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# Separate sites for binding and nicking of bacteriophage λ DNA by terminase

(bacteriophage DNA packaging/DNA–protein interactions/*in vitro* mutagenesis/*cos* mutation/DNA site recognition)

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**ABSTRACT** **The cohesive end site (*cos*) is the site of action of bacteriophage λ terminase, the enzyme that introduces staggered nicks to generate the 12-base cohesive ends of mature λ DNA. Deletion mutations that remove the λ cohesive end sequence have been isolated after *in vitro* mutagenesis. The deletions were obtained by digesting the DNA of a *cos* duplication phage with S1 nuclease to remove the cohesive ends and adjacent base pairs, followed by blunt end ligation and DNA packaging into phage particles. *cos*2 is the result of a 22-base-pair deletion that exactly removes the segment of rotational symmetry that includes the cohesive end sequence. The *cos*2 mutation abolishes nicking by terminase but does not affect terminase binding. We conclude that *cos* contains two sites that interact with terminase: *cos*N, the nicking site; and *cos*B, a binding site for terminase.**

Viruses assemble through specific interactions between the components. Bacteriophage head assembly, for example, requires specific recognition of the virus chromosome by the packaging apparatus. We report here on the nature of the DNA–protein interactions involved in the selection of bacteriophage λ chromosomes for packaging.

λ chromosomes are generated by cleavage of multichromosomal lengths of λ DNA that are primarily the result of rolling circle replication (1). The cleavage reaction occurs at a specific site and results in the introduction of staggered nicks 12 base pairs (bp) apart, which generates linear molecules of unique sequence with complementary single-stranded projections (cohesive ends) that are 12 bp long (2). The cohesive ends hybridize upon injection of the DNA into a cell, cyclizing the molecule.

The cleavage reaction can be carried out *in vitro*; cleavage requires λ terminase, a host factor(s), and small molecules (3). Terminase is the product of the *Nu1* and *A* genes of λ (4). *In vivo*, the cleavage reaction is observed only when packaging can occur, so that molecules with cohesive ends are not found in cells infected by prohead mutants (5–8). It is not known why *in vivo* cleavage is dependent on packaging and *in vitro* cleavage is not (see ref. 9 for discussion).

The site of action of terminase is called the cohesive end site (*cos*); hence, *cos* includes all the base pairs with which terminase interacts to bind to the DNA and introduce the nicks (10, 11). A prominent structural feature at the nicking site is a segment of rotational symmetry: 20 of 22 bp show either strict or purine:pyrimidine rotational symmetry and the nick sites are also symmetrically disposed (12, 13) (Fig. 3). We will designate this symmetry box "the cohesive end symmetry segment." By analogy with type II restriction enzymes it has seemed reasonable to equate this symmetry segment with *cos*, but recent results with λ and phage 21 show that *cos* includes base pairs outside the symmetry box. Phages 21 and λ have packaging systems that have diverged from a common ancestor: each packages the other's DNA with approximately 1% efficiency (14, 15). Viable λ–21 hybrids have been isolated in which the left chromosome end (which contains the terminase and prohead genes) is derived from phage 21 and the rest of the chromosome is λ DNA. Such hybrids have the packaging specificity of phage 21 even though the cohesive end symmetry segment is identical to that of λ (15). This indicates that *cos* includes base pairs at the left chromosome end, outside the symmetry box, that are important for packaging and for which *cos*λ and *cos*φ21 differ. We have recently shown that λ terminase cannot bind to *cos*φ21, even though λ terminase can cleave *cos*φ21 under specific conditions in which the binding defect is bypassed (16). Thus, *cos*φ21 can function as a nicking site for λ terminase but not as a binding site.

Here we report the isolation and characterization of several *cos* mutations in which the cohesive end sequence and neighboring sequences are deleted. The *cos* deletion mutations were isolated as part of studies on the interaction of *cos* with the Chi sequence, the stimulator of *rec*B,C-mediated recombination (ref. 17; for review of Chi, see ref. 18). One of the mutations, *cos*2, has been studied in detail. We find that the *cos*2 mutation is a deletion of the complete cohesive end symmetry segment. The *cos* site with the *cos*2 deletion binds λ terminase but is not nicked by terminase.

