Transfer of Chromosomal Mutations to Plasmids via Hfr-Mediated Conduction

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Nonconjugative plasmids can be transferred from an Hfr donor to a recipient by Hfr-mediated conduction. We found that this phenomenon can be employed to obtain a plasmid in which a mutation in a chromosomal gene has been transferred to the copy of that gene contained in the hybrid plasmid.

Our laboratory is studying the Trg protein, the chemosensory transducer of *Escherichia coli* that mediates tactic responses to galactose and ribose (12, 16). In the course of these studies, it was necessary to obtain cloned copies of mutant *trg* genes that were present in the chromosome of *trg* mutants. We reasoned that Hfr-mediated conduction could be utilized as a means of enriching for hybrid plasmids that had undergone recombination with chromosomal DNA. In this report, we describe in vivo cloning of chromosomal *trg* mutations by using Hfr-mediated conduction transfer.

Most plasmids lacking their own transfer function can be mobilized by the F plasmid (5). Plasmids that lack homology with both the F replicon and the *E. coli* chromosome appear to be transmitted as a result of *recA*-independent formation of a cointegrate of two replicons during translocation of a transposable genetic element from one to the other. For example, conduction of γ - δ (Tn1000) to pBR322 (9). In addition, other nonconjugative plasmids can be transferred in an unknown manner by F-prime plasmids without Tn1000 insertion (8). High-frequency transfer of plasmids occurs from Hfr strains containing hybrid plasmids consisting of pBR322 and a segment of chromosomal DNA of *E. coli*. This efficient transfer requires the *recA* function in both donor and recipient, and the frequency of transfer is dependent upon the distance between the origin of Hfr transfer and the chromosomal piece cloned in the plasmid (21). This observation implies that transfer involves homologous recombination between the hybrid plasmid and the Hfr chromosome. In fact, homologous recombination has been observed between hybrid plasmids and the chromosome (17), and Hfr transfer of plasmids has been used to obtain stable plasmid integrates in a *polA* recipient (19). If conducted plasmids undergo two recombinational events, one for integration into the chromosome before transfer and the other for resolution after transfer, then a proportion of conducted plasmids should have acquired chromosomal DNA.

Three chromosomal *trg* mutants, *trg-8*, *trg-19*, and *trg-21*, obtained from J. Adler (University of Wisconsin) were used. The relative locations of the mutations (Fig. 1) in these strains were determined by deletion mapping (Park and Hazelbauer, manuscript in preparation). The mutations were introduced by P1 transduction (18) into the strain KL16-21 (Hfr KL16 Rec⁺) with the Tn5 insertion *zdb::Tn5* (11) near

trg. Transductants were transformed with the wild-type *trg* plasmid pCP31 (Fig. 1), a derivative of pTH105 (2). Plasmid pCP31 has chromosomal flanking regions on both sides of *trg* that provide sufficient homology for recombination with the chromosome. Each mutant Hfr strain containing the wild-type *trg* plasmid was mated with an F⁻ *trg* deletion strain (CP177; Δ *trg-100 rpsL his Rec*⁺). The method of Hfr transfer was slightly modified from that of Miller (18). Donor strains with plasmids were grown overnight in Luria broth containing ampicillin (50 μ g/ml) and then diluted 1:500 in fresh Luria broth. The recipient culture was prepared by a 1:10 dilution of an overnight culture grown in Luria broth. After 3.5 h at 35°C, donor and recipient cultures were mixed at a ratio of 1:5 and incubated for 1 h. To select for recipients with transferred plasmids, each mating mixture was serially diluted and spread on a Luria broth plate containing both streptomycin (100 μ g/ml) and ampicillin (50 μ g/ml). For plates in high cell densities on which ampicillin-sensitive cells might grow, replicas were made onto the same antibiotic plates.

pCP31 was transferred by conduction with a frequency of approximately 10⁻⁵ per donor cell (Table 1), comparable to conduction rates reported for other hybrid plasmids (21). The values are much greater than conduction frequencies for pBR322 alone (21). The Trg phenotype of plasmid recipients was examined on minimal chemotactic swarm plates containing either ribose or galactose as an attractant (11). Approximately 5% of the transferred plasmids did not confer a wild-type Trg phenotype, but instead conferred a mutant phenotype related to the mutation carried on the chromosome of the donor strain (Table 1). From this set of plasmids, we obtained the desired clones of the mutated *trg* genes.

Plasmid DNA was prepared (13) from the nine mutant isolates. Analysis by electrophoresis in 0.7% agarose gels revealed that six plasmids exhibited mobility indistinguishable from that of supercoiled pCP31. Three exhibited a slower mobility, corresponding higher apparent molecular weights, and will be considered below. Plasmid DNA from each isolate was used to transform the *trg* deletion strain CP177, and one transformant for each of the three *trg* mutations was chosen for detailed characterization.

