The *Escherichia coli* *serA*-linked capsule locus and its flanking sequences are polymorphic, genetic evidence for the existence of more than two groups of capsule gene clusters

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Two families of *Escherichia coli* capsules, termed groups I and II, have been defined previously on the basis of a number of biochemical and genetic criteria. Recently, a third group of capsules, termed I/II has been suggested on the basis of chemical structure and mode of expression. In this paper, we show that group I capsule-producing strains lack the *serA*-linked group II capsule genes. In addition, group I/II capsule-producing strains lack the group II capsule genes despite the former genes also mapping near to *serA*. Therefore, the genetic data presented in this paper support the existence of three groups of capsule gene clusters, two of which are linked to *serA*. Sequences flanking the K4 capsule genes were found in the chromosome of all *E. coli* strains examined and were sometimes present in multiple copies at different loci, indicating that this chromosomal region is highly polymorphic.

Introduction

The enormous diversity of *E. coli* capsular polysaccharide (K antigen) is striking with over 70 chemically different structural types known (Ørskov *et al.*, 1977). On the basis of chemical structure, size, mode of expression and chromosomal location of genetic determinants the capsular polysaccharides can be divided into two broad groups, I and II (Jann & Jann, 1987). A given isolate produces a single K antigen and co-expression or switching of capsule types either within or between groups has not been observed (Jann, 1985).

The expression of group I capsular polysaccharides is restricted to O8 and O9 and occasionally O20 and O101 serotypes (Jann & Jann, 1990). They have a low charge density and are expressed at all growth temperatures (Jann & Jann, 1990). Some group I polysaccharides are anchored in the outer membrane by linkage to core-lipid A and can be thought of as extended lipopolysaccharide

(LPS) (Jann & Jann, 1987, 1990; Jann *et al.*, 1992). Genetic determinants for group I capsule production map near the *his* and *rfb* (O antigen synthesis) loci (Ørskov *et al.*, 1977) but have not been analysed in detail. Studies using the K27 antigen indicate that a second *trp*-linked locus is necessary for surface expression of a complete capsule, although this is not the case for the K30 antigen (Schmidt *et al.*, 1977; Laasko *et al.*, 1988).

The group II capsular polysaccharides have a higher charge density than those of group I and are not expressed at low growth temperatures (17–20 °C). Group II capsules are co-expressed with a variety of O antigens (excluding the four above) (Jann & Jann, 1987). In all group II capsules so far analysed, the reducing end of the polysaccharide is substituted with phosphatidic acid and this substitution may act as the membrane anchor (Schmidt & Jann, 1982; Jann & Jann, 1990). Unlike group I, group II capsule-producing strains have a high CMP-KDO (2-keto-3-deoxy-manno-octulosonic acid) synthetase activity (CKS) at the capsule permissive temperature (Finke *et al.*, 1989, 1990). Genetic determinants for the expression of some group II capsules have been mapped at 64 minutes near *serA* on the *E. coli* chromosome (Ørskov *et al.*, 1976; Vimr, 1991) and have been termed *kps* (Silver *et al.*, 1984; Vimr *et al.*, 1989), formerly *kpsA* (Ørskov & Nyman, 1974). Gene clusters encoding several chemically different group II capsular polysaccharides have been cloned and shown to have a common segmental organization (Roberts *et al.*,

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Abbreviations: KDO, 2-keto-3-deoxy-manno-octulosonic acid; CKS, CMP-KDO synthetase.

Table 1. *Bacterial strains and plasmids*

Strain/plasmid	Characteristics	Source/reference*
LE392	F ⁻ <i>hsdR514</i> (<i>r_K⁺ m_K⁺</i>) <i>supE44 supF58 lacY1 galK2 galT22 metB1 trpR55</i>	Echarti <i>et al.</i> (1983)
512	O1:K1:H7	G. J. Boulnois ^a
20022	O6:K2:H1	
20242	O4:K3:H4	
U1-41	O5:K4:H4	
B18337-41	O10:K5:H4	
2667	O9:K9:H12	
21454	O11:K10:H ⁻	
21455	O13:K11:H11	
21456	O25:K19:H12	
2904	O9:K29:H ⁻	K. Jann ^b
B22	O9:K30:H12	
A12b	O6:K54:H10	
pACYC184	Cm ^r Tc ^r	C. Whitfield ^c F. Ørskov ^d
pGB110	Carries the K5 antigen gene cluster	Chang & Cohen (1978)
pKT172	Carries the K1 antigen gene cluster	Roberts <i>et al.</i> (1986)
pRD1	Carries the K4 antigen gene cluster	Echarti <i>et al.</i> (1983)
pRD10	Flanks the K4 antigen gene cluster	Drake <i>et al.</i> (1990)
		See text

