JOURNAL OF BACTERIOLOGY, Oct. 2006, p. 7186–7194 Vol. 188, No. 20
0021-9193/06/$08.00+0 doi:10.1128/JB.00571-06

Differential Effects of *yfgL* Mutation on *Escherichia coli* Outer Membrane Proteins and Lipopolysaccharide

Emily S. Charlson,[1] John N. Werner,[2] and Rajeev Misra[1]*

Faculty of Cellular and Molecular Biosciences, School of Life Sciences, Arizona State University, Tempe, Arizona,[1] and Department of Molecular Biology, Princeton University, Princeton, New Jersey[2]

Received 22 April 2006/Accepted 25 July 2006

YfgL together with NlpB, YfiO, and YaeT form a protein complex to facilitate the insertion of proteins into the outer membrane of *Escherichia coli*. Without YfgL, the levels of OmpA, OmpF, and LamB are significantly reduced, while OmpC levels are slightly reduced. In contrast, the level of TolC significantly increases in a *yfgL* mutant. When cells are depleted of YaeT or YfiO, levels of all outer membrane proteins examined, including OmpC and TolC, are severely reduced. Thus, while the assembly pathways of various nonlipoprotein outer membrane proteins may vary through the step involving YfgL, all assembly pathways in *Escherichia coli* converge at the step involving the YaeT/YfiO complex. The negative effect of *yfgL* mutation on outer membrane proteins may in part be due to elevated sigma E activity, which has been shown to downregulate the synthesis of various outer membrane proteins while upregulating the synthesis of periplasmic chaperones, foldases, and lipopolysaccharide. The data presented here suggest that the *yfgL* effect on outer membrane proteins also stems from a defective assembly apparatus, leading to aberrant outer membrane protein assembly, except for TolC, which assembles independent of YfgL. Consistent with this view, the simultaneous absence of YfgL and the major periplasmic protease DegP confers a synthetic lethal phenotype, presumably due to the toxic accumulation of unfolded outer membrane proteins. The results support the hypothesis that TolC and major outer membrane proteins compete for the YaeT/YfiO complex, since mutations that adversely affect synthesis or assembly of major outer membrane proteins lead to elevated TolC levels.

The envelope of gram-negative bacteria is comprised of inner and outer membranes and an aqueous compartment, called the periplasm, sandwiched between the two membranes. Unlike the inner membrane, which is composed of a phospholipid bilayer, the outer membrane is unique in that its inner leaflet consists of phospholipids while the outer leaflet, facing the external medium, is made up of lipopolysaccharide (LPS) (31). To reach the outer membrane, proteins synthesized in the cytoplasm must first traverse the inner membrane through the SecYEG translocon (43). In several instances, unassembled outer membrane proteins (OMPs) have been reported to transiently exist in the periplasm after translocation (for a review, see reference 12). These nascent OMP polypeptides are thought to interact with periplasmic folding factors such as chaperones, foldases, and LPS to attain a folding status necessary for their proper insertion into the outer membrane (12). Inability to correctly fold, either due to alterations in an OMP's primary structure or a defective periplasmic folding environment, often leads to the degradation of misfolded OMPs by the periplasmic protease DegP (10, 29).

OMP biogenesis studies have often used trimeric β-barrel porins (OmpF, OmpC, and PhoE), a maltoporin (LamB), or a monomeric OMP (OmpA) as a model. Biogenesis of some OMPs is affected in an LPS mutant background (3, 21, 24, 34) or in the absence of de novo lipid synthesis (5, 34, 47). For this reason, these OMPs are referred to as lipid-dependent OMPs. A recent study on another OMP, TolC, which has a unique three-dimensional α/β-barrel fold (20), showed that its biogenesis proceeds independent of previously characterized folding factors and lipids (45, 46). Yet another group of OMPs, which includes lipoproteins (42) and pilus proteins (41), requires specific chaperones or outer membrane factors for their biogenesis.