## MATERIALS AND METHODS

**Strains.** *Escherichia coli* strains were IC202 (*rec*A1; 19); NS428, a *rec*A *sup*$^0$ lysogen of λAam11 *b*2 *red*3 *c*I857 Sam7 (20); NS433, a *rec*A *sup*$^0$ lysogen of λEam4 *b*2 *red*3 *c*I857 Sam7 (20); MF871, a *sup*$^0$ lysogen of λ*gal*805 *c*I857 Sam7 (21); 594, a *sup*$^0$ strain (22); QD5003 (λ$^+$), a *sup*F strain (23); MF211, a λ*imm*21 lysogen of C600 (22); and MF611, a *rec*A1 *sup*$^0$ strain (24).

Phage λ strains included IM91, which carries a 1.7-kilobase-pair (kbp) *Bgl* II segment that includes *cos*λ. The *cos*λ segment is inserted in reverse orientation into the *Bgl* II site in *Eco*RI fragment B of λ (at 0.47, where 0 is at the *Nu1-A* end and 1.0 is at the *R* end of the λ DNA molecule); IM91 is also *c*I857 *sri*4$^0$ *nin*5 *sri*5$^0$ (see ref. 17 for complete details). The other phages used were λ*imm*434 Aam11 and λ*imm*21 *c*I *b*538 *red*3 *p*4 (15). The methods of strain construction involving mutant *cos* have been described (25).

**Isolation of *cos* Mutants.** S1 nuclease digestion was carried out as follows. A solution containing IM91 DNA (*cos*$^+$/*cos*$^+$ *imm*λ *am*$^+$) at 90 μg/ml and λ *cos*$^+$ *imm*434 *c*I Aam11 DNA at 9 μg/ml in 0.1 M NaCl/0.01 M Tris·HCl/0.1 mM EDTA, pH 8.0, was heated at 66°C for 5 min and then quickly chilled. An equal volume of ice-cold 0.5 M NaCl/0.05 M sodium acetate/4 mM $ZnSO_4$, pH 4.5, was added. To 80 μl of this, 12 μl (288 units) of S1 nuclease (Miles) or 12 μl of S1 diluent (0.3 M NaCl/0.025

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Abbreviations: *cos*, cohesive end site, specificities noted for phage λ or phage 21, as in *cos*λ; bp, base pair(s); kbp, kilobase pair(s).

M sodium acetate/0.1 mM $ZnSO_4$, pH 4.5) without nuclease was added. The mixture was incubated at 30°C for 30 min. To stop the reaction, we added 24 μl of 0.44 M potassium phosphate/0.025 M EDTA, pH 8.0, and heated at 66°C for 5 min. A 2-μl sample was removed into 20 μl of calf thymus DNA solution (200 μg/ml) in TEK buffer (0.01 M Tris·HCl/1 mM EDTA/0.05 M KCl, pH 8.0), and 10 μl of the resulting mixture was used for *in vitro* packaging. The phage particles from IM91 and its derivatives were detected on 594 ($Sup^0$), and those from λ*imm*434 *c*I Aam11 were detected on QD5003(λ) ($Sup^+$).

The rest of the sample (114 μl) was mixed with 5 ml of 0.07 M Tris·HCl/0.01 M $MgCl_2$, pH 7.8, and centrifuged in a polyallomer tube in a SW 50.1 rotor at 30,000 rpm for 16 hr in a Beckman centrifuge. The DNA was suspended in 20 μl of 0.07 M Tris·HCl/0.01 M $MgCl_2$/0.01 M dithiothreitol/0.4 mM ATP, pH 7.8, containing 50 μg of bovine serum albumin per ml. A 2-μl sample was taken for packaging as before. To the remaining solution, we added 3 μl of T4 DNA ligase (kindly supplied by Karen Sprague) and incubated at 15°C for 16 hr (1 μl of this ligase preparation was sufficient to ligate 2 μg of intact λ DNA under this condition). A 2-μl sample of this was added to calf thymus DNA solution and packaged as before. Seven plaques on 594 were purified, and DNA was prepared from each and analyzed by restriction enzyme digestion. One strain, LIK525, was defective in the primary *cos*, and we called the responsible mutation *cos*2 (17). Another strain, LIK518, had a defect in the secondary *cos*, and we called the mutation *cos*3 (17). Four more strains had a defect in the secondary *cos*. One remaining strain had two active *cos* sites.