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1986, 1988*a*). Regions 1 and 3 are homologous between different group II capsule gene clusters and are thought to encode products that perform identical functions in the expression of chemically different group II capsules (Boulnois *et al.*, 1987; Roberts *et al.*, 1988*a*; Boulnois & Roberts, 1990). Region I is about 7 kb and, in part, encodes proteins for the transport of mature, phosphatidic-acid-linked polysaccharide from the periplasm to the cell surface. In addition, the structural gene encoding CKS is located within region 1 (C. Pazzani & I. S. Roberts, unpublished results). The role of this enzyme is unclear, but since KDO has been found at the terminus of some group II capsular polysaccharides that do not have KDO in the repeat unit (Jann & Jann, 1990) CKS has been postulated to play a part in the initiation of polymer biosynthesis (Finke *et al.*, 1989). Region 3 is 1.6 kb and encodes two proteins believed to be involved in the translocation of group II polysaccharide across the inner membrane (Kroncke *et al.*, 1990; Smith *et al.*, 1990; Pavelka *et al.*, 1991). Region 2 appears to be unique for each group II capsule gene cluster and is positioned between regions 1 and 3 (Boulnois & Jann, 1990). The available evidence suggests that region 2 encodes enzymes for the synthesis of the group II polysaccharide in question (Boulnois *et al.*, 1987; Roberts *et al.*, 1988*a*). In the case of the K1 antigen gene cluster, region 2 encodes for the synthesis and activation of CMP-sialic acid, the sole precursor for the polymerization of the K1 polysaccharide (Vimr *et al.*, 1989). An isolate expressing a given group II capsule carries region 2 determinants for the biosynthesis of that polysaccharide only and silent region 2 determinants are

not present on the *E. coli* chromosome (Roberts *et al.*, 1988*b*).

The *serA*-linked group II capsule genes were originally thought to encode a distinct group of capsules. However, recent biochemical evidence has shown that some capsules previously assigned as members of group II resemble those of group I on two counts. First, K3, K10, K11, K54 and K98 antigens are expressed at all growth temperatures (Ørskov *et al.*, 1984). Second, the K2, K3, K10, K11, K19 and K54 antigen-expressing strains do not have the elevated CKS activity at 37 °C typical of group II capsule-producing strains (Finke *et al.*, 1990). In all other respects, these capsules have typical group II characteristics and the mapping of the K10 and K54 capsule genes near *serA* was originally used to define the chromosomal location of *kpsA* (Ørskov & Nyman, 1974). However, in the light of this more recent evidence Finke *et al.* (1990) tentatively classed these former group II capsules as group I/II. The nature of the group I/II capsule genes and their relationship to the group II capsule genes is not clear.

In this paper we report a Southern blot analysis of group I, group I/II and group II capsule-expressing *E. coli* strains to determine the relationship between genes for the production of different capsular polysaccharides.

Methods

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. Bacteria were grown in L-broth (with 1.5% agar as required). Plasmids were propagated in *E. coli* LE392 using L-broth supplemented with 100 µg ampicillin (Ap) ml⁻¹

Fig. 1. Physical map of the recombinant plasmid pKT172 (carrying the biosynthesis genes for the K1 antigen), pRD1 (cloned K4 biosynthesis genes) and pRD10 (see text). The black bars refer to vector sequences. The labelled boxes 1 through 3 refer to functional gene blocks involved in the production of the K1 and K4 antigens (see text). The DNA probes are represented by the lines labelled A through K. The lettered boxes denote regions of homology with the appropriate probe. Restriction enzyme target sites: B, *Bam*HI; C, *Cl*aI; E, *Eco*RI; H, *Hpa*I.

or 25 µg of either chloramphenicol (Cm) or tetracycline (Tc) ml⁻¹ as needed.

