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Gene Regulation in Plasmid RK2: Positive Control by *korA* in the Expression of *korC*

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The broad-host-range plasmid RK2 encodes three host-lethal *kil* genes whose actions are controlled by specific *kor* genes. We have shown previously that the 0′ to 5.5′ region of RK2 encodes both *kilA* and *korC*. Because of the lethal effect of *kilA*, plasmids with this region cannot be maintained in *Escherichia coli* unless the RK2 *korA* gene is also present. To investigate *korC* in the absence of *kilA* and therefore of *korA*, we first mapped *kilA* and *korC* to specific segments of the cloned 0′ to 5.5′ region. This allowed us to construct a *korC*$^+$ plasmid missing the *kilA* region and thereby removed the need to have *korA* in the cell. We found that this *korC*-encoding plasmid alone is insufficient to control *kilC*. The *korA* function is required, and it can be supplied in *trans*. We also constructed a *kilA*$^+$ *korC*$^-$ plasmid and found that *korA* is sufficient to control *kilA*. Thus, in addition to acting negatively to control *kilA*, *korA* acts positively to allow *korC* control of *kilC*. This *korA* dependence of *korC* is bypassed in a *rho-115* mutant of *E. coli*. We consider the possibility that *korA* product acts as an antiterminator of transcription in *korC* expression.

Plasmids of incompatibility group P (IncP) have an intriguing potential for stable maintenance in virtually any gram-negative bacterial species (8, 24). This is not a common property of plasmids (8), and it implies the existence of novel genetic determinants on the IncP plasmids.

Genetic studies designed to identify the determinants essential for replication and maintenance of IncP plasmids have focused on a group of closely related, if not identical (5, 18, 33, 38), plasmids designated RK2, RP1, RP4, R68, and R18 (for review, see reference 34) and R751 (23). For RK2 it is clear that at least two determinants are required for replication: *oriV*, the origin of replication (21), and *trfA*, a gene whose diffusible product is required for *oriV*-dependent replication (11, 37). Schmidhauser et al. (29) have demonstrated that a plasmid encoding only the RK2 *oriV* and *trfA* and a selective marker will replicate in *Escherichia*, *Pseudomonas*, *Rhizobium*, *Azotobacter*, and *Acinetobacter* species. Thus the basic unit of replication is capable of functioning in a variety of hosts.

In addition, there is evidence that other plasmid determinants may be important to the host range of the parental plasmid. Several workers have isolated plasmid mutants with altered host ranges (3, 7, 39). Some of the mutations map in determinants clearly distinct from *trfA* and *oriV*. Also, Thomas et al. (36) reported that a third region of RK2 is required in *cis* for certain derivatives to be maintained in *Pseudomonas aeruginosa*. The functions of these additional determinants are not known.

Plasmids RK2 (12) and RP4 (9) have also been shown to encode several genes which affect the ability of an *Escherichia coli* cell to host the plasmid. Uncontrolled expression of any of three *kil* genes (*kilA*, *kilB*, or *kilC*) can lead to death of the *E. coli* host unless the required control gene (*korA*, *korB*, or *korC*) is also present in the cell (12). All of a variety of IncP plasmids examined were found to specify *kor*-like functions able to control RK2 *kil* genes (*kilA* and *kilB*). This implies that the IncP plasmids also have the corresponding *kil* genes and that these *kil* and *kor* determinants are closely related to those of RK2. Furthermore, determinants able to control RK2 *kil* genes have not been detected on any plasmids tested from 19 other incompatibility groups. Thus, these genes are unique to IncP plasmids. We have speculated that perhaps the *kil* and *kor* genes, discovered by their inadvertent disruption of *E. coli* growth, are actually involved in the replication or maintenance of these plasmids in other hosts. Meyer and Hinds (22) have suggested that *kilA* may influence RK2 maintenance even in *E. coli*. At present, these ideas remain unsubstantiated.