***In Vitro* Packaging of λ DNA.** The earlier procedure (26, 27) was slightly modified. Bacterial strains NS428 and NS433 were used. After the first centrifugation, the cell pellets were suspended in 9 ml of 40 mM Tris·HCl/10 mM spermidine/10 mM putrescine, pH 7.8, at room temperature. The pellet from the second centrifugation was suspended in 0.7 ml of this buffer. λ DNA bounded by a pair of covalently closed *cos* is packaged much more efficiently than linear monomers in this system (28).

**DNA Sequence Analysis.** The base-specific cleavage method (29) was used. pBW8 DNA was 5′ end-labeled at the *Ava* II sites by using [$\gamma$-$^{32}$P]ATP (ICN) and T4 polynucleotide kinase (P-L Biochemicals) (30) or 3′ end-labeled by using [$\alpha$-$^{32}$P]dATP, dGTP, and the Klenow fragment of *E. coli* DNA polymerase I (New England BioLabs) (29). After digestion with *Hin*cII, DNA fragments were separated on an 8% polyacrylamide gel. The *cos*2-containing fragment was recovered by electroelution.

**Plasmids.** All plasmids used are derivatives of pBR322 (31) carrying inserts of λ DNA in the tetracycline-resistance determinant. The $cos^+$ test plasmid used for terminase binding experiments was pMF517; this plasmid has the 1.7-kbp *Bgl* II *cos*λ segment inserted into the *Bam*HI site of pBR322 (32). The other $cos^+$ plasmid used was pSF1, which contains an insert of λ DNA extending from the *Hin*dIII site at 0.912 through *cos* to the *Bam*HI site at 0.113. pBW8 is isogeneic to pSF1 except that it carries the *cos*2 mutation instead of $cos^+$. The *cos*φ21 plasmid pBW3 carries an *Hin*dIII insert of λ–21 hybrid 19 DNA (15) extending from 0.912 through *cos*φ21 to 0.06; pMF2 is a *cos*Δ plasmid carrying a *Bam*HI insert extending from 0.97 to 0.113 but containing a 1.7-kbp *Bgl* II-generated *cos* deletion extending from 0.973 to 0.010. Construction of pSF1, pBW3, pMF2, and pMF517 was described elsewhere (16, 32). The construction of the *cos*2 plasmid pBW8 from IM91 and pBR322 was as described for pSF1 (32). Plasmid DNA was isolated from IC202 derivatives by the method of Clewell (33).

***cos* Cleavage.** Reactions were carried out as described (16). The 70-μl reaction mixture contained 20 μl of 2× buffer A (40 mM Tris·HCl, pH 8.0/6 mM $MgCl_2$/0.1% 2-mercaptoethanol/2 mM EDTA), 4 μl of buffer M2 [6 mM Tris·HCl, pH 7.4/60 mM spermidine/18 mM $MgCl_2$/15 mM ATP/20 mM 2-mercaptoethanol), 0.75 μg of *Pst* I-cut pMF517 DNA, 20 μl of sonic extract of MF611 cells (the source of host factor), and 20 μl of partially purified terminase [prepared as described by Blattner *et al.* (34)]. Incubation was for 15 min at 20°C. The reaction was stopped by addition of 20 μl of 0.25 M EDTA at pH 8.0. RNA was digested for 15 min at 37°C after addition of 2 μl of pancreatic RNase (2 mg/ml). Then, 10 μl of 1% sodium dodecyl sulfate containing proteinase K at 500 μg/ml was added, and digestion was continued for 1 hr at 37°C, followed by ethanol precipitation. After agarose gel electrophoresis, the reaction products were transferred to nitrocellulose (35) and hybridized to a $^{32}$P-labeled pSF1 plasmid probe (36). Autoradiograms were analyzed by densitometry. The *cos* cleavage reaction is a modification of the conditions of Becker and Gold (3).

