Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 1935–1939, March 1982
Genetics

Broad host range plasmid RK2 encodes multiple *kil* genes potentially lethal to *Escherichia coli* host cells

(incompatibility group P plasmids/plasmid–host interaction/regulation/gene cloning)

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Communicated by Donald R. Helinski, December 4, 1981

ABSTRACT **Cloning of specific regions of RK2, a broad host range incompatibility group P plasmid, has revealed three genes: *kilA*, *kilB*, and *kilC*. Each of these genes can cause loss of viability of an *Escherichia coli* host. This effect on the host is normally prevented by the functions of three additional RK2 genes: *korA*, *korB*, and *korC*. Each *kor* gene is specific for a particular *kil* gene. The *kil* and *kor* genes are located in four distinct regions of the RK2 genome. The three *kil* genes are not clustered and, with the possible exception of *kilA*, they are also well separated from their corresponding *kor* genes. We have found that the *korA* and *korB* determinants are not peculiar to RK2 but instead are highly conserved throughout the incompatibility group P plasmids.**

Bacterial plasmids rely on replication control and partitioning mechanisms for survival in proliferating bacterial hosts. To be efficient, these functions must interact intimately with host cell components, and this very likely contributes to host specificity. Nevertheless, incompatibility group P (IncP) plasmids have an exceptionally broad host range among Gram-negative bacteria (1, 2). We are investigating these plasmids because this potential to interact productively with diverse hosts may be indicative of special functions or unique strategies for gene expression.

RK2 is a 56.4 kilobase (kb) (3) self-transmissible IncP R plasmid (4) probably identical (5) to RP1 (6) and RP4 (7). Several genetic determinants important for maintenance have been identified. In *Escherichia coli* RK2 uses a single origin (*ori*) for unidirectional replication (8). *Ori* is closely associated with incompatibility and stability determinants (9). A separate region encodes a *trans*-acting function required for replication at *ori* (refs. 10–12; this work). Genetic studies have also suggested that a third region is involved in RK2 maintenance in *E. coli* (3, 11, 13).

Earlier work showed that RK2 has a gene whose expression can affect the viability of an *E. coli* host (10). In this study, we have cloned both the gene responsible for this phenotype and the gene involved in its control. In addition we have found that RK2 encodes at least two more genes capable of affecting *E. coli* growth. These genes or their products are specifically controlled by the expression of additional RK2 genes. Fourteen different IncP plasmids were tested for two of the control genes, and all were found to code for both.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. *E. coli* MV10 and C2107 have been described (10). DF4063 is MV10 resistant to nalidixic acid (Nal^r). Plasmids containing RK2 DNA are presented in Fig. 1. IncP group plasmids R26, R527, R702, R751, R906, R839, R934, R938, and R1033 (30), pUZ8 (31), R91A (32), and R995 (isolated by R. W. Hedges) were supplied by G. A. Jacoby. R772 (33) was provided by T. V. Potts; and pJP4 (34), by J. M. Pemberton. pJP4.1 (pJP4::Tn*3*) was isolated in this laboratory.

Media and Reagents. LB and LB-glu media (14) were used, except for selection of tryptophan-independent or Tp^r strains. For these, M9-CAA (13) was used with Tp at 100 μg/ml, L-tryptophan at 50 μg/ml, or both, as required. Antibiotics were used as follows: 20 μg/ml for Sm and Nal; 30 μg/ml for Tc; 50 μg/ml for Km, Nm, Cm, and Ap; 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.

Procedures. Plasmid DNA purification and electrophoresis have been described (13, 15). Transformation of *E. coli* was essentially by the method of Cohen *et al.* (35), but with the buffers of Kushner (36). All recombinant DNA procedures were carried out in accordance with the National Institutes of Health guidelines for recombinant DNA research, part II.

RESULTS

***kilA* and *korA*.** At least two RK2 functions must be provided in *trans* for pRK2067 (Fig. 1) to exist in *E. coli* (10). One permits replication from the RK2 *ori*. The second prevents cell stasis or death induced by pRK2067. We have named the gene responsible for this effect on cells "*kilA*" and the required control gene, "*korA*" (*kil*-override).†

The 30.4- to 50.4-kb region of RK2 is not essential for maintenance in *E. coli* (13). Therefore *korA* must be in the 14- to 30.4-kb or the 50.4- to 56.4-kb region. As discussed below, it is not possible to clone the 14- to 30.4-kb region separately. However, the 50.4- to 56.4-kb segment (region II, Fig. 1) can be cloned (24), and we inserted this region into appropriate plasmid vehicles to test for *korA* function (Fig. 1).