Recent genetic, biochemical, and computational analyses have led to the identification of additional proteins that are likely to be involved in OMP biogenesis (16, 17, 32, 36, 46, 47). These include YaeT, YfiO, YfgL, and NlpB, all of which are OMPs themselves, and, with the exception of YaeT, they are lipoproteins (30). The role of a YaeT homolog, Omp85, in OMP biogenesis was first described in *Neisseria meningitidis* (44), and YaeT's biogenesis role was subsequently confirmed in *Escherichia coli* (16, 46, 47). The role of the essential YaeT protein in *E. coli* appears to facilitate the proper insertion of OMPs into the outer membrane (46). Biogenesis pathways for β-barrel OMPs and TolC appear to converge at the step involving YaeT, because its depletion affects the biogenesis of both groups of OMPs (46). YfiO is also essential, but YfgL and NlpB are not (7, 17, 32). Depletion of YfiO reduces the level of both the β-barrel OMPs and TolC (25). Cells deleted for the *yfgL* gene have reduced levels of OMPs (32, 36) and elevated σ^E activity (32), suggesting a possible role of YfgL in OMP biogenesis. Unlike the *yfgL* mutant, cells lacking *nlpB* neither show any significant reduction in the level of β-barrel OMPs (32, 47) or TolC (J. Werner and R. Misra, unpublished data) nor have elevated σ^E activity (32). The roles of these and additional lipoproteins identified through computational motif analysis (32) remain unknown.

Genetically, *yfgL* was identified among suppressor mutations that partially reversed the hypersensitive phenotype of the

* Corresponding author. Mailing address: School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501. Phone: (480) 965-3320. Fax: (480) 965-6899. E-mail: rajeev.misra@asu.edu.

mutant *imp* alleles, *imp208* (E. Charlson, E. Coon, and R. Misra, unpublished data) and *imp4213* (17). The *imp* alleles were first identified through a genetic selection that sought mutants with an increased outer membrane permeability defect (37). Subsequently, mutations in *imp* were also shown to affect the cell's organic solvent tolerance and hence *imp*'s synonym, *ostA* (1). Recently, *imp*/*ostA* was shown to code for an essential OMP (8) involved in LPS transport (6). Although it is unclear at this point as to how null mutations in *yfgL* partially reverse the hypersensitivity phenotype of the *imp* alleles, the suppressors do genetically link the components of the OMP and LPS biogenesis pathways (17, 47).

The aim of this study was to further dissect the role of *yfgL* in OMP biogenesis. The data showed differential effects of Δ*yfgL* on OMP and LPS levels. The effect of Δ*yfgL* on OmpF and TolC was most pronounced: OmpF levels went down roughly 70%, whereas the level of TolC rose more than 180%. Like TolC, LPS levels also rose significantly in *yfgL* mutants. Although the effect of Δ*yfgL* may partly involve σ^E-mediated modulations in OMP and LPS synthesis (33), the data presented here also show an effect of Δ*yfgL* mutation on OMP assembly and outer membrane integrity.

MATERIALS AND METHODS

Bacterial strains, growth media, and chemicals. Bacterial strains and plasmids used in this study are listed in Table 1. Rich media (Luria broth [LB] and LB agar [LBA]) were prepared as described by Silhavy et al. (38). When required, ampicillin (25 μg/ml), chloramphenicol (20 μg/ml), kanamycin (20 μg/ml), or tetracycline (5 μg/ml) was added to the media. Enhanced chemifluorescence (ECF) substrate was purchased from Amersham Pharmacia Biosciences, Immun-star horseradish peroxidase substrate was from Bio-Rad Laboratories, and a SilverQuest silver staining kit was from Invitrogen Life Technologies. All chemicals were of analytical grade.

