

## Cloning, Expression, and Characterization of the *icd* Gene in the *immI* Operon of Bacteriophage P1

H.-D. RIEDEL,† J. HEINRICH, AND H. SCHUSTER\*

Max-Planck-Institut für Molekulare Genetik, Ihnestrasse 73, 1000 Berlin 33, Germany

Received 21 December 1992/Accepted 7 March 1993

The *immI* operon of P1 contains the genes *c4*, *icd* (formerly called *orfx*), and *ant* which are constitutively transcribed in that order from a single promoter, P51b. C4 is an antisense RNA which is processed from the precursor transcript. C4 RNA acts as a translational repressor of *icd*, thereby also inhibiting antirepressor (*ant*) synthesis. We have cloned the *icd* and the overlapping *icd* and *ant* genes. We show, by means of plasmid deletion analysis, that *icd* is translationally coupled to *ant*. An internal in-frame deletion of *icd* making up 65% of the codons still allows antirepressor synthesis at a reduced rate, indicating that a functionally active *icd* gene product is dispensable for *ant* expression. We identify the product of the *icd* gene as a 7.3-kDa protein which interferes with cell division. The results suggest that constitutive expression of *icd*, in the absence of a functionally active antirepressor, prevents P1 lysogen formation because of its detrimental effect on the host cell.

The temperate bacteriophage P1 is maintained as a unit-copy plasmid in the bacterial host. Maintenance of lysogeny requires the continuous expression of two genes, *c1* and *c4*, that code for repressors of lytic growth (for a review, see reference 33). The *c1* gene is located in the immunity region *immC*, and the C1 repressor acts on at least 14 operators (Ops) which are widely distributed over the P1 genome (2, 5, 6, 29). The *c4* gene is located in the *immI* region, and the C4 repressor prevents expression of the closely linked antirepressor gene *ant* (21). The antirepressor antagonizes the effect of the C1 repressor by an as yet unknown mechanism. A third regulatory gene, *bof*, encoding a corepressor of C1 is located in the *immT* region (18, 28, 30).

A detailed analysis of the P1 *immI* operon revealed the following organization (see Fig. 1 and 2). A C1 · Bof-controlled operator-promoter element, Op51-P51a; a non-regulated promoter, P51b; and the genes *c4*, *icd* (formerly called *orfx*), and *ant* are arranged in that order in clockwise orientation of the P1 map (1, 8, 12). The *ant* gene encodes two antirepressor proteins, Ant1 and Ant2, the latter initiating at an in-frame start codon (9, 12). The product of the *c4* repressor gene is a 77-base antisense RNA (4), acting on a target *ant* mRNA. Interaction depends on the complementarity of two pairs of short sequences, a'-a2 and b'-b2, respectively. The sequences a' and b' are contained within loops of a stem-loop secondary structure of C4; that of a2 and b2 are contained in the target RNA and encompass the *vir*<sup>s</sup> mutation and the ribosome-binding site of *icd*, respectively (3). Thus, C4 acts as a translational repressor of *icd*, thereby also inhibiting *ant* expression. In a P1 lysogen, *c4*, *icd*, and *ant* are cotranscribed in that order. Transcription starting from P51b is sufficient to express *c4*, *icd*, and *ant*, the last two only when *c4* is inactive. The transcript has to be processed to yield mature C4 RNA (4). The organizational feature of P1 *immI* is confirmed by the identification of three *ant*-deficient mutants: (i) *ant(reb)22*, an amber mutation in the *ant* structural gene (22); (ii) P<sub>ANT17</sub> (formerly called *ant17*), a promoter-down mutation of P51b (12, 24); and (iii)

*icd16* (formerly called *ant16*), a frameshift mutation in *icd* (12, 24). The latter result indicated that intactness of the open reading frame *icd* is a prerequisite for the expression of *ant*.

So far, the following questions remained open. Does *icd* encode a protein? If so, does *ant* expression depend on the intactness of the *icd* protein or only on that of the reading frame? What is the function of *icd*? Here we show that *icd*, indeed, encodes a protein. *icd* is translationally coupled to, but dispensable for, *ant* expression. Expression of *icd* alone is detrimental to the host cell because it interferes with cell division.

Before the organizational feature of the *immI* operon of P1 was revealed, antirepressor-deficient mutants were uniformly named *ant* or *reb* (for repressor bypass) irrespective of their (at that time unknown) location (22, 24). To avoid confusion, the term *ant* is now restricted to the *ant* structural gene. Promoter mutations which affect the synthesis of the antirepressor are called p<sub>ANT</sub>, keeping in mind that they affect expression of *c4* and *icd* at the same time (see above). The term *icd* (for interference with cell division) is used here to rename *orfx*. Therefore, the original *ant16* mutant (24), which was later identified as an *orfx* mutation (12), is now called *icd16*.

