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Genetics

ρ-Dependent transcription termination of a bacterial operon is antagonized by an extrachromosomal gene product

(plasmid P4/satellite phage P4/*Escherichia coli*/*trp* operon/*lac* operon)

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ABSTRACT **The *psu* gene product of "phasmid" (phage-plasmid) P4 acts as a transcription antitermination factor in *trans* and in *cis*, respectively, within the morphogenic operons of its P2 phage helper during lytic viral development and on P4 itself during the establishment stage of its alternative mode of propagation as a plasmid. Here we show that *psu* also antagonizes activity of the *Escherichia coli* transcription termination factor ρ at the terminator of the *trp* operon. Such a finding provides to our knowledge the first direct evidence for antitermination activity at a known ρ-dependent site by the *psu* gene product. It also reveals an example of an extrachromosomal gene product that acts on specific sites of three different genomes to regulate expression of unlinked families of genes.**

Genetic approaches to analyses of the regulation of transcription termination have led to the discovery and characterization of specific protein factors [such as ρ (1) and nus (2)] that affect termination and antitermination. Certain bacteriophage λ functions provide the most clearly understood example of antitermination activity (3). There are several transcription terminators within each of the early λ operons. The *N* gene product (gp) of the phage prevents transcription termination at each of these sites. *N*-mediated antitermination enables RNA polymerase to transcribe genes distal to the various terminators and is essential for lytic development of the phage.

Less well understood is the mechanism and role of transcription antitermination that occurs during either the lytic helper-dependent or the plasmid modes of propagation of "phasmid" (phage-plasmid) P4 *vir*1. P4 codes for two apparent antitermination factors, psu and sid. Because initial studies with P4 focused on its lytic development as a "satellite" virus dependent on a "helper" such as temperate *coli*phage P2 (4), both of these functions were first identified with respect to their regulatory effects in *trans* on expression of the heterologous genome of the P2 helper. The *psu* (polarity suppression) gp suppresses the transcriptional polarity of amber mutations on downstream genes located in the polycistronic morphogenic operons of P2 (5). Although mutation in the *psu* gene causes a significant reduction in the burst of P4 progeny phage (5), the actual role of gp *psu* during helper-dependent lytic development is unknown. The *sid* (capsid size determination) gp redirects the normal P2 assembly pathway such that it yields a new class of small capsids of proper size to encapsidate the 11.5 kilobase (kb) P4 genome (6) while excluding complete encapsidation of the 33-kb P2 genome (7). The *sid* gp activity apparently regulates the assembly process by altering normal ρ-mediated transcriptional termination patterns in the late morphogenic operons of the P2 helper (8). The *psu* and *sid* gps also act in *cis* as well to regulate expression of P4 itself during P4 *vir*1 infection in the absence of helper (9). However, neither *sid* nor *psu* is essential for the subsequent propagation of P4 *vir*1 as a plasmid (10). Rather, plasmid stability appears to require repression of their expression to avoid lethal perturbation of plasmid/host commensalism (11).

In this paper we directly demonstrate that the *psu* gp acts to antagonize the activity of the essential host transcription termination factor ρ at a known ρ-dependent terminator sequence. Our results also indicate that gp *psu*, rather than gp *sid*, is responsible for homeostatic perturbation. A model, based on the mutual antagonism of factors ρ and psu, is proposed to account for the repression of *psu* gene expression during the stable maintenance of P4 *vir*1 as a high copy number plasmid.

MATERIALS AND METHODS

Bacterial Strains. Strain X8605 *ilv*$^-$ is the *Escherichia coli* K-12 derivative F$^-$, *trp*R, *lac*U169, *str*A(*ton*B-*lac*P) (12, 13). The *ilv*$^-$ marker, nearby to *rho* on the *E. coli* chromosome, remains present in this strain based on its use as a selectable marker in the construction of the strain X8605 *rho*201 (12, 13). P1 transduction was used in the present study to transduce strain X8605 *ilv*$^-$ pJG76 from *trp*R to *trp*R$^+$ followed by selection for tetracycline resistance expressed by a Tn*10* insertion closely linked to *trp*R$^+$ (E. Brickman and J. Beckwith, personal communication) and checked for sensitivity to 5-methyltryptophan.

