A site required for termination of packaging of the phage λ chromosome

(DNA encapsidation/virus assembly/viral DNA processing)

DAVID CUE AND MICHAEL FEISS

Department of Microbiology, College of Medicine, University of Iowa, Iowa City, IA 52242

Communicated by Howard A. Nash, June 28, 1993 (received for review April 12, 1993)

ABSTRACT A chromosomes are cut and packaged from concatameric DNA by the phage enzyme terminase. Terminase initiates DNA packaging by binding at a site called cosB and introducing staggered nicks at an adjacent site, cosN, to generate the left cohesive end of the DNA molecule to be packaged. After DNA packaging terminase recognizes and cuts the terminal cosN, an event that does not require a wild-type cosB. In this work a site, called cosQ, has been identified that is required for termination of DNA packaging. cosQ, defined by mutations in a sequence called R4, is located ~30 bp upstream from cosN. The order of sites is cosQ-cosN-cosB. Helper packaging of repressed, tandem prophage chromosomes demonstrated that a cosQ point mutation affects DNA packaging only when placed at the terminal cos site, whereas cosB mutations only affect packaging initiation. In vivo packaging studies confirmed that cosQ mutations do not affect packaging initiation. In vitro packaging studies indicated that cosQ mutations do not affect cutting of initial cos sites but do cause a defect in packaging termination. cosQ mutants accumulated expanded phage heads, indicating that cosQ mutations affect a step that occurs after packaging of a substantial length of phage DNA. These results show that cosQ mutations define a site required for use of cos sites present at the ends of λ chromosomes undergoing packaging. Available evidence suggests that other viruses, including phages T3 and T7 and the herpesviruses, may ultimately prove to use cosQ-like sites for packaging termination.

The DNA-replication cycle for many viruses, including herpesviruses and phage λ and its relatives, produces end-to-end multimers of virus chromosomes, called concatemers. DNA encapsidation by these viruses involves the specific cutting of concatemers to generate virion DNA molecules (1). For phage λ, concatameric DNA is recognized and subsequently cut by the phage-encoded enzyme terminase. Terminase is a heterotrimer, composed of 20-kDa gpNu1 (the product of gene Nu1) and 74-kDa gpA (the product of gene A) subunits. Terminase binds λ DNA at cosB (Fig. 1 B) and introduces staggered nicks at an adjacent site, cosN, to generate the cohesive ends found on virion DNA. Terminase also binds a prohead, the head shell precursor, to form the ternary DNA–terminase–prohead complex that leads to DNA packaging (3). Terminase likely binds the portal vertex of the prohead, a special vertex through which DNA is thought to be translocated into the prohead. Terminase is also an ATPase and has been speculated to participate in translocation, using ATP as an energy source (4).

A DNA packaging is polarized, and the left end, which contains cosB, is packaged first (5). During packaging, the terminase involved in initiation of packaging remains in contact with the DNA being packaged and scans for and

Fig. 1. Genetic organization of the cos-terminase segment of uncut λ DNA. (A) Genes Nul and A are shown; cos is represented by the filled box; X and R indicate positions of XbaI and EcoRI sites, respectively, used in strain construction. (B) cos structure. Positions of binding sites in cosB for gpNu1 (R3, R2, and R1) and integration host factor (IHF) (I1) are shown. The open box represents cosN, and the staggered vertical lines indicate positions at which nicks are introduced by terminase to generate cohesive ends. The rectangle at left of cosN represents the cosQ sequence. Initiation of packaging by terminase cutting of cosN generates a right DNA end (cosQ-containing) and a left (cosB-containing) end of a DNA to be packaged. (C) Sequence of right end of virion λ DNA; numbering is according to Daniels et al. (2). The mutation generating the XbaI site and the cosQI mutation are shown above the sequence; the ΔcosQ deletion of bp 48,470–48,493 is shown below.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: cosB, binding site for terminase; cosN, nicking site for terminase; gpNu1, the product of gene Nu1; IHF, integration host factor.

*To whom reprint requests should be addressed.
Biochemistry: Cue and Feiss

MATERIALS AND METHODS

Media. Tryptone broth, agar, and soft agar were as described (15) except MgSO_4__ was added to 10 mM. Luria broth, Luria agar, 2× yeast/trypotone, and SOB were as described (16). When required, kanamycin and ampicillin were added to 50 and 200 μg/ml, respectively.

