**kdsA mutations affect FtsZ-ring formation in *Escherichia coli* K-12**

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No one has, as yet, addressed the relationship between the nature of the outer membrane and cell division. *kdsA* encodes 3-deoxy-D-manno-octulosonic acid (KDO) 8-phosphate synthetase which catalyses the first step in the synthesis of KDO, the linker between lipid A and oligosaccharide of lipopolysaccharide (LPS). Seven temperature-sensitive mutants containing missense mutations in *kdsA* were affected in the production of KDO and all mutants stopped dividing at 41 °C and formed filaments with either one or no FtsZ ring. All observed defects were reversed by the plasmid-borne wild-type *kdsA* gene. Western blotting analysis, however, demonstrated that the amount of FtsZ protein was not affected by the mutation. The mutants were more susceptible to various hydrophobic materials, such as novobiocin, eosin Y and SDS at 36 °C. Methylene blue, however, restored *kdsA* mutant growth. Plasmid-borne wild-type *msbA*, encoding a lipid A transporter in the ABC family, partially suppressed *kdsA* mutation. A mutation of *lpxA*, functioning at the first stage in lipid A biosynthesis, inhibited both cell division and growth, producing short filaments. These results indicate that the instability of the outer membrane, caused by the defect in KDO biosynthesis, affects FtsZ-ring formation.

**Keywords**: *kdsA* mutants of *E. coli*, KDO biosynthesis, membrane structure, cell division, lipopolysaccharide synthesis

**INTRODUCTION**

The outer membrane of Gram-negative bacteria, such as *Escherichia coli*, plays a central role in controlling the interaction of the bacteria with the external environment, serving as a barrier, controlling the absorption of ions and nutrients and transmitting signals to the inside of the cell. Instabilities of the outer membrane adversely affect multiple cellular responses. Proper cell division requires both the construction of division machinery, physically separating internal cellular components, and the division of cell membrane structures. Both processes are closely related; instabilities in membranes may affect both the construction of division machinery and the expression of gene(s) essential for cell division. However, the mechanism controlling the relationship between cell division and the nature of the cell membrane remains unknown. We have created seven unique *fts/kdsA* mutants allowing analysis of the discrimination mechanism governing membrane construction and cell division.

The outer membrane of Gram-negative bacteria is composed principally of lipopolysaccharide (LPS) and phospholipids. LPS comprises approximately 30% of the outer membrane by gross weight (Smit *et al*., 1975). LPS, comprising hydrophobic lipid A, the hydrophilic core oligosaccharide chain and 3-deoxy-D-manno-octulosonic acid (KDO), connecting lipid A to the oligosaccharide chain (Raetz *et al*., 1985; Rietschel, 1984), is important in determining outer-membrane barrier function (Leive, 1974; Nikaido & Vaara, 1985).

KDO is synthesized by the condensation of D-arabinose-5-phosphate and phosphoenolpyruvate, followed by dephosphorylation. The first step of the reaction is catalysed by KDO 8-phosphate synthetase, encoded by the *kdsA* gene in both *Salmonella typhimurium* (Rick *et al*., 1977) and *E. coli* (Ray, 1980). *E. coli kdsA*, however, was first identified as a gene complementing the *kdsA* mutation of *Salmonella* (Woisetschlager & Hogenauer, 1987), although no similar mutants have yet been identified in *E. coli*. Mutations in *kdsA* of *Salmonella*

Abbreviations: DAPI, 4',6-diamino-2-phenyl-indole; KDO, 3-deoxy-D-manno-octulosonic acid.
typhimurium cause the accumulation of lipid A in the periplasm, also resulting in the disappearance of LPS from the outer membrane (Rick & Osborn, 1977; Osborn et al., 1980). LPS is an essential membrane component (Strohmaier et al., 1995) and only conditional lethal mutants in KDO biosynthesis can be isolated in Salmonella (Lehmann et al., 1977; Rick et al., 1977; Rick & Young, 1982). The biosynthesis of LPS is growth-phase-regulated at the transcriptional level in E. coli (Strohmaier et al., 1995); no evidence, however, has uncovered a relationship between LPS biosynthesis and cell division. In this study, we isolated temperature-sensitive mutants of kdsA and demonstrated that membrane instability resulting from the defect in KDO biosynthesis affected the FtsZ-ring formation.