P4 Mutants. *Vir*1 is a "promoter-up" point mutation (14, 15) allowing for the stable maintenance of P4 as a plasmid (10, 16). *Sid*1 is an amber mutation in the gene determining the assembly of small P4-size capsids from gene products supplied by helper phage P2 (6–9). δ 35 is a point mutation in the gene required for trans-activation of the morphogenic operons of a mutant P2 helper that is unable to express these operons (17). *Psu*1 is a double amber mutation in the gene necessary for the suppression of transcriptional polarity of amber mutations in the polycistronic morphogenic operons of helper phage P2 (5). *Psu* also mediates the reinitiation of expression of genes α and *sid* (9). δ *inv* (inversion) 11 is a pleiotropic mutant defective for δ, *psu*, and *sid* functions, based on an "inversion" of the *Bam*HI:B fragment of P4 DNA (Fig. 1) (18).

Construction of pP4 *vir*1 *Eco*RI Fragment AD:Km^r (Kanamycin Resistance) Derivatives. Plasmid DNA was isolated as in Goldstein *et al.* (16) and digested with *Eco*RI

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Abbreviations: gp, gene product; kb, kilobases; β-gal, β-galactosidase; Km^r, kanamycin resistance; Cm, chloramphenicol.

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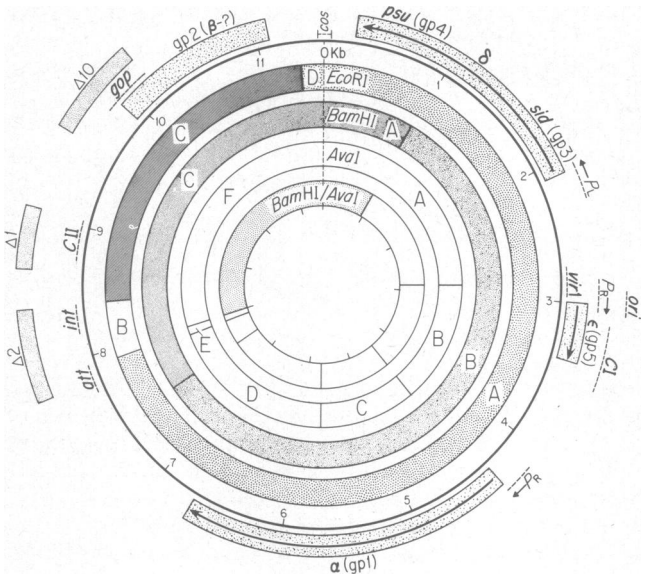

FIG. 1. Genetic and restriction maps of pP4 *vir*1. The 11.6-kb genome of P4 is divided into 1-kb increments starting from the right cohesive end at the *cos* notation. The two-fifths of the P4 genome that is nonessential for helper-dependent lytic phage development resides in the region immediately after the C terminus of gene α, extending to but not including, the *cos* site. An arrow within a gene or operon denotes direction of transcription. Fragment A from any particular digest is that which occurs immediately to the right of the *cos* site. The stippled fragments indicated in both the *Eco*RI map and the *Ava* I–*Bam*HI double-digest map were used in the construction of the plasmids subsequently analyzed for their effect on β-gal activity in strain X8605 *ilv*$^-$ (see Table 2). The P4 *vir*1 δ *inv*11 plasmid (see Tables 1 and 2) was constructed by "inverting" the *Bam*HI fragment B (stippled) within the larger *Eco*RI fragment AD thereby deliberately interrupting the *sid*-δ-*psu* circuitry (18) (R.-Z.J. and R.G., unpublished data). *Ori*, origin of replication; gene α, essential for P4 DNA replication (19); gene ε, necessary for derepression of an integrated heteroimmune P2 helper (20); *vir*1, promoter-up mutation (4, 14, 15) necessary for the normal propagation of P4 as a plasmid (10, 11, 16); gene *sid*, essential for directing the assembly of small P4-size capsids using morphogenic operons of the P2 helper (6–8); gene δ, necessary for P4-mediated trans-activation of the late morphogenic operons of a mutant P2 helper that is unable to turn on these operons (5); gene *psu*, necessary for the suppression of transcriptional polarity of amber mutations in the polycistronic morphogenic operons of helper phage P2 (5); cI and cII, putative P4 repressors (21); *att*, the attachment site for integration into the *E. coli* chromosome (14, 21); gp2, a nonessential gene product expressed early during phage infection (9, 22) and constitutively expressed during plasmid propagation (23). P_R, promoter rightward; P_L, promoter leftward.