Strains. λ-P1:5R Kn_1 c1857 nin5 (where Kn_1 represents kanamycin resistant), hereafter called λ-P1, is a derivative λ carrying the P1smid maintenance genes of phage P1; as a consequence, the λ-P1 prophage is a plasmid (17, 18). The cosQ1 mutation, a C→T transition at λ bp 48,477 (Fig. 1C, numbering convention from ref. 2, was previously described as the R4- mutation (14). Variants of λ-P1 carrying the mutants cosQ1, cosN (a 22-bp deletion of cosN), and R3- R2- R1- Nal_1Ms_1 have been described (14, 19). A new derivative of λ-P1 containing the ΔcosQ mutation, a deletion of bp 48,470–48,483, was constructed by using standard methods (14). A series of pUC19 derivatives carrying the λ segment from bp 47,492 to bp 194 containing cos alleles cos*, cosQ1, ΔcosQ and ΔcosB (deleted to the right of bp 52) were used for in vitro packaging experiments. Host strains used for construction of dilysogens were MF711 and MF532; the helper phage was λ b538 red3 imm21 cl p4 (15). Terminase and prohead extracts were obtained from induced cultures of the E. coli C strain MF1427 carrying, respectively, the terminase expression vector pCM101 (20) and λ c1857 Sam7 Δ(cos-A)-:Kn_1 (Y. Hwang and M.F., unpublished work). MF1427 (λ c1857 att_+ gal_+ cos2) was described (21). The host for in vivo DNA-processing studies was the E. coli C strain C1100. pSF1 (22) was used as hybridization probe.

General Recombinant DNA Techniques. DNA manipulations were done as described (16). Phage and prophage DNAs were isolated by standard methods (21, 23).

Helper Packaging of Prophages from Dilysogens (15). Standard crossing procedures were used to introduce cos’ or cosQ1 into λ c1857 att_+ cos2 gal_+ (14, 21); the resulting att_+ gal_+ phages with cos’ or cosQ1 were used to lysogenize MF713 and MF532. MF713 carries a prophage deleted for attL and the early genes, and MF532 carries an imm434 prophage deleted for the late genes and attR. Integration of an att_+ gal_+ prophage into the attR site in MF173 generates a strain equivalent to a dilysogen with cos’ at the initial position and the cos of the att_+ phage (cosQ1 or cosQ1) in the terminal position (15). Integration of an att_+ gal_+ prophage into the attL site of MF532 generates a strain equivalent to a dilysogen with the cos of the att_+ phage (cosQ1 or cosQ1) in the initial position and the terminal cos of the MF532 prophage. Infections of the MF713 and MF532 dilysogens with the heteroimmune helper phage, λ imm21 cl b538 red3 p4, were done as before (15). Yields of helper and prophage were determined by standard platings, and the markers expected for packaged prophages were verified by standard genetic tests.

In Vitro DNA Packaging Initiation Assay. Cosmid containing various cos alleles, linearized with HindIII and end-labeled by using [α-32P]dATP and Klenow DNA polymerase, were packaging substrates. The 75-μl packaging mixture contained 500 ng of DNA, 15 μl of prehead extract, 5 μl of terminase extract diluted in prehead extract, 6 μl of 6 mM Tris, pH 7.5/18 mM MgCl_2/30 mM spermidine/60 mM putrescine/15 mM ATP and 59 μl of 20 mM Tris, pH 8.0/3 mM MgCl_2/7.3 mM 2-mercaptoethanol. Experiments were prepared as described by Chow et al. (20). After incubation for 30 min at 23°C, 2 μl each of DNAse I (1 mg/ml) and RNase A (10 mg/ml) was added, followed by incubation for 30 min at 23°C. EDTA was added to 25 mM, and the DNA was extracted twice with phenol/CHCl_3 and once with CHCl_3, followed with ethanol precipitation and resuspension in 10 mM EDTA/0.1% SDS. After heating to 65°C for 10 min, the DNA was subjected to agarose gel electrophoresis, the gel was dried, and the bands, located by autoradiography, were cut out and counted by liquid scintillation spectrometry.