DNA procedures. Chromosomal and plasmid DNA was extracted as described by Saito & Miura (1963) and Birnboim & Doly (1979), and cleaved with restriction endonucleases according to the manufacturer's recommendations. DNA fragments were separated by agarose gel electrophoresis, photographed and transferred to nylon filters (Hybond-N, Amersham) in preparation for DNA hybridization, as described by Southern (1975) and Sambrook *et al.* (1989). The conditions for DNA radiolabelling have been described by Feinberg & Vogelstein (1983). The hybridization solution used was 3×SSC (1×SSC is 0.15 M-NaCl and 0.15 M-sodium citrate, pH 7.0) and 2×Denhardt's Solution [50×Denhardt's solution is 1% (w/v) each of Ficoll, bovine serum albumin and polyvinylpyrrolidone], 0.1% SDS, 6% (v/v) polyethylene glycol 6000 containing 200 µg salmon sperm DNA ml⁻¹. Hybridizations were performed at 65 °C in duplicate and washed at 65 °C in either 2×SSC, 0.1% SDS or 0.5×SSC, 0.1% SDS allowing 30% or 15% mismatch respectively. Washing conditions requiring 95% similarity were 0.1×SSC, 0.1% SDS at 65 °C. Fragment sizes were calculated from multiple autoradiographs with reference to size markers on a gel photograph. All strains were checked by serotyping following experimentation.

Results

The K9, K29 and K30 antigens were chosen as representative of the group I capsules and the K1, K4 and K5 antigens as typical group II capsules (Table 1).

The K2, K3, K10, K11, K19 and K54 antigens are members of group I/II (Finke *et al.*, 1990). Gene clusters for the K1, K4 and K5 antigens have already been cloned and characterized (Echarti *et al.*, 1983; Roberts *et al.*, 1988a; Drake *et al.*, 1990).

Group I and I/II capsule-expressing E. coli strains lack group II capsule genes

Southern blot analysis was used to determine whether strains which express a group I capsule also carry the group II capsule genes on the chromosome. Two radiolabelled DNA probes, A and B (Fig. 1) from the cloned K1 antigen gene cluster were used to probe chromosomal DNA from group I capsule-producing *E. coli*. Probe A carries the majority of the region 1 genes (Roberts *et al.*, 1988b) and probe B carries the region 3 genes (Fig. 1). Both probes hybridized to group II chromosomal DNA fragments of the size predicted from the known restriction enzyme cleavage maps of the respective capsule gene clusters, yet failed to hybridize with the chromosomal DNA of all strains expressing a group I capsule. The results are summarized in Table 2 and those obtained with probe A illustrated in Fig. 2(a).

Table 2. Summary of a series of Southern blot analyses of 11 different *E. coli* strains using radiolabelled DNA probes A, B, D, E and F (Fig. 1)

The K antigen expressed by each strain and the capsule group to which it belongs are listed. Chromosomal DNA from each strain was digested with the restriction endonuclease *Bam*HI. The same results were achieved when the filters were washed at either high ($0.5 \times \text{SSC}$, 0.1% SDS at 65°C) or low ($2 \times \text{SSC}$, 0.1% SDS) stringency. The size of chromosomal DNA fragments homologous to each probe are listed in kilobases and a dash indicates that no fragments bound the probe, ND = not done.

Group...	I			II			I/II				
K antigen...	K9	K29	K30	K1	K4	K5	K2	K3	K10	K11	K19
Probe A	—	—	—	5	5	5	5	—	—	—	—
Probe B	—	—	—	> 12	8	12	> 12	—	—	—	—
Probe D	8	> 12	8	> 12	ND	ND	ND	8	> 12	11	ND
Probe E	> 12	> 12	> 12	> 12	ND	ND	ND	> 12	8	—	ND
Probe F	> 12	> 12	> 12	3	ND	ND	ND	> 12	12	—	ND

Fig. 2. Southern blot analysis using radiolabelled probes A and J (Fig. 1) of chromosomal DNA from 11 *E. coli* strains each expressing a different K antigen. (a) Chromosomal DNA digested with restriction endonuclease *Bam*HI and hybridized with probe A. (b) Chromosomal DNA digested with *Hpa*I and hybridized with probe J. DNA was loaded as follows: Lane 1, K1; 2, K4; 3, K5; 4, K2; 5, K3; 6, K10; 7, K11; 8, K19; 9, K9; 10, K29; 11, K30. The filters shown were washed at high stringency ($0.5 \times \text{SSC}$, 0.1% SDS at 65°C). Prolonged autoradiography of filter (a) did not reveal additional bands.

