Rough Mutants of *Salmonella typhimurium*: Immunochemical and Structural Analysis of Lipopolysaccharides from *rfaH* Mutants

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Lipopolysaccharides, extracted by phenol/chloroform/petroleum ether, from two rough mutants of *Salmonella typhimurium* of class *rfaH* were studied by passive haemagglutination inhibition and by methylation analysis. The structural and immunochemical analyses showed that (i) formation of the galactose I unit of the core is defective, but the defect is not complete, and (ii) of those core chains which do receive the galactose I residue, many are not continued to form complete core, but instead terminate at intermediate points. This suggests that the *rfaH* gene, though involved in formation of the galactose I unit, is not the structural gene for the galactosyltransferase which adds this unit. The *rfaH* product may be a positive regulator for several *rfa* genes specifying glycosyltransferases, or it may be a protein needed for the efficient action of several such transferases.

INTRODUCTION

Lipopolysaccharides (LPS) of *Salmonella* consist of three different regions: an outer region, the O-specific chain, built up of polymerized oligosaccharide repeating units linked to an inner region, the basal core chain, which, in turn, is linked to lipid A (Fig. 1) (Lüderitz *et al.*, 1971). The structure of the O-specific chain of *Salmonella typhimurium* LT2 has been elucidated by methylation analysis of extracted LPS (Hellerqvist *et al.*, 1969). Mutants defective in synthesis of the repeating units (*rfb* mutants) or in synthesis of the basal core (*rfa* mutants) were used for structural analysis of the basal core (Hellerqvist & Lindberg, 1971). However, it is a time-consuming process to classify rough mutants by structural analysis of their LPS, and so other methods have been used: mutants have been classified by their sensitivity to a variety of bacteriophages (Wilkinson *et al.*, 1972) and their isolated LPS have been classified by immunochemical methods such as passive haemagglutination inhibition (Beckmann *et al.*, 1964). These methods permit the establishment of certain criteria for the classification of rough mutants of *S. typhimurium* (Lindberg & Hellerqvist, 1971).

An incomplete LPS core may result either (i) from deficiency in a glycosyltransferase activity, due to mutation of an *rfa* gene, or (ii) from inability to synthesize UDPgalactose or UDPglucose (the precursors of, respectively, the galactose and glucose units of the core), because of loss of function of gene *galE*, which specifies UDPgalactose epimerase, or of gene *galU*, which specifies UDPglucose pyrophosphorylase. Mutants of class *galE* make LPS of type Rc (Fig. 1), with a core chain which terminates in a glucose I unit lacking both the galactose units normally attached to it. Such mutants have a characteristic pattern of phage sensitivity, including sensitivity to phages C21 and Br2 (Wilkinson *et al.*, 1972). Two mutants, SL1033 and SL1060, with this phage sensitivity pattern, but which fermented

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Fig. 1. Main features of *Salmonella* lipopolysaccharide structure, and genes concerned with the formation of different units. Genes marked by an asterisk are located between *cysE* and *pyrE*. *rfaH*(?) denotes that the gene affected was thought to specify an enzyme for the transfer of galactose I (Wilkinson & Stocker, 1968). The present paper makes this hypothesis implausible. Genes *galE* and *galU* are indicated in parentheses because they have functions other than LPS synthesis. Entries in Roman type (Ra, Rb1 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.

galactose normally and so were deficient in neither *galE* nor *galU* function, were isolated by Wilkinson & Stocker (1968). Osborn (1968) found that cell-free extracts of these two mutants lacked the wild-type ability to catalyse transfer of galactose from UDPgalactose to LPS of type Rc, and also that the LPS of these two mutants accepted galactose from UDPgalactose when incubated with extracts of *rfa*⁺ bacteria. These observations suggested that the mutations in these two strains affected a gene concerned with the attachment of galactose to C-3 of glucose I of the core (because the galactose transferred to LPS *in vitro* was attached to this carbon to form the main-chain galactose I unit, and not to C-6, to form the galactose II side-branch). The affected gene was designated *rfaH* (Wilkinson & Stocker, 1968) and was surmised to be the structural gene for the galactosyl-transferring enzyme. Genetic analysis of the two original *rfaH* mutants, and of several additional mutants of similar phenotype (Kuo & Stocker, 1972), showed that the *rfaH* locus is not between *cysE* and *pyrE*, where nearly all other identified *rfa* genes are clustered.

