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Entry Exclusion Determinant(s) of IncN Plasmid pKM101

STEPHEN C. WINANS AND GRAHAM C. WALKER*

Biology Department, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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pKM101 renders its host a poor recipient in conjugal matings with genetically distinguishable derivatives of itself. The gene(s) primarily responsible for this, denoted *eex*, is located in between genes required for both conjugal transfer and sensitivity to donor-specific bacteriophage, although it itself is not necessary for transfer. A gene linked to, or coincident with, the region needed for vegetative plasmid replication also inhibited establishment of related plasmids under certain conditions. Construction of an operon fusion between *eex* and the *Escherichia coli lac* promoter has shown that this gene is transcribed in a clockwise fashion on the circular map of pKM101. To date, we have not been able to visualize a protein product(s) of the *eex* gene(s).

A number of conjugal plasmids create a cell surface barrier to conjugal entry of genetically distinguishable derivatives of themselves (28). This phenomenon is designated entry exclusion or surface exclusion and is quite distinct from plasmid incompatibility, which operates at post-DNA penetration stages (24). Plasmid F codes for two such genes, *traS* and *traT*, which inhibit transfer by two independent mechanisms (1, 2). Although these genes are not themselves required for conjugal transfer, they are localized within the main transfer operon and are cotranscribed with flanking transfer genes (12). The *traT* product also plays a role in the resistance of a cell to the bactericidal effects of serum (17).

IncP plasmid RP4 has also been shown to have an entry exclusion system (11). Barth (3) screened a set of Tn*7* insertion derivatives of RP4 for entry exclusion deficiency and obtained four mutants. Three of these were closely linked and had residual levels of entry exclusion. The fourth insertion was localized about 4 kilobases (kb) away from the other three and was totally deficient. All four of these insertion derivatives were also transfer deficient. The entry exclusion-determining region of a third plasmid, R144 (a member of the I incompatibility group), has also been cloned and characterized (10).

The conjugal transfer system of IncN plasmids is being studied in a number of laboratories (7, 15, 26, 29). Such plasmids have a broad host range (25) and render their hosts sensitive to the bacteriophage IKe, PRD1, and PR4, the latter two phage also infecting cells containing IncP and IncW plasmids (5, 13). They elaborate pili of the "short brittle" type (5) and inhibit the fertility of coresident IncP plasmids (31). pKM101 was derived from the clinically isolated IncN plasmid R46 (18) by an in vivo deletion of 14 kb, which removed all of the drug and metal resistance determinants except *bla* (7, 15). pKM101 has been extensively studied due to its ability to increase its host's susceptibility to UV and chemical mutagenesis. It codes for two genes, *mucA* and *mucB*, which are analogs of the chromosomally encoded *umuD* and *umuC* genes (9, 21, 27). In the present study, we describe our analysis of its system of entry exclusion.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phages. TK610 (obtained from T. Kato) is an F^- derivative of *Escherichia coli* having the mutations *thr-1*, *leu-6*, *proA2*, *his-4*, *thi-1*, *lacY1*, *galK2*, *ara-14*, *xyl-5*, *mtl-1*, *tsx-33*, *rpsL31*, *supE44*, *ilv-325*, *uvrA6*, and *umuC36*. ES689 (obtained from E. Seigel) is a *trpA540 metE46 lacZ*(ICR36) *rpsL31* derivative of *E. coli* K-12. DB6432 (obtained from D. Botstein) is an *argE*(Am) *metB* Nal^r Rif^r Δ(*lac-pro*) *supP* derivative of *E. coli* K-12. GW4216 (provided by W. Gilbert) is a *lacI*^{q} derivative of W3110. pKB444 (provided by K. Backman) is a derivative of pBR322 which contains, in place of that plasmid's *Pvu*II site, the *E. coli lac* promoter ("P_{lac}") oriented such that transcription proceeds counterclockwise and a *Kpn*I site directly downstream from it. λ *c*I857 *b*221 *rex*::Tn*5* (Km^r) *O*am29 *P*am80 (henceforth denoted λ::Tn*5*) was obtained from M. Fox.

Mutagenesis of plasmids with Tn*5*. Transposition of Tn*5* from λ::Tn*5* to a plasmid-containing host was performed as described previously (19), except that cells were plated on LB plates containing 20 μg of kanamycin and 2.5 mM sodium pyrophosphate. A sufficient number of infected cells were plated to give rise to approximately 10^5 to 10^6 Km^r colonies per plate. After overnight growth, transductants were pooled and plasmid DNA purified from them was used to transform a recipient strain to Km^r. Virtually every colony that appeared after transformation contained a derivative of the original plasmid bearing a Tn*5* insertion.

