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Map location and nucleotide sequence of *korA*, a key regulatory gene of promiscuous plasmid RK2

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ABSTRACT

From our earlier work, we know that the korA gene of broad host range plasmid RK2 is located within the 50.4'-56.4' region. By additional subcloning of this region, we have mapped korA to the segment between the HaeII site at 55.0' and the HincII site at 55.6'. The direction of korA transcription (55.6' to 55.1') was determined by two methods: (1) inactivation of korA expression signals and fusion of the structural gene to other promoters; and (2) hybridization analysis of korA-specific RNA's synthesized in vivo. We have determined the nucleotide sequence of the korA region. A potentially strong promoter overlaps the HincII site at 55.6', and there is a coding region which specifies the putative korA polypeptide. That this is the korA gene was supported by sequence analysis of Bal31-generated deletion mutants of korA. The sequence shows the korA product to be a small, basic polypeptide of 101 amino acids.

INTRODUCTION

The kil genes of plasmid RK2, an incompatibility group P (IncP) plasmid (1-3), can be lethal to an Escherichia coli host (4). This potential resides in any one of three distinct determinants: kilA, kilB, and kilC. Normally, their lethal actions are controlled by three other plasmid genes: korA, korB, and korC (each specific for the corresponding kil determinant). The true functions of these genes are not yet known. They are not essential for plasmid replication in E. coli, and yet the kor (and probably the kil) genes are highly conserved among plasmids of the IncP group (4). We have speculated that these unique determinants are related to another unusual property of these plasmids: an extraordinary host range which includes virtually all gram-negative bacteria (5, 6).

In our studies on the regulation of the kil genes, we have found that korA specifies an important regulatory function. By itself, korA acts negatively to inhibit the lethality of kilA (4). In addition, korA function is a trans-acting positive regulator for the expression of korB (Bechhofer and Figurski, in preparation) and of korC (Young, Bechhofer, and Figurski,

submitted). These findings lead us to propose a central role for korA in the control of kil and kor genes and, by implication, in RK2 host range.

In this report, we present our work on the structure of the korA gene. By genetic analysis of mutations generated in cloned segments of RK2, we were able to map the precise location of korA and to determine its nucleotide sequence. From the sequence, we conclude that the korA gene product is a small, basic polypeptide.

MATERIAL AND METHODS

Nomenclature and abbreviations

Coordinates of the RK2 physical map are designated with a prime (') (e.g., 55.6') and reflect distance in kilobases from the EcoRI site. The following abbreviations are used in the text: bp (base pair); kb (kilobase pairs), Ap (ampicillin), Cm (chloramphenicol), Km (kanamycin), Tc (tetracycline), IPTG (isopropyl-β-D-galactopyranoside).

Bacterial strains and plasmids

E. coli MV10 (C600 ΔtrpE5) (7) is the host strain for all plasmids described in the text. The P15A plasmids pACYC177 and pACYC184, which are the basis for plasmids constructed in this work, have been described (8). All other plasmids are described in the text.

Media and reagents

LB and LB-glu media (9) were used. Antibiotics were used as follows: 30 μg/ml for Tc; 50 μg/ml for Km, Nm, and Cm; 150 μg/ml for penicillin (to select Ap^r). Enzymes were purchased from New England Biolabs or Bethesda Research Laboratories and used according to the conditions suggested by the suppliers. EcoRI linkers were obtained from Collaborative Research.

Procedures

Plasmid DNA purification and electrophoresis have been described (9, 10). Transformation of E. coli was by the protocol of Cohen et al (11), but with the buffers described by Kushner (12). All recombinant DNA procedures were carried out in accordance with the National Institutes of Health guidelines for recombinant DNA research.

Preparation of end-labeled restriction fragments

Plasmid DNA was digested with appropriate restriction endonucleases. Restriction fragments were treated with calf intestinal alkaline phosphatase (Boehringer-Mannheim) to dephosphorylate the 5' ends. The 5' ends were radioactively labeled with γ-^{32}P-ATP (3,000 Ci/mmol; Amersham), using T4 polynucleotide kinase (Bethesda Research Laboratories), as described by Maxam

and Gilbert (13). The fragments were cleaved with a second restriction enzyme to obtain unique 5' labeled ends. The resulting end-labeled fragments were separated by electrophoresis in 6% polyacrylamide gels in Tris-borate buffer (90 mM Tris, pH 8.0, 90 mM boric acid, 2.5 mM EDTA), from a stock of 30% acrylamide, 0.8% N,N'-methylene-bisacrylamide. Gel slices containing the desired fragments were crushed and the DNA was eluted (13).