In this paper, we describe structural analysis and serological investigation of the LPS of the two original *rfaH* mutants, SL1033 and SL1060. Their LPS proved to be heterogeneous, some core chains terminating in glucose I but others ending at various more distal points. This suggests that the *rfaH* mutations cause partial rather than complete failure to form galactose I units, and that they also cause partial defects in several of the reactions which add successive monosaccharide units distal to glucose I. In the preceding paper, Stocker *et al.* (1980) show that in the same two *rfaH* mutants the gene affected is cotransducible with *metE*, at unit 84 on the current *S. typhimurium* linkage map (Sanderson & Hartman, 1978). Stocker *et al.* (1980) also describe the 'curing' of the LPS defect of *rfaH* mutants by an F' factor which includes the *metE* region of the *Escherichia coli* chromosome, and the effect of RfaH⁻ character on sensitivity to various antibiotics.

METHODS

Bacterial strains. The strains used are listed in Table 1. The two *rfaH* mutants used are derived from parents which are *pmi* (deficient in phosphomannoisomerase and therefore unable to synthesize the mannose-containing O repeating unit except from exogenous mannose); it was therefore not expected that O chains would be added even to any completed core chains. Each strain was reisolated from a single colony and shown to behave as expected when tested for nutritional characteristics and phage sensitivity before and after cultivation.

Extraction of LPS. The bacteria were grown as described by Holme *et al.* (1968). LPS was extracted with an aqueous phenol, chloroform and petroleum ether mixture (the PCP method) from ethanol-, acetone- and ether-dried bacteria (Galanos *et al.*, 1969).

Table 1. *Derivatives of Salmonella typhimurium* LT2 used

Strain no.	LPS genotype*	LPS chemotype	Reference(s)
SH180	<i>his-658</i>	Ra	Nikaido <i>et al.</i> (1967)
SL733	<i>rfaK953</i>	Rb1	Yamamoto & Anderson (1961); Naide <i>et al.</i> (1965)
SL805	<i>gal-417</i> †	Rc	Wilkinson (1966)
SL1027	Wild-type (= smooth)		Wilkinson <i>et al.</i> (1972); Kuo & Stocker (1972)
SL1033	<i>pmi-404 rfaH481</i>	‡	Wilkinson & Stocker (1968); Osborn (1968); Kuo & Stocker (1972); Stocker <i>et al.</i> (1980)
SL1060	<i>pmi-404 rfaH487</i>	‡	Wilkinson & Stocker (1968); Osborn (1968); Kuo & Stocker (1972); Stocker <i>et al.</i> (1980)
SL3660	<i>rfa(R-res-2)674</i>	Rb3	Kuo (1969)
SL3661	<i>rfa(R-res-2)675</i>	Rb3	Kuo (1969)
TV148	<i>rfa(R-res-2)432</i>	Rb3	Subbaiah & Stocker (1964)
TV160	<i>rfaJ418</i>	Rb2	Subbaiah & Stocker (1964)
TV161	<i>rfaJ417</i>	Rb2	Subbaiah & Stocker (1964); Kuo & Stocker (1972)
TV166	<i>rfaJ419</i>	Rb2	Subbaiah & Stocker (1964); Kuo & Stocker (1972)

* For origin and genotypic characters not relevant to LPS character, see the preceding paper (Stocker *et al.*, 1980) and the cited references.

† SL805 is deficient in all three enzymes of galactose metabolism, including UDPgalactose epimerase (Wilkinson, 1966).

‡ LPS is heterogeneous, see text.

Phage attachment studies. Bacteria from exponential phase broth cultures were washed and resuspended to 2×10^8 cells ml⁻¹ in 0.05 M-Tris/HCl buffer, pH 7.2, with 0.005 M-CaCl₂. Rates of irreversible attachment of phage C21 were measured by the chloroform method (Lindberg & Svensson, 1975).

