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Cell Wall Receptor for Bacteriophage Mu G(+)

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The invertible G segment in phage Mu DNA controls the host range of the phage. Depending on the orientation of the G segment, two types of phage particles, G(+) and G(−), are produced which recognize different cell surface receptors. The receptor for Mu G(+) was located in the lipopolysaccharide (LPS) of gram-negative bacteria. The analysis of different LPS core types and of mutants that were made resistant to Mu G(+) shows that the primary receptor site on *Escherichia coli* K-12 lies in the GlcNAcβ1 · · · 6Glcα1–2Glcα1– part at the outer end of the LPS. Mu shares this receptor site in *E. coli* K-12 with the unrelated single-stranded DNA phage St-1. Phage D108, which is related to Mu, and phages P1 and P7, which are unrelated to Mu but contain a homologous invertible DNA segment, have different receptor requirements. Since they also bind to terminal glucose in a different configuration, they adsorb to and infect *E. coli* K-12 strains with an incomplete LPS core.

The invertible G segment in phage Mu DNA belongs to a class of genetic elements which control gene expression by DNA rearrangement (9). Inversion of the G segment is promoted by the phage-encoded, site-specific recombination function Gin (13). Depending on the orientation of the G segment, two types of phage particles which differ in their host range are released (12, 25). The commonly used laboratory strains of *Escherichia coli* K-12 are infected only by the Mu G(+) type, whereas Mu G(−) phage particles are infectious for other species such as *Enterobacter cloacae*, *Citrobacter freundii*, and more. The basis for the switch in the host range lies in the fact that the two phage types, G(+) and G(−), recognize different cell surface receptors, which in both cases are located in the lipopolysaccharide (LPS) part of gram-negative bacteria (14).

In this paper we report on the receptor structure which is required for Mu G(+) adsorption to the *E. coli* K-12 cell wall. We took advantage of the fact that the structure of the *E. coli* K-12 LPS has been determined already (18), so the receptor could be located more precisely by analysis of Mu-resistant mutants of *E. coli* K-12 and by the comparison with known LPS core structures of other bacterial strains in which the absence or presence of the Mu receptor could be demonstrated.

Mu-resistant mutants of *E. coli* K-12 were further characterized by typing with E. coli phages, including D108, P1, and P7, which are of interest with respect to the phenomenon of G inversion (3, 4, 7, 24).

MATERIALS AND METHODS

Bacterial growth conditions. Bacteria (Table 1) were grown at 37°C in dYT medium (15) except for the *E. coli* K-12 *galE* and *galU* strains, which were cultivated in minimal medium M9 (15) with 1% glucose as the carbon source.

Preparation of LPS. LPS was extracted from acetone-dried bacteria by the method of Galanos et al. (6) and purified by ultracentrifugation at 100,000 × *g*. The overall yield of LPS was approximately 1% of the dry weight of the bacteria.

Preparation of phage lysates. Different phage strains are listed in Table 1. Phage Mu, D108, and P1 lysates were prepared by heat induction of lysogenic bacteria. The lysogens were grown in dYT broth to a cell density of 10^8 cells per ml at 32°C, shifted to 42°C for 10 min, and further incubated at 37°C until lysis occurred. Lysed cultures were sterilized by the addition of a few drops of chloroform and were centrifuged to remove cellular debris. $MgSO_4$ was added to a final cencentration of 10 mM. P7, T4, and St-1 were grown by infection of C600. To determine the relative efficiency of plating. 0.1-ml samples from serial dilutions in TM (10 mM Tris-hydrochloride, 10 mM $MgSO_4$) of a given bacteriophage stock containing 10^9 PFU/ml were incubated at 37°C for 15 min with 0.1 ml of an overnight bacterial culture. The mixture was plated with 2.5 ml of NA soft agar on YT plates (15). Plaques were counted after overnight incubation at 42°C.

