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# 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

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**ABSTRACT** **λ chromosomes are cut and packaged from concatemeric 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 vitro* packaging studies confirmed that *cosQ* mutations do not affect packaging initiation. *In vivo* 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, concatemeric DNA is recognized and subsequently cut by the phage-encoded enzyme terminase. Terminase is a heteromultimer, composed of 20-kDa gpNul (the product of gene *Nul*) 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).

λ 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 cleaves the terminal *cos* site (6). Packaging is processive along the concatemer; after the packaging of a chromosome, terminase remains bound to the left end of the next chromosome and initiates packaging of the next chromosome (7).

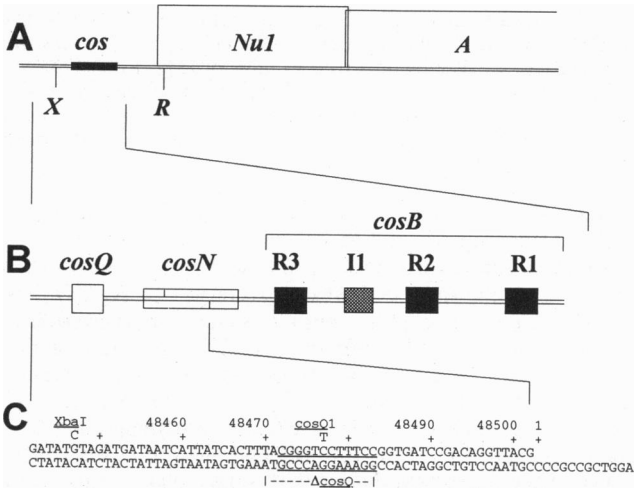


FIG. 1. Genetic organization of the *cos*-terminase segment of uncut λ DNA. (*A*) Genes *Nul* and *A* are shown; *cos* is represented by the filled box; X and R indicate positions of *Xba* I and *Eco*RI sites, respectively, used in strain construction. (*B*) *cos* structure. Positions of binding sites in *cosB* for gpNul (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 λ 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 *Xba* I site and the *cosQ1* mutation are shown above the sequence; the Δ*cosQ* deletion of bp 48,470–48,493 is shown below.

*cosB* contains three binding sites (R1, R2, and R3; Fig. 1*B*) for the gpNul subunit of λ terminase (8) and a single binding site (I1) for IHF, the *Escherichia coli* integration host factor (9, 10). R4 is a sequence that lies across *cosN* from *cosB*. Because R4 resembled the R sequences of *cosB*, it was speculated that R4 might be a gpNul-binding site that is part of *cosB* (11, 12), and deletion mutations indicate that base pairs at R4 are required for packaging cosmid DNA into infectious virions (13). However, purified gpNul, which binds R1, R2, and R3, does not bind to R4 *in vitro* (8).

In an earlier study (14), we introduced a transition mutation into each of the R sequences at a base pair found in all four R sequences. The R3, R2, and R1 mutants, unlike λ$^+$, were

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

*To whom reprint requests should be addressed.

each found to be IHF-dependent for plaque formation, producing nearly normal yields in IHF$^+$ cells. In contrast, the yield of the R4 mutant was ≈10% that of λ$^+$ in both the presence and absence of IHF. Here we report studies showing that mutations affecting the R4 sequence define a site, *cosQ*, required for termination of λ DNA packaging. The R4 point mutation, renamed *cosQ*1, causes a partial defect in termination of DNA packaging; a second mutation, a 14-bp deletion called Δ*cosQ*, causes a profound defect in termination of λ DNA packaging.

