Genetic Evidence That Recognition of \textit{cosQ}, the Signal for Termination of Phage \(\lambda\) DNA Packaging, Depends on the Extent of Head Filling

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ABSTRACT

Packaging a phage \(\lambda\) chromosome involves cutting the chromosome from a concatemer and translocating the DNA into a prohead. The cutting site, \textit{cos}, consists of three subsites: \textit{cosN}, the nicking site; \textit{cosB}, a site required for packaging initiation; and \textit{cosQ}, a site required for termination of packaging. \textit{cosB} contains three binding sites (\textit{R} sequences) for gpNu1, the small subunit of terminase. Because \textit{cosQ} has sequence identity to the \textit{R} sequences, it has been proposed that \textit{cosQ} is also recognized by gpNu1. Suppressors of \textit{cosB} mutations were unable to suppress a \textit{cosQ} point mutation. Suppressors of a \textit{cosQ} mutation (\textit{cosQ1}) were isolated and found to be of three sorts, the first affecting a base pair in \textit{cosQ}. The second type of \textit{cosQ} suppression involved increasing the length of the phage chromosome to a length near to the maximum capacity of the head shell. A third class of suppressors were missense mutations in gene \(B\), which encodes the portal protein of the virion. It is speculated that increasing DNA length and altering the portal protein may reduce the rate of translocation, thereby increasing the efficiency of recognition of the mutant \textit{cosQ}. None of the \textit{cosQ} suppressors was able to suppress \textit{cosB} mutations. Because \textit{cosQ} and \textit{cosB} mutations are suppressed by very different types of suppressors, it is concluded that \textit{cosQ} and the \textit{R} sequences of \textit{cosB} are recognized by different DNA-binding determinants.

Many large dsDNA viruses, such as the tailed bacteriophages and the herpes viruses, produce multichromosomal lengths DNA as a result of replication and recombination. During virion assembly, the multimeric DNA must be processed to generate unit-length virion DNA. For viruses such as phage \(\lambda\) that have unique (non-permutated) chromosomes, specific chromosome ends must be generated by recognition and cleavage of specific DNA sites by viral packaging proteins. DNA from \(\lambda\) virions is a linear duplex, 48,502 bp long, with complementary, 12-base-long extensions staggered by 12 bp, into the concatemeric DNA. The segment of DNA required for efficient packaging of a chromosome is called \textit{cos}; nicking sites are introduced at a subsite of \textit{cos}, \textit{cosN}. The nicks are introduced by a phage-encoded, multifunctional DNA packaging enzyme, terminase. Terminase is a heteromultimer of two subunits, gpNu1 (21 kD) and gpA (74 kD), the products of the \(\lambda\) \textit{NuI} and \(\lambda\) \textit{A} genes, respectively. The endonuclease activity resides in gpA (DAVIDSON et al. 1992; RUBINCHIK et al. 1994). Many of the base pairs at the site of nicking are rotationally symmetric, suggesting that symmetrically disposed terminases are involved in nicking \textit{cosN}. Early studies indicated that the sequences needed for packaging a \(\lambda\) chromosome covered ~200 bp, an extent much greater than \textit{cosN} (FEISS et al. 1983; HOHN 1983; MIWA and MATUSBARA 1983). In 1984, BEAR et al. described a \(\lambda\) mutant, \(\lambda\) \textit{cos154}, that was unable to form plaques on a host lacking integration host factor (IHF), the \textit{Escherichia coli} site-specific DNA binding protein that sharply bends DNA when bound (reviewed in FRIEDMAN 1988; RICE et al. 1996). \textit{cos154} was a C-to-T transition mutation affecting bp 160 of \(\lambda\) DNA. The \textit{cos154} mutation was located in a sequence, called \textit{R1}, that was repeated nearby (Figure 1; BEAR et al. 1984). BEAR et al. identified four repeats of the sequence, called \textit{R1}–\textit{R4}. \textit{R4} was found near the right end of \(\lambda\) virion DNA, and \textit{R3}, \textit{R2} and \textit{R1} were between \textit{cosN} and the start of the \textit{Nul} gene at the left end of virion DNA. Additionally, a match to the consensus IHF binding site was found between \textit{R3} and \textit{R2}. It was proposed that the \textit{R} sequences were terminase binding sites, and that the \textit{cos154} mutation weakened binding of terminase to \textit{R1}. BEAR et al. (1984) further proposed that IHF assisted in the binding of terminase to the \textit{R} sequences, perhaps by facilitating cooperative interactions between terminases bound at the \textit{R} sequences. In this view, the \textit{cos154} mutation weakened terminase binding to \textit{cos} so that IHF was required for stable binding to the mutant \textit{cos}. More recent work has supported the speculations of BEAR et al. The small subunit of terminase, gpNu1, has been shown to bind to \textit{R3}, \textit{R2} and \textit{R1} (though not \textit{R4}), and the \textit{cos154} mutation has been shown to weaken

