Genetical Studies on the Lipopolysaccharide Structure of *Escherichia coli* K12

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SUMMARY

Escherichia coli K12 strain 2578 was crossed with an *E. coli* O8 donor. Most of the recombinants selected for the donor *his* allele were agglutinable by anti-O8 serum. This indicated that the recombinants can synthesize O8-specific units and attach them to the K12 lipopolysaccharide (LPS) core in consequence of the transfer of the *his*-linked donor *rfb* cluster determining O8-chain synthesis. In serological tests and phage sensitivity tests most O8-reactive recombinants behaved like typical smooth *E. coli* O8 strains. This implies that *E. coli* K12 strain 2578 synthesizes a complete core structure, which is a prerequisite for the formation of smooth recombinants.

Some O8-reactive recombinants displayed a partly rough phenotype. In addition, the typical smooth recombinants segregated partly rough forms. Thus the smooth recombinants appear to be partial diploids for some donor gene(s) involved in LPS synthesis.

Serological tests and phage typing showed that the *Escherichia coli* K12 core is different from other known core types of *Salmonella* (Ra) and *E. coli* (R1-R3).

The genes (*rfa*) for the K12 core type were mapped by conjugation at approximately 72 min on the *Escherichia coli* linkage map.

INTRODUCTION

The O-antigenic lipopolysaccharides (LPS) of Enterobacteriaceae consist of O-specific polysaccharide chains, composed of repeating oligosaccharide units, linked to a core oligosaccharide which in turn is bound via 2-keto-3-deoxyoctonate (KDO) to a lipid (Lüderitz, Jann & Wheat, 1968; Lüderitz, Westphal, Staub & Nikaido, 1971). The structure of the *Salmonella* LPS is presented in Fig. 1. The biosynthesis of LPS is genetically determined by various *rfa* genes. Genes *rfa* and *rfb* are responsible for synthesis of the core oligosaccharide and the O-specific polysaccharide chains, respectively (Stocker & Mäkelä 1971). Mutation at any of the various *rfa* loci, most of which are clustered in the *cysE-pyrE* region leads to rough (R) mutants with an incomplete core which lacks the normal site for O-specific chain attachment. Mutation of any of the *rfb* genes, all of which form a cluster closely linked to the histidine (*his*) operon blocks the synthesis of O-specific chains and results in R mutants having a complete core structure. Most of today's knowledge on the genetics of LPS synthesis results from studies with *Salmonella*. But there are sound indications that equivalent loci exist in *Escherichia coli* (Ørskov & Ørskov, 1962; Schmidt, Jann & Jann 1970).

Escherichia coli K12 is a phenotypically rough strain which is widely used for biochemical and genetic studies. This strain has no detectable O-antigen specificity (Ørskov & Ørskov, 1962) and its smooth parent strain is unknown. The polysaccharide portion of the lipopolysaccharide (LPS) extracted from *E. coli* K12 contains glucose, galactose, heptose,

Fig. 1. LPS structure of *Salmonella* (Lüderitz *et al.* 1971; V. Lehmann, unpublished). Glc, glucose; Gal, galactose; GlNAc, N-acetyl-glucosamine; Hep, heptose; KDO, 2-keto-3-deoxyoctonic acid.

KDO and small amounts of rhamnose (Nikaido, Nikaido & Rapin, 1965; Rapin & Mayer, 1966; Sugimoto & Okazaki, 1967), but the structural arrangement of these components is still unknown.

Crosses described by Ørskov & Ørskov (1962) suggested that *Escherichia coli* K12 has defective *rfb* genes. They crossed an *E. coli* K12 F⁺ donor (*His*⁺) with a smooth *E. coli* of serotype O100 (F⁻, *his*). Recombinants selected for *His*⁺ behaved like rough forms. This suggests that defective *rfb* genes of *E. coli* K12 had replaced the intact recipient *rfb* genes determining the synthesis of O100 specific polysaccharide chains.

In crosses recently described by Jones, Koeltzow & Stocker (1972) introduction of the *his-rfb* region from an *Escherichia coli* O8 donor into *E. coli* K12 led to hybrids with serological O8 specificity, but unlike typical smooth *E. coli* strains they retained sensitivity to various rough-specific phages. Several explanations for the R-specific phage sensitivity of the hybrids were suggested by the authors including the possibility of a leaky *rfa* mutation in the recipient.

