal medium (20) was used. Lysis medium is 2 M Tris medium without glucose and with 0.2 ml of 0.2 M EDTA (pH 8), 40 μl of 1 M KCl, and 110 μl of 10 N NaOH.

[3H]Thymidine incorporation into T4 DNA. For [3H]thymidine incorporation into T4 DNA, we used [3H]thymidine (73.5 Ci/mmol) from ICN Pharmaceuticals Inc. and performed the procedure as described by Mosig and Bock (46). Results for cells grown in M9 and Tris minimal medium were similar.

[32P] incorporation into T4 DNA. Cells were grown in Tris medium at 37°C to 2 × 10⁹/ml. At 20 min before infection, 32P-phosphate (carrier-free; Du Pont, NEN Research Products) was added to a final concentration of 100 to 200 μCi/ml to allow incorporation into bacterial DNA whose degradation products are used preferentially for the first T4 DNA replicated after infection (20). Infection with T4 was carried out at a multiplicity of infection of 7. At various times after infection, 1-ml samples of the infected bacteria were added to 0.1 ml of 1.0 M KCl on ice. Infected bacteria were pelleted in an Eppendorf centrifuge for 2 min, suspended in 0.2 ml of lysis medium, and boiled for 15 min (to lyse cells and degrade RNA). The hydrolysate was spun in a tabletop centrifuge through a 1.0-ml column of G50 Sephadex (Pharmacia, Inc.) suspended in 10 mM Tris (pH 8) to remove 32P in low-molecular-weight compounds.

**Hybridization of [32P]-T4 DNA to T4 genome digests.** T4 dC-containing DNA from the multiply mutant strain GT7 (70), grown in *E. coli* B834 (galU50) (58, 71), was phenol extracted from CsCl gradient-purified phage, diazylized, and cut to completion with restriction endonucleases (from Bethesda Research Laboratories, Inc., or New England Biolabs, Inc., and used as specified by the manufacturer). A 14-μg sample of the restricted DNA was loaded in a long trough on 30-cm-long, 0.4% agarose (SeaKem; FMC Corp., Marine Colloids Div.) gels and electrophoresed at 60 V in a horizontal tray submerged in Tris-acetate buffer (38), with buffer recirculation, until the bromphenol blue tracking dye had traveled 18 to 19 cm (18 to 20 h). The DNA was transferred from the gels to nitrocellulose sheets (Schleicher & Schuell, Inc.) by the method of Southern (61). After the sheets had been dried and baked at 80°C, 0.4-cm strips were cut lengthwise from the nitrocellulose for hybridizations. All the strips for a given experiment were cut from the same nitrocellulose sheet.

Strips were prehybridized for 4 h in 2× Denhardt solution (38) at 65°C and hybridized overnight at 42°C in 34% formamide-0.2 M sodium phosphate (pH 7.0)-4× SSPE (38)-0.3% sodium dodecyl sulfate, plus 96 μg of sonicated denatured salmon sperm DNA per ml to which the 32P-labeled nascent T4 DNA was added. After hybridization, the strips were washed in 2× SSPE-0.1% sodium dodecyl sulfate at 42°C four times for 10 min and then twice for 10 min each in a 10-fold dilution of the same solution at room temperature. The strips were air dried and used with Cronex Lightning-Plus intensifying screens (Du Pont Co.) to expose Kodak RP X-Omat X-ray film at −70°C.

**3H-amino acid incorporation into T4 proteins.** Cells were grown in Tris medium or M9 (no differences in results were observed) to 3 × 10⁹ to 4 × 10⁹/ml at 37°C and infected with phage at a multiplicity of infection of 7.5. At various times after infection, 1.0 ml of the infected culture was added to 1 μCi [3H]-amino acids ([3H]-L-amino acid mixture [146 mCi/ml] from ICN), and 2 min later the sample was poured into a 1.5-ml microcentrifuge tube on ice. The tube contained 10 μl of 1 M NaCN and 20 μl of 20% Casamino Acids (Difco Laboratories). The cells were pelleted in an Eppendorf centrifuge for 2 min, suspended in 0.1 ml of gel electrophoresis sample buffer (32), and boiled for 2 min. Samples not immediately used were stored frozen (−20°C) and boiled again prior to use.