endonuclease. Km^r derivatives of pP4 (plasmid P4) were constructed using T4 ligase to ligate and circularize the pP4 *vir*1 8.2-kb *Eco*RI fragment AD (Fig. 1) with a 1.2-kb fragment of pUC4K (24) containing a Km^r (kanamycin resistance) gene. The derivative P4 *vir*1 Km^r plasmids were confirmed based on digestion patterns resolved by electrophoresis (10, 11, 16). All are capable of autonomous replication and contain the Km^r gene ligated in the same direction to the P4 *vir*1 *Eco*RI fragment AD as determined by *Hin*dIII cleavage patterns. Unlike pP4 *vir*1, they do not integrate into the host chromosome because the P4-coded *int* (integration) function is deleted (11). Maintenance of such *int*$^-$ plasmids requires kanamycin selection because an integrated "master copy" of pP4 *vir*1 is otherwise necessary for stability in the absence of direct selection (11). All of the Km^r pP4 *vir*1 derivatives can also be propagated in the phage mode of development following superinfection rescue with helper phage P2.

RESULTS

***Trp-Lac* and P4 Constructions for Assay of Transcription Antitermination.** Although it is apparent that gps *psu* and *sid* can act in *trans* to regulate P2 and in *cis* to control P4 (see *Introduction*), neither sufficient genomic sequence data nor studies on transcripts yet exist from which to deduce the possible terminator sites for their activities. Likewise, a rapid and convenient assay for the gene products that they induce is not available. To directly observe their antitermination activities, we have selected a well-characterized bacterial operon within which to study the potentially analogous roles of *psu* and *sid* gps in *trans*. For this purpose, the specially constructed *E. coli* host strain X8605 was chosen in which a chromosomal deletion brings the *lac* operon adjacent to the *trp* operon (12, 13). The ρ-dependent transcription termination signal at the end of the *trp* operon remains intact‖ while the normal *lac* promoter is partially deleted. Because of this transcription circuitry, the ρ protein in strain X8605 prevents transcription of the *lac* operon by acting at the terminator located at the end of the *trp* operon. Antagonism of ρ-mediated transcription termination can then be directly detected since readthrough into the adjacent *lac* operon results in production of the readily assayable enzyme β-galactosidase (β-gal).

P4 plasmid derivatives were established in X8605 *ilv*$^-$ by using the *vir*1 mutation of P4 that allows for its propagation as a plasmid in the absence of a helper (10, 16). For these experiments, the kanamycin resistance gene from pUC4K (24) was ligated into pP4 *vir*1 in the region normally occupied by the contiguous P4 *Eco*RI fragments B and C (see Fig. 1). The remaining *Eco*RI fragment AD supplies all necessary functions required for development as a helper-dependent phage (4, 11). The relevant phenotypes of pP4 *vir*1 mutant derivatives so constructed are summarized in Table 1. The locations of genes in which mutations are found are displayed in Fig. 1.