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gpNu1 binding to R1 (Shinder and Gold 1988). Muta-
tional work has shown that the C-to-T transition of cos154, when placed in the analogous bp positions of R2 or R3, causes IHF dependence (Cue and Feiss 1992a,b; 1993a). A strong IHF binding site, I1, has been demon-
strated to be located between R3 and R2 (Xin and Feiss 1988, 1993; Kosturko et al. 1989; Mendelson et al. 1991; Xin et al. 1993). The DNA segment from R3 through R1 has been named cosB, the terminase binding site. cosB has been shown to stimulate the efficiency and accuracy of the cos cleavage reaction, and to be essential for a post-cleavage step required for initiation of DNA packaging (Higgins et al. 1988; Cue and Feiss 1993a; Higgins and Becker 1994a,b; Cai et al. 1997). A triple mutant $\lambda$ cosB R3 'R2' R1', was found to be un-
able to form plaques, and viable pseudorevertants were found to have suppressing mutations in Nul (Cue and Feiss 1992a,b). Many studies have led to the follow-
ing model of $\lambda$ DNA packaging (see Becker and Mu-
rialdo 1990; Catalano et al. 1995). Terminase binds cosN and cosB to form an initial complex, followed by nicking of the DNA to generate the nicked complex. Separation of the cohesive ends, by terminase, leads to formation of complex I in which terminase is tightly bound to the left chromosome end (Sippy and Feiss 1992; Kuzminov et al. 1994). Terminase then binds a prohead, the empty protein shell into which DNA is to be packaged, and DNA packaging ensues. Transloca-
tion of DNA requires extensive ATP hydrolysis, perhaps by terminase or some other component of the transloca-
tion complex. When the translocation complex encoun-
ters the downstream cos along the concatemer, cosN nicking and cohesive and separation complete packaging of a $\lambda$ chromosome. Unlike R3, R2, and R1, R4 is not required for initiation of DNA packaging. The C-to-T transition mutation (C1847T), when placed in R4, was found not to affect initiation of DNA packaging, but rather to cause a partial defect in cleavage of the
downstream \textit{cos} site, \textit{i.e.}, to affect termination of DNA packaging (Cue and Feiss 1993b). Furthermore, a 14-bp deletion of R4 was found to not affect initiation of DNA packaging and to cause a lethal defect in terminal \textit{cos} cleavage (Cue and Feiss 1993b). These results, together with the inability of gpN1 to bind R4, suggested a different function for R4, and the site was renamed \textit{cosQ}. The R4 point mutation, C48,477T, was renamed \textit{cosQ}, and the 14-bp deletion was named the \textit{\Delta cosQ} mutation (Cue and Feiss 1993b). Further work shows that the \textit{cos} cleavage defect of \textit{cosQ} mutants is the failure to nick the bottom strand of \textit{cosN} during termination (D. Cue and M. Feiss, unpublished results). In the present work, we describe additional experiments indicating that \textit{cosQ} function is distinct from that of \textit{cosB}, and describe the nature of suppressors of the \textit{cosQ} mutation.

**MATERIALS AND METHODS**

**Media:** Luria Broth (LB), Luria agar (LA), 2× YT broth and SOB were prepared as described in Sambrook et al. (1989). When required, kanamycin and ampicillin were used at final concentrations of 50 and 100 \(\mu\)g/ml, respectively. X-gal plates were LA plus isopropyl-\(\beta\)-thiogalactopyranoside (IPTG) and X-gal at final concentrations of 0.1 mg/ml and 0.02% (w/v), respectively.

**Strains:** The standard \(\lambda\) strain used was \(\lambda\)-P1:5R \textit{d857 Kn}\textsuperscript{r} \textit{nin}\textsuperscript{5}; this phage will be referred to as \(\lambda\) or \(\lambda\textit{cos}\) in the text. \(\lambda\)-P1:5R \textit{d857 nin}\textsuperscript{5} carries a 10-kb segment of phage \(\Pi\) DNA encoding functions for plasmid replication and partitioning (Sternberg and Austin 1983). The \(\lambda\)-P1:5R prophage replicates as a single copy plasmid using the \(\Pi\) replication machinery. Upon inactivation of the \textit{d857} repressor at 42\(^\circ\), the prophage is induced to carry out the lytic cycle. The position of each restriction cut site is given as the first nucleotide of the left cohesive end and continues to the manufacturer's recommendations. DNA sequencing (Sanger et al. 1977) was performed with reagents purchased from Promega and Pharmacia. Plasmid DNA and \(M\)\textsubscript{13} replicative-form DNA were prepared as described by Birnboim and Doly (1979). DNA fragments were purified from agarose gels as described by Vogelstein and Gillespie (1979). Preparation of competent cells and transformation were performed as described by Hanahan (1983). For cloning segments of \(\lambda\) DNA, the commercial vectors pBlues and pBlues were used (IBI, Inc.). Single-stranded DNA from pBlues plasmids was prepared as described by Vieira and Messing (1987).

**RESULTS**

**Test of suppression of the \textit{cosQ} mutation by \textit{Nul} missense mutations:** Pseudorevertants of mutant phages contain suppressor mutations that provide information about the nature of the original defect. In previous studies, \textit{Nul} missense mutations that were second-site suppressors of \textit{cosB} mutations were described (Granton et al. 1988; Cue and Feiss 1992b). The \textit{Nul}\textsubscript{Im1} and \textit{Nul}\textsubscript{Im2} mutations were isolated as suppressors of the lethal \textit{cosB R3 R2 R1} mutation; these \textit{Nul} mutations increased the burst size of the \textit{R3-R2 R1} phage to a level that closely approximates the burst of \(\lambda\) in an \textit{IHF}\textsuperscript{r} host. Plaque formation by the \(\lambda\textit{cosB R3 R2 R1}\) \textit{Nul}\textsubscript{Im} phages was dependent upon \textit{IHF}, however. The \textit{Nul}\textsubscript{Im1} and \textit{Nul}\textsubscript{Im2} mutations cause the changes \textit{LagF} and \textit{LagI} in gpN1, respectively (Cue and Feiss 1992b). The effects of the \textit{Nul}\textsubscript{Im} suppressors on the \textit{cosQ} mutation were examined to see if the \textit{cosQ} mutation is suppressed by the \textit{Nul}\textsubscript{Im} suppressors in a manner analogous to the \textit{R3}, \textit{R2} and \textit{R1} mutations. To quantitate the effects of the \textit{Nul}\textsubscript{Im1} and \textit{Nul}\textsubscript{Im2} mutations on the burst of \(\lambda\textit{cosQ}\), we compared the bursts of \textit{cosQ} and \textit{cosQ} phages that were \textit{Nul}\textsuperscript{+}, \textit{Nul}\textsubscript{Im1} and \textit{Nul}\textsubscript{Im2} (Table 1). Our standard \(\lambda\textit{phage}, \lambda\)-P1:5R \textit{d857 nin}\textsuperscript{5} \textit{Kn}\textsuperscript{r}, hereafter simply called \(\lambda\), is a kanamycin-transducing phage that carries the plasmid replication and partition functions of phage \(\Pi\) (Sternberg and Austin, 1983; Pal and Chattoraj, 1988). For \(\lambda\textit{cos}\) in the \textit{IHF}\textsuperscript{r} host, the \textit{Nul}\textsubscript{Im1} and \textit{Nul}\textsubscript{Im2} mutations increase the yields of progeny phage 3.5-fold and greater than fourfold, respectively. The bursts of the \textit{Nul}\textsubscript{Im1} and \textit{Nul}\textsubscript{Im2} phages, on the \textit{IHF}\textsuperscript{r} host, are approximately equal to the burst of the wild-type phage on the \textit{IHF}\textsuperscript{r} host. In contrast, the burst of \(\lambda\textit{cos}\) in an
IHF⁻ host is reduced about twofold by the NuIms1 and ms2 mutations. The NuI missense mutations affect the burst of the cosQ phage in a manner that closely parallels the effects the NuI mutations have on the cos⁻ phage; under IHF⁻ conditions, the NuI mutations increase the burst of the cosQ phage to an extent sufficient for plaque formation. Under IHF⁻ conditions, as with the cos⁺ phage, the NuI mutations lead to a small decrease in burst size.

