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Analysis of the Host Ranges of Transposon Bacteriophages Mu, MuhP1, and D108 by Use of Lipopolysaccharide Mutants of *Salmonella typhimurium* LT2

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The lipopolysaccharide receptors for the mutator bacteriophages Mu, MuhP1, and D108 were investigated with lipopolysaccharide mutants of *Salmonella typhimurium* LT2. Mu adsorbed only to mutants lacking the terminal O antigen but retaining the main chain sugars of the core; the side chain *N*-acetylglucosamine was not required. MuhP1 and D108 adsorbed partially to cells with the same receptors but adsorbed well only to cells with shorter lipopolysaccharides of the Rc and Rd1 chemotypes.

The *Escherichia coli* bacteriophage Mu and related mutator phages are the largest and most efficient transposable elements known (19). The transposition and DNA packaging properties of these phages make them ideal for many genetic procedures, including insertion mutagenesis, transduction, gene fusing, and DNA translocation and cloning (21). An understanding of the receptors for these phages is important for extending these applications to other species of bacteria.

Mu can grow in all genera of the family *Enterobacteriaceae* that have been tested (6, 13), but many strains are resistant because of a lack of appropriate lipopolysaccharide (LPS) receptors (9). *Salmonella typhimurium* LT2 is resistant to Mu and related phages, but mutations have been reported in the *rfb* region that make it sensitive to Mu (5, 12) and in *galE* that make it sensitive to MuhP1 (2).

The LPS requirements for Mu, the related phage D108 (3), and phage P1, which is not a mutator phage but has a related tail gene region (20), have been examined by using partially characterized *E. coli* K-12 mutants that are resistant to Mu (18). Here we use *S. typhimurium* mutants that are affected in the synthesis of the LPS and that have been well characterized genetically and biochemically (Fig. 1) (10) to study the LPS requirements for Mu, D108, and MuhP1. MuhP1 is a particular recombinant (complete name, MuhP1#1) (2) of Mu with the terminal part of the Mu *S* tail gene (and an unknown amount, if any, of the *U*, *U'*, and *S'* genes) replaced by the corresponding part of bacteriophage P1 (8).

The nature of the LPS receptors was investigated by using three assays to determine phage binding to cells: efficiency of plating (lysis), phage adsorption, and transduction (also called lysogenic conversion) (Table 1). The efficiency of plating was measured by determining the titers of phage induced from *E. coli* lysogens (11). Titers were 10^4 to 10^5-fold less for sensitive *S. typhimurium* strains than for *E. coli* because of host restriction, since *hsd* restriction mutations (1) nearly removed this difference (data not shown). Adsorption was measured as the percentage of PFU removed from the phage suspension after 30 min of incubation with the cells (17). Transduction (21) was measured with the ampicillin-resistant (Ap^r) phage MupAp5 (4) and recombinants of it that were constructed with the host range of MuhP1 and D108: MupAp5hP1 and MupAp5hD108, respectively (unpublished data).

The Mu phage formed plaques on *S. typhimurium* strains only if they possessed the Ra (*rfb* or *rfaL*) or Rb1 (*rfaK*) LPS chemotypes. It also had high rates of adsorption and transduction with these strains. Significant adsorption and intermediate levels of transduction were also seen with the Rb2 (*rfaJ*) chemotype. Thus, the receptor for Mu in *S. typhimurium* requires Glcα1-2Gal for full activity (Fig. 1). The low levels of transduction with the wild type may be due to spontaneous LPS mutants or to failure to cap enough of the LPS cores to block the receptors. The low transduction levels for the shorter LPS Rb3, Rc, and Rd1 structures may be due to partial adsorption of Mu to cells with these structures or leakiness of these mutations.

Sandulache et al. (18) suggested that the Mu receptor in *E. coli* K-12 is GlcNAcβ1-6Glcα1-2Glc, but that the *N*-acetylglucosamine (GlcNAc) may be dispensable. Our data prove that the GlcNAc is dispensable, since all three assays indicate that Mu adsorbs to the *rfaK* mutant as efficiently as to the strains of Ra chemotype. Additionally Sandulache et al. (18) stated that the terminal glucose must be α1-2 linked; however, our data show that a terminal galactose in α1-3 (*rfaJ*) also functions but with lower efficiency.

MuhP1 formed plaques on *S. typhimurium* strains with the Rc (*galE*) and Rd1 (*rfaG*) chemotypes; the same strains adsorbed the phage well and were efficiently transduced.

