Domains for Protein-Protein Interactions at the N and C Termini of the Large Subunit of Bacteriophage λ Terminase

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ABSTRACT

The large subunit of phage λ terminase, gpA, the gene product of the phage A gene, interacts with the small subunit, gpNu1, to form functional terminase. Terminase binds to λ DNA at cosB to form a binary complex. The terminase:DNA complex binds a prohead to form a ternary complex. Terminase also binds the prohead, the empty protein shell into which DNA is packaged. In binding DNA, including 593 codons of the A gene. The chimeric-gp2/A of hybrid 67 binds gp1 to form functional terminase. It is concluded that the specificity determinants for small subunit binding reside in the N-terminal 48 amino acids of gpA and gp2. The primary structures of these 48 amino acid segments of gpA and gp2 are quite different, especially in the first half and the predicted secondary structures are also quite different. The DNA sequence of the prohead binding domain of gp2 was determined. The derived amino acid sequence was found to be quite different from the λ prohead binding domain for the C-terminal 32 amino acids, but the predicted secondary structures were found to be similar. It is suggested that these small functional domains, found at the termini of the gpA polypeptide chain, act as "straps" in protein-protein assemblies.

Bacteriophages have proved to be good objects for study of the formation of biological structures (Casjens 1985). Such studies show that the structural components generally self-assemble in an ordered series of reactions. Ordered assembly is achieved by sequential activation of subunits on the growing structure, as follows. When a soluble subunit binds to a growing structure it undergoes a conformational change making it reactive toward the next soluble subunit. While there is detailed information for a number of phages about the order of assembly during formation of a structure, little is known about the molecular interactions involved in assembly.

Terminase, the DNA packaging enzyme of phage λ, plays a central role in DNA packaging during head assembly (Feiss 1986). In the first step of DNA packaging, terminase binds to concatemeric λ DNA, the DNA packaging substrate. Concatemeric λ DNA, the product of rolling circle replication and recombination, consists of end-to-end multimers of λ chromosomes. The mature λ DNA molecule, as isolated from the virion, is a linear duplex of 48,502 base pairs (Sanger et al. 1982). At the 5' ends of the strands are single-stranded and complementary segments, 12 bases long, called cohesive ends (Wu and Taylor 1971). Upon injection of the λ DNA into a cell the cohesive ends anneal, cyclizing the molecule. At late times, concatemeric DNA accumulates and is packaged. In vitro assembly of λ DNA requires 12 bases of cohesive ends, and the DNA is packaged. In binding λ DNA and prohead, terminase brings together the components necessary for packaging. Another function of terminase is to introduce staggered nicks to generate the cohesive ends (Becker and Gold 1978). The λ DNA site where terminase binds and nicks is called the cohesive end site, cos. cos is actually composed of two sites, cosB, where terminase initially binds, and cosN, where the nicks are introduced (Miwa and Matsubara 1983; Feiss et al. 1983; see Figure 1). Terminase also binds the prohead, the empty protein shell into which DNA is packaged. In vitro assembly...
studies indicate terminase first binds λ DNA at cosB and then binds the prohead (Becker, Murialdo and Gold 1977). The timing of cos cleavage relative to prohead binding is not clear (Murialdo and Feiss 1984).

Terminase is a heterooligomer. The small subunit, with 181 residues as deduced from the DNA sequence (Sanger et al. 1982), is gpNul (the product of gene Nu1, etc.), and the large subunit, with 641 residues as deduced from the DNA sequence, is gpa. Studies with λ-21 hybrids indicate terminase is organized into an array of functional domains. Phage 21 is a relative of λ, and the head gene regions of the two phages are descended from a common ancestor (Simon, Davis and Davidson 1971). 21 DNA has cohesive ends identical to those of λ, and the 21 terminase consists of a small subunit, gp1, and a large subunit, gp2. Gp1 has the same number of amino acids as gpNu1 and has ca. 50% sequence identity with gpNu1. Gp2 has ca. 70% sequence identity with gpA in the N-terminal half that has been compared (Miller and Feiss 1985). In spite of the strong similarities of the λ and 21 terminases, they have different specificities for binding to cosB and for prohead binding (Hohn 1975; Siegele et al. 1983). For example, λ terminase will bind to λ DNA at cosB but not to 21 DNA, which presumably has a different cosB (Feiss and Widmer 1982). Formation of the terminase multimer is also specific, so that the small subunit of one phage cannot form functional terminase with the large subunit of the other phage. Only the cosN specificity of the two phages is the same, as expected since the cohesive ends are the same.

