A switch in translation mediated by an antisense RNA

Koustubh Ranade and Anthony R. Poteete

Department of Molecular Genetics and Microbiology and Program in Molecular Medicine University of Massachusetts, Worcester, Massachusetts 01655 USA

Antisense RNAs regulate expression of target genes in a variety of ways—transcription termination, translation initiation, and mRNA stability. We describe a case in which the target gene encodes two polypeptides, and antisense RNA causes a switch in its translation by selectively inhibiting synthesis of one of the polypeptides. Bacteriophage P22 is a temperate Salmonella phage; in the prophage state it expresses only a handful of its genes. One of these genes, sieB, aborts the lytic development of some phages. P22 itself is insensitive to the lethal effect of SieB because it harbors a determinant called esc. We show that the sieB gene encodes two polypeptides—SieB, which is the exclusion protein, and Esc, which is a truncated version of SieB that inhibits its action. Superinfecting P22 synthesizes an antisense RNA, sas, that inhibits synthesis of SieB but allows continued synthesis of Esc, thus allowing P22 to bypass SieB-mediated exclusion. This translational switch induced by sas RNA is essential to vegetatively developing P22; a mutation that prevents this switch causes P22 to commit SieB-mediated suicide. Finally, we show that P22’s Esc allows it to circumvent the SieB-mediated exclusion system of bacteriophage λ.

[Key Words: Superinfection exclusion; antisense RNA; translational switch]

Received April 26, 1993; revised version accepted June 7, 1993.

In prokaryotes, antisense RNAs have been shown to regulate gene expression in a variety of ways. In some cases, antisense RNAs regulate expression of the target gene by base-pairing with its ribosome-binding site and preventing translation initiation; in other cases, they have been shown to cause premature transcription termination by hybridizing with the target RNA and forming a structure that mimics a transcription terminator. In another case, the antisense RNA has been shown to destabilize the target mRNA by creating a site for RNase III cleavage (for reviews, see Simons and Kleckner 1988; Takayama and Inouye 1990). On this theme of regulation by antisense RNA, we present a variation. We describe a case in which the target gene encodes two polypeptides, and the antisense effector RNA causes a switch in phenotype by selectively inhibiting synthesis of one of them.

Bacteriophage P22 is a temperate Salmonella phage that closely resembles coliphage λ in its genetic structure and the regulation of expression of its genes (for a review of P22 biology, see Poteete 1988). Like other temperate phages, P22 expresses only a handful of its genes in the prophage state; their function, at least in part, is to prevent the growth of superinfecting phages. One of these genes, sieB, aborts the lytic development of some superinfecting phages (Susskind et al. 1974). In a SieB+ host, vegetative development of sensitive phages proceeds normally for ~20 min, but then there is an abrupt cessation of all—phage and host—macromolecular synthesis; as a result, release of progeny phage is prevented and the host cell dies. Susskind and co-workers showed that P22 itself is not susceptible to SieB-mediated exclusion because it harbors a determinant esc [for escape from SieB-mediated exclusion]; they showed further that this P22 function can act in trans and is negatively regulated by the prophage repressor acting at the major leftward promoter of P22 [see Fig. 1]. Below, we present the likely molecular mechanism that P22 utilizes to escape SieB-mediated exclusion.

In a previous study we identified the sieB gene and its promoter (Ranade and Poteete 1993). The sieB gene, which encodes a polypeptide of 192 amino acid residues, is embedded in the pL operon, unlike other genes in this operon, which are read leftward and repressed in the prophage state, sieB is read rightward and expressed in a lysogen. We now show that the sieB gene encodes another polypeptide (referred to as Esc), which is an inhibitor of the exclusion protein SieB. The inhibitor, Esc, is a truncated version of SieB itself; it lacks the amino-terminal 25 amino acid residues and is synthesized from an internal initiation codon in sieB. We present genetic evidence to show that superinfecting P22 synthesizes an antisense RNA that causes ribosomes to switch from synthesizing both the exclusion and inhibitor proteins [SieB and Esc, respectively] to synthesizing only Esc, thereby allowing P22 to escape SieB-mediated exclusion. There is redundancy in the system because...
functional amounts of Esc can be made either by the prophage or the superinfecting phage. We show further that in the presence of SieB, this switch in translation is essential for the lytic development of P22; a mutation that likely prevents this switch is lethal to vegetatively growing P22. Finally, we present evidence indicating that P22’s Esc can disarm the *sieB* exclusion system of bacteriophage λ.

**Results**

**esc as antisense RNA**

Preliminary experiments designed to detect post-transcriptional regulation of the major leftward operon (*pL*) of bacteriophage P22 revealed that the *pL* transcript is processed in the vicinity of the *sieB* gene. This processing was investigated further using an RNase protection assay, and the results are presented in Figure 1.

