Transcription Termination Signals in the nin Region of Bacteriophage Lambda: Identification of Rho-Dependent Termination Regions

Sheau-wei C. Cheng,* Donald L. Court† and David I. Friedman*

*Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan 48109-0620 and †Molecular Control and Genetics Section, Laboratory of Chromosome Biology, ABL-Basic Research Program, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland 21702

Manuscript received December 23, 1994
Accepted for publication April 17, 1995

ABSTRACT

The ~3-kb nin region of bacteriophage λ, located between genes P and Q, contains transcription termination signals as well as 10 open reading frames. Deletions in the nin region frees phage growth from dependence on the λ-encoded N-transcription antitermination system, conferring a Nin phenotype (N-independence). A subregion of nin, roc, is defined by a 1.9-kb deletion (∆roc) which partially frees λ growth from the requirement for N antitermination. The roc region has strong transcription termination activity as assayed by a plasmid-based terminator testing system. We report the following features of the roc region: the biologically significant terminators in the roc region are Rho dependent, deletion analysis located the biologically significant termination signals to a 1.2 kb-segment of roc, and analysis of other deletions and point mutations in the roc region suggested at least two biologically significant regions of termination, tr3 (extending from bp 42020 to 42231) and tr4 (extending from bp 42630 to 42829).

TRANSCRIPTION termination signals (or terminators) play important roles in the control of gene expression (Platt 1986; Friedman et al. 1987). The terminators at the ends of genes or groups of genes define the termini of the transcripts and serve to separate genes or operons into independently regulated genetic units. Thus, transcription terminators can prevent the unintended read-through of transcription into adjacent downstream genes. In addition to ends of operons, transcription terminators can be found within operons; between genes, within genes, and even before the first gene (Richardson 1990; Platt and Richardson 1992). Transcription terminators within genes appear to function when translation and transcription are uncoupled; e.g., when nonsense mutations prematurely terminate translation (Adhya et al. 1976) or under conditions of starvation where a limitation in amino acids slows translation (Landick and Yanofsky 1987). Richardson (1991) proposed that termination within genes might serve to conserve cellular resources in hard times by preventing synthesis of unused transcripts when translation aborts or is significantly reduced. Strategically placed terminators within operons can serve as components of regulatory systems controlling gene expression. There are numerous examples, both in eukaryotes and prokaryotes, of regulation of gene expression by transcription termination and associated antitermination mechanisms (Friedman et al. 1987; Proudfoot 1989; Spencer and Groudine 1990). Two well characterized examples being the N-antitermination system of phage λ (see below) and the attenuation systems found in many bacterial biosynthetic operons (Landick and Yanofsky 1987; Landick and Turnbough 1992).

Studies in Escherichia coli have led to the division of transcription terminators into two classes, intrinsic and Rho dependent (Platt 1986; Friedman et al. 1987; Yager and Von Hippe1 1987). Both signals are recognized at the level of RNA (Adhya and Gottesman 1978; Sharp and Platt 1984). Intrinsic terminators function in a minimal in vitro transcription system, consisting of RNA polymerase and a DNA template, without a transcription termination protein (Reynolds et al. 1992). The features common to this class of terminators are a region of dyad symmetry rich in G and C residues followed by a run of U residues in the RNA (Platt 1986; Yager and Von Hippe1 1987). Rho, an essential protein for E. coli, with RNA-dependent ATPase as well as helicase activity (Platt and Richardson 1992), functions as a hexamer to cause transcription to terminate with site specificity (Court et al. 1980; Ciampi et al. 1989; Richardson 1990). Although Rho-dependent terminators, unlike the intrinsic terminators, do not have an identifiable consensus sequence (Bear and Peabody 1988), analyses of regions of Rho-dependent termination suggest that an upstream region of RNA (80–100 nucleotides) that is relatively high in C and poor in G content and lacking stable secondary structure contributes to the signal (reviewed in Richardson 1990; Platt and Richardson 1992). Based partially on this

Corresponding author: David I. Friedman, Department of Microbiology and Immunology, Medical School, University of Michigan, Ann Arbor, MI 48109-0620. E-mail: davidfri@umich.edu

Genetics 140: 875–887 (July, 1995)
information, the following is the current view of Rho-dependent termination. Rho recognizes and binds to unstructured RNA, in a C-rich region, and then interacts with RNA polymerase by tracking along the RNA towards the 3′ end where it disrupts the ternary RNA Polymerase-RNA-DNA complex to terminate transcription (Adhya et al. 1976; Platt and Richardson 1992; Steinmetz and Platt 1994).

Bacteriophage λ employs systems of transcription termination and antitermination to temporally regulate gene expression (Friedman 1988; Das 1992, 1993; Greenblatt et al. 1993; Roberts 1993). Transcription of λ “early” genes initiates at two promoters, P5 and P4, and partially terminates at Rho-dependent terminators tR1 and tL1, respectively. The leftward early transcript encodes the N-antitermination protein. We focus our discussion on transcription from P5. About 50% of the P5-initiated rightward transcription continues through tR1 but terminates in the nin region without reaching gene Q (Court et al. 1980). In the presence of the N-antitermination complex that includes the phage encoded protein N, phage RNA site nut, and several host Nus factors, the RNA polymerase is modified into a transcription termination-resistant form (Greenblatt 1992). The modified polymerase is able to pass through tR1 and the terminators in the nin region and extend into the Q gene. The Q gene product is a second λ-encoded antitermination factor that activates late genes expression (Roberts 1992).

The nin region is defined by deletions found in λ derivatives that grow in the absence of a functional N protein (Court and Sato 1969). The best characterized nin deletion is nin5, a 2.8-kb deletion extending from bp 40501 to 43306 (Fandt et al. 1971; Kroger and Hobom 1982). The 3-kb nin region encodes 10 ORFs, some having identified function, but none, obviously, being essential for phage growth under laboratory conditions (Figure 1). The N-independence conferred by nin deletions suggests that the 50% termination occurring at tR1 in the absence of N is not sufficient to prevent lytic growth (Court et al. 1980). These deletions in the nin region remove termination signals to allow sufficient P5 read-through into Q. The first terminator in the nin region identified and shown to have terminator activity, tR2, was recognized because it has features common to Rho-independent terminators (Kroger and Hobom 1982). Subsequently, the identification of a mutation in the tR2 sequence, that contributes to N independence, confirmed that tR2 functions as a terminator in situ (Leason 1986; Cheng et al. 1991).

