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Suppression of Polarity of Insertion Mutations in the *gal* Operon and *N* Mutations in Bacteriophage Lambda

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Bacterial mutations (*psuA* and *psu*) known for their ability to suppress the polarity of nonsense mutations are shown to suppress the polarity of certain insertion mutations in the *gal* operon. The short insertion, IS1 (800 nucleotide pairs), is about 15 to 50% suppressed, whereas longer insertions, IS2 (1,400 nucleotide pairs) and IS3 (1,200 nucleotide pairs), are not. Some of the polarity suppressor mutations (*psu-1*, *psu-2*, and *psu-3*) are at least partially permissive for *N*-gene mutations (*N*7 and *N*53) of bacteriophage λ, suggesting a relationship between natural and mutational polar signals. That this relationship may be complex is indicated by the fact that other suppressor mutations, effective in suppressing nonsense or insertion polarity, fail entirely to permit the growth of λ *N* mutants.

Insertion and certain nonsense mutations depress the activity of cistrons located promoter-distal to the mutation in an operon. This effect, known as polarity, can in some cases be corrected by an unlinked suppressor mutation, *psuA* or *psu* (5, 8, 14; L. Korn and C. Yanofsky, J. Mol. Biol., in press). Unlike transfer ribonucleic acid suppressors, polarity suppressors do not correct the defect of the mutated cistron. The polarity suppressor mutation is thought to represent a change in the structural gene for the *Escherichia coli* transcription termination factor rho, resulting in a partial loss of activity of the rho protein (15, 16). The *N* function of bacteriophage λ releases the polarity of all polar mutations whose transcription is initiated at a λ promoter (1, 11). λ *N*-function evidently acts to antagonize rho action at natural polar, i.e., transcription termination signals; the *N* function is essential for phage development in wild-type *E. coli*, whereas λ N^- phage show some degree of growth in *psuA* and similar strains (6; C. Dambly and D. Court, manuscript in preparation; L. Korn, personal communication).

These observations indicate that, in the absence of translation, rho acts abnormally, terminating transcription within, rather than at the end, of an operon (1; S. Adhya, M. Gottesman, B. de Crombrugghe, and D. Court, manuscript in preparation).

We report here a systematic study of polarity release by a series of polarity suppressor mutations, as well as their ability to support the growth of λ *N* mutants. We find that all suppressors tested release the polarity of nonsense and one class of insertion mutations, IS1 (containing 800 nucleotide pairs); some are partially permissive for λ N^- mutants, and none appears to have any suppressing activity on the longer insertion types, IS2 (1,400 nucleotide pairs) and IS3 (1,200 nucleotide pairs). (The IS3 insertion mutation, *gal-3*, used here has the same length as the IS3 insertion mutation, lac_{MS505}, but whether *gal-3* is homologous to lac_{MS505} is not known [4, 10].)

MATERIALS AND METHODS

Bacterial strains. The strains of *E. coli* K-12 used in this study are listed in Table 1. Strains carrying a wild-type or mutant polar suppressor were deleted for the *gal-pgl-att-bio* region by isolating heat-resistant survivors of λc1857*xis*1 lysogens. Transduction of *gal* mutations to the above deletions was performed using phage P1*clr*100CM^R (18) lysates made on a donor *gal* mutant and selecting for bio^+ character. Only transductants not carrying P1 prophage were further used. They were also tested for the presence of the wild-type *pgl* allele (2) and for the corresponding *gal* mutations. Transduction of polar suppressors was also performed with page P1*clr*100CM^R, selecting for ilv^+ transductants on an ilv^- recipient. The transductants were scored for the presence of a polar suppressor by examining the suppression of polarity of a *trpE* ochre mutation present on the recipient strain (14).