Analysis with appropriate restriction endonucleases revealed no differences between any of the three conducted plasmids carrying *trg* mutations and the original pCP31, indicating the absence of any substantial DNA rearrangements (data not shown). Analysis of proteins synthesized in

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FIG. 1. Diagram of plasmid pCP31 and the relative locations of *trg* mutations on the chromosome. pCP31 contains a 4-kilobase-pair (kb) chromosomal fragment including *trg* (1.6 kilobase pairs) and the pBR322 replicon. The arrow represents the transcriptional direction of *trg*. Abbreviations: Ap^r, ampicillin-resistant determinant; ori, the origin of DNA replication. Sites for restriction endonucleases: BglII (B), BssHII (Bs), HindIII (H), MstII (M), NruI (N), and PvuII (P).

minicells containing each of the plasmids demonstrated that all three mutant genes coded for an intact Trg protein exhibiting an apparent molecular weight indistinguishable from that of the wild-type protein (Fig. 2). The minicell-producing host strain, TH1326 [*minA minB* Δ (*tap-cheB*)2231 *rpsL recA*], lacked both the CheR methyltransferase and the CheB demethylase-deamidase enzymes that catalyze covalent modification of all transducers including Trg. Since the modifications generate multiple electrophoretic forms of Trg (10), unambiguous comparison of products of mutant and wild-type genes required a host unable to modify the protein. Like wild-type Trg, protein synthesized from the three mutant genes was also able to accept methyl groups as demonstrated by the appearance of Trg-specific methyl-³H-labeled bands on sodium dodecyl sulfate-polyacrylamide gels (6) of samples of cells harboring the respective plasmids (data not shown). The host used for these experiments was CP362 [Δ *tsr-7028* Δ (*tar-tap*)5201 Δ *trg-100*], which contains active methyltransferase and demethylase but carries deletions in the chromosomal copies of all four transducer genes. In this background, the only potential methyl-accepting transducer protein is coded for by plasmid-carried *trg*.

The mutant nature of the cloned genes was confirmed by examination of tactic behavior on chemotactic swarm plates and of changes in the pattern of methyl-³H-labeled bands of Trg upon tactic stimulation and adaptation. On swarm plates, the tactic phenotype of strains carrying a single chromosomal copy of the mutant gene was ribose negative, weakly galactose positive for *trg-8*, and ribose positive, galactose negative for *trg-19*. The specific phenotypic defects were preserved in cells lacking a chromosomal copy of *trg* and harboring the respective plasmids (Table 2). Adap-

TABLE 1. Hfr-mediated transfer of hybrid plasmids carrying *trg*

Mutation in Hfr donor	Transfer frequency of pCP31 ^a	<i>trg</i> mutants/total Ap ^r transconjugants
<i>trg-8</i>	8.4×10^{-6}	2/40
<i>trg-19</i>	6.7×10^{-6}	4/65
<i>trg-21</i>	5.0×10^{-6}	3/40

^a Values are the number of ampicillin-resistant transconjugants per input donor cell. His⁺ transconjugants occurred 1,000-fold more frequently than ampicillin-resistant transconjugants. In similar conditions, pBR322 alone was transferred about 1,000 times less frequently than hybrid plasmids (21). Hfr KL-16 transfers the chromosome counterclockwise from its origin at 61 min on the *E. coli* linkage map. *trg* is located at 31 min (1, 11); *his* is located at 44 min.

FIG. 2. Trg protein synthesized in minicells containing wild-type and mutant plasmids. The Trg polypeptide occurred as a single unmodified form, since the TH1326 cells from which the minicells were derived lacked both the CheR methyltransferase and the CheB methyltransferase-deamidase. The figure is an autoradiogram of a sodium dodecyl sulfate-9% polyacrylamide gel containing [³⁵S]methionine-labeled protein (10). Only the region surrounding the Trg polypeptide (arrow) at an apparent molecular weight of 60,000 is shown. The first lane shows the protein synthesized in minicells lacking any plasmid, and the other lanes contain protein synthesized in minicells containing pCP31 (*trg*⁺) or its derivatives (mutant *trg* alleles).