### MATERIALS AND METHODS

**Bacteria, bacteriophage, and plasmids.** Bacteria (with relevant markers) used were *Escherichia coli* JM83F' *sup*<sup>+</sup> Str<sup>r</sup> Tet<sup>r</sup> (12, 31) and WM874 *ara* Δ(*lac-pro*) *thi* (original name, CSH26 [13]). Bacteria were grown in TY (19) or M9 (13) medium. Phage mGP1-2 and plasmid vectors pT7-6 (26, 27) and pJF118EH (7) were used for the controlled expression of P1 *immI* proteins by the T7 RNA polymerase/φ10 promoter and *E. coli* RNA polymerase/*tac* promoter systems, respectively (see below).

**Construction of plasmids.** Plasmids with P1 DNA fragments inserted into pT7-6 and pJF118EH carry the prefixes pAH and pAT, respectively. Deletion derivatives of *icd* were constructed by replacing in plasmid pAH1018 the *EcoRI* fragment 14 (nucleotides 411 to 2293) by an *EcoRI* linker-supplemented *DdeI* fragment (nucleotides 552 to 1679) of the same plasmid (Fig. 1). The phosphorylated *EcoRI* linker

\* Corresponding author.

† Present address: Abt. für Gastroenterologie des Zentrums für Innere Medizin, Universitätskliniken, Heinrich-Heine-Universität Düsseldorf, D-4000 Düsseldorf 1, Germany.