Inactivation of the phage by LPS. LPS (0.1 ml, 0.5 to 500 μg) was added to 0.1 ml of phage lysate (diluted to 5×10^3 PFU/ml in TM) and incubated 1 h at 37°C. The mixture was incubated with 0.2 ml of indicator cells (*E. coli* K-12 C600 grown in dYT to stationary phase, sedimented, and suspended in TM) and plated with 2.5 ml of NA soft agar on YT plates. Receptor activity was expressed as the number of the plaques after overnight incubation at 42°C. When *E. coli* K-12 LPS was oxidized with sodium metaperiodate and then subjected to mild hydrolysis as described by Spiro (22), it lost its ability to bind Mu G(+). The oxidation or hydrolysis alone did not destroy the activity of LPS.

Isolation of phage-resistant mutants. Single colonies of *E. coli* K-12 were grown in dYT broth, and 0.2 ml of each culture was mixed with 2.5 ml of NA soft agar and then plated on YT plates (15). Phage lysates at a concentration of 10^9 PFU/ml were spotted on the lawn. After overnight incubation at 42°C, the resistant colonies, one from each plate, were picked from the lysed area and purified by two cycles of restreaking. They were also streaked out on eosin-methylene blue plates with 1% galactose to determine their Gal phenotype.

Quantitative sugar analysis. LPS (1 mg) was hydrolyzed in 1 ml of 0.5 N HCl in sealed tubes for 24 h at 100°C. For the quantitative analysis, a known amount of xylose was added to the hydrolysate as an internal standard. The aldoses were converted to the corresponding alditolacetates for the gas chromatographic analysis (8). The mixture of the alditolacetates was suspended in chloroform (5 μl), injected into a gas chromatograph (Hewlett-Packard model 5840 A) equipped with a 3% ECNSS-M on Gaschrom Q column (8). The alditol acetates were identified by their retention times by using a

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TABLE 1. Bacteria and phages

Bacterium or phage	Description	Reference or source
Bacteria		
E. coli K-12 C600		1
E. coli K-12 PL-2	*galE*	18
E. coli K-12 Gal-23	*galU*	18
E. coli K-12 H165	Hfr H	H. Hoffmann-Berling
E. coli K-12 S1652	Δ*gal*	21
E. coli B		19
E. coli C	Core type R1	Freiburg collection
E. coli F576	Core type R2	Freiburg collection
E. coli F635	Core type R3	Freiburg collection
E. coli F2556	Core type R4	Freiburg collection
S. arizonae	Core type Ra	Max von Pettenkofer Institut, Munich
Phages		
Mu	*c*Its62 *gin*⁻ 445-5 *G(+)*	13
P1	*Cm cts*100	20
D108	*cts*10	10
P7		27
St-1		2
T4	am82	W. Schultz

mixture of authentic alditolacetates as references, and the response factors of these standards were determined. The hexosamine was estimated after hydrolysis of the LPS with 6 N HCl for 18 h by the method of Strominger et al. (23). 3-Deoxy-D-manno-octulosonic acid (KDO) was liberated from LPS by hydrolysis with 0.25 N H_2SO_4 at 100°C for 8 min and determined by the method of Warawdekar and Saslaw (26).

RESULTS

Inactivation of Mu G(+) phage by LPS of different core structures. LPS were extracted and purified from *Salmonella arizonae*, *E. coli* C, *E. coli* F576, *E. coli* F635, *E. coli* F2556 (R forms), *E. coli* B, and *E. coli* K-12 C600, all representing different core types (11), and were tested for the presence of Mu receptor activity (Table 2). Only LPS from *Salmonella arizonae* Ra and *E. coli* F576, which are structurally similar to LPS from *E. coli* K-12, inactivated Mu G(+) phage. However, Ra LPS was 50 times less efficient that *E. coli* K-12 LPS. These results are consistent with the plating efficiency of Mu G(+) on these strains (14), and therefore the inability of Mu G(+) to plate on strains of other core types is due to the absence of receptor on the cell surface and not to the failure of propagation in these strains.

To localize the receptor site for phage adsorption on the LPS of *E. coli* K-12, we tested LPS from two mutant strains with incomplete cores (18). One of these strains has a mutation in *galE*, and the other has a mutation in *galU*. Neither LPS was active, indicating that the receptor is missing (Table 3). This result was unexpected since Mu infects *galE* mutants normally. The explanation of this inconsistency lies in experimental conditions which affect the synthesis of UDP-galactose, necessary for the assembly of the complete LPS core of *E. coli* K-12. UDP-galactose is generated from galactose present in the growth medium.