**Competition *cos* Cleavages.** These reaction mixtures were identical to those in the above cleavage reaction except that 0, 2, or 4 μg of competitor plasmid DNA (added as a mixture of approximately 60% supercoils and 40% open circles) was present in the reaction mixture. The cleavage products of the pMF517 test substrate were small enough to be separable electrophoretically from the competitor DNAs. Nucleic acids were isolated by phenol extraction and ethanol precipitation as described by Becker and Gold (3). For this method, nucleic acid recovery was somewhat variable and was followed by addition of nick-translated $^{32}$P-labeled pMF517 DNA to a specific activity of $3 \times 10^3$ cpm/μg. Extent of *cos* cleavage was determined by ethidium bromide staining of agarose electrophoresis gels of reaction products. Gels were illuminated with UV light and the visible fluorescence was photographed. Densitometer tracings of the negatives were integrated.

## RESULTS

**Isolation of *cos* Mutants by *in Vitro* Mutagenesis.** *cos* duplication phage IM91 (17) was used so that *cos* mutations inactivating one site would not render the phage inviable [IM91 has a secondary *cos* site, a 1.7-kbp *cos* segment inserted into the *b* region (in the middle of the chromosome) in reverse orientation]. Virion DNA of IM91 contains two types of molecules, one type cut at the normal *cos* site and one type cut at the secondary *cos* site. IM91 DNA was mixed with control λ*imm*434 *c*I Aam11 DNA and the mixture was digested with S1 nuclease to remove the cohesive ends (and additional duplex base pairs). The DNA molecules were then ligated and packaged *in vitro*. Among the various ligated forms, the packaging system used selectively packages multimeric DNA (28) (Fig. 2). To monitor the reactions we packaged DNA *in vitro* at each step and mea-

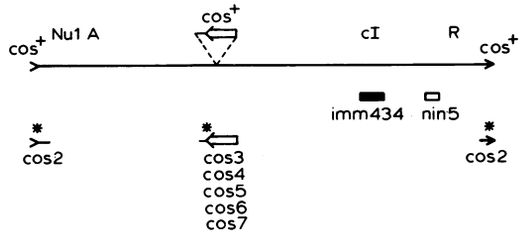


FIG. 1. λ map. (*Upper*) Sites and genes. (*Lower*) Mutations. The natural primary *cos* is indicated as the ends of the arrow. *cos*2 inactivates this primary *cos*. Secondary *cos*, made up of φ80 right end and λ left end, is placed in the middle of the λ IM91 chromosome. *cos*3, *cos*4, *cos*5, *cos*6, and *cos*7 deletions inactivate this secondary *cos*. The genes *Nu1* and *A* code for terminase. *imm*434 substitution replaces the immunity region of λ. *nin*5 is a deletion.

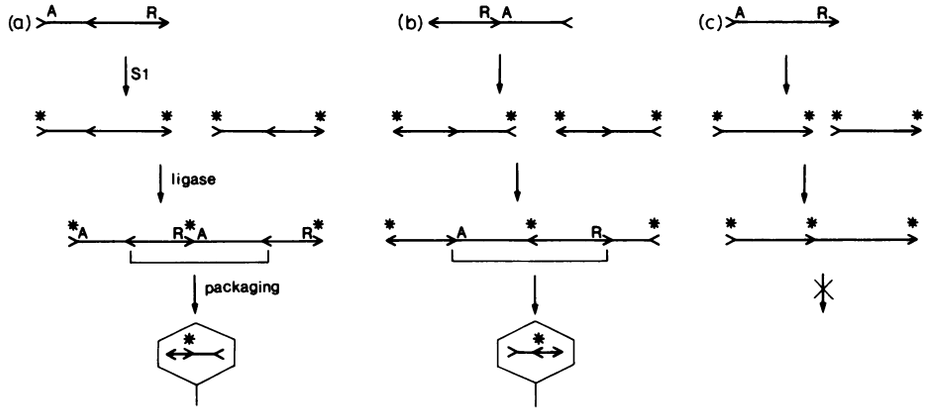


FIG. 2. Strategy in isolation of *cos* deletions. IM91 carries two *cos* sites. Its virion DNA has been packaged either from the primary *cos* (*a*) or from the secondary *cos* (*b*). When S1 nuclease removes nucleotides from the ends, the DNA no longer will be packaged *in vitro* into phage particles. When the ends are properly ligated together, the DNA can be packaged from another intact *cos* pair, and a *cos* mutant will be recovered as a phage particle. Ligation will not revive single *cos* λ (*c*). The ratio of the packaging efficiencies of the two kinds of λ DNA will be a measure of successful ligation.