Electron Microscopy. With standard methods (24), negatively stained preparations of concentrated phage lysates were examined at ×40,000.

RESULTS

Effect of cosQ1 Mutation on Initiation and Termination of DNA Packaging. A helper packaging assay was used to study the effect of the cosQ1 mutation on DNA packaging. The bacterial host for a helper packaging experiment is a dilysogen containing two prophages in tandem. Repressed, tandem prophage chromosomes contain a λ chromosome that is bracketed by two cos sites; this chromosome is analogous to a λ chromosome in a concatamer and can be packaged in situ during lytic growth of a heteroimmune helper phage. Because packaging is polarized in a cosB to cosQ direction, dilysogens could be constructed with tandem double prophages in which either the initial or terminal cos site contained the cosQ1 mutation. These dilysogens, along with control dilysogens, were infected with a heteroimmune helper phage, and the resulting lysates were measured to determine the yield of the packaged prophage. When cosQ1 was placed at the initiating cos, prophage packaging was not significantly affected; whereas packaging of the prophage was reduced to 10-fold when cosQ1 was located at the terminal cos (Table 1). This experiment suggested that cosQ1 might affect a site involved in termination of packaging.

Table 1. Effect of cosQ1 mutation at the initial and terminal cos positions

<table>
<thead>
<tr>
<th>Prophage</th>
<th>Initial cos</th>
<th>Terminal cos</th>
<th>Prophage yield*</th>
</tr>
</thead>
<tbody>
<tr>
<td>cosQ1</td>
<td>MF532 lysogens†</td>
<td>cos*</td>
<td>0.30 (0.04)</td>
</tr>
<tr>
<td>cos2</td>
<td>cos*</td>
<td>cos*</td>
<td>0.20 (0.04)</td>
</tr>
<tr>
<td>cosQ1</td>
<td>MF713 lysogens‡</td>
<td>cos*</td>
<td>0.64 (0.09)</td>
</tr>
<tr>
<td>cos2</td>
<td>cos*</td>
<td>cos*</td>
<td>0.08 (0.02)</td>
</tr>
</tbody>
</table>

*Yields are helper-packaged prophages per helper-infected cell. Numbers in parentheses are variation from the mean for two determinations for MF532 and SDs for MF713.

†Effect of cosQ1 at initial cos position.
‡Effect of cosQ1 at terminal cos position.
Effect of a cosQ Deletion on Packaging Initiation. A second cosQ mutation was constructed so that the effects of a null mutation on DNA packaging could be examined. We noted that for phages λ, 21, and A80, a 17-bp sequence, found in λ from bp 48,468 to 48,484 (Fig. 1), is conserved. Because the right chromosomal ends of these viruses can be interchanged, we surmised that the conserved segment likely marks the extent of cosQ (27). Additionally, cosmid-packaging experiments (13, 28) indicate that no λ DNA sequences to the left of bp 48,471 (Fig. 1) are required for DNA packaging. Accordingly, the ΔcosQ mutation, a 14-bp deletion of bp 48,470–48,783, was constructed and found to be lethal, causing >104-fold reduction in the phage yield (data not shown).

It was of interest to determine whether the effect of the severe ΔcosQ mutation differed only in magnitude from the effect of the cosQ1 mutation. The effect of the ΔcosQ mutation on packaging initiation was of particular interest because cosQ1 had no apparent effect on this packaging step. Due to the lethality of ΔcosQ, the in vitro helper packaging assay could not be used to address the question. To determine the effect of the ΔcosQ mutation on DNA-packaging initiation, an in vitro DNA-packaging assay was used. When cos+ cosmid DNA was used, cleavage of cosN and subsequent packaging of the 2.8-kb-long, cosB-containing segment required the presence of both proheads and terminase (Fig. 2A, lane 4). For cosmid DNA containing the ΔcosQ mutation, packaging of the cleaved substrate DNA was found at a level about the same as for the cos+ DNA (compare lanes 4 and 5). Note that a small amount of 3.4-kb substrate DNA, not cleaved at cosN, was packaged when terminase was present (lanes 4 and 5). Nonspecific packaging was found for linear DNAs lacking cos; because the cleaved product of specific cleavage is separable, nonspecific packaging does not interfere with the determination of specific packaging. The results of a series of packaging-initiation assays with different levels of terminase showed that whereas the ΔcosB DNA gave no specific packaging, the cosQ1 and ΔcosQ DNAs were efficiently packaged, indicating the cosQ mutations have no effect on packaging initiation (Fig. 2B).