**Polyacrylamide gel electrophoresis.** We used 20% polyacrylamide-0.067% bisacrylamide gels (5) specifically to separate proteins in the 50- to 80-kilodalton range. The stacking gel, electrode buffer, and running conditions were as described by Laemmli (32). A 10-μl volume of sample was applied per well, and the samples were electrophoresed until the bromphenol blue tracking dye reached the bottom of the separating gel.

**Fluorography.** Fluorography with sodium salicylate (Aldrich Chemical Co., Inc.) as the fluor was performed as described by Chamberlain (9). Before being exposed to the dried gel, RP X-Omat X-ray film was partially exposed by a filtered photographic flash unit, as described by Laskey and Mills (33), to increase its sensitivity to [3H] and to achieve a linear response to the radioactivity. Each gel was exposed to several films for different lengths of time to obtain optimal exposures of both heavily and lightly labeled bands.
**RESULTS**

**T4 early protein synthesis.** A comparison of T4 proteins in rho<sup>+</sup> and rho026 cells (Fig. 1) shows many changes. The accumulation of at least five early proteins (marked × in Fig. 1) required for normal DNA synthesis is reduced and delayed in the mutant host; these proteins are gp41 (DNA helicase/primase), gp43 (DNA polymerase), gp30 (DNA ligase), gp46 (recombination nuclease), and a protein of apparent molecular weight (M<sub>r</sub>) 36,000 that is probably gp44 (DNA polymerase-associated ATPase), based on the absence of the band in a gene 44 amber mutant (data not shown). The T4 goF1 mutation restores the accumulation of these proteins. These results suggest that the effect of the host rho mutation on T4 DNA replication, and its alleviation by the T4 goF1 mutation, are due, at least in part, to delayed and reduced expression of DNA replication and recombination genes. As discussed below, the reduction of T4 gp41 in the rho host is more significant than that of the other affected gene products. Oishi (50) has shown that gene 41 mutations allow considerable residual T4 DNA replication, which, however, generates predominantly single-stranded DNA. Deficiencies in the synthesis of gene 41 protein should have similar consequences. We therefore also expected, and found, that gp32, the T4 single-stranded DNA-binding protein whose expression is regulated in response to single-stranded regions (31, 40, 68), is overproduced in rho026-infected cells (Fig. 1) (64).

At least eight other proteins (marked △ in Fig. 1) are more abundant in rho026 cells infected with goF1 than with wild-type T4. Polyacrylamide gel electrophoresis of proteins from T4 amber or deletion mutant infections indicate that these include gprIIA (M<sub>r</sub> 95,000), gp39 (M<sub>r</sub> 58,500), and possibly gp42 and/or gp45 (M<sub>r</sub> 25,000). Three additional proteins (marked ⋄ in Fig. 1) show increased levels during goF1 mutant infections, regardless of the host. Since the levels of these proteins are not reduced during wild-type T4 infections of rho026 hosts (which are nonproductive) as compared with rho<sup>+</sup> hosts (which are productive), the differences in abundance of these proteins do not seem to be critical to T4 growth. They might, however, contribute to the ability of goF1 mutant phage to produce progeny in rho026 cells. At least 10 T4 proteins (marked □ in Fig. 1) are more abundant in rho026 than in rho<sup>+</sup> hosts, in both goF1 (productive) and wild-type T4 (nonproductive) infections. Therefore, these differences also do not seem to be critical to the differences in DNA replication that we discuss here.
Overall DNA synthesis. Consistent with earlier results (64), wild-type T4 DNA synthesis, measured by cumulative 
\(^3\)H thymidine incorporation, in infected rhoO26 E. coli bacteria appears to be delayed and reduced compared with that in T4-infected parental rho\(^+\) cells (Fig. 2). The T4 goFl mutation partially compensates for the E. coli rho mutation. Similar results (data not shown) were obtained with other rho mutants of the NusD type.