Southern blot analysis with the same DNA probes was used to determine whether the group I/II capsule-encoding genes are similar to, or distinct from, the group

II capsule genes. Neither probe A nor probe B hybridized to the chromosomal DNA of K3, K10, K11, K19 (Fig. 2a) and K54 capsule-expressing strains (Table 2). However, both probes A and B did hybridize to genomic DNA of the K2 capsule-producing *E. coli*.

Analysis of sequences flanking region 3 of the group II capsule genes

It is apparent that the chromosome of group I and I/II capsule-expressing strains lack the group II capsule determinants. This chromosomal difference may not be restricted to the group II capsule genes but may also include the sequences either side of them. Plasmid pKT172 carries 17 kb of DNA not involved in capsule expression but adjacent to region 3 of the K1 capsule gene cluster (Echarti *et al.*, 1983) and probes D, E and F (Fig. 1) span these sequences. First, to determine whether the sequences adjacent to region 3 in different group II capsule gene clusters are homologous, probes D, E and F were used in Southern blot analysis of pRD1 and pGB110, which encode the K4 and K5 capsule genes respectively. The filters were washed under conditions of high stringency requiring 95% sequence similarity for hybridization. Probes D, E and F hybridized to the DNA fragments in both plasmids which are predicted from the restriction endonuclease cleavage map to lie beyond region 3 of the capsule genes (data not shown). In similar experiments, probes D, E and F hybridize to single fragments in the chromosomal DNA from the group I, group II and group I/II capsule-expressing strains, with the exception of the K11 capsule-expressing strain to which probes E and F failed to hybridize (Table 2). These experiments with probes D, E and F indicate that the K1, K4 and K5 capsule gene clusters are at the similar chromosomal location and that unlike group II capsule genes, sequences adjacent to region 3 of the group II

Table 3. Summary of a series of Southern blot analyses of chromosomal DNA of different encapsulated *E. coli* strains with various probes (Fig. 1)

Refer to the legend of Table 2 for details. Subscript H or B indicates that chromosomal DNA was cleaved with either *Hpa*I or *Bam*HI respectively.

Group ...	I			II			I/II				
K antigen ...	K9	K29	K30	K1	K4	K5	K2	K3	K10	K11	K19
Probe C	—	—	—	7	> 12	> 12	12	—	—	—	—
Probe G	—	—	—	7	> 12	> 12	12	—	—	—	—
Probe K _B	—	—	—	> 12	> 12	12	> 12	> 12	> 12	> 12	> 12
				> 12	> 12	8	12	> 12	> 12	11	
								10			
								7			
Probe K _H	—	—	—	> 12	3	> 12	> 12	> 12	2.5	10	12
				> 12	2.5	3	3	7	2	3	
				8				4			
								2.5			
Probe J _B	> 12	—	> 12	> 12	> 12	> 12	> 12	> 12	> 12	> 12	> 12
				> 12	> 12	> 12	> 12	> 12	> 12		
						9	> 12	12	> 12		
								8			
Probe J _H	3.5	—	3.5	12	> 12	> 12	12	> 12	6.5	7	10
				10	12	> 12	8	11	5.5		
				5.5	6	5	4	8	5		
				3.5	5	4.5	4	7	3.5		
					4	3.5		3.5			
Probe I _B	4	—	4	> 12	> 12	> 12	> 12	> 12	> 12	> 12	—
				4	> 12	> 12	12	> 12	> 12	> 12	
						12	8	9			
							6	8			
							4	7			
								4			
Probe I _H	> 12	—	< 12	6.5	> 12	> 12	> 12	> 12	9	8	—
					12	> 12	> 12	9	6		
					9	9	9	6.5	3		
					6	6	6.5	3			
					3.5	2	5.5				

capsule gene clusters may be present on the chromosome of most, but not all, *E. coli* isolates.