sured the packaging efficiency (phage yield per input phage chromosomes). S1 nuclease digestion decreased the packaging efficiency of both IM91 and λ*imm*434 *c*I Aam11 DNA by a factor of $10^4$ (Table 1). We interpreted this as indicating successful digestion of the cohesive ends. The subsequent ligation step revived the double *cos* IM91 DNA but did not revive the single *cos* DNA of λ*imm*434cI Aam11. We inferred that IM91 DNA was packaged from the other *cos* as shown in Fig. 2. We analyzed the DNA and found *cos* mutants.

**Location and Size of *cos* Deletions.** Digestions of phage DNAs with *Sma* I and *Eco*RI showed that *cos*2 inactivates the primary *cos* and that *cos* mutations 3, 4, 5, 6, and 7 inactivate the secondary *cos* site of IM91 (data not shown; see Fig. 1). *Ava* II–*Pvu* II fragments spanning *cos* are of the same size for the primary and the secondary *cos* sites (ref. 13; Andrew Becker, Toronto, personal communication). We compared the *Ava* II/*Pvu* II digests of IM91 ($cos^+/cos^+$) and the *cos* mutants. All the *cos* mutations shortened one fragment. With *Ava* II/*Hin*cII double digests, we again found the *cos* mutations shortened one fragment. From comparison of the mobilities we estimated the size of the deletions as follows; *cos*2, 20 bp; *cos*3, 25 bp; *cos*4, 20 bp; *cos*5, 25 bp; *cos*6, 20 bp; *cos*7, 30 bp. To study further *cos*2, we inserted *cos*2 and $cos^+$ DNA segments (from the *Hin*dIII site at 0.912 through *cos* to the *Bam*HI site at 0.113) into pBR322. We compared *Ava* II/*Hin*cII digests of the $cos^+$ (pSF1) and *cos*2 (pBW8) plasmids; *cos*2 was estimated to be a deletion of 21 ± 4 bp.

Table 1. Isolation of *cos* mutants

| Step | Packaging efficiency ratio, double *cos* DNA/single *cos* DNA | |
|---|---|---|
| | S1 omitted | Complete |
| After S1 nuclease | $\frac{1.21 \times 10^{-4}}{1.79 \times 10^{-4}} = 0.68$ | $\frac{<1 \times 10^{-8}}{<4 \times 10^{-8}}$ |
| Before ligase | $\frac{1.13 \times 10^{-5}}{1.44 \times 10^{-5}} = 0.78$ | $\frac{7.5 \times 10^{-8}}{8.8 \times 10^{-8}} = 0.85$ |
| After ligase | $\frac{1.04 \times 10^{-5}}{1.39 \times 10^{-5}} = 0.74$ | $\frac{5.2 \times 10^{-7}}{5.0 \times 10^{-8}} = 10.4$ |

IM91 ($cos^+/cos^+$, see Fig. 1) DNA was mixed with λ*imm*434 Aam11 DNA, treated with S1 nuclease, concentrated into a different buffer, and ligated. In a parallel run, nuclease was omitted. At each step, a portion was packaged *in vitro*. The resulting IM91 (and its derivative) phage particles and the λ*imm*434 Aam11 phage particles were detected on different indicator bacteria. The packaging efficiencies (output phage particles/input phage chromosomes) were calculated for IM91 DNA (and its derivatives) and for λ*imm*434 Aam11 DNA (single *cos* site) separately and then compared.