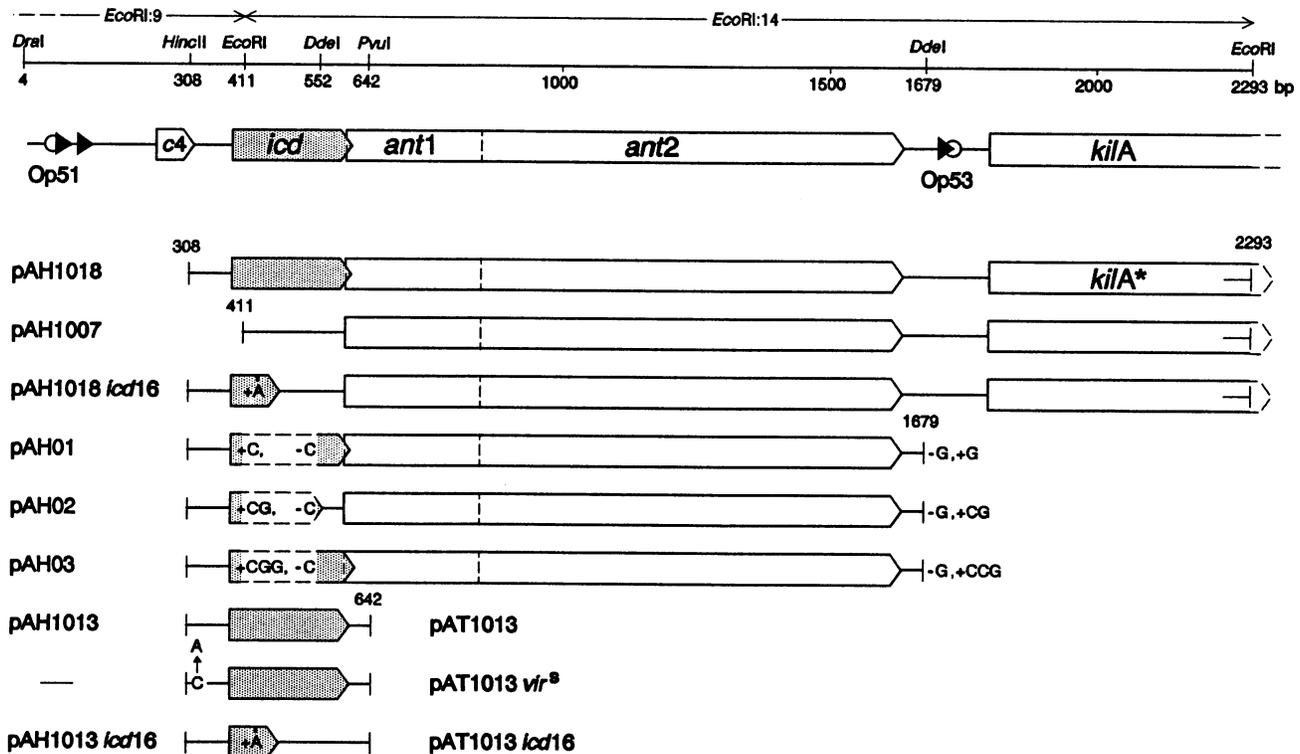


FIG. 1. *immI* operon of P1 and plasmids used. (Upper) The *immI* operon is located on the neighboring P1 *EcoRI* fragments 9 and 14. It contains the  $c1$ -controlled operator Op51 (open circle), the tandem promoter P51a, b (triangles), and the genes *c4*, *icd*, and *ant1/2* (arrowheaded bars with a vertical dashed line marking the start of *ant2*). The adjacent lytic replicon starts with the operator Op53, the promoter P53, and the *kilA* gene (8, 25), the latter extending into P1 *EcoRI* fragment 25 (32). The numbering of bases follows the scheme outlined earlier (12). Relevant restriction enzymes are indicated, with the nucleotide number following the enzyme-cutting site. (Lower) P1 DNA fragments inserted into pT7-6 (pAH plasmids) or pJF118EH (pAT plasmids) are indicated by horizontal lines and corresponding bars. Upon induction, all pT7-6 plasmids carrying P1 *EcoRI* fragment 14 (nucleotides 411 to 2293) yield a Kila\* fusion peptide which terminates in the vector (12). The reading frames of *icd* wild type and mutants are displayed by hatched, arrowheaded bars. *icd* wild type overlaps *ant1* by four nucleotides. It terminates prematurely by the insertion of A in P1 *icd16* (plasmids pAH1018*icd16* and pAH1013*icd16*; see also Fig. 2). *icd* deletion derivatives pAH01, pAH02, and pAH03 were constructed by replacing the *EcoRI* fragment 14 of pAH1018 by the *DdeI* fragment (nucleotides 552 to 1679) supplemented with various *EcoRI* linkers at both ends (see Materials and Methods). The deleted *EcoRI-DdeI* fragment (nucleotides 411 to 552) is indicated by interrupted horizontal lines. The resulting additions (+) and deletions (-) of nucleotides as well as the alterations of the reading frames (hatched area) are shown correspondingly.

d(pGGAATTC), d(pCGGAATTC), or d(pCCGGAATTC CGG) from New England BioLabs was ligated to the *DdeI* fragment, which had been converted to a blunt-ended molecule by T4 DNA polymerase and deoxynucleoside triphosphates. Following treatment with *EcoRI*, the supplemented *DdeI* fragments were inserted into *EcoRI*-treated pAH1018 to yield plasmids pAH01, pAH02, and pAH03 (Fig. 1). The construction of other plasmids (Fig. 1) was described earlier (10, 12).

**Enzymes and radiochemicals.** T4 DNA polymerase, T4 DNA ligase, and restriction enzymes were obtained from Boehringer, Mannheim, Germany. The enzymes were used in accordance with the recommendations of the manufacturers and Sambrook et al. (16).  $^{35}\text{S}$ -methionine (>1,000 Ci/mmol),  $^{35}\text{S}$ -cysteine (>600 Ci/mmol), and  $^{14}\text{C}$ -labeled methylated proteins (molecular weight, 2,350 to 30,000; 10 to 50  $\mu\text{Ci}/\text{mg}$  of protein) were obtained from the Radiochemical Centre, Amersham, England.

**T7 RNA polymerase/promoter system for controlled expression of P1 *immI* proteins.** The T7 RNA polymerase/promoter system consists of (i) plasmid pT7-6 containing the T7 RNA polymerase promoter  $\phi 10$  of phage T7, a polylinker region,

the  $\beta$ -lactamase gene, and the ColE1 origin; and (ii) phage mGP1-2 containing the T7 RNA polymerase gene *l* inserted into M13mp18 and under the control of the *lac* promoter (26, 27). F' bacteria carrying a P1 *immI*-pT7-6 recombinant plasmid are infected with mGP1-2, and expression of T7 RNA polymerase is induced by IPTG (isopropyl- $\beta$ -D-thiogalactopyranoside). Transcription by T7 RNA polymerase starting at  $\phi 10$  is limited exclusively to the cloned genes. We used the protocol described by Tabor and Richardson (26) for the production of gene products.