Analysis of a λ derivative with the 1.9-kb roc deletion extending from bp 41883 to 438825, called Δroc, identified a second region of termination downstream of tR2 (Leason and Friedman 1988). Δroc was isolated because it grows better in E. coli with mutant nus genes than λ wild type. Unlike the nin5 deletion, which removes all of the terminators between P and Q, the roc deletion leaves the tR2 terminator intact and does not render λ completely N independent. Consequently, while the nin5 deletions permits λ to form plaques on lawns of E. coli with any of the nus mutations, the roc deletion permits λ to form plaques on lawns of E. coli with the nusC60 mutation, strain K554, but not hosts with the other nus mutations. nusC60 confers resistance to rifamycin and is composed of three separable mutations in the rpoB gene, encoding the β subunit of RNA polymerase (Friedman et al. 1984). Burst experiments, however, revealed that λ derivatives with the roc deletion grow significantly better in E. coli carrying the other nus mutations than does λ wild type. The regions covered by Δroc and nin5 overlap by 1.4 kb (see Figure 1) with Δroc extending 0.5 kb farther downstream (Leason and Friedman 1988). The roc terminators are thought to be located in the 1.4-kb overlapped region.

We report studies on transcription termination in the roc region showing that the biologically significant transcription termination in the roc region is Rho dependent and that multiple terminators are present that define two segments in the roc region, tR3 (extending from bp 42020 to 42231) and tR4 (extending from bp 42630 to 42825).

**MATERIALS AND METHODS**

**Bacteria, phage and plasmids:** The bacterial strains and previously constructed plasmids used in this study are listed in Tables 1 and 2. Phages not constructed for this study were either obtained from our collections or the National Institutes of Health collection. Details of constructions of plasmids new to this work are detailed below. The media and methods for cultivation of bacteria and phage have been previously described (Leason and Friedman 1988).

**Marker rescue experiment:** Bacteria with plasmids were grown overnight in LB supplemented with ampicillin (32 µg/ml). Overnight cultures were diluted into LBMM (LB with 10 mM MgSO4 and 0.2% maltose) at 37° and grown to a density of (∼2 × 106 cells/ml). One milliliter of the culture was pelleted and resuspended in 0.1 ml of 10 mM MgSO4 and infected with phage at a multiplicity of 5-10 phages per bacterium. The bacteria-phage mixture was incubated at room temperature for 20 min to allow phage adsorption, then diluted with 5 ml of TMG (10 mM Tris-Cl pH 7.4, 10 mM MgSO4, 0.01% gelatin) and pelleted by centrifugation. The bacteria were then resuspended in 5 ml LB with 10 mM MgSO4, and grown at 37° for 2-3 hr. The resulting lysate was treated with chloroform and the phage titers were determined using the appropriate bacterial lawns.

**Nucleic acid techniques:** DNA preparations, restriction digests, ligations, transformations and gel electrophoresis were performed essentially as previously described (Sambrook et al. 1989). Dideoxy DNA sequencing was performed according to the published method (Biggin et al. 1983).

**Plaque hybridization:** Plaque hybridization was performed according to the established procedures (Benton and Davis 1977). Phages were plated to yield 15,000 plaques per 90-mm
petri dish. The plates were then incubated at 37° for 10–12 hr and stored at 4° for ≥1 hr. Plaques were transferred to nitrocellulose filters, lysed and denatured with 0.2 M NaOH/1.5M NaCl, neutralized with 0.4 M Tris-Cl (pH 7.6) and 2X SSC, and baked in a vacuum oven at 80° for 120 min. The nonradioactive detection kit from BRL was used for labeling the DNA probe.

Galactokinase assay: The method of ADHYA and MILLER (1979) was employed. Galactokinase units are nanomoles of galactose phosphorylated per minute per unit of cell density.

Plasmid construction: Construction of pKL500 derivatives: The restriction sites used in generating these constructs as well as the structures of pKL500Δroc97, pKL500BglIIroc+, pKL500rocR, and pKL500rocL are shown in Figure 2. pKL500Δroc97 was isolated by screening a set of nested deletions generated by cleaving pKL500roc’ at the unique AflI site (bp 42630) and treating with exonuclease Bal31 for various lengths of time. BglII linkers were added and the resulting DNA was digested with BglII, gel purified, rejoined with ligase and transformed into the galK+ strain K3448. Plasmid DNAs from GalK+ transformants were isolated and treated with restriction enzymes to screen for the smallest deletion within the roc region. The resultant plasmid was named pKL500Δroc97. pKL500BglIIroc+ was also constructed from pKL500roc’ by digesting at the unique AflI site and ligating the ends with BglII linkers. The DNA was digested with BglII, gel purified as linear, and rejoined using ligase. pKL500rocR was constructed by replacing the BglII-EcoRI region in pKL500roc’ with the BglII-EcoRI fragment from pKL500BglIIroc’. The resulting plasmid, pKL500rocR, retains the deletion, Δroc, to the left of the BglII site and has the wild-type region to the right of the BglII site. pKL500rocL was constructed by replacing the BamHI-BglII region in pKL500roc’ with the BamHI-BglII region from Anin+ (bp 41732–44141).

TABLE 1

<table>
<thead>
<tr>
<th>E. coli strains</th>
<th>Relevant genotype</th>
<th>Source</th>
<th>Bacterial strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>K37</td>
<td>nus+</td>
<td>NIH collection</td>
<td></td>
</tr>
<tr>
<td>K95</td>
<td>nusA1</td>
<td>This laboratory</td>
<td></td>
</tr>
<tr>
<td>K450</td>
<td>nusB5</td>
<td>This laboratory</td>
<td></td>
</tr>
<tr>
<td>K554</td>
<td>nusC60 rif+</td>
<td>This laboratory</td>
<td></td>
</tr>
<tr>
<td>K556</td>
<td>nusE71</td>
<td>This laboratory</td>
<td></td>
</tr>
<tr>
<td>K99 (C600)</td>
<td>supF</td>
<td>This laboratory</td>
<td></td>
</tr>
<tr>
<td>K1066 (SA1030)</td>
<td>rho+</td>
<td>S. ADHYA</td>
<td></td>
</tr>
<tr>
<td>K1024*</td>
<td>rho13(ts)</td>
<td>S. ADHYA</td>
<td></td>
</tr>
<tr>
<td>K3443</td>
<td>galK2 recA− pro−</td>
<td>K. MCKENNEY</td>
<td></td>
</tr>
<tr>
<td>K1617</td>
<td>marl35</td>
<td>E. FLAMM</td>
<td></td>
</tr>
<tr>
<td>K4446</td>
<td>λ1857Q::cat</td>
<td>This laboratory</td>
<td></td>
</tr>
</tbody>
</table>

* A derivative of SA1030.