Thus it remained uncertain whether *E. coli* K12 also has a *rfa* defect in addition to the *rfb* lesions. This paper describes results which show that the *rfa* genes necessary for the complete LPS core synthesis are intact in the *Escherichia coli* K12 strain investigated.

On the basis of serological tests and phage typing the *Escherichia coli* K12 LPS core does not belong to one of the already established LPS core types of *Salmonella* (Ra; Lüderitz *et al.* 1971) and *E. coli* (*E. coli* R1-R3; Schmidt, Fromme & Mayer, 1970), thus it represents a newly identified core type. Moreover the genes for the K12 core synthesis were transferred to *E. coli* R1-type mutants. The locus was mapped at a site which is apparently equivalent to that of the *Salmonella rfa* gene cluster (Kuo & Stocker, 1972; Sanderson & Saeed, 1972).

METHODS

Bacterial strains. All strains used for genetic experiments are derived from *Escherichia coli* K12 or from *E. coli* O8:K27:H⁻ (E56b). The strains and their genetic markers are listed in Table 1. The *E. coli* K12 donor HfrC was provided by Drs I. and F. Ørskov and the *E. coli* K12 donor KL25 was obtained from Dr B. Low (Low, 1968). *Escherichia coli* K12 strain 2578 represents a mutant (*his*, *trp*, *str-r*) of *E. coli* K12 strain W3100 originally from Drs E. and I. Lederberg's collection provided by Dr A. Rapin.

Escherichia coli F504 is an R mutant with the complete *E. coli* R1 core structure derived from *E. coli* O8:K27⁻. The *E. coli* Hfr59 (O8:K27⁻) has been previously described (Schmidt, 1969). The origin and direction of chromosome transfer of the different donors used are indicated in Fig. 2.

Bacteriophages. The phages and their host strains are listed in Table 2. The phages Felix O (FO), Br10, FP1, C21 and 6SR have proved useful in characterization of *Salmonella*

Fig. 2. Linkage map of *Escherichia coli* with some relevant markers. Origin and transfer direction of Hfr strains are indicated by arrows.

Table 1. *Escherichia coli* strains used

Strain no.	Serotype	Genetic markers	Fertility type
W3100	K12	Prototroph	F ⁻
2578	K12	<i>his</i> , <i>trp</i> , <i>str-r</i>	F ⁻
KL25	K12	<i>thi</i> , <i>str-s</i>	Hfr
HfrC	K12	<i>met</i> , <i>str-s</i>	Hfr
F504	<i>E. coli</i> R1 core	<i>mtl</i> , <i>xyl</i> , <i>ara</i> , <i>met</i> , <i>his</i> , <i>str-r</i>	F ⁻
F459	O8:K27 ⁻	<i>ilv</i> , <i>str-s</i>	Hfr59

Symbols: *his*, *trp*, *met*, *ilv*, *thi*, for histidine, tryptophane, methionine, isoleucine/valine, thiamine requirement; *mtl*, *xyl*, *ara* for non-fermentation of mannitol, xylose, arabinose; *str-s/r* for streptomycin sensitivity/resistance.

and *E. coli* R mutants (Schmidt & Lüderitz, 1969; Schmidt, Jann & Jann, 1970). Phage U₃ was kindly provided by Dr K. Paigen, through Dr A. Rapin of this Institute. This phage lyses only wild-type K12 strains, but not *galE* or *galU* mutants which have galactose or glucose-deficient LPS (Watson & Paigen, 1971). Phage Ω8 is specific for *Escherichia coli* strains belonging to serotype O8 (Jann, Schmidt & Wallenfels, 1971).

The phages were propagated on their respective host strains in meat extract broth (Adams, 1959).

Table 2. *Bacteriophages and their host strains*

Phage	Host strain
Felix-O (FO)	<i>Salmonella paratyphi</i> B
Ø8	<i>Escherichia coli</i> O8:K27 ⁻
Br10	<i>S. paratyphi</i> B 1/2
FP1	<i>S. minnesota</i> R5
C21	<i>S. typhimurium</i> SL869
6SR	<i>E. coli</i> F576 (R2 core mutant)
U3	<i>E. coli</i> K12
T1-T7	<i>E. coli</i> B

Phage sensitivity of strains was tested using suspensions containing about 10^8 plaque-forming units/ml. Drops of phage suspension were placed on dried bacterial lawns on agar plates and incubated overnight at 37 °C.