Construction of pGW2205. pGW1653 *eex-1232*::Tn*5*, which contains three *Hin*dIII sites, was digested with *Hin*dIII and ligated under conditions favoring intramolecular ligation. This procedure resulted in isolation of pGW2205, which has a deletion of the internal *Hin*dIII fragment of Tn*5* as well as the *Hin*dIII fragment containing the pKM101 DNA to the right of the Tn*5* insertion.

Entry exclusion assays. (i) Qualitative assays. A lawn of ES689(R46) or DB6432(R46) was spread on an LB plate, and derivatives of TK610 containing no plasmid, or pKM101, or any insertion, deletion, or cloned derivative of pKM101 were applied to these plates with a toothpick. The plates were incubated at 37°C for 6 h to allow transfer of R46 to occur. They were replica plated onto media selective for Tc^r transfer.

(ii) Quantitative assays. Sm^r strains containing plasmids were subcultured 50:1 from the stationary phase into 5 ml of broth and grown to mid-log phase. To these cells was then added 50 μl of a mid-log-phase culture of DB6432(R46), and 3 to 5 ml of these cultures was collected onto membrane filters (Millipore Corp.) by vacuum filtration and placed on prewarmed LB plates. After incubation at 37°C for exactly 1 h, the filters were placed in test tubes containing 5 ml of saline, vortexed, and plated on rich media containing 500 μg

* Corresponding author.

of streptomycin per ml and tetracycline in the amounts indicated in Results.

RESULTS

pKM101-mediated superinfection inhibition. As a first step in determining whether pKM101 can inhibit the conjugation-mediated establishment of related plasmids, we measured the efficiency of transfer of R46 (the Tc^r parent of pKM101) from DB6432 into strains TK610 and TK610(pKM101). These matings were carried out on Millipore filters with the recipient present in 100-fold excess over the donor. Since DB6432 is Sm^s and TK610 is Sm^r, selection for transconjugants was made by plating cells on rich media containing 500 μg of streptomycin and 2 μg of tetracycline per ml. R46 itself codes for resistance to low levels of streptomycin, but it cannot protect its host from the high levels used in these experiments.

In these matings, the recipient strain not containing any plasmid gave rise to over 11 transconjugants per donor per hour, whereas a recipient containing pKM101 gave rise to only 3.1×10^{-3} transconjugants per donor per hour. The degree to which the presence of pKM101 in the recipient interfered with mating is referred to as "superinfection inhibition" (SI) (20) and is expressed quantitatively as the number of transconjugants obtained per donor per hour with a plasmid-free recipient divided by the number obtained with a recipient containing pKM101 (or other plasmid). This "SI coefficient" provides a measure of the proficiency with which the plasmid in question can interfere with the conjugal entry and subsequent establishment of R46 in the recipient. Under the conditions used in this experiment, a strain containing pKM101 has an SI coefficient of 3,600.

Table 1 shows the ability of pKM101 and a set of deletion derivatives (shown in Fig. 1) to prevent establishment of R46. Transconjugants were selected with 2 μg of tetracycline per ml. It is apparent that, under these conditions, all deletion derivatives tested were almost completely deficient in this property, suggesting that a locus involved in SI lay within the region deleted by all of these derivatives. This conclusion is confirmed below.

Table 1 also shows the SI coefficients of the same plasmids when transconjugants were selected with higher levels of tetracycline. Under these conditions, each of the deletion derivatives retained a residual level of SI. The region of pKM101 required for the additional SI observed at high tetracycline levels was coincident with Rep, the region of pKM101 required for vegetative replication (15). A possible explanation for the SI mediated by the Rep region is considered below.

Localization of the *eex* locus, using cloned fragments. The observation (Table 1) that pGW279 was deficient in the SI observed at low tetracycline levels suggested that a locus involved in this phenomenon lay within the region of DNA deleted in this plasmid. We therefore measured the SI coefficient of a number of plasmids containing cloned pKM101 fragments that were derived from this region. We have shown that the gene(s) responsible lies wholly within a 0.7-kb sequence flanked by insertions *traC1134*::Tn*5* and *traD1141*::Tn*5* (Fig. 2). This can be illustrated by comparing four derivatives of plasmid pKB444 which contain cloned pKM101 fragments (Fig. 2).

The first pair of pKB444 derivatives, pGW1652 and pGW1653, contain pKM101 DNA from the *Kpn*I-1 site of pKM101 to the proximal *Hin*dIII sites of the Tn*5*'s of *traC1134*::Tn*5* and *traD1141*::Tn*5*, respectively. Therefore, pGW1653 contains the same pKM101 DNA as pGW1652 and an additional 0.7-kb sequence to the right. pGW1653 had an SI coefficient of 3,600, whereas pGW1652 had an SI coefficient of 0.9.