DNA manipulation. The chromosomal *yfgL* gene was deleted using the λ-red recombination-mediated gene deletion technique described by Datsenko and Wanner (15). Primers for PCR were designed to delete 1,137 bases of the 1,179-bp-long *yfgL* open reading frame and replace it with a kanamycin resistance (Km^r) cassette. The Km^r cassette was amplified from pKD4 using the forward primer 5′-GGAGAAGGACAGCGTGGAAATTTACGAGAACGAAAACGACAGGCTGGAGCTGCTTCG-3′ and the reverse primer 5′-GATGGACAAATTATTAATTTTCATCTGCATCATTTCGCTCATATGAATATCCTCCTTAG-3′. Purified PCR-amplified DNA was electroporated into RAM1134, and transformed cells were incubated overnight in a 30°C water bath. The next day cells were spread on LBA kanamycin plates. The chromosomal *yfgL* deletion in Km^r colonies was checked by PCR analysis using a forward *yfgM* primer (5′-GAGAAGGACACCATGGAAATTTACGAG-3′) and a reverse *yfgL* primer (5′-CAGACAACGCAAGCTTTATTCGCG-3′) complementary to the remaining *yfgL* sequence. Amplified PCR fragments had the size expected for the Δ*yfgL*::Km^r allele, thus confirming the deletion of the *yfgL* open reading frame. The antibiotic-resistant (Km^r) gene from the chromosomal Δ*yfgL*::Km^r allele was flipped out by flippase recombinase which was expressed from a plasmid (pCP20) with a temperature-sensitive replicon.

The *araCBAD* genes were deleted in the same way as *yfgL*. They were replaced by a Km^r cassette or chloramphenicol resistance (Cm^r) cassette. The DNA sequences of the Km^r and Cm^r cassettes were amplified from either pKD4 (Km^r) or pKD3 (Cm^r) using the forward primer 5′-GTAATCGACGCCGGAAGGTTTGATCACAAAGACGCCGCGCTAGGCTGGAGCTGCTTCG-3′ and the reverse primer 5′-GCCGTCAATTGTCTGATTCGTTACCAATTATGACAACCATATGAATATCCTCCTTAG-3′. Purified PCR product was electroporated into RAM1134 and incubated as described above. Cells were plated on LBA kanamycin or chloramphenicol plates. PCR amplification with primers flanking the deletion site was performed to verify the deletion.

To clone *yfgL* into pBAD24, specific cloning primers were designed for PCR amplification. The forward cloning primer 5′-ATCTGCTAGCCTGAGAGGGACCCGATG-3′ creates an NheI site (underlined) 14 bases before the *yfgL* start codon. The reverse cloning primer 5′-CAGACAACGCAAGCTTTATTCGCG-3′ creates a HindIII site (underlined) downstream of the *yfgL* stop codon. DNA was amplified from the chromosome using the *yfgL* cloning primers, digested with NheI and HindIII, and ligated into appropriately digested pBAD24 plasmids. Expression of YfgL from the plasmid clone was verified by complementation analysis.

Two primers were designed to clone *surA* into pTrc99A, a forward PCR primer, 5′-CACGAATTCCGCAGTGCGGTTAATTGAAATG-3′, creating an EcoRI site (underlined), and a reverse PCR primer, 5′-GCTGTGCAATCTAGACAACTAAGTCCG-3′, creating an XbaI site (underlined). Amplified PCR products were digested and ligated into appropriately digested pTrc99A plasmid. Expression of SurA from the plasmid clone was verified by genetic (complementation) and biochemical (Western blots) tests.

Protein analysis. To obtain whole-cell protein samples, cell pellets were resuspended in 10 mM Tris, pH 7.5, 2% sodium dodecyl sulfate (SDS) and heated at 95°C for 5 min. Periplasmic protein samples were obtained by the gentle osmotic shock method described by Arié et al. (4) with a modification: after incubation with the osmotic shock buffer, samples were centrifuged for 1 h at