Mating experiments. These were performed as previously described (Schmidt, Jann & Jann, 1970). Recombinants were first transferred to the media used for selection and then streaked on complete agar medium from which single colonies were isolated.

Slide agglutination. The presence of the O8 antigen in recombinants was tested by slide agglutination tests in *Escherichia coli* O8 antiserum. In addition an *E. coli* K12 antiserum containing antibodies specific to the K12 core was used.

The antisera were diluted in 0.3 % instead of 0.9 % NaCl solution to avoid a non-specific agglutination of rough forms.

Media. Solid complete medium was D_{1.5}-agar (Schlecht & Westphal, 1967) with 0.3 % glucose and 1.5 % agar. Meat extract broth of the following composition was used (g/l distilled water): Difco Bacto-Peptone, 10; NaCl, 3; Na₂HPO₄. 12 H₂O, 2; glucose, 1; Difco Beef-Extract, 5; pH 7.3. For selection of recombinants from mating mixtures Davis minimal agar (Lederberg, 1950) with 0.3 % glucose was supplemented with the appropriate amino acids (20 µg/ml agar). For selection of fermentation markers glucose was replaced by the appropriate sugar.

Serologic methods. Production of antisera and passive haemagglutination tests were performed as previously described (Schmidt, Fromme & Mayer, 1970). Lipopolysaccharides used for haemagglutination were extracted by a mixture of phenol-chloroform-petrol ether as described by Galanos, Lüderitz & Westphal (1969).

RESULTS

Characterization of Escherichia coli K12 strains. The *E. coli* K12 strains listed in Table 1 showed rough colonial morphology and cells grown on D_{1.5}-agar were strongly agglutinated by 3.5 % NaCl or 0.3 % auramine solution (Schmidt, Schlecht, Lüderitz & Westphal, 1969) but only slightly by 0.9 % NaCl. No agglutination was observed in 0.3 % saline. Therefore the antisera used for testing recombinants in slide agglutination (see below) were diluted 1:10 in 0.3 % saline.

In addition the sensitivity of the *Escherichia coli* K12 strains to a number of rough-specific phages was tested. All four strains were sensitive to phages U3, Br10, FP1, and to the seven T-phages (T1-T7). Thus these K12 strains showed the typical traits of rough (R) mutants.

No lysis was observed with the R-specific phages FO, C21 and 6SR. Of special interest is the sensitivity of the strains to phage U3. This phage is known to attack 'wild-type'

Table 3. Serological analysis and phage sensitivity of *his*⁺ recombinants from cross *Hfr*59 × 2578

No. of recombinants	Slide agglutination* in			Phage sensitivity†				
	Antisera		NaCl 3.5 %					
	O8	K12		Ω8	Br10	FP1	U3	T‡
78	+	—	—	+	—	—	—	—
18	+	+	—	+	+	+	+	+
4	—	+	+	—	+	+	+	+
Parent strains								
<i>Hfr</i> 59	+	—	—	+	—	—	—	—
2578	—	+	+	—	+	+	+	+

* +, agglutination; —, no reaction. † +, confluent lysis; —, no action. ‡ T for T1–T7 phages.

Escherichia coli K12 strains but not mutants which have glucose or galactose deficient lipopolysaccharides (Watson & Paigen, 1971). This suggests that the strains tested here have the unimpaired LPS of *E. coli* K12. It should be noted that sensitivity of *E. coli* K12 towards phage 6SR recently observed by Jones *et al.* (1972) reflects the use of phage 6SR-J propagated on *E. coli* K12 and later found to differ in respect of host range from other stocks of phage 6SR (Dr Stocker, personal communication). Phage 6SR-J was also active on the *E. coli* K12 strains used here.