**METHODS**

**Bacterial strains and bacteriological procedures.** The E. coli strains used in this paper are catalogued in the NIG collection (http://shigen.lab.nig.ac.jp/ecoli/strain/). L broth contained 1% tryptone, 0.5% yeast extract, 0.5% NaCl and 0.1% glucose. The pH was adjusted to 7.0 using 1 M NaOH. Thymine was added to media at a concentration of 100 µg ml⁻¹. Tetracycline, when needed, was utilized at a concentration of 20 µg ml⁻¹. Transduction with P1 and T4G17 phages was performed as described by Buxton & Holland (1973) and by Wilson et al. (1979), respectively. Selection plates used for P1-phage-mediated transduction contained 1% sodium citrate. Cell number was monitored with a Coulter multisizer II (Coulter Electronics) equipped with a 30 µm orifice and accommodating a volume of 100 µl.

**Fluorescence microscopy.** To observe cells and nucleoids by microscopy, cells were stained by 4',6-diamino-2-phenylindole (DAPI) as described by Hiraga et al. (1989). Localization of FtsZ was visualized by immunofluorescence microscopy, as described by Hiraga et al. (1989) using a rabbit anti-FtsZ antibody, kindly provided by Dr J. Lutkenhaus. Western blots were visualized using an ECL Western blotting kit (Amersham). Kindly provided by Dr J. Lutkenhaus (Addinall et al., 1996), as the first antibody and Cy3-labelled anti-rabbit IgG antibody as a second antibody.

**Site-directed mutagenesis.** We constructed a plasmid by site-directed mutagenesis substituting a G and A for T696 and G699 of kdsA, respectively, carried by pTN18H. We designed four primers to prepare PCR products containing either the 5′ or 3′ half of kdsA using pTN18H as the template. The sequence of the forward primer to amplify the 5′ half region of kdsA was 5′-TCATCGATAAGCTTATAGCGGTAG-3′, homologous to the HindIII flanking region of pTN18H. The sequence of the reverse primer was 5′-AGGCCGGCTAGCCCTAC-3′, the complementary sequence from G690 to T707 of kdsA containing the T696G and G699A substitutions. The resulting PCR products contained both the 2 base substitutions and an additional Nhel site, 5′-GCTAGC-3′. To amplify the 3′ region of kdsA, the forward primer sequence 5′-CGGGTGAGGGCTAGCGG-3′ was used to create 2 base substitutions and an Nhel site. The reverse primer 5′-GGCGCAGGCGCTAGCGGTAAA-3′ contained the PvuII flanking region of PB322, the parent vector of pTN18H. The PCR product, containing the 5′ half region, was cut by Nhel and HindIII; the 3′ half region was cut by Nhel and PvuII. Both were ligated to the 2 kb HindIII–PvuII fragment of PB322 concurrently. The resulting plasmid, pTN18HX, was used for the complementation test of the fts mutants.

**Quantitative analysis of KDO.** Quantitative analysis of KDO was performed as described by Karkhanis et al. (1978) with the following modifications. Cultures growing exponentially (OD₆₀₀ = 0.2) in t-broth at 30 °C were diluted 1- to 10-fold with fresh medium [for JE10830/p(kdsA)10 µM IPTG was added] and incubated at 41 °C for 0.5, 1, 2 and 3 h, respectively. At each time point, OD₆₀₀ was measured, the values of which were corrected for the dilution factors and are shown relative to the value of the 0 h sample. This makes it possible to give the same nutritional condition for each point of analysis. Cells were harvested from 12 ml of each culture and washed twice with 0.5 ml 10 mM HEPES (pH 7.4) by centrifuging at 3500 r.p.m. for 10 min. Cells were broken with an Ultrasonic Cleaner USC-1 (Pasorina) for 30 s in an Eppendorf tube. A 10 µl aliquot of the sample was utilized to quantify the total proteins. We added 60 µl 0.9 M H₂SO₄ to the remaining sample and boiled in a water bath for 40 min to separate the KDO from LPS. Samples were allowed to cool to ambient temperature, then centrifuged at 15000 r.p.m. for 5 min. A 0.25 µl aliquot of the supernatant was transferred to a new tube, 125 µl 40 mM HIO₄ was then added and incubated at room temperature for 20 min to form KDO-COOH. After 5 min incubation with 125 µl 2.5% NaAsO₂ in 0.5 M HCl, 250 µl 0.6% TBA was added. Samples were then boiled for 15 min. Following the addition of 500 µl undiluted DMSO, the OD₄₄₀ was measured. Purified KDO (Sigma) was used as a standard.