The cosQ phage responds to the NuI missense mutations in the same manner and to nearly the same extent as the cos⁺ phage. This is the result that might be anticipated if the NuI mutations affect the terminase-cosB interaction without affecting the terminase-cosQ interaction; both λ⁺ and λ cosQ have wild-type cosB regions. These experiments indicate that the NuI missense mutations, which were isolated as suppressors of cosB mutations, do not directly suppress the cosQ mutation. We conclude that either terminase binding to cosQ occurs via a different DNA recognition determinant than does terminase binding to R1, R2 and R3 and/or that the cosQ sequence is required at a different step in the DNA packaging process than is cosB.

**Suppression of the cosQ mutation by a second cosQ mutation:** To see what sorts of mutations would allow suppression of the cosQ mutation, IHF-independent variants of the cosQ mutant were selected for by plating λ cosQ on the IHF⁻ host, MF1493. Variants of the cosQ mutant were found at a frequency of ~10⁻⁷. Twenty-five of these variants were isolated and replated on MF1427 (IHF⁻) and MF1972 (IHF⁻); the plating characteristics of this group of variants were very uniform; all formed plaques that were approximately wild-type in size on the IHF⁻ host and moderate-sized on the IHF⁻ host.

Two of these variants, Rev10 and Rev24 of λ cosQ, were further characterized. Small plasmid libraries were constructed and marker rescue experiments were performed to map the suppressors. The results indicated that DNA segments carrying the cosQ site were able to rescue the cosQ mutation (Table 2).

The sequences of the Rev10 and Rev24 DNAs were determined from bp 48,299 to bp 194; Rev10 and Rev24 were found to have retained the cosQ mutation (G₄₈,₄₇₇T) and to have an additional G-to-A transition at bp 48,473 (also within the cosQ sequence). We designate the G₄₈,₄₇₇A change as the cosQ₁ mutation. The net effect of the two mutations is to alter the wild-type cosQ sequence from 5'-CGGGTCTCTTTCC-3' (top strand) to 5'-CAGGTCTCTTTCC-3'.

The bursts of the Rev10 and Rev24 (λ cosQ₁ cosQ₂) phages were found to be ~67 pfu/induced lysogen under IHF⁻ conditions and 14 pfu/induced lysogen in the absence of IHF, as compared with bursts of 6.3 pfu/induced IHF⁺ lysogen and 0.8 pfu/induced IHF⁻ lysogen for λ cosQ₁.

**Suppression of the cosQ₁ mutation by an increase in chromosome length:** During the course of performing the marker rescue experiments described above, it appeared that λ DNA fragments that were cloned from the cosQ₁ Rev10 and cosQ₁ Rev24 phages carried mutations that were responsible for the suppression of the cosQ₁ mutation. These DNA fragments, corresponding to the wild-type λ bp 17,791–19,996, carried the λ lom gene and portions of the J and orf401 coding sequences. When these plasmids were crossed with the cosQ₁ phage, recombinant phages that formed large plaques on MF1427 were readily obtained; these recombinant

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**TABLE 1**

<table>
<thead>
<tr>
<th>Phage</th>
<th>Yield on IHF⁺ host</th>
<th>Relative yield</th>
<th>Yield on IHF⁻ host</th>
<th>Relative yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ cos⁺</td>
<td>104.8 (11.5)</td>
<td>1.0</td>
<td>27.3 (6.1)</td>
<td>1.0</td>
</tr>
<tr>
<td>λ cos⁺ NuIms1</td>
<td>46.5 (13.9)</td>
<td>0.44</td>
<td>95.7 (0.5)</td>
<td>3.5</td>
</tr>
<tr>
<td>λ cos⁺ NuIms2</td>
<td>54.9 (16.7)</td>
<td>0.52</td>
<td>118.0 (4.0)</td>
<td>4.3</td>
</tr>
<tr>
<td>λ cosQ₁</td>
<td>6.3 (0.06)</td>
<td>0.06</td>
<td>0.8 (0.2)</td>
<td>0.08</td>
</tr>
<tr>
<td>λ cosQ₁ NuIms1</td>
<td>3.6 (0.3)</td>
<td>0.03</td>
<td>3.0 (0.8)</td>
<td>0.11</td>
</tr>
<tr>
<td>λ cosQ₁ NuIms2</td>
<td>3.5 (0.7)</td>
<td>0.03</td>
<td>4.8 (0.18)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Numbers in parentheses are standard deviations.