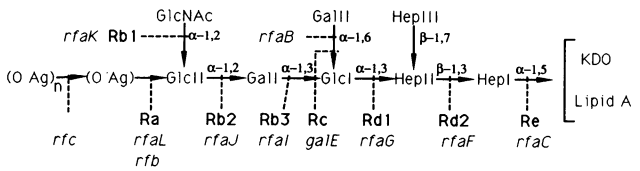

FIG. 1. Structure of the LPS of *S. typhimurium* and genes required for its synthesis (7, 10). The LPS is synthesized by transfer of carbohydrate units onto the lipid A as shown by the arrows. The genes required for each step are shown. Mutations in the genes give rise to LPSs that are blocked at the sites of the dashed lines. The chemotypes of the LPSs produced by mutations are indicated. There are three differences in the *E. coli* K-12 LPS: there is no O antigen (O Ag), GlcNAc is linked as β1-6, and galactose I (GalI) is replaced by glucose (GlcII) (15).

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TABLE 1. Sensitivity of *S. typhimurium* LPS mutants to Mu and related phages[a]

Bacterial strains[b]	Chemotype	Mu			MuhP1			D108			Mutant group	Sensitivity of analogous *E. coli* mutants[f]		
		Efficiency of plating[c]	Adsorption[d]	Transduction[e]	Efficiency of plating	Adsorption	Transduction	Efficiency of plating	Adsorption	Transduction		Mu	P1	D108
S. typhimurium LT2														
SA1355 (*rfa*$^+$)	S	−	0	1.1×10^{-4}	−	0	6.3×10^{-6}	−	0	1.1×10^{-5}				
SL428 (*rfc-458*)	SR	−	10	$<2 \times 10^{-6}$	−	0	5.9×10^{-6}	−	1	1.4×10^{-4}				
SA1627 Δ(*his-rfb*)*642*	Ra	3.7×10^{-4}	89	1.2	−	4	2.2×10^{-2}	8.6×10^{-5}	0	7.7×10^{-2}	Wild type	S	S	S
SL3749 (*rfaL446*)	Ra	3.7×10^{-4}	67	1.1	+	3	4.5×10^{-2}	1.5×10^{-5}	2	0.16				
SL 733 (*rfaK953*)	Rb1	2.0×10^{-5}	90	0.7	−	0	7.9×10^{-6}	−	0	2.2×10^{-4}				
SL3750 (*rfaJ417*)	Rb2	−	22	2.6×10^{-2}	−	63	1.2×10^{-5}	−	41	1.2×10^{-4}	1	R	S	S
SL3748 (*rfaI432*)	Rb3	−	0	5.7×10^{-5}	−	0	7.5×10^{-6}	−	5	1.1×10^{-5}	3	R	R	R
SL1306 (*galE503*)	Rc	−	0	7.7×10^{-3}	1.5×10^{-4}	88	1.38	3×10^{-4}	84	2.35	4	R	S	S
SL3769 (*rfaG471*)	Rd1	−	0	3.1×10^{-5}	2.1×10^{-4}	93	0.62	2.1×10^{-4}	48	0.30				
SL3789 (*rfaF511*)	Rd2	−	0	$<2 \times 10^{-6}$	+	75	1.4×10^{-3}	3×10^{-4}	47	0.35				
SA1377 (*rfaC630*)	Re	−	6	$<2 \times 10^{-6}$	−	2	$<4 \times 10^{-7}$	−	1	$<9 \times 10^{-7}$	6	R	R	R
E. coli K-12 MC4100	Wild type	1	73	5.5×10^{-3}	1	74	1.7×10^{-2}	1	63	5.8×10^{-3}				

[a] The data are the averages of at least two experiments.

[b] *S. typhimurium* strains are from the *Salmonella* Genetic Stock Centre, Calgary, Canada. Only the relevant LPS genotype is presented. Most of the strains designated SL are part of an isogenic set constructed by Roantree et al. (16).

[c] Efficiency of plating of phage, induced from *E. coli* K-12 lysogens, relative to the titers on *E. coli* K-12 strain MC4100 (where the titers were 10^9 to 10^{10} PFU/ml). +, lysis with high phage concentrations, with no plaques at lower concentrations; −, no clearing with over 10^9 PFU of phage. The efficiency of plating was measured by the standard phage technique in LB–2 mM $MgSO_4$ agar medium overlaid with soft agar (11).