**Construction of plasmids.** A 2.3 kb DNA fragment containing intact msaB was amplified from genomic DNA of wild-type strain MG1655 by PCR using a pair of primers: 5′-CAGGCAAATTGTCCAGATCCTC-3′ and 5′-CAGCCCTTACACCGGATCCCCGGA-3′. Amplified DNA was digested with BamHI and cloned at the BamHI site on the vector plasmid pMW118 (Nippon Gene Co.). One of the resulting plasmids, in which the msaB gene is located downstream of the lac promoter on the vector plasmid in the same direction, was named as pMsA8.

**Western blotting.** Western blotting was performed as described by Sambrook et al. (1989). Pelleted cells were resuspended in 100 µl SDS sample buffer to give 1 OD₆₀₀ unit, then boiled for 3 min. Ten microlitres of each sample was electrophoresed on a 10% SDS polyacrylamide gel, transferred to membranes and immunostained using an anti-FtsZ polyclonal antibody. The anti-FtsZ polyclonal antibody was kindly provided by Dr J. Lutkenhaus. Western blots were visualized using an ECL Western blotting kit (Amersham). The resultant membranes were exposed to X-ray films.

**RESULTS**

**Effects of the seven fts mutations on cell growth**

Some 356 fts mutants, screened from ‘Hirot’a’s temperature-sensitive mutant bank’ and defective in septation, were roughly mapped by complementation (Nishimura et al., 1991) using the bank of 2200 E. coli strains carrying pLC-plasmids (Clarke & Carbon, 1976). Seven of these fts mutants, JE10446, JE10705, JE10830, JE11167, JE11171, JE11212 and JE11241, were defined as carrying mutations in new genes involved in cell division because they were complemented by the pLC13-27 plasmid, carrying the 27 min region of the E. coli chromosome (Nishimura et al., 1992). This region does not contain any of the known fts genes and hence the seven fts mutants were chosen for further study.

We examined the effects of the fts380 mutation on cell division and growth. Cultures of both JE10830 and the
parent bacterium, PA3092, growing exponentially at 30 °C, were diluted to 5 × 10⁷ cells ml⁻¹ and incubated at 41 °C. We monitored cell numbers utilizing a Coulter counter and cell growth as OD₆₀₀. The OD₆₀₀ of JE10830 bacteria continued to increase after the temperature shift, although the increase was lower than that of the wild-type strain. Cell division, however, stopped completely 2 h after the temperature shift to 41 °C (Fig. 1). DAPI staining demonstrated that chromosomal replication and segregation appeared normal for at least 3 h at 41 °C (Fig. 2). We examined the six remaining strains similarly, using the same medium, LB containing 0–5 % NaCl. JE10446, JE11171, and JE11241 demonstrated similar phenotypes to JE10830 (data not shown). JE10705 and JE11212 appeared to have a leaky phenotype since cell number per OD₆₀₀ after 3 h incubation at 41 °C was about three times more than that of JE10830. Cells, however, stopped dividing completely at 41 °C when grown in LB medium without NaCl. Many cell division mutants recover cell division when the growth medium has an NaCl concentration higher than a critical concentration specific to osmotic effects (Reeves et al., 1970; Ricard & Hirota, 1973). The critical concentration of NaCl for these two strains may be lower than 0·5 %.