## 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/tryptone, 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$^r$ *c*I857 *nin*5 (where Kn$^r$ represents kanamycin resistant), hereafter called λ-P1, is a derivative λ carrying the plasmid maintenance genes of phage P1; as a consequence, the λ-P1 prophage is a plasmid (17, 18). The *cosQ*1 mutation, a C → T transition at λ bp 48,477 (Fig. 1*C*, numbering convention from ref. 2), was previously described as the R4$^-$ mutation (14). Variants of λ-P1 carrying the markers: *cosQ*1, *cos*2 (a 22-bp deletion of *cosN*), and R3$^-$ R2$^-$ R1$^-$ *Nul*ms1 have been described (14, 19). A new derivative of λ-P1 containing the Δ*cosQ* 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*$^+$, *cosQ*1, Δ*cosQ* and Δ*cosB* (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 *b*538 *red*3 *imm*21 *c*I *p*4 (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 *c*I857 *S*am7 Δ(*cos–A*)::Kn$^r$ (Y. Hwang and M.F., unpublished work). MF1427 (λ *cI857 att*$^+$ *gal*$^+$ *cos*2) 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 *cosQ*1 into λ *cI857 att*$^+$ *cos*2 *gal*$^+$ (14, 21); the resulting *att*$^+$ *gal*$^+$ phages with *cos*$^+$ or *cosQ*1 were used to lysogenize MF713 and MF532. MF713 carries a prophage deleted for *attL* and the early genes, and MF532 carries an *imm*434 prophage deleted for the late genes and *attR*. Integration of an *att*$^+$ *gal*$^+$ prophage into the *attR* site in MF713 generates a strain equivalent to a dilysogen with *cos*$^+$ at the initial position and the *cos* of the *att*$^+$ phage (*cos*$^+$ or *cosQ*1) 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 (*cos*$^+$ or *cosQ*1) 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 *imm*21 *c*I *b*538 *red*3 *p*4, 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.** Cosmids containing various *cos* alleles, linearized with *Hin*dIII and end-labeled by incubation with [α-$^{32}$P]dATP and Klenow DNA polymerase, were packaging substrates. The 75-μl packaging mixture contained 500 ng of DNA, 15 μl of prohead extract, 5 μl of terminase extract diluted in prohead 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. Extracts 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.

***In Vivo cos* Cutting.** The assay described by Murialdo and Fife (25) was used to study *cos* cutting *in vivo*. *E. coli* C1100 lysogens were grown in 25 ml of LB medium at 31°C to a cell density of ≈2 × 10$^7$ cells per ml. The cultures were transferred to 42°C for 20 min and then incubated at 37°C, with aeration, for 30 min. The cells were harvested, and nucleic acids were extracted as described (26). *Bst*XI-digested DNAs were subjected to electrophoresis through 1.0% agarose and transferred onto a GeneScreen*Plus* (New England Nuclear) membrane. DNA hybridizations were done by using $^{32}$P-labeled, *Hin*dIII-digested pSF1 as probe.

## RESULTS

**Effect of *cosQ*1 Mutation on Initiation and Termination of DNA Packaging.** A helper packaging assay was used to study the effect of the *cosQ*1 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 concatemer 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 *cosQ*1 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 *cosQ*1 was placed at the initiating *cos*, prophage packaging was not significantly affected; whereas packaging of the prophage was reduced ≈10-fold when *cosQ*1 was located at the terminal *cos* (Table 1). This experiment suggested that *cosQ*1 might affect a site involved in termination of packaging.

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

| Prophage | Initial *cos* | Terminal *cos* | Prophage yield* |
|---|---|---|---|
| | MF532 lysogens† | | |
| λ *att*$^+$ *cos*$^+$ | *cos*$^+$ | *cos*$^+$ | 0.30 (0.04) |
| λ *att*$^+$ *cosQ*1 | *cosQ*1 | *cos*$^+$ | 0.20 (0.04) |
| | MF713 lysogens‡ | | |
| λ *att*$^+$ *cos*$^+$ | *cos*$^+$ | *cos*$^+$ | 0.64 (0.09) |
| λ *att*$^+$ *cosQ*1 | *cos*$^+$ | *cosQ*1 | 0.08 (0.02) |

*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 *cosQ*1 at initial *cos* position.
‡Effect of *cosQ*1 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 φ80, 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 Δ*cosQ* mutation, a 14-bp deletion of bp 48,470–48,783, was constructed and found to be lethal, causing $>10^5$-fold reduction in the phage yield (data not shown).