In Vivo cos Cutting by cosQ Mutants. cosN cutting in vivo can be followed by examining intracellular DNA. cos cleavage cuts a 4.9-kb BstXI restriction fragment spanning cos into two lower bands (indicated by filled rectangles). (Middle) Cleavage of an intact cos site generates one mature right and one mature left chromosomal end; packaging of the chromosome to the right of the cleaved cosN site can then proceed. (Bottom) Restriction of mature λ DNA at BstXI sites (indicated by the Bs) generates the short restriction fragments shown. The BstXI restriction sites are at λ bp 46,434 and 2,845. The mature right (R, 2.1 kb) and left (L, 2.9 kb) chromosomal ends can be resolved by electrophoresis through agarose and detected by Southern blotting. (B) Effect of cos mutations on the in vivo processing of λ DNA. Total nucleic acids were isolated from λ-infected C1100 cells, digested with BstXI, subjected to electrophoresis through 1.0% agarose, transferred to a membrane, and hybridized with radiolabeled pSF1. Lanes: 1, uninfected C1100; 2–6, C1100 infected with λ-P1 cos+ (lane 2), λ-P1 cosQ1 (lane 3); λ-P1 R3−R2−R1− (lane 4); λ-P1 Aam42 (lane 5); or λ-P1 cos2 (lane 6). Mature λ-P1 cos+ DNA, digested with BstXI, was in lane 7. (C) C1100 infected with λ-P1 ΔcosQ (lane 1); λ-P1 Aam42 (lane 2); λ-P1 cos+ (lane 3); and mature λ-P1 cos+ DNA, digested with BstXI (lane 4). Positions of bands of the mature left (L) and right (R) chromosomal ends are indicated. The other BstXI fragments that hybridize to the pSF1 probe extend from λ bp 38,292 to 46,434 (upper band); bp 46,434 to 2,855 (second band, not resolved in B), and bp 2,855 to 6,706 (third band).

Fig. 2. Effects of cosQ mutations on DNA-packaging initiation in vitro. (A) Effect of ΔcosQ mutation on packaging initiation. In this assay 32P-labeled, linearized cosmid DNA and ATP were mixed with extracts containing terminase and proheads; after incubation, DNase I was added to hydrolyze any cosmid DNA not protected by packaging. The linearized plasmid DNAs used were ~3.4 kb long, with cos placed 600 bp from the end so that, upon cleavage of cosN during initiation of DNA packaging, a segment ~2.8 kb long was packaged. The level of packaged DNA was determined by digestion of the proheads followed by agarose gel electrophoresis. Lane 1, linearized cos+ substrate DNA cut with terminase—top band is the 3.4-kb uncleaved DNA, lower band is 2.8-kb, cosB-containing product of cutting at cos [the other product band (600 bp) is not shown]. Lanes: 2, untreated, linear cos+ substrate DNA; 3, cos+ substrate DNA in packaging reaction lacking terminase; 4, cos+ substrate DNA in complete packaging reaction; 5, ΔcosQ substrate DNA in complete packaging reaction. No DNase I was added to reactions in lanes 1 and 2. (B) Packaging initiation with cos+, cosQ1, ΔcosQ, and ΔcosB DNAs. Terminase concentrations represent addition of 2.5, 1.0, and 0.5 μl of undiluted terminase extract to reaction mixtures. Vertical lines are SDs.