Introduction of the Escherichia coli O8 *rfb* region into *E. coli* K12. The question arises whether *E. coli* K12 synthesizes a complete or an incomplete LPS core structure. Incomplete core oligosaccharides lack the normal site for the O-specific polysaccharide attachment. To test whether the K12 core can attach O-specific chains the intact *rfb* locus of an *E. coli* O8 donor was introduced into *E. coli* K12. The *rfb* locus is closely linked to *his* loci (Ørskov & Ørskov, 1962). Therefore *str-r*, *his*⁺ recombinants were selected from crosses of the *E. coli* donor *Hfr*59 (O8:K27[—], *his*⁺, *str-s*) with *E. coli* K12 strain 2578 (*his*, *trp*, *str-r*) on suitably supplemented minimal agar containing streptomycin at 100 µg/ml agar to counter-select the streptomycin-sensitive donor. The *his*⁺ recombinants were tested serologically by slide agglutination and also for phage sensitivity (see Table 3). Of 100 *his*⁺ recombinants, 96 were agglutinated by O8 antiserum, a result which suggests that introduction of the intact donor *rfb* region of *Hfr*59 enables the K12 recipients to synthesize a LPS carrying O8-specific chains. Of the 96 O8-agglutinable recombinants, 78 were not agglutinated by K12 antiserum and were sensitive to phage Ω8, but not to phages U3, Br10, FP1 and to the T phages. They behaved like fully smooth *E. coli* strains; this indicates the presence of a complete core in K12 recipient strain 2578. The remaining 18 O8-agglutinable *his*⁺ recombinants were also agglutinated by K12 antiserum but not by auramine or 3.5 % saline. They were sensitive to phage Ω8 and to rough-specific phages. These recombinants have a partly rough phenotype. In addition the sensitivity of two representatives of each recombinant group was tested by Dr Stocker, to other rough-specific phages (Br60, Br2, Ffm not used here) and to a series of colicins active on *E. coli* K12 (see Jones *et al.* 1972). As expected, the fully smooth recombinants were resistant and the partly rough strains were, like the parental *E. coli* K12, also sensitive to these phages and colicins.

The partly rough recombinants were stable in their phenotypic expression, for no other classes segregated out following single colony isolations. In contrast, the fully smooth recombinants were unstable. Thus in some cases they segregated colonies which resemble

Table 4. *Phage sensitivity patterns of Escherichia coli R mutants with different core types*

Strain no.	Derived from <i>E. coli</i>	Core type	FO	Br10	FP1	C21	6SR	U3
F470	O8:K27	R1	—	+	+	+	+	—
F576*	O8:K42	R2	+	+	+	—	+	—
F653	O111:K58	R3	—	+	+	—	+	—
2578	K12	K12	—	+	+	—	—	+

* *Salmonella* Ra mutants show the sensitivity pattern of F576 using these phages. This correlates with the close structural relationship between these core types (see Hämmerling, Lüderitz, Westphal & Mäkelä, 1971).

the partly rough recombinants in phage patterns and serological properties, and which retain the *His*⁺ phenotype of the donor.

Studies comparing the Escherichia coli K12 core with the known core types of E. coli and Salmonella. The formation of fully smooth *his*⁺ recombinants in the crosses described above suggests the presence of a complete LPS core structure in *E. coli* K12 strain 2578. Three different complete LPS core types (*E. coli* R1–R3) are known in *E. coli* serotypes which are distinct from the core present in *Salmonella* serotypes (Schmidt, Fromme & Mayer, 1970). The relationship of the *E. coli* K12 core to already known core types was studied by passive haemagglutination tests. Comparing the serologic properties of the LPS isolated from representative core-type mutants and *E. coli* K12 it was found that the LPS of *E. coli* K12 strain 2578 did not react in the heterologous antisera against *E. coli* R1, R2, R3 and *Salmonella* Ra-type mutants. Conversely the LPS of the *E. coli* and *Salmonella* core-type mutants showed no haemagglutination in anti-K12 serum. This suggests that the *E. coli* K12 core is serologically unrelated to the other already known core types of *E. coli* and *Salmonella*. This result is in full agreement with the finding that representative core type mutants and *E. coli* K12 exhibit distinct sensitivity patterns to a number of rough-specific phages (see Table 4).

