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Interference with Phage λ Development by the Small Subunit of the Phage 21 Terminase, gp1

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Bacteriophage λ development is blocked in cells carrying a plasmid that expresses the terminase genes of phage 21. The interference is caused by the small subunit of phage 21 terminase, gp1. Mutants of λ able to form plaques in the presence of gp1 include *sti* mutants. One such mutation, *sti*30, is an A · T-to-G · C transition mutation at base pair 184 on the λ chromosome. The *sti*30 mutation extends the length of the ribosome-binding sequence of the *Nul* gene that is complementary to the 3′ end of the 16S rRNA from GGA to GGAG. The *sti*30 mutation causes a ~50-fold increase in the level of expression of a *Nul-lacZ* reporter gene, indicating that the *sti*30 mutation overcomes the gp1 inhibition by increasing the level of expression of gpNul. Although the *Nul* and *A* genes of λ overlap, the *sti*30 mutation has little effect on the level of gpA expression, indicating that translational coupling does not occur.

Phages λ and 21 have the same overall genome organization and particle morphology and are members of the lambdoid family of temperate bacteriophages. The head genes of λ and 21, which specify the DNA-packaging enzyme terminase and the head shell, have partial sequence identity, indicating descent from a common ancestor phage (27). λ terminase consists of a small subunit, gpNul, the predicted 181-amino-acid product of the *Nul* gene, and a large subunit, gpA, the predicted 641-amino-acid product of the *A* gene (gene structure diagrammed in Fig. 1). The corresponding small and large subunits of phage 21 terminase are gp1 and gp2, respectively (26). Terminase is a multifunctional enzyme that acts to (i) recognize phage DNA by specific binding of a site, *cosB*, near the left end of the chromosome, (ii) cleave *cosN* to generate the cohesive ends, and (iii) bind the prohead, the empty protein shell into which DNA is to be packaged (2, 8) (Fig. 1). In addition, terminase is a DNA-dependent ATPase and is speculated to be a DNA translocase that pumps the DNA into the prohead.

We have found that λ grows poorly in cells carrying plasmids that express the terminase genes of phage 21, and this report describes an analysis of the interference.

MATERIALS AND METHODS

Strains. The phages, described before (9), were λ *imm*^{21} *c*I *b*538 *red*3, λ *imm*^{21} *c*I *b*538 *red*3 *qsr′*, and λ-21 hybrid 19 *imm*^{21} *c*I *b*538 *red*3. λ *c*I857 *red*3 and λ *c*I857 *red*3 *W*am403 were from our collection. λ-21 hybrid 33 *I*am5 was described before (16); λ *A*am11am32 and λ-21 hybrid 51 *I*am5 were described before (17). Other phage 21 amber mutations used were described before (26).

Escherichia coli strains were C600, a *supE44 rec*$^+$ strain (3); IC202, a *recA1* derivative of C600 (14); R594, a *sup*$^+$ (nonsuppressing) strain (3); MF894, a *supE44 polA15* strain (12); and MC1061, a Φ(*lacPOZY A*) strain (4).

Plasmid constructions. Plasmid pWXI was prepared by ligation of a segment of λ *c*I857 *red*3 *W*am403 *sti*30 DNA, extending from the *Hin*dIII site at 44141 through *cos* to the *Ava*I site at 4720, into pBR322 that had been digested with *Hin*dIII and *Ava*I (Table 1). pWX2 was derived from pWX1 by digestion with *Hin*dIII and *Hpa*I, followed by digestion with S1 nuclease, ligation, and transformation. An isolate, pWX2, was identified that was deleted for the segment extending from position 44141 through *cos* to position 732 near the *Nul-A* boundary. Detailed restriction analysis showed that the S1 digestion did not remove many base pairs of λ DNA to the right of 732. Plasmid pSF1 (11) contains the λ DNA segment extending from the *Hin*dIII site at 44141 through *cos* to the *Bam*HI site at 5505 inserted into the homologous sites of pBR322. Plasmid pBW3 contains a segment of λ-21 hybrid 19 DNA extending from the *Hin*dIII site at 44141 to the phage 21 *Hin*dIII site at ~3050 (12). Plasmids pVJ5, pVJ149, and pVJ40 are homologous to pBW3 except that each contains an amber mutation, in genes *1*, *2*, and *3*, respectively (17). Plasmid pBW4 is a derivative of pBW3 made by digestion of pBW3 with *Eco*RI, followed by ligation to delete the late promoter $p_{R'}$ (located between the pBR322 *Eco*RI site and the λ *Eco*RI site at 44932).