Sequence analysis

DNA sequencing was essentially as described by Maxam and Gilbert (13), except that piperidinium-formate, pH 4.0, was used in the G+A reaction. For cleavage products larger than 20 nucleotides, the final lyophilizations were replaced by precipitations with ethanol (14). Cleavage products were electrophoresed in 8% or 20% polyacrylamide/7M urea gels (40 cm x 20 cm x 0.4 mm). Autoradiography was at -70°C with Kodak XR-5 film. Sequences were analyzed on a PDP11/45 computer using programs written by R. Staden (MRC Laboratory, Cambridge, U.K.) (15). The nucleotide sequence was determined on both strands for the entire sequence, except for the first 18 nucleotides.

Nucleic acid hybridizations

After cleavage with appropriate restriction enzymes, DNA was electrophoresed in 0.8% agarose gels in Tris-borate buffer (90mM Tris pH 8.0, 90mM boric acid, 2.5mM EDTA). DNA in the gel was depurinated, denatured, and transferred onto nitrocellulose filters (Schleicher & Schuell) (16, 17, 18). DNA on filters was annealed with the appropriate ^{32}P-labeled nick translated DNA fragments.

RNAs were dissolved in a buffer containing 50% formamide, 2.2 M formaldehyde, and 1X running buffer (20 mM morpholinopropanesulfonic acid, pH 7.0, 5mM sodium acetate, 1 mM EDTA). The RNAs were electrophoresed on 0.8% agarose gels containing 2.2 M formaldehyde (19) and then transferred to nitrocellulose filters (20). Hybridization was with ^{32}P-end labeled DNA restriction fragments or with nick-translated DNA fragments.

RESULTS

Genetic mapping of korA

We showed previously that the cloned 50.4'-56.4' region of RK2 expresses *korA* (4). To determine the exact position of *korA*, smaller fragments from the 50.4'-56.4' segment (Fig. 1) were subcloned, and the resulting plasmids were tested for expression of *korA* (Table 1). A plasmid is considered *korA*$^+$ if its presence in the cell allows that cell to form colonies after transformation with a *kilA*$^+$ test plasmid, whereas cells carrying

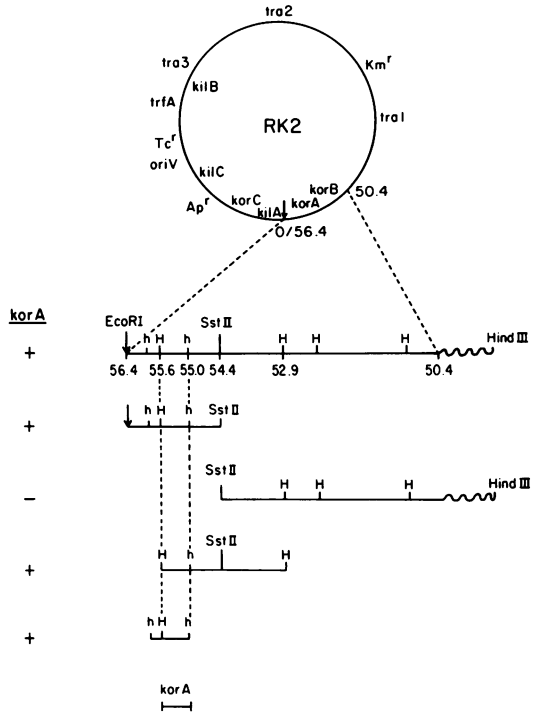

FIG. 1. Subcloning of the 50.4'-56.4' region of RK2. The RK2 map (4) includes transfer regions (tra), trans-acting replication function (trfA), and kil and kor genes. The EcoRI-HindIII fragment (7.75 kb) was cloned (4) from an RK2::Mu derivative, pRK214.1 (9). The wavy line indicates Mu DNA. KorA phenotypes of the subcloned fragments are shown. "H" designates a HincII site; "h", a HaeII site. Only the relevant HaeII sites are marked.

korA$^-$ plasmids will not form colonies. From the results in Table 1, it is clear that korA lies between the HincII site at 55.6' and the HaeII site at 55.0' (see Fig. 1).

