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Distance between Alleles as a Determinant of Linkage in Natural Transformation of *Acinetobacter calcoaceticus*†

DOROTHEE-URSULA KLOOS,[1,2] ANTHONY A. DiMARCO,[1] DAVID A. ELSEMORE,[1] KENNETH N. TIMMIS,[3] AND L. NICHOLAS ORNSTON[1]*

Biology Department, Yale University, New Haven, Connecticut 06520,[1] and Department of Microbiology, National Research Centre for Biotechnology (GBF), Braunschweig,[3] and Institute for Plant Genetics and Crop Plant Research, (IPK), Gatersleben,[2] Germany

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Cotransformation frequencies of 16, 39, 51, and 60% were observed when donor alleles were separated by distances of 9.2, 7.4, 6.3, and 5.1 kb, respectively, in donor *Acinetobacter calcoaceticus* DNA. A different and unexpected pattern was observed when the distance between recipient alleles was reduced from 9.2 to 5.1 kb. Ligation of unlinked chromosomal DNA fragments allowed them to be linked genetically through natural transformation.

Natural transformation (12) has been useful in the analysis of catabolic pathways in *Acinetobacter calcoaceticus* (1, 3–8, 10, 14, 15, 18), but, as yet, there has been little evidence correlating cotransformation frequencies with the physical distance between alleles (11). An opportunity to investigate such correlations was presented by elucidation of the *pca-qui-pob* gene cluster. The *pca* genes encode enzymes required for growth with protocatechuate (15), and this metabolite can be produced by either the action of *qui* gene products on quinate (7, 8) or the *pobA*-encoded metabolism of *p*-hydroxybenzoate (3). Null *pca* mutations prevent growth with both *p*-hydroxybenzoate and protocatechuate (9), whereas null *pob* mutations allow growth with protocatechuate (1). Cotransformation frequencies can be determined by transforming recipient strains, blocked in both *pca* and *pob*, with DNA containing the wild-type alleles. Wild-type *pca* DNA can be selected by demanding growth with protocatechuate, and cotransformation of wild-type *pob* DNA can be assessed as the frequency of the selected transformants that grow with *p*-hydroxybenzoate. Engineered deletions removing segments of *qui* DNA do not impede the growth of cells with either protocatechuate or *p*-hydroxybenzoate (7, 8). Therefore, we were able to examine how variations in distance caused by the deletion of DNA between *pca* and *pob* influenced their cotransformation frequencies.

Organization of wild-type and mutant genes in the investigated strains is presented in Fig. 1. In the wild-type background of strain ADP4021, *pcaH19* and *pobR5* are separated by about 9.2 kb, and transformation with donor DNA in which the wild-type alleles were identically separated yielded a cotransformation frequency of 16% (Fig. 2). The frequency increased to 39, 51, and 60% when the donor alleles were separated by 7.4, 6.3, and 5.1 kb, respectively (Fig. 2). This information may provide a rough guide to the correlation between cotransformation frequencies and the linear distances between transformed alleles in a wild-type background, but the results must be regarded with caution because a different pattern was observed when DNA from the same donors was provided to recipient strain ADP699, in which *pcaH19* and *pobR5* are separated by only 5.1 kb. As shown in Fig. 2, the cotransformation of markers in ADP699 was essentially invariant at 35% when the separation of donor alleles ranged between 5.1 and 7.4 kb (Fig. 2). The most remarkable finding was the difference in the cotransformations of ADP4021 and ADP699 when donor DNA provided alleles separated by 5.1 kb. In this comparison, shortening the distance between the alleles in the recipient strain by 45% reduced the frequency of cotransformation by 42% (Fig. 2). There is no obvious reason why an engineered increase in the chromosomal linkage of two alleles would result in a decrease in their linkage as observed by transformation, but it is possible that the observation is a consequence of a change in chromosomal conformation brought about by the designed deletion.

The results show that 9-kb transforming DNA fragments are readily assimilated by *A. calcoaceticus*, and further analysis gives an indication of the extent to which recombination may segregate alleles from donor DNA. Selection for growth of strain ADP4021 with *p*-hydroxybenzoate demands acquisition of wild-type DNA corresponding to both *pcaH19* and *pobR5*. Strains containing *qui* deletions were used as donors; the segregation of *qui* deletions from donor DNA was determined with growth of the recombinants with 5 mM quinate. The frequency of segregation of the nonselected *qui* alleles increased through observed levels of 9, 24, and 27% as the distance between the selected markers increased through distances of 5.1, 6.3, and 7.4 kb, respectively.

To explore further the segregation of alleles introduced in a single donor DNA fragment, genes known to be unlinked in the wild-type chromosome were joined by ligation. A 1.6-kb *Sal*I-*Kpn*I fragment in pIB1345 (16, 17) contains *A. calcoaceticus catA*. The insert was removed as a *Sal*I fragment and introduced into the *Xho*I site of pZR405 (3), giving rise to *pobRpobA1*::*catA* within an insert of 4.9 kb of *A. calcoaceticus* DNA in pZR439. DNA released by linearization of pZR439 with *Eco*RI and *Hin*dIII was used to transform strain ADP 4022, which contains both *catA3139* (17) and *pobR5* (3). Of strains selected for the wild-type *catA* allele, 3% had acquired wild-type *pobR*. After selection for *pobR*, 7% of the transformants had acquired wild-type *catA*. Such transformants were not observed when a strain from which the *catA*-containing

* Corresponding author. Mailing address: Department of Biology, Yale University, P.O. Box 208103, New Haven, CT 06520-8103. Phone: (203) 432-3498. Fax: (203) 432-6161.

