Sites and Gene Products Involved in Lambdaoid Phage DNA Packaging

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21 is a temperate lambdaoid coliphage, and the genes that encode the head proteins of λ and 21 are descended from a common ancestor bacteriophage. The sequencing of terminase genes I and 2 of 21 was completed, along with that of a segment at the right end of 21 DNA that includes the R4 sequence. The R4 sequence, a site that is likely involved in termination of DNA packaging, was found to be very similar to the R4 sequences of λ and φ80, suggesting that R4 is a recognition site that is not phage specific. DNA packaging by 21 is dependent on a host protein, integration host factor. A series of mutations in gene I (her mutations), which allow integration host factor-independent DNA packaging by 21, were found to be missense changes that affect predicted α-helices in gp1. gp2, the large terminase subunit, is predicted to contain an ATP-binding domain and, perhaps, a second domain important for the cos-cutting activity of terminase. orf1, an open reading frame analogous in position to FI, a λ gene involved in DNA packaging, shares some sequence identity with FI. orf1 was inactivated with nonsense and insertion mutations; these mutations were found not to affect phage growth. 21 was also not able to complement a λ FI mutant.

The morphogenesis of bacteriophage λ has been studied for many years as a model of viral development. Head assembly involves the packaging of viral DNA into an empty protein shell, the prohead (for reviews, see references 5 and 18). The viral DNA substrate for packaging is end-to-end polymers (concatemers) of λ chromosomes. A viral DNA-packaging enzyme, terminase, binds the DNA at a site called cosB and introduces staggered nicks at an adjacent site, cosN, to generate the cohesive ends of mature λ chromosomes. The small subunit of the heterooligomeric terminase binds cosB, and the large subunit, gpA, has the nicking activity (15). GpA also has a domain for binding of the prohead. The prohead is a spherical shell composed primarily of gpE, the major shell subunit; in addition, there is a special structure, called the portal vertex, composed primarily of gpB (for a review, see reference 25). In binding the prohead, terminase is thought to interact with the portal vertex. Following nicking of the DNA and prohead binding, DNA is packaged into the prohead; both subunits of the λ terminase have ATPase activity, and terminase has been proposed to act as a translocase during DNA packaging.

The head genes of 21 and λ are descended from a common ancestor, and the head gene products share ca. 50% sequence identity (35, 44). Comparison of the λ and 21 head gene products has been very useful, because the gene products are similar in size, function, and domain structure. Because of this similarity, features of one protein are expected to be retained in the analogous protein of the other virus. As an example, the small subunit of the λ terminase, gpNu1, contains amino acids characteristic of the helix-turn-helix domain for binding of DNA (32; Andrew Becker, cited in reference 18), and these characteristic amino acids were found to be present in gp1, the small subunit of the 21 terminase (Andrew Becker, cited in reference 18), suggesting that gp1 has the helix-turn-helix domain at the analogous position.

Because the sequence available for 21 has been valuable in understanding lambdaoid phage assembly, we have completed sequencing of the terminase genes of 21, along with the right DNA end. Sequence analysis of the prohead assembly genes is reported elsewhere (44). Genetic studies on orf1, an open reading frame analogous in position to λ FI, were also carried out.

MATERIALS AND METHODS

Media. Tryptone broth, soft agar, and bottom agar, each supplemented with 10 mM MgSO₄, were prepared as described by Arber et al. (1). L broth, 2× YT broth, SOB broth, and MacConkey agar were prepared as described by Maniatis et al. (35). Kanamycin, ampicillin, and tetracycline were added to media at final concentrations of 50, 200, and 12.5 μg/ml, respectively. LB agar containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and isopropyl-β-D-thiogalactopyranoside (IPTG) was prepared as described by Vieira and Messing (45).