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**TABLE 2**

<table>
<thead>
<tr>
<th>Cloned DNA segment</th>
<th>Phage yield (pfu/ml)</th>
<th>On IHF⁺ cells</th>
<th>On IHF⁻ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>47,942–194</td>
<td>4.6 × 10⁷</td>
<td>5.0 × 10⁶</td>
<td>1.4 × 10⁵</td>
</tr>
<tr>
<td>1–2819</td>
<td>1.9 × 10⁸</td>
<td>&lt;10⁴</td>
<td>&lt;10³</td>
</tr>
<tr>
<td>Rev24 of λ cosQ₁</td>
<td>44,141–194</td>
<td>1.0 × 10⁹</td>
<td>2.7 × 10⁶</td>
</tr>
<tr>
<td>48,299–194</td>
<td>3.1 × 10⁹</td>
<td>1.4 × 10⁵</td>
<td>1.0 × 10⁵</td>
</tr>
<tr>
<td>1–2819</td>
<td>1.5 × 10⁹</td>
<td>&lt;10⁴</td>
<td>&lt;10³</td>
</tr>
</tbody>
</table>

Restriction enzyme sites used to generate λ DNA inserts were HpaII (44, 141), BclI (λ bp 47,942), HindII (48,296), EcoRI (194), and EcoRI (2815). The end point at bp 1 was produced by filling in the left cohesive end of virion DNA with DNA polymerase.
phages, however, did not form plaques on an IHF+ host (data not shown).

As a control for these experiments, the corresponding DNA fragment was cloned from the wild-type phage. Crosses performed between the plasmid carrying the wild-type DNA and the cosQ1 phage also yielded large-plaque forming recombinants. The results of these crosses indicated that while the plasmids carrying the cloned phage DNA somehow suppressed the cosQ1 mutation, this suppression was not due to the presence of a mutation within the DNA fragments that had been cloned from the cosQ1 variants.

A likely explanation for these results was that, because a portion of the λ DNA segment carried by these plasmids is from the “nonessential” b region of the λ genome (COURT and OPPENHEIM 1983), the plasmids would be free to integrate within the cosQ1 phage chromosome, increasing its length from 46.2 to 51.3 kb. Suppression of the cosQ1 mutation could be the result of either an increase in the length of the phage chromosome or the duplication of a λ gene.

If the idea that suppression of the cosQ1 mutation resulted from plasmid integration was correct, then the phages forming large plaques that resulted from the crosses would be predicted to transduce ApR (the ApR determinant being carried by the plasmid vector) with high efficiency. Several of the phages that formed large plaques were isolated and the abilities of the phages to transduce ApR were tested. All of the recombinant phages that were tested were found to be capable of efficiently transducing ApR (data not shown). The ability of the recombinant phages to transduce ApR was however readily lost; when this loss occurred, the resulting phages invariably formed small plaques, indistinguishable from plaques of the cosQ1 parent phage.

The results of these experiments verified that it is the insertion of the b region-bearing plasmids into the phage chromosome that results in suppression of the cosQ1 mutation. It seemed that the most likely effect these insertions would have is to result in an increase in the length of the cosQ1 phage chromosome and that suppression of the cosQ1 mutation was a direct result of the increase in length. The possibility remained, however, that other effects, such as the duplication of the λ tom gene or the activation of a cryptic gene by the insertions, could be responsible for suppression of the cosQ1 mutation.

To test whether the suppression effect was directly due to an increase in chromosome length, plasmids were constructed that carried various subfragments of the 17,791–19,996 λ DNA fragment and the abilities of these plasmids to suppress the cosQ1 mutation were tested. The inserts carried by these plasmids, the sizes of the plasmids and the results of the crosses with the cosQ1 phage are listed in Table 3.

Plasmid insert A, in Table 3, is the original 17,791–19,996 cloned fragment; inserts B–E represent various subcloned segments of insert A. Crosses performed with plasmids carrying inserts B, D and E also resulted in the recovery of recombinant phages that formed large plaques on IHF+. E. coli. Plasmid inserts D and E carry the 19,400–19,996 and 18,387–19,399 λ DNA segments, respectively; these two plasmids do not carry any known λ DNA sequences, nor does either plasmid code for any known λ protein, yet both plasmids are capable of suppressing the cosQ1 mutation. Therefore, the likeliest explanation for the suppression of the cosQ1 mutation is that suppression is due to the integration of the plasmid into the phage chromosome; the resulting increase in chromosome length is directly responsible for suppression of the cosQ1 mutation. The plasmid carrying insert C (Table 3) would not be expected to lead to the isolation of recombinant phages, since the integration of the plasmid carrying this insert would result in the disruption of the essential λ gene. Additional studies showed that the cosQ1 mutation was suppressed by insertions elsewhere in the chromosome, i.e., at the extreme left and right ends (D. Cue, unpublished observations).

The fact that the cosQ1 mutation is partially suppressed by the insertion of DNA within the central region of the λ chromosome, suggested that the cosQ1 mutation has an effect on the terminal step of DNA packaging (see DISCUSSION). The question arises then as to how a cosB mutant would respond to an increase in chromosome length.

To test the response of a cosB mutant phage to an increase in chromosome length, a cross was performed between the b region-bearing plasmid and λ cosB R3′R2′R1′ Nu1ms3. This phage was chosen because it

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Suppression of the cosQ1 mutation by plasmid integration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid 'insert'</td>
<td>Plasmid size</td>
</tr>
<tr>
<td>J</td>
<td>lom 401</td>
</tr>
<tr>
<td>J</td>
<td>lom 401'</td>
</tr>
<tr>
<td>J</td>
<td>3,477</td>
</tr>
<tr>
<td>J'</td>
<td>3,484</td>
</tr>
<tr>
<td>J'</td>
<td>3,928</td>
</tr>
<tr>
<td>No insert</td>
<td>2,923</td>
</tr>
</tbody>
</table>