TABLE 2

<table>
<thead>
<tr>
<th>Plasmids not constructed in the course of this work</th>
<th>Vector</th>
<th>Insert</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>pLR31</td>
<td>pBR325</td>
<td>BamHI-EcoRI fragment (bp41732–44972)</td>
<td>This laboratory</td>
</tr>
<tr>
<td>pKL50</td>
<td>pBR325</td>
<td>BamHI-EcoRI fragment (bp41732–44972)</td>
<td>K. MCKENNEY</td>
</tr>
<tr>
<td>pKL600</td>
<td>pBR325</td>
<td>BamHI-HindIII fragment (bp41732–44141)</td>
<td>K. MCKENNEY</td>
</tr>
<tr>
<td>pKL500roc+</td>
<td>pKL500</td>
<td>BamHI-HindIII fragment (bp41732–44141)</td>
<td>This laboratory</td>
</tr>
<tr>
<td>pKL500roc−</td>
<td>pKL500</td>
<td>BamHI-HindIII fragment (bp41732–44141)</td>
<td>This laboratory</td>
</tr>
</tbody>
</table>

FIGURE 1.—Features of the reconstructed λ nin region relevant to this study. The upper line indicates restriction sites, including the BglII site that replaced the AflI site (AflI/BglII), the locations of the tR2 terminator and tR3 mutation as well as the Q::cat insertion. The middle area shows the extents of the deletions. The numbers give the base pair coordinates from the standard X sequence (DANIELS et al. 1983). The borders of Aroc97 (not shown) extend from the left boundary of AtR3 and the right boundary of ΔR4. The bottom line shows the translation products and overlaps of reading frames indicated by overlapping boxes. The number below each translation frame represents the number of amino acids in each gene product.
BglII fragment from pKL500BglIIroc'. The resulting plasmid, pKL500rocL, retains the deletion to the right of the BglII site, ΔtR3, and has the wild-type region to the left of the BglII site. pKL500SB(tR3'), pKL500CB(tR3'), and pKL500RB(tR3') were constructed from pKL500rocSS(tR3'-tR4') (see Figure 3). The latter was made by cloning the SspI-SspI fragment (bp 41905–42825), from the wild-type tR3/tR4 region, into the Smal site located in the pKL500 polycloning region. The orientation of the inserts places tR3 adjacent to plac. pKL500SB(tR3') was constructed from pKL500rocSS by digesting at the BglII site within the cloned fragment and the Sph site in the vector, filling in the 5' overhang and joining the blunt ends of the large fragment, which included pKL500 and a region (bp 41905–42630) containing tR3. pKL500CB (tR3') and pKL500RB(tR3') were constructed by isolating respectively the ClaI-BglII region (bp 42020–42630) with subsequent blunt-ending of the ClaI site and the EcoRV-BglII region (bp 42231–42630). These fragments were cloned, respectively, in pKL500 between the Smal and EcoRI sites.

The same strategies employed in cloning tR3' derivatives were used to construct analogous plasmids with the tR3 mutation. The resulting plasmids were named pKL500SB(tR3'),

FIGURE 2.—Construction of pKL500 derivatives. pKL500roc' has been previously described (LEASON and FRIEDMAN 1988). The details of construction of pKL500roc97, pKL500BglIroc', pKL500rocL, and pKL500rocR are outlined in MATERIALS AND METHODS. Termination activity of cloned segments in the pKL500 derivatives was assessed by measuring levels of expression of the downstream galK gene; i.e., GalK activity reflects the level of transcription read-through. The numbers in parenthesis represent the level of galK expressed by that construct relative to the level of galK expressed by the vector with no insert.
A nin Region Termination Signals

Figure 3.—pKL500 derivatives with inserts from the roc region. Top of the figure is a restriction map of the roc BamHI-HindIII fragment from pKL500-BglIroc'. The patterned rectangles represent the various fragments cloned from this region. The length of the rectangle provides a relative idea of the size of the fragments. Listed below are the pKL500 derivatives with schematic representations of the inserts from the roc region. The black lines represent vector DNA and cloned fragments are indicated by the patterned rectangles corresponding to the fragments in the restriction map above. Indicated are the restriction sites used for cloning on the plasmid and at the ends of the cloned fragments. * Overhangs were blunted before cloning.

pKL500CB(R3') and pKL500RB(R3'), pKL500SB(R3'+), pKL500SS were constructed by cleaving pKL500SB(R3') with Clal, filling in the Clal ends, and religating. By filling in the GC overhang at the Clal site in the cloned R3' region, 2 bp were added that also created a new NraI site, pKL500SB(R3') and pKL500SB(R3'+) shown in Figure 3.

Construction of pKL600 derivatives: pKL600roc', pKL600-BglIroc', and pKL600Δroc fragments were constructed (Figure 4) by cloning the BamHI-EcoRI fragments from the appropriate pKL500 derivatives into pKL600 at the BamHI and XbaI sites (both the EcoRI site on the fragment and the XbaI site were filled in before digestion with BamHI). These pKL600 constructs are not diagrammed in the figure. pKL600CR(R3') contains the Clal-EcoRV fragment with R3' (bp 42020-42231), isolated from pKL500roc'. This fragment was made blunt-ended and cloned into the Smal site in M13mp18. The M13mp18 clones were sequenced to determine the orientation of the cloned fragment. A clone with the correct orientation was digested with Smal and XbaI and the 211-bp fragment carrying the Clal-EcoRV region was ligated into the Smal and XbaI sites of pKL600. pKL600BS(R3') was constructed by cloning the R3' BglII-SmaI fragment (bp 42630-42825) isolated from pKL500BglIroc' into pKL600 between the BamHI and XbaI sites.

Construction of pBR322 and pBR325 derivatives A pBR325 derivative, pLR31, containing the λ BamHI-EcoRI fragment extending from bp 41372 to 44972 (Leason 1986) was used to generate pLR31ΔR3' and pLR31ΔR4' by replacing the λ BamHI-HindIII region (bp 41372-44141) in pLR31 with the corresponding regions from pKL500rocR or pKL500rocL. pBBE(AΔR3'ΔR4') was constructed by cloning the BamHI-EcoRI fragment (bp 41372-44972) from λΔR3'ΔR4' into pBR325. These plasmids are not diagrammed in the figures. pLR31 Q:cat was constructed by cloning the HindIII-linkered FnuDI fragment encoding chloramphenicol resistance (Cam') from pBR328 into the HindIII site of λQ (Figure 1).

pSA is a pBR322 derivative in which the SstI to ApaI segment is deleted. Schematic representations of pSA derivatives are shown in Figure 5. pSAB-Bg was constructed by cloning the IR3 region in a BamHI-BglII fragment (bp 41372-42630) from pKL500BglIroc' into a linearized pSA that was prepared by digesting with EcoRI, ligating BglII linkers, and redigesting with BglII and BamHI. pSAB-BgΔCR was constructed by completely digesting pSAB-Bg with Clal and EcoRV, blunting the ends, and religating.