**Sequence of the *cos*2 Deletion.** To determine the exact location and size of the *cos*2 deletion, we determined the sequence of the 5′ strand of *cos*2 from the *Ava* II site located to the left of *cos* and proceeded rightward (data not shown). We compared the sequence with the $cos^+$ sequence (13). We found that *cos*2 is a continuous 22-bp deletion; the deletion removes the 12 bp of the cohesive ends, 5 bp to their left, and 5 bp to their right (Fig. 3). The sequence outside of this deletion was the same as that determined by Nichols and Donelson (13) from −32 to +44. To confirm the *cos*2 sequence, the 3′ strand was also analyzed from the *Ava* II site and the same 22-bp deletion was deduced.

***In Vivo* Defectiveness of Deleted *cos* Sites.** The restriction enzyme analysis indicated that the deleted *cos* sites do not function *in vivo*; for each mutant the $cos^+$ fragment appears to be always cleaved and the deleted *cos* site fragment appears not to be (see above and ref. 17).

A helper packaging experiment also indicated that the *cos*2 mutation renders *cos* nonfunctional *in vivo*. We studied the ability of a helper phage to package the $cos^+$ and *cos*2 plasmids (pSF1 and pBW8). Under recombination-blocked conditions, cosmid packaging depends on a functional *cos* (32). We found that pSF1 was packaged by a helper, but pBW8 was not (data not shown).

**The *cos*2 Site Is Not Efficiently Cleaved by Terminase *in Vitro*.** The *cos*2 substrate was pBW8 plasmid DNA made linear with *Hin*dIII. The $cos^+$ control DNA was linear pSF1 DNA, which differs from pBW8 only at *cos*. Under the reaction conditions used, the DNA concentration was just sufficient to saturate the terminase. Forty percent of the $cos^+$ DNA molecules were cleaved in the reaction, whereas cleavage of the *cos*2 substrate was not detected (<4%) (Fig. 4). The *cos*2 mutation thus causes a defect in *cos* cleavage.

**The *cos*2 Site Binds Terminase.** Cleavage by terminase of a small $cos^+$ test substrate (pMF517, made linear by *Pst* I cleavage) was studied in the presence of *cos*-containing competitor DNAs (added in circular, predominantly supercoiled form). If a competitor DNA binds terminase, the cleavage of the test substrate is reduced (as detected by separation of cleavage products on agarose gels, followed by measuring ethidium bromide fluorescence). Fig. 5 shows the results of the experiment. The $cos^+$ control curve (pSF1) shows the reduction of cleavage of the test substrate. The *cos*Δ control curve (pMF2) shows the absence of competition for a competitor plasmid lacking *cos* altogether. An additional negative control is a *cos*φ21 competitor previously shown not to bind terminase (16). The *cos*2 competitor DNA competed for terminase nearly as well as the $cos^+$ control DNA. The reason for the reproducibly small difference between the $cos^+$ and *cos*2 DNAs is not understood. The difference may be due in some way to the difference in the reactions; that is, extensive *cos* cleavage only occurs in the $cos^+$ reaction, or the *cos*2

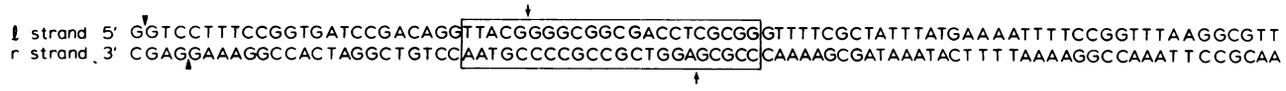


FIG. 3. Sequence of *cos*$^+$ and *cos*2. *cos*2 lacks the boxed sequence. Arrows, terminase cuts to make cohesive ends; ▲, ▼, *Ava* II site used for end labeling. The *cos*$^+$ sequence inside the box is from Nichols and Donelson (13).

mutation may delete base pairs that play a minor role in terminase binding. The *cos*$^+$ and *cos*2 plasmids competed more strongly than expected from the ratio of competitor to substrate sites. The competition greater than expected is due to the supercoiled state of the competitor DNAs. When linear or open circular *cos*$^+$ competitor DNA was used, there was agreement between observed and expected (data not shown).

We conclude that the major determinants for terminase binding are not deleted by the *cos*2 mutation.