**Seven fts mutants have mutations in the kdsA gene**

We performed P1-phage-mediated transduction to verify that the *fts830* mutation lies at approximately 27 min within the *E. coli* genetic map and that pLC13-27 does not contain a multicopy suppressor. The temperature-sensitive phenotype of JE10830 was eliminated by cotransduction of either *trp*::Tn₁₀ or *fadR*::Tn₅ with an efficiency of 13·0 % and 23·8 %, respectively, using P₁ phage. On the contrary, the *fts830* mutation was cotransduced out with *trp*::Tn₁₀ into the wild-type strain, MG1655, with a similar efficiency using T₄GT₇ phage. The six other mutations were also cotransduced with *trp*::Tn₁₀ and *fadR*::Tn₅ with similar efficiencies.

To clone the wild-type *fts830* gene, we constructed the plasmids pTN₁₈ and pTN₂₃ by insertion of the 4·8 kb *SspI* segment of pLC13-27 into the *EcoRV* site within pBR322 in both directions. As shown in Fig. 3(a), both plasmids complemented the *fts830* mutation. After testing subclones containing various deletions for their ability to complement *fts830*, we determined that a
1.5 kb HindIII–PvuII segment, carried by the pTN18H plasmid, which lacks 3.3 kb of the original SspI fragment, complemented fts830. Sequencing analysis demonstrated that this segment contained both kdsA and ORF-X, on the opposite strand. ORF-X may encode a protein composed of 315 aa. The six remaining fts mutants were also complemented by the pTN18H plasmid.

We isolated and sequenced PCR products from this region using genomic DNA of mutants as a template. All seven mutants contained missense mutations in kdsA, causing the expected amino acid substitutions (Fig. 4a). No amino acid changes were found, however, in the ORF-X sequence of four mutants, JE10446, JE10705, JE11167 and JE11171. Strong polarity changes of amino acid substitutions in KdsA of the mutants resulted in greater inhibition of cell division, as seen with the JE10446, JE10830, JE11171 and JE11241 mutants, although JE11167 was an exception (Fig. 4b).

To confirm the phenotype of the mutants was caused by
mutations in kdsA, not ORF-X, we constructed a plasmid, pTIN18HX, through site-directed mutagenesis, altering T696 and G699 of kdsA to G and A, respectively (Fig. 3b). These base substitutions do not alter the KdsA amino acid sequence, but change the 70th glutamic acid of ORF-X to a nonsense codon. The plasmid complemented all seven fts mutants, displaying colony formation at 41 °C. These results suggest that the seven fts mutations are alleles of kdsA.

We found recently that the ts20 mutant, defective in cell division at the restrictive temperature (Nagai & Tamura, 1972), carried the same allele as fs705.

**The kdsA mutations affect KDO production and sensitivity to hydrophobic materials**

LPS serves as a barrier against hydrophobic materials and mutations involved in LPS synthesis result in an increased sensitivity to hydrophobic materials (Hancock & Reeves, 1976). To examine membrane stability, we compared the sensitivity of both the mutants and the wild-type strain to hydrophobic drugs. Exponentially growing cultures of both JE10830 and the parent, PA3092, were diluted and plated on LB medium containing variable concentrations of novobiocin, a hydrophobic antibiotic inhibitor of the gyrB protein (Drlica, 1984). Incubation was carried out at 36 °C, the maximum temperature at which the mutants were able to form colonies. The colony-forming ability of JE10830 decreased inversely with increasing concentrations of novobiocin; growth of the parent strain was not affected (Fig. 5). We also examined the effects of other substances on mutant growth. Cultures of the JE10830 mutant and the parent strain were diluted to 5 × 10⁵ cells ml⁻¹, and 1 µl was spotted onto LB agar containing varying concentrations of substances that destabilize, including SDS, eosin Y, EDTA and methylene blue. These cultures were incubated at 36 °C for 36 h. JE10830 was highly sensitive to eosin Y and SDS; the minimum concentration of eosin Y and SDS inhibiting colony-forming ability at 36 °C in JE10830 was 0.1% and 1%, respectively, whilst that in PA3092 was more than 0.5% and 4%, respectively. The mutant was less sensitive to EDTA than the wild-type strain; JE10830 was sensitive up to 10 mM EDTA while PA3092 was sensitive up to 8 mM. On the contrary, the colony-forming ability of JE10830 was enhanced by the addition of methylene blue; JE10830 colony size increased with the addition of 0.1% methylene blue whilst PA3092 colony size decreased compared to the control at 36 °C. Although JE10830 did not grow on LB medium without NaCl at 36 °C, the addition of methylene blue restored growth under these conditions. Sensitivity to hydrophilic materials, such as kanamycin, was not altered by the mutation. These results suggest that the kdsA mutation may cause instability in membrane structures, possibly affecting gene expression.