Construction of λQ:cat phage and lysogen: Strain K37 was lysogenized with the defective phage, λQ:cat creating strain
K4446. The defective phage $\lambda Q$:cat was constructed as a recombinant from a cross between $\lambda$dl857 and plasmid pLR31Q::cat. The resulting lysate, which contains $\lambda$dl857Q::cat, was used to infect strain K37, selecting chloramphenicol resistant defective lysogens that were immune to K4446. The defective phage with the other ing series of crosses. Aimm21/R2$'^{'}$ was constructed by a cross Ad857Q::enf, by appropriate genetic tests for the markers. prophage in the lysogen K4446. Aimm21/R2$'^{'}$ was then crossed PSA

![Figure 4](image-url)

**Figure 4.**—pKL.600 derivatives with inserts from the roc region. Top of the figure is a restriction map of the roc BamHI-HindIII fragment from pKL500BgIroc$^{'}$. The patterned rectangles represent the various fragments cloned from this region. The length of the rectangle provides a relative idea of the size of the fragments. Listed below are the pKL.600 derivatives with schematic representations of the inserts from the roc region. The black lines represents vector DNA and cloned fragments are indicated by patterned rectangles corresponding to the fragments in the restriction map above. Indicated are the restriction sites used for cloning on the plasmid and at the ends of the cloned fragments. * End was blunted before cloning.

$\Delta$roc 97 was constructed by growing $\lambda$ (nin') in hosts with either pKL500$\Delta$roc 97 or pKL500roc$^{'}$. Because the identifying phenotype of $\Delta$roc is the ability to form plaques on a lawn of a nusC60 host, K554 (a host that does not support growth of the parent wild type $\lambda$, which is nin'), we tested the phage resulting from the crosses in K554. The cross with pKL500$\Delta$roc 97 yielded phages ($\Delta$roc 97) that formed plaques on the restrictive hosts at a frequency of ~0.02%, while the crosses with pKL500roc$^{'}$ failed to yield such plaques. $\lambda$IR2$^{218}$ $\Delta$roc 97 was constructed similarly but $\lambda$IR2$^{218}$ instead of $\lambda$ was used in the initial cross.

$\Delta$IR3 was constructed by crossing pLR31IR3 and $\lambda$dl857Q::cat, plLR31IR3, which contains a fragment that includes the mutation $\Delta$IR3 as well as the wild-type $\lambda$ Q gene, was transformed into K4446 containing the Q defective $\lambda$dl857 prophage. Transformants were grown at 32° to log phase and then shifted to 42° to induce the $\lambda$dl857Q::cat prophage. Only recombinant phages with a wild-type $\lambda$ can form plaques and many of these recombinants will have picked up $\Delta$IR3. Viable phage with the deletion were identified by appropriate restriction digestions. A similar procedure was employed to construct $\lambda$IR4 using plasmid pLR31IR4. $\lambda$IR2$^{218}$ $\Delta$IR4 was constructed in a cross between pSAB-BgACR and $\lambda$IR4 selecting for plaque forming plaques on a lawn of the nusC60 host K554.

$\lambda$IR3$^{12}$ $\Delta$IR4, a MR31 derivative with the additional GC nucleotides filling in the ClaI site was created by a cross between $\lambda$IR3$^{12}$ IR4 and pKL500BgI roc$^{'}$SB. Phages with the IR3 ClaI-EcoRV region containing the extra GC bases were identified by plaque hybridization using a IR3 ClaI-EcoRV DNA fragment as the probe.

**RESULTS**

**Rho-dependent termination in the roc region:** Termination signals in the $\lambda$ nin region were assessed for Rho dependency using an E. coli strain carrying the rhoi5(18) mutation, K1024 (Das et al. 1976) and special $\lambda$ derivatives. These $\lambda$ derivatives share the following features that made them useful for the study: two amber mutations in the N gene, which results in $\lambda$ not being able to produce N-mediated antitermination complexes in nonsuppressing hosts; the bio10 deletion-substitution, which eliminates functions that can interfere with growth of N-independent derivatives growing in the absence of N (Court and Oppenheim 1983); and $\lambda$IR2$^{218}$.

**Figure 5.**—pSA derivatives with inserts from the roc region. Top of the figure is a restriction map of the roc BamHI-HindIII fragment from pKL500BgIroc$^{'}$. The patterned rectangles represent the various fragments cloned from this region. The length of the rectangle provides a relative idea of the size of the fragments. Listed below are the pSA derivatives with schematic representations of the inserts from the roc region. The black lines represents vector DNA and cloned fragments are indicated by the patterned rectangles corresponding to the fragments in the restriction map above. Indicated are the restriction sites used for cloning on the plasmid and at the ends of the cloned fragments. * End was blunted before cloning.

![Figure 5](image-url)
the deletion removing the previously identified Rho-independent terminator in the nin region. As shown in Table 5, λbio10Nam7, am53 fails to form plaques on lawns formed from either the rho15(ts) or isogenic rho+ strain. λbio10Nam7, am53 tr2Δ18, on the other hand, does form plaques on a lawn formed from the rho15(ts) host, but not on a lawn formed from the rho+ strain. In contrast, λbio10Nam7, am53nin5 makes plaques on both the rho15(ts) mutant and rho+ hosts, suggesting that the requirement for N action in the nin region is imposed by both Rho-dependent and -independent terminators. The tr2 terminator previously has been shown to be Rho independent (KROGER and HOBOM 1982). This is supported by the observation that λbio10Nam7, am53 does not form plaques on the rho mutant. The fact that deletion of the tr2 Rho-independent terminator is sufficient to permit λbio10Nam7, am53 to form plaques on the rho15(ts) mutant, suggests that the other terminators in the roc portion of the nin region beyond tr2 are likely to be Rho dependent.