Cells with pRK2108 (Fig. 1) were tested for the ability to be transformed by *kilA*$^+$ plasmids (Table 1). pRK2067 will not transform this strain, unlike the pRK2067–ColE1 hybrid plasmid (pRK2067.1; Fig. 1), which is not dependent on the RK2 replication function. Because pRK2067.1 is *kilA*$^+$, region II must contain the *korA* gene. These results also demonstrate that region II is not sufficient to support the replication of pRK2067.

pRK2102 (Fig. 1) contains the 0- to 8.5-kb region of RK2 and

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Abbreviations: Ap, ampicillin; Cm, chloramphenicol; Gm, gentamicin; IncP, incompatibility group P; kb, kilobase(s); Km, kanamycin; Nal, nalidixic acid; Nm, neomycin; ^{r}, resistant; ^{s}, sensitive; Sm, streptomycin; Su, sulfonamide; Tc, tetracycline; Tp, trimethoprim.

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† The nomenclature for *kil* and *kor* genes supersedes that used in a preliminary report of this work (37).

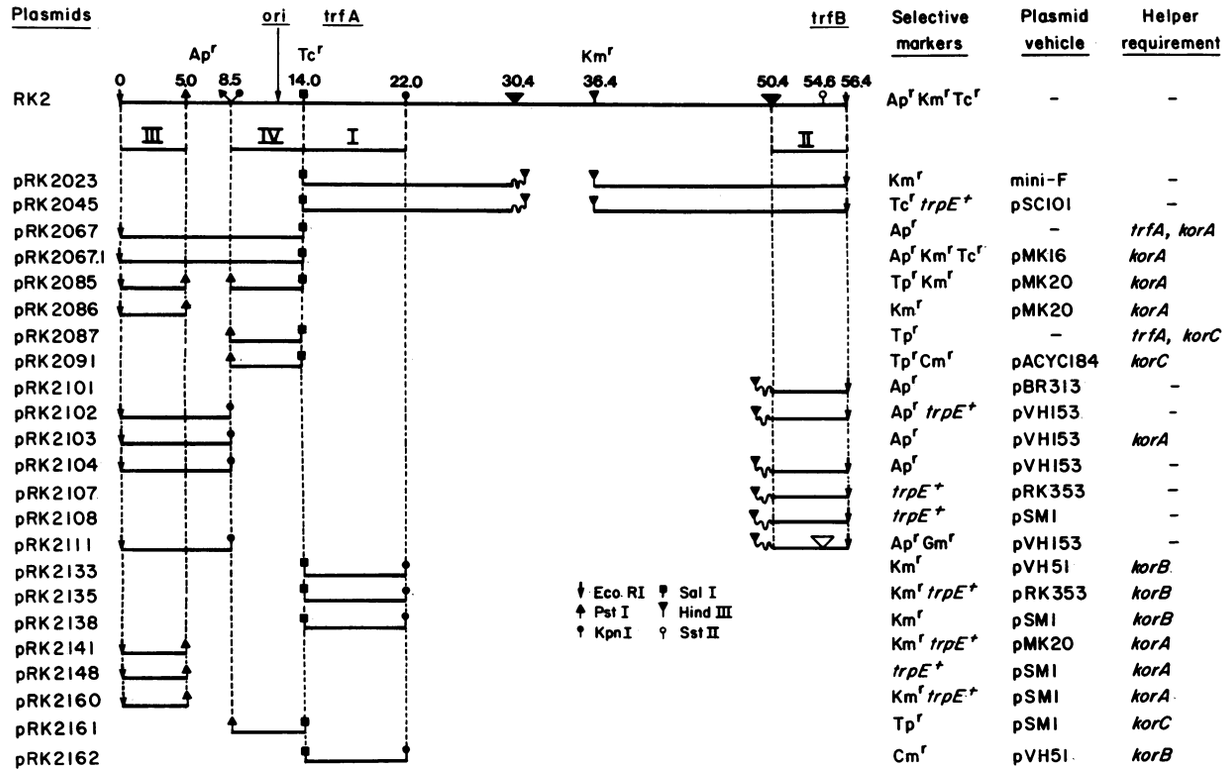

FIG. 1. Physical and genetic characteristics of plasmids derived from RK2. The RK2 map (3, 11, 14), linearized at the *Eco*RI site, shows coordinates (kb) of relevant nuclease cleavage sites. Triangles at 30.4 and 50.4 kb depict phage Mu insertions in pRK212 and pRK214, respectively (13). Regions I, II, III, IV are described in the text. Regions of RK2 in the plasmids and the cleavage sites bordering these regions are shown below the map. Mu remnants are represented by wavy lines. The triangle in pRK2111 shows the insertion of an *Sst* II Gm^r fragment (see abbreviation footnote for names of antibiotics). If no vehicle is listed, replication is from RK2 *ori*. The helper genes must be present in the cell to permit maintenance of the plasmid. pMK16 (Km^r Tc^r) and pMK20 (Km^r) (15), pVH51 (16), pVH153 (*trpE*$^+$) (17) are derivatives of ColE1. pBR313 (Ap^r Tc^r) is a ColE1-like plasmid (18). Mini-F (19), pSC101 (Tc^r) (20), pRK353 (*trpE*$^+$) (21), pSM1 (22), and pACYC184 (Tc^r Cm^r) (23) are compatible with one another and with ColE1 plasmids. RK2 derivatives described previously are pRK212.2 and pRK214.1 (13), pRK2023, pRK2045, and pRK2067 (10); and pRK2101 (24). Plasmids were constructed as follows: pRK2067.1, ligation of pRK2067 and pMK16 at their *Eco*RI sites (transformation of a pRK2045$^+$ host); pRK2102, insertion of the *Kpn* I/*Hin*dIII fragment of pRK214.1 that contains regions II and III into pVH153; pRK2103, transformation with *Eco*RI-cleaved pRK2102 (pRK2023$^+$ host); pRK2104, replacement