In this paper, we report a second regulatory function of the RK2 *korA* gene and the first evidence that the expression of the various *kil* and *kor* genes may be interconnected. We have found that *korA* is required for the ability of *korC* to inhibit *kilC*. This positive role of *korA* in *korC* function contrasts with the apparent negative role of *korA* in the control of *kilA* action (12). In addition, the dependence of *korC* on *korA* is abolished in an *E. coli* mutant defective in *rho*, a factor involved in the termination of transcription (27). This indicates strongly that the effect of *korA* is on *korC* expression, and we suggest that the *korA* product may do this by interfering with the termination of transcription. We discuss the implications of such a mechanism on the regulation of other RK2 genes.

MATERIALS AND METHODS

Nomenclature. If a relevant plasmid gene is not present in a bacterial strain, we indicate this with a superscript 0 (e.g., *korA*^{0}). Coordinates of the RK2 physical map are defined by the clockwise distance from the *Eco*RI site in kilobases and are designated by a prime (′) (e.g., 0′ to 5.5′ region).

Bacterial strains and plasmids. The *E. coli* strains used in this study are listed in Table 1. Strains DW319 and M41 were provided by C. Squires, and the *rho* markers were tested as previously described (17). Briefly, melibiose (Mel) utilization depends on *lacY* permease at 42°C. Because the IS*1*-MS319 mutation in *lacZ* has a polar effect on *lacY*, the presence of the *rho* mutation can be monitored as a Mel$^+$ phenotype at 42°C. The plasmids used are listed in Table 2.

A variety of insertion and deletion mutants of pRK2086 were constructed in vitro by ligating a Cm^r-encoding *Hae*II

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TABLE 1. *E. coli* strains

Strain	Relevant phenotype	Relevant genotype	Description	Reference
MV10	Trp$^-$	Δ*trpE5*	C600 strain	Bachmann (2)
RP1770	Trp$^+$	*trpE*$^+$ *korA*$^+$ *korB*$^+$	*trpE*$^+$ *korA*$^+$ *korB*$^+$ region of pRK2108 integrated into MV10 chromosome	Pohlman and Figurski, unpublished data
RP1894	Trp$^+$ Ap^r	*trpE*$^+$ *korA*$^+$ *korB*$^+$ *korC*$^+$ *kilA*$^+$	*trpE*$^+$ *korA*$^+$ *korB*$^+$ *korC*$^+$ *kilA*$^+$ *Ap*^{r} region of pRK2102 integrated into RP1770 chromosome	Pohlman and Figurski, unpublished data
DW319	Mel$^-$ at 42°C	*rho*$^+$	*lacZ*::IS*1*-MS319	Fiandt et al. (10); Malamy (20)
M41	Mel$^+$ at 42°C	*rho-115*	*lacZ*::IS*1*-MS319 *rho-115*	Fiandt et al. (10); Malamy (20)

fragment into random *Hae*II sites. *Hae*II digestion of pRK2086 was carried out in the presence of ethidium bromide to obtain a population of partially (and presumably randomly) cleaved pRK2086 (see below). As a source of the *Hae*II Cm^r-encoding fragment, completely digested pKJ11 was added in large excess to the partially cleaved pRK2086. The mixture was then ligated and used to transform the *korA*$^+$ strain MV10(pRK2107), with selection for Km^r Cm^r colonies. The *korA* gene is needed to control *kilA* (12), and this strain allowed us to isolate mutant plasmids which were still *kilA*$^+$.

We found three classes of mutations in the mutant plasmids: (i) insertion mutations, with the Cm^r-encoding fragment at one of the *Hae*II sites; (ii) substitutions, with the Cm^r-encoding fragment at the position of a deletion which had lost one or more *Hae*II fragments; and (iii) simple deletions of one or more *Hae*II fragments, in which Cm^r was the result of a double transformation with pKJ11. Two such plasmids used in this work are pRK2260 (a class iii mutant) and pRK2261 (a class ii mutant). Their structures are shown in Fig. 1.