Fig. 3. DNA processing in vivo. (A) Rationale of in vivo cos cutting assays. (Top) Coscatenomeric λ DNA. Right (cosQ) and left (cosB) ends of successive chromosomes are indicated. cosN sites are indicated by open boxes with staggered vertical lines; cosB segments are represented by filled rectangles. (Middle) Cleavage of an intact cos site generates one mature right and one mature left chromosomal end; packaging of the chromosome to the right of the cleaved cosN site can then proceed. (Bottom) Restriction of mature λ DNA at BstXI sites (indicated by the Bs) generates the short restriction fragments shown. The BstXI restriction sites are at λ bp 46,434 and 2,845. The mature right (R, 2.1 kb) and left (L, 2.9 kb) chromosomal ends can be resolved by electrophoresis through agarose and detected by Southern blotting. (B) Effect of cos mutations on the in vivo processing of λ DNA. Total nucleic acids were isolated from λ-infected C1100 cells, digested with BstXI, subjected to electrophoresis through 1.0% agarose, transferred to a membrane, and hybridized with radiolabeled pSF1. Lanes: 1, uninfected C1100; 2–6, C1100 infected with λ-P1 cos+ (lane 2), λ-P1 cosQ1 (lane 3); λ-P1 R3−R2−R1− (lane 4); λ-P1 Aam42 (lane 5); or λ-P1 cos2 (lane 6). Mature λ-P1 cos+ DNA, digested with BstXI, was in lane 7. (C) C1100 infected with λ-P1 ΔcosQ (lane 1); λ-P1 Aam42 (lane 2); λ-P1 cos+ (lane 3); and mature λ-P1 cos+ DNA, digested with BstXI (lane 4). Positions of bands of the mature left (L) and right (R) chromosomal ends are indicated. The other BstXI fragments that hybridize to the pSF1 probe extend from λ bp 38,292 to 46,434 (upper band); bp 46,434 to 2,855 (second band, not resolved in B), and bp 2,855 to 6,706 (third band).
two new fragments representing the mature right (R; 2.1 kb) and left (L; 2.9 kb) DNA ends (Fig. 3A). We assayed in vivo \textit{cos} cutting by isolation of total DNA from induced lysogens, digestion of the DNA with \textit{BstXI}, and detection of the mature chromosomal ends by Southern blotting (25, 26).

Two negative controls were used in these experiments: because the R3- R2- R1- and \textit{cos2} mutations, the latter a deletion of \textit{cosN}, cause defects in initiation of DNA packaging, no mature DNA was expected, and none was found (Fig. 3B, lanes 4 and 6, respectively). An additional control was \textit{A-P1 Aam42}; the \textit{Aam42} mutation in a nonsense mutation in the fifth-to-last codon of the \textit{A} gene. \textit{Aam42} mutation is lethal, due to the failure of the mutant terminase to bind \textit{A} proheads; the initial \textit{cos} cleavage that is required for initiation of DNA packaging occurs, but in the absence of prohead binding, packaging does not occur (26). The initial \textit{cosN} cleavage should generate one mature right chromosomal end and one mature left end. The \textit{Aam42} DNA contained mature left ends, but no right ends were detected (Fig. 3B, lane 5).

Failure to observe mature right ends of chromosomes in \textit{Aam42}-infected cells suggests that DNA packaging is required to protect the mature right ends from nuclease digestion. The mature right chromosomal ends that are the products of initial \textit{cos} cleavage events are known to be accessible to exonuclease \textit{V} in vivo (26, 29). In contrast, terminase is thought to remain bound to \textit{cosB} of the mature left end, protecting against nuclease attack (26, 29). DNA isolated from \textit{\lambda-P1 cosQ1}-infected cells contained mature left-end fragments and a faint band representing right end fragments (Fig. 3B, lane 3), a result consistent with efficient initiation and inefficient termination. The intracellular DNA of \textit{\lambda-P1 \Delta cosQ} was also examined and found to contain only mature left ends, a result consistent with a profound defect in terminal \textit{cos} cleavage (Fig. 3C, lane 1).

\textbf{Structures in \textit{cosQ} Mutant Lysates.} Before packaging, \textit{\lambda} proheads are empty spherical particles that are 25 nm in radius (30). Accompanying packaging of a DNA length between 6 and 22 kb of DNA is a rearrangement of the subunits of the prohead shell that results in a hexagonal shape and expansion to a radius of 32 nm (33). If \textit{cosQ} mutations affect packaging termination, then \textit{cosQ} mutant lysates are predicted to contain a significant fraction of expanded phage heads; these expanded heads would result from DNA-packaging events that initiated normally, but for which packaging was not completed. To test this prediction, head-related structures in phage lysates were examined in the EM. In our preparations for microscopy the DNA is lost from expanded heads containing abortively packaged DNA, so only the expanded empty head is seen.