Mapping the locus determining the Escherichia coli K12 core. To map the genes for the K12 core oligosaccharide synthesis matings were performed between *E. coli* K12 donors and the multiple marked *E. coli* R mutant F504 as recipient which has the complete coli R1 core. After single colony-purification recombinants were tested for unselected markers. Their core type (*E. coli* R1 or K12) was inferred from their sensitivity pattern to appropriate phages. Thus recombinants which have inherited the genes for K12 core synthesis should be sensitive to the K12-specific phage U3. The presence of the *E. coli* R1 core in the recombinants could be detected by their sensitivity to phages C21 and 6SR and by their resistance to phage U3 (see Table 4).

Escherichia coli F504 was first mated with the *E. coli* K12 donor KL25, which has a point of origin between *pyrE* and *ilv* and injects its chromosome clockwise (Fig. 2). Selections were made for *met*⁺, *ara*⁺, *str-r* and *his*⁺, *str-r* recombinants. Fifty recombinants of each class were isolated and tested by phage typing. None of these recombinants were able to synthesize a K12 core; they all showed the typical phage pattern of *E. coli* R1 mutants. Therefore the genes for the K12 core oligosaccharide do not lie between the origin and the *his* locus of the donor chromosome. In another cross HfrC was used as donor which injects its chromosome counterclockwise with *purE* as the leading locus (Fig. 2). In this case *mtl*⁺ *str-r* and *xyl*⁺ *str-r* recombinants were selected. Among these recombinants a large number showed the typical phage pattern of *E. coli* K12, indicating that they have inherited the genes for K12 core synthesis from the donor.

Table 5. *Passive haemagglutination of different R lipopolysaccharides*

R-LPS from	Reciprocal titre in antisera	
	<i>E. coli</i> R1	K12
HfrC (K12)	< 20	1280
F504 (<i>E. coli</i> R1)	5120	< 20
F870 (recombinant)	< 20	640

Table 6. *Inheritance of donor K12 rfa locus among different recombinant classes of a cross between HfrC and Escherichia coli F504*

Sensitivity to phage 6SR and C21 indicates the presence of the *E. coli* R1 core; sensitivity to phage U3 was taken as evidence for the presence of the *E. coli* K12 core resulting from a transfer of the donor *rfa* locus.

Recombinant class	Total no. of recombinants	No. of recombinants with core type		Transfer frequency of K12 <i>rfa</i> locus (%)
		K12	<i>coli</i> R1	
<i>xyl</i> ⁺ <i>mtl</i> ⁻	54	0	54	0
<i>xyl</i> ⁺ <i>mtl</i> ⁺	176	100	76	57
<i>xyl</i> ⁻ <i>mtl</i> ⁺	38	24	14	63

The LPS of one of the K12-like recombinants (F870, *his*, *xyl*, *mtl*⁺, *ara*, *met*) was extracted and used in passive haemagglutination tests with *E. coli* R1 and K12 antisera. For comparison the results with LPS of the parent strains HfrC (K12) and *E. coli* F504 (*E. coli* R1) are included (see Table 5). The haemagglutination test shows that in addition to the phage sensitivity pattern the recombinant strain F870 synthesizes a LPS which is serologically identical with that of *E. coli* K12.

Results with three recombinant classes *mtl*⁺*xyl*⁻, *mtl*⁻*xyl*⁺ and *mtl*⁺*xyl*⁺ from the cross between HfrC and F504 are presented in detail in Table 6.

About 60 % of the *mtl*⁺ recombinants have received from the donor the genes for the K12 core synthesis. This clearly shows that this gene locus (termed *rfa* according to usual nomenclature) is located close to *mtl*. Analysis of the different recombinant classes also gives information about the order of the three loci. *Xyl*⁺*mtl*⁻ recombinants had not obtained the donor K12 *rfa* locus. Furthermore all those recombinants selected for *xyl*⁺ which have inherited the K12 *rfa* locus were also *mtl*⁺. Therefore the three loci are most probably in the order *xyl*-*mtl*-*rfa*. Moreover no quadruple crossover would be required among the 268 recombinants tested by this order, in contrast to other possible orders. Together with the negative results of the cross with Hfr K125 this suggests that the K12 *rfa* locus lies between *mtl* and the point of origin of the Hfr K125 (see Fig. 2). The distance between *mtl* and the *rfa* locus can be estimated as follows. On the linkage map of *Escherichia coli* (Taylor, 1970) there is a 1 min distance between the *mtl* and the *xyl* loci. In the crosses described here the linkage observed between these loci is about 65 % and between *mtl* and *rfa* about 60 % (see Table 6, column 5). Therefore one can estimate that *rfa* maps about 1 min distance from *mtl* - that is, about 72 min on the *E. coli* linkage map.