**Identifying termination signals within the roc region:** We have been unable to generate simple point mutations in λbioNam7, am53 tr2Δ18 that eliminate termination in the roc region leading us to suspect that roc could contain multiple termination signals. Because the roc deletion covers 1942 bp (LEASON and FRIEDMAN 1988), further characterization was simplified by analyzing segments of the region for terminators. Deletions were generated by BaB1 in the 2.4kb fragment cloned in pKL500roc+ (Figure 2), and termination activity was assessed in strain K3443. The level of galK expression inversely reflects the strength of termination signals cloned between the plac promoter and the galK reporter gene of pKL500 (MCKENNEX et al. 1981). The parent pKL500roc+ scores as Gal+ because of the strong termination signals in the roc region. Screening of the transformants generated from the BaB1-treated plas-
mids on MacConkey galactose plates revealed that 43 of 107 transformants were Gal+. The DNAs from the gal+ colonies were analyzed employing digestions with restriction enzymes to characterize the size of the deleted region (data not shown). We identified Δroc 97 as the smallest deletion yielding a Gal+ phenotype. DNA sequencing revealed that the deletion in this clone extends from bp 42117 to bp 43329. Quantitative measurement of galK expression showed that pKL500-Aroc97 exhibited 78% read-through relative to pKL500 (set at 100% read-through), approximately the same as that of pKL500Δroc (Figure 2 and Table 4).

To establish that Δroc 97 covers biologically significant terminator regions, we tested its effect on λ derivatives with different requirements for N antitermination. The Δroc 97 mutation was crossed to two λ derivatives: λnin+ with a wild-type nin region and λR2Δ3/4 with a deletion of the Rho-independent terminator, tR2 (see MATERIALS AND METHODS). As shown in Figure 6, Δroc 97 confers the same level of N independence to λ as does the original Δroc deletion (assessed by plating on E. coli with nus mutations).

To more precisely define the termination signals in this 1212-bp fragment deleted by Δroc 97, we exploited the central location of the AflII site to divide the roc region. Two plasmids derived from pKL500Δroc 97 were constructed as described in MATERIALS AND METHODS and detailed in Figure 2. The first, pKL500rocR, has wild-type sequences to the right of the AflII site and part of Δroc 97, a deletion of 500 bp to the left of the AflII site. The second, pKL500rocL, has wild-type sequences to the left of the AflII site and part of Δroc 97, a deletion of 700 bp to the right of the AflII site. The terminator activities of pKL500rocR and pKL500rocL were tested by measuring galK expression (Table 4). Both sides, rocR and rocL, must contain termination signal(s) because read-through in both plasmids, as shown by the levels of galK expression, is low, 3 and 8%, respectively. The termination regions on the left and right sides of the AflII site will be referred to, respectively, as the tR3 and tR4 regions. Phages with deletions of tR3 (λΔtR3) or tR4 (λΔtR4) form plaques on a lawn of the nus+ host, K37, but not on a lawn of the nusC60 host, K554 (Figure 6) confirming that either termination region functions independently in the phage.

**Strategy for isolating mutations in tR3 and tR4:** To identify the tR3 and tR4 termination signals, point or small deletion mutations in the roc region influencing termination were selected. Because λΔtR3 and λΔtR4 fail to form plaques on a lawn of the K554 nusC60 host, while λΔroc (ΔtR3-ΔtR4) does, we reasoned that a mutation in the undeleted portion (e.g., a point mutation in tR3 coupled with ΔtR4) of the roc region inactivating the termination signal(s) coupled with the deletion in the other portion should result in a phage that forms plaques on a lawn of K554.

Mutants forming plaques on a lawn of K554 were selected from lysates of λΔtR3 and λΔtR4 following one round of growth in a mutD5 mutator strain of E. coli (K1617) (Echols et al. 1983). The DNA from mutant phages were analyzed to determine the nature of the mutation. A series of restriction enzyme generated fragments from the mutant phage DNA, all of which included the roc region, were subcloned into pBR322-derived plasmids (Figure 5). Further digestion of these constructs with restriction enzymes provided a series of small fragments that could be compared with analogously cloned fragments derived from wild-type phage DNA. We found no evidence of deletions (data not shown). From these subclones of mutant phage DNA, we identified those containing the new mutations by marker rescue; recombinants that formed plaques on K554 were limited to fragments containing the tR3 region when the cross was with λΔtR4 and to fragments containing the tR4 region when the cross was with λΔtR3. These new mutations were named according to their position on the λ genetic map as tR3+ and tR4+.

**Table 4**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Insert (kb)</th>
<th>galK expression*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pKL500</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>pKL500roc*</td>
<td>BamHI-HindIII (bp 41732-44141) (2.4)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>pKL500Δroc</td>
<td>BamHI-HindIII (bp 41732-44141) with Δroc (0.4)</td>
<td>81</td>
</tr>
<tr>
<td>pKL500Δroc 97</td>
<td>BamHI-HindIII (bp 41732-44141) with Δroc 97 (1.2)</td>
<td>78</td>
</tr>
<tr>
<td>pKL500BglIIroc*</td>
<td>BamHI-HindIII (bp 41732-44141) from Δroc, AflII site replaced with BglII (2.4)</td>
<td>3</td>
</tr>
<tr>
<td>pKL500rocR (tR4+)</td>
<td>BamHI-HindIII (bp 41732-44141) with ΔtR3 (1.9)</td>
<td>8</td>
</tr>
<tr>
<td>pKL500rocL (tR3+)</td>
<td>BamHI-HindIII (bp 41732-44141) with ΔtR4 (1.7)</td>
<td>3</td>
</tr>
<tr>
<td>pKL500SB (tR3+)</td>
<td>SphI-BglII (bp 41905-42630) (0.9)</td>
<td>2</td>
</tr>
<tr>
<td>pKL500CB (tR3+)</td>
<td>ClaI-BglII (bp 42020-42630) (0.6)</td>
<td>2</td>
</tr>
<tr>
<td>pKL500RB (tR3+)</td>
<td>EcoRI-BglII (bp 42231-42630) (0.4)</td>
<td>12</td>
</tr>
<tr>
<td>pKL500SB (tR3+)</td>
<td>SphI-BglII (bp 41905-42630) from λR3Δ (0.9)</td>
<td>5</td>
</tr>
</tbody>
</table>

