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Location on RP4 of a Tellurite Resistance Determinant Not Normally Expressed in IncPα Plasmids

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The tellurite resistance (Te^r) determinant of RP4 is not normally expressed unless variants are selected on medium containing tellurite. The determinant was mapped in the variant plasmid RP4Te^r by Tn*7* insertion mutagenesis. Based on a 56.4-kilobase (kb) replicon, it covered the region from 56 kb, across the *Eco*RI site at 0 kb, to 1.5 kb.

Bacterial resistance to tellurium in the form of tellurite (TeO_3) is generally plasmid mediated (11) but comparatively rare. Until recently the phenotype was restricted in *Escherichia coli* K-12 to H complex plasmids (4, 15, 17) and one unclassified plasmid (7), but it has now been found in the majority of IncP plasmids (3). IncPα plasmids (6, 19) do not express transferable tellurite resistance (Te^r) unless bacteria are grown on plates containing tellurite; IncPβ plasmids do not express it at all (3).

The specific mutation in RP4 to generate RP4Te^r is unknown. The restriction maps of RP4 and RP4Te^r appear identical, and there were no obvious inversions, deletions, or duplications in the latter (D. E. Taylor, unpublished data). Moreover, RP4Te^r specifies stable constitutive expression of Te^r. To determine the location of the normally unexpressed Te^r determinant on the RP4 map, we used Tn*7* insertion mutagenesis. We started with *E. coli* J62::Tn*7*(RP4Te^r) from which small loopfuls of different clones were mixed in 0.1 ml of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) on a nutrient agar plate with approximately an equal quantity of *E. coli* JE2571-2 (Leu^- Thr^- Str^r Rif^r). After overnight incubation at 30°C, the mixture was streaked for single colonies on tryptic soy agar with rifampin (200 μg/ml)–streptomycin (200 μg/ml)–trimethoprim (10 μg/ml). Eight independent Tn*7* insertion mutants in which the phenotype of RP4Te^r became tellurite susceptible were obtained from 600 RP4::Tn*7* derivatives isolated. Insertion of Tn*7* in RP4 to produce mutants 1 to 7 rendered RP4 incapable of regenerating Te^r when replated on tellurite-containing medium. However, the other RP4::Tn*7* derivative (mutant 8) was able to grow on tellurite after reexposure to the antimetabolite.

DNA was isolated from the eight RP4::Tn*7* plasmids as described previously (16, 20). Restriction endonucleases *Eco*RI, *Kpn*I, *Pst*I, *Sma*I, *Sst*II, and *Xho*I (Boehringer Mannheim Biochemicals, Montreal, Quebec, Canada) were used for mapping, both singly and in combinations. Phage lambda DNA digested with *Hin*dIII (8) was used as a size standard.

DNA from the eight plasmids was analyzed by restriction endonuclease digestion to give the location of the Te^r determinant (Fig. 1). Our results are based on the restriction map of Thomas (18) with a size of 56.4 kilobases (kb) (Fig. 1a) rather than that of Lanka and co-workers (12) at 60 kb because the locations of cloned RP4 fragments provided by Figurski et al. (10) were based on the former (see below). The position of the Tn*7* insertion site with respect to the *Sma*I site at 1.2 kb on the RP4 map was determined by digestion with *Sma*I, which does not cut Tn*7*. *Eco*RI digestion was used to determine the insertion site of Tn*7* with respect to the *Eco*RI site at 0 kb, taking into consideration that *Eco*RI cuts Tn*7* into two segments. The smaller one (5.0 kb) contained drug resistance genes, and the larger one (9.0 kb) contained transposon functions (5). All eight Tn*7* insertion mutants of RP4Te^r contained Tn*7* in the alpha orientation; i.e., the end closest to the *Eco*RI site was the clockwise end of the inserted transposon. Barth and Grinter (1) showed that Tn*7* inserted preferentially in this orientation in RP4. From the restriction endonuclease analysis we concluded that the Tn*7* insertion sites extended from 56.0 kb, across the *Eco*RI site at 0 kb, to 1.5 kb (Fig. 1).

Plasmids of the P group, unlike those of most other groups, are able to transfer to and replicate in a wide range of gram-negative bacterial species (13). This broad host range phenotype is likely to be controlled by special plasmid-encoded maintenance genes. The P group plasmids also specify unusual *kil* determinants (e.g., *kilA*) which are lethal for host cells. Other kill override genes (e.g., *korA*) prevent the lethal action of *kil* genes (10). These genes appear to be an essential feature of the P group plasmid replication mechanism. The Te^r determinant appeared to be located between the *korA* and *kilA* genes (Fig. 1b).

To confirm the above results, we tested the Te^r-generating ability of several cloned fragments of RK2 (=RP4) which were isolated from the *korA* or *kilA* region by Figurski and co-workers (10). All clones were tested in *E. coli* MV10, a Trp^- derivative of C600 (9). Table 1 shows that strain MV10(RK2) generated Te^r clones when plated on tellurite medium. Likewise, pRK2102, whether extrachromosomal or integrated, generated stable Te^r. However, when pRK2102 had the portion between 50.4 and 56.4 kb (=0 kb, the *Eco*RI site) inverted (fragment pRK2104), Te^r was not generated. We presumed that the reversal had cleaved the Te^r determinant, which must, therefore, have spanned the *Eco*RI site of the plasmid as indicated by the Tn*7* insertion described above. pRK2108 (50.4 to 56.4 kb) did not generate Te^r, confirming that DNA clockwise from the *Eco*RI site was necessary for generation of Te^r.