TABLE 1. Bacteria strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)[a]	Reference or source
Strains		
MC4100	F⁻ *araD139* Δ(*argF-lac*)*U139 rpsL150 relA1 fibB5301 ptsF25 deoC1 thi-1 rbsR*	9
DME553	MC4100 Δ*lamB106* Δ*ompF80*	S. Benson
RAM123	DME553 *cog192* ($OmpG^+$)	27
RAM725	MC4100 Φ*ompF′*::*lacZ*$^+$	R. Misra
RAM726	MC4100 Φ*ompC′*::*lacZ*$^+$	R. Misra
RAM789	MC4100 *rcsB*::Km^r	R. Misra
RAM791	RAM789 Δ*rfa3*::Cm^r	R. Misra
PLB3260	MC4100 Δ*lamB106* Φ*ompF′*::*lacZ*$^+$	S. Benson
PLB3261	MC4100 Δ*lamB106* Φ*ompC′*::*lacZ*$^+$	S. Benson
B2045	RAM123 Φ*ompC′*::*lacZ*$^+$	D. Fajardo
RAM1118	B2045 $OmpA^-$ (phage K3-resistant isolate)	R. Misra
RAM1134	BW25113 pKD46	15
RAM1292	MC4100 Δ*ara714*	46
RAM1313	MC4100 Δ*yfgL*::Km^r	This study
RAM1314	RAM1292 Δ*yfgL*::Km^r	This study
RAM1315	RAM1292 Δ*yfgL*-scar	This study
RAM1316	RAM1292 *degP*::Tn*10*	This study
RAM1317	RAM1314 *degP*::Tn*10*	This study
RAM1318	RAM1315 *degP*::Tn*10*	This study
RAM1319	RAM1314 pACYC184	This study
RAM1320	RAM1314 pCS10	This study
RAM1321	RAM1317 pBR322-*skp*$^+$	This study
RAM1322	RAM1317 pTrc-*surA*$^+$	This study
RAM1323	RAM1317 pACYC184	This study
RAM1324	RAM1317 pCS10	This study
RAM1325	RAM1315 *degP*::Km^r	This study
RAM1326	RAM1325 *ompC′*::*lacZ*$^+$ *ompA610*	This study
RAM1327	RAM1325 *ompF′*::*lacZ*$^+$ *ompA610*	This study
Plasmids		
pACYC184	Cm^r; expression vector	11
pCS10	Cm^r; pACYC-$degP_{S210A}$	39
pBAD24	Ap^r; expression vector	19
pBAD24-*yfgL*$^+$	Ap^r	This study
pBR322-*skp*$^+$	Ap^r	R. Misra
pTrc99A-*surA*$^+$	Ap^r	This study
pKD4	Km^r	15
pKD3	Cm^r	15
pCP20	Ap^r, temperature-sensitive replicon	15

[a] Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin.

100,000 × *g*. Periplasmic protein samples in the supernatant were collected and run on SDS-polyacrylamide (11%) gels. Proteins were transferred to polyvinylidene difluoride membranes (Immobilon-Millipore). Once the proteins had been transferred, the membranes were incubated with appropriate primary antibodies for 1.5 h, followed by incubation with goat anti-rabbit alkaline phosphatase-conjugated immunoglobulin G secondary antibodies for 1 h. Finally, membranes were incubated with ECF or luminol substrate for approximately 5 min, and protein bands were visualized using a phosphorimager or a chemiluminescence imager, respectively.

Membrane analysis. Bacterial cell lysis was achieved by the French press method as described by Misra (26). Whole-cell envelopes were isolated by centrifuging cell-free lysates for 1 h at 105,000 × *g*. Envelopes were separated into inner and outer membranes by centrifugation through 30 to 55% (wt/vol) sucrose density gradients, as described by Misra et al. (28). Membrane buoyant densities were calculated by measuring the refractive index of each fraction at room temperature. Samples from each fraction were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE). To better resolve OmpC and OmpF bands, 4 M urea was added to the SDS-polyacrylamide running gel. LPS samples were prepared by incubating the sucrose gradient fractions with 0.25 mg/ml proteinase K for 1 h at 60°C. Proteinase K-digested membrane samples were analyzed by SDS-PAGE, and the LPS bands were visualized with silver staining.

RESULTS

The absence of YfgL reduces levels of several OMPs but increases TolC levels. YfgL has been implicated in the biogenesis of several OMPs, including OmpA, OmpC, OmpF, and LamB (32, 36). Furthermore, it has been shown that YfgL forms a complex with YaeT, YfiO, and NlpB (47), of which YaeT and YfiO have been demonstrated to be involved in OMP biogenesis (16, 25, 44, 46, 47). Since YaeT and YfiO appear to be general assembly factor, meaning that when either is depleted in the cell, assembly of both β- and α/β-barrel OMPs with distinct folding requirements is affected (25, 45, 46, 47), we asked whether the absence of YfgL would also produce broad OMP biogenesis defects.