## RESULTS

**The *icd* gene codes for a 7.3-kDa protein.** A reading frame, named *icd* and made up of 73 codons, starts with an ATG (position 380) and terminates with a TGA stop codon (position 599; Fig. 2). To test the expression of *icd*, bacteria carrying plasmid pAH1018 or pAH1013 (Fig. 1) were infected with mGP1-2 phage. Following the induction by IPTG of T7 RNA polymerase, *icd* is transcribed from the T7 promoter  $\phi 10$ . Because most of the *c4* gene is deleted in these plasmids (Fig. 1), translation of *icd* can proceed



FIG. 2. DNA sequence of the P1 *c4 icd* regulatory region. The numbering of bases follows the scheme outlined in the legend to Fig. 1. The 17-bp operator Op51 is framed, and the locations of promoters P51a and P51b are indicated by the corresponding -35 and -10 regions. Transcription from P51b starts at position +1. The *c4* gene encoding the C4 antisense RNA is represented by the 77-base-long nucleotide sequence in brackets (4). Interaction of C4 and target RNA depends on the complementarity of the boxed b'/b2 and a'/a2 sequence elements (3). The consensus sequence for binding of *E. coli* ribosomes (23) overlaps the b2 element. The positions showing where the P1 sequence is identical to, or divergent from, the consensus sequence are indicated by uppercase and lowercase letters, respectively. The *icd* and the beginning of the *ant1* gene are framed. The TGA stop codon of *icd* overlaps the ATG start codon of *ant1* by two nucleotides. Stop codons created by the insertion mutation *icd16* and the *icd* deletion derivatives (Fig. 1) are underlined and marked 16, 02, 01 (identical to the stop codon of *icd* wild type), and 03. The point mutations  $p_{ANT17}$ , *c4.32*, and *vir<sup>s</sup>* and the deletion mutant *ant44* are explained in the text.

unimpeded. Four polypeptides of decreasing size (with molecular weights in parentheses) are synthesized from pAH1018: Ant1 (42,000), Ant2 (32,000), Kila\* (23,000), and a 7.3-kDa polypeptide (Fig. 3). The latter is also synthesized from plasmid pAH1013 which only contains the *icd* gene, but synthesis is not observed with pAH1013*icd16* in which translation of *icd* terminates prematurely (Fig. 1). It is also not found in bacteria carrying the vector pT7-6 alone (Fig. 3). These results prove that the 7.3-kDa polypeptide is the product of the *icd* gene.

**Expression of *icd* inhibits bacterial cell division.** Heat induction of a Plc4ts lysogen causes bacterial filamentation (20). Filaments were also observed when antirepressor was synthesized from a multicopy plasmid (12). Therefore, it was suggested that inhibition of cell division is caused by the action of antirepressor. Here, we show that expression of *icd*, in the absence of *ant*, is sufficient for filament formation. Upon addition of IPTG to bacteria carrying plasmid pAT1013, cell division ceases (Fig. 4, top) and filaments are formed. When an aliquot of the bacterial culture is resuspended in fresh medium without IPTG after 90 min of incubation with IPTG, most of the filaments divide and normal growth resumes (Fig. 4, middle). However, when IPTG incubation is continued for a longer time, filament formation becomes irreversible. Bacteria carrying plasmid pAT1013*vir<sup>s</sup>* (Fig. 1) also form filaments upon addition of IPTG. This is to be expected because the *vir<sup>s</sup>* and the wild-type character of pAT1013 are indistinguishable in the absence of an active C4 RNA. However, cell division occurs unimpeded when bacteria carrying plasmid pAT1013*icd16* (Fig. 1) are treated with IPTG (Fig. 4, bottom). In pAT1013*icd16*, translation of *icd* is terminated prematurely as shown above for plasmid pAH1013*icd16*.

**Icd protein is translationally coupled to, but dispensable for, antirepressor synthesis.** A translational coupling of *icd* and *ant1* is indicated by two findings. (i) A nucleotide sequence

in which six (of nine) positions are identical to the consensus sequence of the ribosome-binding site of *E. coli* (23) is only found in front of the *icd* gene (Fig. 2). (The next ribosome-binding site further downstream of *icd* is located eight nucleotides upstream of the in-frame ATG start codon of *ant2* [12]). (ii) The TGA stop codon of *icd* overlaps the ATG start codon of *ant1* (Fig. 2). Again, in using the T7 RNA polymerase/promoter system as described above, it is found that the presence of *icd* (wild type) is a prerequisite for *ant1* expression (12). As shown in Fig. 5, Ant1 is only expressed by plasmid pAH1018 (*icd* wild type) and not from plasmids pAH1018*icd16* and pAH1007 in which the ribosome-binding site and the beginning of *icd* are deleted (Fig. 1). However, Ant2 (and also Kila\*) is synthesized by the last two plasmids (Fig. 5), indicating that the ribosome-binding site in front of *ant2* is functionally active.