TABLE 1. Effect of tetracycline concentration on ability of pKM101 and its deletion derivatives to inhibit establishment of R46[a]

Plasmid	Amt of tetracycline used to select transconjugants (μg/ml)	Transconjugants per donor-h	SI coefficient[b]
None	2	11.4	1
pKM101	2	3.1×10^{-3}	3,600
pGW279	2	3.1	3.6
pGW278	2	3.4	3.3
pGW271	2	4.1	2.8
pGW268	2	6.2	1.8
pGW281	2	5.5	2.1
None	8	6.2	1
pKM101	8	1.6×10^{-3}	3,900
pGW279	8	7.2×10^{-2}	86
pGW278	8	1.2×10^{-1}	50
pGW271	8	9.3×10^{-2}	67
pGW268	8	1.0×10^{-1}	62
pGW281	8	1.0×10^{-1}	60
None	32	8.6×10^{-2}	1
pKM101	32	1.0×10^{-6}	87,000
pGW279	32	$<3.4 \times 10^{-6}$	>10,000
pGW278	32	3.8×10^{-5}	2,300
pGW271	32	1.4×10^{-5}	6,300
pGW268	32	$<3.4 \times 10^{-6}$	>10,000
pGW281	32	$<3.4 \times 10^{-6}$	>10,000

[a] DB6432(R46) was used to introduce R46 via conjugation into TK610 containing the plasmid indicated. Recipients were present in 100-fold excess. After 1 h of mating on Millipore filters, cells were vortexed into saline and plated on rich media containing 500 μg of streptomycin per ml and tetracycline at the levels indicated.

[b] Expressed as the number of transconjugants per donor obtained with a plasmid-free recipient divided by the number obtained with a recipient containing the plasmid indicated.

The second pair of pKB444 derivatives, pGW1655 and pGW1664, contain pKM101 sequences from the *Kpn*I-2 site of pKM101 to the *Bam*HI sites of the Tn*5*'s of *traC1134*::Tn*5* and *traD1141*::Tn*5*, respectively (Fig. 2). Strains containing either of these plasmids also contain pGW2130 (Fig. 1), which is a derivative of pACYC184 containing pKM101 DNA between the *Sal*I-1 site and the proximal *Hin*dIII fragment of *traA1114*::Tn*5* cloned into the gap in the vector created by digestion with the same two enzymes. The presence of pGW2130 was necessary because pGW1655 and pGW1664 contain *kilB*, a gene coding for a product which is potentially lethal to the host and whose effect is suppressed by pGW2130 (30). pGW1655 has an SI coefficient of 54,000, whereas pGW1664 had an SI coefficient of 5, confirming that the locus responsible for entry exclusion lies between *traC1134*::Tn*5* and *traD1141*::Tn*5*. This locus has been designated *eex* (for entry exclusion).

SI coefficient of pKM101 derivatives having Tn*5*'s in or near *eex*. Since no Tn*5* insertion had been isolated in pKM101 lying within this 0.7-kb region, we isolated such an insertion in pGW1653 (insertion *eex-1232*::Tn*5*) and crossed it into pKM101 by homologous recombination (Winans et al., J. Bacteriol., in press). pKM101 *eex-1232*::Tn*5* was as deficient in SI as the eex^- deletion pGW279 (Table 2), whereas all other Tn*5* insertions in that region of pKM101 that we tested