*Galactokinase levels are presented as values relative to that expressed by the pKL500 vector without an insert.
Identification of the \( \text{tR}^3 \) mutation: Marker rescue employing plasmids containing cloned fragments from the \( \lambda \text{R}^3 \) mutant were used to locate the \( \text{tR}^3 \) mutation (see Figure 1). Crosses of \( \lambda \Delta \text{tR}^4 \) with both pKL500SB(\( \text{tR}^3 \)), containing the SspI-BgII fragment (extending from bp 41905 to 42630), and pKL500-CB(\( \text{tR}^3 \)), containing the Clal-BgII fragment (extending from bp 42020 to 42630), yielded recombinants that plated on K554. However, crosses with pKL500-RB(\( \text{tR}^3 \)), containing the EcoRV-BgII fragment (extending from bp 42231 to 42630) did not yield these recombinants. Comparison of the sequences of the Clal-BgII fragments from phages either with a wild-type \( \text{roc} \) region or the \( \text{tR}^3 \) mutation revealed one difference, the mutant has an insertion of a G in a run of four Gs located in \( \lambda \) sequence 42055–42058 (Figure 7). Thus, the mutant phage \( \lambda \Delta \text{tR}^3 \text{tR}^4 \) has a frameshift that fuses the \( \text{ninD} \) and \( \text{ninE} \) reading frames. The failure in transcription termination could result either from a change in the sequence signaling transcription termination or from occlusion of the transcription termination signal by ribosomes translating the message beyond the frameshift mutation.

Translation read-through occludes the \( \text{tR}^3 \) terminator: To test if the relief of termination caused by the \( \text{tR}^3 \) G insertion results from translation readthrough from \( \text{ninD} \) into \( \text{ninE} \), a second mutation was used to restore the \( \text{ninD} \) reading frame without changing the \( \text{tR}^3 \) mutation. Plasmid pKL500SB(\( \text{tR}^3^{+2} \)) was constructed by filling in the Clal site (bp 42020) in a cloned \( \text{tR}^3 \) fragment. This added two extra base pairs to the \( \text{ninD} \) region with the \( \text{tR}^3 \) mutation (Figure 7). \( \lambda \Delta \text{R}^3^{+2} \Delta \text{tR}^4 \), created by recombination with this plasmid (see MATERIALS AND METHODS), fails to form plaques on a lawn of the K554 lawn while the reconstructed \( \lambda \Delta \text{R}^3 \Delta \text{tR}^4 \) forms plaques on this \( \text{nusC60} \) host. Thus, the two bases reestablishing the \( \text{ninD} \) reading frame restored the wild-type terminator phenotype, a result consistent with the argument that the \( \text{tR}^3 \) mutation changes the translation reading frame of \( \text{ninD} \), fusing it to \( \text{ninE} \), causing interference with the \( \text{tR}^3 \) termination signal within the \( \text{ninE} \) open reading frame. Because \( \text{ninD} \) overlaps \( \text{ninE} \) and \( \text{ninE} \) has a GUG initiation codon, we assume that \( \text{ninE} \) is, at best, poorly translated.

If translational read-through from \( \text{ninD} \) into \( \text{ninE} \) is responsible for the failure in termination imposed by the \( \text{tR}^3 \) mutation, then the mutation should not influence termination if translation from \( \text{ninD} \) is eliminated. Indeed, we find that the inserts in pKL500SB(\( \text{tR}^3 \)) and pKL500SB(\( \text{tR}^3^{+2} \)), cloned so that \( \text{ninD} \) is not translated, exhibit similar high levels of termination (data not shown).

Identification of termination signals in \( \text{ninE} \): To confirm the implication of the previous experiments and show that there is a termination signal in the \( \text{ninD-ninE} \) region, we tested whether the Clal-EcoRV fragment (bp 42020–42231) in pKL500CR(\( \text{tR}^3 \)), which includes the region implicated in termination, has transcription termination activity. pKL500 is a vector similar to pKL500, but contains translation stop codons in all three reading frames upstream of the multicloning site. As shown in Table 5, pKL500CR(\( \text{tR}^3 \)) reduces readthrough confirming that the Clal-EcoRV fragment has terminator activity. For comparison, results of GalK assays with control plasmids having intact or deleted \( \text{roc} \) regions are also shown Table 5.

Analysis of \( \text{tR}^4 \): Mutational analysis failed to provide any definitive information about the \( \text{tR}^4 \) termination signal(s). In an attempt to locate the \( \text{tR}^4 \) termination signal(s), we analyzed the \( \text{tR}^4 \) mutation. The \( \text{tR}^4 \) mutation together with \( \Delta \text{tR}^3 \) was cloned in a BamHI-EcoRI fragment (bp 41792–44972) into pBR325 creating pBBE(\( \Delta \text{tR}^3 \text{tR}^4 \)). The \( \text{tR}^4 \) mutation could be rescued from this fragment because recombinant phages forming plaques on a lawn of \( \text{nusC60} \) were obtained from a cross between this plasmid and \( \lambda \Delta \text{tR}^3 \). The \( \text{tR}^4 \) region from the \( \text{tR}^4 \) mutant (from bp 42630 to 43329) which is covered by the \( \Delta \text{roc}^97 \) deletion and should contain the biologically significant termination signals) was sequenced and the result was compared with the wild-type sequence (data not shown). Surprisingly, no sequence
Therefore, it is likely that the mutation we have called within the termination for X lytic growth (COSTANTINO et al. 1990). For the tR4 terminator, could be deduced from the observation that the Rho-independent tR2 signal was the first terminator identified in the nin region (KROGER and HOBOM 1982). The roe region, located downstream of tR2, is defined by a 1.9-kb deletion that reduces the dependence on N antitermination for λ lytic growth (LEASON and FRIEDMAN 1988).

In addition to transcription termination signals, the nin region also encodes 10 ORFs: ren and ninA-ninI, each preceded by a ribosome recognition signal (KROGER and HOBOM 1982) (Figure 1). These 10 ORFs together with genes O, P, and Q are arranged in an overlapping pattern; except for ren and ninA, there is no intercistronic region between genes or ORFs. In the junctions between most of the consecutive ORFs in the nin region, the sequence ATGA is found. These ATGs could serve as translation initiation codons and the TGAs could serve as potential termination codons for the preceding genes. The exceptions are the ninD/E, ninF/G, and ninH/I junctions. The ninDand ninEORFs overlap with each other by 10 codons, ninF and ninG by three codons, and ninH and ninI by seven codons.