Media, enzymes, and standard procedures. Media for growth and selection of bacteria have been described previously (12). Restriction enzymes and T4 DNA ligase were purchased from commercial suppliers and used as suggested. The preparation of plasmid DNA (19), agarose gel electro-

TABLE 2. Plasmids

Strain	Relevant phenotype	Relevant genotype	Description	Reference
pCY2	Tp^r		pSM1 replicon	Figurski et al. (12)
pKJ11	Cm^r		P15A replicon with *Hae*II Cm^r-encoding fragment	Figurski, unpublished data
pLB2	Tp^r		Mini-plasmid with replication region of R388	Babiss and Figurski, unpublished data
pMK20	Km^r		ColE1 replicon	Kahn et al. (19)
pRK2086	Km^r	*kilA*$^+$ *korC*$^+$	ColE1 replicon with 0′ to 5.5′ region of RK2	Figurski et al. (12)
pRK2091	Cm^r Tp^r	*oriV*$^+$ *kilC*$^+$	P15A replicon with 8.5′ to 14.0′ region of RK2	Figurski et al. (12)
pRK2102	Trp$^+$ Ap^r	*korA*$^+$ *korB*$^+$ *korC*$^+$ *kilA*$^+$	ColE1 replicon with 0′ to 8.5′ and 50.4′ to 56.4′ regions of RK2	Figurski et al. (12)
pRK2107	Trp$^+$	*korA*$^+$ *korB*$^+$	pRK353 replicon with 50.4′ to 56.4′ region of RK2	Figurski et al. (12)
pRK2108	Trp$^+$	*korA*$^+$ *korB*$^+$	pSM1 replicon with 50.4′ to 56.4′ region of RK2	Figurski et al. (12)
pRK2161	Tp^r	*oriV*$^+$ *kilC*$^+$	pSM1 replicon with 8.5′ to 14.0′ region of RK2	Figurski et al. (12)
pRK2216	Ap^r KorA$^+$	*korA*$^+$	P15A replicon with 800-bp region (55.1′ to 55.9′) of RK2	Bechhofer and Figurski, in press
pRK2219	Ap^r KorA$^-$	*korA*$^-$	P15A replicon with 700-bp region (55.2′ to 55.9′) of RK2	Bechhofer and Figurski, in press
pRK2240	Tc^r KorA$^+$	*korA*$^+$	P15A replicon with 500-bp region (55.1′ to 55.6′) of RK2	Bechhofer and Figurski, in press
pRK2241	Tc^r KorA$^-$	*korA*	P15A replicon with 500-bp region (55.1′ to 55.6′) of RK2	Bechhofer and Figurski, in press
pRK2260	Km^r	*kilA*$^+$ *korC*$^-$	ColE1 replicon with 0′ to 2.3′ region of RK2 (Fig. 1)	This work
pRK2261	Km^r Cm^r	*kilA*$^+$ *korC*$^+$	ColE1 replicon with 0′ to 2.3′ and 3.3′ to 5.5′ region of RK2; contains *Hae*II Cm^r-encoding fragment inserted at site of deletion (Fig. 1)	This work
pRK2262	Km^r	*kilA*$^-$ *korC*$^+$	ColE1 replicon with 3.3′ to 5.5′ region of RK2 (Fig. 1)	This work