Because of the partial sequence identity of the λ and 21 terminase genes, it is possible to cross λ and 21 to generate recombinant terminase genes that encode chimeric subunits. One recombinant phage, λ-21 hybrid 51, produces a chimeric gp1/Nu1, such that the N-terminal half is homologous to gp1 and the C-terminal half is homologous to gpNu1 (Frackman, Siegele and Feiss 1985). The hybrid 51 small subunit is specific for 21 DNA, indicating that N-terminal half contains a DNA binding domain. It is assumed that both gp1 and gpNu1 have a DNA binding domain in the N-terminal half, since the chimeric proteins studied so far have either one specificity or the other, as though the specificities are alternatives. The domain thus defined is a specificity domain, in which reside the specificity determinants by which λ and 21 differ. Whether a specificity domain proves to also be a structural domain remains to be shown. The hybrid 51 small subunit assembles with gpA to form functional terminase, indicating that the C-terminal half contains a domain for subunit-subunit interactions. Two other hybrids produce a chimeric large subunit. λ-21 hybrid 33 produces a terminase identical to the 21 terminase except that the C-terminal 38 amino acids of the large subunit are from λ (Frackman, Siegele and Feiss 1984). The gp2/A of hybrid 33, which is identical to gp2 except for the C terminus, binds λ proheads, indicating that the C-terminal 38 amino acids contain a domain for prohead binding. A third hybrid, λ-21 hybrid 54, produces a chimeric large subunit for which the N-terminal third is derived from 21 and the remainder from λ (Frackman, Siegele and Feiss 1985). This gp2/A assembles functional terminase with gp1, indicating that in the N-terminal third there is a specificity domain for interaction with the small subunit.

In the present work we have extended the previous experiments. Because the prohead binding domain at the C terminus of the large subunit was so small, we were curious whether the N-terminal domain for binding the small subunit was also small. We also wanted to compare the amino acid sequences of these domains to see whether the specificity differences would be reflected in sequence differences.

MATERIALS AND METHODS

Bacteria, phages and plasmids: Escherichia coli K12 strains used were the C600, (supE; Campbell 1961), R594 (supC1 or nonsuppressing; Campbell 1961); MF611, a recA1 strain (Feiss et al. 1977); and JM103, the host for the M13mp19 cloning vector (Messing, Crea and Seeburg 1983).

Phages were used λ Aam32 (Campbell 1961), λ cos57 Nu1lam3 (the Nu1lam3 marker is the lam3 marker of φ80: the cos57 I gene is functionally equivalent to the Nu1 I gene of λ (Weisberg, Stenberg and Gallay 1979); λ-21 hybrid 19 and amber mutant derivatives (hybrid 19 contains the entire head gene region of phage 21, Siegele et al. 1983); λ-21 hybrid 33 and amber derivatives (Frackman, Siegele and Feiss 1984), and M13mp19 (Messing 1983). Isolation of λ-21 hybrid 67 is described in results. λ-21 hybrid 67 lam3 was isolated from a cross of λ-21 hybrid 67 vs. PVJ5.
Plasmids were used derivatives of pBR322 (Rodriguez et al. 1977) carrying the inserts shown in Figure 2. With the exception of pWX2, all the inserts were cloned into the homologous restriction enzyme sites of pBR322. The plasmids were constructed using methods of Maniatis, Fritsch and Sambrook (1982). pSF1 contains a HindIII to BamHI insert of λ DNA that includes the late promoter and the Nul and A genes (Feiss et al. 1982). pW3 is an analogous plasmid that contains the late promoter and the phage 21 1 and 2 genes; pVJ5 is a derivative of pW3 containing the lam5 mutation (Siegele et al. 1983). pWX1 was made by digesting pSF1 with AatI enzyme, followed by ligation. pWX2 was made by digesting pWX1 with HindIII, removal of the cohesive ends with S1 nuclease, digestion with HpaI, and ligation of the blunt ends, a procedure that deleted the Nul gene. pWF1 consists of a HindIII-to-BamHI insert that includes the λ-21 hybrid 67 terminase genes.