It was of interest to determine whether the effect of the severe Δ*cosQ* mutation differed only in magnitude from the effect of the *cosQ*1 mutation. The effect of the Δ*cosQ* mutation on packaging initiation was of particular interest because *cosQ*1 had no apparent effect on this packaging step. Due to the lethality of Δ*cosQ*, the *in vivo* helper packaging assay could not be used to address the question. To determine the effect of the Δ*cosQ* 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. 2*A*, lane 4). For cosmid DNA containing the Δ*cosQ* 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 Δ*cosB* DNA gave no specific packaging, the *cosQ*1 and Δ*cosQ* DNAs were efficiently packaged, indicating the *cosQ* mutations have no effect on packaging initiation (Fig. 2*B*).

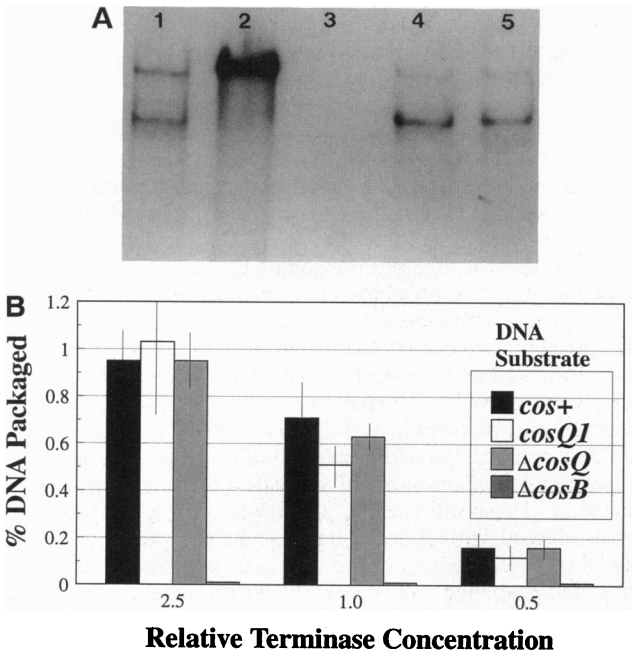


FIG. 2. Effects of *cosQ* mutations on DNA-packaging initiation *in vitro*. (*A*) Effect of Δ*cosQ* mutation on packaging initiation. In this assay $^{32}$P-labeled, linearized cosmid DNA and ATP were mixed with extracts containing terminase and proheads; after incubation, DNaseI was added to hydrolyze 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 disruption of the proheads followed by agarose gel electrophoresis. Lane 1, linearized *cos*$^+$ substrate DNA cut with terminase—top band is the 3.4-kb uncut 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, Δ*cosQ* substrate DNA in complete packaging reaction. No DNaseI was added to reactions in lanes 1 and 2. (*B*) Packaging initiation with *cos*$^+$, *cosQ*1, Δ*cosQ*, and Δ*cosB* 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.