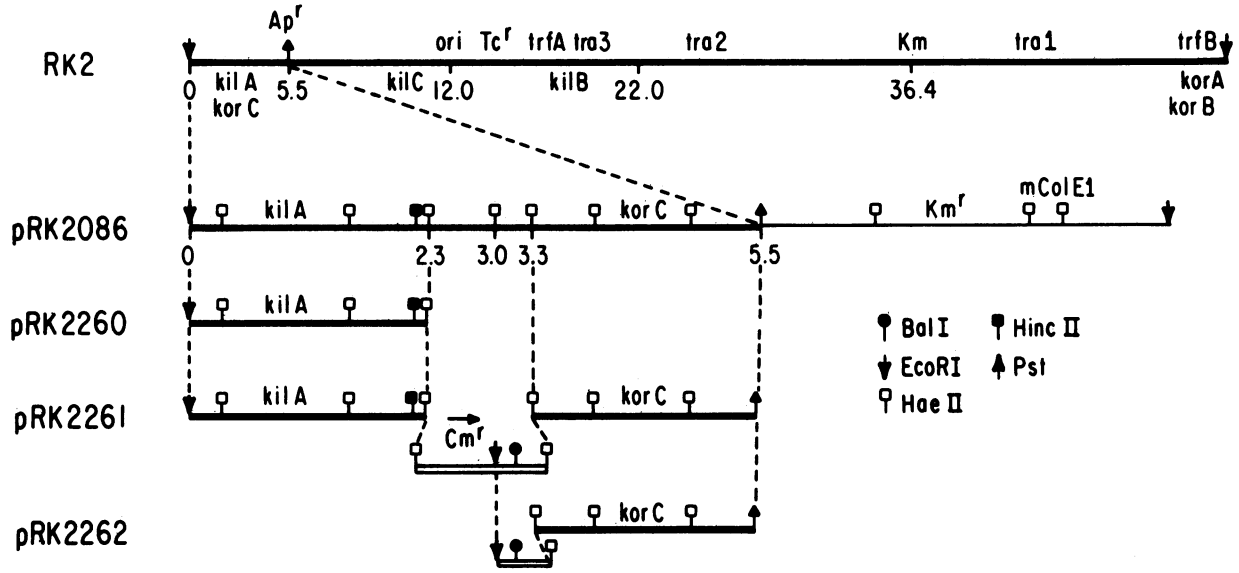

FIG. 1. Relationship of RK2 to pRK2086 and its derivatives. The genetic and physical map of RK2 is linearized at its *Eco*RI site (0'/56.4'). The numbers refer to RK2 coordinates in kilobase units from the *Eco*RI site. Only relevant restriction endonuclease cleavage sites are depicted. The segment of RK2 present in pRK2086 (12) is expanded and flanked by dashed lines. The *Hae*II sites were mapped by the order of *Hae*II fragment loss in a BAL 31 exonuclease reaction on *Hin*cII-cleaved pRK2086 DNA. pRK2260 is missing the 2.3' to 5.5' region of RK2 DNA and the adjoining *Pst*I-*Hae*II fragment from the vehicle. pRK2261 has a deletion of the 2.3' to 3.3' region of RK2 DNA, with insertion of an *Hae*II Cm^r-encoding fragment at the site of the deletion. pRK2262 is missing the 0' to 3.3' region of RK2 DNA.

phoresis (19), polyacrylamide gel electrophoresis (19), and transformation of *E. coli* with plasmid DNA (6) were done by previously published procedures. To obtain a population of pRK2086 cleaved partially with *Hae*II for the construction of insertion mutants, ca. 5 μg of plasmid was digested in the presence of 80 μg of ethidium bromide per ml. After the reaction was stopped by treatment at 65°C for 10 min, the DNA was precipitated, pelleted by centrifugation, and resuspended in DNA ligation buffer.

RESULTS

Location of *kilA*. Our earlier studies have shown that both *kilA* and *korC* are encoded by the 0' to 5.5' region, which is present as a cloned *Eco*RI-*Pst*I fragment in plasmid pRK2086 (Fig. 1) (12). This plasmid was the parental plasmid for the manipulations described below.

Our first objective was to verify that *korC* and *kilA* were indeed separate genes. Our approach was to construct a variety of mutants by inserting a *Hae*II Cm^r-encoding fragment at random into the *Hae*II sites of pRK2086, as described above. Two such mutants important to this work are pRK2260 and pRK2261 (Fig. 1).

These two plasmids were tested for their ability to confer KilA$^+$ and KorC$^+$ phenotypes (Table 3). The KilA$^+$ phenotype was observed as the inability of a *korA*^{0} host to be transformed by the plasmid being tested, whereas a *korA*$^+$ host was transformed efficiently. Both pRK2260 and pRK2261 are clearly *kilA*$^+$ because the ability of the cell to tolerate their presence depends on *korA*.

The KorC$^+$ phenotype was observed as the ability of a test plasmid in *E. coli* to allow the cell to be transformed subsequently by a *kilC*$^+$ plasmid (12). Here we used pRK2091 (*kilC*$^+$) because it has the P15A replicon, which is compatible with the ColE1 replicon of pRK2260 and pRK2261. In this experiment, the host strain must also be *korA*$^+$ to allow the *kilA*$^+$ pRK2260 and pRK2261 to be maintained. The results (Table 4) show that pRK2261 is *korC*$^+$, whereas pRK2260 lacks *korC*.