To test whether synthesis of an intact Icd protein is required or whether a translational coupling per se would be sufficient for *ant1* expression, a series of *icd* deletion derivatives was constructed in which about 47 codons (of a total of 73) were deleted. By incorporating different *EcoRI* linkers, in-frame (pAH01) and out-of-frame deletion derivatives (pAH02 and pAH03) were obtained (Fig. 1). As can be seen in Fig. 5, plasmid pAH01 expresses 8.5% of the amount of Ant1 synthesized by plasmid pAH1018 (*icd* wild type). However, Ant1 is not expressed when the translation is terminated prematurely (pAH02), as observed with plasmid pAH1018*icd16*. When the translation is terminated within the reading frame of *ant1* (pAH03), only 1% of Ant1 is synthesized. On the contrary, Ant2 is synthesized irrespective of whether a translational coupling between the truncated Icd and Ant1 exists (Fig. 5). These results show that a translational coupling per se is sufficient for *ant1* expression, although the coupling is not as effective as under *icd<sup>+</sup>* conditions. Instability of the truncated mRNA transcribed from plasmid pAH01 cannot be the reason for the strongly

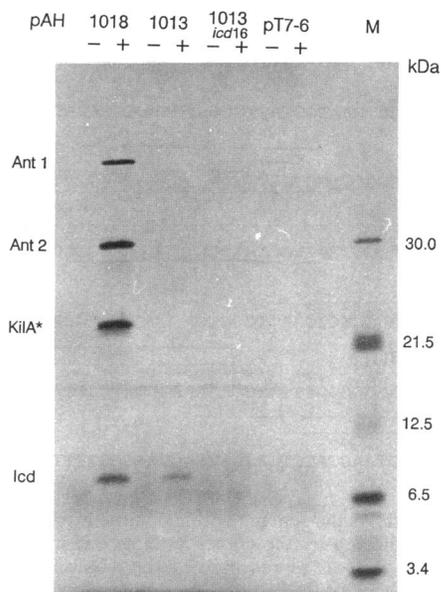


FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of P1 Icd protein. *E. coli* JM83F' bacteria carrying the plasmid indicated at the top were grown in M9 medium supplemented with thiamine (20  $\mu$ g/ml) and 18 amino acids (1 mg/ml each, minus cysteine and methionine). Bacteria (8 ml) were grown at 37°C to an optical density at 600 nm of 0.35. One-half of the culture was infected with mGP1-2 (multiplicity of infection = 50), and T7 RNA polymerase synthesis was induced by IPTG (2 mM final concentration). After 25 min, rifampin (200  $\mu$ g/ml) was added, and after an additional 25 min at 37°C,  $^{35}$ S-cysteine (10  $\mu$ Ci/ $\mu$ l) was added for 10 min. The second half of the culture was not infected (-) but was otherwise treated identically. Bacteria were then centrifuged, and the pellet was dissolved in 250  $\mu$ l of buffer (26), heated to 95°C for 3 min, and loaded onto a 20% polyacrylamide gel containing 0.1% NaDodSO<sub>4</sub>. M =  $^{14}$ C-labeled, methylated proteins (molecular weight in parentheses), in descending order: carbonic anhydrase (30,000), soybean trypsin inhibitor (21,500), cytochrome *c* (12,500), aprotinin (6,500), and insulin chain B (3,400). By scanning the X-ray film with a Personal Densitometer (Molecular Dynamics), the molar ratios of Ant1, Ant2, and Icd were found to be 1.0:3.6:1.3.

reduced amount of Ant1 protein, because 42% of Ant2 protein is still synthesized. Rather, the interaction of the truncated mRNA with the protein-synthesizing machinery is impaired, or, more likely, an improper synthesis and/or folding of the truncated Icd polypeptide inhibits the effective translation of Ant1.

## DISCUSSION

The analysis of the *immI* operon of P1 has revealed a complex regulatory system for expression of the P1 antirepressor gene *ant*. The *c4* and *icd* genes are essential elements of this system. It was suggested earlier that *c4* would encode a reading frame for a repressor protein composed of 66 amino acids (1). However, recently it was shown that *c4* encodes an antisense RNA which functions as a repressor (3, 4). To substantiate the suggestion that the reading frame *icd* encodes a protein different from the antirepressor (12) (and to exclude the possibility that it might hide another nonproteinous regulatory element), we have cloned and expressed the *icd* gene in a protein expression system. By that means, evidence is presented that *icd* encodes a protein of 7.3 kDa. This value is in reasonable agreement with the molecular