To test whether origin initiation, recombination-dependent initiation, or both, were affected by the rhoO26 E. coli mutation, we measured DNA replication of a recombination-deficient T4 gene 46-47 mutant, in which DNA replication is arrested prematurely, and of the quadruple mutant. 4\(\times\), in which this arrest is alleviated by additional mutations in genes 33 and 55 (36). DNA replication in these recombination-deficient mutants is largely initiated from primary or tertiary origins. Because in the quadruple mutant the RNA polymerase is not reprogrammed for late T4 transcription, more reinitiation from primary origins can occur than in the gene 46-47 double mutant (36). DNA synthesis in both the double and quadruple mutants is reduced in rhoO26 cells compared with rho\(^+\) hosts, implying that origin initiation is at least partially defective (Fig. 2). The residual DNA synthesis of these T4 mutants, especially of the quadruple mutant, in the rhoO26 host indicates, however, that this defect is not absolute and that some origin initiation does occur.

In addition, recombination-dependent initiation of wild-type T4 appears to be (partially) defective, an expected consequence of reduced synthesis of the recombinational proteins discussed above. If there were no recombination-dependent initiation in wild-type T4 in the rho mutant host, we would expect the wild-type DNA synthesis pattern to resemble that of the gene 46-47 double mutant. If recombination-dependent replication were unaffected, we would expect higher incorporation than is seen, especially at late times.

The data also show that the DNA arrest phenotype of recombination-deficient T4 in the rho\(^+\) host is not as evident as it is in E. coli B (compare Fig. 2 with the results of reference 36). It has been suggested (53) that recombination functions of some resident prophages can partially compensate for defective recombination functions of T1 and perhaps of T4; defective lambda prophages are well known to occur in E. coli K-12 (12). Such a putative prophage function could be responsible for the more extensive DNA synthesis in the rho\(^+\) host strain used here as compared with E. coli B. If that were the case, the rhoO26 mutation might also affect the expression of that putative prophage function in a way similar to that in which it affects lambda gene expression (10), and this might lead to a more severe defect for the recombination-deficient T4 mutant in the mutant host. Regardless, the \(^3\)H thymidine incorporation experiments suggest that there are abnormalities in both origin initiation and recombination-dependent initiation of T4 DNA synthesis in rhoO26 hosts, but that both types of initiation are still occurring.

Use of origins. Several different primary and tertiary origins of initiation have been identified in T4 (26, 27, 29, 43, 72). To determine whether the same origins are used in wild-type and rhoO26 cells, nascent T4 DNA was labeled with \(^32\)P early after infection and hybridized to restriction enzyme digests of whole T4 GT7 DNA. The same restriction fragments corresponding to oriA, oriE, and oriF were labeled first in both rhoO26 and rho\(^+\) bacteria (Fig. 3, lanes A and B), indicating that initiation from these origins is similar in both hosts. However, oriG was not labeled in rhoO26 cells (restriction fragment 1 in Fig. 3), and the level of the oriF fragment (restriction fragment 12 in Fig. 3) was reduced under these conditions. Since oriG and oriF both function in
the tertiary mode (29), these results suggest that tertiary initiation is directly or indirectly affected by rho026. In addition, there is slightly less 32P label in the origin-containing T4 restriction fragments in rho026 than in rho + cells, and T4 replication forks have not progressed from the origin-containing fragments into adjacent restriction fragments in rho026 at the same early time as they have in the rho + cells (Fig. 3, compare lanes A and B). These results are expected as a consequence of the deficiencies in gp41 and gp46: leading-strand initiation at primary origins occurs, but lagging-strand synthesis and recombination-dependent initiation are considerably delayed (19, 45; A. Luder, Ph.D. thesis, Vanderbilt University, 1981). Deficiencies in gp41 are also expected to affect tertiary origin initiation (30, 41). In addition, replication forks initiated at origins pause at certain distances from the origins when lagging-strand DNA synthesis is impaired (G. Lin, Ph.D. thesis, Vanderbilt University, 1988; M. Gruidl, Ph.D. thesis, Vanderbilt University, 1988; G. Mosig, unpublished data; G. Lin, M. Gruidl, and G. Mosig, manuscript in preparation).

Consistent with the idea that the later initiations occur predominantly at random from recombinational intermediates, at late times after infection all T4 restriction fragments were labeled, in proportion to their size, in both rho026 and
rho+ cells (Fig. 3, lanes C and D), but there was less total T4 DNA synthesis in rho026 than in rho+ cells.

**DISCUSSION**

Our results show that wild-type phage T4 infection of a rho mutant host with the NusD phenotype results in delayed and diminished synthesis of at least five early T4 proteins that are important in DNA replication and recombination: gp41 (DNA helicase, subunit of the T4 primase), gp43 (DNA polymerase), gp30 (T4 DNA ligase), gp46 (recombination nuclease), and probably gp44 (DNA polymerase-associated ATPase). Similar results (data not shown) were obtained with other NusD rho mutants.