To map korA more precisely, we generated deletion mutants in this region by the strategy outlined in Figure 2. Plasmid pRK2211 contains the cloned HaeII fragment which encodes korA, and the Cm^r-specifying HaeII fragment of pACYC184 inserted as shown. In this plasmid the asymmetric EcoRI site of the Cm^r-encoding fragment is proximal to korA. To produce deletions, pRK2211 was first digested with EcoRI and then for various times with exonuclease Bal31. Because of the orientation of the Cm^r-encoding HaeII fragment, we reasoned that korA would be disrupted before the Ap^r

TABLE 1. Test for expression of korA activity by various plasmid derivatives.[a]

Resident plasmid[b]	Efficiency of transformation by a kilA$^+$ plasmid
pRK2104	1.0
pRK2178	1.1
pRK2186	0.8
pRK2216	1.0
pRK2218	0.02[c]
pRK2219	<0.001
pRK2240	1.0
pRK2241	<0.001
none	<0.001

a MV10 is the host in each case. The kilA$^+$ plasmid is pRK2160 (4) (pSM1 vehicle, Km^r) or pRK2123 (pRK353 vehicle, Trp$^+$ and Ap^r). The efficiencies of transformation are normalized to that of the pRK2104-containing strain. Values include a correction for the slight differences in transformation competence as measured by transformation with a standard amount of pSM1 or pRK353 plasmid with no RK2 sequences.

b Plasmid pRK2104 is a mini-ColE1 replicon with the 50.4'-56.4' region of RK2 (4; see Fig. 1); pRK2178, a pACYC177 derivative with the 52.9'-55.6' HincII fragment (Fig. 1); pRK2186 is a pACYC177 derivative with the 55.0'-55.9' HaeII fragment (Fig. 1); the structures of pRK2216, pRK2218, and pRK2219 are given in Fig. 2; pRK2240 and pRK2241, in Fig. 3.

c Colonies were smaller than those of strains containing pRK2104, pRK2186, pRK2216, or pRK2240.

determinant was lost. EcoRI linkers were ligated to the reduced molecules and the DNA was used to transform E. coli.

Plasmids from 100 Ap^{r}Cm^s clones were examined. The amount of korA region DNA remaining in the plasmids was measured by determining the distance of the newly attached EcoRI site to the HincII site at 55.6'. Three plasmids were studied in detail (Fig. 2). Plasmid pRK2216 was found to have about 500 bp of RK2 DNA remaining between the HincII site and EcoRI site, and thus 120 bp of RK2 DNA was deleted. In the genetic test for korA (Table 1), pRK2216 is korA$^+$. Plasmid pRK2218 is deleted for an additional 100 bp in the korA region. Cells containing pRK2218 can be transformed with a kilA$^+$ test plasmid, but at a frequency 50-fold lower than that of pRK2216-containing cells, and the colonies are smaller. We refer to this as a KorA$^\pm$ phenotype. The korA region of the third derivative, pRK2219, has lost about 20 bp more than pRK2218, and this plasmid is korA$^-$.

From these results we suggest the following: (1) that korA is encoded

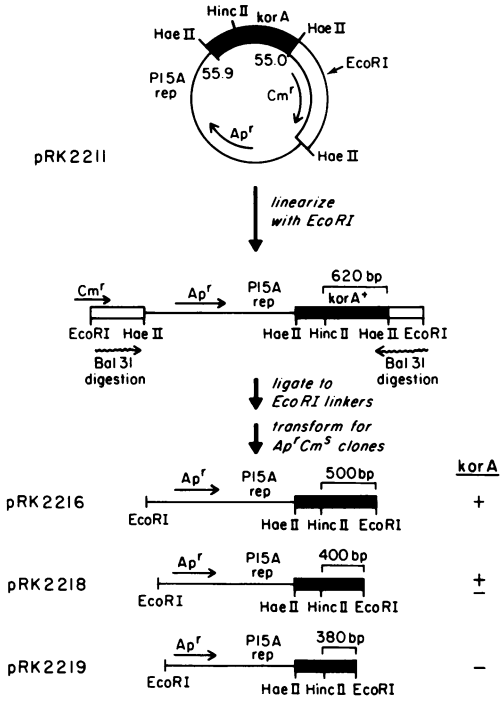

FIG. 2. Construction of deletion mutants in the korA region using exonuclease Bal31. Shaded box indicates RK2 DNA. Numbers are RK2 map coordinates in kb. Open box indicates the inserted Cm^r-specifying HaeII fragment. Position of the P15A replicator region is marked. Arrows show the direction of transcription of antibiotic resistance genes.

within a 500 bp region defined by the HincII site at 55.6' and the end point of the pRK2216 deletion at 55.1' and (2) that the deletion endpoints in pRK2216, pRK2218, and pRK2219 bracket one end of the korA gene.