In the absence of exogenous galactose, UDP-galactose originates from the epimerization of UDP-glucose. A mutation in *galE* resulting in the loss of epimerase causes an LPS core without galactose. We observed that in minimal synthetic medium without any galactose, the *galE* strain synthesized an incomplete LPS core, whereas in broth medium it shows the complete wild-type LPS. Apparently, trace amounts of galactose which provide for adequate synthesis of UDP-galactose are present in broth. Consequently, *galE* bacteria grown in dYT medium were sensitive to Mu, whereas the same strain cultivated in minimal medium was resistant.

Another correlation between Gal phenotype and Mu sensitivity exists in the case of *E. coli* K-12 S1652. Because of a deletion in the *gal* operon, both pathways of UDP-galactose synthesis are abolished in this strain, and S1652 was found to be resistant to Mu independent of growth medium. The sensitivity to Mu could be restored by crossing this strain

TABLE 2. Mu G(+) inactivation by LPS of different core types

LPS core type	Structure[a]	ID_{50} of LPS (μg/ml)[b]
Ra *S. arizonae*	GlcNAcα1-2Glcα1-2Galα1-3Glcα1-3Hepα1- | α1,6 Gal	500
R1 *E. coli* C	Galα1-2Galα1-2Glcα1-3Glcα1-3Hepα1- | β1,3 Glc	>5,000
R2 *E. coli* F576	GlcNAcα1-2Glcα1-2Glcα1-3Glcα1-3Hepα1- | α1,6 Gal	22
R3 *E. coli* F635	Glcα1-2Glcα1-2Galα1-3Glcα1-3Hepα1- | α1,3 GlcNAc	>5,000
R4 *E. coli* F2556	Galα1-2Galα1-2Glcα1-3Glcα1-3Hepα1- | β1,4 Gal	>5,000
E. coli K-12	GlcNAcβ1---6Glcα1-2Glcα1-3Glcα1-3Hepα1- | α1,6 Gal	4
E. coli B	Glcα1-3Glcα1-3Hepα1-	>5,000

[a] The LPS structures are as given by Jansson et al. (11). Abbreviations: GlcNAc, *N*-acetylglucosamine; Glc, glucose; Gal, galactose; Hep, L-glycero-D-mannoheptose. In the LPS of *E. coli* K-12 the last glucose is partly substituted (in our preparation 15%) with GlcNAc.

[b] ID_{50} is the LPS dose by which half of the phage was inactivated.

TABLE 3. Mu G(+) inactivation by LPS from core-defective *E. coli* K-12 mutants

Strain	Structure[a]	ID_{50} of LPS (μg/ml)
Wild type	GlcNAc Gal :β1,6 | α1,6 Glcα1-2Glcα1-3Glcα1-3Hepα1-3Hep1-KDO- | 1,7 Hep	2.5
galE	Glcα1-3Glcα1-3Hepα1-3Hep1-KDO- | 1,7 Hep	>5,000
galU	Hepα1-3Hep1-3KDO-	>5,000

[a] For abbreviations, see Table 2, footnote *a*. KDO, 3-deoxy-D-manno-octulosonic.

TABLE 4. Sugar composition of the LPS from Mu^r mutants[a]