*Diagrams indicate extents of λ genes cloned into pBIB1. Apostrophes indicate gene truncations. The coordinates of the inserts are as follows: A, 17,791 (MluI) to 19,996 (MluI); B, 18,556 (KpnI) to 19,996 (MluI); C, 17,791 (MluI) to 18,385 (EcoRV); D, 19,397 (Smal) to 19,996 (MluI); E, 18,385 (EcoRV) to 19,397 (Smal).
Sustained from the fact that the mutation at bp 4184 particles, but no large-plaque forming phages were obtained. The mutation, Bms6, was identified, a C-to-T transition at bp 3826 that changes codon 331 of the gene from proline to serine. In crosses analogous to those used to map the Rev29 mutation, the P$_{3826}$ change in gpB was also found to be a suppressor of the cosQ1 mutation. [In the reciprocal experiment, the Bms6 mutation was found not to suppress the terminase mutation affecting the prohead binding domain of gpA (A. Yeo, personal communication).] The effects of the Bms suppressors are mild; the burst size data of Table 5 show that Bms6 and Bms8 increase the yield about three- and twofold, respectively, both in the presence and absence of IHF. The weakness of the Bms8 suppression may be due in part to the fact that the Bms8 mutation has been shown to cause a threefold decrease in the efficiency of interaction of Bms8 proheads with wild-type terminase (Yeo and Feiss 1995). Additional crosses showed that the Bms6 suppressor was unable to suppress the IHF-dependence of λ cosB R1, λ cosB R3, and λ cosB R1 R2 R3 NuIms3; nor did the Bms6 suppressor allow λ cosB R3 R2 R1 or λ ΔcosQ to form plaques on IHF+ or IHF− cells.

Suppression of the defects of λ cosQ1 cosB R3 R2 R1 by the NuIms1 and Bms6 suppressors: Finding the cosQ1 mutation is suppressed by suppressors distinctly different from suppressors of cosB mutations makes the prediction that a phage with both cosQ and cosB defects would require two suppressors, one for the cosQ defect and one for the cosB defect. This prediction was confirmed by examining the suppression requirements of λ cosQ1 cosB R3 R2 R1. λ cosQ1 cosB R3 R2 R1 was crossed with a series of plasmids containing inserts and the resulting lysates examined for viable recombinants. Crossing λ cosQ1 cosB R3 R2 R1 with a plasmid carrying an insert extending from 194 to 5505 and containing the NuIms1 and Bms6 mutations resulted in viable recombinants that formed tiny plaques, whereas crosses with plasmid inserts lacking one

### Table 4

<table>
<thead>
<tr>
<th>Cloned DNA segment</th>
<th>Phage yield (pfu/ml)</th>
<th>Recombinants (pfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ cosQ+ DNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>458-5548</td>
<td>4.4 x 10⁷</td>
<td>&lt;10⁴</td>
</tr>
<tr>
<td>47 942-194</td>
<td>9.2 x 10⁷</td>
<td>1.9 x 10⁶</td>
</tr>
<tr>
<td>Rev29 of λ cosQ1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>458-5548</td>
<td>3.0 x 10⁸</td>
<td>2.4 x 10⁶</td>
</tr>
<tr>
<td>2815-5548</td>
<td>1.0 x 10⁸</td>
<td>1.0 x 10⁴</td>
</tr>
<tr>
<td>2815-4198</td>
<td>3.8 x 10⁸</td>
<td>&lt;10⁴</td>
</tr>
<tr>
<td>3800-5548</td>
<td>3.5 x 10⁸</td>
<td>4.0 x 10⁵</td>
</tr>
<tr>
<td>2815-4470</td>
<td>4.6 x 10⁸</td>
<td>3.0 x 10⁴</td>
</tr>
<tr>
<td>458-2560</td>
<td>8.45 x 10⁸</td>
<td>&lt;10⁴</td>
</tr>
<tr>
<td>458-2815</td>
<td>7.0 x 10⁸</td>
<td>&lt;10⁴</td>
</tr>
<tr>
<td>4198-5548</td>
<td>3.3 x 10⁸</td>
<td>&lt;10⁴</td>
</tr>
</tbody>
</table>

Restriction enzyme sites used to generate λ DNA inserts, in addition to those given in Table 2 were as follows: MseI (458), PstI (2530), BglII (3800), CdeI (4198), AscI (4470), and MluI (5548).

Although the segment from 2815 to 4198 carries the Bms6 mutation, no recombinant phage was isolated. This likely resulted from the fact that the mutation at bp 4184 is only 17 bp from the end of the cloned DNA fragment.

has a phenotype that is very similar to that of the cosQ1 mutant; it forms small plaques on an IHF+ host and does not make plaques on IHF− cells (Cue and Feiss 1992b). The Nulms3 mutation causes a C-to-T transition at the site of tail attachment (Bazinet and King 1985). Recently another missense mutation was identified in B as a suppressor of a mutation altering the prohead binding domain of terminase (Yeo and Feiss 1995). This mutation, Bms8, is a C-to-T transition mutation at bp 3826 that changes codon 331 of the B gene from proline to serine. In crosses analogous to those used to map the Rev29 mutation, the P$_{3826}$ change in gpB was also found to be a suppressor of the cosQ1 mutation. [In the reciprocal experiment, the Bms6 mutation was found not to suppress the terminase mutation affecting the prohead binding domain of gpA (A. Yeo, personal communication).] The effects of the Bms suppressors are mild; the burst size data of Table 5 show that Bms6 and Bms8 increase the yield about three- and twofold, respectively, both in the presence and absence of IHF. The weakness of the Bms8 suppression may be due in part to the fact that the Bms8 mutation has been shown to cause a threefold decrease in the efficiency of interaction of Bms8 proheads with wild-type terminase (Yeo and Feiss 1995). Additional crosses showed that the Bms6 suppressor was unable to suppress the IHF-dependence of λ cosB R1, λ cosB R3, and λ cosB R1 R2 R3 NuIms3; nor did the Bms6 suppressor allow λ cosB R3 R2 R1 or λ ΔcosQ to form plaques on IHF+ or IHF− cells.