Phage strains. The amber mutants of λ used in this work originally came from A. Campbell (7) and R. Thomas et al. (20). $\lambda_{vir}N500$ was isolated by D. Court. *N*500 is an amber mutation that fails to recombine with *N*53 (D. Court, personal communication). The *vir* marker came from J. Lederberg via

TABLE 1. *Bacterial strains*

Designation	Suppressor allele	Other genotypes	Source or reference
OR518	+	F⁻*lacZ*118 *trpR* *val*^{r} *str*^{s} exW3110[a]	Korn and Yanofsky
OR519	*psu-1*	F⁻*lacZU*118 *trpR* *trp* *val*^{r} *str*^{s} exW3110	Korn and Yanofsky
OR520	*psu-2*	F⁻*lacZU*118 *trpR* *str*^{s} exW3110	Korn and Yanofsky
OR754	*psu-3*	F⁻*lacZU*118 *trpR* *trpE*ts9777 *trpA*am9761 *val*^{r} *str*^{s} exW3110	Korn and Yanofsky
DM2055	*psuA*120[b]	F⁻*leu*am *trpR* *trpE*och *str*^{s} exW3110	14
DC45	*psuA*1	F⁻*trpE*och *his*am *str*^{s} exW3100	Dambly and Court, in preparation
OR697		F⁻*lacZU*118 (*gal-bio*)$^\Delta$ *trpE* *his*am *str*^{r} *ilv* exW3110	This study
OR778		OR697 *psu-1* *ilv*$^+$	This study
OR779		OR697 *psu-2* *ilv*$^+$	This study
OR780		OR697 *psu-3* *ilv*$^+$	This study
N720	*sup-3*	F$^+$	NIH Collection
N3005	+	*gal*306 exW3110	19
N3004	+	*gal*128 exW3110	19
N2790	+	F⁻*galT*N102	12
MS726	+	F⁻*galT*104	3
SA695	+	HfrH *galE*amUV39	3
SA631	+	F⁻*galE*och95	3
KB308	+	F⁻*gal*308	19
SA1030	+	F⁻*gal*-3	3
SA696	+	HfrH *galE*490	9
SA1090	+	F⁻*galE*S148	3
SA1241	+	F⁻*galT*S101	3

[a] exW3110 means the strain is a derivative of W3110 strain of J. Lederberg.

[b] Although originally characterized as an amber mutation, *psuA120* does not respond to *sup-3* in our hands.

A. Campbell. The amber mutants of λ carry the *c*I857 marker except λ*c*II*R*5 and λ*c*II2002*R*221.

Media. Tryptone broth, nutrient broth, tryptone agar, and MacConkey galactose agar were made as described in the Difco Manual. M56 media contained per liter: 8.2 g of Na_2HPO_4, 2.7 g of KH_2PO_4, 0.25 mg of $FeSO_4 \cdot 7H_2O$, 5 mg of $Ca(NO_3)_2 \cdot 4H_2O$, 100 mg of $MgSO_4 \cdot 7H_2O$, 1 g of $(NH_4)_2SO_4$, 0.3% glycerol, and appropriate nutritional supplements.

Enzyme assays. Galactokinase assays were performed essentially according to Wilson and Hogness (21) using toluene-lysed cells. Uridine diphosphate galactose-4-epimerase was determined according to Wilson and Hogness (21) as modified by Merrill and Das (manuscript in preparation). One enzyme unit is defined as nanomoles of product per minute per milliliter of cells of optical density at 590 nm of 1.0.

RESULTS

Suppression of *gal* polar mutations. The polarity suppressor mutations were originally selected by their ability to suppress polarity due to nonsense mutations in *lac* (*psuA1*, reference 5; *psuA78*, reference 8), in *trp* (*psuA120*, reference 14), or in *trp* and *lac* simultaneously (*psu-1*, *psu-2*, and *psu-3*, Korn and Yanofsky, in press). To test their effect on polar *gal* mutations, derivatives of the polarity suppressor strains *psuA1*, *psuA120*, and *psu-2* deleted for the *gal-bio* region were isolated (see Materials and Methods). These strains were then transduced to *bio*$^+$ by P1 phage grown on a variety of donors which bore insertion or nonsense mutations in the *gal* operon. The structure of the operon and the map positions of the polar mutations used are shown in Fig. 1. The resultant strains were assayed for galactokinase, the product of gene *galK*, which is the last cistron of the *gal* operon. The level of kinase is a measure of the polarity of the mutations located earlier in the operon (Table 2).

(i) Effects of polarity suppressors on a wild-type *gal* operon. Lines 1 and 2 of Table 2 show that polarity suppressors *psu-2*, *psuA1*, and *psuA120* all decrease the induced level of galactokinase of a wild-type *gal* operon to 60 to 80% of the level observed in an unsuppressed strain. This inhibition, at present unexplained, has been taken into account in calculating the efficiency of polarity suppression. The galactokinase levels of the induced *gal*$^+$ *psuA120* or *gal*$^+$ *psu-2* cells are taken as 100%.