## DISCUSSION

The *cos* deletion mutants were initially isolated to analyze stimulation of Rec-mediated recombination by the Chi sequence. In λ lytic infections, Chi was fully active in combination with a functional *cos* site (of one particular orientation), but the *cos*2 deletion eliminated this activity (17). These observations together with the present results indicate that the terminase–*cos*2 interaction revealed in this paper is not sufficient to activate Chi.

The *cos*2 mutation clearly inactivates *cos* function *in vivo*, as shown by: (*i*) the restriction enzyme study showing that the *cos*$^+$/*cos*2 duplication phage uses only the *cos*$^+$ site for cleavage (17), and (*ii*) the inability of the *cos*2 cosmid pBW8 to be packaged by helper λ. The detailed study of terminase–*cos*2 interactions shows that the *cos*2 mutation affects nicking by terminase but terminase binding is not greatly altered. This leads to a model for *cos* with two separate sites: a nicking site (*cos*N) and a binding site (*cos*B). The *cos*2 site is thus $N^-B^+$.

The behavior of the phage 21 cohesive end site is also consistent with a *cos*N and *cos*B structure (16). The *cosφ*21 site can be nicked by λ terminase but does not bind λ terminase ($N^+B^-$). The ability of λ terminase to nick *cosφ*21 was demonstrated under special conditions in which the requirement for a binding site was bypassed: A chromosome can be packaged by λ when the initial *cos* site is *cos*λ ($N^+B^+$) and the terminal *cos* site is *cosφ*21 ($N^+B^-$). The λ terminase that cleaves the terminal *cos* site must bind at the initial *cos* site and be brought into contact with the terminal *cos* site by packaging. Thus, the terminal *cos* need not have a binding site (16).

***cos*N Structure.** The cohesive end symmetry segment is a 22-bp sequence in which many of the base pairs show either strict or Pu·Py 2-fold rotational symmetry, and the nicking sites are also symmetrically arranged. It has been assumed that the symmetric bases represent the base pairs involved in specific interactions with identical subunits of a multisubunit terminase during nicking as in the case of *cro* protein (37). The results with *cos*2 and *cosφ*21 are consistent with but do not prove this model. The *cosφ*21 site is identical to *cos*λ in the cohesive end symmetry segment and can be nicked by λ terminase. The *cos*2 mutation removes the 22-bp symmetry segment and blocks nicking by terminase, showing that sequence specificity is required for nicking. More detailed statements about *cos*N structure cannot be made, however, because the distance between base pairs on either side of the *cos*2 deletion is changed. Were such base pairs part of the nicking site, they could be functionally inactivated by the deletion.

***cos*B Structure.** Several experiments indicate that *cos*B is located at the left chromosome end, that is, to the *Nul* side of the cohesive end symmetry segment.

First, linear mature DNA can be packaged by λ *in vitro* and the packaging is terminase dependent. Because packaging proceeds in a *Nul*-to-*R* direction, it is logical that the terminase binding site be at the *Nul* end of the chromosome, a point first made by Hohn (14). Second, the *cos*2 mutation eliminates the cohesive end symmetry segment without abolishing the terminase binding site. Third, the *cosφ*21 site has base pair changes only to the *Nul* side of the cohesive end symmetry segment,

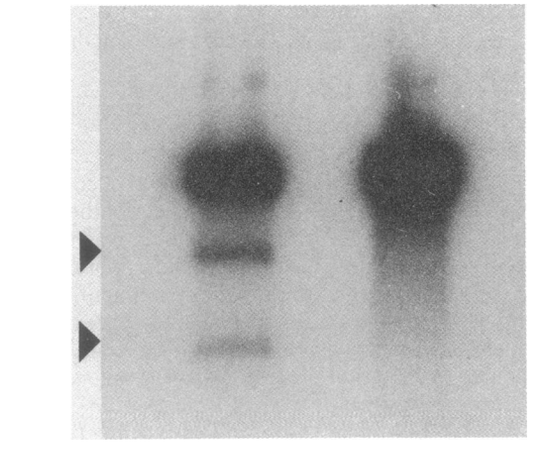

FIG. 4. The *cos*2 site is not efficiently cleaved by terminase. Autoradiogram of terminase cleavage reactions with *Hin*dIII-cut substrate plasmids. Lanes: left, *cos*$^+$ substrate (pSF1); right, *cos*2 substrate (pBW8). Cleavage of the 14-kbp substrate molecules at *cos* generates 9.7-kbp and 4.3-kbp fragments (►).