DISCUSSION

Previous investigations indicated that phenotypically rough *Escherichia coli* K12 has defective *rfa* genes resulting in a lack of O-chain synthesis (Ørskov & Ørskov, 1962; Jones *et al.* 1972).

The introduction of the *his*-linked *rfb* region from the *Escherichia coli* O8 donor Hfr59 into *E. coli* K12 strain 2578 led to smooth recombinants which have serological O8 specificity and resemble the parental *E. coli* O8. This suggests that *E. coli* K12 has a complete LPS core structure which can attach O8-specific chains. Moreover, recent studies (Jann, unpublished) established that in recombinants the O8-specific polymer, which is a mannan (Reske & Jann, 1972), is covalently bound to the K12 core oligosaccharide chain.

Some O8-reactive *his*⁺ recombinants from matings between the *Escherichia coli* O8 donor Hfr59 and the *E. coli* K12 strain 2578 were partly rough (see Table 3). Moreover, in some cases smooth *his*⁺ recombinants can segregate partly rough forms, which remain stable with respect to the histidine character. Therefore it is likely that some or possibly all of the smooth recombinants are partial diploids for donor gene(s) involved in LPS synthesis, and that they segregate haploids showing a partly rough phenotype as a result of the loss of this donor gene(s). The partly rough forms remained agglutinable by anti-K12 serum and retained sensitivity to various rough-specific phages characteristic of the parental *E. coli* K12 strain. This suggests that part of the LPS core stubs is not substituted by O8 repeat units and therefore remains accessible to K12 core-specific antibodies. Similarly, one can suggest that O chains, which obviously mask the receptor sites for rough-specific phages in fully smooth strains, are not present in adequate numbers on the cell surface of partly rough forms to prevent rough-specific phages from attaching to their respective receptor sites. Therefore the partly rough forms might have a partial defect in the enzymatic transfer of O8 units to the core.

Jones *et al.* (1972) also found partly rough but not fully smooth *his*⁺ recombinants in crosses between the *Escherichia coli* O8 donor Hfr59 and an *E. coli* K12 strain. Therefore it may be that *E. coli* K12 strain 2578 used in this laboratory maintains partial diploidy more readily than the K12 strain used by Jones *et al.* (1972).

Escherichia coli K12 shows a phage pattern which is distinct from those of other core-type mutants (see Table 4). The phage resistance of a given strain may result from DNA restriction rather than from failure of the phage to adsorb. But phage U3, which is a K12 core-specific phage, failed to adsorb to resistant strains (unpublished observation), thus indicating the absence of corresponding receptor sites in these strains. These data suggest that the differences in phage sensitivity between *E. coli* K12 and the other core-type strains is due to differences in their LPS core structures. The outcome of passive haemagglutination tests confirmed this suggestion.

In an earlier study *rfa* genes for different steps of the core synthesis of *Escherichia coli* O8:K27 were found in the upper left quadrant of the chromosome near to *mtl* (Schmidt, Jann & Jann, 1970). In addition Eriksson, Grennberg, Nordström & Englund (1971) have mapped the mutation site for ampicillin resistance of two *E. coli* K12 mutants near to *mtl*. The LPS composition of both these mutants differed from each other and from that of the original *E. coli* K12 and the genes were designated *LpsA* and *LpsB*, respectively. These mutations may affect different *rfa* genes concerned with the K12 core synthesis. In the present investigation with *E. coli* K12 a *rfa* locus determining the K12-type core oligosaccharide was located in the region *xyl-mtl-rfa*, with *rfa* about 1 min from *mtl*. Together with recent results mentioned above concerning *E. coli* O8:K27 and *E. coli* K12 it is assumed that this locus represents a cluster of different genes governing the synthesis of the core oligosaccharides in *E. coli* and is most likely equivalent to the *Salmonella rfa* gene cluster which has been mapped between *cysE* and *pyrE* (Kuo & Stocker, 1972; Sanderson & Saeed, 1972).

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