Strains. Phage 21gp c (2) was used as a source of DNA for sequencing gene 2, and 21gp DNA (generously given by Stephanie Schneider, Stanford University) was used to sequence the right end of 21 DNA. The lac5 and her variants of λ-21 hy33 were previously described (21). λ-21 hy19 and λ-21 hy19 gal⁺ att⁺ cl857 were also previously described (19). λ F1am471 was from our collection. Cloning vectors included M13mp19 and pUC19 (49), VCSM13 (Stratagene Cloning Systems, Inc.), a variant of M13, was used to produce single-stranded DNAs of vectors pBluescriptIIKS+ and pBluescriptIIKS* (Stratagene Cloning Systems, Inc.). The bacterial strains used were as follows: MF1427, galK100; C600, supE (10); R594, sup⁺ (10); MF894, galK2 supE44 polA59I (our collection); CJ236, dut-1 ung-1 (31); XL-1Blue, lacF’ proAB lacY1 lacZAM15 Tn10; KL1269, supF (glycine inserting; 37); XAC-1, a strain carrying an alanine-inserting amber suppressor (30). A cassette contain-

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ing a kanamycin resistance gene (Kn\textsuperscript{r} GenBlock) was obtained from Pharmacia, Inc.

**Cloning and DNA sequencing.** Standard methods were employed for cloning (33), and transformation was done as described by Hanahan (27). Plasmid DNA and M13 replicative-form DNA were prepared as described by Birnboim and Doly (8). M13 single-stranded DNA was prepared as described by Messing (34). Single-stranded DNA was prepared as described by Vieira and Messing (45), and sequencing (40) was performed with commercial reagents.

**Sequence designations.** The numbering convention of Daniels et al. (13) was used. Numbering of the \( \lambda \) and 21 sequences begins with the first base of the left cohesive end and continues to the right along the 1 strand (the upper strand of the phage genome) in the 5'-to-3' direction. Base pairs to the left of bp 1 are numbered leftwards as \(-1, -2, \) etc. The positions of restriction enzyme cleavage sites are given as the first nucleotide of the restriction sequence.

**Oligonucleotide-directed mutagenesis.** Mutagenesis of \( \text{o}rf1 \) was performed by the method described by Kunkel (31). The restriction fragment extending from the \( \text{BamH} \) site at 7291 to the \( \text{BamHI} \) site at 7968 of 21 was cloned into M13mp19. Two primers, \( 5'\text{-GCTATCTCAGCCACT-3'} \), complementary to bp 7510 to 7527 of 21, and \( 5'\text{-CGGATTCATAG GTTTCAC-3'} \), complementary to bp 7701 to 7718 of 21, were used for mutagenesis. Each primer creates an amber mutation and an \( \text{AvrII} \) site; amber mutations were verified by DNA sequencing.

**Phage crosses and complementations.** Cells to be infected were grown to \( 10^8 \) per ml in Tryptone broth supplemented with 0.2% maltose and infected at a multiplicity of 5 of each phage per cell. Following adsorption (15 min at 22\( ^\circ \))C, the infected cells were diluted into Tryptone broth and incubated at 37\( ^\circ \)C. For crosses, the infected cells were diluted into 0.01 M \( \text{MgSO}_4 \), irradiated with UV light (ca. 300 ergs/mm\( ^2 \)), and further diluted in Tryptone broth. Following lysis, the lysates were sterilized with \( \text{CHCl}_3 \) and plated.

**Computer analysis of sequence information.** The sequence of the 21 head genes was analyzed with the program DNASIS (version 3.0 [1984]) and PROSIS (version 1.0 [1987]) of Hitachi Software Engineering Co., Ltd. Secondary-structure predictions for this package use the methods of Chou and Fasman (11). Additional programs used were from the University of Wisconsin Genetics Computer Group sequence analysis software package (version 7.0 [April 1991]; 17).

**Nucleotide sequence accession number.** The nucleotide sequence described here (see Fig. 1) has been assigned GenBank accession no. M81255.

**RESULTS**

**Sequence of the right chromosomal end of 21 DNA and completion of the sequence of the terminase genes.** Miller and Feiss (35) reported the sequence of the left end of the 21 DNA molecule, from bp 1 (numbering is rightward from bp 1, the first base of the left cohesive end) to bp 1635; Wu et al. (47) sequenced a short segment at the end of gene 2 and the beginning of gene 3. To complete sequencing of cos, we sequenced a segment at the right end of the 21 DNA, from bp 1 (the first bp to the left of bp 1) to \(-166, \) and sequenced rightwards from bp 1635 by using the chain termination method (40). Figure 1 shows the sequence from \(-166 \) to 2700, which includes terminase genes 1 and 2.