Envelopes from *yfgL*$^+$ and Δ*yfgL* cultures were isolated and fractionated into inner and outer membranes via centrifugation in sucrose density gradients (Fig. 1). We noted that the buoyant densities of inner (1.1513 g/cm^3) and outer (1.2296 g/cm^3) membranes from the *yfgL*$^+$ culture were identical to those of the Δ*yfgL* culture, reflecting that the combined mass of OMPs and LPS is similar in both strains. Even though the outer membrane densities were identical, levels of OmpF, LamB, and, to a lesser degree, OmpA from the outer membrane fractions of the Δ*yfgL* strain were significantly lower than those of the *yfgL*$^+$ parental strain (Fig. 2). In contrast, there was only a slight decrease in the OmpC level, and surprisingly, the level of TolC in the outer membrane fractions of the Δ*yfgL* strain was significantly higher than that of the parental strain (Fig. 2). We noted that in an OmpC-minus strain, which has a greatly elevated level of OmpF compared to that of the isogenic OmpC$^+$ OmpF$^+$ strain, the effect of Δ*yfgL* on OmpF was significantly reduced (only a 20% reduction in OmpF level; data not shown), suggesting that OMP expression levels can influence the extent of Δ*yfgL*'s adverse effect on an OMP.

We asked whether elevated TolC levels observed in the Δ*yfgL* strain were accompanied by an increase in the expression of AcrAB, the major efflux pump proteins with which TolC interacts to pump out antibiotics (18). Western blot analysis of proteins from the inner membrane fractions showed similar AcrA levels in the wild-type and Δ*yfgL* strains (Fig. 2), thus eliminating the possibility that elevated TolC levels in the Δ*yfgL* strain were due to a general increase in the efflux pump

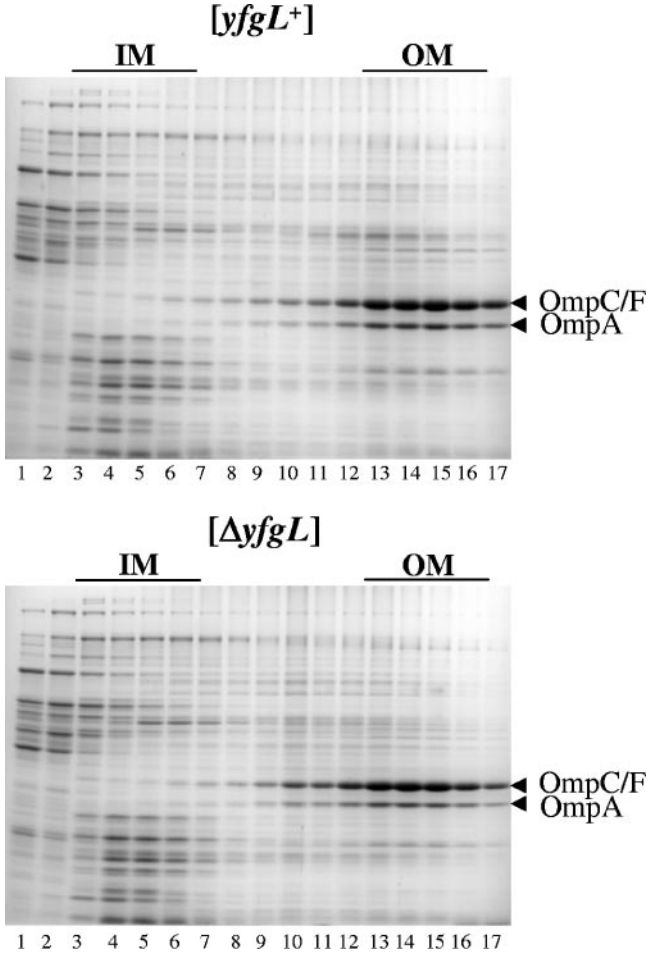

FIG. 1. SDS-PAGE analysis of proteins from outer membranes (OM) and inner membranes (IM) fractionated by sucrose density gradients. Envelopes, prepared from cultures of *yfgL*$^+$ and Δ*yfgL* strains grown overnight in LB, were resuspended in lysis buffer. After centrifugation, 20 fractions of 0.25 ml each were removed from the top of the gradient. Samples from fractions 1 to 17 were analyzed by SDS-PAGE, and protein bands were visualized after staining the gel with Coomassie blue. Because the running gel did not contain urea, OmpC and OmpF bands were not separated.