Nul-lacZ translational fusions were constructed as follows. A derivative of pBR322 lacking a *Bal*I site (see following section) was made by digesting pBR322 DNA with *Bal*I and *Pvu*II restriction enzymes, followed by ligation and transformation into C600. A transformant with a plasmid (designated pPR2) deleted for the *Bal*I-*Pvu*II segment was identified and retained. λ DNA segments, either *sti*$^+$ or *sti*30, extending from the *Hin*dIII site at 44141 through *cos* to the *Sph*I site at 2212, were obtained by digestion of pSF1 (*sti*$^+$) and pWX1 (*sti*30) with *Hin*dIII and *Sph*I. The λ DNA segments were ligated into *Hin*dIII- and *Sph*I-digested pPR2, and transformants containing the desired plasmids were identified and designated pJQ8 (*sti*$^+$) and pJE3 (*sti*30). The *lacZ* segment was obtained by *Bam*HI digestion of pMC1871 (4) and ligated into *Bgl*II-digested pJQ8 and pJE3 to generate pJZ23 (*sti*$^+$) and pJRE4 (*sti*30), respectively. The *Bgl*II site in pJQ8 and pJE3 is at bp 412 of λ, and insertion of the *lacZ* segment in the *Bgl*II site creates a chimeric gene in which the first 76 codons are from *Nul*, followed by the *lacZ* segment.

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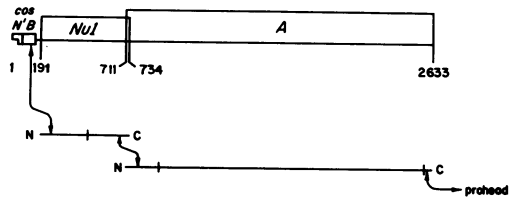

FIG. 1. Schematic diagram of the left end of the λ chromosome. Terminase is encoded by the *Nul* and *A* genes. *cosB* includes binding sites for gpNul (see reference 2 for a detailed description of *cos*). *cosN'* represents the left cohesive end, generated by cleavage of *cosN*. Numbering of the λ sequence follows Daniels et al. (6), so that bp 1 is the first base of the left cohesive end. The two specificity domains of the gpNul subunit are for *cosB* binding (amino terminus) and multimer assembly (carboxy terminus), and the two specificity domains for the gpA subunit are for multimer assembly (amino terminus) and prohead binding (carboxy terminus). The phage 21 terminase subunits gp1 and gp2 contain analogous domains with specificities distinct from those of gpNul and gpA.

Detailed restriction enzyme digestions were done to verify that pJZ23 (*sti*$^+$) and pJRE4 (*sti*30) had the proper structures.