To confirm the relationship between instability of outer membrane and KDO synthesis, we examined the effect of fts830 on the synthesis of KDO. JE10830, the kdsA transformant JE10830/p(kdsA⁺) and their parent PA3092 cells, growing exponentially at 30 °C, were diluted in fresh medium and allowed to continue growing at 41 °C for 0.5, 1, 2 or 3 h. We monitored cell growth by measuring the OD₆₀₀ and KDO concentrations were measured as described in Methods. As shown in Fig. 6, the specific content of KDO decreased approximately 75% for each doubling of the OD₆₀₀.

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**Fig. 5. Effect of fts830 on sensitivity to novobiocin.** JE10830 and the parent, PA3092, growing exponentially at 30 °C, were diluted and spread on LB-agar medium containing variable concentrations of novobiocin. Following incubation at 30 or 36 °C for 36 h, the number of colonies was counted. ○, PA3092 at 30 °C; □, PA3092 at 36 °C; ■, JE10830 at 30 °C; ▬, JE10830 at 36 °C.

**Fig. 6. Effect of fts830 on KDO synthesis.** JE10830 (▲), JE10830 transformed by a plasmid-borne wild-type kdsA, fts830/p(kdsA⁺) (▲), and the parent (PA3092; ●) cells growing exponentially at 30 °C were diluted 1- to 150-fold with fresh medium and incubated at 41 °C for 0.5, 1, 2 and 3 h, respectively. For fts830/p(kdsA⁺), 10 µM IPTG was added at time zero. OD₆₀₀ was monitored at each time interval and cells were harvested and used for measuring KDO concentrations. The values of OD₆₀₀ were corrected for the dilution factors and are shown relative to 0 h. The relative amounts of KDO were plotted against the relative amount of OD₆₀₀.
value. The plasmid carrying the wild-type kdsA restored the specific content of KDO in the mutant cells to a level equal to that of the parent. These results indicate that the defect in KDO synthesis in JE10830 causes the instability of the outer membrane.

The htrB gene encodes KDO-dependent lauroyltransferase and a defect in htrB causes the accumulation of lipid A (Clementz et al., 1996). Enhanced export of lipid A precursors mediated by extra copies of msbA permits cell growth in the htrB mutant (Karow & Georgopoulos, 1993; Zhou et al., 1998). Therefore, we also examined whether the defects in fts830/kdsA could be corrected by plasmid-borne wild-type msbA. As shown in Fig. 7(a, b), cell length of the mutant harbouring the msbA plasmid JE10830/pMsbA was apparently shorter than that of the mutant harbouring only vector, JE10830/pMW118, at 41 °C. The growth defect of JE10830 was also corrected by transformation of pMsbA; the growth rate of JE10830/pMsbA was higher than that of JE10830/pMW118. The colony-forming ability of JE10830 was not restored, however. The result demonstrates that the defect in the cell division of JE10830 is corrected by the extra copies of wild-type msbA, and the growth defect of JE10830 is alleviated partially, although colony-forming ability is not recovered.

To investigate whether the accumulation of lipid A precursors in the cytoplasmic membrane or the instability of the outer membrane affects cell division, we examined the effect of the lpxA mutation on cell division. lpxA functions at the first stage in lipid A biosynthesis (Raetz, 1986), so lipid A is not synthesized in these mutants. Cells of an lpxA mutant, SM101, growing exponentially at 30 °C, were diluted in fresh medium, allowed to continue growing at 41 °C for 3 h and observed by phase-contrast microscopy. The lpxA mutation apparently affected cell division at 41 °C (Fig. 7c, d), although the resulting filaments were very short because the mutation also affected cell growth severely. The cells were very thick and many ghost cells were found, indicating the instability of the membrane.