To prove that the 500 bp region of pRK2216 encodes korA, we subcloned it into pACYC184 (Fig. 3). First, EcoRI linkers were ligated to the blunt end generated by HincII cleavage of pRK2216. (We designate the new EcoRI site, which replaces the HincII site at 55.6' as "$EcoRI^H$". The other EcoRI site, which marks the endpoint of the Bal31-generated deletion at 55.1', is named "$EcoRI^B$".) The 500 bp korA-encoding region was then excised with EcoRI and inserted into the EcoRI site of pACYC184. Plasmids were recovered with the fragment in opposite orientations. Two examples are pRK2240 and pRK2241 (Fig. 3).

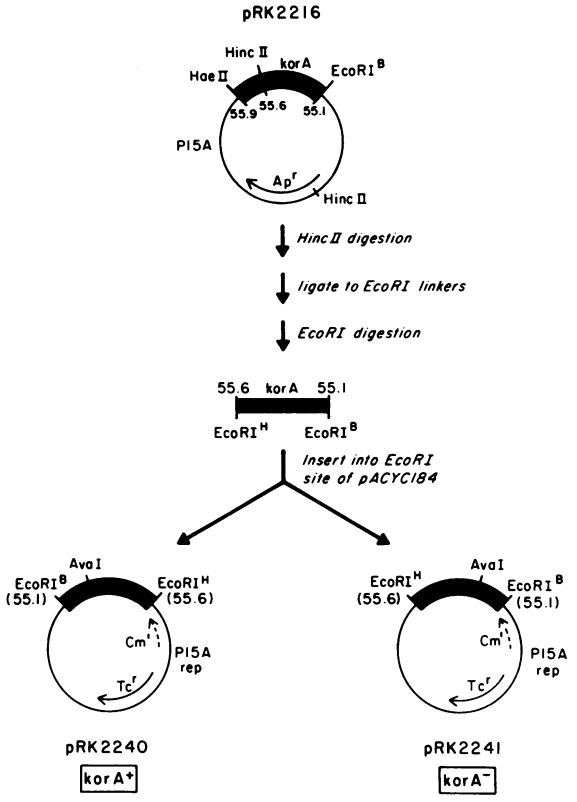

FIG. 3. Cloning of the 500 bp korA region into pACYC184 in two orientations. $EcoRI^B$ marks the endpoint of the Bal31-generated deletion in pRK2216. $EcoRI^H$ designates the new EcoRI site which replaces the HincII site. Dashed arrow in pRK2240 and pRK2241 indicates the direction of transcription into the 500 bp cloned fragment.

With these plasmids, we found that expression of korA did occur from the 500 bp fragment, but it was dependent on orientation. Cells with pRK2240 are $KorA^+$; with pRK2241, $KorA^-$. These inserts are within the structural gene for chloramphenicol acetyltransferase (cat), and in pRK2240 the $EcoRI^H$ site is proximal to the cat promoter (21). The region was also tested under conditions in which no exogenous transcription entered the cloned segment. For this, the 500 bp EcoRI fragment was cloned into pLc28 (22) in both orientations and downstream of the λP_L promoter. In the presence of λ repressor, this new plasmid was found to be $korA^-$.

These results suggest that the 500 bp region between coordinates

55.1' and 55.6' does encode the korA structural gene, but not the korA promoter. Furthermore, the finding that korA is expressed in the pRK2240 orientation led us to predict that the direction of korA transcription is from 55.6' (EcoRI^H) to 55.1' (EcoRI^B).

Direction of korA transcription

We tested this directly by hybridization analysis of RNA synthesized from the korA region in vivo. Total RNA was prepared from a strain carrying RK2 and from another strain carrying pRK2108 (a plasmid with the cloned 50.4'-56.4' region of RK2). The molecules were separated by electrophoresis through formaldehyde-agarose gels and transferred to nitrocellulose for hybridization with ^{32}P-labeled korA-specific DNA fragments. The hybridization probes were DNA fragments (AvaI-EcoRI^H and AvaI-EcoRI^B, see Fig. 4) labeled at the 5' terminus of only one strand, and the labeled strands were of opposite polarity. The autoradiogram in Fig. 4 shows clearly that only one strand gives detectable hybridization to the RNA. In a parallel experiment, the EcoRI^H-AvaI fragment was labeled at the 5' terminus of either the EcoRI-generated end or the AvaI-generated end. Only the latter probe detected transcripts (data not shown), and this is consistent with the results in Fig. 4. The hybridization pattern shows that at least four distinct RNA species are homologous to the probe. The same pattern is observed if the DNA probe is the complete 500 bp EcoRI^H-EcoRI^B fragment which has been uniformly labeled in both strands by nick translation (data not shown).