In outline, the *A-lacZ* translational fusion plasmids were constructed by insertion of *lacZ* into pJQ8 and pJE3 at a site downstream of the *Nul* and *A* genes, followed by replacement of a segment extending from a site in *A* to a site in *lacZ* with an analogous segment from a plasmid containing an in-frame *A-lacZ* fusion, described below. Specifically, the *Sal*I site of pJQ8 and pJE3 is at pBR322 bp 651, ~100 bp downstream of the *A* gene segment in these plasmids. The *lacZ* segment obtained by *Sal*I digestion of pMC1871 was ligated into *Sal*I-digested pJQ8 and pJE3, and isolates containing *lacZ* with the same polarity as the *Nul* and *A* genes were identified and designated pJQ82 (*sti*$^+$) and pJE3I (*sti*30), respectively. Segments of pJQ82 and pJE3I extending from the *Bal*I site in the *A* gene (λ bp position 1328) to the *Sac*I site at the amino terminus of *lacZ* were replaced with a *Bal*I-*Sac*I segment from the *A-lacZ* fusion plasmid pTVS-A1 of Sampson et al. (24). (Sampson et al. constructed an in-frame *A-lacZ* fusion in plasmid pTVS-A1 by ligation of λ DNA, cut at the λ *Sau*3A site at 1606, to the beginning of the *lacZ* segment of *Bam*HI-cut pMC1871, a construction that generates a hybrid *A-lacZ* gene containing 401 codons of the *A* gene followed by the *lacZ* segment.) The resulting plasmids, pJQ82F (*sti*$^+$) and pJE31F (*sti*30), had a replaced, in-frame *A-lacZ* fusion fragment from the *Bal*I site in λ at 1328 to the *Sac*I site in *lacZ*. Detailed restriction enzyme digestions were done to verify that pJQ82F and pJE3IF had the proper structures.

TABLE 1. Plasmid structures

Plasmid	Gene arrangement
pBW3	$p_{R'}$ *S R Rz cos 1 2 3*
pVJ5	$p_{R'}$ *S R Rz cos 1*am5 *2 3*
pVJ149	$p_{R'}$ *S R Rz cos 1 2*am149 *3*
pVJ40	$p_{R'}$ *S R Rz cos 1 2 3*am40
pBW4	$\Delta p_{R'}$ *S R Rz cos 1 2 3*
pSF1	$p_{R'}$ *S R Rz cos sti*$^+$ *Nul A W B C'*
pWX1	$p_{R'}$ *S R Rz cos sti*30 *Nul A W B'*
pWX2	$\Delta(p_{R'}$ *S R Rz cos sti*30 *Nul) A W B'*
pJZ23	$p_{R'}$ *S R Rz cos sti*$^+$ *Nul-lacZ*
pJRE4	$p_{R'}$ *S R Rz cos sti*30 *Nul-lacZ*
pJQ82F	$p_{R'}$ *S R Rz cos sti*$^+$ *Nul A-lacZ*
pJE31F	$p_{R'}$ *S R Rz cos sti*30 *Nul A-lacZ*

TABLE 2. Effect of plasmids on plaque formation by λ strains

Phage	Relative efficiency of plating[a] on host carrying:		
	pBW3	pBW4	pSF1
λ *imm*^{21} cI *b538 red3*	3×10^{-5}	0.8	0.6
λ-21 hybrid 19 *imm*^{21} cI *b538 red3*	0.7	0.9	0.6
λ *imm*^{21} cI *b538 red3 qsr'*	0.8	0.9	0.7
λ *imm*^{21} cI *b538 red3 sti30*	1.0	0.9	0.8

[a] The host bacterium was IC202; plating efficiencies are relative to that with IC202 lacking a plasmid.

Assay of *lacZ* fusion proteins. β-Galactosidase activity was measured with the assay of Miller (22). Units of activity are as defined by Miller (22), except the correction for cell turbidity was omitted and the cell density was measured at the time of infection. For infections, MC1060 cells containing the appropriate fusion plasmid were grown at 37°C in LB plus maltose and ampicillin to 10^8/ml and infected at a multiplicity of 10 with λ *imm*^{21} cI *b538 red3*. After adsorption at room temperature for 15 min, the infected cells were incubated with shaking at 37°C for 60 min. The lysate was chloroformed and clarified and then assayed for β-galactosidase activity. Depending on the level of fusion protein in the lysate, the incubation period of the assay varied from 1 to 12 h. Controls showed that the assay was linear with time for 12 h and that cells lacking a *lacZ* plasmid gave a background level of <1 U.