proteins. Interestingly, when TolC was expressed under the control of the pTrc99A plasmid, a roughly twofold increase in its level was observed in the Δ*yfgL* strain (data not shown). This indicated that a postsynthesis step of TolC biogenesis is affected in the absence of YfgL. It is conceivable that in the *yfgL* mutant, TolC levels go up because reduced OmpF, OmpA, and LamB levels allow TolC to now effectively compete for the limited number of the YaeT-YfiO complexes. If this is the case, we should also see a rise in TolC levels in genetic backgrounds lacking major OMPs. To test this hypothesis, TolC levels were examined in eight different genetic backgrounds either expressing all majors OMPs or lacking one or more of them. In genetic backgrounds lacking two or more major OMPs (OmpA, OmpC, and OmpF), TolC levels rose more than 150% compared to strains lacking no or one major OMP (Fig. 3). Thus, an increase in TolC levels in the *yfgL* mutant may be the result of reduced competition for YaeT/YfiO-mediated mem-

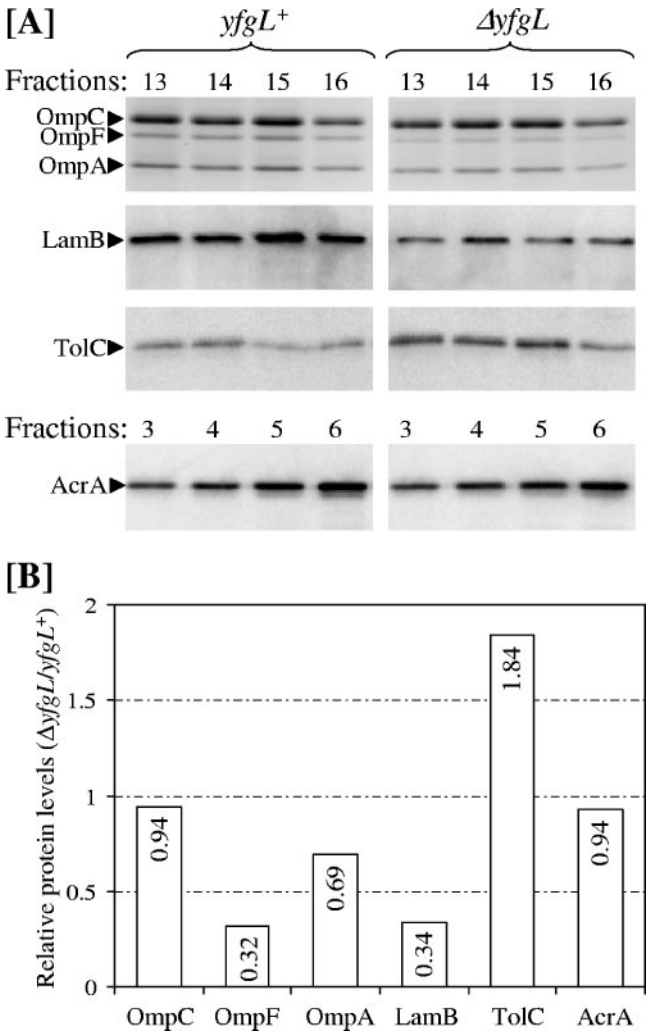

FIG. 2. Analysis of proteins from the outer and inner membrane fractions of *yfgL*$^+$ and Δ*yfgL* strains. Membrane fractions were obtained from sucrose density gradients, as described in the legend to Fig. 1. (A) Protein samples from the four peak outer membrane and inner membrane fractions, 13 to 16 and 3 to 6, respectively, in Fig. 1, were analyzed by SDS (urea)-PAGE. OmpC, OmpF, and OmpA were detected in the outer membrane fractions after staining the gel with Coomassie blue. LamB and TolC from the outer membrane fractions and AcrA from the inner membrane fractions were detected by Western blot analysis using antibodies specific to these proteins. (B) Protein levels from all four fractions were quantified, averaged, and graphed. Multiple fractions were analyzed to eliminate any fraction-specific bias in protein quantification.