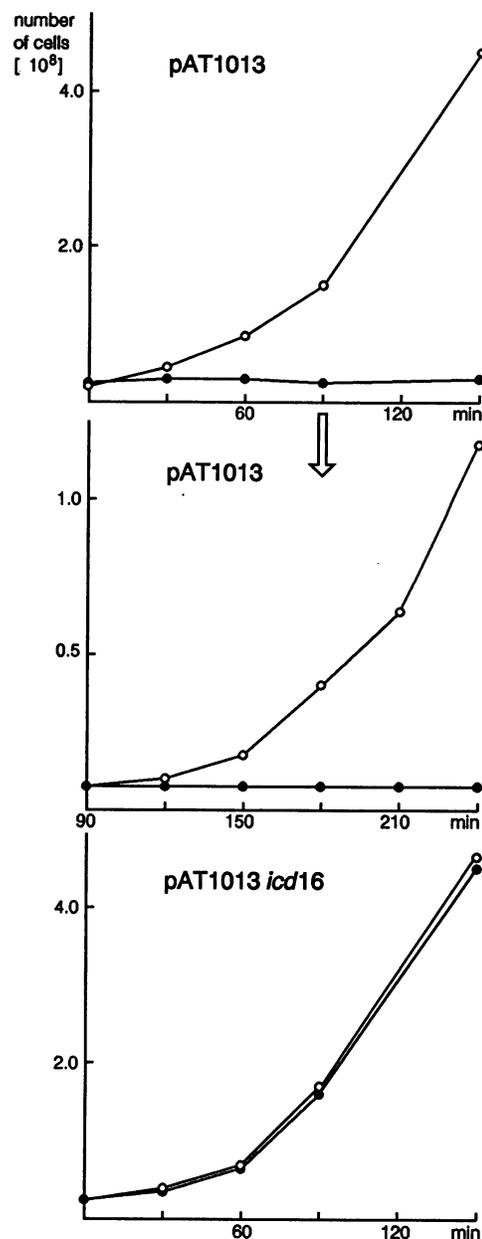


FIG. 4. Inhibition of cell division by P1 Icd protein. *E. coli* WM874 bacteria carrying the plasmid indicated at the top were grown in TY medium at 37°C to  $2 \times 10^7$  cells per ml. The culture was then divided into halves, and growth was continued in the presence (●) or absence (○) of 1 mM IPTG. After 90 min, an aliquot of the IPTG-treated WM874/pAT1013 bacteria was centrifuged and resuspended in a fivefold volume (vertical arrow). Growth was then continued at 37°C  $\pm$  IPTG as before.

weight of 8,398 predicted from the amino acid composition of Icd.

Syntheses of Icd and Ant1 proteins are translationally coupled. Generally, translational coupling of two genes requires that termination of translation of the first gene overlaps (or is very close to) the translation initiation site of the second gene (for a review, see reference 14). Such overlaps have frequently been found for bacterial operons and for bacteriophages. For example, of 30 overlapping

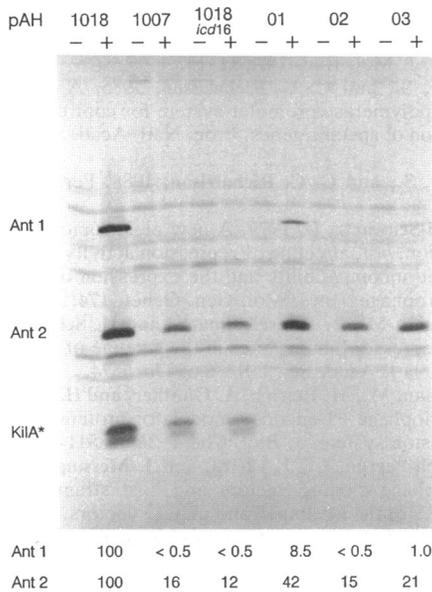


FIG. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of P1 antirepressor proteins. *E. coli* JM83F' bacteria carrying the plasmid indicated at the top were treated as described in the legend to Fig. 3 except that  $^{35}\text{S}$ -methionine ( $10 \mu\text{Ci}/\mu\text{l}$ ) was used instead of  $^{35}\text{S}$ -cysteine. Samples were subjected to sodium dodecyl sulfate-17.5% polyacrylamide gel electrophoresis. The relative amounts of the proteins were determined by scanning the X-ray film. Ant1 and Ant2 from pAH1018 were synthesized in a molar ratio of 1.3:1.

termination initiation sites of  $\lambda$  proteins, 15 are of the ATGA type (17) identified for the *icd ant1* overlap. Translational coupling may be a way to ensure proper molar ratios of two proteins which function as a complex. Icd and Ant1 were synthesized in a molar ratio of 1.3:1 in the T7 expression system (Fig. 3). However, in contrast to the Ant1 and Ant2 proteins which copurify during the preparation of P1 antirepressor, Icd protein was not found associated with Ant1 (15). Therefore, it appears unlikely that the function of Icd or Ant requires complex formation of the two proteins.

