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Transformation of *Salmonella typhimurium* with Plasmid DNA: Differences Between Rough and Smooth Strains

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Lipopolysaccharide-defective mutants of *Salmonella typhimurium* were transformed by plasmid DNA with a Ca^{2+} treatment method. Only those mutants with an Rc or Rd_2 chemotype, due to *galE* or *rfaF* mutations, respectively, gave efficiencies greater than 10^5 transformants per μg of DNA, frequencies 8- to 630-fold higher than with smooth strains or other rough mutants.

Unlike several other bacterial species, members of the enteric group are not naturally competent for transformation (31). However, a large number of methods derived from the Ca^{2+} shock method of Mandel and Higa (22) have been developed to induce levels of DNA uptake sufficient for use in molecular cloning (7, 9, 12, 15, 16, 24) and, to a more limited extent, for the mapping of chromosomal loci (10, 25, 26). Transformation occurs in two stages: uptake, which includes both binding of the DNA to the cell surface and its transport across the cell envelope, and establishment. The particular surface components involved in DNA binding and the means by which transport is effected are currently unknown, although outer membrane proteins involved in cobalamin transport (9) and the *ompA* (35) protein have been implicated. The structure of lipopolysaccharide (LPS), a major surface component of the gram-negative cell, affects the transfection of both *Escherichia coli* (34) and *Salmonella typhimurium* (5). (See Fig. 1 for the detailed structure of the LPS of *S. typhimurium*.) No difference between smooth strains, which possess the O-somatic side chains, and rough strains, which lack them, was found for transformation by plasmid DNA in either *E. coli* or *S. typhimurium* (16), although data cited by Humphreys et al. (12) and the more recent work of van Die et al. (37) indicate that *E. coli* mutants with rough LPS due to a galactose-epimerase (*galE*) mutation are better plasmid recipients than Gal^+ strains. We undertook an analysis of the effect of LPS on plasmid transformation in *S. typhimurium* to determine whether LPS influences transformation by plasmid DNA and to identify those strains most suitable as recipients for molecular cloning.

The transformation method was adapted from Lederberg and Cohen (16) and incorporates a number of changes that improve the transformability of *S. typhimurium*. Cells (0.5 ml) from a saturated overnight L-broth culture (18) were inoculated into 100 ml of L-broth and grown at 37°C with shaking for aeration. A 40-ml sample of early logarithmic phase cells (absorbancy at 640 nm, 0.2) was chilled for 15 min on ice, harvested by centrifugation at 4°C, washed with 1 volume of 0.1 M $MgCl_2$, suspended in 0.5 volume of 0.1 M $CaCl_2$ in 50 mM 3-*N*-morpholinepropanesulfonic acid buffer (pH 6.5), incubated on ice for 20 min, pelleted, and suspended in 6.25×10^{-3} volume (0.25 ml) of the $CaCl_2$ solution. Samples of the concentrated cell suspension were transformed as follows: pBR322 (usually 76 ng) was added in 10 μl of TES buffer (50 mM Tris-hydrochloride, 10 mM EDTA, 50 mM NaCl, pH 8.0) to 0.1 ml of the Ca^{2+}-treated cells, the cell-DNA mixture was then heat-pulsed for 2 min in a 42°C water bath, chilled on ice for 5 min, and diluted 1:10 with chilled L-broth. To determine the number of antibiotic-resistant transformants, cells were added to 4 ml of soft L-agar and poured onto L-agar plates containing either 25 μg of tetracycline per ml or 30 μg of ampicillin per ml, incubated overnight, and counted. All glassware and solutions were chilled to 4°C, and all operations before the heat-pulse step were performed in a 4°C cold room.

Strains bearing LPS mutations representing most of the LPS chemotypes were tested for their transformability with pBR322 DNA (Table 1). These strains included isogenic *rfa* mutants (27) and Gal^- mutants from several sources.

Bursztyn et al. (5) found that *S. typhimurium* strains could be divided into three classes with respect to transfectability. Only strains lacking galactose-4-epimerase (*galE*) activity were efficiently transfected with P22 DNA. Significantly lower levels of transfection were obtained with the rough mutants *rfaH*, *rfaG*, and *rfaF*. Genetically smooth strains, *galE* strains made smooth by growth with glucose plus galactose (8), and various other rough mutants were not competent. We also were able to distinguish three classes with respect to transformation by pBR322. However, all LPS chemotypes were transformable, and the composition of the classes is slightly different from that for transfection.

SL1306 (*galE503*) has an Rc-chemotype LPS and is a very efficient transformation recipient, yielding better than 4×10^5 transformants per μg of DNA, levels 8- to 36-fold higher than those of Gal^- strains with smooth LPS (SA2881 *galK9*, SL3856 *gal-851*), wild-type smooth strains (SL3770), or *galE* smooth phenocopies. The *galE503* allele was transduced by P22 from SL1306 to yield SA2380, which gave equally high levels of transformation. Since several different alleles yielding a galactose-epimeraseless phenotype (SL3684, *galE855*; SA2381, *galE409*; SA2444, *gal-5*, a regulatory mutant with low levels of *galEKT* operon expression) are also efficiently transformed, the effect of *galE* on transformation is neither strain nor allele specific.