Passive haemagglutination inhibition studies. The immunochemical specificity of the extracted LPS fractions was studied in a haemagglutination inhibition system using sheep red blood cells sensitized with alkali-treated LPS and sera chosen to represent chemotypes Ra, Rb1, Rb2, Rb3 and Rc of the *Salmonella* core (Fig. 1). The serum dilutions used were two to four haemagglutinating units. The tests were performed using a Takatsy microtitrator (Cooke Engineering Co., Alexandria, Va., U.S.A.) using 25 µl loops and 1% erythrocyte suspensions. Agglutination was recorded after incubation at room temperature for 1 h and in the cold for 18 h.

Methylation analysis of LPS. Treatment of the LPS with aqueous acetic acid at pH 3.2 for 1 h at 100 °C cleaved the acid-labile 3-deoxy-D-manno-octulosonic acid (KDO) linkages. After centrifugation (4000g, 4 °C, 20 min), the precipitate containing lipid A was discarded. The supernatant contained a polysaccharide with no or diminished content of lipid. The polysaccharides were then hydrolysed to give a mixture of monosaccharides which were analysed as alditol acetates by gas-liquid chromatography (Holme *et al.*, 1968). The different sugars were identified solely by their retention times and mass spectra (Golovkina *et al.*, 1966). The lipid-free polysaccharides were also subjected to methylation analysis (Hellerqvist *et al.*, 1968) to elucidate the structural features of the core polysaccharide chain. The mixture of methylated sugars obtained was transformed into the corresponding alditol acetates and analysed by gas-liquid chromatography/mass spectrometry (Björndal *et al.*, 1967a, b).

RESULTS

Passive haemagglutination inhibition

LPS extracted from the two *rfaH*, three chemotype Rb2 and three chemotype Rb3 *S. typhimurium* mutants were tested for their ability to inhibit haemagglutination in a set of LPS/homologous anti-bacterial serum systems representing complete core LPS and four different incomplete core types (Table 2 and Fig. 1). LPS from the two *rfaH* mutants (SL1033 and SL1060) inhibited all five of the haemagglutination systems, at concentrations ranging from 2 to 128 µg ml⁻¹. In contrast, LPS from chemotype Rb3 mutants inhibited only the homologous system, and LPS from chemotype Rb2 mutants inhibited the homologous and, more weakly, the Rb1 system. This indicates that whereas LPS from the chemo-

Table 2. *Passive haemagglutination inhibition tests with LPS of rough mutants of S. typhimurium LT2*

The results show the minimum concentration of LPS needed to inhibit haemagglutination visible to the naked eye in the indicated system (sheep red blood cells coated with LPS plus homologous anti-bacterial serum; two haemagglutination units). — indicates no inhibition by LPS at 256 $\mu\text{g ml}^{-1}$, the highest concentration tested.

Inhibitor, LPS from strain no.	Phage pattern (and chemotype)	Minimum inhibitory concentration of LPS ($\mu\text{g ml}^{-1}$) against homologous LPS/antiserum systems				
		SH180 (GlcNAc) Ra	SL733 (GlcII) Rb1	TV160 (GalII, GalIII) Rb2	TV148 (GalIII) Rb3	SL805 (GlcI) Rc
TV160	R-res-1	—	128	2	—	—
TV161	(Rb2)	—	64	4	—	—
TV166		—	64	4	—	—
SL1033	Epi-1	4	16	4	4	2
SL1060	(heterogeneous)	8	4	8	4	128
TV148	R-res-2	—	—	—	0.5	—
SL3660	(Rb3)	—	—	—	4	—
SL3661		—	—	—	4	—

Table 3. *Methyl ethers from the hydrolysate of methylated, degraded lipopolysaccharide**

Sugar	Retention time†	Molar proportions of methyl ethers			
		TV166	TV148	SL1033	SL1060
2,3,4,6-Tetra- <i>O</i> -methyl-D-glucose	1.00	3.0	2.0	12.1	8.5
2,3,4,6-Tetra- <i>O</i> -methyl-D-galactose	1.25	41.2	20.0	40.0	25.0
3,4,6-Tri- <i>O</i> -methyl-D-glucose	1.98	ND	ND	2.9	1.6
2,4,6-Tri- <i>O</i> -methyl-D-galactose	2.28	ND	ND	2.9	ND
2,3,4-Tri- <i>O</i> -methyl-D-glucose	2.48	ND	19.0	15.0	12.4
3,4,6-Tri- <i>O</i> -methyl-D-galactose	2.50	ND	ND	10.4	5.6
4,6-Di- <i>O</i> -methyl-D-galactose	3.64	ND	ND	2.5	1.4
2,4-Di- <i>O</i> -methyl-D-glucose	5.10	20.7	ND	18.9	10.8

ND, Not detected.