From these experiments, we conclude the following: (i) *kilA* is located within the 0' to 2.3' region of RK2; (ii) *kilA* and *korC* are very likely separate genes, because inactivation of *korC* does not destroy *kilA* activity; and (iii) *korC* is not required to control *kilA* because pRK2260, which is *kilA*$^+$ *korC*$^-$, can be maintained in a *korA*$^+$ host.

Separation of *korC* from *kilA*. Knowing the location of *kilA* allowed us to delete *kilA* specifically from a *korC*$^+$ plasmid. For this, we chose pRK2261 because the inserted Cm^r-encoding fragment introduced a second *Eco*RI cleavage site between *kilA* and a 2.2-kilobase segment which might encode *korC*. This permitted the simple deletion of the *kilA*-encoding region by digesting pRK2261 with *Eco*RI and transforming *E. coli* cells. Colonies which were Km^r were

TABLE 3. Relative efficiency of transformation by derivatives of pRK2086[a]

Transforming plasmid	Resident plasmid recipient strain (genotype): None (*kor*^{0})	pRK2108 (*korA*$^+$ *korB*$^+$)	pRK2240 (*korA*$^+$)	pRK2241 (*korA*$^-$)
pRK2086	<0.001	1.0	1.0	<0.001
pRK2260	<0.001	1.0	1.3	<0.001
pRK2261	<0.001	1.0		
pRK2262	1.1	1.0		

[a] MV10 strains with the indicated helper plasmids were transformed with the test plasmids, and kanamycin-resistant colonies were selected. The relative competence of each strain was monitored by transformation with pMK20 as described previously (12). Values are adjusted for competence differences, which were never more than twofold. Efficiencies of transformation for each plasmid are normalized to that of strain MV10(pRK2108).

TABLE 4. Test of pRK2086 derivatives for expression of *korC*

Resident plasmid[a]	Relative efficiency of transformation by a *kilC*$^+$ plasmid[b]
None	<0.001
pRK2086	1.0
pRK2260	<0.001
pRK2261	0.1[c]
pRK2262	1.4

[a] Plasmids pRK2086, pRK2260, and pRK2261 require *korA* to be maintained (see Table 3). Therefore, the host strains in this experiment all contained pRK2108.

[b] The strains were transformed with *kilC*$^+$ plasmid pRK2091, and Tp^r colonies were selected. The relative competence of each strain was measured by transformation with pLB2. No difference was greater than twofold. Efficiencies of transformation are normalized to that of the pRK2086-containing strain.

[c] These colonies varied in size.

selected and screened for Cm^s. We again used a *korA*$^+$ host, strain MV10(pRK2108), because it was possible that another *kil* gene dependent on *korA* was located in the remaining region. One clone gave pRK2262, which had lost the small *Eco*RI fragment and no longer contained the promoter region of the chloramphenicol acetyltransferase gene (1) found in pRK2261.

pRK2262 was tested for its KilA and KorC phenotypes (Tables 3 and 4). pRK2262 was *kilA*, as expected, and showed no evidence of any other *kil*-like function. In addition, it was *korC*$^+$. Thus, *korC* maps in the 3.3′ to 5.5′ region of RK2.

Two genes required for the KorC phenotype. The construction of pRK2262 allowed us to test whether *korC* alone is sufficient to protect cells from the lethal *kilC* function. This was now possible because pRK2262 is *kilA*$^-$ and can therefore be maintained in a *korA*^{0} host.

Strains carrying both pRK2262 and pRK2108 were KorC$^+$ (Table 5). As expected from our earlier work (12), pRK2108 itself did not confer a KorC$^+$ phenotype (Table 4). However, pRK2262 alone was unable to provide *korC* activity against the *kilC*$^+$ test plasmid.

We conclude that two genes are required to express a KorC$^+$ phenotype: *korC* from pRK2262 and another, as yet unidentified gene in the 50.4′ to 56.4′ region of RK2 that is present in pRK2108.

***korA* required for *korC* activity.** There are two known *kor* genes (*korA* and *korB*) in the 50.4′ to 56.4′ region of RK2 (12). We first tested whether *korA* was also needed for the KorC$^+$ phenotype.