of the *trpE*$^+$-encoding *Eco*RI/*Hin*dIII fragment of pRK2102 with the 0.3-kb *Eco*RI/*Hin*dIII fragment of pMB9 (25); pRK2107, insertion of the region II-containing *Eco*RI/*Bam*HI fragment from pRK2101 into pRK353; pRK2108, linkage of pSM1 and the region II/*trpE*$^+$-encoding *Eco*RI fragment of pRK2102; pRK2111, insertion of an *Sst* II fragment with the Gm^r of pMAC20 (26) into the *Sst* II site of pRK2104 (pRK2107$^+$ host); pRK2133 and pRK2134, linkage of the Km^r/region I-containing *Kpn* I fragment of pRK212.2 to *Kpn* I-cleaved pVH51 in opposite orientations (pRK2107$^+$ host); pRK2162, substitution of the *Eco*RI/*Hin*dIII fragment of pRK2134 by a Cm^r-encoding fragment of pKT214 (27) (pRK2108$^+$ host); pBP11 and pBP16, linkage of a Km^r-encoding *Eco*RI fragment (28) to pRK353 and pSM1, respectively; pBP13, deletion of the *Kpn* I fragment of pBP11 to give a Km^s plasmid with a single *Kpn* I site between two *Eco*RI sites (17); pRK2135, insertion of the Km^r/region I-containing *Kpn* I fragment of pRK212.2 into pBP13 (pRK2101$^+$ host); pRK2138, digestion of pRK2135 with *Eco*RI and ligation of the Km^r/region I-containing fragment to pSM1 (pRK2101$^+$ host); pRK2083, replacement of the *trpE*$^+$-encoding *Eco*RI fragment of pRK2080 (10) with one that encodes the Tp^r of pFE332 (29) (pRK2023$^+$ host); pRK2085, ligation of *Pst* I-cleaved pRK2083 and pMK20 (pRK2107$^+$ host); pRK2086, transformation with *Eco*RI-cleaved pRK2085 (pRK2107$^+$ host); pRK2141, insertion of a *trpE*$^+$-encoding fragment of pDF71 (15) at the *Pst* I site of pRK2086; pRK2148, ligation of the *trpE*$^+$/region III-containing *Eco*RI fragment of pRK2141 to pSM1 (pRK2101$^+$ host); pRK2160, insertion of the Km^r-encoding *Eco*RI fragment of pBP16 into partially digested pRK2148 (pRK2101$^+$ host); pRK2087, see text; pRK2091, ligation of pRK2087 to pACYC184 at their *Bam*HI sites (pRK2086$^+$ pRK2107$^+$ host); pRK2161, linkage of the region IV-containing *Bam*HI fragment of pRK2091 to *Bgl* II-cleaved pSM1 (pRK2104$^+$ host).

region II. To determine if *kilA* is present on pRK2102, the *korA*-containing region II was deleted. The new plasmid, pRK2103 (Fig. 1), will not transform *E. coli* cells, unless they are carrying a *korA*$^+$ helper plasmid (Table 1). Thus a gene responsible for the Kil$^+$ phenotype is in the 0- to 8.5-kb region. Because the 5.0- to 8.5-kb portion is from a transposon (38), it is not likely to contain *kilA*. This was verified with pRK2086 (Fig. 1). Therefore, *kilA* is located within RK2 region III.