E. coli K-12 strain	Sugar composition of the LPS (%)[b]					Molar ratio (Gal:Glc:Hep)[c]	Proposed structure
	Gal	Glc	Hep	KDO	GlcN		
C600 (wild type)	3.4	10.7	10.5	10.5	11.0	1:2.7:3.2	Glc-Glc-Glc-Hep-Hep-KDO- ⋮ | | GlcNac Gal Hep
Group 1							
Mu^r#4	3.6	6.0	6.5	9.9	8.0	1:1.7:2	Glc-Glc-Hep-Hep-KDO- | |
Mu^r#10	2.9	6.9	7.1	8.7	7.6	1:2.3:2.8	Gal Hep
Group 2							
S1652	0	6.2	8.8	9.7	ND	0:2:3.2	Glc-Glc-Hep-Hep-KDO- |
							Hep
Group 3							
D108^r#10	1.1	1.2	2.0	5.3	7.7	1:1:2.1	Glc-Hep-Hep-HDO-
D108^r#13	3.1	4.2	7.3	8.3	9.7	1:1.4:2.7	| |
D108^r#25	3.5	5.0	8.0	8.3	8.5	1:1.4:2.7	Gal Hep
Group 4							
Mu^r#31	0	5.1	8.4	8.8	8.9	0:1:1.7	Glc-Hep-Hep-KDO-
Mu^r#35	0	4.8	7.1	10.0	8.9	0:1:2.2	
Group 5							
Mu^r#38	0	0.2	7.9	9.5	9.4	0:0:2.1	Hep-Hep-KDO-
Mu^r#12	0	0.2	6.5	8.3	9.7	0:0:1.6	
Mu^r#102	0	0.2	9.3	7.2	8.2	0:0:2.8	
Group 6							
S1652 T4^r#4	0	0	0	12.1	ND		KDO-

[a] For abbreviations, see Tables 2 and 3, footnote *a*; GlcN, glucosamine; ND, not determined.
[b] Values which were obtained by gas chromatography (see text) expressed as percentage of the dry weight of each preparation.
[c] The molar ratios of the constituents were based on three KDO and on two glucosamines from lipid A.

with HfrH165. All of 20 Gal$^+$ recombinants selected on minimal Gal streptomycin plates were Mu-sensitive.

The absence of galactose from LPS can be caused also by a defect in the *rfa* cluster, which controls LPS core assembly (17). *E. coli* B, which is Gal$^+$, synthesizes an incomplete LPS core that is identical to that of a *galE* mutant or S1652 (19) and is also unable to adsorb Mu G(+). Thus, the presence of galactose in the LPS core is tightly correlated with the sensitivity to Mu G(+) phage.

Isolation of *E. coli* K-12 mutants which lack the receptor for Mu and D108. Mutants of the *E. coli* K-12 strain C600 were selected for resistance to Mu G(+) phage. Of 40 independently isolated mutants, 2 were also D108-resistant; on the other hand, all 50 mutants selected for D108 resistance were also Mu G(+) resistant. Our observations confirm previous results that Mu and D108, which are largely homologous, have different host ranges (7, 10). To characterize the receptor for D108, we also isolated D108-resistant mutants of strain S1652. S1652 belongs to that class of strains which is resistant to Mu but sensitive to D108.

That the vast majority of these phage-resistant mutants indeed represent receptor mutants was confirmed after purification of LPS from a sample of these mutant strains by demonstrating that LPS did not inactivate Mu G(+). The fact that LPS of S1652 inactivated D108 and LPS of a D108-resistant mutant of S1652 did not shows furthermore that the receptor of D108 is also LPS. The mutant S1652 D108^r#3 was the only apparent exception because it synthesized an active LPS. This mutation did not affect the receptor structure but rather affected phage multiplication.

Biochemical characterization of receptor mutants of *E. coli* K-12. To establish the structural cause for resistance to Mu G(+), LPS of several mutants was analyzed by gas chromatography for sugar composition. Six classes of mutants with various degrees of incomplete core could be distinguished, and hypothetical structures based on sugar ratios were established (Table 4). All types of incomplete cores were obtained except the one lacking the branching galactose. This confirmed previous results that galactose is a prerequisite for incorporation of the distal glucose into LPS (17). Mutants which synthesized LPS with one glucose and one galactose (Table 4, group 3) were earlier described in *Salmonella typhimurium* (16), and strains showing LPS with one glucose only (Table 4, group 4) were previously isolated in *E. coli* K-12 and *E. coli* B (18, 19). Another group of mutants was represented by group 5, which carried LPS without the outer core; only traces of glucose were present.

Because of the Mu^r phenotype of S1652, we expected some Gal$^-$ strains among *E. coli* K-12 mutants selected for Mu resistance. Indeed, 3 of 90 mutants proved to be Gal$^-$. On the basis of the LPS structure, they must be *galU* mutants and were included in group 5 of the mutants.