***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 *Bst*XI restriction fragment spanning *cos* into

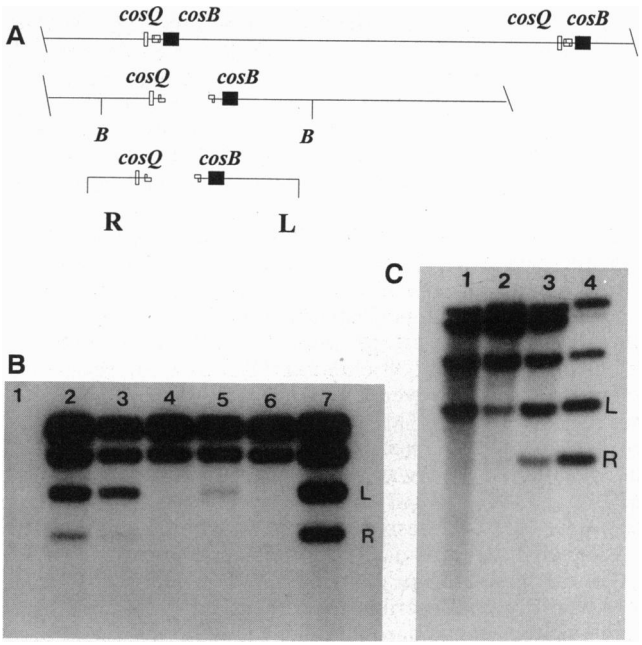


FIG. 3. DNA processing *in vivo*. (*A*) Rationale of *in vivo cos* cutting assays. (*Top*) Concatemeric λ DNA. Right (*cosQ*) and left (*cosB*) ends of successive chromosomes are indicated. *cosN* sites are indicated by open boxes with staggered vertical lines; *cosB* segments are represented by filled rectangles. (*Middle*) Cleavage of an initial *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 *Bst*XI sites (indicated by the *Bs*) generates the short restriction fragments shown. The *Bst*XI 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 *Bst*XI, 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 *cosQ*1 (lane 3); λ-P1 R3$^-$ R2$^-$ R1$^-$ (lane 4); λ-P1 *A*am42 (lane 5); or λ-P1 *cos*2 (lane 6). Mature λ-P1 *cos*$^+$ DNA, digested with *Bst*XI, was in lane 7. (*C*) C1100 infected with λ-P1 Δ*cosQ* (lane 1); λ-P1 *A*am42 (lane 2); λ-P1 *cos*$^+$ (lane 3); and mature λ-P1 *cos*$^+$ DNA, digested with *Bst*XI (lane 4). Positions of bands of the mature left (L) and right (R) chromosomal ends are indicated. The other *Bst*XI 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. 3*A*). We assayed *in vivo cos* cutting by isolation of total DNA from induced lysogens, digestion of the DNA with *Bst*XI, 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 *cos2* mutations, the latter a deletion of *cosN*, cause defects in initiation of DNA packaging, no mature DNA was expected, and none was found (Fig. 3*B*, lanes 4 and 6, respectively). An additional control was λ-P1 *A*am42; the *A*am42 mutation in a nonsense mutation in the fifth-to-last codon of the *A* gene. *A*am42 mutation is lethal, due to the failure of the mutant terminase to bind λ proheads; the initial *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 *cosN* cleavage should generate one mature right chromosomal end and one mature left end. The *A*am42 DNA contained mature left ends, but no right ends were detected (Fig. 3*B*, lane 5). Failure to observe mature right ends of chromosomes in *A*am42-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 *cos* cleavage events are known to be accessible to exonuclease V *in vivo* (26, 29). In contrast, terminase is thought to remain bound to *cosB* of the mature left end, protecting against nuclease attack (26, 29). DNA isolated from λ-P1 *cosQ*1-infected cells contained mature left-end fragments and a faint band representing right end fragments (Fig. 3*B*, lane 3), a result consistent with efficient initiation and inefficient termination. The intracellular DNA of λ-P1 Δ*cosQ* was also examined and found to contain only mature left ends, a result consistent with a profound defect in terminal *cos* cleavage (Fig. 3*C*, lane 1).

**Structures in *cosQ* Mutant Lysates.** Before packaging, λ 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 (13). If *cosQ* mutations affect packaging termination, then *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.

λ-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. 4*A*; Table 2). Expanded heads can be distinguished from proheads by their larger diameter and hexagonal shape (30). λ-P1 *cos*2 (Fig. 4*C*) and λ-P1 R3⁻ R2⁻ R1⁻ (data not shown) lysates contained only proheads, as expected for phages with initiation defects.

The λ-P1 *cosQ*1 lysates contained the same structures as the λ-P1 *cos*⁺ lysates (Fig. 4*B*), but the relative proportions differed. Lysates of the *cosQ*1 mutant contained phages representing 5% of the total structures present; this value is ≈10-fold lower than was found for λ-P1 *cos*⁺, consistent with the ≈10-fold lower yield of λ-P1 *cosQ*1. The ratio of expanded heads to phages for λ-P1 *cosQ*1 was ≈20-fold higher than the ratio for λ-P1 *cos*⁺. The λ-P1 Δ*cosQ* lysate was similar to the λ-P1 *cosQ*1 lysate, except that the level of phages was much lower (0.4% vs. 5%, respectively). The accumulation of expanded heads by *cosQ*-containing mutants is consistent with the idea that *cosQ* mutations affect packaging termination.