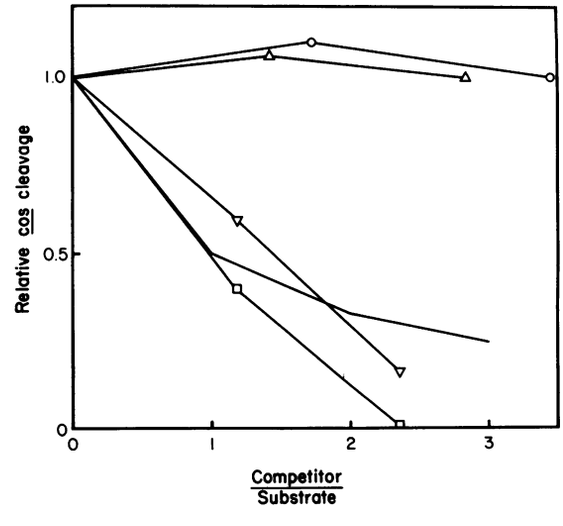


FIG. 5. λ terminase binds the *cos*2 site. The relative *cos* cleavage of the *cos*$^+$ substrate in the presence of various competitor DNAs is plotted. The expected competition for a *cos*$^+$ competitor, assuming equal affinity of terminase for substrate and competitor *cos* sites, is shown as a line without symbols. The other lines show competition results as follows: □, *cos*$^+$ plasmid pSF1; ▽, *cos*2 plasmid pBW8; △, *cosφ*21 plasmid pBW3; ○, *cos*Δ plasmid pMF2. The level of cleavage without competition was 52% for the experiments with pSF1 and pBW8 (terminase preparation 1) and 15% for the experiments with pBW3 and pMF2 (terminase preparation 2). The ratio of competitor *cos* sites (or molecules in the case of pMF2) to substrate *cos* sites is plotted.

and terminase binding is abolished.

The present work with *cos*2 indicates that the *cos*B site is confined to a segment extending toward *Nul* from the end of the cohesive end symmetry segment (+12, starting from the center of the dyad). The *cos*B segment cannot extend beyond +180, because a cosmid carrying a λ segment ending at the Charon 4a *Eco*RI site (38) at approximately +180 is packagable *in vivo* and is cleaved *in vitro* by terminase (16, 32). Unpublished work by us indicates that *cos*B occupies only a fraction of this length.

**Other Viruses.** Separate binding and cleavage sites may be general for terminases. For P22, chromosomes are packaged processively from concatemeric replicating DNA. Packaging of the first chromosome of a series initiates unidirectionally from a site called *pac*. The first cut is at *pac* and the second cut occurs when the head is full with approximately 103% of the unique sequence length of DNA, generating a terminally redundant molecule (39). The site of the second cut generates the start point of the next chromosome in the packaging sequence, so that the remaining chromosomes are packaged without sequence specificity. Casjens and Huang (40) have found that the initial cuts made at *pac* occur in six regions (called *end* sites) within a 120-bp segment of DNA. They proposed models in which the P22 terminase binds at a unique site, *pac*, and cleaves one of the nearby *end* sites. The obvious parallels between λ and P22 are that the terminase of each phage has separate binding and cutting sites. The differences are that the P22 terminase does not generate cohesive ends and does not invariably cleave the same point.

The DNA cutting apparatus of phage Mu acts in a manner analogous to that of P22. Mu virion DNA has segments of bacterial DNA at both ends, and the bacterial segment at the end from which packaging is postulated to initiate varies between 55 and 110 bp in length (41). Within this range are blocks of preferred cleavage 5–6 bp long with 5-bp spacers between the blocks. George and Bukhari (41) postulated a specific binding site for the cleavage enzyme in the nearby Mu sequences.

Hammarskjöld and Winberg (42) identified an encapsidation recognition DNA sequence near the left end of the adenovirus 16 DNA molecule, suggesting that recognition sites near chromosome termini may be a general feature among viruses.

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