Wild-type *Salmonella* was infected with virulent P22, and RNA was isolated 10, 20, and 30 min after infection. The riboprobes used in the experiment are shown in Figure 1A; two of the riboprobes (Hpa and Nde) have a common 5′ end that is 75 bp upstream of the transcription start site of *sieB/esc*. They have different 3′ ends; the Hpa probe extends to the *HpaI* site and has ~426 nucleotides of complementarity with the *pL* transcript. The Nde probe has ~175 nucleotides of complementarity and extends to the *NdeI* site in *sieB/esc*. The third probe (Xmn) has a different 5′ end; it extends from the *HindIII* site in *ral* for 426 nucleotides to the *XmnI* site in *sieB/esc*. As can be seen in Figure 1B, the Hpa probe is fully protected by RNA extracted 10 min postinfection (where observed, the difference in size between the fully protected and the untreated probes is the result of the presence of vector sequences in the probe), between 10 and 20 min after infection, however, there is a decrease in the full-length probe with a concomitant increase in a 105-nucleotide protected fragment. This pattern is mirrored in the experiment with the Nde probe, suggesting that the 5′ end of the 105-nucleotide fragment is to the left of the *NdeI* site. A 105-nucleotide protected riboprobe is obtained even when one uses a probe with a different 5′ end (Xmn probe). Taken together, these results indicate that the *pL* transcript (in this region) is processed into a 105-nucleotide RNA species, and this processed fragment lies within the boundaries of the Nde probe. In addition to the 105-nucleotide fragment, the Xmn probe yields three other protected species. The largest of these is the full-length probe. We suspect that the other two (280 and 300 in Fig. 1B) are partially processed forms of the *pL* transcript; their likely provenance is diagramed in Figure 1A. These two partially processed RNAs also allow one to identify the most likely ends of the 105-nu-

---

**Figure 1.** RNase protection experiment to detect *sas* RNA. ([A]) The riboprobes used in the experiment are indicated as solid lines. Arrowheads under the Xmn probe indicate the likely processing sites in the *pL* transcript. (300 and 280) The singly processed forms of *pL* RNA and (105) the antisense effector RNA, *sas*; the numbers refer to the number of nucleotides in each species, inferred from the gel shown in B. Broken lines indicate that the RNA species is longer than that detected by the Xmn probe. (B) Autoradiograph of a 6% polyacrylamide/8M urea gel used to separate riboprobes protected from nucleases by RNA extracted from phage-infected cells. Xmn, Hpa, and Nde refer to the three riboprobes shown in A. ([P]) Untreated riboprobe; ([U]) RNA extracted from uninfected cells. The numbers 10, 20, and 30 indicate that RNA was extracted 10, 20, and 30 min after phage infection. The 300-, 280-, and 105-nucleotide fragments discussed in the text are marked at right. Markers ([M]) are *HindIII* fragments of pBR322, filled in by the Klenow fragment of *E. coli* DNA polymerase I in the presence of [α^32P]dATP. The numbers at left indicate the length of each fragment (in nucleotides).
wild type + + +
Substitution 1 + + +
am4 + + +
am14 + + +
am22 + + +
am29 + + +
am115 + + +
P_{sas-10} + + +
Substitution 1 am115 - - +
Substitution 2 am115 - - +

Salmonella strain MS1868 was transformed with plasmid pKR682 bearing the wild-type allele of sieB, or with derivatives of pKR682 bearing the substitution 2 or am115 alleles of sieB. Plasmid-bearing cells were grown to late log phase in LB + ampicillin, and 0.1 ml of culture was used to make lawns on LB-ampicillin plates. Tenfold serial dilutions of P22vir3 bearing the indicated alleles of sieB/esc were spotted on such lawns, and the plates were incubated overnight at 30°C.

*A minus sign indicates that the plating plates 100- to 1000-fold less efficiently on a particular host as compared with its plating efficiency on Salmonella expressing the am115 allele of sieB/esc; a plus sign indicates that the plating efficiency is ~1.

cleotide fragment: The 5’ end of this RNA is at or very near the sieB initiation codon, and the 3’ end is ~70 nucleotides to the left of the sieB/esc transcription initiation site. This processed fragment of the pL transcript is referred to, from now on, as sas (sieB antisense) RNA.

Studies by Susskind and co-workers (1974) showed that esc—the determinant in P22 that allows it to bypass SieB-mediated exclusion—is part of the pL operon and is tightly linked to sieB. Our deletion mapping studies revealed that a critical esc determinant maps almost precisely to the region encompassed by the sas RNA (data not shown). Taken together, these observations suggest that esc is sas RNA. Given the small size of this RNA and the fact that it has the potential to base-pair with the ribosome-binding site of sieB, it seemed plausible that sas RNA might be involved in negatively regulating sieB expression. If sieB antisense RNA, synthesized by superinfecting P22, acting on the untranslated region of sieB mRNA, were responsible for allowing P22 to grow in a SieB+ cell, one might expect that mutations in the antisense effector that abolish base-pairing with the target sieB mRNA would render P22 susceptible to SieB-mediated exclusion. To test this possibility, a large deletion/substitution was introduced in the untranslated region of sieB. This mutation—substitution 1 (substitution 1 in Fig. 3B, below)—replaces almost the entire untranslated region of sieB mRNA (24 of 31 nucleotides). The substitution was crossed into virulent P22, and its Esc phenotype was tested. As indicated in Table 1, substitution 1-bearing P22 had no phenotype; P22 sub1 plates as well as does wild-type P22 on cells expressing wild-type sieB. This result, prima facie, would rule out base-pairing between sas RNA and the untranslated region of sieB mRNA as a necessary part of the mechanism by which P22 bypasses SieB-mediated exclusion. As is shown below, however, this result reflects redundancy in the devices that P22 elaborates to circumvent SieB-mediated exclusion—knocking out the antisense effector reveals the action of the inhibitor Esc.