### Table 5

<table>
<thead>
<tr>
<th>Phage Yield (pfu/ml)</th>
<th>Recombinants (pfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phage</td>
<td>Yield (pfu/ml)</td>
</tr>
<tr>
<td>Phage</td>
<td>Yield (pfu/ml)</td>
</tr>
<tr>
<td>λ cosQ+ B+</td>
<td>83 (10.5)</td>
</tr>
<tr>
<td>λ cosQ− Bms6</td>
<td>51.1 (8.0)</td>
</tr>
<tr>
<td>λ cosQ+ Bms8</td>
<td>47.2 (12.8)</td>
</tr>
<tr>
<td>λ cosQ+ B+</td>
<td>5.4 (1.4)</td>
</tr>
<tr>
<td>λ cosQ+ Bms6</td>
<td>17.3 (1.0)</td>
</tr>
<tr>
<td>λ cosQ+ Bms8</td>
<td>9.7 (3.9)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are standard deviations.
or both of the suppressor mutations failed to generate recombinants (Table 6). An additional study showed that the defects of \( \lambda \) *cosQ* and *cosB* could be suppressed with a combination of the Nu1ms1 suppressor and a chromosomal insertion (D. CUE, unpublished observations).

### DISCUSSION

**Evidence that cosQ and cosB perform distinctly different roles in \( \lambda \) DNA packaging:** An abundance of genetic and molecular evidence indicates that *cosQ* and *cosB* play distinctly different roles in \( \lambda \) DNA packaging reactions. *cosB* stimulates the efficiency and accuracy of nicking of *cosN* and is essential for initiation of DNA packaging (CUE and FEISS 1992a, 1993a; HIGGINS and BECKER 1988, 1994a, b). Mutations of *cosB* that are quite defective for packaging initiation do not affect termination of packaging. *cosQ* is not required for initiation of DNA packaging, but is required for termination of packaging (CUE and FEISS 1993b). GpNul binds the three R sequences of *cosB*, but not *cosQ* (SHINDER and GOLD 1988). Here we present further genetic evidence, derived from suppressors, that *cosQ* and *cosB* play different roles in DNA packaging.

The Nu1ms1 and Nu1ms2 mutations were isolated as suppressors of *cosB* mutations (CUE and FEISS 1992b). The Nu1ms1 and Nu1ms2 mutations are strong suppressors of all of the *cosB* mutations tested; both mutations permit plaque formation by a phage, \( \lambda \) *cosB* R5"-R2"-R1", carrying a transition mutation in each of the R sequences of *cosB* and promote IHF-independent growth by all IHF-dependent *cosB* mutants that have been tested.

Despite the fact that the Nu1ms1 and ms2 mutations function as efficient suppressors of *cosB* mutations, neither of these Nu1 mutations has a large effect on the plating characteristics of the *cosQ1* phage. The *cosQ1* mutant, like the *cos*" phage, has a wild-type *cosB* region and responds to the terminase mutations in a manner that closely parallels the response of the *cos" phage; the Nu1ms1 and Nu1ms2 mutations both decrease the burst of the *cosQ1* phage about twofold on an IHF" host and increase the phage burst several fold on an IHF" host. The increase in burst size is sufficient to allow the *cosQ1* Nu1ms1 and Nu1ms2 phages to form plaques in the absence of IHF, but the ability of the Nu1 missense mutations to allow IHF-independent plaque formation by the *cosQ1* mutant cannot be regarded as direct suppression, but rather reflects the response of a *cosB"* phage to the Nu1 mutations. Why the Nu1ms1 and Nu1ms2 suppressors decrease the burst size of both *cosQ1* and *cos"* phages is not clear; it has been speculated that the interaction of the suppressor gpNul with *cosB* may be strengthened such that a necessary disassembly step is less efficient (CUE and FEISS 1992b).

In contrast, the Nu1ms1 and Nu1ms2 mutations increase the burst of \( \lambda \) *cosB* R3"-R2"-R1" phage nearly 1000-fold on an IHF" host. These same *Nu1* mutations also increase the burst of the R3"-R2"-R1" phage on an IHF" host by \~100-fold, an increase insufficient to allow plaque formation (CUE and FEISS 1992b). In sum, the *Nu1* missense mutations can be regarded as specific suppressors of *cosB* mutations.

The fact that the *Nu1* mutations can lead to suppression of point mutations in R3, R2 and R1 (CUE and FEISS 1992b), but do not suppress the *cosQ1* mutation suggests that *cosQ* unlike R1, R2 and R3, is not a gpNul binding site. SHINDER and GOLD (1988) demonstrated that the small terminase subunit does bind to R1, R2 and R3; these same researchers (SHINDER and GOLD 1989) later reported that gpNul did not bind to *cosQ*, but that, somehow, *cosQ* may aid in gpNul binding to the other three R sites. Our results suggest a different role for *cosQ* in DNA packaging.

Although *cosQ* was originally identified by its sequence homology to the other R sequences (BEAR et al. 1984), the *cosQ* sequence shares homology with *cosN* half-sites as well as with the R sequences (Fig. 2), suggesting that terminase may recognize *cosQ* by the same specificity determinant that is used to recognize *cosN*. This suggestion is supported by our finding that the related lambdoid phage, 21, has a *cosQ* sequence that is identical to the \( \lambda \) *cosQ* sequence (SMITH and FEISS 1993). \( \lambda \) and 21 have terminase genes that are \~60% homologous (reviewed in FEISS 1986). Although \( \lambda \) and

### TABLE 6

**Suppression of the cosQ and cosB mutations by the Bms6 and Nu1ms1 suppressors**

<table>
<thead>
<tr>
<th>Phage</th>
<th>Plasmid</th>
<th>( \lambda ) insert</th>
<th>Suppressor</th>
<th>Titer on MF844 (pfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda ) <em>cosQ1</em></td>
<td>pSX1</td>
<td>47,942-194</td>
<td><em>cosQ</em></td>
<td>( 1.4 \times 10^5 )</td>
</tr>
<tr>
<td><em>Bms6</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \lambda ) <em>cosQ1</em></td>
<td>pSX102</td>
<td>194-5505</td>
<td><em>Nu1ms1</em></td>
<td>&lt;10</td>
</tr>
<tr>
<td>pRV122</td>
<td>458-5548</td>
<td></td>
<td><em>Bms6</em></td>
<td>&lt;10</td>
</tr>
<tr>
<td>( \lambda ) <em>cosQ1</em></td>
<td>pRV5</td>
<td>194-2819</td>
<td><em>Nu1ms1</em></td>
<td>&lt;10</td>
</tr>
<tr>
<td>pRV181</td>
<td>194-5505</td>
<td></td>
<td><em>Bms6</em></td>
<td>( 1.6 \times 10^4 )</td>
</tr>
</tbody>
</table>