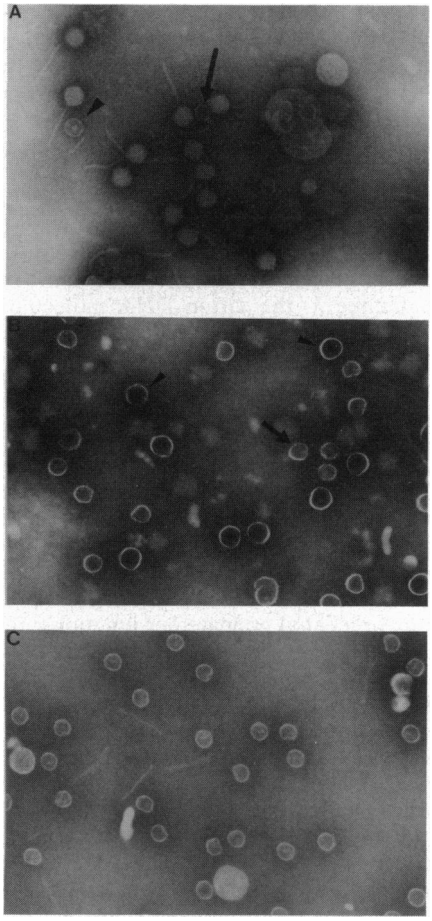


FIG. 4. Electron micrographs of head-related structures in phage lysates. Proheads, present in each micrograph, are 25 nm in radius. (*A*) λ-P1 *cos*⁺ structures. The arrow indicates a λ prohead; an expanded phage head is indicated by the arrowhead. (*B*) λ-P1 *cosQ*1 structures. The arrow indicates a λ prohead; the arrowheads indicate expanded λ heads. (*C*) Proheads in a λ-P1 *cos*2 lysate.

## DISCUSSION

**The Role of *cosQ*.** For packaging of a λ chromosome, initiation requires *cosN* and *cosB*. Our results show that a unique site (*cosQ*), as well as *cosN*, is required for termination of DNA packaging. What is the defect for *cosQ* mutants? Preliminary evidence indicates that *cosQ* mutations result in failure to cut the terminal *cos*, as follows. The Δ*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 *cos* has been packaged into the head (unpublished data). These particles are similar to those obtained during packaging

Table 2. Phage, proheads, and expanded heads present in phage lysates

| Phage lysate | Structures | | |
|---|---|---|---|
| | Phages | Proheads | Expanded heads |
| λ-P1 *cos*⁺ | 47.5 | 42.3 | 10.1 |
| λ-P1 *cos*2 | 0 | >99 | <1 |
| λ-P1 *cosQ*1 | 5 | 76.7 | 18.3 |
| λ-P1 Δ*cosQ* | 0.44 | 76.6 | 22.9 |
| λ-P1 R3⁻ R2⁻ R1⁻ | 1 | 98 | 1 |

Numbers are percentage of total identifiable phage head-related particles. Number of particles examined were as follows: λ-P1 *cos*⁺, 1659; λ-P1 *cos*2, 1757; λ-P1 *cosQ*1, 1588; λ-P1 Δ*cosQ*, 2493; and λ-P1 R3⁻ R2⁻ R1⁻, 449.

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 pause 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 λ, these viruses make specific cuts in concatemeric DNA during packaging. Unlike λ, 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 *a* sequence. The *a* 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 concatemer junctions; regeneration by local replication has recently been found for T7 (33).

Well-developed genetics and biochemistry make the λ DNA-packaging system ideal for dissecting the molecular mechanism of packaging termination. Further study of λ-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. λ 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 *A*am42 mutation only inactivates the prohead-binding activity of terminase; *A*am42 terminase still binds *cosB* and cuts *cosN* (26). Unlike *cosQ* mutants, lysates of λ *A*am42 contain only proheads, indicating no attempt to package DNA (26). The intracellular DNA of λ-P1 *A*am42 contains mature left ends but has no right ends (ref. 26; Fig. 3 *b* 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 λ-P1 Δ*cosQ* also has only left ends (Fig. 3*C*), 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 λ-P1 *cosQ*1, 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 λ-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 *cosQ*1 lysates, the levels of expanded heads (18%) and completed phages (5%) give an estimate of 0.22 for the efficiency of packaging completion, and for λ-P1 Δ*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.

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