eesc as protein

When expression of proteins was analyzed, using maxicells from the sieB region of P22, we discovered that two proteins are expressed from this region [see Fig. 2B, lane 3]. On the basis of the phenotype of an amber mutation [am4 in Fig. 2A], we inferred that the larger polypeptide is expressed from the sieB gene (Ranade and Poteete 1993). An examination of the sequence of the sieB gene revealed four in-frame initiation codons downstream of the sieB initiation codon (indicated as 2, 3, Esc ATG, and 5 in Fig. 2A). To test the possibility that the smaller polypeptide is the result of initiation at one of these ATGs, amber mutations were introduced between consecutive ATGs; an amber mutation was introduced

![Figure 2](https://example.com/figure2.png)

**Figure 2.** (A) The sieB/esc gene is shown with the amber mutations (am) discussed in the text. The deduced initiation codons of sieB and esc are indicated as SieB ATG and Esc ATG, respectively. (B) Autoradiograph of 35S-labeled proteins expressed in maxicells and separated by SDS-PAGE (10%) [tricine-urea system; Schagger and Jagow 1987]. Lane 1) No plasmid; (lane 2) pBR322 with its tetracycline resistance-conferring gene deleted. Lanes 3-7 are derivatives of pKR682 expressing different alleles of sieB/esc. (Lane 3) Wild type; (lane 4) am4; (lane 5) am14; (lane 6) am22; (lane 7) am115. Arrows indicate positions of β-lactamase, SieB, and Esc. The numbers at left indicate molecular mass markers (Pharmacia) (in kilodaltons).
toward the carboxyl terminus of sieB as well. Derivatives of pKR682 bearing these amber alleles were constructed, and expression of proteins from these plasmids was analyzed using maxicells. (Plasmid pKR682 is a derivative of pBR322 bearing the HindIII–MluI­tailed-sieB region of P22; see Fig. 1.) All five amber mutations confer a SieB− phenotype [data not shown] and, as shown in Figure 2C, prevent synthesis of the larger, SieB, polypeptide. Only am29 and am115 prevent synthesis of the smaller, Esc, polypeptide; am14 and am22, on the other hand, result in slight overproduction of Esc, perhaps as a result of translation reinitiation by ribosomes terminating at these amberes. Two conclusions can be drawn from these results: First, SieB and the smaller polypeptide (Esc) are synthesized from the same open reading frame; and second, Esc is initiated, most likely, from the fourth ATG in the sieB gene.

By analogy with other systems where a truncated protein inhibits the function of the full-length polypeptide [e.g., p1 and p2 proteins of transposon Tn5 (Isberg et al. 1982; Johnson et al. 1982) S107 and S105 proteins of bacteriophage λ (Young 1992)], we guessed that Esc may be an inhibitor of SieB-mediated exclusion. When a plasmid expressing the am22 allele of sieB, which abolishes synthesis of SieB but not Esc, was introduced into a SieB− lysogen, it converted the cell to a SieB− phenotype [data not shown]. We interpreted this result to mean that Esc expressed from the plasmid inhibited the action of endogenous full-length SieB. If Esc were responsible for neutralizing endogenous SieB in a P22-superinfected cell, one would predict that superinfecting P22 bearing amber mutations in sieB upstream of the esc initiation codon [am4, am14, and am22; see Fig. 2A] would be able to bypass SieB-mediated exclusion. P22 bearing amber mutations within sequences encoding both sieB and esc [am29 and am115], however, would be sensitive to SieB. To test this prediction, all five amber mutations were crossed into virulent P22, and the ability of P22 bearing these amber mutations to grow on a host expressing wild-type sieB was tested. As shown in Table 1, not one of the amber mutations has an effect on P22’s ability to grow on a SieB− cell; P22 strains bearing amber mutations that prevent synthesis of the inhibitor Esc—am29 and am115—plate with the same high efficiency as do P22 strains with amber mutations that do not diminish synthesis of Esc—am4, am14, and am22. These results would appear to rule out a role for Esc in P22’s ability to bypass SieB-mediated exclusion. However, as pointed out earlier, this reflects redundancy in the devices that P22 uses to circumvent SieB-mediated exclusion; knocking out the inhibitor, Esc, reveals the action of sieB antisense RNA.