These results agree with and extend those reported by Stitt et al. (64), who found that the levels of gp43 and a band of apparent molecular weight 58,000 were reduced early after T4 infections of rho026 cells. The present gels, containing a lower concentration of bisacylamide, have resolved the 58,000-molecular-weight band into at least four T4 proteins, which we identified as gp46, gp41, gp30, and gp39. A possible additional protein comigrating with gp41 may also be reduced under these conditions. We also believe that the level of gp44 is reduced (our identification is based on comparisons with other gels [data not shown]). Four of the affected proteins participate in DNA replication in general and two play a role in initiation of replication forks from recombinatorial intermediates. The defective expression of these proteins, in particular of the DNA helicase/primase, is sufficient to explain the effect of the rho026 mutation on DNA replication.

**gp41.** Of the proteins we have identified, gp41 is one of the most severely affected by the rho026 mutation. gp41 and gp58/61 together constitute the T4 primase/helicase (51), which initiates lagging-strand synthesis and, via coupling to the replisome, stimulates leading-strand synthesis in vitro. When T4 primase is mutationally inactivated, leading-strand DNA synthesis in one direction only is initiated from primary origins, resulting in displacement synthesis in vivo (48; Luder, Ph.D. thesis) and accumulation of single-stranded T4 DNA (19, 50). Primase is also probably required for the tertiary mode of initiation (31, 41). Thus, several different consequences for DNA metabolism and also overproduction of the single-stranded DNA-binding protein (gp32), all of which we found, are readily explained by deficient synthesis of gp41 helicase in rho026 mutant hosts. These are as follows: (i) Leading-strand synthesis initiated from primary origins is retarded and pauses excessively. These effects are probably accentuated by gp44 deficiencies discussed below. (ii) Tertiary origins are used rarely, if at all. Of the two tertiary origins characterized by Kreuzer et al. (29), oriG appears to be completely defective (Fig. 3, lane A). The residual functioning of oriF under these conditions would be consistent with observations from our laboratory that oriF can also function in the primary mode, which does not require primase/helicase (17; Gruidl, Ph.D. thesis; Lin and Mosig, in preparation). (iii) The DNA strands that are displaced by leading-strand synthesis from primary origins are not copied efficiently and titrate the single-stranded DNA binding protein, gp32, thus inducing more synthesis of this autoregulated protein (68).

**gp43.** In their analysis of T4-infected rho tabC mutants, Caruso et al. (8) found gp43 (DNA polymerase) to be reduced, and they stated that this reduction is sufficient to account for the observed depressed DNA synthesis. In our strains, however, gp43 accumulation was less severely affected than in tabC mutants, and Jensen and Susman (24) have isolated similar host mutants in which gp43 synthesis is indistinguishable from that in wild-type cells. The aberrant DNA synthesis phenotype, however, is common to all strains. It therefore seems that the quantity of gp43 may not be critical to the defective DNA synthesis phenotype.

**gp44.** gp44, an accessory protein of DNA polymerase which has ATPase activity, is a component of the replication machinery that, among other activities, confers processivity on DNA polymerase (51).

**gp30.** Although the production of gp30 (T4 DNA ligase) is strongly affected in rho026 cells (Fig. 1), this defect may not be critical for primary-mode T4 DNA replication, because in this mode the host ligase can substitute for T4 DNA ligase (22, 25). Ligase is not essential for the initial leading-strand (displacement) synthesis (see above) that occurs in rho026 cells as a result of deficiencies in primase/helicase synthesis.

**gp46.** The reduced and delayed accumulation of the recombination protein gp46 (Fig. 1) is expected to accentuate the defective DNA replication in the rho026 hosts (Fig. 2), for the same reason that additional gene 46 mutations accentuate defects in DNA replication of T4 primase/helicase mutants: in primase-deficient situations, recombination has to provide primers to copy the displaced strands (44, 48; Luder, Ph.D. thesis).