*These \( \lambda \) *cosQ1* *cosB* R1"-R2"-R3" Nu1ms1 Bms6 recombinants formed tiny plaques on MF844 and were unable to form plaques on MF1427.*
the cosQ1 mutation (C48,477T) is suppressed by a second mutation within cosQ, i.e., by the cosQ2 mutation (G48,473A). While the cosQ2 mutation is proposed to restore cosQ function, we have no information on how the cosQ1 or cosQ2 mutations affect cosQ function. The speculative proposal that cosQ and cosN half-sites are recognized similarly leads to an examination of the effects of the cosQ mutations on sequence identity between cosQ and the cosN half-sites (Figure 2b). cosQ has 5-bp matches when aligned with the 8-bp of cosNL, and the cosQ1 and cosQ2 mutations each eliminate a bp match, so that cosQ with the cosQ1 and cosQ2 mutations has only a 3-bp match with cosNL. cosQ has 4-bp matches when aligned with the 8-bp of cosNR. The cosQ1 mutation increases the cosQ/cosNR match to 5 bp, and the cosQ site with both the cosQ1 and cosQ2 mutations has a match of 4 bp. Thus, in suppressing cosQ1, the cosQ2 mutation restores the number of matching bp to the wild-type value of 4. It is possible that the bp match between wild-type cosQ and cosNR is optimal for cosQ function, and that optimal matching is restored in the cosQ2 cosQ1 doubly mutant site. It is hoped these highly speculative suggestions will encourage further study into cosQ recognition. It has recently been found that a lethal cosQ mutation blocks nicking of cosNR, while having little effect on cosNL nicking, so there is evidence of a functional link between cosQ and cosNR (D. Cue and M. Feiss, unpublished results).

Suppression of the cosQ1 mutation by DNA insertions: The effect of the cosQ1 mutation has been found to be largely suppressed by the insertion of DNA segments within the chromosome of the cosQ1 phage; plasmids carrying a portion of the λ b region can integrate within the phage genome and promote the formation of near wild-type size plaques on an IHF+ host. The λ b region is an 8.6-kb DNA segment in the central region of the λ genome, between the λ f gene and the λ att region (Court and Oppenheimer 1983). Although the b region encodes for several proteins, the region contains no genes that are essential for λ growth (Hendrix and Duda 1992).

Since the b region is nonessential, plasmids that carry segments of this region can integrate within the phage chromosome without disrupting essential genes. The net effect of the integration of the plasmids listed in Table 3 is to increase the length of the λ cosQ1 chromosome from 46.2 kb to ~50–51 kb. The experiments summarized above demonstrate that plasmid integration is both necessary and sufficient for suppression of the cosQ1 mutation. The results presented in Table 3 demonstrate that a variety of plasmids can suppress the cosQ1 mutation, despite the fact that the plasmids do not carry any common λ DNA sequences or any intact λ genes; thus the suppression effect is the direct result of an increase in chromosome length. A chromosome insertion does not result in an enhanced plaque-forming ability for the cosB mutant λ cosB R3”R2”R1”
Termination of λ DNA Packaging

**NuImS**, a phage that forms plaques that are of approximately the same size as λ cosQ1 plaques. Thus, the ability of DNA insertions to suppress cos mutations appears to be specific for the cosQ1 mutant. The fact that an insertion in the central portion of the λ chromosome can suppress the effects of the cosQ1 mutation argues that the cosQ1 mutation affects the termination of DNA packaging. The termination of λ DNA packaging, or the cutting of the downstream cos site, is influenced by chromosome length and is independent of cosB specificity, i.e., λ 21 (Feiss et al. 1981; Feiss and Widner 1982; Feiss and Becker 1983). Feiss and Becker (1983) speculated about the molecular explanation for the effect of chromosome length on terminal cos cleavage. One proposed model states that the velocity of DNA entry into the phage head is dependent upon DNA length and that the rate of DNA packaging slows progressively as increasing amounts of DNA are packaged into the head; the ability of terminase to cut at the terminal cos could be inversely dependent on the packaging rate. There is some in vitro evidence to support the idea that the rate of DNA packaging does decrease as packaging proceeds (Shibata et al. 1987). The cosQ1 mutant has a chromosome that is 46.2 kb in length, a size that would not be predicted to influence the efficiency of DNA packaging (Feiss et al. 1977; Feiss and Siegele 1979), yet insertions of 3.5–5.1 kb of DNA result in the partial suppression of the cosQ1 mutation. A reasonable explanation for the cosQ1 defect is that the cosQ1 mutation interferes with the ability of terminase to cut the terminal cos site. Increasing the size of the cosQ1 chromosome from 46 to ≈50 kb might suppress the cosQ1 defect by slowing DNA packaging to a rate such that terminase can recognize the mutant cosQ and cut the terminal cos site.

Suppression of the cosQ1 mutation by DNA insertions is heavily dependent upon IHF. The recombinant cosQ1 phages with DNA insertions form plaques that are nearly wild type in size on an IHF+ host, but fail to form plaques on an IHF– host. Thus, IHF contributes to suppression of the cosQ1 mutation. Apparently, neither an increase in chromosome size nor the presence of IHF alone is sufficient to alter the phenotype of λ cosQ1.