* The methyl ethers of the heptose and *N*-acetylglucosamine residues are not included.

† Retention times of the corresponding alditol acetates on the ECNSS-M S.C.O.T. column, relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

type Rb2 and Rb3 mutants have core polysaccharide chains with one, or at most two, immunochemical specificities, *rfaH* LPS displays five specificities.

Phage C21 attachment

The attachment rate constant for phage C21, which attaches maximally to strains in which the core terminates with the glucose I residue (Fig. 1) (Lindberg, 1973), was determined using the two *rfaH* mutants and also chemotype Ra, Rb1, Rb2, Rb3 and Rc mutants. High attachment rate constants were observed only for two *galE* (chemotype Rc) mutants (mean $117 \times 10^{-11} \text{ ml}^{-1} \text{ min}^{-1}$, range 113 to 120×10^{-11}). No attachment ($< 5 \times 10^{-11} \text{ ml}^{-1} \text{ min}^{-1}$) was seen for chemotype Ra, Rb1, Rb2 and Rb3 strains. Attachment of C21 was, however, demonstrated for both *rfaH* mutants (21×10^{-11} and $30 \times 10^{-11} \text{ ml}^{-1} \text{ min}^{-1}$ for SL1033 and SL1060, respectively). These data suggest that in the *rfaH* mutants uncapped glucose I residues are accessible to the phage.

Structural studies of lipopolysaccharides

LPS preparations, with no or little lipid A, from the two *rfaH* mutants (SL1033 and SL1060), one Rb2 (TV166) and one Rb3 (TV148) mutant were subjected to sugar and

Table 4. *Inferred structure of core polysaccharide chains*

Structure of side-chain	No. of core chains with indicated structure/100 core chains					
	SH180* Ra	SL733* Rb1	TV166 Rb2	SL1033	SL1060	TV148 Rb3
$\begin{array}{c} \text{GlcNAc} \\ \downarrow 1 \\ \text{GlcII } 1 \longrightarrow 2 \text{ GalI } 1 \longrightarrow 3 \text{ GlcI } 1 \longrightarrow \end{array}$	83	< 1	< 1	8	7	< 1
$\begin{array}{c} \text{GalII} \\ \downarrow 1 \\ \text{GlcII } 1 \longrightarrow 2 \text{ GalI } 1 \longrightarrow 3 \text{ GlcI } 1 \longrightarrow \end{array}$	< 1	61	< 1	20	14	< 1
$\begin{array}{c} \text{GalII} \\ \downarrow 1 \\ \text{GalI } 1 \longrightarrow 3 \text{ GlcI } 1 \longrightarrow \end{array}$	< 1	< 1	87	22	18	< 1
$\begin{array}{c} \text{GalII} \\ \downarrow 1 \\ \text{GlcI } 1 \longrightarrow \end{array}$	< 1	< 1	< 1	38	43	91
$\begin{array}{c} \text{GlcI } 1 \longrightarrow \end{array}$	< 1	< 1	13	12	18	9

* Calculation based on data from Hellerqvist & Lindberg (1971). In analyses for LPS from strains SH180 and SL733, the missing number of core chains (17 for SH180 and 39 for SL733) have a structure identical to the given core chain except for the absence of the galactose II residue.

methylation analysis (Table 3). The glucose:galactose:heptose molar ratios in LPS from SL1033, SL1060, TV166 and TV148 were 29:33:38, 29:31:40, 22:36:43 and 25:26:49, respectively, as determined by sugar analyses. When the LPS were subjected to methylation analysis the hexose methyl ethers found in SL1033 and SL1060 LPS included all of the methyl ethers expected from a mixture of LPS of chemotypes Ra, Rb1, Rb2, Rb3 and Rc (Fig. 1). In contrast, the number of methyl ethers found in the LPS of strains TV166 and TV148 was much smaller and they were of the types expected to arise from the LPS of chemotypes Rb2 and Rb3, respectively.