Why is expression of these specific genes, but not of other prereplicative T4 genes, affected by the rho026 mutation? The simplest interpretation is based on the complex regulation of prereplicative, delayed-early genes mentioned in the Introduction. Only genes whose expression depends largely on antitermination (and not on alternative initiation from middle promoters) are expected to be seriously affected by NusD-type rho mutations. We propose that genes 41, 43 (but also see below), and 46, and probably 44 and 30, may be of this type. For three of these genes, promoters have been
identified or proposed (3, 14, 16, 21, 63), and in these cases there is a palindromic sequence with an interspersed CUU CGG sequence between the promoter and the structural gene of interest (Fig. 4). (No such stem-loop system has been found for gene 30, but the sequence upstream of this gene is not well known [4]. For gene 44, the CUUCCGG sequence is present, but the length of its stem is uncertain [62, 67].) Such sequences have been proposed to have regulatory functions (67). These sequences could, for example, be involved in transcription antitermination, permitting efficient expression of downstream genes (60). Consistent with this idea, evidence for reduced amounts of the gene 41 transcripts in T4<sup>+</sup>-infected rho026 cells compared with the rho<sup>+</sup> parent has been obtained from analysis of in vivo transcripts (D. M. Hinton, J. Biol. Chem., in press).

The antitermination requirement for gene 43 (DNA polymerase) expression is expected to be less severe than for gene 41 expression, because gene 43 has an additional weak motA-dependent middle promoter downstream of the CUUC GG loop and it is also autoregulated at the translational level (3, 63). We would not expect rho mutations to affect the latter type of regulation.

We assume, in agreement with previous proposals (8, 54, 55, 64), that the wild-type goF gene product is an antitermination factor which does not function normally in the NusD-type rho mutants. The consequences, when this factor is defective, are expected to depend on the strength of the termination signal that must be overcome, on the precise defect in the putative antitermination factor, and perhaps on the presence of certain recognition signals.

We postulate that in the NusD rho mutants, antitermination is defective because the interactions among rho protein, RNA polymerase, the T4-encoded antiterminators, and/or the nucleic acid target sequences are altered, resulting in poor expression of certain genes. The goF1 mutation partially restores the proper interaction or competition with Rho and thus allows antitermination and better expression of genes downstream of the affected terminators (64). In support of this proposal, Das et al. (10) have shown directly that the rho026 mutation affects antitermination by the N protein of phage lambda.

The fact that deletions of the goF region of T4 are viable suggests either that a host antitermination factor is sufficiently active to permit necessary transcription through the factor-dependent termination regions or that T4 has more than one such factor with overlapping specificities. The existence of an <i>E. coli</i> antitermination function has been suggested (2, 42, 59) and perhaps found (34). This putative host factor would also be unable to interact with the mutationally altered rho026 protein. In addition, there is evidence for several different T4 antiterminators (64; B. Stitt, Ph.D. thesis, California Institute of Technology, 1978). One or more of these factors might substitute if the goF product is deleted.

We have noted two different patterns of protein overproduction in Fig. 1, one that is seen when comparing rho<sup>+</sup> and rho026 hosts and one that is present in goF1-infected cells but not in T4<sup>+</sup> infections. The first type of overproduction is evidently not critical to T4 growth, since it occurs in both wild-type (unproductive) and goF1 (productive) infections of the rho mutant cells. It is possible that the increase in production of certain other, as yet unidentified, early proteins in goF1-infected cells contributes to the ability of this mutant phage to produce progeny in rho026 hosts.

Despite the considerable residual DNA replication of wild-type T4 in rho026 hosts, no viable progeny particles are produced. Results from several laboratories agree that essential late proteins are missing in these hosts (8, 23, 64). Thus, transcription termination and antitermination involving Rho must play important roles in both early and late phage T4 gene expression.

In summary, the effects of DNA synthesis in nonproductive wild-type T4 infections of NusD-type rho hosts can be accounted for by the deficient or reduced synthesis of certain replication and recombination proteins that we have identified here.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM 13221 (to G.M.) and Biomedical Research grant RR07201 from the National Institutes of Health and by the National Science Fund of Vanderbilt. Preparation of the manuscript was also supported by National Science Foundation grant DMB88-06747 (to B.L.S.).

We thank Ruth Senby and Doris Powell for excellent technical assistance, Paul Macdonald and Robert Thompson for helpful discussions, and Cindy Young for help in preparation of the manuscript.

LITERATURE CITED