[d] Percentage of PFU removed from suspension. Numbers below 10% are not significant.

[e] The number of Ap^r transductants relative to the average number of transductants obtained in the strains sensitive to the phage. The phages were induced from lysogens of *S. typhimurium* SL733(MupAp5) and SL3769(MupAp5hP1 and MupAp5hD108).

[f] Sensitivity of mutants of *E. coli* to each phage, summarized from the data of Sandulache et al. (18).

Rd2 (*rfaF*) cells were lysed with high titers of phage but did not form visible plaques. They adsorbed the MuhP1 phage and gave a low level of transduction. Strains with the Ra chemotype were also partially sensitive; they gave significant transduction but, surprisingly, no significant adsorption. Other strains were resistant. P1 phage was previously shown to transduce *galE* and *rfaG* mutants of *S. typhimurium* (14); the adsorption of MuhP1, as expected, resembled that of P1.

The adsorption properties of D108 resembled MuhP1, but there were quantitative differences. Strains carrying the *galE*, *rfaG*, or *rfaF* mutations were fully sensitive based on lysis, adsorption, and transduction tests (Table 1). In addition, the strains of Ra chemotype (*rfb* and *rfaL*) gave lysis (although the plaques were turbid) and transduction, although, surprisingly, significant adsorption was not detected. Thus, as in MuhP1, LPS with terminal glucose or heptose in the inner core of the LPS is a good receptor for D108, but LPS of the Ra chemotype (terminal glucose) is a less efficient receptor (Fig. 1). However, the much higher (by over 2 orders of magnitude) levels of transduction of MupAp5hD108 into Rd2 (*rfaF*) strains strongly suggests that the receptors of these phages are different, in contrast to what has been seen with P1 and D108 with *E. coli* mutants (18).

In contrast to their adsorption to *S. typhimurium*, P1 and D108 phages adsorbed well to wild-type strains of *E. coli* K-12 (analogous to the Ra chemotype) and to *E. coli* mutant groups 1 and 4, which have proposed structures that are analogous to Rb2 and Rc (Table 1) (18). Thus, whereas P1 (or MuhP1) and D108 do not discriminate between the two genera in the inner core components of the LPS (terminal heptose or glucose), the differences in the absorption to Ra chemotypes suggest that differences between the Ra chemotypes of these genera (GlcNAc linkages or the glucose and galactose content of the main chain; Fig. 1) may affect adsorption.

The three assays we used were generally consistent, but two exceptions deserve comment. The *rfaJ* mutant, which adsorbed MuhP1 and D108 efficiently, did not give lysis or significant levels of transduction (Table 1). However, the equivalent mutant group 1 of *E. coli* K-12 (Table 1) was fully sensitive to these phages, suggesting that the terminal Galα1-3Glc in the LPS of *S. typhimurium rfaJ* is equivalent to the corresponding terminal Glcα1-3 in *E. coli* for binding but not for productive infection. Since Mu binding to free polysaccharides can irreversibly trigger the phage (18), it is tempting to speculate that Mu may trigger but fail to inject its DNA when bound to *S. typhimurium rfaJ* LPS.

The other exception is the efficient transduction but low adsorption of *S. typhimurium rfb* and *rfaL* strains by MuhP1 and D108. Perhaps the low adsorption level is sufficient if enough time is allowed, as is the case when incubating to form plaques or for transduction. These strains have terminal GlcNAc linked α1-2 in contrast to the β1-6 of *E. coli*. Although GlcNAc is not required for Mu adsorption in *E. coli* or *S. typhimurium*, it is necessary for MuhP1 or D108 when present in the α1-2 as in *S. typhimurium*, since *rfaK* mutants do not adsorb these phages.

The three phages used here have an invertible DNA region for the synthesis of phage with two alternative types of tail fibers, (+) and (−) (9). Our absorption tests measure the binding of only (+) Mu and MuhP1 phage, since only the (+) type of Mu and the original P1 phage can infect *E. coli* K-12, which was used for determining titers (9). The results of the other two tests in Table 1 correlate well with the absorption results, which is consistent with the use of only the (+) orientation for all of these phages. Also consistent with this is the observation that the D108 phage, which plaques well on two different groups of LPS mutants, forms plaques equally efficiently on strains in each group after being grown lytically on strains of either group (data not shown). This would not be expected if different orientations were being used, since, at least for Mu and P1 (5, 9), lytic growth selects for phage predominantly in the infective orientation.

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