Characterization of receptor mutants by phage typing. A simple way of characterizing receptor mutants is phage typing. In addition to D108, we employed phages P1, P7, St-1, and T4, which also bind to LPS from *E. coli* K-12 (Table 5). No Mu^r mutants were infected by St-1, a single-stranded DNA phage unrelated to Mu. Conversely, all St-1^r mutants were shown to be Mu^r. Mutants of groups 1, 2, and 4 were sensitive for D108, P1, and P7, which showed that terminal

TABLE 5. Sensitivity of Mu^r mutants to other phages

E. coli K-12 strain	Relative efficiency of plating[a]				Inactivation by LPS (% surviving phage)[b]		
	D108	P1	P7	T4	D108	P1	P7
Wild type	1	1	1	1	0	2	3.8
Group 1							
Mu^r#10	0.5	0.3	1	1	0	1.6	34
Group 2							
S1652	1	1	0.2	1	5.5	0	0.8
Group 3							
D108^r#10	$<10^{-6}$	$<10^{-9}$	$<10^{-8}$	1	100	100	81
D108^r#13	$<10^{-6}$	$<10^{-9}$	$<10^{-8}$	1	77.8	100	124.5
D108^r#25	$<10^{-6}$	$<10^{-9}$	$<10^{-8}$	1	69.4	100	74.5
Group 4							
Mu^r#31	0.3	1	1	1	6.9	3.5	3.8
Mu^r#35	1	0.5	1	1	1.4	0	0
Group 5							
Mu^r#38	1	1	$<10^{-6}$	$<10^{-9}$	70.9	72	100
Mu^r#12	0.3	1	$<10^{-6}$	$<10^{-9}$	43	45	100
Mu^r#102	0.4	1	$<10^{-6}$	$<10^{-9}$	62	89	100
Group 6							
S1652T4^r#1	$<10^{-6}$	$<10^{-9}$	$<10^{-8}$	$<10^{-9}$	100	80	100

[a] Mu G(+), G(−), and St-1 did not make plaques on *E. coli* K-12 Mu G(+)^r mutants (less than 10^{-8} relative to the titer on wildtype strain C600).
[b] Inactivation of the phage was carried out with 500 μg LPS per ml.

glucose was required for adsorption of these phages. Mutants of group 3 were only sensitive for T4. Mutants of group 5 were not infected by P7, whereas mutants of group 6 were resistant to all the phages we tested. Apart from the S1652 D108^r#3 mutant referred to above, inconsistencies were noticed in several resistant mutants in which normal efficiency of plating was not correlated with receptor activity of LPS. The example of class 5 mutants suggests that the difference is due to the leakiness of these mutants since traces of glucose which could be demonstrated by LPS analysis might be sufficient for adsorption of D108 and P1 under plating conditions but not in the LPS binding assay. On the other hand, these traces are obviously not enough to allow adsorption of P7. This would also explain the failure of P7 to plate on a particular mutant, C600 Mu^r#4, which we placed in group 1 on the basis of its LPS structure but which could in fact be a leaky mutant of group 3.

Carbohydrates as inhibitors of Mu adsorption. Since Mu phage recognizes the polysaccharide part of LPS, we tested the specificity of different mono-, di-, and trisaccharides as inhibitors of Mu adsorption. Disaccharides such as gentobiose and threhalose had a stronger effect than glucose alone did (Table 6).