RESULTS

Inhibition of λ growth by phage 21 head gene expression. While doing routine spot tests, we observed that some λ strains were unable to form plaques on bacteria harboring pBW3. Table 2 shows the basic phenomenon: λ *imm*^{21} cI *b538 red3* plated very poorly on cells harboring pBW3. The same phage plated well on cells with pBW4, a plasmid derived from pBW3 which lacks the late promotor (see Table 1 for plasmid structures). This result indicates that the inhibition phenomenon is due to transactivation of phage genes on the plasmid by the infecting phage. Support for this interpretation comes from the results for λ *imm*^{21} cI *b538 red3 qsr'*, a phage which carries a DNA substitution for the *QSR* segment derived from a defective prophage (20, 30). The *qsr'* segment is functionally analogous to the *QSR* segment of λ, but *q*, the *Q* analog, has a different late promotor specificity. Thus, a λ *qsr'* phage is unable to transactivate the λ late promotor on pBW3. λ *imm*^{21} cI *b538 red qsr'* grew well on cells with pBW3 (Table 2); this is consistent with the previous interpretation that the inhibition is due to transactivation of phage genes carried by pBW3.

The nature of the inhibition is suggested by the ability of λ-21 hybrid 19 *imm*^{21} cI *b538 red3* to plate on cells carrying pBW3 (Table 2). λ-21 hybrid 19 strains carry the head gene region from phage 21 and have phage 21 packaging specificity (9). Since a λ-21 hybrid 19 phage could form plaques on a host with pBW3 whereas λ could not, it was tentatively concluded that the inhibition is due to expression of phage 21-derived head genes on pBW3. The inhibition then could be some incompatibility or interference between phage 21 head gene products from pBW3 and analogous head gene products from an infecting λ phage. Another observation

consistent with this interpretation is the lack of inhibition of the λ phage (Table 2) by cells carrying pSF1, a plasmid which carries part of the head gene region of λ but is otherwise structurally analogous to pBW3. The head gene region of pSF1 is larger (from *cos* to the *Bam*HI site at 5.5 kbp) than the analogous segment of pBW3 (from *cos* to the *Hin*dIII site at 3.05 kbp), so pSF1 contains and expresses more of the head gene region than pBW3. The total size of pSF1 is greater than that of pBW3, too, and this may affect plasmid copy number and thus gene dosage. Conclusions drawn from comparisons of pBW3 and pSF1 are subject to these reservations.

The inhibition phenomenon has been termed Its, for the apparent inhibition by phage 21 terminase subunits of λ growth by pBW3. The inhibition phenomenon seems not to be reciprocal, in that cells with pSF1 allowed λ-21 hybrid 19 strains to grow (Table 2). Whether the lack of reciprocity is due to the trivial differences between pBW3 and pSF1 listed above or to some more fundamental difference has not been studied. The presence of the imm^{21}, cI, and *b*538 markers is not important for the results presented in Table 2; similar results were obtained with strains carrying *imm*λ (cI857) and the region deleted by *b*538.

The inhibition of λ by pBW3 was strongest in the absence of general recombination. Phages λ red^+ imm^{21} cI *b*538 and λ *red*3 imm^{21} cI *b*538 had relative plating efficiencies of 0.53 and 0.15, respectively, in rec^+ *E. coli* C600(pBW3) and of 0.015 and 3×10^{-5}, respectively, in *recA1 E. coli* IC202 (pBW3) (relative to titer on hosts without pBW3). Severe depression of the efficiency of plating only occurs when the general recombination systems of the phage (Red) and the bacterium (Rec) have been inactivated by mutation. In the absence of both the Red and Rec systems, the burst size of λ is reduced 10- to 20-fold (32). The action of recombination systems in counteracting the inhibition is likely to be simply an increase of the λ burst size above the minimum necessary to form a plaque, but this point has not been investigated further.