Translation of *ant1* does not require the preceding translation of an intact *icd*. Only the proper reading frame has to be retained to ensure termination at the normal stop codon. This raises the question of whether *icd* is an essential gene in the P1 life cycle. So far, filament formation is the only effect we can attribute to the *icd* gene product. We do not know yet whether Icd protein interferes directly with the cellular division pathway or whether filament formation is due to a pleiotropic effect of *icd*. In any case, constitutive expression of *icd* should be incompatible with the establishment or maintenance of the lysogenic state because such cells would not survive. This assumption implies that lysogens of P1  $c4^- icd^+ ant^-$  and P1  $vir^s icd^+ ant^-$  cannot exist. Indeed, all attempts to isolate the latter mutant type have failed. (i) When bacteria carrying plasmid pAH1018  $vir^s icd16 ant^+$  or pAH1018  $vir^s icd^+ ant44$  (a mutation in which two nucleotides at position 640 to 641 in the *ant1* gene are deleted [Fig. 2]) were superinfected with P1  $vir^s$ , only the *icd16* and not the *ant44* marker could be rescued in the form of a P1  $vir^s$  suppressor lysogen (11). (ii) When *ant*-defective P1 mutants were isolated as lysogen-proficient suppressor mutants of P1  $vir^s$ , 14 isolates carried frameshift mutations of the type P1  $vir^s icd^- ant^+$ , but no P1  $vir^s icd^+ ant^-$  mutant was found

(11, 12, 24). One suppressor mutant, P1 $_{ANT2}vir^s$ , contained a P51b promoter-down mutation identical to p $_{ANT17}$  (Fig. 2) (11). P1 $_{ANT17}$  was isolated as a suppressor mutation of P1c4.32 (12, 24). Both mutants, P1 $_{ANT2}vir^s$  and P1 $_{ANT17}c4.32$ , can exist as lysogens because the promoter mutation down-regulates transcription of *c4*, *icd*, and *ant* simultaneously (12).

All *ant*-defective, lysogen-proficient suppressor mutants of P1 $c4^-$  isolated so far derive from P1c4.32 (22, 24). Only one, P1c4.32 *ant(reb)22*, was analyzed and identified as a P1  $c4^- icd^+ ant^-$  mutant type (12). We suspect that in this mutant lysogen the expression of *icd* is not totally derepressed, for the following reason. The *c4.32* mutation which is located at the very end of stem I of the C4 RNA (Fig. 2) (4) affects the stability of the RNA molecule rather than the interaction of C4 with the target RNA: *c4.32* plasmid-carrying bacteria still contain 10 to 30% of the antisense RNA found in bacteria with the corresponding  $c4^+$  plasmid (4). This residual amount of C4.32 RNA may be sufficient to down-regulate the expression of *icd^+* to a level tolerable to the P1c4.32 *ant(reb)22* lysogen. Therefore, we expect that only *c4* mutations which severely affect either the antisense RNA stability or the C4-target RNA interaction (as the *vir^s* mutation does in reverse) cannot exist as P1 $c4^- icd^+ ant^-$  lysogens.

#### ACKNOWLEDGMENTS

We are grateful to Ute Peters for expert technical assistance. We thank D. Vogt for the preparation of plasmid DNA.

This work was supported by Fonds der Chemischen Industrie.