Analysis of sequences flanking region 1 of the group II capsule genes

Since DNA sequences flanking region 3 of the group II capsule genes are present in many *E. coli* isolates (see above), similar studies were performed to see whether this also applies to those sequences flanking region 1 of the group II capsule genes. Probe C (Fig. 1), which is 4 kb in length, extends from a *Bam*HI site 1 kb within region 1 of the K1 gene cluster to a *Bam*HI site fortuitously generated in the original cloning of the K1 genes in the construction of pKT172 (Echarti *et al.*, 1983) (Fig. 1). This probe was used in Southern blot analysis of the genome of various encapsulated *E. coli*. Homologous sequences were only detected in those strains which carry the group II capsule genes (Table 3), as well as the K2

capsule-expressing strain. The equivalent probe taken from pRD10 (probe G, Fig. 1) gave identical results (Table 3). From this, we conclude that at least 3 kb of DNA adjacent to region 1 of the K1 and K4 antigen gene clusters is not present on the chromosome of strains which do not carry the group II capsule determinants, although there is no evidence to suggest that is essential or involved in group II capsule biogenesis.

To obtain probes extending beyond the sequences covered by probe G, a previously constructed cosmid library of *E. coli* U1-41 (Drake *et al.*, 1990) was screened by colony hybridization using probe A. Ten recombinants were identified that carry sequences homologous to probe A (Fig. 1) yet failed to produce the K4 antigen (Drake *et al.*, 1990). Plasmid DNA was extracted from each recombinant, cleaved with *Bam*HI and subjected to Southern blot analysis using radiolabelled probe C. A 15.5 kb *Bam*HI fragment of U1-41 genomic DNA, present within seven of the recombinants,

Fig. 3. Chromosomal DNA from *E. coli* K9 was cleaved with the restriction endonuclease indicated and subjected to Southern blot analysis. A single nylon filter was hybridized with (a) probe D and then stripped and hybridized with (b) probe J. Lane 1, *Bam*HI; 2, *Eco*RI; 3, *Pst*I; 4, *Hind*III; 5, *Kpn*I; 6, *Cla*I; 7, *Pvu*II; 8, *Dra*I; 9, *Hinc*II; 10, *Eco*RV; 11, *Hpa*I.

hybridized to probe C (data not shown). This fragment was subcloned from one recombinant using the vector pACYC184 to generate the plasmid pRD10. A restriction

endonuclease cleavage map of pRD10 was constructed and the extent of homology with probe C established by Southern blot analysis (Fig. 1).

Three DNA probes, I, J and K taken from pRD10 (Fig. 1), were used in Southern blot analysis of *Hpa*I- or *Bam*HI-cleaved chromosomal DNA of the test strains. The results are summarized in Table 3 and those obtained with probe J illustrated in Fig. 2(b). In the case of group II and group I/II probes I, J and K hybridized to multiple DNA fragments.

Analysis of K9 chromosomal DNA

Probes J and D represent the sequences closest to regions 1 and 3 respectively of the group II K antigen gene clusters that also both hybridize to group I capsule-expressing strains (Tables 2 and 3). To determine whether probes D and J derive from contiguous sequences on the chromosome of group I capsule-expressing strains, chromosomal DNA from *E. coli* K9 was cleaved with a variety of restriction enzymes and used in Southern blot analysis with probes D and J. A comparison of the pattern of hybridization of the two probes revealed that probes D and J always hybridized to different fragments, indicating that the sequences that flank the group II capsule genes are not contiguous on the K9 chromosome (Fig. 3).

Discussion

DNA probes taken from regions 1 and 3 of the cloned K1 antigen gene cluster (Fig. 1) detect highly homologous sequences in all group II capsule-producing *E. coli* so far tested (Roberts *et al.*, 1988b). Sequences homologous to regions 1 and 3 were not detected in the chromosomal DNA of the group I capsule-producing strains, even at low stringency (Table 2 and Fig. 2a). Therefore, whilst the transport mechanisms encoded by regions 1 and 3 are capable of handling a diversity of group II polysaccharides ranging from the homopolymer K1 to the substituted heteropolymer K4 (Drake *et al.*, 1990), the results of this study would suggest that group I capsule expression is independent of these mechanisms and that silent copies of the group II capsule genes are not present near *serA*.