Superinfecting P22 requires sieB antisense RNA or Esc protein to circumvent SieB-mediated exclusion

Because mutations that would [1] prevent sieB antisense RNA from interacting with its sense partner [substitution 1], or [2] prevent synthesis of the SieB inhibitor, Esc [sieB/esc am29 and am115], were found not to confer an Esc− phenotype, it seemed possible that superinfecting P22 requires either a function encoded by the pL operon (sieB antisense RNA) or Esc protein, but not both, to bypass SieB-mediated exclusion. To test this possibility, a P22 strain bearing substitution 1 in combination with the sieB/escam115 allele was constructed, and its ability to grow on a SieB+ host was tested. The results are presented in Table 1. As can be seen, P22 sub1 sieB/escam115 plates inefficiently on cells expressing wild-type sieB. Neither mutation alone has any effect on the plating behavior of P22. On the control SieB− host (expressing the sieB/escam115 allele), however, the sub1 sieB/escam115 double mutant and wild-type P22 plate with the same high efficiency. These results strongly indicate that a P22 strain unable to synthesize its own Esc relies on sieB antisense RNA to bypass SieB-mediated exclusion.

In the experiment described above, the antisense effect of the infecting phage was wrecked by mutation, thus revealing the role of Esc. We reasoned that the converse experiment should also yield the same result, that is, destroying, by mutation, the ability of the endogenous target sieB mRNA to interact with the wild-type antisense effector should reveal superinfecting P22’s dependence on Esc for bypassing SieB-mediated exclusion. We could not use substitution 1 to test this because it confers a SieB− phenotype [data not shown]. Consequently, another mutation was introduced in the untranslated region of sieB. This mutation—substitution 2 in Figure 3B—replaces 9 nucleotides (2–11) in the sieB untranslated region with unrelated sequence. A derivative of pKR682 was constructed carrying this mutation, and the SieB phenotype of cells bearing this derivative was tested. Salmonella expressing sub2 sieB is phenotypically SieB−; it excludes an Esc− phage but not wild-type P22 [Table 1; data not shown]. The plating behavior of P22 bearing various amber alleles of sieB/esc was

![Figure 3](#)

**Figure 3.** (A) The sieB/esc gene is shown, and the antisense pL transcript is indicated as a broken arrow below. (Sub1 and Sub2) Substitutions 1 and 2, respectively. (B) The untranslated sequence of P22 sieB is shown (+1). The transcription start site of sieB, the sieB initiation codon is underlined. Shown below the wild-type sequence are the changes present in the two substitutions.
tested on cells expressing sub2 sieB, and the results are presented in Table 1. As expected, P22 strains bearing amber mutations upstream of the esc initiation codon, am4, am14, and am22, plate as efficiently as does wild-type P22. P22 bearing mutations in esc, am29 and am115, however, plate inefficiently. The plating behavior of P22 bearing another mutation, P_sieB-10, was also tested. This mutation alters the ~10 hexamer of the sieB promoter, thus preventing synthesis of sieB mRNA [Ranade and Poteete 1993]. P22 P_sieB-10 plates efficiently on cells expressing wild-type sieB, suggesting that the P_sieB-10 mutation has no effect on the antisense effector. On cells expressing sub2 sieB, however, the mutant plates with low efficiency. This latter result indicates that Esc is synthesized from the same transcript as SieB.

One might expect that mutations that restore the ability of the antisense effector RNA to base-pair with the target sub2 sieB mRNA should restore the ability of P22 escam115 to plate efficiently on cells expressing sub2 sieB. To test this possibility, substitution 2 was combined with sieB/escam115 on a plasmid and then introduced into P22 by homologous recombination. As expected, this plasmid plates inefficiently on cells expressing wild-type sieB, but it also grows poorly on cells expressing sub2 sieB [see Table 1]. P22 sieB/escam115 bearing the larger substitution 1 also plates inefficiently on both cell types. On a SieB− cell, however, both plasmid plates with high efficiency. These results suggest that substitution 2 wrecks the ability of the sense/antisense pair to interact with each other even when it is borne by both effector and target RNAs. This hypothesis is discussed below.

### A P22 mutant that commits suicide

Attempts to cross substitution 2 alone (in the absence of am115 in sieB/esc) into P22 failed; few progeny phage were recovered from the cross by plating on wild-type Salmonella, and among these the few tested were non-recombinants [data not shown]. In the light of results presented above—the inability of P22 sub2 sieB/escam115 to grow on cells expressing sub2 sieB—it seemed possible that substitution 2 by itself is lethal to P22, by preventing the phage from shutting off synthesis of its own SieB. We reasoned that substitution 2-carrying P22 might be prevented from committing SieB-induced suicide if the host were constitutive for the SieB inhibitor Esc. This is indeed the case. Substitution 2-carrying P22 (see Materials and methods for a description of how this was obtained) was tested for its plating efficiency on cells that do or do not express Esc. P22 sub2 plates 100-fold more efficiently on Salmonella that expresses the SieB inhibitor, Esc, than on cells that are deficient in Esc. (The host was constitutive for Esc by virtue of harboring a derivative of pKR682 that carried the sieB/escam22 allele, and the control Esc− cells carried another derivative of pKR682 bearing the sieB/escam115 allele.) The lethality of substitution 2 is attributed solely to SieB made by the infecting phage because P22 bearing substitu-
A switch in translation mediated by an antisense RNA