Possible antitermination activity at the *trp* terminator might be due to the action of P4-coded gps in *cis* because P4 *vir*1 type plasmids are known to integrate (10, 11, 16) within the *E. coli* chromosome near 96 min (14, 21). To observe possible activity in *trans*, the P4 plasmids used for these studies were deliberately constructed so as to delete the P4-coded *int* function (Fig. 1) thereby precluding integration and any subsequent activity in *cis* on the chromosome (11).

Identification of a P4-Coded Antiterminator That Antagonizes ρ-Mediated Transcription Termination. The ability of P4 to prevent transcription termination, in *trans*, at the bacterial *trp* operon termination site was measured by determining the β-gal activity of X8605 *ilv*$^-$ strains that harbored different pP4 plasmids. The results in Table 2 indicate that pP4 *vir*1 *Eco*RI fragment AD:Km^r antagonizes termination at the *trp* terminator since ≈20-fold more β-gal activity is found than that seen for the control X8605 *ilv*$^-$. In contrast, when a P4 plasmid contains an amber mutation in the *psu* gene, the ability of P4 to antiterminate, as indicated by β-gal activity, is nil (Table 2). This assay also demonstrates that a δ*inv*11 derivative plasmid, constructed *in vitro* through an inversion of the *Bam*HI fragment B of P4 (18) (Fig. 1), which expresses neither gp *psu* nor *sid*, has lost the ability to antiterminate. The results in Table 2, likewise, reveal that a plasmid containing the *sid*1 amber mutation, which negatively affects gp *psu* levels (9), causes only a very low level of antitermi-

‖Both a ρ-independent and a ρ-dependent terminator have been found to exist at the 3′ end of the *trp* operon. The latter is located ≈250 base pairs after the former. The downstream ρ-dependent terminator is the major termination site for *trp* mRNA while the proximal ρ-independent site acts as a minor terminator and as a protective barrier to 3′-exonucleolytic degradation (25, 26).

Table 1. Expression of gene products *psu* and *sid* by P4 mutants

P4 mutant	Phenotypes	
	gp *psu*	gp *sid*
*vir*1	+	+
*vir*1 *psu*1	−	+
*vir*1 *δinv*11	−	−
*vir*1 *sid*1	±	−
*vir*1 *δ*35	+	−

All of the mutants carry the *vir*1 mutation allowing for plasmid maintenance (10, 11, 16). +, Expression; ±, low level expression; −, no expression.

nation activity. In contrast, it can be seen from Table 2 that a P4 plasmid containing the δ35 mutation and known to produce almost normal levels of the *psu* gp (9), expresses 13-fold more β-gal activity than the control. Under similar conditions in the absence of the P2 helper, this mutant produces no gp *sid* (9). Such a result suggests that the *psu* gp, rather than the *sid* gp, is responsible for the ability of P4 to prevent termination at the *trp* terminator.

Table 2. Expression of β-gal activity as a measure of antitermination of the *trp* terminator

Strain	β-gal units
X8605 *ilv*$^-$	5.9 ± 0.1
X8605 *ilv*$^-$ pUC4K	3.8 ± 0.1
X8605 *ilv*$^-$ pBR325	6.5 ± 0.6
X8605 *ilv*$^-$ pP4 *vir*1 *Eco*RI fragment AD:Km^r	90.0 ± 7
X8605 *ilv*$^-$ pP4 *vir*1 *psu*1 *Eco*RI fragment AD:Km^r	4.9 ± 0.6
X8605 *ilv*$^-$ pP4 *vir*1 *δinv*11 *Eco*RI fragment AD:Km^r	5.5 ± 0.7
X8605 *ilv*$^-$ pP4 *vir*1 *sid*1 *Eco*RI fragment AD:Km^r	11.1 ± 0.9
X8605 *ilv*$^-$ pP4 *vir*1 δ35 *Eco*RI fragment AD:Km^r	65.0 ± 0.6
X8605 *ilv*$^-$ pJG76	163.0 ± 9*
X8605 *ilv*$^-$ pJG76 *psu am*1	6.9 ± 0.5
X8605 *rho*201	178.0 ± 5
X8605 *ilv*$^-$ *trp*R$^+$ pJG76 (minus tryptophan)	125.0 ± 13
X8605 *ilv*$^-$ *trp*R$^+$ pJG76 (tryptophan at 100 μg/ml)	13.9 ± 1.4