***kilB* and *korB*.** To isolate the replication gene encoded by RK2 region I, we attempted to clone from pRK212.2 (10) the *Kpn* I fragment that has this region and an inserted gene for Km^r. Regardless of the plasmid vehicle used, all Km^r transformants yielded plasmids with extensive deletions in the RK2 DNA. This failure is similar to the failure to clone the 0- to 14-kb region of RK2 (10) and suggested the existence of another *kil* gene. Any putative *kor* gene would necessarily be encoded between 22 and 28.5 kb or between 50.4 and 56.4 kb on RK2 because all known RK2 derivatives that retained the 14- to 22-kb region also carried both of these regions.

Because we had constructed hybrid plasmids with the 50.4- to 56.4-kb sequences (region II), the attempt to clone region I was repeated with host cells carrying a region II-containing plasmid (pRK2107; Fig. 1). This strategy was successful. One such hybrid is pRK2133 (Fig. 1); and, as predicted, pRK2133 transforms *E. coli* cells only if a region II-containing helper plasmid is present in the cells (Table 1). Therefore, a second RK2 gene, distinct from *kilA*, is responsible for a Kil$^+$-like phenotype. This gene maps in region I and is designated "*kilB*."

The gene that controls *kilB* is in region II. Because *korA* maps in this region, we asked if *korA* itself, or another gene, is re-

Table 1. Relative transformation efficiencies by plasmid derivatives of RK2

Transforming plasmid	Resident plasmids in the recipient strain: None	pRK2045	pRK2108	pRK2045 + pRK2086	pRK2108 + pRK2086
pRK2067	<0.005	1.0	<0.005		
pRK2067.1	<0.005	1.0	1.2		
pRK2102	1.6	1.0			
pRK2103	<0.005	1.0	1.5		
pRK2086	<0.005	1.0	2.8		
pRK2133	<0.005	1.0	1.1		
pRK2087	<0.005	<0.005	<0.005	1.0	<0.005
pRK2091	<0.005	<0.005	<0.005	1.0	2.6

MV10 is the parent for all strains. Plasmid DNA was digested with restriction endonucleases that selectively cleaved the helper plasmid. The number of transformants per ml is normalized to the pRK2045 strain or the pRK2045, pRK2086 strain. The values include a correction for differences of transformation competence measured by transformation with a saturating amount of pACYC184. The greatest variation was 5-fold.

sponsible for the control of *kilB*. Region II has an *Sst* II site (Fig. 1), which we interrupted with a Gm^r-encoding fragment. pRK2111 is identical to pRK2104, except for this insertion (Fig. 1). These two plasmids were tested for their ability to control *kilA* and *kilB*.

The data in Table 2 show that, unlike pRK2104, pRK2111 cannot permit establishment of a $kilB^+$ plasmid. However, it retains a functional *korA* gene. We conclude that a gene other than *korA* is required for the control of *kilB*. We have named this gene "*korB*."

***kilC* and *korC*.** Initial attempts to clone the 8.5- to 14-kb region (region IV, Fig. 1) were unsuccessful. Because the 12- to 14-kb segment, which contains *ori*, has been cloned (39), our failure to clone the larger fragment suggested a third *kil*-like gene between 8.5 and 12 kb. The required *kor* gene would be confined to region II or region III because pRK2085 (which already carries region III) can exist in cells that have the region II plasmid, pRK2107 (Fig. 1).

pRK2085 was partially digested with *Eco*RI to delete region III. The DNA was used to transform cells carrying pRK2045 (which has not only region II but also region I to provide the RK2 replication function in *trans*) and pRK2086 (which contains region III). Plasmid DNA prepared from one of the Tp^r transformants revealed three separate plasmids: pRK2045, pRK2086, and a new plasmid, pRK2087 (Fig. 1), which consists of the two *Eco*RI fragments known to specify *ori* and Tp^r.