Plasmid pRK2240 carries a 500-base-pair (bp) fragment which encodes *korA* and expresses it from the chloramphenicol acetyltransferase promoter. pRK2241 has the same fragment in the opposite orientation, and it does not express *korA*. pRK2216 also carries *korA* but on an 800-bp segment. In this plasmid, *korA* is expressed from its own promoter. pRK2219 is similar to pRK2216, but it contains an exonuclease BAL 31-generated deletion mutation. This mutant is *korA*$^-$ because of a deletion at the carboxy-terminus of *korA*. Genetic and nucleotide sequence analysis of these plasmids is presented elsewhere (D. H. Bechhofer and D. H. Figurski, Nucleic Acids Res., in press).

We showed that both *korA*$^+$ plasmids successfully allowed pRK2262 to provide a KorC$^+$ phenotype (Table 5). The *korA*$^-$ plasmids were unable to do so. Therefore, we conclude that the *korA* gene is the additional factor required for the KorC$^+$ phenotype.

***korA* requirement for *korC* activity bypassed in a *rho* mutant of *E. coli*.** From other results concerning an interaction of *korA* and *korB* (D. Bechhofer and D. Figurski, manuscript in preparation), we guessed that *korA* may be involved in *korC* transcription. Two possible models for positive regulation of *korC* transcription are: (i) *korA* is required to activate the *korC* promoter, and (ii) *korC* transcription is terminated prematurely unless *korA* function is present. To get an indication of whether the second mechanism might be operating, we tested whether the *korC* dependence on *korA* is eliminated in a *rho* host strain in which normal *rho*-dependent termination does not occur.

For this experiment, we used strains DW319 and M41, which are isogenic except for the *rho-115* mutation in M41 (Table 6). As expected, the KorC$^+$ phenotype was not expressed by the *rho*$^+$ strain DW319(pRK2262) because no *korA* was present. In contrast, the *rho* strain M41(pRK2262) showed efficient transformation by the *kilC*$^+$ plasmid in the absence of *korA*. Therefore, in the *rho* strain, the *korA* function is not required for *korC* to be expressed.

DISCUSSION

Our experiments revealed a functional relationship between the *korC* and *korA* genes of plasmid RK2 in the negative control of *kilC*. Both *kor* genes are shown here to be required for the KorC$^+$ phenotype. Thus, *korA* has two distinct functions on RK2. One is positive (activation of *korC*), and the other is negative (inhibition of *kilA*). This result also indicates that the interactions of *korA*, *korB*, and *korC* with *kilA*, *kilB*, and *kilC*, respectively, are not independent of each other.

The additional finding that a mutation in the *rho* gene of *E. coli* makes *korC* independent of *korA* has the following significance. (i) It shows that once the *korC* product is available, it is sufficient to control *kilC*. Therefore, in normal *rho*$^+$ *E. coli* cells, *korA* function is very likely required for expression of the *korC* gene rather than for activity of the *korC* product. (ii) It suggests a possible mechanism for *korA* action. The *rho* effect indicates that transcription may terminate in a *rho*-dependent manner before or within *korC*. Thus *korC* can be expressed if the *rho* factor is missing. Since *korA* allows *korC* expression even in *rho*$^+$ cells, it is possible that the function of *korA* is to prevent transcription termination.

Positive regulation by interference with the termination of transcription has been demonstrated in other systems. It is

TABLE 5. Test for expression of KorC$^+$ phenotype in presence and absence of *korA*[a]

Test plasmid	Genotype	Relative efficiency of transformation by *kilC*$^+$ plasmid
None	*korA*^{0}	<0.001
pRK2108	*korA*$^+$	1.0
pRK2240	*korA*$^+$	0.8
pRK2241	*korA*$^-$	<0.001
pRK2216	*korA*$^+$	0.9
pRK2219	*korA*$^-$	<0.001

[a] All transformations were done in strain MV10 with pRK2262 and the additional test plasmid indicated. Experiments were done exactly as described in Table 4, footnote *b*. Efficiencies of transformation are normalized to that of the pRK2108-containing strain.