Surprisingly, *rfa* mutants that have defects in the core region of the LPS do not display a clear pattern of increased transformation relative to smooth strains (Table 1). In the *rfa* mutants, only the allele *rfaF511* (chemotype Rd_2: SL3789; SL3790) gave transformation efficiencies (2.9×10^5 to 3.3×10^5 transformants per μg of DNA) comparable to those of the *galE* lines. A second allele, *rfaF537* (SL3855), was slightly less efficient as a transformation recipient. The rough mutants with LPS core lengths longer than the *galE*

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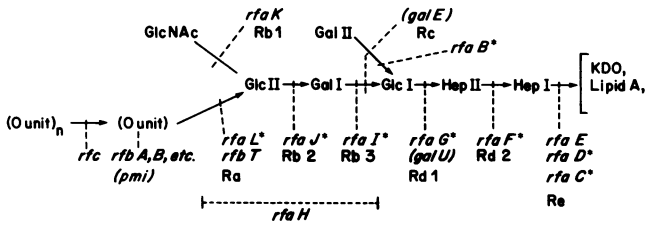

FIG. 1. Structure of the LPS of *S. typhimurium* and the genes required for its synthesis (21). Genes are shown in italics. Genes marked with an asterisk are located between *cysE* and *pyrE* in a cluster of *rfa* genes at 79 min on the linkage map (14, 30). Genes within parentheses have functions in addition to LPS synthesis. Entries in Roman type (Ra, Rb, etc.) are symbols for the chemotype of LPS produced by mutants of the indicated class, i.e., for LPS completed up to the indicated block. The *rfaH* mutation produced LPS of heterogeneous composition (19).

Rc chemotype (SL3749, *rfaL*, chemotype Ra; SL3750 *rfaJ*, chemotype Rb_2; SL3748 *rfaI*, chemotype Rb_3; SL1277 *rfaH*, heterogeneous chemotype Ra-Rc) are transformable but at much lower frequencies (1.2×10^3 to 7.4×10^3 transformants per μg of DNA). These levels are similar to the smooth Gal^+ and Gal^- strains discussed above. Two different *rfaG* mutants, SL3769 (*rfaG471*) and SL3854 (*rfaG489*), have an LPS core length between the Rc chemotype of *galE* and the Rd_2 chemotype of *rfaF* mutants, yet they, like most other *rfa* mutants, were poorly transformed.

The *rfaC* and *rfaE* deep rough mutants (heptoseless, chemotype Re) were transformed extremely poorly (less than 10^3 transformants per μg of DNA). However, SL3149, a deep rough mutant which produces some Re-chemotype LPS and some smooth LPS (17), gave 0.6×10^4 to 1.6×10^4 transformants per μg of DNA, a level significantly higher than that of the other deep rough mutants.

Rough mutants affected in the inner core of the LPS differ from smooth strains by being more permeable to antibiotics and other agents (23, 29), they leak periplasmic enzymes (6, 20), and they are deficient in some outer membrane proteins (1), but enriched for LPS and phospholipids (11). Rough mutants in the outer core, including those with an Rc chemotype, are not markedly more sensitive to antibiotics or dyes (23), nor is their outer membrane architecture severely perturbed (1). This suggests that abnormal outer membrane permeability is not principally responsible for determining transformability.

TABLE 1. Transformability of smooth and rough *S. typhimurium* strains[a]

Strain no.	Genotype[b]	LPS chemotype	No. of transformants per μg of pBR322 DNA	% Survival[c]	Strain source or reference
SL3770	*rfa*$^+$	Smooth	4.7×10^3	20	(27)
SL3856	*rfa*$^+$ *gal-851* LT7	Smooth	1.3×10^4	31	(27)
SA2881	*rfa*$^+$ *galK9*	Smooth	3.5×10^4	52	Fukasawa and Nikaido
SL1306	*galE503*	Smooth[d]	1.1×10^4	43	G. F.-L. Ames
SL3749	*rfaL446*	Ra	7.4×10^3	39	(27)
SL1905	*rfaJ4041*[e]	Rb_2	1.2×10^3	52	(27)
SL1909	*rfaJ4041*[e] *ompA201*	Rb_2	5.2×10^3	62	(33)
SL3750	*rfaJ417*	Rb_2	2.5×10^3	55	(27)
SL3748	*rfaI432*	Rb_3	3.9×10^3	88	(27)
SL1277	*rfaH481*[f]	Mixed[g] Rc-Ra	2.4×10^3	26	(32)
SL1306	*galE503*	Rc	4.0×10^5	36	G. F.-L. Ames
SA2380	*galE503*	Rc	4.6×10^5	38	*galE503* in SL3770[h]
SA2381	*galE409*	Rc	5.0×10^5	35	*galE409* in SL3770[h]
SL3684	*galE855*	Rc	8.0×10^5	53	J. R. Roth
SA2444	*gal-5* LT7	Rc	2.3×10^6	63	Fukasawa and Nikaido
SL3769	*rfaG471*	Rd_1	4.4×10^3	37	(27)
SL3854	*rfaG489* *gal-851* LT7	Rd_1	7.5×10^3	50	(27)
SL3789	*rfaF511*	Rd_2	2.9×10^5	39	(27)
SL3790	*rfaF511* *gal-851* LT7	Rd_2	3.3×10^5	33	(27)
SL3855	*rfaF537* *gal-851* LT7	Rd_2	7.3×10^4	41	(27)
SL3149	*rfaD657*[e]	Re	1.1×10^4	48	(17)
SL1102	*rfaE543*[e]	Re	116	25	(39)
SA1377	*rfaC630*	Re	152	33	(29)

