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Plasmid Transformation in *Haemophilus influenzae*

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Purified 34-megadalton-plasmid deoxyribonucleic acid from antibiotic-resistant strains of *Haemophilus influenzae* transforms competent strains of *H. influenzae* more efficiently if the recipient strains contain certain other 30-megadalton plasmids.

Studies of transfection and transformation of chromosomal markers in *Haemophilus influenzae* suggest that stable expression of transforming DNA requires the presence of a specific base sequence on the donor DNA for uptake (8, 18), a periplasmic DNA binding protein (22), and sufficient homology between the donor and recipient DNAs to allow recombination (5, 14, 19). Transformation with plasmid DNA in *H. influenzae*, however, has not been extensively studied. Elwell et al. (10) were unable to transform strains of *H. influenzae* with DNA preparations of a 31-megadalton plasmid (pUB701) from *H. influenzae*, but were able to transform them with DNA preparations of another 30-megadalton plasmid (RSF007) sharing 65 to 70% homology with the pUB701 plasmid. Stuy (20, 21) has recently reported the *rec-1*-dependent transformation of plasmid antibiotic resistance markers with crude DNA preparations from Rd strains containing chromosomally integrated plasmids. Transformation with crude DNA preparations from Rd strains containing nonintegrated plasmids was markedly inefficient. As part of an ongoing study into the mechanisms of antibiotic resistance transfer, we have studied the transformation by purified plasmid DNA of *H. influenzae* strains containing integrated or free plasmids.

H. influenzae strain Rd is a transformable rough derivative of the capsular type d strain originally isolated by Alexander and Leidy (2). Strain BC200 is derived from Rd (3), but is lacking an inducible defective prophage (6). Strain 9(N3) is a lysogenic derivative of the transformable strain JC9 containing the prophage N3 and is defective in chromosomal transformation (16). The mutants *rec-1* and *rec-2* are recombination-deficient strains derived from Rd and have been previously described (15, 17). The ampicillin resistance plasmid from BC200-amp90383 (4), designated p(amp90383), was transferred to a rifampin-resistant clinical isolate of *H. influenzae* by conjugation and then transferred to strains BC200, 9(N3), *rec-1*, and *rec-2* by conjugation. Transconjugants from these matings contained a nonintegrated 30-megadalton plasmid when cleared lysates of these strains were screened by agarose gel electrophoresis (13). DNAs of two incompatible 34-megadalton antibiotic resistance plasmids (p1002, carrying resistance determinants for ampicillin and tetracycline, and p2265, carrying resistance determinants for tetracycline and chloramphenicol) were purified by CsCl centrifugation. Partial characterizations of these plasmids have been previously given (1). Chromosomal DNA was prepared from a strain resistant to novobiocin and streptomycin by the method of Marmur (12).

Transformation was carried out with cells made competent in MIV medium (19), and plasmid transformants were selected for resistance to tetracycline. Chromosomal transformants were selected for resistance to novobiocin [BC200, 9(N3), and *rec-1*] or streptomycin (*rec-2*). The frequencies of transformation for plasmid and chromosomal antibiotic resistance markers were determined, and the results are presented in Table 1.

Results were similar for the two purified plasmid DNA preparations and demonstrated a marked influence of the presence and type of resident plasmid in the recipient strain. Strain BC200, although highly competent for transformation with chromosomal markers, was inefficiently transformed with plasmid DNA, as reported previously for strain Rd by Stuy (21). The presence of either free or integrated plasmid p(amp90383) increased the frequency 3×10^3 to 4×10^3 above that seen with the parent BC200 strain. Strains 9(N3) and 9(N3)(p(amp90383)), although considerably less transformable than the BC200 strains, showed the same effect of stimulation of plasmid transformation by the resident plasmid. However, the *rec-1* and *rec-2* strains showed no plasmid transformation even in the presence of the p(amp90383) plasmid.