**The R4 sequence.** The sequence from the \( \text{S}a\text{pl} \) site at bp \(-166 \) to the cosN cleavage site includes the segment of DNA known as the R4 sequence. R sequences (3, 18) are repeated DNA sequences found in the cos regions of \( \lambda \) and 21 DNAs (Fig. 2a). Shinder and Gold (42) showed that gpNu1 bound to the R sequences of \( \lambda \) cos\( B \) (R1, R2, and R3); however, the R4 sequence of \( \lambda \) was not bound by gpNu1 in vitro. The R3, R2, and R1 sequences of the cos\( B \) sites of \( \lambda \) and 21 differ (5, 35), as do the DNA-packaging specificities of the \( \lambda \) and 21 terminases (19, 29). Viable hybrids of \( \lambda \) and 21 with chimeric terminases indicated that the DNA specificity of the terminases resides in the amino termini of the small subunits, gpNu1 and gp1, respectively (24). Later analysis suggested that putative helix-turn-helix domains at the N termini of gpNu1 and gp1 determined the DNA-binding specificities of the \( \lambda \) and 21 terminases (32; Andrew Becker, cited in

**FIG. 1.** Sequence of the terminase genes of phase 21. Shown is the complete sequence extending from bp \(-166 \) to 2700. The numbering system used is that of Daniels et al. (13). bp 1 is the first base of the left cohesive end and numbering proceeds rightwards, 5' to 3'. The rightmost duplex base pair of virion DNA (adjacent to bp 1) is bp \(-1, \) and numbering proceeds to the left (\(-2, -3, \) etc.) to 5'. The recognition sites underlined are as follows: R3, R2, and R1, presumptive binding sites for gp1, the small terminal subunit, in cos\( B \); II, the strong IHB-binding site for gp1 (48); and cosN, the site at which nicks are introduced to generate the cohesive ends. See the text for a discussion of R4. Predicted gene products, identified by similarity to analogous genes of \( \lambda \), are indicated above the DNA sequence, translation start codons are underlined, and translation termination codons are indicated with asterisks.
(b) Alignment of the right ends of the λ, 21, and φ80 DNA molecules. The underlined segment is the highly conserved R4 sequence, and gaps indicate possible sequence shifts to maximize alignment.

(c) Alignment of R sequences of λ and 21.

R4 is a sequence found on the other side of cosN from cosB, and in λ DNA the R4 sequence resembles that of the other R sequences in cosB (3), raising the possibility that the R4 sequence is part of cosB (3, 5, 18). There is evidence that a site important for DNA packaging is present at the right end of λ DNA, as follows. Mutations resulting in deletion of R4 (22, 29, 36) and a point mutation (12a) resulted in DNA-packaging defects. If R4 of λ were a gpN1-binding site, then one would expect R4 of 21 to resemble the R sequences of cosB of 21. Accordingly, R4 of 21 was sequenced; the sequence in the R4 segment differs from that of λ R4 at only one position (Fig. 2b and c). Alignment of the 21 R4 sequence with the 21 R1, R2, and R3 sequences, however, shows that R4 has little identity with the other 21 R sequences and is much more similar to the R λ sequences (Fig. 2c). It is possible to obtain better alignment of the R4 sequence of 21 with the R1, R2, and R3 sequences of 21 by altering the spacing between R4 and cosN, but the strongest alignment is that shown in Fig. 2c.

Gp1 and the her mutations. The small terminase subunits of λ and 21 have been previously shown to contain a presumptive helix-turn-helix motif for binding DNA, comprising amino acids 5 to 24 (double underlined in Fig. 3; 32; Andrew Becker, cited in reference 18), an ATP-reactive center starting at amino acid 29 (underlined in Fig. 3; 4), and a domain to the right of amino acid 90 for interaction with the large subunit (24). A series of mutations (her mutations) that allow 21 DNA packaging to be independent of integration host factor (IHF) were previously found to be in the I gene (21). IHF is thought to assist the binding of terminase to cosB (5). We determined the sequence changes of several of the her mutations, along with that of an amber mutation, Jam5. Two her mutations are to a basic amino acid; her-23 changes amino acid 54 from E to K, and her-21 changes amino acid 90 from T to K. Two additional her mutations, her-9 and her-10, were found to be identical to her-23. The third mutation, her-20, is a Y-to-C change at amino acid 87, and the Jam5 mutation affects codon 23 of the I gene. The her mutations affect segments of gp1 predicted to form the third and fourth α-helices of the protein (Fig. 3).