[a] Cells grown in L-broth were transformed with nonsaturating amounts of pBR322 DNA (0.076 μg).

[b] All strains are LT2, and genotypes are complete unless otherwise indicated.

[c] Percent survival is calculated by comparing the number of viable cells at the time of plating after transformation to the number expected from viable cell numbers in the broth cultures.

[d] When *galE* mutants are grown in the presence of 1% glucose plus 1% galactose, they make smooth LPS (8). The smooth phenotype of the cells was confirmed by testing phage sensitivity (39). Transformation of SL1306 grown in the presence of 1% glucose is the same as that when grown with 0.1% glucose (data not shown).

[e] Indicates alleles are present in an SL1027 background, namely, *metA22 trp-2 H1-b H2-e,n,x* "cured of Fels2" *flaA22 rpsL120 xyl-404 metE551*.

[f] Full genotype is *proA pepP1* (P22 *sie*)$^+$ *metE*::Tn*10 rfaH481*.

[g] *rfaH* mutants make LPS side chains of various lengths (19).

[h] SA2380 and SA2381 result from transfer of *galE* alleles into SL3770 as follows. SL3770 was transduced with bacteriophage P22HT105/1 *int*, which had been grown on JL2688 (*bio203*::Tn*10*) with selection for Tc^r; the resulting strain was Bio^- Tc^r. This strain was then transduced to Bio^+ with P22HT105/1 *int* grown on SL1306; the resulting transductant (SA2380 *galE503*) was Bio^+ Tc^s $GalE^-$. SA2381 (*galE409*) was derived in the same way with transduction by P22HT105/1 *int* grown on SL869 (*galE409*).

Ca^{2+} treatment reduces cell viability (7, 25), and transformability is correlated with variations in sensitivity to Ca^{2+} treatment that occur during growth (4). The survival of the different rough mutants spans the range 20 to 90% (Table 1). Since no correlation between LPS structure, transformability, and survival after transformation was evident, the poor transformation efficiency of some rough mutants, particularly the deep rough mutants, is not due to killing during the course of transformation. Conversely, neither is the high transformability of the *galE* lines due to altered survival after Ca^{2+} treatment.

Several outer membrane components are known to be more accessible in rough mutants. Rough mutants are fully competent recipients for F-mediated conjugation, whereas smooth strains are not (28). Lipoprotein is exposed to antibodies only in Rb_2 or deeper rough mutants (3), and phospholipid vesicle fusion is possible only with rough mutants (13). The very high transformability of *galE* or *rfaF* mutants may be due to increased exposure of sites responsible for DNA binding. The most probable DNA binding sites are the outer membrane proteins (9, 35, 36, 38). However, the lack of a correlation between LPS core chain length and transformability, especially among the Rc, Rd_1, and Rd_2 chemotypes, argues strongly against simple steric hindrance as the limiting determinant. The restoration of transformability by the production of some smooth LPS in the *rfaD* mutant suggests that membrane architecture rather than steric hinderance by LPS is responsible for the effect of LPS core length on transformation. LPS-outer membrane protein interaction within the outer membrane can be highly specific (2). Changes in these interactions caused by changes in LPS structure may well alter the exposure of particular membrane components involved in DNA binding without markedly perturbing overall membrane structure. However, it is also possible that DNA binding per se is not affected by LPS structure, but rather the productivity of such binding is affected. Membrane rearrangements occurring after DNA binding, for example, possible phase transitions induced by heat treatment, may be affected by LPS core length such that transport of the bound DNA across the cell envelope is more efficient.

Although both *galE* and *rfaF* mutants are efficient transformation recipients, we recommend the use of *galE* lines, since these can be converted to smooth, P22-sensitive strains by growth in glucose plus galactose, thus permitting efficient transduction. *galE* mutations are easily introduced by any of several means. They can be isolated by direct selection for phage Felix O-resistance (39) or by using transposons closely linked to *galE* such as *bio-203*::Tn*10* (Table 1) or *zbi*::Tn*10* (30).

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