Figure 4. (A) Plasmids used to analyze the effect of antisense RNA on expression of sieB and esc. The HindIII-MluI region present in the parent plasmid pKR682 is shown as a solid line below. Arrows indicate the extent of antisense RNAs that would be synthesized from the three plasmids. \( P_{\text{lacUV5}} \) The lacUV5 promoter, \( t_{\text{ant}} \) a transcription terminator. The SieB phenotype conferred by these plasmids is also shown. (+) Cells bearing that plasmid plate an Esc phage \( (L) \) 100- to 1000-fold less efficiently as compared with its plating efficiency on cells expressing an amber allele (am115) of sieB. (-) The plating efficiency is \(-1\). (B) Autoradiograph of 3SS-labeled proteins expressed in maxicells and separated by SDS-PAGE. (Lane 1) pBR322 with its tetracycline resistance-conferring gene deleted; (lane 2) pKR682; (lane 3) pKR687; (lane 4) pKR688; (lane 5) pKR689. The positions of \( \beta \)-lactamase, SieB, and Esc are indicated at right.

A switch in translation mediated by an antisense RNA

Figure 5. (A) Map of the \( \lambda \) genome in the vicinity of the sieB gene. Sequences present in the sieB-expressing plasmids constructed for these studies are shown below. (B) The sequence of the untranslated region of \( \lambda \) sieB is indicated, and the sequence of the untranslated region in the \( lac \) fusion plasmid is shown below.
Ranade and Poteete

Table 2. Esc phenotypes of P22 bearing different alleles of sieB/esc on cells expressing AsieB

| sieB/esc allele in host* | sieB allele in host |
|--------------------------|---------------------|
| pTP462 (PsieB-AsieB)    | pTP482 (PsieB-AsieB) |
| Wild type                 | +                     |
| am22                     | +                     |
| am115                    | -                     |
| P_sieB-10                 | -                     |

This experiment was performed essentially as described in Table 1, except that IPTG (at a final concentration of 1 mM) was added to lawns of cells bearing plasmid pTP482.

A minus sign indicates that the phage plates 100- to 1000-fold less efficiently on a particular host as compared with its plating efficiency on cells bearing the am115 allele of P22 sieB. A plus sign indicates that the plating efficiency is ~1.

A lysogen, the sieB/esc genes are expressed from a weak promoter just upstream of the sieB initiation codon, the strong leftward promoter pl is repressed by the action of c2 repressor. The cell is phenotypically SieB- at this ratio of SieB (the exclusion protein) to Esc (the inhibitor protein); the prophage does not exclude itself, presumably because the trigger that activates the lethal action of SieB is present only during the lytic phase. Upon superinfection by virulent P22, the situation changes dramatically. There is a burst of transcription from the two major promoters of the superinfecting phage, pl and pr, and low-level transcription from the sieB/esc promoter as well. The region of the pl transcript, which is antisense with respect to sieB/esc mRNA, is processed into a 105-nucleotide sieB antisense effector RNA, sas. Sas RNA then binds sieB/esc mRNA (synthesized from the prophage and superinfecting phage) and causes a switch in translation—from directing synthesis of both SieB and Esc, sieB/esc mRNA now directs synthesis of only Esc. In essence, superinfecting P22 circumvents SieB-mediated exclusion by altering the ratio of SieB to Esc. This mechanism has redundancy built into it because functional amounts of Esc can be made by the prophage or the superinfecting phage.

Several lines of evidence support this model. Amber mutations in sieB/esc have no effect on the plating efficiency of P22 on cells expressing wild-type sieB (Table 1), presumably because these phages are proficient at making sas RNA. Similarly a mutation that would hinder base-pairing between sas RNA and the target sieB mRNA (substitution 1 in Fig. 3) has no effect on P22's ability to grow in a SieB- host because the phage can still make Esc. When superinfecting P22 can make neither sas RNA nor Esc (sub1 sieB/escam115 and sub2 sieB/escam115; Table 1), it is no longer able to grow in a host-expressing wild-type sieB. Alternatively, if the target sieB mRNA is rendered unresponsive to the translational switch induced by sas RNA, superinfecting P22 depends on its own Esc to bypass SieB-mediated exclusion (P22 sieB/escam29 and am115; P_sieB-10; Table 1). The switch induced by sas RNA is essential to vegetatively developing P22; substitution 2, which renders sieB mRNA unresponsive to its antisense partner, causes P22 to SieB-exclude itself upon infection of a nonlysogen. When sieB is inactivated by an amber mutation (am115), however, substitution 2 is innocuous. Finally, artificial antisense RNAs inhibit the synthesis of SieB but not Esc [Fig. 4].

sas RNA

The status of the effector that causes the translational switch as an RNA molecule seems reasonably secure. (1) Mutations that render P22 susceptible to the lethal effect of SieB (in the absence of Esc) map to the untranslated region of sieB which is where many antisense RNAs act to regulate gene expression. (2) The pl transcript is processed into a small 105-nucleotide molecule, and this molecule encompasses the region identified, by mutational studies, as being important for P22's ability to bypass SieB. The small size of sas is in harmony with other reported antisense RNA effectors, which are 50–110 nucleotides in length (Simons and Kleckner 1988).