The large subunit of the 21 terminase. Completion of sequencing of the 2 gene indicated that gp2 is 642 amino acids long, 1 longer than gpA. The amino acid identity between gp2 and gpA is 64%. The N-terminal 48 amino acids and the C-terminal 32 amino acids, which correspond to phage-specific domains for binding of the small terminase subunit (24, 47) and the prohead (23), respectively, show little sequence identity. Other previously described motifs include a possible metal-binding segment suggested for gpA (7) of the form H-X₄-H-X₄-C-X₇-C, between amino acids 232 and 243 (HFMRHVACH). However, as noted by Guo et al. (26), the motif is not conserved in gp2, which has the predicted sequence HFMRFYVCHGC, with a Y residue at the location occupied by the second H residue in λ, a residue that should coordinate the metal ion. Hence, the
FIG. 4. Motifs proposed for the large terminase subunits of 21 (gp2) and λ (gpA). (a) Putative A-type ATP-binding domain in gp2 and gpA. The sequence for adenylate kinase is labeled AK, and amino acids strongly conserved in ATPases are in bold (46). (b) Alignment of the endonuclease segment of gpA, the corresponding segment of gp2, and the polymerase motif A that is involved in nucleotide binding. Sequences for DNA polymerase I of E. coli (PolI) and the 629 DNA polymerase (629 PolI) are aligned below; periods indicate gaps introduced to improve alignment. Highly conserved residues are in bold.

existence of a conserved metal-binding site in gp2 and gpA is problematical.

A putative leucine zipper was described for gpA and gp2 by Davidson and Gold (15), who found mutations that affect amino acids in the motif that specifically abolished the nicking activity of the λ terminase. The putative leucine zipper was noted to be present in gp2 between residues 565 and 599 (15).

Guo et al. (26) noted an amino acid sequence in gpA related to the A-type ATP-binding motif (46), between residues 485 and 509; we also found this motif in gp2 at the same position (Fig. 4a); the sequence includes a glycine-rich segment thought to form a flexible loop that is involved in nucleotide binding. It is likely that gpA and gp2 have ATP-binding centers at this position.

Additional mutations that affect the endonuclease activity of the λ terminase were found by Davidson and Gold (15) to change residues 401 and 403 of gpA; these residues were shown to reside in the sequence DSQDRR, a sequence that, with adjustments, matches the motif DXSYL/ID/EKR, where X indicates any amino acid. The sequence motif is found in many primases and DNA polymerases (16; Fig. 4b).

We found that the corresponding sequence for gp2, also amino acids 400 to 405, is DSQRNN; the 21 sequence differs from that of gpA in residues 4 and 5. For the Escherichia coli polymerase I Klengow fragment, the known and predicted secondary structures are a segment of β-sheet with D-705 at the C terminus, followed by a β-turn and then an α-helix beginning at I-709 (16). For gp2 and gpA, the segments ending with D-400 were also predicted to be β-sheet, followed by a turn, in agreement with the polymerase I structure. Following the turn, an ambiguous structure prediction was found for both gpA and gp2 (data not shown).

Genetic analysis of orf1. The FI protein of λ is an assembly catalyst that assists binding of the prohead to complex I, the complex of terminase with λ DNA (6, 14). No mutation in an FI analog was found in previous genetic analysis of the 21 head genes. Sequencing revealed that 21 has an open reading frame, orf1, in the position analogous to that of FI in λ (44). The predicted orf1 product (Fig. 5) is 124 amino acids long and has only 22% sequence identity with gpFI (132 amino acids; 13). To understand whether orf1 is essential for 21, we mutagenized orf1.

First, amber mutations were introduced at two sites (Fig. 5). Derivatives of pUC19 carrying orf1 with one or the other amber mutation were constructed. Crosses between λ-21 hy19, a hybrid between λ and 21 with the 21 head genes (19), and the orf1 plasmids carrying one of the amber mutations yielded no suppressor-dependent recombinants among 2,000 progeny tested.

In a second experiment, we prepared cointegrants of λ-21 hy19 and the PUC19 orf1am plasmids. These cointegrants were viable, suggesting that an insertion in orf1 was not lethal, and attempts to isolate suppressor-dependent segregants of the λ-21hy19::pUC19 orf1am cointegrants were not successful.

