Location on RP4 of a Tellurite Resistance Determinant Not Normally Expressed in Inc\(\alpha\) Plasmids

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The tellurite resistance (Te') determinant of RP4 is not normally expressed unless variants are selected on medium containing tellurite. The determinant was mapped in the variant plasmid RP4\(\mathrm{TeF}^+\) by Tn7 insertion mutagenesis. Based on a 56.4-kilobase (kb) replicon, it covered the region from 56 kb, across the EcoRI 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 Inc\(\alpha\) plasmids (3). Inc\(\alpha\) plasmids (6, 19) do not express transferable tellurite resistance (Te') unless bacteria are grown on plates containing tellurite; Inc\(\beta\) plasmids do not express it at all (3).

The specific mutation in RP4 to generate RP4\(\mathrm{TeF}^+\) is unknown. The restriction maps of RP4 and RP4\(\mathrm{TeF}^+\) appear identical, and there were no obvious inversions, deletions, or duplications in the latter (D. E. Taylor, unpublished data). Moreover, RP4\(\mathrm{TeF}^+\) specifies stable constitutive expression of Te'. To determine the location of the normally unexpressed Te' determinant on the RP4 map, we used Tn7 insertion mutagenesis. We started with E. coli J62::Tn7(RP4\(\mathrm{TeF}^+\)) 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" Rif") After overnight incubation at 30°C, the mixture was streaked for single colonies on tryptic soy agar with rifampin (200 \(\mu\)g/ml)—streptomycin (200 \(\mu\)g/ml)—trimethoprim (10 \(\mu\)g/ml). Eight independent Tn7 insertion mutants in which the phenotype of RP4\(\mathrm{TeF}^+\) became tellurite susceptible were obtained from 600 RP4::Tn7 derivatives isolated. Insertion of Tn7 in RP4 to produce mutants 1 to 7 rendered RP4 incapable of regenerating Te' when replated on tellurite-containing medium. However, the other RP4::Tn7 derivative (mutant 8) was able to grow on tellurite after reexposure to the antimetabolite.

DNA was isolated from the eight RP4::Tn7 plasmids as described previously (16, 20). Restriction endonucleases EcoRI, KpnI, PstI, SmaI, SstII, and XhoI (Boehringer Mannheim Biochemicals, Montreal, Quebec, Canada) were used for mapping, both singly and in combinations. Phage lambda DNA digested with HindIII (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' 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 Tn7 insertion site with respect to the Smal site at 1.2 kb on the RP4 map was determined by digestion with Smal, which does not cut Tn7. EcoRI digestion was used to determine the insertion site of Tn7 with respect to the EcoRI site at 0 kb, taking into consideration that EcoRI cuts Tn7 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 Tn7 insertion mutants of RP4\(\mathrm{TeF}^+\) contained Tn7 in the alpha orientation; i.e., the end closest to the EcoRI site was the clockwise end of the inserted transposon. Barth and Griner (1) showed that Tn7 inserted preferentially in this orientation in RP4. From the restriction endonuclease analysis we concluded that the Tn7 insertion sites extended from 56.0 kb, across the EcoRI 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 kil 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' determinant appeared to be located between the korA and kilA genes (Fig. 1b).

To confirm the above results, we tested the Te'-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' clones when plated on tellurite medium. Likewise, pRK2102, whether extrachromosomal or integrated, generated stable Te'. However, when pRK2102 had the portion between 50.4 and 56.4 kb (=0 kb, the EcoRI site) inverted (fragment pRK2104), Te' was not generated. We presumed that the reversal had cleaved the Te' determinant, which must, therefore, have spanned the EcoRI site of the plasmid as indicated by the Tn7 insertion described above. pRK2108 (50.4 to 56.4 kb) did not generate Te', confirming that DNA clockwise from the EcoRI site was necessary for generation of Te'.

An unusual feature of the Te' 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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Seven of eight Tn7 insertion mutants of RP4 Te' which had lost Te' became incapable of expressing Te' 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' after growth on tellurite. Perhaps Tn7 in mutant 8 is inserted into a regulatory locus, the positive function of which is required for constitutive Te'. The site of Tn7 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'. The Te' determinant appears to constitute a transposable element since it can be translocated from RP4Te' 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' carried by P group plasmids, the reason that this determinant normally remains unexpressed in IncPα plasmids is not yet understood.

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