From these data and from the genetic results in the preceding section, we conclude the following: (1) that all korA region-specific RNA molecules we detect are of the same polarity and (2) that transcription of korA proceeds away from the EcoRI^H site (i.e., RK2 coordinate 55.6') and towards EcoRI^B (coordinate 55.1'). We have not yet determined the reason for the multiple RNA species, although it is clear that they are larger than the korA-encoding region and contain sequences downstream of the korA region (Bechhofer and Figurski, in preparation).

Attempts to visualize the korA polypeptide

Since korA functions in trans and since it is encoded by a 500 bp fragment, we tried to detect a small (approximately 15,000 dalton) korA polypeptide. We used three different methods. (1) Polypeptides specified by pRK2240, pRK2241, and several other korA$^+$ plasmids were examined in minicells from strain DS410 (23). (2) The 500 bp EcoRI fragment was cloned into an expression plasmid, pLc28 (22), which carries the inducible P_L

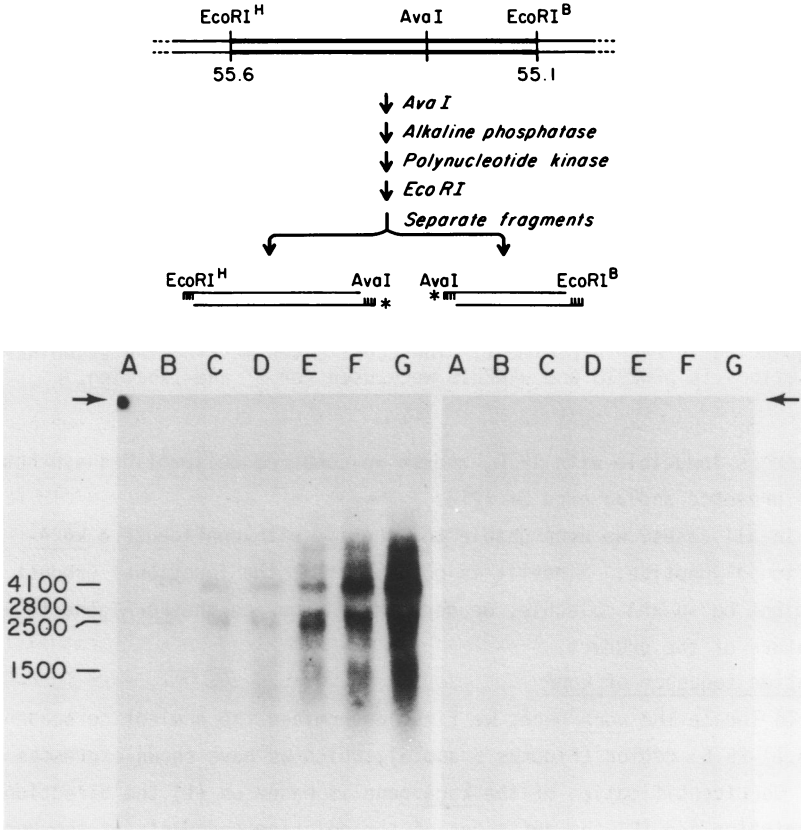

FIG. 4. Direction of transcription in the korA region. Top line depicts the 500 bp korA fragment in pRK2240. Asterisks in the separated fragments show positions of the ^{32}P-phosphate groups. End-labeled probes were hybridized to RNA which was electrophoresed in a 0.8% agarose gel and blotted to nitrocellulose filters. RNA samples are as follows: Lane A has 10 µg RNA from MV10; lanes B, C, and D have 5, 10 and 15 µg, respectively, from MV10(RK2); lanes E, F, and G have 5, 10, and 15 µg from MV10(pRK2108). The arrows mark the sample origin, and the numbers refer to approximate lengths of the RNA in nucleotides.

promoter of bacteriophage λ. In this construction, as mentioned above, korA function was genetically confirmed to be under λ repressor control. Using a host which specified a temperature-sensitive λ repressor, we examined polypeptides synthesized at various times after inducing λ P_L with a temperature shift. (3) The same korA-encoding EcoRI fragment was cloned into the expression vehicle, pKK223-3, which has the "tac" promoter (J. Brosius, unpublished). In the proper orientation this new plasmid is korA$^+$. The

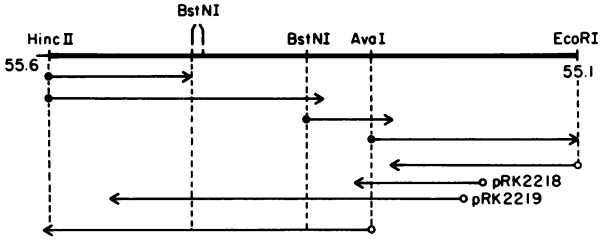

FIG. 5. Sequencing strategy. A cleavage site map of the korA region is shown on the top line. Arrows show the direction and approximate extent of sequencing in each experiment. The EcoRI sites marking the endpoints of deletions in pRK2218 and pRK2219 were used for 5' end-labeling.

promoter is inducible with IPTG, and so we compared polypeptides synthesized in the presence and absence of IPTG.