The third attempt to mutate the orf1 gene of the phage was made by insertion of a Kn' cassette into orf1 as follows. The Kn' cassette was ligated into the EcoRV site in the middle of the orf1 gene (Fig. 5) carried on PUC19 orf1am1, creating PUC19 orf1am1 orf1::Kn'. The lysogenic MFI427 (λ-21 cI857 gal") (PUC19 orf1am1 orf1::Kn') was induced, and the lysate was used to transduce a galK2 polA1-carrying strain to Gal" AP' Kn' to select for prophages of λ-21 hy19 into which the PUC19 orf1am::Kn' plasmid had integrated.

To isolate derivatives of these cointegrants harboring the PUC19 plasmid but retained the Kn' cassette, the transductants were then induced and the lysates were used to transduce the galK2 polA1 strain to Gal' and Kn'. Of 75 Gal' Kn' transductants, 2 were Ap'; these were induced and were found to be stable Kn' transducing phages. DNAs from the two phages were analyzed by restriction enzyme digestion and Southern hybridizations to various probes. Such DNA studies showed that (i) each phage carried no pUC19 sequences; (ii) each phage carried only one copy of the orf1 gene; (iii) in each phage, the orf1 gene contained the Kn' insertion mutation; and (iv) one of the two isolates also carried the orf1am1 mutation. These derivatives of λ-21 hy19 formed plaques that were indistinguishable in size from those of the orf1' phage on a number of hosts.

These results suggest that orf1 is dispensable, but the possibility that the λ-21 hy19 orf1::Kn' isolates contained a mutation suppressing an orf1 defect was considered. In the case of the λ FI gene, there are fin mutations that allow λ FI amber mutants to grow on sup' bacteria. fin mutations are located in the terminase genes (38). A cross, diagrammed in Fig. 6, was performed between λ-21 hy19 orf1::Kn' and λ-21 hy19 lamS to test whether λ-21 orf1::Kn' contains a fin-like mutation in the terminase genes. If a fin-like mutation were present in the terminase genes, then the recombinants that
were X-21 hy19 lam5 orfl::Knr' would be expected to be unable to form plaques on any host, including a host containing supE, which suppresses lam5, because the fin-like mutation would be expected to be crossed out of the X-21 hy19 orfl::Knr' phage when the lam5 marker was crossed in (Fig. 6). Alternatively, if no fin-like mutation were present in X-21 hy19 orfl::Knr', then X-21 hy19 lam5 orfl::Knr' recombinants would be expected to form plaques on a supE host but not on a supE host. The cross lysate was used to transduce recipient cells to Knr'. Of 100 of these Knr' prophages, 91 produced phages able to form plaques on both supE and supE hosts and were thus X-21 hy19 orfl::Knr' parental prophages. Nine prophages produced phages able to form plaques on the host carrying a suppressor for the lam5 mutation, but not on the supE host; these were scored as X-21 hy19 lam5 orfl::Knr' recombinants. Since these nine recombinants behaved as expected if there had been no fin-like mutation in X-21 hy19 orfl::Knr', and no recombinant that was unable to form plaques on supE cells was found, it was concluded that X-21 hy19 orfl::Knr' does not contain a fin-like mutation in the terminase genes. With this possibility eliminated, we concluded that orfl is not an essential gene.

Can 21 complement \( \lambda \) Flam471? Although orfl is not an essential gene for 21, complementation tests were performed to determine whether the putative orfl gene product is able to complement an FI mutant of \( \lambda \) (Table 1). In a positive control, a X-21 hybrid that has the X FI gene, X-21 hy33 lam5, efficiently complemented Flam471, but neither X-21 lam5 nor X-21 hy19 lam5 orfl::Knr' complemented Flam471. Hence, 21 does not complement \( \lambda \) Flam471.

**TABLE 1.** Complementation tests between \( \lambda \) Flam471 and X-21 by 19

<table>
<thead>
<tr>
<th>Infection</th>
<th>Phage</th>
<th>Yield (no. of phage/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda ) Flam471</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>X-21 hy19 lam5</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>X-21 hy33 lam5</td>
<td>3</td>
<td>0.2</td>
</tr>
<tr>
<td>X-21 hy19 lam5 orfl::Knr'</td>
<td>4</td>
<td>0.1</td>
</tr>
<tr>
<td>X-21 hy33 lam5</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>X-21 hy19 lam5 orfl::Knr'</td>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td>X-21 hy33 lam5</td>
<td>7</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*In previous work (20), the yield of X-21 by 33 showed ca. 10-fold dependence on Fl.*