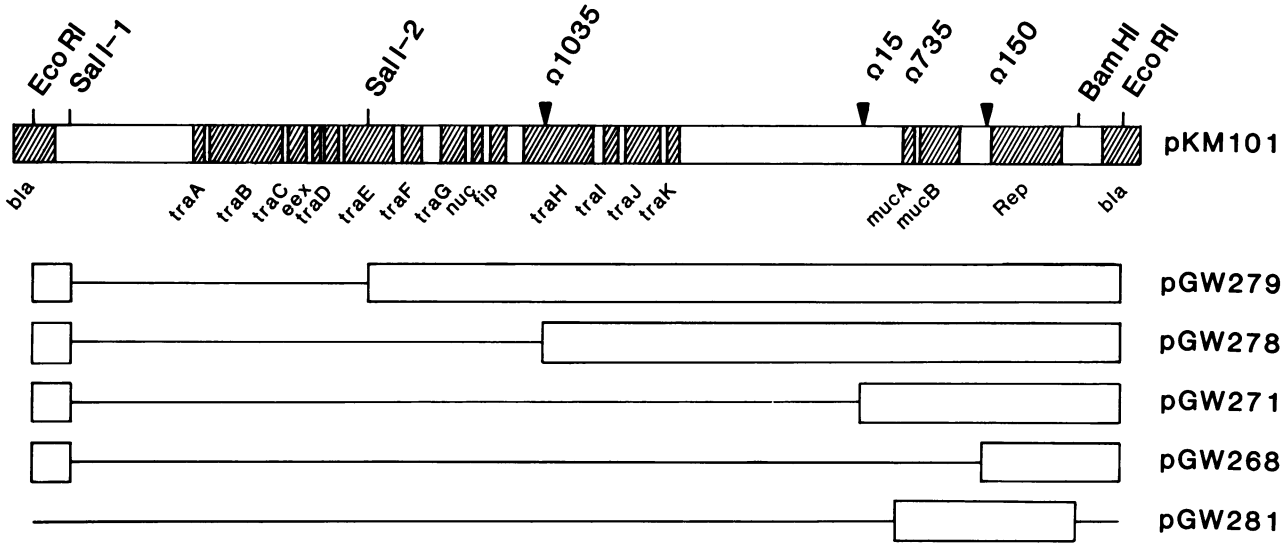

FIG. 1. Deletion derivatives of pKM101 tested for their ability to render their hosts inefficient conjugal recipients in matings with R46. Strains carrying no plasmid, or pKM101, or one of these deletion derivatives were used as conjugal recipients of R46 as described in the text. Open boxes refer to pKM101 sequences present in the deletion derivatives; horizontal lines indicate regions deleted. Construction of these deletion derivatives is described by Langer et al. (15).

were proficient in SI. pKM101 *eex-1232*::Tn*5* is about 4-fold deficient in conjugal transfer compared with pKM101 itself, whereas all neighboring Tn*5* insertions are at least 10^6-fold deficient (29).

pGW1653 can block the entry of R46 only if the latter plasmid is introduced by conjugation; it has no effect on entry of R46 mediated by transformation (data not shown). Furthermore, pGW1653 does not affect the stability of a coresident R46 to any degree greater than the vector pKB444 (data not shown). We have also observed that the efficiency of transfer of R46 is not decreased by preincubating either the donor or the recipient with a strain containing pGW1653. Therefore, *eex*-containing strains protect only themselves from entry by R46; they cannot confer protection upon neighboring cells. These results suggest that the *eex* gene product(s) acts in some fashion to block the effective conjugal transfer of plasmid DNA.

The direction of transcription of *eex* is clockwise. Plasmid pKB444 used to create pGW1653 and pGW1655 has a P_{lac} promoter adjacent to its *Kpn*I site. This promoter reads toward the *eex* gene in both clones (Fig. 2). We transformed these two plasmids into GW4216 (a *lacI*-overproducing strain) and measured the ability of these plasmids to mediate entry exclusion in the presence or absence of the gratuitous