All assays were performed twice. β-gal units are expressed and the assay performed according to Miller (27). For this purpose cells were grown in LBG (10) (with the exception of the *trp*R$^+$ derivatives) plus appropriate antibiotic and then pelleted and resuspended in PB (27). The *trp*R$^+$ strains were grown in glucose minimal media (12) supplemented with isoleucine and valine, with and without tryptophan. Each of the results given for the *trp*R$^+$ derivatives represent the average level of β-gal expression found for eight independently constructed *trp*R$^+$ strains. pP4 (plasmid P4) *vir*1 *Eco*RI fragment AD:Km^r derivatives of X8605 *ilv*$^-$ were established by either phage infection (16) or plasmid transformation (11, 18), followed by kanamycin selection (50 μg/ml). The plasmids pUC4K (24) and pBR325 (28) were each established in X8605 *ilv*$^-$ by transformation followed by selection for kanamycin (50 μg/ml) and chloramphenicol (30 μg/ml), respectively. Likewise, pJG76, a pBR325 vector carrying the *psu* gene under expression control of the pBR325 Tc^r promoter (see Fig. 1) (18), was established in X8605 *ilv*$^-$ following transformation and selection for chloramphenicol (Cm) (30 μg/ml). A *psu*$^-$ mutant derivative, pJG76 *psu am*1, containing two amber mutations in the *psu* gene (15), was established in X8605 *ilv*$^-$ in the same manner.

*In the *trp*R constitutive strain X8605 *ilv*$^-$ pJG76, we could also observe a 2.8-fold increase in attenuation upon addition of tryptophan at 100 μg/ml as shown by the decrease in β-gal activity (data not shown). All of the above such assays for β-gal were carried out in strains first grown in LBG because this medium induces the high copy number state (n = 40–50) of pP4 *vir*1 and mutant derivatives (10). This rich growth medium results in high levels of attenuation in the *trp* operon. However, when strain X8605 *ilv*$^-$ pJG76 is grown under conditions of "minimal attenuation", i.e., glucose minimal media plus isoleucine, valine, and the tryptophan analogue 3β-indoleacrylic acid (20 μg/ml; Roberto Kolter, personal communication), β-gal expression was found to be 410 units.

Resolution of gp Psu Activity. When the P4 *vir*1 *Eco*RI fragment AD is inserted into the *Eco*RI site of the plasmid vector pBR325, β-gal is still poorly expressed in X8605 *ilv*$^-$ at levels similar to that of the strain without P4 (data not shown). Such P4-pBR325 hybrids are known to produce lowered levels of the P4-coded α DNA primase (R.-Z.J. and R.G., unpublished results) that normally drives P4 DNA replication (19). Because gp α appears to act pleiotropically affecting both P4 DNA replication and expression (17, 22), it was necessary to determine if α is also directly or indirectly involved in antitermination activity. A hybrid plasmid, pJG76, was transformed into X8605 *ilv*$^-$ for this purpose. This pBR325 vector carries a P4 fragment defined by *Bam*HI and *Ava* I sites (Fig. 1) and within which exists only the *psu* gene plus several nonessential P4 phage functions. Neither gene α nor gene *sid* is present (18). The *psu* gene carried by pJG76 is under control of the Tc promoter of pBR325 thereby resulting in constitutively higher than normal levels of expression of *psu*. As indicated in Table 2, the *psu* activity expressed by pJG76, in the absence of gps α and *sid*, induces significant expression of β-gal. Moreover, being nearly 2-fold greater than that found for pP4 *vir*1 *Eco*RI fragment AD:Km^r, the high level of β-gal expression as induced by pJG76 approximates that found for the host X8605 containing the severely defective *rho* allele 201 (12, 13). In contrast, the level of β-gal expression elicited by the *psu*$^-$ mutant derivative of pJG76 can be seen from Table 2 to approximate the background level found for X8605 *ilv*$^-$.