**The kdsA mutations affect FtsZ-ring formation but not the production of FtsZ**

The seven mutants examined in this study did not demonstrate constriction at the potential division site (Fig. 2), suggesting cell division was inhibited at an early step. We therefore analysed the effect of the fts830 mutation on FtsZ-ring formation by immunofluorescence microscopy using an anti-FtsZ antibody. As shown in Fig. 8, although the cell length of JE10830 continued to increase after temperature shift, the FtsZ ring increased marginally. After 3 h incubation at 41 °C, 65% of the cells contained only one FtsZ ring at one end of the filaments and the remaining 35% did not have an FtsZ ring. The ftsI mutant, RP41, contained FtsZ rings,
**kdsA affects FtsZ-ring formation in *E. coli***

**Fig. 8.** FtsZ-ring localization in *fts830* and *ftsI* cells. Immunofluorescence micrographs of JE10830 (*fts830*) grown at 30 (a) or 41 °C for 1 (b), 2 (c) or 3 h (d), and RP41 (*ftsI*) (e) grown for 3 h at 41 °C.

**Fig. 9.** Immunodetection of FtsZ by Western blotting in both JE10830 and the parent (PA3092) cells, grown at 41 °C for 3 h.

A decrease in FtsZ to 60–70% of normal levels results in the inhibition of cell division (Dai & Lutkenhaus, 1991). We also examined the total levels of FtsZ in whole cells. Both JE10830 and the parent cells were cultured as in the above experiment, then the total amounts of FtsZ were measured by Western blotting. The levels of FtsZ in the mutant cells did not change with increasing time of incubation at 41 °C, similar to the parent (Fig. 9). These data indicate that the *kdsA* mutation affects FtsZ-ring formation, but does not affect the production of FtsZ.

**DISCUSSION**

*kdsA* mutations not only affect the production of KDO and stability of the outer membrane, but also have profound effects on cell division. The cell division defect is caused by deficiencies in FtsZ-ring formation. A plasmid, carrying the wild-type *kdsA*, reversed all observed defects: KDO production, cell division and cell growth at 41 °C. Therefore, we conclude that decreases in LPS synthesis affect FtsZ-ring formation, resulting in aberrant cell division. Western blotting demonstrated that *kdsA830* (*fts830*) did not affect FtsZ production, however.

We concluded that instability of the outer membrane, due to a defect in the synthesis of complete LPS molecules, affects cell division. Complete LPS molecules may be required for some essential physiological func-
tion of the outer membrane or assembly of a functional outer membrane. Rick & Osborn (1977) also demonstrated in S. typhimurium that a defect in KDO synthesis causes pleiotropic effects on growth, LPS synthesis and accumulation of lipid A precursor in the isolated cytoplasmic membrane (Osborn et al., 1980). A defect in htrB, encoding KDO-dependent lauroyltransferase which acts after KDO addition during lipid A biosynthesis (Clementz et al., 1996), also inhibits cell division, producing filamentous cells at the non-permissive temperature, and inhibition of cell division of the htrB mutant is suppressed by msbA which may encode an ATP-dependent translocator (Karow & Georgopoulos, 1993). It is proposed that accumulation of lipid A precursors is toxic to cell growth (Rick & Young, 1982) and that enhanced export of these precursors mediated by extra copies of msbA may permit cell growth (Zhou et al., 1998). The defect in cell division of JE10830 is also suppressed by the presence of extra copies of msbA. The defect in colony-forming ability, however, was not suppressed at all. Moreover, a mutant of lpxA, functioning at the first stage in lipid A biosynthesis to affect LPS synthesis (Raetz, 1986), also inhibits cell division in E. coli, producing short filamentous cells, although the growth was very poor at the restricted temperature. Our results, therefore, demonstrate that the inhibition of cell division by the kdsA mutation is not caused by the accumulation of lipid A in the inner membrane. It seems more likely that the presence (or indeed the transport) of the lipid A precursor to the outer membrane may be essential for the stability of this membrane and that its instability in turn affects cell division.