In all cases, we were unable to identify with confidence a korA-specific polypeptide. Since it is possible that the functional product of korA might be an RNA molecule, we decided to sequence the korA gene to learn the nature of the product.

Nucleotide sequence of korA

To locate the korA gene, we first determined the nucleotide sequence of the 55.1'-55.6' region (Figures 5 and 6), which we have shown expresses korA. Our identification of the korA gene is based on (1) the direction of transcription and (2) the positions of the deletion endpoints of the korA$^{\pm}$ plasmid pRK2218 and the korA^{-} plasmid pRK2219. These deletions enter the korA region from the promoter-distal end, and we reasoned that if korA specifies a polypeptide, the deletions should affect the carboxy-terminus of any putative korA gene product.

Only one reading frame specifies a polypeptide whose termination signal occurs within the 510 bp region and whose carboxy-terminal end is changed by the deletion mutations in pRK2218 and pRK2219. This open reading frame begins with an ATG initiation codon at position 115 and ends with a TGA termination signal at position 418.

The hypothetical korA polypeptide specified by this region would be affected by the deletions in pRK2218 and pRK2219. In both cases the termination codon is lost, and a fusion product would result from continued translation for an unknown distance into the adjoining sequence. However, pRK2218 would give a polypeptide which is wild-type to its final two carboxy-terminal amino acids, whereas the polypeptide from pRK2219 is defective in

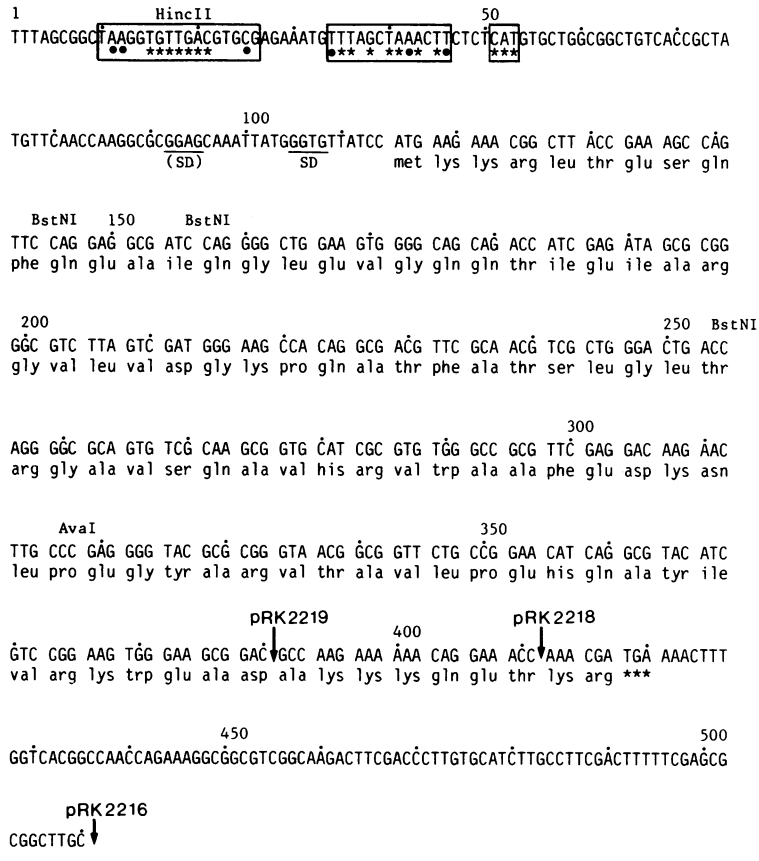

FIG. 6. Nucleotide sequence of the 510 bp korA region. Both strands were sequenced except for the first 18 nucleotides. Transcriptional signals are boxed. Asterisks indicate complete homology with consensus sequence (25). Dots indicate acceptable alternative nucleotides used in transcriptional signals. Possible Shine-Dalgarno sequences are underlined and indicated with "SD". The endpoints of the deletions in pRK2216, pRK2218, and pRK2219 are marked by the arrows. Numbers refer to nucleotide position and increments of ten are noted with a dot above the nucleotide.

its last nine amino acids. It is reasonable that this difference is responsible for the difference in phenotypes shown by pRK2218 (KorA$^{\pm}$) and pRK2219 (KorA^{-}).