pRK2087 is dependent upon regions I, II, *and* III provided in *trans* (Table 1). Region I is needed for the replication function because pRK2087 can replicate only via the RK2 *ori*. This requirement was removed by linking pRK2087 to another plasmid replicon, pACYC184 (Fig. 1). The new plasmid, pRK2091, still requires helper plasmids with regions II and III. The inability to transform helperless cells by a functional replicon (Table 1) suggests that a third *kil* gene, "*kilC*," is present in region IV and is at least partly encoded by the 8.5- to 12-kb region of RK2. The need for region III indicates that "*korC*" maps in the 0- to 5-kb region. The region II requirement is probably an indirect consequence of the need to control *kilA* of region III by *korA*, although it may encode an additional determinant for *korC*.

Table 2. Relative transformation efficiencies of a pRK2111-containing strain by kil^+ plasmids

Incoming plasmid	*kil* determinant	Resident plasmid in recipient strain: None (*korA*$^-$ *korB*$^-$)	pRK2104 (*korA*$^+$ *korB*$^+$)	pRK2111
pRK2148	$kilA^+$	<0.005	1.0	1.1
pRK2138	$kilB^+$	<0.005	1.0	<0.005

The experiment was done as described for Table 1. Transformation efficiency is compared to the pRK2104 strain.

***kil* Genes Cause Loss of Cell Viability.** There are two explanations for the failure to recover transformants with kil^+ plasmids: (*i*) *kil* genes inhibit replication of any plasmid on which they reside; or (*ii*) the *kil* genes inhibit cell proliferation and the transformants fail to form colonies. To distinguish between these, each *kil* gene was linked to pSM1, which does not require *E. coli* DNA polymerase I. The *kor* genes were each inserted into ColE1-like plasmids, whose replication is strictly dependent upon DNA polymerase I (40). For each *kil* gene, the kil^+ plasmid and its corresponding kor^+ plasmid were established together at 30°C in C2107, an *E. coli polA* mutant temperature-sensitive for DNA polymerase I.

Table 3 shows the results of plating these strains at 42°C, which does not allow the kor^+ plasmid to replicate. Daughter cells with only the kil^+ plasmid are eventually generated. If the kor^+ plasmid is needed only to provide a function that allows replication or maintenance of the kil^+ plasmid, then colonies will form at 42°C in the absence of selection. These cells will have lost both plasmids. In contrast, if the *kor* function is required to prevent *kil*-mediated cell death, then no colonies will form at 42°C, even in the absence of selection. The results indicate that *kilA*, *kilB*, and *kilC* each prevent colony formation of *E. coli* when the cells are cured of the kor^+ helper plasmid. Only 4–6% of plated cells form colonies. These colonies are either missing the kil^+ plasmid (pRK2160 and pRK2161) or carry plasmids from which the *kil* region has been deleted (pRK2138) (unpublished results). Presumably, kil^- cells can emerge in the time it takes for the *kor* function to be diluted out after the shift to 42°C. Thus this experiment probably gives a minimal measurement of killing.

Table 3. Effect of kil^+ plasmids on colony-forming ability of host cells

Resident plasmids: Test plasmid	*kil*	Helper plasmid	Does helper require pol I?	Selection: None	Test plasmid	Helper plasmid
—	—	—	—	1.0	—	—
—	—	pRK2101	Yes	0.91	—	<0.005
pRK2160	$kilA^+$	pRK2101	Yes	0.04	<0.005	<0.005
pRK2138	$kilB^+$	pRK2101	Yes	0.04	0.04	<0.005
pBP16	—	pRK2101	Yes	0.92	0.99	<0.005
—	—	pRK2104	Yes	0.98	—	<0.005
pRK2161	$kilC^+$	pRK2104	Yes	0.06	<0.005	<0.005
pCY2	—	pRK2104	Yes	0.87	1.1	<0.005
—	—	pRK2130	No	1.0	—	0.67
pRK2160	$kilA^+$	pRK2130	No	0.97	1.5	1.1
pRK2138	$kilB^+$	pRK2130	No	0.78	0.70	0.96

C2107 (*polA*ts) strains were grown under selection at 30°C to 5 × 10^7 cells per ml, then plated at 30°C and 42°C on media that were nonselective, selective only for the helper plasmid, and selective only for the test plasmid. Values are the ratios of the number of colony-forming units per ml at 42°C to the number appearing at 30°C. Control plasmids are pBP16 (pSM1-Km^r), pCY2 (pSM1-Tp^r), and pRK2130 (mini-F and pRK2101 linked at their *Eco*RI sites). Like pSM1, mini-F is not dependent on DNA polymerase I (pol I).