Psu Activity in Strain X8605 During Repression by Tryptophan. The results described above do not rule out the unlikely possibility that gp *psu* might be acting directly to promote expression of the *lac* operon rather than indirectly by suppressing ρ-mediated termination at the adjacent *trp* terminator. A *trp*R$^+$ derivative of strain X8605 *ilv*$^-$ pJG76 was, therefore, constructed to demonstrate that gp *psu* allows readthrough from the *trp* operon. The presence of the *trp*R$^+$ allele renders the *trp* operon in this previously constitutive strain repressible by the addition of tryptophan. In the *trp*R$^+$ strain, the presence of tryptophan should also result in lowered levels of β-gal activity if gp *psu* activity is at the level of the *trp* terminator, whereas no effect on β-gal expression should occur if gp *psu* acts directly on the *lac* operon. As can be seen in Table 2, expression of the *lac* operon in the *trp*R$^+$ strain is sensitive to the addition of tryptophan as evidenced by a reduction in β-gal activity by a factor of 9.2.

DISCUSSION

Our results make no distinction between models for a direct or indirect mechanism of ρ antagonism by gp *psu*. It may be that *psu* directly competes with ρ for an RNA polymerase binding site, inactivates ρ protein, competes for a DNA terminator binding site, or indirectly antagonizes ρ activity by controlling *rho* expression. Likewise, we do not yet know whether *psu* acts to antiterminate at ρ-independent sites for transcription termination.

The antitermination activity of gp *psu* is likely to be due to an antagonistic effect of gp *psu* on normal ρ activity. Consistent with this hypothesis is the observation that P4 *vir*1 cannot be stably established as a plasmid in a host containing the partially defective *rho* allele 102 (29). Infection of this strain with P4 *vir*1 results in 99% killing of recipient cells, and none of the survivors carry pP4 *vir*1 (R.L. and R.G., unpublished data). Since it is known that a completely defective *rho* phenotype is lethal to *E. coli* (30, 31), this killing appears to be due to the synergistic effects of *psu* antagonism and the defective *rho*102 allele. Further support for this explanation is derived from the finding that the P4 *vir*1 *δinv*11

mutant, which does not produce gps *psu* and *sid* (Table 1 and Fig. 1), is readily established as a stable plasmid in this ρ-defective strain (R.L. and R.G., unpublished data).

Perturbation of normal patterns of cell growth, as shown by filamentation and a substantial increase in doubling time, is a phenotype associated with *E. coli* containing a severely defective *rho* allele (30, 31). Since poor rates of cell growth and filamentation are an immediate consequence of P4 *vir*1 phage infection of *E. coli* (10, 11, 16), *psu*-mediated interference with ρ likely mediates this phenomenon during the initial "establishment" of P4 *vir*1 in the plasmid state. If this hypothesis is correct, curtailment of *psu* expression or activity might be expected during the subsequent "maintenance" of P4 *vir*1 as a high copy number plasmid because cells stably carrying pP4 *vir*1 exhibit normal doubling times and do not filament (10, 11, 16). As described below, significant differences in the levels of gp *psu* are indeed found in the establishment and maintenance stages. The central element to the model that we favor to account for these results is the existence of a ρ-sensitive regulatory switch for the negative control of *psu* gene expression. A notable implication of this model is the resultant mutual antagonism of the transcription termination factor ρ and the transcription antitermination factor psu, i.e., psu activity interferes with normal ρ-mediated transcription termination (this report) while ρ activity blocks psu expression by interfering with transcription through the polycistronic *sid-δ-psu* operon.