TABLE 1. *Frequencies of transformation by purified plasmid DNAs of H. influenzae strains containing integrated or free plasmids*

Strain	Plasmid	Plasmid state	Plasmid transformation frequency		Chromosomal transformation frequency
			p1002 DNA	p2265 DNA	
BC200	None		2.0×10^{-7}	5.6×10^{-8}	3.6×10^{-2}
BC200	p(amp90383)	Integrated	9.5×10^{-4}	2.8×10^{-4}	
BC200	p(amp90383)	Free	5.2×10^{-4}	1.7×10^{-4}	
BC200	p2193	Free	1.2×10^{-3}	2.0×10^{-4}	
BC200	p2316	Free	3.2×10^{-6}	2.6×10^{-6}	
BC200	p2365	Free	2.1×10^{-5}	1.6×10^{-6}	
9(N3)	None		$<1.4 \times 10^{-9}$	$<1.3 \times 10^{-9}$	6.0×10^{-6}
9(N3)	p(amp90383)	Free	2.5×10^{-7}	7.0×10^{-8}	
rec-1	None		$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$	4.0×10^{-8}
rec-1	p(amp90383)	Free	$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$	
rec-2	None		$<6.0 \times 10^{-10}$	$<6.0 \times 10^{-10}$	4.0×10^{-9}
rec-2	p(amp90383)	Free	$<6.0 \times 10^{-10}$	$<6.0 \times 10^{-10}$	

These data suggest that the wild-type *rec-1* and *rec-2* genes are required for plasmid transformation, although an unlikely alternative is that if the ratio of plasmid transformation to chromosomal transformation were the same in the Rec$^-$ as for the BC200 strains, the frequency of plasmid transformation could be below the levels measurable in these experiments.

The enhanced frequency of transformation seen in the isogenic strains carrying 30-megadalton free or integrated plasmids could be due either to an increased efficiency of DNA uptake or to an increased efficiency of marker rescue after uptake. Differences in DNA uptake alone are very unlikely to account for the enhanced frequency of transformation, since uptake of chromosomal DNA is normal in *rec-1* and *rec-2* strains (17), the number of uptake sites is constant in the donor DNA, and there is no a priori reason to suggest that the presence of an antibiotic resistance plasmid in the cell should increase the number of periplasmic DNA binding sites. Data not shown indicate that competence is required for plasmid transformation, and the presence of small, 3- to 6-megadalton antibiotic resistance plasmids, such as RSF0885 (9) and pJB1 (7), does not increase the frequency of plasmid transformation over that seen in strains without plasmids. The simplest interpretation of the data is that homology between the donor and recipient DNAs plays an important role in determining the efficiency with which antibiotic resistance plasmids are able to transform competent *H. influenzae*. Another 30-megadalton ampicillin resistance plasmid (p2193) which, like p(amp90383), is incompatible with the p2265 plasmid (data not shown), shows similar frequencies of transformation (Table 1). Two other 30-megadalton ampicillin resistance plasmids (p2316 and p2365) which are compatible with the p2265 plasmid, and hence are probably much less homologous (11), show a 100-fold lower frequency of transformation (Table 1).

These studies suggest a model for plasmid transformation in *H. influenzae* in which homologous plasmid DNA in the recipient strain, either free or linearly integrated in the chromosome, serves the same function as recipient chromosomal DNA in chromosomal transformation, and recombination between donor and recipient plasmids, requiring *rec-1* and *rec-2* gene products, is essential for efficient transformation. This model is supported by our observation that transformants cotransfer the donor and recipient resistance markers in conjugative matings. Further studies will be necessary, however, to establish the fate of the non-antibiotic resistance portions of the donor and recipient plasmids during transformation.

Finally, it is noted that the frequencies of transformation seen in this system are as high as or higher than those reported for other *Haemophilus* plasmid transformation systems, which suggests that efficient gene cloning with this plasmid system may be possible.

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