Table 4. Relative transformation efficiencies of IncP plasmid strains with *kil*$^+$ plasmids

Plasmid*	Markers†	Original host	Geographical origin	Transforming plasmid‡	
				kilA$^+$	*kilB*$^+$
None	—	—	—	<0.005	<0.005
RK2	AKT	*Klebsiella*	England	1.00	1.00
R26	ACGKSSuTH	*Pseudomonas*	Spain	0.51	0.87
R91A	AKT	*Pseudomonas*	England	0.53	0.56
R527	ACGKSSuTH	*Serratia*	Spain	0.37	0.97
R702	KSSuT	*Proteus*	USA	2.26	1.72
R751	Tp	*Klebsiella*	England	1.10	0.92
R772	K	*Proteus*	USA	0.47	1.41
R839	AKSSuT	*Serratia*	England	0.81	0.77
R906	ASSuH	*Bordetella*	Japan	0.50	0.38
R934	AKT	*Serratia*	France	2.10	1.36
R938	ACKSSuTSp	*Serratia*	France	0.43	0.69
R995	KST	*Proteus*	Hong Kong	0.40	0.64
R1033	ACGKSSuTH	*Pseudomonas*	Spain	0.39	0.85
pJP4.1	2,4-D, HA§	*Alcaligenes*	Australia	1.02	0.74
pUZ8	KTH	*Pseudomonas*	Spain	0.93	1.38

* Plasmids were tested in DF4063. The *kilA*$^+$ plasmid was pRK2148 and the *kilB*$^+$ plasmid was pRK2135, except for R772, which was tested with pRK2162. Helper plasmids were eliminated as in Table 1. Selection was for tryptophan independence (Cm^r for pRK2162) and the underlined marker.

† A, Ap^r; C, Cm^r; G, Gm^r; H, $HgCl_2{}^r$; K, Km^r; S, Sm^r; Sp, spectinomycin^r; Su, Su^r; T, Tc^r; Tp, Tp^r; 2,4-D, metabolism of 2,4-dichlorophenoxyacetic acid.

‡ Transformation efficiency is normalized to the RK2 strain. DF4063 competence was verified.

§ Ap^r was introduced by insertion of Tn*3*.

Occurrence of *korA* and *korB* on Other IncP Plasmids. Are the *kil* and *kor* genes present on other IncP plasmids? It is possible to test for *korA* and *korB* because cloned *kilA* and *kilB* are separated from IncP incompatibility determinants. *kilC* is still linked to the *ori* region, which encodes incompatibility (9), and this prevented testing for *korC*.

We tested 14 different IncP plasmids (Table 4). Although the host specificity has not been tested for each of these, the broad host range is already reflected in the variety of bacterial species that originally hosted the plasmids. It is noteworthy that one of these, pJP4, carries no antibiotic resistance determinants but instead codes for enzymes that metabolize the pesticide 2,4-dichlorophenoxyacetic acid (2,4-D) (39). Cells carrying these IncP plasmids were transformed with *kilA*$^+$ and *kilB*$^+$ plasmids. Successful transformation is indicative of the presence of *korA*-like and *korB*-like functions in the cell. Table 4 shows that all 14 plasmids encode both of these functions.

DISCUSSION

We have found that three separate regions of RK2 encode unique plasmid genes whose functions are potentially lethal to *E. coli* host cells. Because *E. coli* is a natural host for RK2, the action of these *kil* genes must necessarily be controlled. The *kor* genes specifying these control functions map in two segments of RK2. One of the regions (50.4–56.4 kb on the map) encodes the determinants *korA* and *korB*, whose functions prevent loss of cell viability by *kilA* and *kilB*, respectively. These are probably separate genes because *korB* can be inactivated without affecting *korA*. Recent experiments place *korA* in the 54.6- to 56.4-kb region (unpublished results). *korC* maps in the 0- to 5-kb region and is specific for *kilC*. The positions and interactions of these genes are summarized in Fig. 2.