Identification of gp1 as the cause of the inhibition of λ. Plasmids analogous to pBW3 were constructed except that each carried an amber mutation in one of the phage 21 genes carried by pBW3. The plasmids carried the *1*am5 (pVJ5), *2*am149 (pVJ149), and *3*am40 (pVJ40) mutations. The plasmids were placed in MF611 and used as plating bacteria for λ cI857 *red*3. pVJ5, with the *1*am5 mutation, did not inhibit phage growth, while the other plasmids did (data not shown). We conclude that gp1 is the cause of inhibition of λ by pBW3.

λ *sti* mutants insensitive to inhibition by pBW3. The rare phages in a λ *red*3 stock that are able to form plaques on a host carrying pBW3 (Table 2) have mutations that render λ insensitive to the pBW3 inhibition. Three classes of mutants have been identified. The first class consists of red^+ revertants, identified as able to grow on *polA* mutant bacteria, unlike the *red*3 phages. In the second class are delayed-lysis mutants. Phages with lysis delay defects form noticeably smaller plaques than the parent phage, and lysogens of these phages show strikingly delayed lysis following induction in comparison with the parent phage. One lysis delay mutation was localized to the right chromosome arm (from *att* through *Rz*) by genetic crosses; these mutants were not studied further. The third class of mutations map in the left chromosome arm and are called *sti* mutations (the parent is sti^+ [sensitive to phage 21 inhibition]). A variant of λ cI857 *red*3 *W*am403 able to form plaques (Table 2) on IC202(pBW3) was purified and named λ cI857 *red*3 *W*am403 *sti*30. The *sti*30 mutation was mapped to the left chromosome arm in preliminary crosses (data not shown).

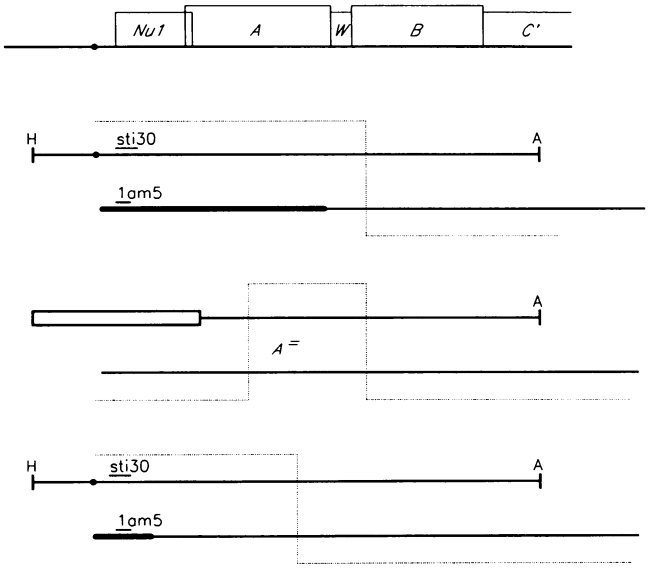

FIG. 2. Crosses to map the *sti*30 mutation. Light lines represent λ DNA; heavy lines represents phage 21 DNA; and the dot represents *cos*. (Top) Gene arrangement at left end of λ chromosome. Dot represents *cos*. (Top cross) Cross between pWX1, which carries the *sti*30 mutation, and λ-21 hybrid 33 *1*am5. H and A indicate *Hin*dIII and *Ava*I sites, respectively, used for cloning the λ DNA segment of pWX1. (Middle cross) Cross between pWX2 and λ *A*am11am32. Open box indicates deleted DNA of pWX2. (Bottom cross) Cross between pWX1 and λ-21 hybrid 51 *1*am5.

To further map the *sti*30 mutation, the segment of λ *sti*30 DNA that extends from the *Hin*dIII site at 44141 through *cos* to the *Ava*I site at 4720 was inserted into the plasmid pBR322 to generate pWX1. The sti^+ control plasmid was pSF1, as described above (11). λ-21 hybrid 33 *1*am5 was used to infect cells carrying pWX1 or pSF1, and the resulting lysates were plated on sup^+ cells to select Am^+ recombinants (cross diagrammed in Fig. 2). The Am^+ recombinant plaques were stabbed into MF611(pBW3) to test for *sti*. The cross λ-21 hybrid 33 *1*am5 × pSF1 was the negative control, and no Am^+ recombinant tested was *sti*30 (Table 3). The cross λ-21 hybrid 33 *1*am5 × pWX1 is the experimental cross, and all Am^+ recombinants tested were *sti*30, indicating that *sti*30 maps between 1 and 2525, the segment of λ-21 hybrid 33 that