#### REFERENCES

- Baumstark, B. R., and J. R. Scott. 1987. The *c4* gene of phage P1. *Virology* **156**:197-203.
- Baumstark, B. R., S. R. Stovall, and S. Ashkar. 1987. Interaction of the P1 c1 repressor with P1 DNA: localization of repressor binding sites near the c1 gene. *Virology* **156**:404-413.
- Citron, M., and H. Schuster. 1990. The *c4* repressors of bacteriophages P1 and P7 are antisense RNAs. *Cell* **62**:591-598.
- 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.
- Citron, M., M. Velleman, and H. Schuster. 1989. Three additional operators, Op21, Op68, and Op88, of bacteriophage P1. *J. Biol. Chem.* **264**:3611-3617.
- Eliason, J. L., and N. Sternberg. 1987. Characterization of the binding sites of c1 repressor of bacteriophage P1. *J. Mol. Biol.* **198**:281-293.
- Fürste, J. P., W. Pansegrau, R. Frank, H. Blöcker, P. Scholz, M. Bagdasarian, and E. Lanka. 1986. Molecular cloning of the plasmid RP4 primase region in a multi-host-range *tacP* expression vector. *Gene* **48**:119-131.
- Hansen, E. B. 1989. Structure and regulation of the lytic replicon of phage P1. *J. Mol. Biol.* **207**:135-149.
- Heilmann, H., J. N. Reeve, and A. Pühler. 1980. Identification of the repressor and repressor bypass (antirepressor) polypeptides of bacteriophage P1 synthesized in infected minicells. *Mol. Gen. Genet.* **213**:149-154.
- Heinrich, J. 1991. Ph.D. thesis. Freie Universität Berlin, Berlin, Germany.
- Heinrich, J., A. Günther, and H. Schuster. Unpublished data.
- Heisig, A., H. D. Riedel, B. Dobrinski, R. Lurz, and H. Schuster. 1989. Organization of the immunity region *immI* of bacteriophage P1 and synthesis of the P1 antirepressor. *J. Mol. Biol.* **209**:525-538.
- Miller, J. H. 1972. Experiments in molecular genetics, p. 431-435. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Normark, S., S. Bergström, T. Edlund, T. Grundström, B.

- Jaurin, F. P. Lindberg, and O. Olson. 1983. Overlapping genes. *Annu. Rev. Genet.* **17**:499–525.
15. Riedel, H. D., J. Heinrich, A. Heisig, T. Choli, and H. Schuster. Unpublished data.
  16. Sambrook, J., E. F. Fritsch, and T. Maniatis (ed.). 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
  17. Sanger, F., A. R. Coulson, G. F. Hong, D. F. Hill, and G. B. Peterson. 1982. Nucleotide sequence of bacteriophage  $\lambda$  DNA. *J. Mol. Biol.* **162**:729–773.
  18. Schaefer, T. S., and J. B. Hays. 1990. The *bof* gene of bacteriophage P1: DNA sequence and evidence for roles in regulation of phage *c1* and *ref* genes. *J. Bacteriol.* **172**:3269–3277.
  19. Schuster, H., M. Mikolajczyk, J. Rohrschneider, and B. Geschke. 1975.  $\phi$ X174 DNA-dependent DNA synthesis *in vitro*: requirement for P1 ban protein in *dnaB* mutant extracts of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **72**:3907–3911.
  20. Scott, J. R. 1970. Clear plaque mutants of phage P1. *Virology* **41**:66–71.
  21. Scott, J. R. 1980. Immunity and repression in bacteriophages P1 and P7. *Curr. Top. Microbiol. Immunol.* **90**:49–65.
  22. Scott, J. R., B. W. West, and J. L. Laping. 1978. Superinfection immunity and prophage repression in phage P1. *Virology* **85**:587–600.
  23. Shine, J., and L. Dalgarno. 1975. Determinant of cistron specificity in bacterial ribosomes. *Nature (London)* **254**:34–38.
  24. Sternberg, N. 1979. A characterization of bacteriophage P1 DNA fragments cloned in a  $\lambda$  vector. *Virology* **96**:129–142.
  25. Sternberg, N., and G. Cohen. 1989. Genetic analysis of the lytic replicon of bacteriophage P1. II. Organization of replicon elements. *J. Mol. Biol.* **207**:111–133.
  26. Tabor, S., and C. C. Richardson. 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. *Proc. Natl. Acad. Sci. USA* **82**:1074–1078.
  27. Tabor, S., and C. C. Richardson. 1988. Personal communication.
  28. Touati-Schwartz, D. 1979. A new pleiotropic bacteriophage P1 mutation, *bof*, affecting *c1* repression activity, the expression of plasmid incompatibility and the expression of certain constitutive prophage genes. *Mol. Gen. Genet.* **174**:189–202.
  29. Velleman, M., B. Dreiseikelmann, and H. Schuster. 1987. Multiple repressor binding sites in the genome of bacteriophage P1. *Proc. Natl. Acad. Sci. USA* **84**:5570–5574.
  30. Velleman, M., M. Heirich, A. Günther, and H. Schuster. 1990. A bacteriophage P1-encoded modulator protein affects the P1 *c1* repression system. *J. Biol. Chem.* **265**:18511–18517.
  31. Yanisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**:103–119.
  32. Yarmolinsky, M. 1990. Bacteriophage P1, p. 52–62. *In* S. J. O'Brien (ed.), *Genetic maps*, 5th ed., book 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
  33. Yarmolinsky, M., and N. Sternberg. 1988. Bacteriophage P1, p. 291–438. *In* R. Calendar (ed.), *The bacteriophages*, vol. 1. Plenum Publishing Corp., New York.