An unusual feature of the Te^r determinant is that it is silent, presumably requiring some specific mutation event before it is expressed. It must, therefore, be regulated in some way, but we have not yet identified the mechanism.

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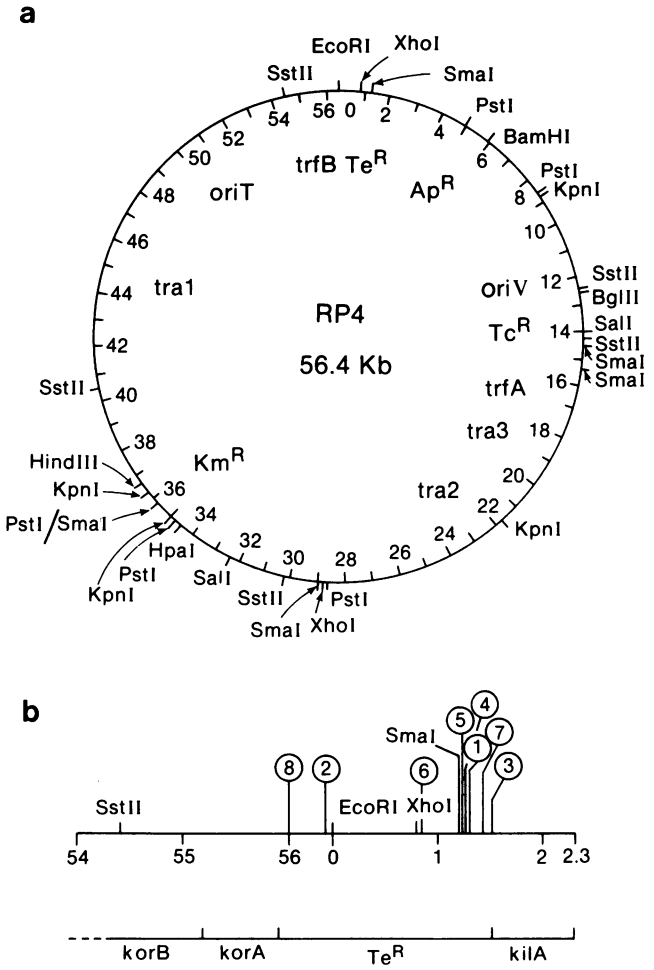

FIG. 1. Location of Te^r determinant: approximate position on restriction map of RP4 (=RP1, RK2) by the coordinates of Thomas (18) is shown in the circular map (a). The linear map (b) shows the position of the Te^r determinant relative to adjacent genes; numbers in circles represent points at which Tn*7* inserted into RP4Te^r to form respective Te^s mutants (see the text).

Seven of eight Tn*7* insertion mutants of RP4 Te^r which had lost Te^r became incapable of expressing Te^r after growth on tellurite. These mutants were located between coordinates 56.3 and 1.5. In contrast, mutant 8 (Fig. 1b) at 56 kb was still able to express Te^r after growth on tellurite. Perhaps Tn*7* in mutant 8 is inserted into a regulatory locus, the positive function of which is required for constitutive Te^r. The site of Tn*7* insertion in mutant 8 is very close to the *korA* gene, and it is possible that the *korA* region is involved in some fashion in regulation of Te^r. The Te^r determinant appears to constitute a transposable element since it can be translocated from RP4Te^r to other plasmids including the F factor (D. E. Bradley and D. E. Taylor, FEMS Microbiol. Lett., in press). Efforts are in progress to measure the putative transposon and prepare a restriction map. It may seem peculiar that resistance to an apparently uncommon element such as tellurium should be determined at all, but in fact tellurium is widespread in nature. Small amounts have been found in soil, plants, and humans (14). Although the ubiquitous nature of tellurium may have selected for Te^r carried by P group plasmids, the reason that this determinant normally remains unexpressed in IncPα plasmids is not yet understood.

TABLE 1. Generation of Te^r by cloned fragments of the RK2 (=RP4) replicon

Strain and plasmid	Location of fragment on RK2[a]	No. of colonies on tellurite medium[b]	No. of colonies surviving on tellurite medium after four subcultures[c]
MV10		1	1
MV10(RK2)	0–56.4	200	15
MV10(pRK2102)	50.4–0–8.5	200	20
MV10.pRK2102Cm[d]	50.4–0–8.5	100	12
MV10(pRK2104)	56.4–50.4; 0–8.5[e]	0	
MV10(pRK2108)	50.4–56.4	3	0

[a] A 56.4-kb RK2 replicon with the *Eco*RI restriction site at 0 kb was used for mapping (10).

[b] Bacteria were spread on plates containing 5 μg of potassium tellurite per ml (2, 3). Small colonies in thickly spread areas which had transient Te^r (not scored) were not always distinguishable from those with stable Te^r; the numbers of the latter are, therefore, approximate save where zero to three were found.

[c] Twenty representative single colonies [except for MV10(pRK2104) and MV10(pRK2108)] from those obtained as described in footnote *b* were streaked on 1.5-cm squares on a tellurite plate for each subculture. Growth was scored when no reduction in the size of most of the colonies occurred from one subculture to the next, even though some susceptibility was indicated by the presence of small colonies.

[d] The pRK2102 fragment was inserted into the MV10 chromosome (strain RP1894 in reference 21).

[e] This fragment is the same as pRK2102 save that the portion between 50.4 kb and the *Eco*RI site (0 kb) has been inverted.

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