Earlier work demonstrated that the presence of wild-type cosB is not required for either terminal cos cleavage or processive DNA packaging (Feiss et al. 1981, 1985; Feiss and Widner 1982). The model proposed (Feiss et al. 1985) as a result of these earlier experiments is as follows. Packaging is initiated at a cosB+ cos site, and DNA translocation delivers terminase to the terminal cos site. The terminal cosN is directly recognized and cut by terminase. Following the packaging of the initial cosB chromosome, terminase remains bound to the left end of the next chromosome, binds a prohead and initiates packaging of the next chromosome. Although λ terminase requires cosB of λ for initiation of packaging, termination and processivity are carried out at a downstream cosBA or cosBd21, indicating that the sequence requirements for termination and processivity differ from those required for packaging initiation (Feiss and Widner 1982; Feiss et al. 1985). More recent results established that cosQ is required for the recognition of terminal cos sites (Cue and Feiss 1992b). The IHF-dependence of suppression of cosQ1 by DNA insertion suggests that cosB may also play a role in packaging termination.

While there are a number of possible mechanisms whereby IHF could contribute to cosQ1 suppression, we favor a model where IHF binding within cosB affects the efficiency of packaging termination. We have recently found that a downstream cos consisting of cosQ and cosN is inadequate to support efficient packaging termination. Rather, we have found that the sequences within cosB function with cosQ and cosN to promote packaging termination and processivity. Thus it is possible that IHF functions similarly during packaging termination and initiation.

We previously reported (Cue and Feiss 1992b) that IHF contributes little to suppression of the cosQ1 mutation. While these results seem to be at odds with the results reported here, it seems likely that IHF plays only a minor role in termination and processive packaging of cos+ chromosomes. It may be only under specific circumstances (i.e., packaging of λ cosQ1 chromosomes that are >50 kb in length) that the contributory role is apparent.

**Suppression of the cosQ1 mutation by a portal protein mutation**: The product of the B gene of λ is the portal protein, which assembles into a gear-shaped dodecamer that serves as the site for assembly of the shell of the prohead and forms the portal vertex of the prohead (Kochan and Muraldo 1983; Kochan et al. 1984; reviewed in Georgopoulos et al. 1983). gpB is predicted to be a protein of 583 amino acids (Sanger et al. 1982). During prohead assembly, a majority of the gpB is processed to gpB* by removal of the N-terminal 20 amino acids (Casjens and Hendrix 1974; Walker et al. 1982). In addition to serving as the site of prohead shell assembly, the portal vertex is the site of tail attachment (Tsui and Hendrix 1980). The portal vertex interacts with the C-terminus of the large terminase subunit, gpA, during DNA packaging (Yeo and Feiss 1995) and is likely the DNA entry and exit site during packaging and injection, respectively (reviewed in Valpuesta and Carrascosa 1994; Catalano et al. 1995). The gpB dodecamer has a central hole sufficiently large to allow DNA passage, and a number of DNA packaging models include packaging of the DNA through this central hole (Hendrix 1978; Earnshaw and Casjens 1980; Dube et al. 1993), although other models involve wrapping of the DNA around the outside of the structure (Turnquist et al. 1992). There is evidence that the portal vertex is intimately involved in the DNA packaging pro-
cess, as follows. Phages P22 and SPP1 are phages that package DNA by a headful mechanism: following an initial, site-specific cut, chromosomes are cut from the concatemeric precursor DNA by nonspecific cuts that appear to be triggered by filling of the head. Mutations affecting the portal proteins of P22 and SPP1 have been found that increase and decrease the length of the packaged DNA molecules, respectively (Casjens et al. 1992; Tavares et al. 1992). These mutations indicate a role for the portal vertex in sensing the amount of DNA that has been packaged, by sensing (1) the packaging density, (2) the rate of DNA translocation, or (3) the energy required to continue packaging (Casjens et al. 1992).

For λ, there is a length-sensing aspect to termination of DNA packaging, in the cleavage of the downstream cos becomes progressively less efficient as the chromosome is shortened. That is, over decreasing chromosome lengths from 0.90 (43.6 kb) to 0.78 (57.8 kb) that of wild type (48.5 kb), the efficiency of terminal cos cleavage declines from 100% to ~75% (Feiss and Siegel 1979). It seems likely that the λ portal protein, like those of P22 and SPP1, may play a role in sensing how much DNA has been packaged into the head. Here we find that mutations affecting the λ portal protein suppress the defect of a leaky cosB mutant. We consider two models for how changes in the portal protein might affect cosQ recognition.

In the first model it is proposed that the sensing mechanism measures the rate of DNA translocation, and that ∆Bms6 and ∆Bms8 slow the rate of translocation, thereby increasing the efficiency at which the weakened cosQ site is recognized by the translocation complex. While speculative, this proposal fits well with the observation that increased DNA length also suppresses the cosQ mutation, since increased DNA length might also alter the rate of translocation at the time cosQ is encountered by the translocation machinery.

In a second model, the B suppressor mutations are proposed to alter the sensing mechanism per se. In this model, again assuming that the sensing mechanism measures the rate of translocation, the B mutations are proposed to alter the threshold of the sensing mechanism, so that the transition to the cleavage mode occurs at a higher translocation rate, such that the cosQ site is recognized more efficiently. The first model predicts that the B suppressors affect the translocation rate, while the second model does not. We plan to test whether the B suppressors alter the rate of DNA translocation.

We thank Andy Becker, Carlos Catalano, Hisao Fujisawa and our coworkers Zhi-Hao Cai, Carol Duffy, Qi Hang, Hillary Johnson, Jenny Meyer, John Randell, Jean Sipp, Michael Smith, Fedor Tereshchenko, Doug Wetterzwer and Ashley Yeo for advice and interest during the course of this work. This work was supported by National Institutes of Health research grants AI-12581 and GM-51611.

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Casjens, S. R., and R. W. Hendrix, 1974 Locations and amounts of DNA molecules, respectively (Casjens et al. 1992; Tavares et al. 1992). These mutations indicate a role for the portal vertex in sensing the amount of energy required to continue packaging (Casjens et al. 1992). It seems likely that the λ portal protein, like those of P22 and SPP1, may play a role in sensing how much DNA has been packaged into the head. Here we find that mutations affecting the λ portal protein suppress the defect of a leaky cosB mutation. We consider two models for how changes in the portal protein might affect cosQ recognition.

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Termination of λ DNA Packaging


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