Media: Tryptone broth (TB), top agar and bottom agar were as described by Arber et al. (1984), except each was supplemented with 0.01 M MgSO₄. L broth (LB) and LB agar were as described by Arber et al. (1984). X-gal plates were prepared as described by Messing (1983). As necessary, ampicillin was added to 100 μg/ml and maltose to 0.2%.

Enzymes and isotopes: Restriction enzymes were purchased from New England Biolabs and Bethesda Research Laboratories. T4 DNA ligase and DNA polymerase 1 (Klenow fragment) were from Boehringer-Mannheim. [α-32P]dATP was from Amersham. M13mp19 RF and the 17-base oligonucleotide primer were obtained from Bethesda Research Laboratories. Mixes of deoxy- and dideoxynucleotides were purchased from New England Biolabs.

DNA sequencing: The method of Messing, Crea and Seeberg (1983), was used to prepare single-stranded template DNA, and the dideoxynucleotide chain-termination method for sequencing was used (Sanger et al. 1980). Sequencing gels were prepared as described by Maxam and Gilbert (1980).

RESULTS

Size of the small subunit binding domain of gpA and gp2: We wished to construct a λ-21 hybrid that would produce a terminase containing a chimeric large subunit, with the amino terminus derived from 21 and the remainder from λ. The phage vs. plasmid cross shown in Figure 3 was done to generate such a hybrid. The phage parent was λ-21 hybrid 33 2am149, a λ-21 hybrid that has terminase genes almost entirely derived from 21. In λ-21 hybrid 33, only the carboxy-terminal 38 codons of the large subunit are from λ. The parent phage has an amber mutation in the large subunit, so it cannot grow on cells lacking a suppressor. The plasmid parent was pWX2, a plasmid carrying the A gene but deleted for the Nul gene. In the cross, homologous recombination on either side of the 2am149 mutation replaces a DNA segment containing the mutation with a corresponding segment of the A gene of pWX2 that does not contain an amber mutation. The crossover to the right of the 2am149 mutation is between homologous segments of λ DNA, and the crossover to the left of the 2am149 mutation is between the 2 gene of the phage and the A gene of the plasmid. Such recombinants were selected as plaque-forming
phages on a nonsuppressing host and one, designated A-21 hybrid 67, was purified for further study.

In order to ask whether A-21 hybrid 67 had the expected structure and to find the site of the crossover, a restriction enzyme site map of the terminase genes of A-21 hybrid 67 was compared with the sites of A and 21. The analysis showed that A-21 hybrid 67 was the result of a crossover that occurred in a segment between an AvaI site of 21 at 849, which the hybrid has, and a HpaI site of 21 at 922, which the hybrid lacked. To determine the crossover site, the appropriate segment of A-21 hybrid 67 DNA was sequenced. As shown in Figure 4, the crossover occurred in a homologous segment extending from 861 to 874 (A coordinates). The 21 DNA extends from the left cohesive end through cosB+21 and the 1 gene, to a site 48 codons into gene 2. A DNA continues from the crossover site through the rest of the chromosome. The gp2lA subunit of A-21 hybrid 67 contains 48 amino acids at the amino terminus that are homologous to the N-terminal 48 amino acids of gp2, followed by a segment of 9 amino acids common to both gp2 and gpA, followed by 584 amino acids homologous to gpA, for a total of 641 amino acids. It should be noted that the amino acid sequences of the two proteins are deduced from the DNA sequences of the presumed 2 and A genes. It is interesting to note that within the 48 amino acids comprising the specificity domain for binding the small subunit, 16 of the first 17 amino acids are different for gp2 and gpA, a point taken up below.

The DNA structure indicates λ-21 hybrid 67 produces a terminase consisting of gp1 and gp2/A, leading to the prediction that hybrid 67 would complement a 1 mutant of 21 but would not complement either a 2 mutant of 21 or an A mutant of λ. λ-21 hybrid 67 should be unable to complement a 2 mutant of 21 because the gp2/A has specificity for the λ prohead, and the hybrid 67 should be unable to complement a A A- mutant because the gp2IA protein should require a gp1 small subunit. Complementation tests were performed to verify this prediction. We used cells carrying plasmids that supplied the terminase (but not the proheads) of A-21 hybrid 67, A, and 21. These cells were infected with A or 21 terminase amber mutants. The plasmids carry the terminase genes under the control of the normal late A promoter, and so expression of the plasmid terminase genes is activated by the gpQ transactivation factor of the infecting phage. The results are shown in Table 1. The plasmid that carries the terminase genes of A-21 hybrid 67 complements a I mutant (infection la) of 21 but not a Nul mutant of A (infection 3a), showing that A-21 hybrid 67 indeed supplies gp1. The A-21 hybrid 67 plasmid does not complement either a 21 2 mutant (line 2a) or a A A mutant (line 4a), a result expected for a hybrid 2/A subunit with specificity for a 21 small subunit and a λ prohead.