TABLE 3. Crosses to map *sti*30

Rescue and infecting page	Plasmid	No. of *sti*30 Am^+ recombinants/no. of Am^+ recombinants
Rescue of *sti*30 from pWX1		
λ-21 hybrid 33 *1*am5	pSF1 (sti^+)	0/17
	pWX1 (*sti*30)	33/33
λ-21 hybrid 51 *1*am5	pSF1 (sti^+)	0/33
	pWX1 (*sti*30)	29/29
Rescue of *sti*30 from pWX1 and pWX2		
λ *A*am11am32	pWX2	0/22
	pWX1	358/670
	pSF1	0/114

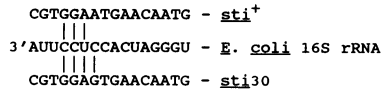

FIG. 3. Complementarity between the ribosome-binding segment of the *Nul* gene and the 3′ end of the 16S rRNA, and the effect of the *sti*30 mutation on complementarity.

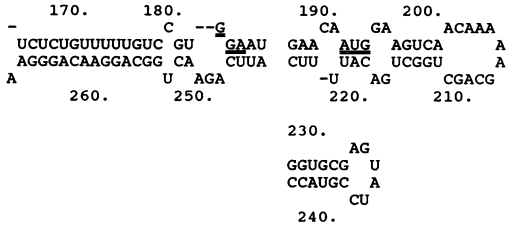

FIG. 4. Possible RNA secondary structure involving the ribosome-binding segment of the *Nul* gene. Structure generated by the folding program of Zuker and Steigler (33). Predicted free energy of formation is −31.0 kcal/mol. The ribosome-binding segment and initiation codon are underlined.

is derived from phage 21. The λ and phage 21 sequences have sufficient sequence divergence to reduce general recombination to insignificant levels. Thus, λ-21 hybrid 33 *1*am5 recombines to become Am^+ by picking up the entire λ segment from 1 to 2520. Since all the Am^+ recombinants are *sti*30, *sti*30 maps in the 1 to 2525 segment.

A derivative (called pWX2) of pWX1 was prepared by deletion of the DNA segment from the vector *Hin*dIII site through *cos* to the *Hpa*I site at 734. The deletion results in the removal of *Nul* but leaves the *A* gene intact (Fig. 2). A cross between pWX2 and λ *A*am11am32 *c*I857 *red*3 was done, and A^+ recombinants were tested to see whether the *sti* marker had been corescued. No (0 of 22) A^+ recombinant was *sti*30 (Table 3), indicating that *sti*30 is located to the left of the *Hpa*I site at 734. In a third set of crosses, the phage used was λ-21 hybrid 51 *1*am5; in this phage, the phage 21 DNA segment extends from 1 to 490. The crosses gave results equivalent to those obtained with λ-21 hybrid 33: all Am^+ recombinants tested from the cross with pWX1 were *sti*30 (Table 3). This maps *sti*30 to the 1 to 490 interval.

Sequence of the *sti*30 mutation. The segment extending from bp 1 to 490 was sequenced. The *sti*30 mutation was found to be a single A · T-to-G · C transition mutation at bp position 184. Bp 184 is part of the proposed ribosome-binding segment of the *Nul* gene (5). The *sti*30 mutation results in better complementarity with the *E. coli* 16S rRNA sequence CUCC (Fig. 3). This complementarity plays a major role in the ribosome-mRNA interaction (25). Since the mutation apparently results in a stronger mRNA-ribosome interaction, we examined the effect of the *sti*30 mutation on *Nul* translation through the use of a *Nul-lacZ* fusion construct.