The putative korA coding region is preceded by a reasonable Shine-Dalgarno sequence (GGTG) for ribosome binding located six nucleotides before the ATG. An in-frame GTG codon is also located three codons prior to the ATG

at 115. We cannot rule out the possibility that this GTG is the actual signal for initiation of translation, particularly since it is preceded by the more common GGAG sequence for ribosome binding (27). However, for this discussion we will consider the more commonly used ATG as the beginning of the structural gene. We do not believe that the other two ATG codons (positions 31 and 52) are part of korA. The first is followed immediately by one of the two TAA termination codons in the region. The second is in a segment with no homology to the Shine-Dalgarno sequence. In addition, it occurs within a potential transcription initiation signal (see below).

The 115 nucleotide region preceding the ATG has sequences homologous to the consensus -35 and -10 transcription initiation signals, and these are noted in Fig. 6. If these signals are aligned with those of Siebenlist et al. (29), the spacing between them is 17 bp. This distance has been shown to be optimal for RNA polymerase binding (30). It is significant that the HincII site at 55.6' occurs within the putative -35 sequence. This is consistent with our finding that destruction of the HincII site by ligation of a linker abolishes korA expression but does not disrupt the structural gene (Table 1). In addition, a strong RNA polymerase binding site has been mapped to this region by electron microscopy (31).

The putative korA product contains 101 amino acids, of which 18 are basic (Arg, Lys, His) and 12 are acidic (Asp, Glu). Thus, the molecule has a net positive charge. The acidic amino acids are distributed more or less randomly throughout the chain, while the basic residues are biased towards the carboxy-terminal end (i.e., 7 of the last 15 amino acids).

The third reading frame shows a long potential coding region, but a termination signal does not occur within the 510 bp segment. This coding region could start with the ATG at position 75 or at 102, although only the latter is preceded by a ribosome binding site (GGAG). Regardless of the start, the coding region would completely encompass the korA structural gene and could be expressed from the same transcriptional signals. Therefore, this open reading frame may be part of another gene which overlaps korA.

The second reading frame has five termination codons, only one of which is preceded by an ATG located 13 codons prior. This reading frame has a high percentage (14.7%) of codons considered rare, at least for E. coli genes (32). The arginine codon (AGG), which is extremely rare, is used 10 times in this reading frame alone, and twice within the thirteen codon space mentioned above. We have also examined the three reading frames which read opposite to what our results indicate is the direction of transcription. In none of the

frames is there a potential coding region for a product larger than 50 amino acids, and none of these products is affected by the deletion in pRK2218 or pRK2219 or by disruption of the HincII site. We conclude that korA cannot be encoded by any of these reading frames.

DISCUSSION

We have determined the nucleotide sequence of a 510 bp region which expresses korA, and we have identified the korA gene. Translation of the sequence reveals that the korA product is a basic polypeptide of 13,000 daltons.

Our identification of the korA gene is based on several criteria: (1) the direction of transcription; (2) evidence that korA is completely encoded by the 510 bp region; and (3) the locations of the deletions in korA mutants and their relationship to the phenotypes.

(1) By hybridization analysis of RNA synthesized in vivo, we detected an abundance of RNA specific for the korA-region. All the RNA species detected were of the same polarity and were synthesized in the 55.6' and 55.1' direction. Furthermore, we showed that disruption of the HincII site at 55.6' destroyed expression of korA but did not disrupt the structural gene. Therefore, the reading sense of korA structural gene should be 55.6' to 55.1'.

(2) The korA activity expressed from the cloned 55.1'-55.6' region is indistinguishable from that specified by larger cloned segments. In this work, we showed that the cloned region will control high copy number kilA$^+$ plasmids. We have also learned that the region will activate korB (Bechhofer and Figurski, in preparation) and korC (Young, Bechhofer, and Figurski, submitted) in trans. Furthermore, the 55.1'-55.6' fragment is able to express normal korA function from three different plasmid vehicles if a promoter is provided. Thus, expression of korA is sensitive to the environment of the cloned fragment, but the korA gene product is not. This indicates that the korA structural gene is completely encoded within the 510 bp region.