With the exception of the K2 strain, sequences homologous to the group II capsule gene cluster were not detected in group I/II capsule-expressing strains (Table 2 and Fig. 2a) even though both the group II (Ørskov *et al.*, 1976) and group I/II capsule genes (Ørskov & Nyman, 1974) have been mapped to the same locus and the capsules share many biochemical characteristics. The hybridization between probes A and B (Fig. 1) and chromosomal DNA from the K2 strain would suggest

that K2 may be a group II capsule with aberrant temperature regulation and not a group I/II capsule as previously suggested by Finke *et al.* (1990). Hence, expression of group I/II capsules would appear not to utilize the group II capsule gene products, nor are these genes present on the chromosome. Studies of CMP-KDO synthetase activity led Finke *et al.* (1990) to postulate the existence of a third group of *E. coli* capsular polysaccharides. The genetic information presented here supports the existence of a third group encoded by genes distinct from the group II capsule genes (at least as assessed by Southern blot analysis) but probably located at, or adjacent to, the same locus near *serA*. Nevertheless, it is possible that these genes do encode for similar proteins that perform common steps in capsule biogenesis. Such a situation exists between the capsule gene clusters of *Haemophilus influenzae*, *Neisseria meningitidis* and *E. coli* group II. Despite a lack of nucleotide sequence homology these capsule gene clusters encode a number of functionally homologous proteins (Kroll *et al.*, 1990; Frosch, *et al.*, 1991). Therefore, further genetic analysis is necessary to determine the functional relationships between *E. coli* group II and group I/II capsule gene clusters.

DNA adjacent to region 3 of the group II genes hybridized to most strains regardless of capsule type (Table 2). Therefore, this DNA is a common component of the *E. coli* chromosome. This is consistent with the findings of Vimr (1991), in which the sequences adjacent to the K1 capsule genes were hybridized to the overlapping set of clones generated by Kohara *et al.* (1987). Since the K1 capsule genes were mapped to 64 minutes on the physical map of the *E. coli* chromosome (Vimr, 1991), it follows that the K4 and K5 capsule genes are also located at this site on the chromosome. This is likely to be true for all group II capsule gene clusters.

At least 3 kb of sequence immediately flanking region 1 of both the K1 and the K4 antigen clusters was present only on the chromosome of *E. coli* strains expressing group II capsule gene clusters as well as the K2 strain (Probe C, Fig. 1, Table 3). This further supports our hypothesis that the K2 strain may actually be a group II strain. This sequence, although only found in association with group II capsule genes, has not to date been implicated in capsule expression (Roberts *et al.*, 1988a). A further 2.5 kb of DNA adjacent to this sequence (Probe K, Fig. 1, Table 3) is present on the chromosome only when group II or group I/II capsule genes are present. Beyond this DNA, sequences were present in most strains tested (Probes J and I, Fig. 1, Table 3). In a group I capsule-expressing strain (K9), although the group II capsule genes are absent, the flanking sequences that hybridize to probes D and J are not contiguous (Fig.

3). Thus, in this strain, DNA which is less than 70% homologous to the group II capsule genes must lie at this site.

A striking observation is that in many cases DNA flanking region 1 of the K4 capsule genes (probes I, J and K) hybridizes to multiple fragments in the chromosomal DNA from all the group II capsule-expressing isolates and two of the four group I/II strains studied (Fig. 2b and Table 3). Given the sizes of the fragments that hybridize to these probes, it is unlikely that these repeated sequences are immediately adjacent to one another. Thus DNA flanking region 1 of the K4 capsule gene cluster is found in most *E. coli* regardless of capsule type and is repeated in group II and group I/II capsule-expressing strains. The function(s) of the product(s) (if any) of this repeated DNA is unclear but it is thought not to play a role in group II capsule expression (Roberts *et al.*, 1988a).

The evidence presented in this paper suggests that *E. coli* may have at least three distinct groups of gene clusters for the production of capsular polysaccharide, two of which appear to map to a locus near *serA*. This *serA*-linked locus is polymorphic and can adopt three allelic forms. Two of these alleles are associated with capsule expression (groups II and I/II), of which group II is known to be internally variable. The third form (group I) has no known association with capsule production. Comparison of the *E. coli* and *Salmonella typhimurium* linkage maps (Riley & Krawiec, 1987) indicates that the *serA* region of the chromosome of these organisms is one of a restricted number of sites at which acquisition of new genetic material has occurred. A predilection to acquire and perhaps reorganize genetic material at or adjacent to *serA* may be one mechanism for the generation of capsule diversity in *E. coli* and explain the polymorphic nature of this locus.

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