In prokaryotes, transcription termination can be greatly influenced by translation because of the coupling of transcription and translation. The translating ribosomes may interfere with transcription termination by altering, either directly or indirectly, the secondary RNA structure of termination signals (WRIGHT and HAYWARD 1987; LANDICK and TURNBOUGH 1992) or by obstructing the movement of Rho along the RNA towards the RNA polymerase paused at the terminator (PLATT and RICHARDSON 1992). Alternatively, ribosomes might directly mask termination signals so that they are not recognized by Rho or other termination proteins. Considering the arrangement of the consecutive open reading frames in the nin region in the absence of N, it is plausible to assume that transcription termination here might depend, in part, on the translation pattern of the nin ORFs. Thus, factors that influence translation could indirectly influence the pattern of termination in the nin region and alter the course of regulation of late gene expression. Moreover, studies by REYNOLDS

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Insert (kb)</th>
<th>galK expression*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pKL600</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>pKL600roc*</td>
<td>BamHI-HindIII</td>
<td>(bp 41732-44141)</td>
</tr>
<tr>
<td>pKL600BglIIroc*</td>
<td>BamHI-HindIII</td>
<td>(bp 41732-44141)</td>
</tr>
<tr>
<td>pKL600Δroc</td>
<td>BamHI-HindIII</td>
<td>(bp 41732-44141)</td>
</tr>
<tr>
<td>pKL600CR (tR3*)</td>
<td>ClaI-EcoRV</td>
<td>(bp 42020-42231)</td>
</tr>
<tr>
<td>pKL600BS (tR4*)</td>
<td>BglII-SspI</td>
<td>(bp 42630-42825)</td>
</tr>
</tbody>
</table>

*Galactokinase levels are presented as values relative to that expressed by the pKL600 vector without an insert.

change was found in this region indicating that the tR4* mutation is located downstream of the right-hand boundary of the Δro97 deletion. The bhp mutation, which creates a new promoter in this downstream region, significantly reduces the requirement for N antitermination for λ lytic growth (COSTANTINO et al. 1990). Therefore, it is likely that the mutation we have called tR4* does not directly affect the tR4 terminator, but may indirectly obviate its action as does the bhp mutation. Therefore, we decided against further analysis of tR4*.

The limits of the right end of the termination signals in the nin region, which should serve as a similar limit for the tR4 terminator, could be deduced from the observation that λ derivatives with the nin3 deletion (see Figure 1) grow in the absence of N antitermination (COSTANTINO et al. 1990). Because the fusion of the upstream ren gene and the ninGORF caused by the nin3 deletion results in a new reading frame that terminates within ninG, translation from ninG into ninH cannot explain the reduction of transcription termination caused by the nin3 deletion. Thus, significant terminators in the nin region must be removed by the nin3 deletion. This means that since the right-hand boundary of nin3 is bp 42828, therefore tR4 is probably located somewhere between the BglII site at bp 42630 and this right-hand boundary; i.e., between bp 42630 and 42828. Studies with pKL600BS (tR4*), a plasmid derivative with the BglII-SspI insert (from bp 42630-42825) support this argument. As shown in Table 5, this insert exhibits significant termination activity.

**DISCUSSION**

In this paper, we present studies on the roe region of the λ genome, a region with a subset of the transcription termination signals found in the nin region. The nin region, located between genes P and Q, has been defined by large deletions, such as the 2.8-kb nin5 and 2.5-kb nin3 deletions, which allow the growth of λ derivatives defective in N antitermination (COURT and SATO 1969). For the wild-type phage, the N-antitermination system composed of the λ-encoded protein N, the λ nut site, and E. coli Nus factors, permits transcription to pass through termination signals including those in the nin region (FRIEDMAN 1988; DAS 1992; GREENBLATT et al. 1993; ROBERTS 1993). Termination in the nin region blocks expression of the Q gene, whose product is a second transcription antitermination function required for effective levels of late gene expression. This explains why the nin deletions, which remove the nin termination signals, obviate the need for N antitermination. The Rho-independent tR2 signal was the first terminator identified in the nin region (KROGER and HOBOM 1982). The roe region, located downstream of tR2, is defined by a 1.9-kb deletion that reduces the dependence on N antitermination for λ lytic growth (LEASON and FRIEDMAN 1988).

In addition to transcription termination signals, the nin region also encodes 10 ORFs: ren and ninA-ninI, each preceded by a ribosome recognition signal (KROGER and HOBOM 1982) (Figure 1). These 10 ORFs together with genes O, P, and Q are arranged in an overlapping pattern; except for ren and ninA, there is no intercistronic region between genes or ORFs. In the junctions between most of the consecutive ORFs in the nin region, the sequence ATGA is found. These ATGs could serve as translation initiation codons and the TGAs could serve as potential termination codons for the preceding genes. The exceptions are the ninD/E, ninF/G, and ninH/I junctions. The ninDand ninEORFs overlap with each other by 10 codons, ninF and ninG by three codons, and ninH and ninI by seven codons.

In prokaryotes, transcription termination can be greatly influenced by translation because of the coupling of transcription and translation. The translating ribosomes may interfere with transcription termination by altering, either directly or indirectly, the secondary RNA structure of termination signals (WRIGHT and HAYWARD 1987; LANDICK and TURNBOUGH 1992) or by obstructing the movement of Rho along the RNA towards the RNA polymerase paused at the terminator (PLATT and RICHARDSON 1992). Alternatively, ribosomes might directly mask termination signals so that they are not recognized by Rho or other termination proteins. Considering the arrangement of the consecutive open reading frames in the nin region in the absence of N, it is plausible to assume that transcription termination here might depend, in part, on the translation pattern of the nin ORFs. Thus, factors that influence translation could indirectly influence the pattern of termination in the nin region and alter the course of regulation of late gene expression. Moreover, studies by REYNOLDS
and coworkers (REYNOLDS and CHAMBERLIN 1992; REYNOLDS et al. 1992) suggest that the activities of terminators may vary depending on the context. It is thus important to consider that the activities of a signal assessed in a terminator testing vector may not faithfully reflect the activity of the terminator in its normal context. With this in mind, we have in most cases tested signals both in the tester vector to quantify the level of termination and in situ to assess biological significance.

Our studies indicate that the biologically significant termination in the roe region are Rho dependent. We infer this from our findings that if tR2 terminator activity is eliminated, N-mediated antitermination is not required for Q expression providing that either the phage genome is deleted for the roe region (Δroe 97) or the host bacterium has reduced Rho activity. The 1.2-kb region defined by Δroe 97, extending from bp 42117 to 43329 downstream of tR2, could further be divided into subregions of terminator activity, tR3 and tR4. Although we have attempted to isolate point mutations that reduce Rho-dependent termination in the tR3-tR4 region, these mutations failed to identify a unique sequence required for Rho action. One of the mutations, tR3Δ, caused a shift in the translation reading frame and the other, tR4*, lies out of the region. The tR4* mutation lies downstream of tR4 and could, like the byp mutation, cause partial N independence by creating a new promoter (COSTANTINO et al. 1990). The inability to exploit mutations to identify a sequence necessary for Rho termination is not surprising. Previous studies (PLATT and RICHARDSON 1992) have concluded that though there is undoubtedly some type of signal or signals in the RNA for Rho-dependent transcription termination, the signal(s) may rely more on structure than sequence. However, ALIFANO et al. (1991) identified one sequence feature common to a number of Rho-dependent terminator regions, RNA rich in cytosine and poor in guanine. Consistent with this observation, we find that both the region of the λ genome containing tR3, nucleotides 42,160 to 42,190, and that identified as containing tR4, nucleotides 42,660 to 42,700, are C rich and G poor.