DISCUSSION

The structural component of the cell wall that is responsible for the adsorption of Mu G(+) phage has been located within the LPS of *E. coli* K-12. Phage-resistant mutants of *E. coli* K-12 were defective in the LPS core, and the loss of the distal part of the core was already sufficient to make an *E. coli* K-12 cell resistant to Mu. Therefore *N*-acetylglucosamine (GlcNAc) and the distal glucose might play an important role in the binding of the phage. However, it is known that the C6 position at the distal glucose in the *E. coli* K-12 core is only partially substituted by GlcNAc (18). Since we also observed only 15% substitution by GlcNAc in our most active LPS preparation, it seems more plausible that terminal glucose in the majority of the LPS molecules is the Mu receptor in *E. coli* K-12. The involvement of GlcNAc in the receptor cannot be ruled out completely at this moment, but it seems unlikely in view of our failure to detect a subclass of Mu-resistant mutants which lacks GlcNAc only. Glucose alone cannot be sufficient because of the absence of receptor activity in *E. coli* C and incomplete cores of *E. coli* K-12. The comparable receptor activity of R2 core type LPS (Table 2) suggests that terminal 1.2-linked glucose can be replaced largely by GlcNAc in the same glycosidic linkage. This is consistent with the presence of terminal GlcNAcα1-2Glcα1- in the Ra core (11), but the poor affinity of the Ra LPS for Mu (Table 2) suggests that additional structural features of the LPS could affect the phage-receptor interaction. For instance, the presence of galactose instead of glucose next to the terminal disaccharide GlcNAcα1-2Glcα1- (Table 2) might decrease the binding of the phage, and the substitution of galactose, as in the R3 core type, could interfere with the phage attachment even more severely. To summarize, we conclude that terminal Glcα-2Glcα1-

TABLE 6. Inhibition of Mu adsorption to *E. coli* K-12 cells by sugars

Sugar[a]	% Inhibition
Glucose	18
1-α-Methylglucose	20
1-β-Methylglucose[b]	<10
Threhalose [Glc(α1-2α)Glc]	30
Gentiobiose [Glc(β1-6β)Glc]	80

[a] Final concentration, 0.6 M by the method of Dawes (5). For abbreviations, see Table 2, footnote *a*.
[b] 3-O-Methyl glucose, 1-α-methyl galactose, 1-β-methyl galactose, maltose, sucrose, melibiose, and raffinose were also without effect on Mu adsorption.

or GlcNAcα1-2Glcα1- is a requirement for adsorption of Mu G(+).

Terminal glucose in a different configuration is also part of the Mu G(−) receptor: our preliminary results suggest that Glcβ1-6Glcα1- is needed for attachment of Mu G(−) to the host cell wall. The genetic information for the receptor specificity must lie in the invertible G segment so that tail fibers with different receptor binding sites are expressed depending on the orientation of the G segment. It is therefore of considerable interest to analyze the receptor of other phages which also have the invertible G segment, such as P1, P7, and D108. In this report, we have shown by phage typing of the *E. coli* K-12 cell wall mutants that in the first approximation, P1, P7, and D108 have the same receptor and that this receptor is different from the Mu receptor. The structural requirements for P1 adsorption have been characterized in more detail (R. Sandulache, W. Ritthaler and D. Kamp, manuscript in preparation). Since P1 cannot discriminate between 1-2 or 1-3 glycosidic linkage, it also binds to incomplete LPS core. Unlike P1 and P7, D108 is largely homologous to Mu (10). However, the D108 receptor seems to be identical to the P1 receptor and not to the Mu receptor.

Convergence may be a plausible explanation why Mu and St-1 have the same cell surface receptor because Mu and St-1 represent totally different types of phages. We would like to point out, however, that an evolutionary relationship between the tail fibers of Mu and St-1, or perhaps even T4, cannot be ruled out because Mu and P1 are also unrelated, except for the homologous G segment.