Figure 6. Molecular mechanism to explain P22's ability to bypass SieB-mediated exclusion. [A] In a lysogen, both SieB and Esc are synthesized from a transcript initiated at P_sieB; pl is repressed by the phage repressor, c2. [B] In an infected lysogen, P_sieB and pl of the superinfecting phage are transcribed. The pl transcript is processed into sas RNA, which then acts on the target sieB/esc mRNA to cause a translational switch, thus allowing synthesis of only Esc. Broken arrows indicate transcripts.
[3] There is no likely translation initiation site in sas RNA. Moreover, an amber mutation in the only substantive leftward open reading frame in this region of the pL operon has no phenotype (data not shown).

The genetic approach to showing definitively that base-pairing interactions between two RNA molecules are important for the system in question is to isolate mutations in one that are compensated by mutations, which restore base-pairing, in the other. We have not yet shown this to be true of sas and sieB RNAs. Even when substitution 2 is present in both sieB mRNA and sas RNA, the sense and antisense RNAs are unable to interact with each other, making P22 susceptible toSieB-mediated exclusion. This could be attributable to at least two reasons. First, substitution 2 is a relatively large mutation (9 nucleotides are changed), and this could affect the structure of the target and effector RNAs. Second, substitution 2 is very near the processing site in the pL RNA, and it may affect the rate of processing or abolish processing completely. We note that most compensating mutations [those that restore sense and antisense pairing] that have been described are single-base changes [Simons and Kleckner 1988].

Several interesting questions about sas RNA and its action remain unanswered. We imagine that sas RNA acts by occluding the ribosome-binding site of sieB mRNA, but it is also possible that it acts by destabilizing this part of sieB/esc RNA. Such an effect has been documented in the case of OOP antisense RNA and the hok/sok plasmid maintenance system [Krinke and Wulf 1990; Gerdes et al. 1992]. Preliminary evidence, however, suggests that the stability of sieB mRNA is unaffected by sas RNA (data not shown). It is not clear whether the processing of pL RNA is absolutely essential for it to be an effective antisense RNA. The unprocessed form of pL may be unable to interfere with the expression of sieB or esc, perhaps because it cannot fold into the appropriate structure. Another point that deserves to be mentioned is that the region of pL RNA 5' to sas RNA is rapidly degraded, one can see only two protected species with the Hpa probe: full-length probe and sas RNA (see Fig. 1B). This observation suggests how P22 can synthesize Esc in the presence of the pL transcript [because the pL transcript is also antisense to esc mRNA]—the region of the pl RNA that can base-pair with esc mRNA is rapidly degraded. The host enzymatic activity that is responsible for processing is unknown. The c4 antisense RNA of bacteriophage P1 is also processed from a larger precursor, in that system, the tRNA processing activity, RNaseP, has been implicated (Citron and Schuster 1992).

Relationship to other exclusion systems

We have presented genetic evidence indicating that P22's Esc can circumvent the SieB-mediated exclusion system of λ [Table 2]. Although the untranslated regions of the sieB genes of the two phages are closely related, sas RNA acting alone evidently is not sufficient to allow P22 to bypass λ SieB-mediated exclusion [Table 2]. In contrast, sas RNA and Esc appear to make individually sufficient contributions to P22's ability to circumvent its own SieB [Table 1]. We can envisage at least two reasons for this difference. (1) It may be that sas RNA can base-pair with λ sieB mRNA but cannot cause a translational switch, perhaps because λ sieB does not encode an Esc-like inhibitor. According to this hypothesis, to disarm SieB, preventing its synthesis is not enough; SieB molecules already present in the cell must be neutralized, and this is accomplished by Esc. (2) It is possible that blocking SieB synthesis is sufficient, but P22 sas RNA might not bind λ sieB mRNA because of the 17-base mismatch in the overlap regions of the two RNAs. If this is true, then it suggests why P22 has evolved Esc; it enables superinfecting P22 to bypass any exclusion system that functions through the same host macromolecule (or molecules) as does SieB. If the first possibility is correct, then it raises the question of how λ is able to bypass its own SieB-mediated exclusion system. The answer may be that λ is naturally insensitive to SieB-mediated exclusion [perhaps it lacks the ability to trigger SieB]. A mutant of λ with a large deletion in its sieB gene [and therefore its putative esc gene and sas RNA] plates efficiently on Escherichia coli expressing plasmid-borne λ sieB [K. Ranade and A. Potete, unpubl.].