(ii) Effect of polarity suppressors on nonsense mutations. The polarity suppressors, as expected, partially release the polarity of nonsense mutations (an amber and an ochre) located early in the *galE* gene (Table 2, lines

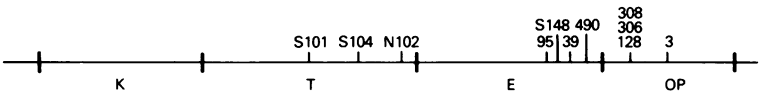

FIG. 1. *Genetic map of the gal operon, showing the relative positions of the gal mutations discussed in this work.*

TABLE 2. *Suppression of polarity*[a]

Gal genotype	Addition of fucose (5 × 10^{-3} M)	Galactokinase levels			
		Unsuppressed	*psu-2*	*psuA120*	*psuA1*
1. *gal*$^+$	+	10.0	6.3 ± 1.9 (5)	6.4 ± 2.4 (5)	8.4 (1)
2. *gal*$^+$	−	1.2 (2)	1.6 (1)	0.7 (1)	
3. *E*amUV39	+	1.0 (2)	2.6 ± 1.3 (3)	4.8 ± 0.4 (3)	
4. *E*amUV39	−	0.7 (2)	0.4 ± 0.2 (3)	0.6 ± 0.2 (3)	
5. *E*och95	+	0.8 (1)			4.4 (1)
6. *E*och95	−				
7. *T*N102 (IS1-I)	+	0.2 (2)	1.3 ± 0.4 (4)	1.3 ± 0.3 (4)	1.9 (1)
8. *T*N102 (IS1-I)	−	0.2 (2)	0.2 ± 0.1 (4)	0.4 ± 0.3 (4)	0.6 (1)
9. *T*S104 (IS1-I)	+	0.2 (2)	1.1 (2)	1.3 ± 0.2 (3)	
10. *T*S104 (IS1-I)	−	0.4 (2)	0.5 (2)	0.5 ± 0.1 (3)	
11. *OP*306 (IS1-I)	+	0.4 (2)	3.2 (2)	3.1 ± 0.6 (3)	
12. *OP*306 (IS1-I)	−	0.1 (2)	1.4 (2)	1.5 ± 0.5 (3)	
13. *OP*128 (IS1-II)	+	0.5 (2)	2.6 (2)	2.5 ± 0.5	
14. *OP*128 (IS1-II)	−	0.2 (2)	1.3 (2)	1.2 ± 0.3	
15. *OP*308 (IS2)	+	0.3 (1)	0.3	0.6	
16. *OP*308 (IS2)	−	0.4 (1)	0.2	0.7	
17. *E*490 (IS2)	+	0 (2)	0.4 (2)	0.7 ± 0.2 (5)	0.3 (1)
18. *E*490 (IS2)	−	0 (2)	0.1 (2)	0.6 ± 0.2 (5)	0.7 (1)
19. *E*S148 (IS2?)	+	0.1 (1)	0.3 (1)	0.4 (2)	0.6 (1)
20. *E*S148 (IS2?)	−	0.1 (1)	0.1 (1)	0.2 (2)	0.5 (1)
21. *OP*3 (IS3)	+	0.2 (2)	0.4 (2)	0.7 ± 0.2 (3)	
22. *OP*3 (IS3)	−	0.2 (2)	0.3 (2)	0.4 ± 0.1 (3)	
23. *T*S101 (IS4?)	+	0.1 (2)	0.1 (2)	0.3 (2)	
24. *T*S101 (IS4?)	−	0.2 (2)	0.1 (2)	0.2 (2)	

[a] The wild-type *gal*$^+$ strains are OR518 (unsuppressed), OR520 (*psu-2*) and DM2055 (*psuA120*), DC45 (*psuA1*). The *gal* mutations were transduced to the corresponding backgrounds as described in Materials and Methods. Galactokinase values obtained were normalized to the specific activity of strain OR518 taken as 10. The actual value for OR518 is 11.7 ± 1.6 units in six experiments. Numbers between brackets are the number of experiments performed to give the average. The numerals I or II after the IS1 designation refer to one or the other of the two possible orientations of the deoxyribonucleic acid insertions.