Effect of the *sti*30 mutation on expression of a *Nul-lacZ* reporter gene. To determine whether the *sti*30 mutation increased the efficiency of translation, we constructed an in-frame fusion of the *Nul* and *lacZ* genes, encoding a hybrid gpNul-LacZ protein. The fusion construction was produced in plasmids containing the λ late operon without (pJZ23) and with (pJRE4) the *sti*30 mutation. Expression of the hybrid *Nul-lacZ* gene was induced by infection of cells harboring the plasmid with λ imm^{21} cI *b*538 *red*3; the infecting phage supplies gpQ to activate the plasmid $p_{R'}$ promotor. The infected cells were incubated until lytic growth of the infecting phage was complete and the cells had lysed. The levels of β-galactosidase activity in lysates indicate that the *sti*30 mutation had a strong effect (~50-fold increase) on production of the gpNul-LacZ protein. pJZ23 and pJRE4 gave 3.1 and 163 U of β-galactosidase activity, respectively. The strong effect of the *sti*30 mutation on *Nul-lacZ* expression is consistent with the notion that the *sti*30 mutation increases the efficiency of *Nul* translation by strengthening the ribosome-mRNA interaction. Because of the large effect of the *sti*30 mutation, we also looked for RNA secondary structure that might, by occluding the ribosome-binding segment, affect *Nul* expression. The segment from bp 165 to 270 is predicted to fold into a structure in which the ribosome-binding segment is base-paired (Fig. 4).

Effect of the *sti*30 mutation on expression of an *A-lacZ* reporter gene. In many instances in which open reading frames overlap, there is translational coupling; that is, translation of a cistron depends on translation of the preceding cistron (23). Translational coupling is thought to be the result of efficient delivery of ribosomes to the ribosome-binding site for the second cistron as a consequence of prior translation, by the ribosomes, of the preceding cistron of the polycistronic mRNA. Since the DNA segment proposed to encode the carboxyl terminus of gpNul overlaps with the beginning of the presumed start of the gpA open reading frame by eight codons, we asked whether translational coupling occurs between *Nul* and *A* by measuring the effect of the *sti*30 mutation on the expression of an *A-lacZ* fusion protein. The *sti*30 mutation had little effect on expression of an *A-lacZ* reporter gene (1.4 and 2.4 U of β-galactosidase activity with sti^+ and *sti*30, respectively), indicating that little translational coupling occurs between the *Nul* and *A* cistrons.

DISCUSSION

Expression of the terminase genes of phage 21 from a plasmid inhibits lytic growth of phage λ. The inhibition is due to interference with λ development by the small subunit of the phage 21 terminase, gp1. gp1 apparently interferes with λ terminase function, because mutations such as *sti*30 that increase the expression of the small subunit of λ terminase, gpNul, allow λ growth in the presence of gp1. The *sti*30 mutation affects the ribosome-binding sequence for the *Nul* coding sequence, resulting in a great increase in expression of a *Nul-lacZ* reporter gene construct. Translation of *A* is not coupled to translation of *Nul*.

Its inhibition. The *1* and *Nul* proteins carry out analogous roles in the functioning of the terminases of phages 21 and λ (17, 21). Studies of the small terminase subunits indicate the presence of three domains. First, the amino terminus of the small subunit is involved in DNA binding (17), and conserved amino acids indicating the presence of a helix-turn-helix DNA-binding motif are present (2). Second, gpNul has an ATPase activity and a conserved ATP-binding motif just after the helix-turn-helix motif (1). Third, the carboxyl terminus is involved in interactions with the large terminase subunit (17). gp1 and gpNul, while having 52% sequence identity, are divergent for DNA-binding specificity and for specificity for binding the large subunit. While we have not identified the mechanism by which gp1 interferes with λ development, it seems likely that gp1 interferes with gpNul action, because overexpression of gpNul reverses the interference. This interference could be due to binding by gp1 of *cosB* of λ or gpA or both. We propose that gp1, while having

diverged from gpNul for specificity of binding of *cosB* and the large terminase subunit, has some affinity for either λ *cosB* or gpA. Binding of *cosB* of λ could interfere with proper binding by λ terminase, and binding of gpA by gp1 would generate a terminase with specificity for the *cosB* of phage 21.