A second complementation test demonstrated that the gp2/A protein of λ-21 hybrid 67 would complement a 21 2 mutant λ proheads were provided. The
TABLE 1

Identification of the terminase genes of λ-21 by 67

<table>
<thead>
<tr>
<th>Infection†</th>
<th>Infecting phage‡</th>
<th>Plasmid§</th>
<th>Yield†</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>λ-21 hy 19 lam5</td>
<td>pWF1'</td>
<td>1.4 +</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>λ-21 hy 19 lam5</td>
<td>pSF1'</td>
<td>1.3 × 10⁻³</td>
<td>-</td>
</tr>
<tr>
<td>c</td>
<td>λ-21 hy 19 lam5</td>
<td>pBW3§</td>
<td>1.5 +</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>λ-21 hy 19 lam5</td>
<td>&lt;1.3 × 10⁻³</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>λ-21 hy 19 lam149</td>
<td>pWF1'</td>
<td>4.9 × 10⁻³</td>
<td>-</td>
</tr>
<tr>
<td>b</td>
<td>λ-21 hy 19 lam149</td>
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<td>&lt;3.0 × 10⁻³</td>
<td>-</td>
</tr>
<tr>
<td>c</td>
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<td>pBW3§</td>
<td>6.3 +</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>λ-21 hy 19 lam149</td>
<td>3.0 × 10⁻³</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>λ lam3</td>
<td>pWF1'</td>
<td>1.12 × 10⁻⁴</td>
<td>-</td>
</tr>
<tr>
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<td>pSF1'</td>
<td>2.3 +</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>λ lam3</td>
<td>pBW3§</td>
<td>1.12 × 10⁻⁴</td>
<td>-</td>
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<tr>
<td>d</td>
<td>λ lam3</td>
<td>&lt;1.12 × 10⁻⁴</td>
<td>-</td>
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</tr>
<tr>
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<td>λ Aam32</td>
<td>pWF1'</td>
<td>&lt;2.1 × 10⁻⁴</td>
<td>-</td>
</tr>
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</tr>
<tr>
<td>c</td>
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<td>-</td>
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<td>d</td>
<td>λ Aam32</td>
<td>&lt;2.1 × 10⁻⁴</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

† Complementations of phages by plasmids were performed as described in MATERIALS AND METHODS.
‡ Infecting phages were cl857 red3 or imm21 cl red3.
§ The host was MF611.

The results of Table 2, line 2 show that λ-21 hybrid 67 lam⁻ complemented 21 R⁻. In the complementation the small terminase subunit is provided by the 21 R⁻ parent and the large subunit is the gp2/A of the hybrid 67 parent. Packaging is assumed to be into the λ proheads of the hybrid 67 parent since a negative result was obtained when λ proheads were lacking (Table 1, line 2a). In a control, λ-21 hybrid 67 lam⁻ was unable to complement λ A⁻ (line 4). These genetic results show that the gp2/A subunit of hybrid 67 has specificity for binding the small subunit of 21, gp1, and the prohead of λ. The results of this section indicate that the specificity for binding the small subunit resides in the amino-terminal 48 amino acids of the large subunit of terminase, a segment much smaller than estimated previously (FRACKMAN, SIEGEL AND FEISS 1985).

**Sequencing the prohead binding domain of gp2:**

Although the prohead binding domain was previously localized to the carboxy-terminal 38 amino acids of gp2, the amino acids of gpA, and the second line the amino acids of gp2. The third and fourth lines show the 21 and λ DNA sequences, respectively. The box shows the segment in which the crossover occurred to generate λ-21 hybrid 33. Asterisks indicate translation stop codons. To the right of the stop codons are the 3 and W genes of 21 and λ, respectively.