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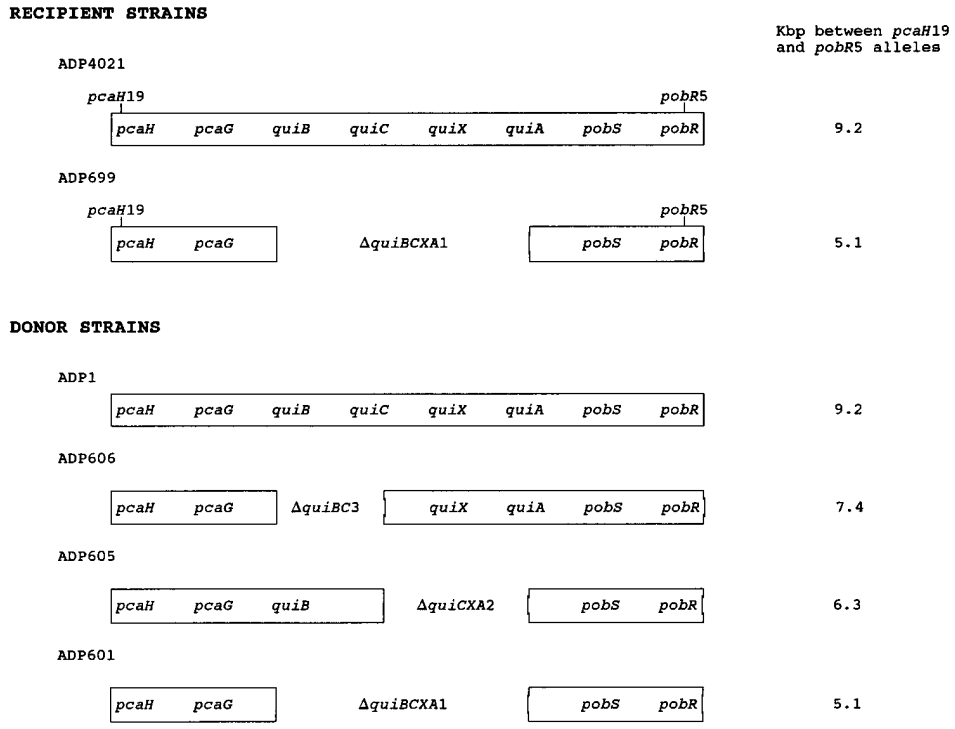

FIG. 1. Organization of genes in recipient and donor strains. Recipient strain ADP4021 contains sequenced null mutations *pcaH19* (9) and *pobR5* (4) separated by 9.2 kb of sequenced DNA (7, 8, 15). The Δ*quiBCXA1* deletion reduces this distance to 5.1 kb in strain ADP699 (7, 8). The distance between wild-type alleles corresponding to *pcaH19* and *pobR5* is 9.2 kb in wild-type donor strain ADP1, and removal of *Pst*I restriction fragments reduced the distances to 7.4, 6.3, and 5.1 kb in donor strains ADP606, ADP605, and ADP601 (7, 8), respectively.

*Sal*I-*Kpn*I chromosomal segment had been deleted was used as recipient. Therefore, the acquisition of wild-type *catA* depends upon homologous recombination in the *catA* region. As expected, no linkage of *catA* and *pobR* was observed when DNA from wild-type strain ADP1 was used as the donor for the transformation of strain ADP4022.

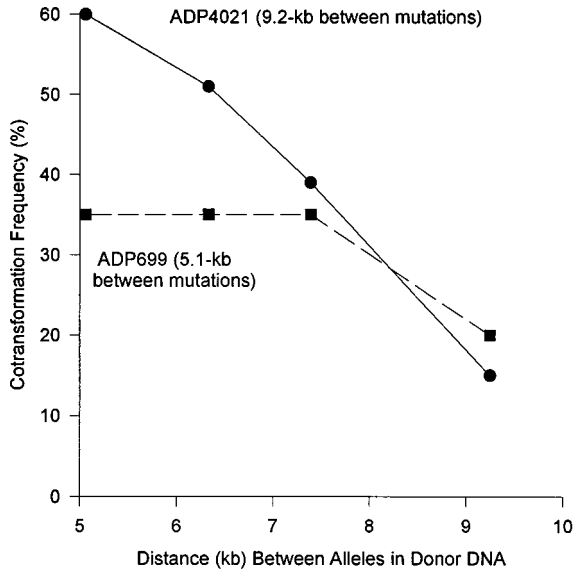

FIG. 2. Influence of linear distance between Δ*pcaH19* and *pobR5* on cotransformation in recipient strains. Cotransformation frequencies were determined by selection for wild-type *pcaH* on plates containing 5 mM protocatechuate followed by screening for the fraction of recombinants that had gained the ability to grow with 5 mM *p*-hydroxybenzoate. At least 100 transformants were screened in each cross. Crude lysates (9) were used for transformation. Similar cotransformation frequencies (13) were observed with purified donor DNA (2).

The creation of cotransforming DNA fragments by the ligation of chromosomally unlinked genes presents opportunities to expand the genetic investigation of *A. calcoaceticus*. A genetic marker for which selection procedures are not available may be linked to a selectable marker, which can then be used as a Trojan horse to introduce DNA into recipient strains by transformation followed by selection. If, as present results suggest, the nonselectable marker is present in several percent of the transformants, its detection by screening should not be arduous.

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