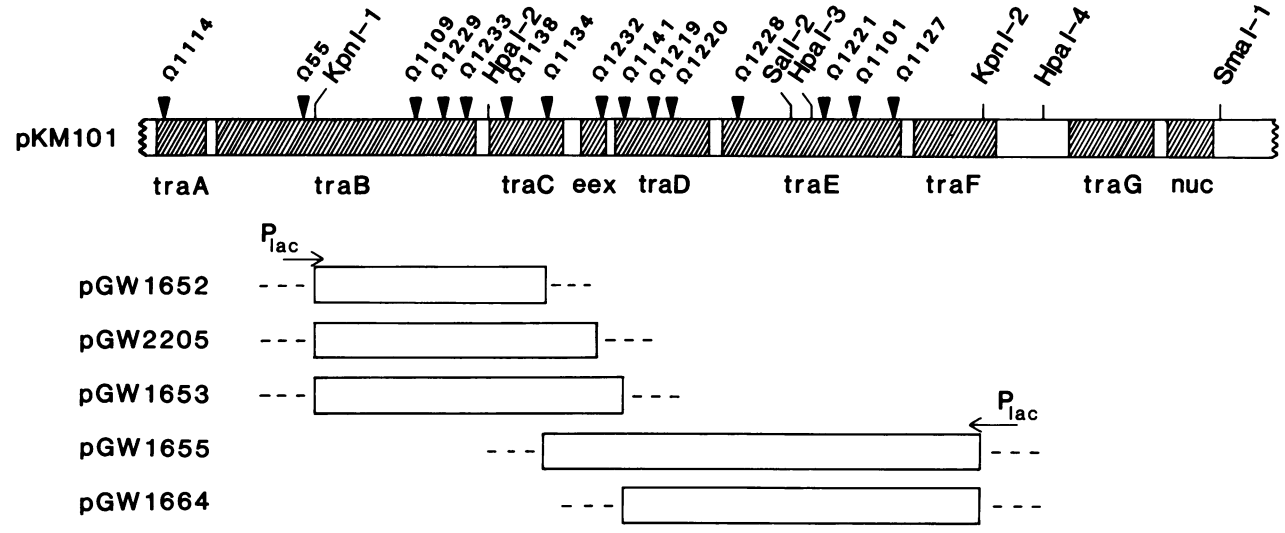

FIG. 2. Localization of *eex*, using cloned fragments of pKM101. Open bars depict pKM101 sequences present on each plasmid. Horizontal dashed lines indicate vector DNA or Tn*5* DNA present in cloned derivatives. pGW1652, pGW2205, pGW1653, pGW1655, and pGW1664 are derivatives of pKB444 containing pKM101 DNA from the *Kpn*I-1 or *Kpn*I-2 site of pKM101 to the points of insertion of *traC1134*::Tn*5*, *eex-1232*::Tn*5*, or *traD1141*::Tn*5*, as shown. Plasmids pGW1652, pGW2205, and pGW1653 make use of the proximal *Hin*dIII sites of their respective Tn*5*'s and are therefore Km^s. pGW1655 and pGW1664 make use of the *Bam*HI sites of the two Tn*5*'s and, due to the orientation of these transposons, both clones include the *neo* gene of Tn*5* and therefore render their host Km^r.

TABLE 2. Ability of derivatives of pKM101 having Tn5 insertions in or near the *eex* locus to render their hosts poor recipients in matings with R46

Plasmid	Location (kb)[a]	SI coefficient[b]
None		1[c]
pKM101		6,400
pGW279[d]		5.1
Insertion derivatives of pKM101		
traB55	6.6	1,300
traB1109	7.55	1,000
traB1229	7.8	3,900
traB1233	8.0	9,300
traC1138	8.4	19,000
traC1134	8.75	74,000
eex-1232	9.25	6.1
traD1141	9.4	4,200
traD1219	9.6	6,800
traD1220	9.7	11,000
traE1228	10.3	31,000
traE1221	11.0	25,000
traE1101	11.35	8,200
traE1127	11.7	22,000

[a] Position to the right of the *Eco*RI site of pKM101 as in Fig. 1.
[b] The number of transconjugants obtained with a plasmid-free recipient divided by the number obtained with a recipient containing the plasmid indicated in column 1.
[c] A total of 11.2 transconjugants per donor-h were obtained with a plasmid-free recipient.
[d] A derivative of pKM101 deleted for pKM101 DNA between the *Sal*I-1 and *Sal*I-2 sites (see Fig. 1).

inducer isopropyl-β-D-thiogalactopyranoside (IPTG). GW4214(pGW1653) had an SI coefficient of 24 in the absence of IPTG, whereas in the presence of IPTG it had an SI coefficient of 3,400, or 150-fold higher (Table 3). In contrast, the SI coefficient of GW4216(pGW1655) was unaffected by IPTG. The IPTG-mediated induction of the *eex* gene(s) of pGW1653 indicates that an operon fusion had indeed been created in this plasmid and, therefore, that the direction of transcription of *eex* is clockwise on the pKM101 circular map (or from left to right on the linear map in Fig. 2). The lack of induction of *eex* in pGW1655 is consistent with this conclusion. Whatever the mechanism of action of the *eex* gene product(s), it is evident that increased amounts of it result in stronger expression of the phenotype.

Attempts to visualize the product of the *eex* gene. We have made a preliminary attempt to identify a putative protein product or products of the *eex* locus by means of the maxicell technique for radiolabeling plasmid-coded proteins (23). Proteins encoded by pKB444, pGW1653, pGW2205, and pGW1652 are shown in Fig. 3. pGW2205 was constructed from pGW1653 *eex-1232*::Tn5 by deletion of two of the three *Hin*dIII fragments of its parent, as described in Materials and Methods, and is entry exclusion deficient. Since pGW1653 is entry exclusion proficient, yet contains only 0.2 kb of additional pKM101 DNA, any protein expressed by pGW1653 and not by pGW2205 would be a good candidate for an *eex* product. Radiolabeling was performed in the presence of 1 mM IPTG to enhance expression of *eex* from the P_{lac} promoter. There are no apparent differences in the proteins coded by pGW1653 (Fig. 3, lane 2) and pGW2205 (Fig. 3, lane 3). Since it seemed possible that the protein might have been sufficiently small to run off the bottom of the gel shown in Fig. 3, we examined the same extracts by using a 16% acrylamide gel and conducted the electrophoresis for a sufficiently short time to allow visualization of extremely small proteins. Even with this gel, no difference between pGW1653 and pGW2205 was detected (data not shown).