These results affect the interpretation of genetic studies defining essential plasmid genes. Previous deletion analyses have indicated that three distinct regions of RK2 are essential in *E. coli* (3, 11, 13). One region contains the RK2 *ori* (8), and another codes for a gene (*trfA*) clearly required for replication (refs. 10–12; this study, Table 1). A putative third replication gene, *trfB*, was reported to map within the region shown in this work to contain *korA* and *korB* (11). However, recent evidence gathered by Thomas (12) and us (unpublished) indicates that *trfB* is not essential for replication. This suggests that the third region may be essential only to control a *kil* gene near *trfA*. Recent results (unpublished) support this hypothesis.

In another study (3) the 0- to 6-kb region could not be deleted from RK2. Yet pRK248, a small RK2 replicon, lacks this and other regions (11). This can now be explained by the finding that *korC* maps within the 0- to 5-kb segment. Deletion of this gene alone is lethal. However, pRK248 can be maintained because it is also missing the 8.5- to 12-kb region (11), which encodes *kilC*.

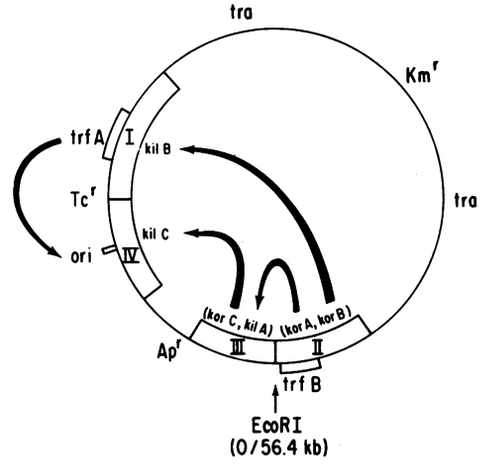

FIG. 2. Location of *kil* and *kor* genes on RK2. All genes are described in the text, except *tra* (transmissibility) (11, 13). Coordinates of regions I, II, III, and IV are in Fig. 1. The locations of *trfA*, *trfB*, and *ori* have been reported (11). Parentheses around the genes in regions II and III indicate that the relative positions of the genes are unknown. Arrows indicate interactions of RK2 functions.

The functions of the *kil* genes are unknown. They are not essential for replication in *E. coli* (ref. 11; unpublished results). We cannot exclude the possibility that the *kil* genes are derived from a cryptic phage in the plasmid, or that they code for bacteriocins, as yet undetected. However, 14 other IncP group plasmids encode *korA* and *korB* functions. (*korC* could not be tested in this system.) These seem to be specific for IncP group plasmids because none of the plasmids from 19 other incompatibility groups we recently tested showed these functions (unpublished results).

The presence of *korA* and *korB* on IncP group plasmids implies that *kilA* and *kilB* have also been conserved. Indeed, one of the plasmids tested here, R751, has been shown to have an "X" region that cannot be cloned, unless another portion of R751 ("S" region) is present in the cell (38). Although it has not been shown that the X region is inhibitory to cell proliferation, it is likely that it contains a *kil* gene and that the S region codes for the required *kor* gene.

One explanation for the ubiquity of these genes in the IncP group is that there is a need for these plasmids to retain the *kil* and *kor* genes. Kil$^-$ variants of each of the cloned *kil* genes are readily isolated. The survival of these genes in nature suggests that it is preferable to retain them. In general, plasmid functions that are highly conserved within an incompatibility group are concerned with replication and, to a lesser extent, transmissibility. *kilA* and *kilC* do not map in the regions required for RK2 self-transmissibility (10). Therefore, it is interesting to suggest that the *kil* genes of RK2, although nonessential and potentially harmful in *E. coli*, may provide functions necessary for existence in other hosts.

We thank L. Babiss, S. Malik, and C. Young, who constructed some of the plasmid intermediates in this laboratory, and H. Spivak, who assisted in some of these experiments. We are grateful to several colleagues for providing bacterial strains with various plasmids: G. Jacoby, J. Pemberton, and T. Potts, for IncP group plasmids; K. Armstrong, for pSM1; M. Bagdasarian, for pKT214; M. Fling, for pFE332; E. Lederberg, for pACYC184; and U. Streips, for pMAC20. This work was supported by National Science Foundation Grant PCM 78-08906 and National Institutes of Health Grant GM 26863 to D.F. and Cancer Center Support Grant CA 13636 to Columbia University. A.P. was a Postdoctoral Fellow and C.K. was a Predoctoral Fellow supported by National Institutes of Health Training Grant AI 07161.

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