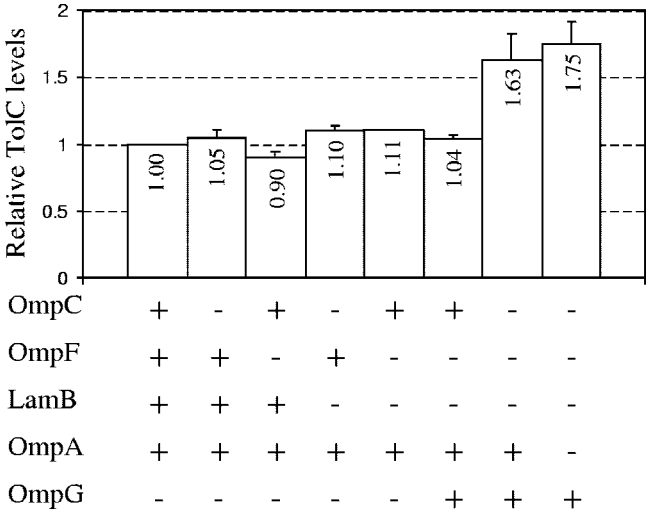

FIG. 3. TolC levels in various strains either expressing (+) or lacking (−) various OMPs. Proteins from whole-cell extracts of cultures grown overnight at 37°C in LB were analyzed using Western blots. TolC levels were quantified relative to maltose binding protein (MBP) and averaged from two independent Western blots. The TolC/MBP ratio from MC4100 (lane 1) was taken as 1, and values from other strains were relative to that of MC4100. Antibodies used in the Western blots were raised against the TolC-MBP chimeric protein; hence, they recognize both proteins.

brane insertion. The differential effect of Δ*yfgL* on certain OMPs suggested that unlike YaeT and YfiO, YfgL may have a restricted role in OMP assembly and that YfgL's interaction with YaeT-YfiO is not obligatory for their broad role in OMP biogenesis.

The absence of YfgL elevates LPS levels. Since the assembly of several OMPs, such as OmpF and LamB, is strongly dependent on LPS (21), it is conceivable that the reduced OMP levels observed in the absence of YfgL might be due to a defect in LPS biogenesis. Initially we examined LPS from whole cells and found greater levels of LPS in Δ*yfgL* cells than in the parental *yfgL*$^+$ cells (data not shown). To further validate these results and to ensure that LPS in Δ*yfgL* cells was properly localized to the outer membrane, we analyzed LPS from membrane fractions obtained through sucrose density gradients. The results presented in Fig. 4 confirmed the whole-cell data and showed there is more LPS in the outer membrane fractions of the Δ*yfgL* strain than in the parental *yfgL*$^+$ strain. Furthermore, no LPS could be detected from the inner membrane fractions of both strains. Quantification of the gel data from Fig. 2 and 4 revealed that in the *yfgL* mutant, LPS and TolC levels rose 1.65- and 1.84-fold, respectively; OmpA, OmpF, and LamB levels collectively dropped 0.45-fold; and OmpC levels showed no significant change. Thus, it appears that despite a decrease in some OMP levels, elevated LPS levels leave the buoyant density of the outer membrane unperturbed in the Δ*yfgL* strain. These data showed that an OMP biogenesis defect in the Δ*yfgL* strain is not due to

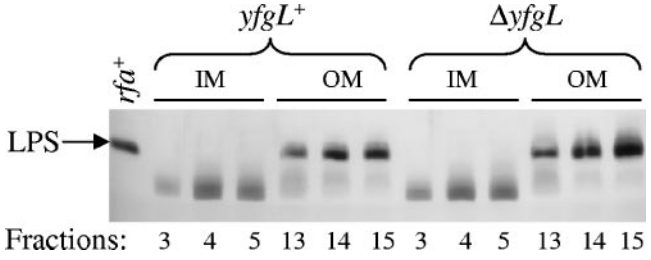

FIG. 4. Analysis of LPS from outer membranes (OM) and inner membranes (IM) of *yfgL*$^+$ and Δ*yfgL* strains. Three sucrose density gradient fractions, corresponding to the peak regions of the outer (fractions 13 to 15) and inner (fractions 3 to 5) membranes in Fig. 1 from each strain, were analyzed. The first lane contains LPS isolated from wild-type (*rfa*$^+$) cells. LPS was visualized by silver staining.