Both pGW1653 and pGW2205 (as well as pGW1653 *eex-1232*::Tn5) strongly express a 25,000-dalton protein which is not expressed by pGW1652. Since pGW1652 was constructed with *traC1134*::Tn5, it ought to lack a portion of the *traC* gene. In contrast, pGW1563 and pGW2205 should contain the entire *traC* gene. Therefore, the 25,000-dalton protein expressed by these two plasmids and not expressed by pGW1652 may be the product of the *traC* gene. We have speculated elsewhere that *traC* may encode the putative subunit protein of the plasmid's sex pilus (29).

TABLE 3. IPTG inducibility of the *eex* locus of pGW1653

Plasmid	IPTG[a]	Transconjugants per donor-h	SI coefficient
None	−	2.1×10^{1}	1
None	+	3.3×10^{1}	0.6
pGW1653	−	8.7×10^{-1}	24
pGW1653	+	6.4×10^{-3}	3,400
pGW1655[c]	−	5.9×10^{-2}	360
pGW1655	+	5.6×10^{-2}	380

[a] "+" and "−" indicate that cultures were grown to mid-log phase before mating in the presence or absence of 1 mM IPTG.
[b] Strain containing pGW1655 also contained pGW2130 to inhibit lethality of the *kilB* gene of pGW1655.

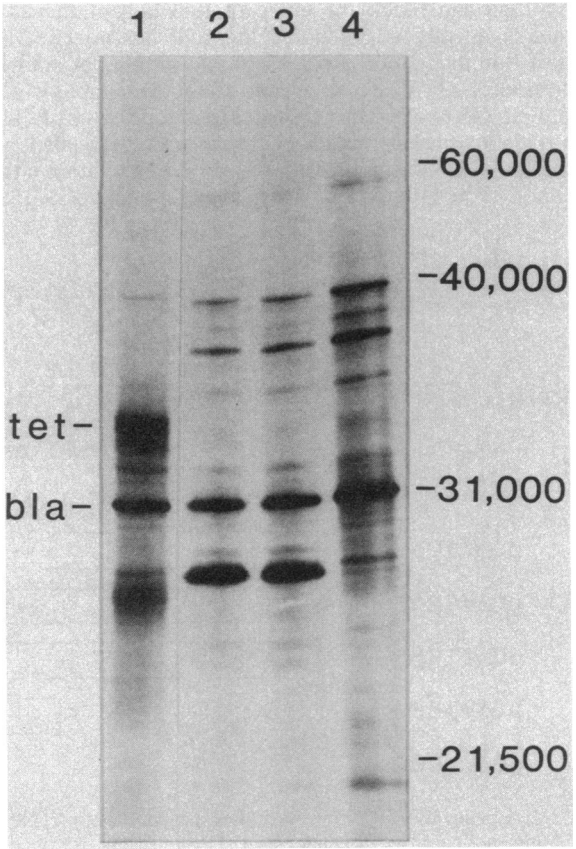

FIG. 3. Radiolabeling of plasmid-coded proteins, using the maxicell technique. Labeling occurred in the presence of 10^{-3} M IPTG. Lane 1, pKB444; lane 2, pGW1653; lane 3, pGW2205; lane 4, pGW1652.

DISCUSSION

In the present study, pKM101 is shown to confer upon its host a deficiency in the ability to act as a conjugal recipient of R46. Two pKM101 loci appear to be involved in this process. One of these loci (*eex*) was localized to a 0.7-kb region of DNA that lies between *traC* and *traD*. The *eex* gene is not required for conjugal transfer. Insertions on either side of *eex* are not strongly polar upon it and, similarly, an *eex*::Tn*5* insertion is not strongly polar upon nearby transfer genes. *eex* is transcribed in a clockwise fashion on the circular map of pKM101. Whatever the mechanism of action of the *eex* gene product(s), it seems that it must block the effective conjugal transfer of the plasmid.