TABLE 6. KorC phenotype of pRK2262 in an *E. coli rho* mutant[a]

Host strain	Relevant genotype	Resident plasmid	Relative efficiency of transformation by *kilC*$^+$ plasmid
DW319	*rho*$^+$ *korA*^{0}	None	<0.001
DW319	*rho*$^+$ *korA*^{0}	pRK2262	0.006
M41	*rho korA*^{0}	None	<0.001
M41	*rho korA*^{0}	pRK2262	0.6
RP1894	*rho*$^+$ *korA*$^+$ *korC*$^+$	None	1.0

[a] The strains listed were transformed with pRK2161. Relative competence was determined by transformation with pCY2. Efficiencies of transformation are normalized to that of the RP1894 strain.

an important aspect of phage λ gene regulation. The λ *N* gene product (14, 27) and very likely the λ *Q* gene product (4, 13, 28, 30) are antiterminators. Recently, it has been proposed that antitermination may be a regulatory mechanism for the *rpsU-dnaG-rpoD* macromolecular synthesis operon in *E. coli* (31, 40).

There are other interpretations of the results, but we regard these as less likely. (i) The *korA* function activates the *korC* promoter, and the *rho* mutation allows fortuitous readthrough from another plasmid promoter whose transcription is normally terminated before *korC*. (ii) The *kilC* region actually encodes two *kil* determinants; one is controlled by *korC*, and the other is controlled by *korA*. This explanation requires that the latter *kil* determinant not be active in the *rho* host strain. (iii) The *rho* mutation allows the expression of a host gene whose function replaces that of *korA* in allowing the *korC* gene to be expressed or the *korC* product to control *kilC*. We have no evidence against these possibilities, but we argue that a direct effect of the *rho* mutation on *korC* expression is the simpler model.

If *korA* is involved in the expression of *korC*, might it also play a role in *korB* control of *kilB*? This possibility was tested, and it was found that *korA* function is indeed involved in the KorB$^+$ phenotype (Bechhofer and Figurski, manuscript in preparation). We are currently trying to determine whether *korA* is required for expression of the *korB* gene.

Considering *korA* as an antiterminator helps to explain the phenotype expressed by pRK2501*ts*3, a mini-RK2 plasmid. This plasmid is temperature sensitive for replication (35), yet the mutation was mapped to a region which has been shown to encode no essential replication determinants (25, 26, 35). The structure of this plasmid makes it possible that the replication gene, *trfA*, is expressed from the promoter of the normally nonessential *korA* gene (35; Bechhofer and Figurski, in press). If a transcription termination sequence were present between *korA* and *trfA*, then expression of *trfA* would depend on the function of the *korA* product. Thus, if the *korA* product were temperature sensitive, the plasmid could not express *trfA* at the nonpermissive temperature and would be unable to replicate. This model predicts that pRK2501*ts*3 would be complemented by *korA*$^+$ in *trans* and by *trfA*$^+$ in *trans*. We have verified both predictions (unpublished data), and Smith and Thomas (32) have recently reported that a small region encoding *korA* complements the *ts*3 mutation. In addition, one predicted class of revertant would contain a deletion of the putative termination signal. Revertants which have deletions in the appropriate location have been reported (35).

Another consideration is the question of how *korA* controls *kilA*. It seems paradoxical that a gene product which serves as a positive regulator of *korC* can also act as a negative regulator of *kilA*. One possibility is that another gene (*korA2*?) next to *kilA* is directly involved in the control of *kilA*. Thus *korA2* could be positively regulated by *korA* in the same way that *korC* is positively regulated. If *korA* is an antiterminator, then a logical location for the putative *korA2* gene is downstream of *kilA* and in the same transcriptional unit. Another possibility is derived from studies on the retroregulation of λ *int* expression (15, 16). In this system, *int* transcription from the λ p_L promoter is extended by *N*-mediated antitermination to allow the synthesis and formation of a new RNase III cleavage site. Cleavage results in rapid degradation of the transcript and thereby prevents *int* expression. By analogy, *korA*-mediated antitermination of a *kilA* transcript might lead to the formation of a new RNA which is unstable or inactive. At present, such mechanisms for RK2 are purely hypothetical.

In summary, we have separated *korC* and *kilA*, and we have shown that *korC* expression depends on *korA* function. We have proposed the idea that *korA* acts through the antitermination of transcription, but additional genetic and biochemical analyses will be required to test this idea rigorously.

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