tation of wild-type cells to stimulation by galactose or ribose is linked to increased methylation of Trg (12, 16). For *trg-8* and *trg-19* mutants, methylation increases were only observed after stimulation with the sugar to which the cells responded on a swarm plate. The same pattern was observed for cells lacking a chromosomal *trg* but containing the appropriate mutant plasmid (data not shown). Determination of nucleotide sequences of cloned *trg-8* and *trg-19* genes has located single-base substitutions in the region to which the chromosomal mutations were mapped (Park and Hazelbauer, in preparation). The situation for *trg-21* is more complex, since there is a difference between the phenotypes conferred by the chromosomal and plasmid-carried mutations (Table 2). It appears that multiple copies of *trg-21* confer a generally nonchemotactic phenotype reminiscent of the dominant effect of specific *tsr* mutations termed *cheD* (4). However, as in the case of the other mutations, nucleotide sequencing of the cloned gene has located a single base substitution in the region to which chromosomal *trg-21* was mapped (Park and Hazelbauer, in preparation). These observations taken together indicate that the specific *trg* mutations were transferred from the gene in the chromosome to

TABLE 2. Chemotactic swarm phenotype conferred by chromosomal and plasmid-carried *trg* mutations

Mutation	Taxis to:			
	Ribose	Galactose	Serine ^a	Aspartate or maltose ^b
Chromosomal				
<i>trg-8</i>	—	(+) ^c	+	+
<i>trg-19</i>	+	—	+	+
<i>trg-21</i>	(+)	(+)	+	+
Plasmid carried				
<i>trg-8</i>	—	+	+	+
<i>trg-19</i>	+	—	+	+
<i>trg-21</i>	—	—	—	—

^a Response mediated by Tsr transducer. The ability to form a serine or aspartate chemotactic ring was tested on a plate containing tryptone

^b Responses mediated by Tar transducer.

^c Very weak response.

the cloned copy of the gene on a plasmid, and thus the procedure worked as postulated.

It was possible that the appearance of larger plasmids among the set of conducted mutant plasmids was related to the process of conduction. Thus we investigated further by examining 50 wild-type plasmids as well as the 9 mutant plasmids that had been transferred by conduction. Analysis by electrophoresis on 0.7% agarose gels revealed that approximately 50% of the plasmids were indistinguishable from supercoiled pCP31 DNA, whereas the others fell into one of three size classes, each of higher apparent molecular weight than pCP31. Digestion of each of the larger plasmids with a restriction endonuclease having a single recognition site in pCP31 produced a single fragment of linear DNA with an apparent molecular weight identical to linearized pCP31 when the supercoiled plasmid was of the intermediate size class and with an apparent molecular weight 25% larger than pCP31 when the supercoiled plasmid was from the smallest or largest size class. We interpret this to mean that the intermediate size class comprised dimers of pCP31 and that the smallest and largest classes were pCP31 plus additional DNA and dimers of the enlarged plasmid, respectively. Detailed analysis with restriction endonucleases of 18 plasmids containing additional DNA revealed that all had an identical 1.5-kilobase insertion to the 140-base-pair region between the *Bgl*II and *Mst*II recognition sites (Fig. 1). This region is within the chromosomal DNA cloned in pCP31, more than 200 base pairs to the 5' side of the initiation codon of *trg*. Since plasmids containing the insertion conferred a wild-type or partially defective *Trg* phenotype characteristic of the particular *trg* mutation, it appears that the insertion does not affect expression of *trg* significantly. A map of restriction endonuclease sites in the 1.5-kilobase-pair insertion reveals no indication of duplication of DNA in pCP31 or any correspondence to published maps of insertion elements IS1 (3), IS2 (7), IS3 (20), IS4 (15), IS5 (3), or IS50 (14). It seems unlikely that the 1.5-kilobase-pair insertion is related to the much larger *Tn1000*. Thus the identity and origin of the inserted fragment are unknown. Since many of the transferred plasmids carrying mutant or wild-type *trg* did not contain the insertion, it is unlikely that the DNA fragment was involved in a crucial way in either conduction or the alternative recombination events that resulted in introduction of a chromosomal mutation into a plasmid-carried gene.

The results described in this report support the notion that Hfr-mediated transfer of a hybrid plasmid containing a chromosomal segment involves formation of a cointegrate between plasmid and chromosome presumably mediated by the *recA* product. After transfer into the recipient, the cointegrate would be resolved by circularization or replication of the plasmid through homologous recombination. However, since the proportion of transferred plasmids that were mutants was lower than expected even after taking into account the asymmetry of the homologous regions flanking the mutations, we cannot exclude the possibility that transposon insertion or another mechanism independent of homologous recombination might be involved (8). The increased size of some transferred plasmids is consistent with this possibility.

The *in vivo* transfer method described in this report should be applicable to other systems where the appropriate hybrid plasmid is available, particularly for cloning chromosomal mutations that do not confer a selectable phenotype.

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