(3) The deletions in pRK2218 and pRK2219 remove 99 bp and 120 bp, respectively, from the 510 bp korA-encoding region. These mutants show changes in korA activity. The deletion in pRK2218 does not destroy korA function, although noticeably less korA activity is specified by this plasmid. The pRK2219 mutant is completely defective in korA function. To be consistent with the direction of transcription of korA, these mutations must

affect the 3' end of the korA gene. Thus, we expected that the mutant phenotypes should be the result of changes in the carboxy-terminus of the korA product.

Only one reading frame specifies a coding region which fulfills each of these requirements. The gene we have identified has a plausible promoter region which overlaps the HincII site at 55.6' (criterion 1). The coding region begins with an ATG preceded by a ribosome binding site and ends with a TGA codon 90 bp from the end of the 510 bp segment (criterion 2). The putative polypeptide loses two carboxy-terminal amino acids in the korA$^{\pm}$ mutation (pRK2218) and nine carboxy-terminal amino acids in the korA^{-} mutation (pRK2219). These alterations seem a reasonable explanation for the differences in phenotypes shown by these mutants (criterion 3). From these data, we conclude that the coding region we have identified specifies korA.

We have noted another potential coding region, which occurs in a different reading frame. However, it does not specify a termination codon within the 510 bp segment. We are intrigued by the possibility that this may be part of another RK2 gene which completely encompasses the korA gene, but we have no function to ascribe to it. We have also considered that this truncated coding region might actually be the korA determinant. However, for this to be true, it would be necessary to postulate that activity of this "korA" polypeptide is unaffected by substantial changes in the carboxy-terminal region. Different plasmids containing the cloned segment would express different korA fusion products by translation into the sequences adjacent to the insert. We have detected no differences in korA activity specified by this region in four different plasmids. Furthermore, the deletion in pRK2218 would cause the loss of an additional 33 amino acids from an already incomplete polypeptide and yet the product would retain some activity. Until we identify the korA product, we cannot unequivocally rule out the possibility that this incomplete coding region is korA, but we regard it as very unlikely.

Studies in our laboratory have demonstrated that korA is involved in a number of kil and kor regulatory phenomena. The first observation was that the korA function acts negatively to inhibit kilA (4). This control is probably at the level of expression because kilA expressed from other promoters (even weak ones) is not regulated by korA (Young, Prince, and Figurski, in preparation). We have also found that korA is a trans-acting positive regulator of both korB (which in turn is required to inhibit kilB) and korC (required to inhibit kilC). We believe these are all properties of

the same gene for the following reasons: (1) The 500 bp region shown here to be capable of controlling *kilA* is completely able to activate *korB* and *korC*; and (2) the mutants shown here to be defective in the control of *kilA* are unable to activate *korB* or *korC* (Young, Bechhofer, and Figurski, submitted; Bechhofer and Figurski, in preparation).

Since *korA* appears to be a key element in the control of RK2 gene expression, it is reasonable that *korA* functions by interacting directly with DNA or RNA. We therefore find it interesting that the *korA* product is inferred to be a small basic protein. Of 101 amino acids, 18 are positively charged, and 12 are negatively charged. For comparison, the λ *cro* product, which binds to specific sequences on λ DNA, is 66 amino acids in length with 12 positively charged residues and 6 negatively charged residues (33). Unfortunately, our attempts to visualize the *korA* product have been unsuccessful, even with it placed downstream of strong promoters. Because of the variety of regulatory functions attributed to *korA*, it is conceivable that its product is unstable. With the nucleotide sequence of the gene, we hope to be able to manipulate the gene to enhance expression so that we may detect the *korA* product and ultimately amplify it for purification.

Why does such a hierarchy of control exist for regulation of the *kil* and *kor* genes of RK2? Since these genes are not required for replication in *E. coli*, the conservation of *kor* functions throughout the IncP group plasmids (4) leads us to surmise that these genes have a functional significance in other bacterial hosts. Our working hypothesis is that specific *kil* genes are essential for replication in certain hosts. In these host cells, the *kil* gene products may interact with the host replication complex to allow RK2 replication. However, in other hosts expression of a *kil* gene product may inadvertently interact with the host replication complex and thereby interfere with host DNA replication. Therefore, the genes must be turned off in certain hosts, and indeed they may be tightly regulated even in hosts where the *kil* gene products are essential for the plasmid.

Because it can affect the expression of *kilA*, *kilB*, or *kilC*, the *korA* gene appears to be central to determining which plasmid genes are allowed to function. It is conceivable that *korA* is, in fact, a "sensing device" which identifies the host and acts to allow expression of the appropriate genes for plasmid maintenance. We are eager to learn the true function of *korA* in the cell and to determine the molecular mechanism by which it operates. We believe that the nucleotide sequence of *korA* is an important first step towards these objectives.

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