3-6). The efficiency of suppression of polarity caused by the amber mutation is somewhat greater for *psuA120* than for *psu-2*.

(iii) Suppression of IS1 polarity. The polarity of two insertions occurring with similar orientations and located at the beginning (N102) and middle (S104) of the *galT* gene is also released by *psu-2*, *psuA120*, and *psuA1* (see Fig. 1 and Table 2, lines 7-10). The efficiency of suppression is about the same in both cases (about 20% of *gal*$^+$ values). This is less than half the suppression observed for 306, an IS1 insertion with the same relative orientation but with a location between *galE* and the operator-promoter region (see below). This might reflect an intercistronic gradient of suppression or merely differences peculiar to these mutational sites.

The polarity of the IS1 insertions 306 and 128 is also released by *psu-2* and *psuA120* to about the same extent (50 and 40% of the *gal*$^+$ values, respectively) (Table 2, lines 11-14). Note that mutations 306 and 128 are present in opposite orientations (10); evidently inversion of the IS1 sequence affects neither its polarity nor the suppression of its polarity by external suppressors. The 306 and 128 insertions are located in or near the *gal* operator-promoter region (19). When suppressed by the polarity suppressors, the strains become weakly Gal$^+$. They are, furthermore, inducible rather than constitutive for galactokinase (Table 2, lines 11-14) and uridine diphosphate galactose-4-epimerase (data not shown), the latter being the product of gene *galE*, the first cistron of the *gal* operon. This suggests that these insertions do not inactivate *galE* nor the *gal* operator promoter. Instead, they appear to act as polar barriers between these regions.

(iv) Effect on long polar insertions. The

long insertions tested (308, 490, S148, 3, and S101) represent at least two insertion sequences. 308 and 490 are IS2 insertions (10). The S148 insertion in *E* has not been classified although, like 490, it contains a rho-sensitive transcription termination signal (9). Insertion S101 differs from IS2 in sequence but not in length (10). Insertion 3, on the basis of its length, is apparently an IS3 type (4).

The longer insertions have in common the fact that they are not efficiently suppressed by polar suppressors (Table 2, lines 15–24). On MacConkey galactose indicator plates, polar suppressor strains bearing the 308 and 3 insertions located in the promoter-operator region of the operon appear Gal$^-$. We shall return to this observation in the Discussion.

Suppression of bacteriophage λ *N*$^-$ mutation. Lambda phage strains with a mutation in gene *N* display a phenotype which resembles polarity in *E. coli*. Expression of λ early and late genes does not occur, a result of termination of transcription at rho-sensitive sites to the left of *N* gene and to the right of *tof* gene (17). The similarity is strengthened by the observation that *psuA1* and a class of mutations similar to *psuA1* partially relieve the requirement of *N* gene product (6; Dambly and Court, manuscript in preparation). We have confirmed and extended these studies, demonstrating the partial suppression of the *N*$^-$ phenotype of λ in the other polar suppressors, *psu-1*, *psu-2*, *psu-3*, and *psuA120*. The efficiency of suppression, however, clearly depends both on the particular suppressor mutation and on the genetic background of the strain.

The efficiency of plating (EOP) of λ*N*7 and λ*N*53 is greatly increased in strains carrying the *str*^{s} allele and the *psu-1*, *psu-2*, or *psu-3* mutation (Table 3, lines 1–5). On the other hand, no suppression of the *N*7 or *N*53 defect is seen if the strain bears the *psuA120* mutation.

Transfer of the polarity suppressor mutations to a different genetic background, which carries a *str*^{r} allele, decreases the EOP of λ*N*7 and λ*N*53 (lines 6–10). The reason for the loss of suppression is not clear. The *str*^{r} allele may play a role in it.

λ_{vir}*N*500 shows significant growth on both *str*^{r} and *str*^{s} derivatives. Since the *N*500 mutation is identical to *N*53, which is suppressed poorly in these strains, the uncharacterized *vir* mutation(s) in the phage must also affect transcription termination elsewhere in the phage genome.