\textit{\lambda-P1 cos+} lysates contained three types of head-related structures: phages (47.5%), proheads (42.4%), and a low level of expanded heads (10.1%) (Fig. 4A; Table 2). Expanded heads can be distinguished from proheads by their larger diameter and hexagonal shape (30). \textit{\lambda-P1 cos2} (Fig. 4C) and \textit{\lambda-P1 R3- R2- R1-} (data not shown) lysates contained only proheads, as expected for phages with initiation defects.

The \textit{\lambda-P1 cosQ1} lysates contained the same structures as the \textit{\lambda-P1 cos+} lysates (Fig. 4B), but the relative proportions differed. Lysates of the \textit{cosQ1} mutant contained phages representing 5% of the total structures present; this value is \approx 10-fold lower than was found for \textit{\lambda-P1 cos+}, consistent with the \approx 10-fold lower yield of \textit{\lambda-P1 cosQ1}. The ratio of expanded heads to phages for \textit{\lambda-P1 cosQ1} was \approx 20-fold higher than the ratio for \textit{\lambda-P1 cos+}. The \textit{\lambda-P1 \Delta cosQ} lysate was similar to the \textit{\lambda-P1 cosQ1} lysate, except that the level of phages was much lower (0.4% vs. 5%, respectively). The accumulation of expanded heads by \textit{cosQ}-containing mutants is consistent with the idea that \textit{cosQ} mutations affect packaging termination.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Phage lysate & Phages & Proheads & Expanded heads \\
\hline
\textit{\lambda-P1 cos+} & 47.5 & 42.3 & 10.1 \\
\textit{\lambda-P1 cos2} & 0 & >99 & <1 \\
\textit{\lambda-P1 cosQ1} & 5 & 76.7 & 18.3 \\
\textit{\lambda-P1 \Delta cosQ} & 0.44 & 76.6 & 22.9 \\
\textit{\lambda-P1 R3- R2- R1-} & 1 & 98 & 1 \\
\hline
\end{tabular}
\caption{Phage, proheads, and expanded heads present in phage lysates. Numbers are percentage of total identifiable phage head-related particles. Number of particles examined were as follows: \textit{\lambda-P1 cos+}, 1659; \textit{\lambda-P1 cos2}, 1757; \textit{\lambda-P1 cosQ1}, 1588; \textit{\lambda-P1 \Delta cosQ}, 2493; and \textit{\lambda-P1 R3- R2- R1-}, 449.}
\end{table}

\section*{Discussion}

\textbf{The Role of \textit{cosQ}.} For packaging of a \textit{\lambda} chromosome, initiation requires \textit{cosN} and \textit{cosB}. Our results show that a unique site (\textit{cosQ}), as well as \textit{cosN}, is required for termination of DNA packaging. What is the defect for \textit{cosQ} mutants? Preliminary evidence indicates that \textit{cosQ} mutations result in failure to cut the terminal \textit{cos}, as follows. The \textit{\Delta cosQ} mutant produces a low yield of noninfectious phages (Table 2). Examination of the DNA in these particles shows they contain molecules in which DNA containing an uncut terminal \textit{cos} has been packaged into the head (unpublished data). These particles are similar to those obtained during packaging.
of DNA lacking a terminal \( \cos \), where the head is simply filled to capacity (31). We speculate that \( \cosQ \) is a recognition site for a component of the packaging apparatus, such as the portal protein of the prohead or terminase. Recognition of \( \cosQ \) might cause DNA packaging to enable efficient terminal \( \cos \) cleavage. Alternatively, recognition of \( \cosQ \) might alter the subunit composition or conformation of the packaging machinery, triggering terminal \( \cosN \) cutting. Similar mechanisms seem likely to be used by a number of viruses, including phages T3 and T7 and the herpesviruses. Like \( \lambda \), these viruses make specific cuts in concatenemic DNA during packaging. Unlike \( \lambda \), the virion DNA molecules for these viruses possess direct terminal repeats. For T3 and T7, in which packaging is polarized right to left, initiation involves a sequence in the right half of the terminal repeat, adjacent to the cut site, and termination involves a sequence in the left half of the terminal repeat, near the terminal cut site (32, 33). A similar arrangement of sites has been proposed for the herpesvirus terminal repeat, the \( \alpha \) sequence. The \( \alpha \) sequence contains two unique sequences, \( Uc \) and \( Ub \), that flank the cut site and are proposed to be signals for initiation and termination of packaging, respectively (34, 35). An additional complexity for T3, T7, and the herpesviruses is regeneration of the terminal repeat at concatamer junctions; regeneration by local replication has recently been found for T7 (33).