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LITERATURE CITED

1. **Appleyard, R. K.** 1954. Segregation of lambda lysogenicity during bacterial recombination in *E. coli* K-12. Genetics **39**:429–439.
2. **Bradley, D. E.** 1970. A comparative study of some properties of the φX174 type bacteriophages. Can. J. Microbiol. **16**:965–971.
3. **Chow, L. T., T. R. Broker, R. Kahmann, and D. Kamp.** 1978. Comparison of the G DNA inversion in bacteriophages Mu, P1, and P7, p. 55–56. *In* D. Schlessinger (ed.), Microbiology—1978. American Society for Microbiology, Washington, D.C.
4. **Chow, L. T., and A. I. Bukhari.** 1976. The invertible DNA segments of coliphages Mu and P1 are identical. Virology **74**:242–248.
5. **Dawes, J.** 1975. Characterization of the bactiriophage T4 receptor site. Nature (London) **256**:127–128.
6. **Galanos, C., O. Luderitz, and O. Westphal.** 1969. A new method for the extraction of R lipopolysaccharides. Eur. J. Biochem. **9**:245–249.
7. **Gill, G. S., A. C. Hull, and R. Curtiss III.** 1981. Mutator bacteriophage D108 and its DNA: an electron microscopic characterization. J. Virol. **37**:420–430.
8. **Holme, T., A. A. Lindberg, P. J. Garegg, and T. Onn.** 1968. Chemical composition of cell-wall polysaccharide of rough mutants of *Salmonella typhimurium*. J. Gen. Microbiol. **52**:45–54.
9. **Howe, M. M.** 1980. The invertible G segment of phage Mu. Cell **21**:605–606.
10. **Hull, R. A., G. S. Gill, and R. Curtiss III.** 1978. Genetic characterization of Mu-like bacteriophage D108. J. Virology **27**:513–518.
11. **Jansson, P., A. A. Lindberg, B. Lindberg, and R. Wollin.** 1981. Structural studies on the hexose region of the core in lipopolysaccharides from Enterobacteriaceae. Eur. J. Biochem. **115**:571–577.
12. **Kamp, D.** 1981. Invertible deoxyribonucleic acid: the G segment of bacteriophage Mu, p. 73–76. *In* D. Schlessinger (ed.), Microbiology—1981. American Society for Microbiology, Washington, D.C.
13. **Kamp, D., R. Kahmann, D. Zipser, T. R. Broker, and L. T. Chow.** 1978. Inversion of the G DNA segment of phage Mu controls phage infectivity. Nature (London) **271**:577–580.
14. **Kamp, D., and R. Sandulache.** 1983. Recognition of cell surface receptors is controlled by invertible DNA of phage Mu. FEMS Microbiol. Lett. **16**:131–135.
15. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Osborn, M. J.** 1968. Biochemical characterization of mutants of *Salmonella typhimurium* lacking glucosyl or galactosyl lipopolysaccharide transferases. Nature (London) **217**:957–960.
17. **Osborn, M. J., and L. I. Rothfield.** 1971. Biosynthesis of the core region of LPS, p. 331–350. *In* G. Weinbaum, S. Kadis, and S. J. Ajl (ed.), Microbial toxins, vol. 4. Academic Press, Inc., New York.
18. **Prehm, P., G. Schmidt, B. Jann, and K. Jann.** 1976. The cell wall lipopolysaccharide of *Escherichia coli* K-12 Eur. J. Biochem. **70**:171–177.
19. **Prehm, P., S. Stirm, B. Jann, and K. Jann.** 1975. Cell wall lipopolysaccharide from *Escherichia coli* B. Eur. J. Biochem. **56**:41–55.
20. **Rosner, J. L.** 1972. Formation, induction, and curing of bacteriophage P1 lysogens. Virology **49**:679–689.
21. **Saedler, H., and P. Starlinger.** 1967. 0° mutation in the galactose operon in *E. coli*. Mol. Gen. Genet. **100**:178–189.
22. **Spiro, R. G.** 1966. Analysis of sugars found in glycoproteins. Methods Enzymol. **8**:44–49.
23. **Strominger, J. L., J. T. Park, and R. E. Thompson.** 1959. Composition of the cell wall of *Staphylococcus aureus*: Its relation to the mechanism of action of penicillin. J. Biol. Chem. **234**:3263–3269.
24. **Toussaint, A., N. Lefebvre, J. R. Scott, J. A. Cowan, F. Bruijn, and A. I. Bukhari.** 1978. Relationship between temperate phages Mu and P1. Virology **89**:146–161.
25. **Van de Putte, P., S. Cramer, and M. Giphart-Gassler.** 1980. Invertible DNA determines host specificity of bacteriophage Mu. Nature (London) **286**:218–222.
26. **Waravdekar, V. S., and L. D. Saslaw.** 1959. A sensitive colorimetric method for the estimation of 2-deoxy sugars with the use of the malonaldehyde-thiobarbituric acid reaction. J. Biol. Chem. **234**:1945–1950.
27. **Yun, T., and D. Vapnek.** 1977. Electron microscopic analysis of bacteriophage P1, P1Cm and P7: determination of genome sizes, sequence homology and location of antibiotic resistance determinants. Virology **77**:376–385.