The *psu* gp is expressed at relatively high levels during most of the first 3 hr following infection by phage P4 *vir*1 (9). The first 50 min of this period is analogous to the time during which P4 would undergo helper-dependent lytic phage development if its P2 helper was present in such cells (4). It is during the initial 3-hr establishment period that filamentation and increased cell doubling time are found for a *rho*$^+$ host (10, 11, 16), while similiar but far more deleterious phenotypes result in 99% lethality for infected cells containing a partially defective *rho* allele (18, 23) (R.L. and R.G., unpublished data). In this latter type of host, both earlier and significantly higher levels of *psu* expression are found (9).

In the subsequent stage of stable high copy number maintenance of pP4 *vir*1 (10, 16), expression of gp *psu* is reduced to very low levels as measured by $NaDodSO_4$/polyacrylamide gel electrophoretic analyses of [^{35}S]methionine-labeled infected cell extracts (11, 23). Likewise, high level reinitiation of expression of the P4-coded α DNA primase, dependent on activity of gp *psu*, does not occur (9, 23).

Analysis of DNA sequences from the *sid-δ-psu* gene cluster (15, 18) (Fig. 1) indicates the presence of an unusual number of sets of hyphenated dyad sequences that would give rise to several mutually exclusive ρ-dependent and -independent RNA transcription termination signals (E. Ljungquist, A. Spadaro, and R.G., unpublished data). Location of these signals places them in position to act in concert with ρ so as to interrupt protein synthesis within this polycistronic operon such that the more proximal *sid* gene is successfully expressed while the more distal *psu* gene is not (Fig. 2). Indeed, the distal *psu* gene is expressed earlier and at higher levels in a partially defective *rho*$^-$ host, whereas expression of the proximal *sid* gene is not analogously affected (9). Further, expression of gp *sid* is only partially turned down during stable maintenance of pP4 *vir*1, whereas the production of gp *psu* is significantly reduced in the same *rho*$^+$ host (23).

One direct test for this model would be the removal of the *psu* structural gene from the putative ρ-sensitive regulatory circuitry found in the *sid-δ-psu* polycistronic operon. Strain X8605 *ilv*$^-$ pJG76 (this report) provides such an example since it carries the *psu* gene under control of the strong Tc promoter of pBR325, thereby eliminating possible negative control of *psu* expression by ρ. In addition to showing higher levels of *psu* activity than are found for strains carrying pP4 *vir*1 (Table 2), it exhibits significant filamentation and a 4-fold increase in doubling time (18). Both of these phenotypes are normally associated with strains carrying a severely defective *rho* allele. The *psu* clone carried in X8605 *ilv*$^-$ pJG76 can only be maintained under stringent selection for Cm^r (chloramphenicol resistance) expressed by the pBR325 vector (this report). The deleterious effect(s) resultant from *psu* antagonism of the essential ρ function is extreme enough to select for both ρ-resistant and/or *psu*-defective mutations following continued maintenance of pJG76 under Cm selection in a *rho*$^+$ host (18). Following transformation of pJG76 into cells carrying a partially defective *rho* allele, no survivors are found that carry the constitutively expressed *psu* clone. When considered together, these results support the proposed model postulating that ρ-mediated turn-down of *psu* expression is a prerequisite for the stable propagation of pP4 *vir*1 as a high copy number plasmid (9, 23) (Fig. 2).

The demonstration in this report that a P4-coded gp antagonizes transcription termination at a known ρ-dependent site provides to our knowledge the first direct evidence for this hypothesized activity. The observation that gp *psu* acts in *trans* to modulate expression of the bacterial chromosome expands our understanding of the levels of its activity. With respect to the generality of this type of regulation, this is a particularly important finding since it indicates that the phasmid P4 encoded gp *psu* acts to alter transcription termination patterns on three essentially heterologous genomes, i.e., in *cis* on P4 itself (9), in *trans* on temperate phage P2 (5), and in *trans* on the bacterial chromosome (this report).