As we have described previously (Hellerqvist & Lindberg, 1971), one can calculate from the relative abundance of the methyl ethers the proportion of core chains ending in different units in the LPS analysed (assuming the chemotype Ra, or complete core structure, shown in Fig. 1). In Table 4, the inferred structures of the LPS of *rfaH* mutants SL1033 and SL1060, deduced from the proportions of methyl ethers (Table 3), are given together with those of mutants SH180 (R-sens, chemotype Ra), SL733 (D-1, chemotype Rb1), TV166 (R-res-1, chemotype Rb2) and TV148 (R-res-2, chemotype Rb3).

The core of LPS from SH180 (chemotype Ra), SL733 (chemotype Rb1), TV166 (chemotype Rb2) and TV148 (chemotype Rb3) all terminated with the expected residue – respectively, *N*-acetylglucosamine, glucose II, galactose I and galactose II. The only observed exceptions to the regular structure were that (i) some of the core chains in TV166 and TV148 terminated with the glucose I residue, and (ii) some of the glucose I residues of SH180 and SL733 lacked the galactose II residue. The LPS of *rfaH* mutants SL1033 and SL1060 was much less homogeneous. Incomplete core chains terminating with the glucose I, galactose I, galactose II and glucose II residues, as well as complete cores terminating with *N*-acetylglucosamine, were observed.

DISCUSSION

The structural and immunochemical analyses of LPS isolated from the two *rfaH* mutants show that (i) the block in formation of the galactose I unit is incomplete, as shown by methylation analysis and passive haemagglutination inhibition, and (ii) some of those core chains which receive the galactose I residue terminate abnormally and the core is not completed. This makes it unlikely that *rfaH* (defined as the gene mutated in strains SL1033 and SL1060) is the structural gene for a polypeptide which is, or is a specific component of, an enzyme whose only function is to catalyse the formation of the galactose I unit of the core.

LPS from *rfa* mutants of four other classes inhibited one, or at most two, of the haemagglutination inhibition systems representing the various core types (Fig. 1) (Table 2 and Lindberg & Hellerqvist, 1971). The LPS from SL1033 and SL1060 showed a broader activity and inhibited all five haemagglutination inhibition systems tested (Table 2).

Lipid-free polysaccharides from the two *rfaH* mutants were subjected to methylation analysis (Tables 3 and 4). The composition of methyl ethers found in the polysaccharides analysed showed a complex picture, in agreement with the haemagglutination inhibition data. Based on the structure in Fig. 1, analysis of the methyl ethers gave the composition of core structures for SL1033 and SL1060 shown in Table 4. All structures representing incomplete cores from chemotype Rc out to complete cores of chemotype Ra were represented. In both LPS, the GalII 1 $\rightarrow$ 6 GlcI 1 $\rightarrow$ structure (chemotype Rb3), expected to result from failure to form the galactose I unit, predominated, as shown by the abundance of the 2,3,4-tri-*O*-methylglucose ether (Table 3) resulting from methylation of C-6 substituted

glucose residues. The next commonest core structure was GalI 1 $\rightarrow$ 3 GlcI 1 $\rightarrow$ (chemotype Rb2), followed by chemotype Rb1 and then chemotype Ra. No such complexity was seen in the core polysaccharides from mutants representing four other *rfa* classes (Table 4).

In quantitative analyses of lipid-free polysaccharide from strains SL1033 and SL1060, the GalII 1 $\rightarrow$ 6 GlcI 1 $\rightarrow$ structure was found by Osborn (1968). This, coupled with the low soluble LPS-galactosyltransferase I activity in extracts from the strains, and the ability of their isolated LPS to act as *in vitro* acceptors of galactose in a soluble galactosyltransferase I system, led to the conclusion that the defect in *rfaH* mutants affected the α -1,3 galactosyltransferase and that *rfaH* was the structural gene for this enzyme. Osborn's (1968) data on the SL1033 and SL1060 core polysaccharides are, however, also compatible with the complex picture found in this investigation. Thus, the reported low galactosyltransferase I activity could be sufficient for addition of the galactose I residue to 39 to 50% of cores in SL1060 and SL1033 (Table 4), and the levels of glucosamine found by Osborn are also consistent with the presence of 7 to 8% of completed cores in these strains (Table 4).