Since Tn*5* is usually strongly polar on downstream genes (14), it seems likely that *eex* may be expressed from a promoter contained within this 0.7-kb region. Furthermore, *eex-1232*::Tn*5* is not polar on flanking *tra* genes, a result suggesting that none of the genes mediating Tra functions is dependent on the putative *eex* promoter. However, it is important to keep in mind that Tn*5* is not always strongly polar (4). In the case of Tn*10*, polarity is dependent upon the presence of a *rho*-dependent termination site lying between the insertion and the gene whose expression is blocked (8). If pKM101 lacks such a site near *eex*, then its expression might not be decreased by Tn*5* insertions lying between it and its promoter.

eex is localized between two groups of genes required for conjugal transfer and for sensitivity to donor-specific phage (Fig. 1) (29). Since these donor-specific phage bind to the sex pilus (5), we have speculated that these two groups of genes may be required for pilus synthesis or retraction. The analogous F-encoded proteins *traS* and *traT*, in contrast, are localized adjacent to, rather than among, the analogous group of F-coded pilus genes. This is one of the several ways in which the transfer region of pKM101 differs from that of F. It is premature to speculate as to whether the mode of action of *eex* is the same as that of *traS* and *traT*.

There are several possible reasons for our inability to visualize an *eex* product. It is possible that the putative *eex* protein is highly unstable in maxicells or that it comigrates with the *bla* gene product or with the 25,000-dalton protein. It also is possible that the mature *eex* product does not contain any methionine residues or that it does but is expressed in extremely small amounts. A final intriguing possibility is that the *eex* gene product is not a protein at all. It is formally possible, for example, that an RNA molecule might be able to interact with entering plasmid DNA in some fashion such that effective establishment of the plasmid is prevented.

The second locus involved in SI is linked to Rep, a region of pKM101 which is necessary and sufficient for vegetative replication of the plasmid (15). The effect of this region was seen only when transconjugants were selected with relatively high levels of tetracycline. Since the Rep region contains the origin of replication, it probably also contains loci controlling plasmid copy number, since in all or most plasmids so characterized loci controlling copy number usually are closely linked or coincident with the origin (24). Therefore, when R46 entered a cell which already contained a plasmid containing Rep, its replication would probably have been inhibited and its copy number would have remained initially at one per cell. The normal copy number of R46 has not been carefully measured, but the amount of DNA routinely recovered from lysed cultures indicates that its copy number is approximately 10 per cell. It is not unlikely that when R46 entered the plasmid-free recipient it would have replicated quickly until it reached this higher copy number. If both of these conjectures are true, then a recipient of R46 which had previously been plasmid-free would, shortly after mating, contain 10-fold more copies of R46 than a recipient which had previously harbored a plasmid containing Rep and would, therefore, probably confer resistance to higher levels of tetracycline.

Although in certain respects the transfer system of pKM101 appears somewhat similar to that of RP4 (6), their systems of entry exclusion seem quite different. Four entry exclusion-deficient mutants of RP4 have been obtained by transposon insertion mutagenesis (3). These lie in two distinct loci rather than the single locus of pKM101. Furthermore, all four of these insertions are *tra*$^-$, whereas the *eex*$^-$ insertion in pKM101 is *tra*$^+$.

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LITERATURE CITED

1. **Achtman, M., N. Kennedy, and R. Skurray.** 1977. Cell-cell interactions in conjugating *Escherichia coli*: role of *traT* protein in surface exclusion. Proc. Natl. Acad. Sci. U.S.A. **74:** 5104–5108.
2. **Achtman, M., P. A. Manning, B. Kusecek, B. Schwuchow, and N. Willetts.** 1980. A genetic analysis of F sex factor cistrons needed for surface exclusion in *Escherichia coli*. J. Mol. Biol. **138:**779–795.
3. **Barth, P. T.** 1979. RP4 and R300B as wide host-range plasmid cloning vehicles, p. 399–410. *In* K. N. Timmis and A. Puhler (ed.), Plasmids of medical, environmental, and commercial importance. Elsevier/North-Holland Biomedical Press, Amsterdam.
4. **Berg, D., A. Weiss, and L. Crossland.** 1980. Polarity of Tn*5* insertion mutations in *Escherichia coli*. J. Bacteriol. **142:** 439–446.
5. **Bradley, D. E.** 1979. Morphology of pili determined by the N incompatibility group plasmid N3 and interaction with bacteriophage PRD1 and IKe. Plasmid **2:**632–636.
6. **Bradley, D. E., and E. L. Rutherford.** 1975. Basic characterization of a lipid-containing bacteriophage specific for plasmids of the P, N and W incompatibility groups. Can. J. Microbiol. **21:**152–163.
7. **Brown, A. M. C., and N. S. Willetts.** 1981. A physical and genetic map of the Inc N plasmid R46. Plasmid **5:**188–201.
8. **Ciampi, M. S., M. S. Schmid, and J. R. Roth.** 1982. Transposon Tn*10* provides a promoter for transcription of adjacent sequences. Proc. Natl. Acad. Sci. U.S.A. **79:**5016–5020.
9. **Elledge, S. J., and G. C. Walker.** 1983. Proteins required for ultraviolet light and chemical mutagenesis. Identification of the products of the *umuC* locus of *Escherichia coli*. J. Mol. Biol. **164:**175–192.
10. **Hartsheerl, R. A., J. E. N. Bergmans, M. C. Kamp, and W. P. M. Hoekstra.** 1983. Cloning of an exclusion-determining fragment of the IncI plasmid, R114. Plasmid **10:**11–20.
11. **Hedges, R. W., and A. E. Jacob.** 1974. Transposition of ampicillin resistance from RP4 to other replicons. Mol. Gen. Genet. **132:**31–40.
12. **Helmuth, R., and M. Achtman.** 1975. Operon structure of DNA transfer cistrons on the F sex factor. Nature (London) **257:**652–656.
13. **Khatoon, H., R. V. Iyer, and V. N. Iyer.** 1972. A new filamentous bacteriophage with sex-factor specificity. Virology **48:**145–155.
14. **Kleckner, N.** 1977. Translocatable elements in procaryotes. Cell **11:**11–23.
15. **Langer, P. J., W. G. Shanabruch, and G. C. Walker.** 1981. Functional organization of plasmid pKM101. J. Bacteriol.