**Figure 5.—DNA sequences of the carboxy terminal ends of the 2 and A genes and the codon assignments.** The top line shows the amino acids of gpA, and the second line the amino acids of gp2. The third and fourth lines show the 21 and λ DNA sequences, respectively. The box shows the segment in which the crossover occurred to generate λ-21 hybrid 33. Asterisks indicate translation stop codons. To the right of the stop codons are the 3 and W genes of 21 and λ, respectively.
of the large subunit, the sequence of the 21 DNA from this region was unknown. Starting with the previously constructed restriction site map of 21 DNA (Frackman, SIEGELE and FEISS 1985), we constructed a more detailed map of the region around the prohead binding domain. An FnuDII fragment thought to span the domain was sequenced. Sequence similarity to the carboxyl terminus of the A gene was found, indicating that the DNA fragment does encode the carboxyl terminus of gp2 (Figure 5). The deduced amino acid sequence shows that there is strong sequence similarity between gp2 and gpA, as expected from previous sequencing results (Miller and Feiss 1985). The similarity decreases markedly for the final 32 amino acids which comprise the specificity domain for prohead binding.

**Predicted secondary structures of specificity domains:** The C and N termini of gp1 and gp2 are specificity domains for protein-protein interactions, and the amino acid sequences are sharply divergent for the two proteins. We examined the predicted secondary structures of the domains to see whether the secondary structures of the domains had been conserved, even though the primary structures were different. The method of CHOU and FASMAN (1978) was used. Figure 6a shows the predicted secondary structures of the N-terminal 50 amino acids of gp2 and gpA. The amino-terminal segments, roughly comprising the first 15 to 18 residues are very different. GpA is predicted to contain a β-turn from residues 4 to 7 and β-sheet from residues 8 to 15, whereas gp2 is predicted to have an α-helix from residues 1 to 12. To the right of residue 18 the predicted structures are similar, as expected from the similarity of the primary structure.

The predicted structures of the C-terminal 38 amino acids of gpA and gp2 are very similar (Figure 6b). The proteins terminate with an α-helix followed by one (gpA) or two (gp2) β-turns.

**DISCUSSION**

λ-21 hybrid 67 is the product of recombination between λ and 21 within the genes coding the large subunit of terminase. The left end of the λ-21 hybrid 67 chromosome is derived from 21 and extends for 48 codons into the 2 gene. The remainder of the λ-21 hybrid 67 chromosome is from λ. The hybrid gp2/A produced by λ-21 hybrid 67 consist of 48 N-terminal amino acids derived from gp2, a segment of 9 amino acids common to both gp2 and gpA, and 584 amino acids derived from λ. The gp2/A protein differs in structure from gpA for only the amino-terminal 48 amino acids, and differs functionally from gpA in specificity for binding the small subunit. The λ-21 hybrid 67 gp2/A binds gp1 whereas gpA binds gpNul. We conclude that the amino-terminal 48 amino acids are a specificity domain for subunit assembly in the λ and 21 terminases.

The 21 DNA encoding the C-terminal amino acids of gp2 has been sequenced and the amino acid sequence of the C terminus of gp2 has been derived from the DNA codon assignments. Earlier work showed that the C-terminal 38 amino acids of gp2 and gpA determined the prohead binding specificities of the 21 and λ terminases (Frackman, SIEGELE and FEISS 1984); the present sequencing results indicate that the first 6 amino acids of the prohead binding domain are identical between λ and 21, so that the specificity determinants reside in the C-terminal 32 amino acids.

Secondary structure predictions indicate that the N-terminal domains for subunit assembly are likely to be quite different between gp2 and gpA, and that the structure of the prohead binding domains may be similar between gp2 and gpA. We discuss next several questions. Why should the specificity domains for protein-protein interactions be localized in C- and N-terminal domains? Are there protein-protein interactions in addition to those indicated by the specificity domains?