Polarity suppressors *psu-1*, *psu-2*, and *psu-3*, even in the *str*^{s} background, do not permit the double mutant λ*N*7*N*53 to form plaques (EOP is 10^{-7}). Weak suppression of the double mutant can, however, be detected as a zone of lysis when the phage is spotted on these suppressor strains at concentrations of 10^6 plaque-forming units/ml. No suppression was observed in any polar suppressor strains for λ*N*ts8 at 39 C (EOP less than 10^{-5}). Other regulatory and structural amber mutants of λ, λ*A*11, λ*B*1, λ*E*43, λ*I*2, λ*J*6, λ*O*29, λ*P*3, λ*Q*21, λ*Q*57, λ*Q*57*Q*501, λ*R*221, and λ*R*5 all showed an EOP of 10^{-6} or less in the presence of polarity suppressor mutations.

DISCUSSION

We find that polar suppressor mutations release the polarity of short (IS1) insertions in the *gal* operon, present in either orientation, but not that of long insertions. It has been mentioned that the polarity of IS1 insertions in the *lac* operon is suppressible by *psuA1* (Torti and Malamy, cited in reference 13); these

TABLE 3. *Suppression of λ N mutants*[a]

Strain	Suppressor	*str* allele	EOP		
			*N*7	*N*53	vir*N*500
1. OR518	+	s	10^{-6}	10^{-6}	0.2–0.9 (*s*)
2. OR519	*psu-1*	s	0.9	0.4	0.9
3. OR520	*psu-2*	s	0.9	1.0	1.1
4. OR754	*psu-3*	s	0.6	0.5	1.0
5. DM2055	*psuA120*	s	10^{-6}	10^{-6}	0.9
6. OR697	+	r	10^{-6}	10^{-6}	10^{-6}
7. OR778	*psu-1*	r	0.009	0.009	0.96
8. OR779	*psu-2*	r	0.009	0.01	0.94
9. OR780	*psu-3*	r	2×10^{-6}	2×10^{-6}	0.06
10. OR806	*psuA120*	r	2×10^{-6}	2×10^{-6}	0.06

[a] EOP is defined as titer on the tester strain over titer on N720 (*sup3*) strain performed on TB plates at 39 C. N720 is permissive for the *N* amber strains used. The titer of each phage stock on N720 strain was 2×10^9 per ml. s, Pin-point plaque when observed.

authors find that IS1 insertions near the promoter-distal end of *lacZ* are suppressed more efficiently than insertions at the promoter-proximal end. We observe equal suppression of two IS1 insertions present in different locations in *galT*, but with the same orientation. This discrepancy might be due to the different polarity suppressor alleles used or to the relative orientations of the insertions (not reported in the cited communication).

The failure of the polarity suppressor mutations to suppress IS2 insertions can be explained as follows. According to our model (Adhya et al., in preparation) all polarity is the consequence of rho-mediated transcription termination within operons, which is provoked by the premature release of polypeptide chains at intracistronic nonsense codons. A site of transcription termination within the *gal* operon has been described (9). This site responds only to relatively high concentrations of rho. We imagine that IS1 insertions either contain nonsense codons or generate nonsense codons through a frameshift. Their polarity would be due to transcription termination at this rho-sensitive site. In contrast, IS2 insertions have been shown to carry, within their sequence, sites of transcription termination sensitive to very low levels of rho; we assume they also carry nonsense triplets upstream to these sites. Since the polarity suppressor mutations studied in this work are only partially rho defective (15, 16; Korn and Yanofsky, in preparation), we expect greater suppression of IS1 polarity than of IS2. A more deficient rho mutation would suppress the polarity of both IS1 and longer insertions, and this, in fact, occurs (A. Das, D. Court, and S. Adhya, Proc. Natl. Acad. Sci. U.S.A., in press).

The observed suppression of single amber (*N7* and *N53*) mutations and not of the double amber (*N7N53*) and the temperature-sensitive mutations in the *N* gene of λ also suggests that some residual *N* activity plays a role in the observed level of suppression in the polarity suppressor strains *psu-1*, *psu-2*, and *psu-3*. Although the various suppressor mutations are similar in their efficiency of suppression of nonsense and IS1 polarity, they vary greatly in their ability to support the growth of λ N^- phage (compare *psu-2* and *psuA120* in Tables 2 and 3). These findings raise the possibility that a mutation in rho might affect its activity at certain termination sites and not at others. This notion is currently under investigation.

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