The gp *N* transcription antiterminator of phage λ (32–34) appears to be limited in its specificity due to dependency on the presence of a *nut* site such as those found on the λ genome (2, 35, 36) or the chromosomal *nut*-equivalent site of the *rpsU-dnaG-rpoD* operon (37, 38). Unlike the activity of *psu* in *trans* on the P2 genome, gp *N* will not act on other phages

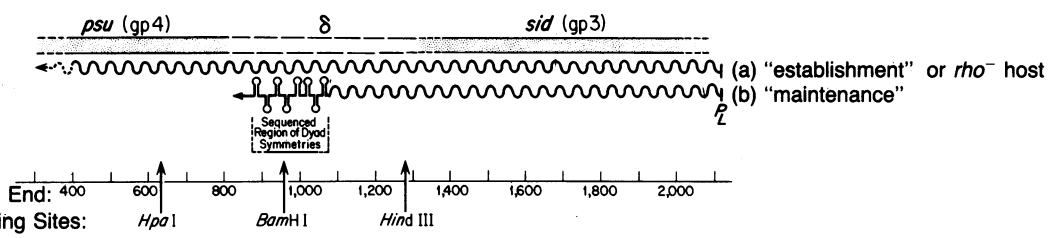

FIG. 2. Model for ρ-mediated "switch" in the central δ region of the polycistronic *sid-δ-psu* gene operon. Putative terminator region negatively controlled by *rho* factor, immediately preceding the *psu* gene, is indicated by hairpin structures. Eight dyads of symmetry exist within a stretch of some 200 base pairs centered around the indicated *Bam*HI site. Because some of these dyads overlap, it is possible only to form four stem-loop structures at a given time. Definition of this unusual sequence that is rich in ρ-dependent and ρ-independent regions is based on DNA sequencing analysis by E. Ljungquist, A. Spadaro, and R.G. (unpublished data). P_L, promoter leftward, for transcription of the polycistronic operon. "Establishment" refers to the initial expression patterns following P4 phage infection. "Maintenance" refers to transcription patterns during the subsequent stable propagation as a plasmid.

such as P22 and 21 (3) or P2 and P4 (18). Nor will it act at the ρ-dependent *trp* terminator (39, 40) as shown in this report for *psu*. These results plus the observation that the *psu* gp will not complement a λ N^- mutant (18) suggest that *psu* gp activity may likewise involve the specific recognition of a unique hyphenated dyad sequence.

Despite differences in operon specificities, the general similarities in the functions of gps *psu* and *N* are striking and may be suggestive of a common mechanism used by some types of extrachromosomal elements to modulate the expression of families of unlinked genes. Particularly intriguing here are the following observations that both gps: (*i*) positively affect the expression of late viral operons involved in capsid assembly and cellular lysis (i.e., helper phage P2 operons for the case of *psu* and λ operons for the case of *N*); (*ii*) positively regulate the expression of an essential DNA primase gene (i.e., reinitiation of gp α of P4 by *psu* and high levels of the host *dnaG* gene by *N*); and (*iii*) negatively affect the essential host regulatory factor ρ, thereby providing a rapid and appropriately timed mechanism for interference with a cellular regulatory function that would otherwise block successful lytic development.

It is also noteworthy that an *E. coli* host containing a defective *rho* allele will suppress the effects of *N* gene mutations in λ (41, 42) and *psu* mutations in P4 (5, 9) despite the apparent differences in the operons regulated by gps *N* and *psu*. Since *psu* activity does not appear to be determined by the essential *nut* site sequence required for *N*-mediated antagonism of ρ, it should be of interest to determine if a divergent but analogous site is required for *psu*-mediated antagonism of ρ, and if so, how this sequence differs from that of *nut*.

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