The loss of LPS during KDO deficiency destabilizes the membrane structure, presumably causing large abnormalities in both the localization of membrane proteins and cell division. We observed a deficiency of FtsZ rings in the JE10830 filaments, although the levels of FtsZ were not affected by the mutation. The FtsZ ring, however, is constructed onto the inner membrane, maintaining a position at the leading edge of the invaginating septum (Bi & Lutkenhaus, 1991; Sun et al., 1998) and the protein(s) governing this cell division at the outer membrane have not been found as yet. The mechanism whereby the instability of the outer membrane results in the loss of FtsZ-ring formation, therefore, remains to be established.

One of the possible explanations is that the instability of the outer membrane might affect the transcription of a cell division gene(s), required for FtsZ-ring formation. The outer membrane functions to communicate information received from the external environment into the cell. Therefore, membrane instability is expected to affect the transcription of genes, including those involved in cell division. We analysed the effect on membrane stability by examining the sensitivity of mutants to various hydrophobic materials. The results demonstrated that although the mutants were more susceptible to various hydrophobic materials, some hydrophobic materials restored kdsA mutant growth, indicating that the instability of the outer membrane creates the altered cellular responses to the environment. Null mutations in either htrB or msbB also enable growth on four times the concentration of deoxycholate relative to wild-type bacteria (Karow & Georgopoulos, 1993). Destabilization of the outer membrane may result in the expression of a subset of genes overcoming the sensitivity to the hydrophobic materials.

LPS comprises approximately 30% of the outer membrane in gross weight (Leive, 1974). Therefore, decreases in LPS may destabilize the outer membrane and may expose phospholipids to the external environment. Phospholipids are integral in the regulation of cell cycle, and interruptions of phospholipid synthesis inhibit both the initiation of chromosome replication and cell division (Norris, 1989). In addition, the physical nature of phospholipids changes dramatically with various growth conditions (Tilcock, 1986). Phospholipids are also important in the transfer of signals from the extracellular environment to the replication and transcription machinery (Sekimizu, 1994). Therefore, loss of LPS may change the physical nature of phospholipids due to exposure to their surroundings, altering the expression of various genes. In S. typhimurium, the rate of OmpA synthesis is proposed to be activated by the kdsA mutation (Rick et al., 1983, 1984), although the mechanism of this activation is not well understood.

All six mutations analysed in this article concerned non-polar amino acids of which four were replaced by polar ones. Mutations substituting these amino acids inhibited cell division severely, except fts1167. Recently, the crystal structure of KdsA was determined by Radaev et al. (2000). The unit cell of the crystal consists of a homotetramer of KdsA. The six mutation sites are not localized in space, but are scattered throughout the three-dimensional structure: two sites, Leu 118 (fts705) and Ala 227 (fts1212), were located on the monomer–monomer interfaces making contacts between the same residues, Leu–Leu and Ala–Ala, of different subunits, and thus might affect the stability of tetramer complexes. Ala 203 (fts446) is close to His 202, which is one of the binding sites of phosphoenolpyruvate (Radaev et al., 2000), and thus might disturb catalytic activity of KdsA. Gly 73 (fts830 and fts1241), sitting at the start of an α-helix, has a positive phi-angle, which is allowed for Gly but may not be adequate for other amino acids such as Asp. Ala 14 (fts1167) and Ala 234 (fts1171) are close to each other in space, suggesting similar effects if any, whereas we cannot see any definite reason for instability caused by mutations at these sites. These data suggest that the reduction of catalytic activity of KdsA might result from the instability of three-dimensional structures at high temperature, rather than any direct effects on the active centre of the enzyme.

**ACKNOWLEDGEMENTS**

We are grateful to Dr. Joe Lutkenhaus who kindly provided anti-FtsZ polyclonal antibody. We also thank Dr Koichi Inouye who kindly advised on the technique for the quan-
titative analysis of KDO. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (A), from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Received 25 May 2001; revised 7 August 2001; accepted 25 September 2001.