**Protein-protein assembly domains at the ends of polypeptide chains:** Among proteins of known tertiary structure, extended terminal arms are com-
monly found to act as straps between domains (Thornton and Sibanda 1983). Five examples of proteins with terminal arms that act as straps are actinidin, with C- and N-terminal arms of 5 and 17 amino acids, respectively (Baker 1980); beef liver catalase with an N-terminal arm of 35 amino acids (Murthy et al. 1981); tomato bushy stunt virus capsid protein (Harrison et al. 1978); mitochondrial aspartate amino transferase with an N-terminal arm of 14 amino acids (Ford, Eickele and Jansonius 1980); and lactate dehydrogenase with an N-terminal arm of 70 amino acids (Holbrook et al. 1983). Because our previous results demonstrated that the large subunit of terminase has N- and C-terminal segments involved in protein-protein interactions, we suggested that these segments are likely to be straps (Frackman, Siegele and Feiss 1984). The present results add further information consistent with the suggestion of straps. First, the C-terminal domains of gp2 and gpA are shown to be sharply divergent in amino acid sequence. This is expected for domains containing divergent prohead binding specificities. Second, the N-terminal domain is shown to be much smaller than indicated by the previous results. The N-terminal domain is shown to be no more than 48 amino acids long, a size that approaches the size of known straps. As is the case for the C-terminal domains, the N-terminal domains of gp2 and gpA are sharply divergent, especially at the ends: 16 of the first 17 amino acids are completely different.

On straps: The N-terminal domains of gp2 and gpA are sharply different in amino acid sequence and in predicted secondary structure, whereas the C-terminal domains, which also differ sharply in sequence, are predicted to be similar in secondary structure. An examination of the primary and secondary structures of the known straps (cited above) shows, not surprisingly, no conserved sequence or structural motif. The known straps have both ionic and hydrophobic interactions with the targets, involving 5 to 10 residues of the strap, and so we may expect the putative straps of gp2 and gpA to have a similar number of residues involved in interactions with the partner.

It is not clear whether the specificity domains constitute the only interactions of the gpA and gp2 with the prohead and the small subunit. Our work shows only that the specific interactions are confined to these domains. Other interactions would be expected to not be phage specific.

The strong conservation of gene structure and sequence for the 2 and A genes makes it very likely that the two genes are diverged from a common ancestor gene. It could be that the DNA segments encoding the specificity domains, which have much less sequence identity, could have been acquired by some mechanism such as mutation or illegitimate recombination, but it seems most likely that these parts of the A and 2 genes have also diverged from the common ancestor gene. Since there is no structural motif for straps, there would seem to be little constraint as regards secondary structure during the evolution of a new specificity. It is not surprising therefore that the predicted structures of the N-terminal domains are quite different. The situation is quite different for interacting macromolecules in which there is a particular motif. In the case of a DNA-binding protein with the helix-turn-helix motif (Pabo and Sauer 1984), one might expect that the easiest path of divergence would involve change in the amino acids that make specific contacts with DNA base pairs and conservation of amino acids involved in maintenance of the structure of the motif. In the case of the C-terminal domain, the predicted structural similarity might be a consequence of the fact that the target is part of the prohead and might be involved in prohead structure or function in a way that limits structural variation. We have discussed the binding of the prohead by terminase as simply involving an interaction of the terminase strap with a prohead protein, but the situation is probably more complex.

First, there may be other molecules that are involved. One is the λ protein gpFI, which has recently been shown to facilitate the binding of the prohead by the binary DNA:terminase complex (A. Becker, personal communication). GpFI plays an accessory role, because mutants of λ, called fin mutants, can be isolated that are able to grow in the absence of gpFI (Murlaldo et al. 1981). Therefore it is unlikely that gpFI plays a direct role in the recognition of the prohead by the DNA:terminase complex. Also, it is possible that RNA might be involved in the terminase:prohead interaction. It has recently been found that a short RNA molecule can be isolated from the phage λ29 prohead, and that the RNA is essential for λ29 DNA packaging (Guo, Erickson and Anderson 1987). It is possible that the involvement of RNA in DNA packaging may be a general phenomenon that will be found in λ.

Second, there is evidence that the terminase:prohead interaction is functionally complex. Available evidence indicates that terminase must first bind to λ DNA before the prohead is bound (Becker, Murlaldo and Gold 1977). This suggests that a conformational change occurs when terminase binds λ DNA, allowing prohead binding. In the strap model, the conformational change might free the strap from interacting with a site on terminase, allowing the strap to interact with the prohead. This speculation provides a physical model for activation of a protein in an ordered series of assembly reactions. An additional functional complexity comes from the work of Murlaldo and Fife (1987), who have shown that the nicking activity of terminase is modulated by an inhibitor that is encoded by a
segment of the prohead genes. They propose that cutting is prevented by the inhibitor, and that the block is released by the binding of a mature prohead. This mechanism might insure that nicking is accompanied by packaging. Since a terminase:prohead interaction is involved, the prohead binding domain may play a role in this mechanism.

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LITERATURE CITED


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