**DISCUSSION**

R4 and termination. Comparison of the R1, R2, and R3 sequences of \( \lambda \) and 21 shows little similarity between the R sequences of these phages (5); this is an expected result, since both phages have cosB-binding reactions that are phage specific for the N-terminal regions of their terminase small subunits. In contrast, the R4 sequences of 21, \( \lambda \), and \( \phi80 \) are highly conserved, suggesting that the R4 sequence has a function that is not phage specific. That R4 is not phage specific agrees well with the finding that X-21 hybrid phages with the 21 terminase are viable, even though the right chromosomal end, including R4, is from \( \lambda \). Evidence that R4 is important for DNA packaging comes from deletion studies with cosmids which showed that deletion of R4 resulted in a packaging defect (22, 29, 36). More recently, an R4 mutation has been shown to cause a defect in termination of DNA packaging (12a). If R4 is a recognition site needed for proper termination of DNA packaging, as seems likely, the recognition specificity is conserved in 21 and \( \lambda \).

**her** mutations. The her mutations permit the 21 terminase to function in the absence of IHF (21) and are likely to increase either the affinity of the terminase for cosB22 or the efficiency of terminase action (5, 12). Since the her-23 (E54K) and her-2 (T90K) mutations introduce basic amino acids that might form electrostatic interactions with DNA, these mutations may increase the strength of binding of gp1 to DNA. Alternatively, the her-23 and her-21 mutations may alter the efficiency of terminase action at a step following binding. Interestingly, these two mutations are dominant to wild-type gp1 in complementation tests in an IHF- host. How the recessive her-20 (Y87C) mutation might affect terminase action is unclear.

**Gp2.** The large subunit of the 21 terminase has been found to conserve amino acids suggested to represent two functional domains in gpA of \( \lambda \); these are motif A (15) and an ATP-binding motif (26). Motif A is present in a large family of proteins that includes DNA and RNA polymerases. Blasco et al. (9) have found that mutations that change amino acids of motif A of \( \phi29 \) DNA polymerase alter the Mg\(^{2+}\) deoxyribonucleotide triphosphate-binding activity of the enzyme. In contrast, Davidson and Gold (15) recently found that mutations in the motif A-like segment of gpA inactivate the endonuclease activity of the \( \lambda \) terminase while not disrupting the DNA-packaging activity. Determination of whether the motif A-like segments of the \( \lambda \) and 21 terminases are homologous to polymerase motif A requires further structural and functional information.

There are putative ATPase sites in both the large and small subunits of the 21 and \( \lambda \) terminases (4, 25), and ATP has been shown to play several roles in \( \lambda \) terminase function. These roles include stimulation of the fidelity and rate of cosN nicking and separation of the cohesive ends (28). Packaging of phage DNA is known to require ATP, on the order of ca. 1 ATP molecule per base pair of DNA packaged (26, 41), and the ATPase activity of terminase is speculated to reflect a translocase activity of terminase (5). Purified gpNu1 of the \( \lambda \) terminase is known to have ATPase activity, and gpA has an ATP-binding site, on the basis of crosslinking studies (12a, 29a; cited in reference 28).

**orf1.** GpFI is an assembly catalyst that facilitates the capture of a prohead to increase the terminase-DNA complex (6, 14). A previous mutant hunt did not generate any mutations in a 21 analog of the X FI gene (43), raising the question of whether 21 has an FI analog. Here we show that orf1, an open reading frame analogous in position to FI, is dispens-
able. Thus, in the hosts we have used, 21 does not need to produce a gpFI-like assembly catalyst. λ-21 hybrid 33 is a viable phage with a terminase derived from 21, except for the C-terminal 32 amino acids, which are derived from λ and which confer λ prohead-binding specificity (23); λ-21 hy33 was shown to be dependent on gpFI (20). It appears from λ-21 hy33 that the segment of terminase that is dependent on gpFI is the C terminus, a conclusion that makes sense, since the C terminus is involved in prohead capture. The question of whether the terminase of 21 is able to capture a prohead without an assembly catalyst or whether the catalyst is supplied by the host remains. Since orfI has been retained by 21, it seems likely that the orfI product is necessary under certain conditions. The hosts we have used may supply an assembly catalyst that is not present in some natural hosts of 21.

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