The tR3 region: To identify biologically significant tR3 terminator(s), we looked for point mutations that might locate the specific sequences signaling termination. The tR3Δ mutation combined with a tR4 deletion confers a phenotype identical to that conferred by roeΔ97. Rather than altering a termination signal, the tR3Δ mutation, a G insertion in a run of four Gs in the ninD region, shifts the reading frame of ninD and fuses it to ninE. This change in reading frame is likely to be the cause of the loss of termination observed with the tR3Δ mutation, because a second mutation near tR3 that restores the original reading frame restores termination. We suggest that the tR3 terminator is in ninE and that in wild type λ ninE is poorly, if at all, translated.

The inference that the ninE region contains terminator(s) of biological significance was substantiated in two ways. First, we cloned the 211-bp Clal-EcoRV fragment covering most of the ninE region in the terminator tester vector pKL600 and demonstrated that the cloned fragment has terminator activity (Table 5). Second, we constructed a λ derivative with a deletion of the 211-bp Clal-EcoRV fragment, ΔtR3ΔCR and ΔtR4 and showed that this phage, λΔtR3ΔCRΔtR4, is partially N independent (Figure 6).

The tR4 region: Deletion analysis showed that a tR4 subregion, the 195-bp BglII-SspI (bp 42630–42825) region, has biologically significant terminator activity. This was shown by the observation that a deletion of 700-bp region to the right of the A/J site in the nin region is necessary but not sufficient for N-independent λ growth. We were unsuccessful in further defining this terminator by any point mutations in the region that reduced termination. However, the nin3 deletion, which fuses the ren and ninG reading frames so that translation terminates in ninG, allows λ to grow independently of N. To allow N-independent growth, the nin3 deletion must remove all of the significant terminators in the nin region. Consequently, tR4 must be located proximal to the right-hand boundary of nin3. Consistent with this argument, studies with the terminator-tester system show that the 195-bp BglII-SspI fragment whose right-hand border is at bp 42825 has significant terminator activity. We speculate that the tR4 terminator lies within ninG. This implies that normally ninG is inefficiently translated.

The genetic makeup of the nin region: The nin region has been considered “nonessential” because nin deletions neither affect lytic growth nor the lysogeny frequency under laboratory conditions (COURT and OPPENHEIM 1983). Among the 10 nin ORFs only ren, ninB, ninG and ninI have been assigned functions (Figure 1). The gene product of ren protects phages from exclusion imposed by rex gene product of other phages (TOOTHAMAN and HERSKOWITZ 1980), while that of ninB is involved in recombination via the host RecF pathway (SAWITZKE and STAHL 1992). The presence of ninG (or rap) increases the frequency of phage-plasmid homologous recombination via the RecBC pathway (HOLLIFIELD et al. 1987) and the ninI product has protein phosphatase activity (COHEN et al. 1988; P. COHEN and P. T. COHEN 1989; P. T. COHEN and P. COHEN 1989). Whether the other nin ORFs are expressed is not known although a protein band associated with the induction of the defective Adv phage of the proper size has been identified with ninG (AKABOSHI and MATSUBARA 1981). Unpublished studies in this laboratory and work in other laboratories (KROGER and HOBOM 1982) have revealed that regions of ninG, ninG, and ninH cannot be cloned under a strong promoter, suggesting that the products from these ORFs are lethal to the host cells. Whether these products or the termination signals defined here play roles in phage growth under conditions that differ from those in the laboratory is currently un-
known. The fact that the nin region has not been lost from λ suggests that it has a biologically significant role. This could be regulatory, involving the transcription termination signals or functional, involving the products of some of the ORFs. Obviously, there could be a combination of both. It is not difficult to conceive of scenarios where products of ORFs with known functions might aid phage development. A phosphatase activity might be useful if there were a phosphorylated protein that interfered with some aspect of phage development. The ren, ninB, and ninG ORFs could provide an advantage to the phage by protecting it from exclusion by other phages (ren), or providing additional recombination functions (ninB and ninG). It is conceivable, that the DNA in the nin region does not play a specific biological role other then, perhaps, provide "stuffer" DNA to assure a correctly sized genome for proper packaging (Bellett et al. 1971; Wei, et al. 1972). According to this scenario, the nin region could carry any set of bacterial genes. Antitermination, would serve, at least in part, to allow transcription to proceed through the acquired DNA if it has transcription termination signals. We think this unlikely, because some of the genes in the nin region encode functions useful to the phage (Hollifield et al. 1987; Sawitzke and Stahl 1992). However, it is of interest to note that another lambdoid phage, H19B, carries the genes for a Shiga-like toxin in the nin-analog of that phage (Weiss et al. 1990). As the name implies, the toxin gene is closely related to the toxin gene found in another enteric bacterium, Shigella, closely related to E. coli. It seems reasonable to assume that the toxin gene and the associated sequences were acquired from a bacterial chromosome. If the nin region marks a site in the lambdoid phage genomes that can acquire bacterial genes, the question arises as to how these genes are acquired. One identified mechanism for such an acquisition is through illegitimate recombination of the integrated prophage (Weisberg and Landy 1983). This recombination results in the excision of the prophage with the acquired bacterial genes adjacent to the integration, or attachment (att), site of the prophage. If a similar model of integration and excision is invoked to explain events at the nin region, it is necessary to entertain the possibility that the nin region can serve as a site for integration, att, of the phage genome into the bacterial chromosome. Unfortunately, beyond this circumstantial argument there is no evidence that the nin region can serve as an attachment site.

This work was supported by National Institute of Allergy and Infectious Diseases grants AI-1459-10 and M01-RR00452 (for sequence analysis) and in part by the National Cancer Institute, DHHS, under contract with ABL. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, organizations imply endorsement by the U.S. Government.

LITERATURE CITED


A nin Region Termination Signals


Communicating editor: M. M. HOWE.