Since little interference is noted in cells coinfected with λ and phage 21 (9), the interference observed here probably depends on a higher level of gp1 expression from the plasmid than occurs during the phage 21 lytic cycle. The level of gp1 expression, either from the plasmid or by phage 21, is not known. The level of expression of the small subunit by lambdoid phages is known to vary greatly. During λ infection, for example, the levels of gpNul and gpA are both quite low (5), whereas during a φ80 infection, the level of gp1 (the small subunit) is about 280-fold higher than the level of gp2 (large subunit) (31).

Control of terminase expression. Chow et al. (5) previously argued that much of the control of the efficiency of translation of *Nul* and *A* was due to the strength of the mRNA-rRNA interaction, because they obtained high-level expression of both gpNul and gpA following replacement of the ribosome-binding segments at the beginning of each gene. Our results with *sti*30 agree with and extend those of Chow et al. (5), because here we show that increasing the complementarity of the natural ribosome-binding sequence of the *Nul* gene with the 3′ end of the 16S rRNA from 3 to 4 bp (Fig. 3) results in an increase in *Nul* expression. Numerous studies show that for a given ribosome-binding site, the efficiency of translation is directly related to the mRNA-rRNA complementarity (7; reviewed in references 18 and 19).

The increased expression of gpNul is therefore likely due, at least in part, to the change in the mRNA-rRNA complementarity caused by the *sti*30 mutation.

Several features of the *Nul* ribosome-binding segment are atypical and may account for the poor expression of the *Nul* gene (discussed in reference 5). In addition to the poor complementarity with the 16s rRNA (3 bp), there is an AUG triplet between the ribosome-binding segment and the initiation AUG, a feature found rarely (29). The *sti*30 mutation changes the intervening AUG to GTG, a change that could improve translation. An additional control of *Nul* expression may be involvement of the ribosome-binding segment of the *Nul* transcript in base-pairing (Fig. 4). The *sti*30 mutation could weaken this possible pairing, making the ribosome-binding segment more accessible to the ribosome.

The *sti*30 mutation, while increasing the level of expression of the *Nul-lacZ* reporter gene, had little effect on expression of the *A-lacZ* reporter gene. We conclude that translation of *A* is not coupled to translation of *Nul* to any significant extent. This result is in agreement with the variable levels of expression of the small subunits of λ and φ80, which are independent of the level of expression of the large subunits in these phages.

Why are the levels of expression of the small subunits of the terminases of λ and φ80 so dramatically different? Since the level of gpNul expression can be strongly increased by mutation without detriment to the phage, the level of expression may be determined by the history of the virus. Perhaps φ80 has experienced a selection for high expression of the small terminase subunit, similar to that imposed by the presence of phage 21 gp1 in the cell. An alternative explanation is suggested by our finding, reported elsewhere (10), that the *sti*30 mutation allows plaque formation by λ *FI* mutants. Perhaps high expression of the small terminase subunit is simply important for the φ80 life cycle.

The effect of the *sti*30 mutation on expression of the *Nul-lacZ* reporter gene was very stong, causing a ~50-fold increase in expression. Premature termination of transcription has been observed within the *lacZ* gene in constructs with very inefficient initiation of translation (28). It is possible that the dramatic increase in *Nul-lacZ* expression by the *sti*30 mutation might be due in part to suppression of premature transcription termination. This point has not been tested, but we note that the expression of the *Nul-lacZ* reporter gene is activated by the λ *Q* gene product, which acts as an antiterminator (15), which may well eliminate the problem of premature transcription in our experiments. Supporting this assertion, the *sti*30 mutation had little effect on expression of the *A-lacZ* reporter gene, a result not expected if the *sti*30 mutation were suppressing upstream transcription termination.

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