When FO phage is applied on *rfaH* mutants, a thinning of the bacterial lawn is seen, but no plaques (Wilkinson & Stocker, 1968). This is interpreted as a consequence of interaction between the FO phage and the small amount of completed cores, i.e. the FO receptor (Lindberg, 1973), in *rfaH* mutants. The *rfaH* mutants are also sensitive to phage C21 (Wilkinson & Stocker, 1968). Phage C21 lyses rough mutants of classes Rd1 (deficient in glucose I, *galU* or *rfaG*) and Rc (deficient in galactose I and II, *galE*), but not Rb3 (deficient in galactose I, R-res-2) and Rb2 (deficient in glucose II, R-res-1). The sensitivity of the *rfaH* mutants to C21 could be readily explained by the presence of uncapped glucose residues (12 and 18% of core chains in SL1033 and SL1060, respectively). The demonstration of attachment of phage C21 to the *rfaH* mutants, although reduced compared with the attachment rate to *galE* mutants, is in agreement with the presence of a reduced number of un-

capped glucose residues in *rfaH* mutants. One should observe, however, that in strains TV148 and TV166, 9 and 13 %, respectively, of the core chains terminate with uncapped glucose residues, yet these strains are resistant to C21.

The nature of the defect in the *rfaH* mutants remains unexplained. The heptose region of the core is capped by glucose residues (Osborn, 1968; our data, not shown). The defect thus affects the reactions for addition of the galactose I, galactose II, glucose II and *N*-acetylglucosamine units. Our data do not test whether *rfaH* mutants can add O chains to their completed core chains because the PCP method used does not extract 'smooth' LPS (Galanos *et al.*, 1969) and because the *pmi* defect of the mutants would have prevented formation of O chains. Both the *rfaH* mutants analysed here, together with two other phenotypically similar mutants (SL3635 and SL3657), mapped elsewhere than the main *rfa* gene cluster (Kuo & Stocker, 1972) and have now been shown to be cotransducible with *metE* (Stocker *et al.*, 1980). It is reasonable to assume that at least these four mutations affect the same gene, *rfaH*. The mutation of one of these four mutants, strain SL3635, is of type amber (B. A. D. Stocker, personal communication), so gene *rfaH* must specify a protein. There are a number of ways in which a single protein could be needed for efficient transfer of several different sugars to form units of the LPS core. (i) The *rfaH* product might be a polypeptide component common to each of the four glycosyltransferases: this is made implausible by the observation (Endo & Rothfield, 1969) that purified UDPgalactose:LPS α ,3-galactosyltransferase contains only a single kind of polypeptide. (ii) The *rfaH* product might be an enzyme which modifies the structure of the inner core in such a way as to increase its ability to accept the various glycosyl units – compare the effect of the absence of phosphorylation of heptose(s) on the formation of the core chain distal to the glucose I residue in *rfaP* mutants of *S. minnesota* (Mühlradt *et al.*, 1968); however, the LPS of *rfaH* mutants SL1033 and SL1060 accepted galactose to the same extent as did chemotype Rc LPS when a preparation from *rfa*⁺ bacteria was used as the enzyme source (Osborn, 1968). (iii) The *rfaH* product might serve as a positive regulator of the activity of the four glycosyltransferase genes. (iv) The *rfaH* product might be a protein which, though not a component of any of the transferases, yet is required for their efficient functioning. In *E. coli* K12 the core unit corresponding in position and linkage to galactose I of the *Salmonella* core (Fig. 1) is a glucose, yet a plasmid-borne fragment of the *E. coli* K12 chromosome transferred to the *rfaH* mutant SL1060 made it phenotypically Rfa⁺, as shown by phage sensitivities and by assay of UDPgalactose:LPS α ,3-galactosyltransferase activity (Creeger *et al.*, 1979). Similarly transfer of an *E. coli* F' carrying the *metE* region of the *E. coli* K12 chromosome to *pepQ*⁺*metE* transductant strains carrying the *rfaH* alleles of SL1033 or of two other *rfaH* mutants restored smooth phage sensitivity and O agglutinability, i.e. the Rfa⁺ phenotype (Stocker *et al.*, 1980). These observations are compatible with gene *rfaH* serving as a positive regulatory gene for several *rfa* loci or with it specifying a polypeptide needed for efficient functioning of several glycosyltransferases. We hope that the isolation of temperature-sensitive *rfaH* mutants may show the nature of the *rfaH* gene function.

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