The SieB/Esc system of exclusion that we have described resembles the RexA/RexB exclusion system elaborated by phage λ [for review, see Court and Oppenheim 1983]. This system is composed of two genes, rexA and rexB, and their gene products abort the growth of certain mutants of phage T4; Rex + K lysogens of Salmonella also exclude P22. Parma et al. [1992] showed that when rexB is overexpressed in a Rex + λ lysogen, it confers a Rex + phenotype, a result reminiscent of the effect of Esc on SieB-mediated exclusion. In that system, however, the biological relevance of this observation is not clear. They speculated that overexpression of rexB may be the mechanism by which an induced λ prophage does not “Rex-exclude” itself.

Materials and methods

Bacteria

E. coli W3110 lacIqL8 [Brent and Ptashne 1981] was used for the propagation of plasmids, except for plasmids pKR687, pKR688, and pKR689, which were propagated in strain HB101 [Sambrook et al. 1989]: Strain CSR603 recA1 uvrA6 [Sancar and Rupert 1978] was used for maxicell analysis. Salmonella typhimurium LT2 strains MS1868 [leuAaam414 r t m + r ] and DBS3 [cysA1348 hisC527 fels] were provided by M. Susskind (University of Southern California, Los Angeles).

Plasmids

Plasmid pTP546 was constructed by cutting pAS474 [Semerjian et al. 1989] at the unique HindIII site followed by digestion with BAL31. The ends were filled in by the Klenow fragment of E. coli DNA polymerase I in the presence of all four deoxynucleotides and ligated in the presence of HindIII linkers. Plasmids pKR687, pKR688, and pKR689 were constructed as follows. The EcoRI–HindIII fragment from pKR682 [Ranade and Potete 1993] was replaced by a λ{sas}-bearing EcoRI–HindIII fragment from pTP50 [Semerjian et al. 1989]; the resulting plasmid was called pKR683. The large ori-containing PstI–EcoRV frag-
ments from plasmids pAS474, pTP546, and pTP520 [Ranade and Poteete 1993] were ligated with the P<sub>salI</sub>-<sub>poly</sub>-bearing PstI–PvuII fragment from pTP30 [Berget et al. 1983]. The resulting plasmids were called pKR684, pKR685, and pKR686, respectively. These plasmids were cut with HindIII, filled in, and digested with PstI. The resulting <i>sieB</i>/<i>esc</i> antisense-expressing small fragments were ligated to the large ori-containing PstI–AFlI (filled-in) fragment from pKR683. To minimize intramolecular recombination, the new plasmids were propagated in strain HB101. The recombinant plasmids are called pKR687, pKR688, and pKR689, respectively.

Mutant alleles of <i>sieB</i>/<i>esc</i> were constructed using site-directed mutagenesis as described [Kunkel et al. 1987; Ranade and Poteete 1993]. Construction of the <i>am</i>4 and <i>P<sub>sieB</sub>+</i>-to alleles of <i>sieB</i>/<i>esc</i> has been described and [Ranade and Poteete 1993]. The mutagenic oligonucleotides (mismatched nucleotides underlined) used to construct the other mutations were as follows: am14, 5′-CATCTTTCTTGCTAGAAAAACG; am22, 5′-CTATAGCTTACATAGTGAAGTACGAGTTTATCC; am15, 5′-CAATACGATCTGATATG; am115, 5′-CATTGCCAACACTAGCTTTGC; substitution 1, 5′-CTCTTTTTACTCTGACATCCCCTGAAGTAAAATAATAAGATGTTACGAGTTTATCC; substitution 2, 5′-CTCTTTTTACTCTGACATCCCCTGAAGTAAAATAATAAGATGTTACGAGTTTATCC; substitution 3, 5′-CTCTTTTTACTCTGACATCCCCTGAAGTAAAATAATAAGATGTTACGAGTTTATCC. (am refers to the amber mutation that was introduced into the <i>sieB</i>/<i>esc</i> gene, and the number that follows refers to that codon of <i>sieB</i>/<i>esc</i> that was mutated to amber; substitutions 1 and 2 refer to the mutations that were introduced into the untranslated region of <i>sieB</i>/<i>esc</i>.) For crossing <i>sieB</i>/<i>esc</i> onto <i>P22</i> [see below], a HindIII–<i>EcOrV</i> fragment, from an M13 clone, carrying the mutation, was used to replace the wild-type HindIII–<i>EcOrV</i> fragment from pTP83 [Poteete 1982]. For maxicell analysis, derivitives of pKR682 were constructed that carried the amber alleles of <i>sieB</i>/<i>esc</i>. This was accomplished by replacing the wild-type HindIII–<i>EcOrV</i> fragment from pKR682 with the mutation-bearing HindIII–<i>EcOrV</i> fragment from the corresponding M13 clone. Substitutions 1 and 2 were combined with am115 as follows. Mutation-bearing HindIII–HpaI fragments were used to replace the corresponding wild-type fragment from a derivative of pKR682 carrying the mutation. Some of these plasmids were used to transform E. coli strain MS1686 that expressed the <i>sieB</i>/<i>esc</i> inhibitor Esc by virtue of having a substitution T2442G that was introduced into the <i>sieB</i>/<i>esc</i> gene (see below). This transformed strain was used as the control Esc- phage [Susskind et al. 1974].