145:1310–1316.

16. **Manning, P. A., and M. Achtman.** 1979. Cell to cell interactions in conjugating *Escherichia coli*: the involvement of the cell envelope, p. 409–447. *In* M. Inouye (ed.), Bacterial outer membrane biogenesis and functions. John Wiley & Sons, Inc., New York.
17. **Moll, A., M. A. Manning, and K. N. Timmis.** 1980. Plasmid-determined resistance to serum bactericidal activity. II. A major outer membrane protein, TraTp, is responsible for plasmid-specified serum resistance in *Escherichia coli*. Infect. Immun. **28**:359–367.
18. **Mortelmans, K. E., and B. A. D. Stocker.** 1979. Segregation of the mutator property of plasmid R46 from its ultraviolet-protecting property. Mol. Gen. Genet. **167**:317–327.
19. **Mulligan, J. T., W. Margolin, J. H. Kreuger, and G. C. Walker.** 1982. Mutations affecting regulation of methionine biosythetic genes isolated by use of *met-lac* fusions. J. Bacteriol. **151**:609–619.
20. **Novick, R. P., R. C. Clowes, S. N. Cohen, R. Curtiss III, N. Datta, and S. Falkow.** 1976. Uniform nomenclature for bacterial plasmids: a proposal. Bacteriol. Rev. **40**:168–189.
21. **Perry, K., and G. C. Walker.** 1982. Identification of plasmid (pKM101)-coded proteins involved in mutagenesis and UV resistance. Nature (London) **300**:278–280.
22. **Rambach, A., and D. Hogness.** 1977. Translation of *Drosophila melanogaster* sequences in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**:5041–5045.
23. **Sancar, A., R. P. Wharton, S. Saltzer, V. M. Kacinski, N. D. Clark, and W. D. Rupp.** 1981. Identification of the *uvrA* gene product. J. Mol. Biol. **148**:45–62.
24. **Scott, J. R.** 1984. Regulation of plasmid replication. Microbiol. Rev. **48**:1–23.
25. **Tardiff, G., and R. B. Grant.** 1980. Characterization of the host range of the N plasmids, p. 351–359. *In* C. Stuttard and K. R. Rozee (ed.), Plasmids and transposons: environmental effects and maintenance mechanisms. Academic Press, Inc., New York.
26. **Thatte, V., and V. N. Iyer.** 1983. Cloning of a plasmid region specifying the N transfer system of bacterial conjugation in *Escherichia coli*. Gene **21**:227–236.
27. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48**:60–93.
28. **Willetts, N. S., and R. Skurray.** 1980. The conjugation systems of F-like plasmids. Annu. Rev. Genet. **14**:41–76.
29. **Winans, S. C., and G. C. Walker.** 1985. Conjugal transfer system of the IncN plasmid pKM101. J. Bacteriol. **161**:402–410.
30. **Winans, S. C., and G. C. Walker.** 1985. Identification of pKM101-encoded loci specifying potentia-ly lethal gene products. J. Bacteriol. **161**:417–424.
31. **Winans, S. C., and G. C. Walker.** 1985. Fertility inhibition of RP1 by IncN plasmid pKM101. J. Bacteriol. **161**:425–427.