Well-developed genetics and biochemistry make the \( \lambda \) DNA-packaging system ideal for dissecting the molecular mechanism of packaging termination. Further study of \( \lambda \)-packaging termination should yield a model for a general mechanism of DNA-packaging termination and also provide insights into other complex, multistep DNA–protein transactions.

**cos Cutting in Vivo.** Studying cos cutting in vivo is complex because unprotected DNA ends are subject to nuclease attack. \( \lambda \) DNA ends, both left and right, are protected by packaging, and unpackaged left ends are presumed to be protected by terminase bound to \( \cosB \) (26, 29). For example, the Aam42 mutation only inactivates the prohead-binding activity of terminase; Aam42 terminase still binds \( \cosB \) and cuts \( \cosN \) (26). Unlike \( \cosQ \) mutants, lysates of \( \lambda \) Aam42 contain only proheads, indicating no attempt to package DNA (26). The intracellular DNA of \( \lambda \)-P1 Aam42 contains many left ends but has no right ends (refs. 27 and 28; Fig. 3b and c). In the absence of degradation, right and left ends should be present in an equimolar ratio; apparently, right ends are preferentially degraded in vivo. Therefore, in the in vivo assays, recovery of mature right chromosomal ends depends upon DNA packaging.

Intracellular DNA of \( \lambda \)-P1 \( \Delta \cosQ \) also has only left ends (Fig. 3C), a result consistent with (i) nuclease digestion of right ends produced by initial cos cutting, (ii) protection of left ends by terminase binding to \( \cosB \) and/or packaging into proheads, and (iii) failure to cut terminal cos sites or, if terminal cutting occurs, failure to stably package right ends. For \( \lambda \)-P1 \( \cosQ \), right ends are found, but at a reduced level, consistent with a partial defect in terminal cos cutting and protection by packaging of the right ends that are generated.

**Efficiency of Packaging Completion.** Phage lysates contain three types of head-related structures: intact phage particles, proheads, and expanded heads. Expanded heads represent DNA packagings that proceeded to the point of prohead expansion but failed before the formation of complete phage. For \( \lambda \)-P1 cos\(^{-} \) lysates, comparison of the levels of expanded heads (10.1%) and complete phages (47.5%) gives an estimate of 0.82 (= 47.5/47.5 + 10.1) for the efficiency of packaging completion. In the cosQ1 lysates, the levels of expanded heads (18%) and complete phages (5%) give an estimate of 0.22 for the efficiency of packaging completion, and for \( \lambda \)-P1 \( \Delta \cosQ \), \(<0.02 of packaging attempts are successful.

\( \cosQ \) mutant lysates also contain increased levels of proheads. One explanation is that the increased prohead levels are from disruption of processive packaging by \( \cosQ \) mutations. Terminase acts processively in DNA packaging, normally packaging two to three chromosomes from a DNA concatemer per initiation event (5, 7). If failure to complete packaging of the initial chromosome of a packaging series is accompanied by disruption of processivity, one expects the accumulation of one expanded head and one or two proheads.

We thank Zhi-Hao Cai, Young Hwang, Gary Johnson, Jean Sippy, and Ashly Yeo for their interest and help; Peter Greenberg for reading the manuscript; and Shelley Grimes (University of Minnesota, Minneapolis) for the in vitro packaging protocol. This research was supported by National Institutes of Health Grant AI-12581. D.C. was supported in part by a Fellowship from the Food and Agricultural Sciences National Needs Program (U.S. Department of Agriculture Grant 87-GRAD-9-0079) and a Traineeship from the University of Iowa Training Program in Cellular and Molecular Biology (National Institutes of Health Grant GM07228).