\[ \text{Phage} \]

In some of the experiments, L was used as the control Esc- phage [Susskind et al. 1974]. Mutant alleles of <i>sieB</i>/<i>esc</i> were introduced into virulent P22 by crossing mutation-bearing plasmids with P22 vir3Δ474. This phage was constructed by crossing P22 Δ474 [Semerjian et al. 1989] with P22 vir3, selecting for virulence, and screening for the deletion. P22 vir3Δ474 has a large deletion in the <i>kil</i>–<i>ral</i> region, because the phage lacks gene 17, it is unable to grow on a fels lysogen [Susskind and Botstein 1978]. <i>Salmonella</i> strain MS1686 was transformed with mutant derivatives of plasmid pTP83, and plasmid by phage crosses [using P22 vir3Δ474] were performed as described [Semerjian et al. 1989]. The lysates from the crosses were plated on <i>Salmo-
A switch in translation mediated by an antisense RNA

lexA gene product. *Proc. Natl. Acad. Sci.* 78: 4204–4208.

Citron, M. and H. Schuster. 1992. The c4 repressor of bacteriophage P1 is a processed 77 base antisense RNA. *Nucleic Acids Res.* 20: 3085–3090.

Court, D. and A.B. Oppenheim. 1983. Phage lambda’s accessory genes. In *Lambda II* (ed. R. Hendrix, J. Roberts, F. Stahl, and R. Weisberg), pp 251–271. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Gerdes, K., A. Nielsen, P. Thorsted, and E.G. Wagner. 1992. Mechanism of killer gene activation. Antisense RNA-dependent RNase III cleavage ensures rapid turnover of the stable Hok, SmrB and PndA effector messenger RNAs. *I. Mol. Biol.* 226: 637–649.

Isberg, R.R., A.L. Lazaar, and M. Syvanen. 1982. Regulation of Tn5 by the right-repeat proteins: Control at the level of the transposition reaction? *Cell* 30: 883–892.

Johnson, R.C., J.C.P. Yin, and W. Reznikoff. 1982. Control of Tn5 transposition in Escherichia coli is mediated by protein from the right repeat. *Cell* 30: 873–882.

Krinke, L. and D.L. Wulff. 1990. RNase III-dependent hydrolysis of λcII-O gene mRNA by λ OOP antisense RNA. *Genes & Dev.* 4: 2223–2233.

Kunkel, T.A., J.D. Roberts, and R.A. Zakour. 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol.* 154: 367–382.

Parma, D.H., M. Snyder, S. Sobolevski, M. Nawroz, E. Brody, and L. Gold. 1992. The Rex system of bacteriophage λ: Tolerance and altruistic cell death. *Genes & Dev.* 6: 497–510.

Poteete A. R. 1982. Location and sequence of the *orf* gene of phage P22. *Virology* 119: 422–429.

——. 1988. Bacteriophage P22. In *The bacteriophages* 2 (ed. R. Calendar), vol. 2, pp 647–682. Plenum Press, New York.

Ranade, K. and A.R. Poteete. 1993. Superinfection exclusion (sieB) genes of bacteriophage P22 and λ. *J. Bacteriol.* 175: [in press].

Rennell, D., S.E. Bouvier, L.W. Hardy, and A.R. Poteete. 1991. Systematic mutation of bacteriophage T4 lysozyme. *J. Mol. Biol.* 222: 67–88.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: A laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Silhavy, T.J., M.L. Berman, and L.W. Enquist. 1984. *Experiments with gene fusions*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Simons, R.W. and N. Kleckner. 1988. Biological regulation by antisense RNA in prokaryotes. *Annu. Rev. Genet.* 22: 567–600.

Susskind, M.M. and D. Botstein. 1978. Molecular genetics of bacteriophage P22. *Microbiol. Rev.* 42: 385–413.

——. 1980. Superinfection exclusion by λ prophage in lysogens of *Salmonella typhimurium*. *Virology* 100: 212–216.

Susskind, M.M., A. Wright, and D. Botstein. 1974. Superinfection exclusion by P22 prophage in lysogens of *Salmonella typhimurium*. IV. Genetics and physiology of sieB exclusion. *Virology* 62: 367–384.

Takayama, K.M. and M. Inouye. 1991. Antisense RNA. *Crit. Rev. Biochem. Mol. Biol.* 25: 155–184.

Young, R. 1992. Bacteriophage lysis: Mechanism and regulation. *Microbiol. Rev.* 56: 430–481.
A switch in translation mediated by an antisense RNA.

K Ranade and A R Poteete

*Genes Dev.* 1993, 7:
Access the most recent version at doi:10.1101/gad.7.8.1498

References

This article cites 21 articles, 5 of which can be accessed free at: http://genesdev.cshlp.org/content/7/8/1498.full.html#ref-list-1

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.