TABLE 2. Bacterial growth, as measured by colony-forming ability, of strains with various genetic backgrounds[a]

Relevant characteristics	Colony growth[a] at:	
	30°C	37°C
yfgL$^+$ *degP*$^+$	++	+++
Δ*yfgL*::Km^r *degP*$^+$	++	+++
yfgL$^+$ *degP*::Tn*10*	++	++
Δ*yfgL*::Km^r *degP*::Tn*10*	+	−
Δ*yfgL*-scar *degP*$^+$	++	+++
Δ*yfgL*-scar *degP*::Tn*10*	+	−
Δ*yfgL*::Km^r *degP*::Tn*10*(pBAD24-*yfgL*$^+$)	++	++
Δ*yfgL*::Km^r *degP*::Tn*10*DegP$_{S210A}$ (pCS10)	++	++
Δ*yfgL*::Km^r *degP*::Tn*10*(pBR322-*skp*$^+$)	+	−
Δ*yfgL*::Km^r *degP*::Tn*10* (pTrc99A-*surA*$^+$)	+	−
Δ*yfgL*-scar *degP*::Km^r	+	−
Δ*yfgL*-scar *degP*::Km^r *ompC′*::*lacZ*$^+$ *ompA610*	++	++
Δ*yfgL*-scar *degP*::Km^r *ompF′*::*lacZ*$^+$ *ompA610*	++	++

[a] Relative colony size was recorded after 18 h of incubation on LB agar plates.

mislocalization or reduced biogenesis of LPS. Our observation of an elevated LPS level in *yfgL* mutants is consistent with reports showing that the synthesis of several LPS genes is under the control of σ^E (13, 33), whose levels go up in the *yfgL* mutant (32).

Conditional lethality of *yfgL* and *degP* null mutations. *yfgL* null alleles were reported to be conditionally lethal in the presence of *imp4213* (36) and produced synthetic phenotypes when the periplasmic foldase SurA or FkpA was absent (32). The synthetic phenotype of *yfgL* and either *surA* or *fkpA* null mutation may reflect the loss of overlapping gene activities required for proper OMP biogenesis. If YfgL assists in OMP folding or membrane insertion, then its absence may lead to partially folded OMPs transiently accumulating in the periplasm. To facilitate their detection, we attempted to introduce a *degP* null allele (Δ*degP*::Tn*10*-Tc^r) into a Δ*yfgL*::Km^r background by P1 transduction. No tetracycline-resistant (Tc^r) transductants could be obtained at 37°C, but the expected number of Tc^r transductants grew at 30°C. The Tc^r transductants purified at 30°C but failed to grow at 37°C (Table 2), demonstrating that the presence of both *yfgL* and *degP* null alleles produces a conditional lethal phenotype. Note that DegP$^-$ cells do not grow on rich medium when incubated at temperatures above 40°C, form smaller colonies than DegP$^+$ cells at 37°C, and grow just like the wild-type strain at 30°C (Table 2). Unlike DegP$^-$ or YfgL$^-$ cells, cells carrying both *yfgL* and *degP* null alleles produced significantly smaller colonies when grown at 30°C (Table 2). (Onufryk et al. reported normal growth of the Δ*yfgL* Δ*degP* mutant at 30°C [32]. At this point, we cannot explain the discrepancy.) It should be noted that the effect of Δ*yfgL*::Km^r was fully complemented by a plasmid expressing *yfgL* only (Table 2), thus, the Δ*yfgL*::Km^r allele did not produce a polar effect on the downstream genes and the downstream genes did not contribute to the observed conditional lethal phenotype of Δ*yfgL* Δ*degP*. Lastly, the Δ*yfgL*-scar allele, in which the Km^r cassette was flipped out, and the Δ*yfgL*::Km^r allele produced identical results, thus, once again showing that the conditional lethal phenotype is solely due to the loss of YfgL and DegP functions.

Detection of soluble unfolded OmpA in the absence of YfgL and DegP. The viability of cells carrying both *degP* and *yfgL* null alleles at 30°C provided a means for detecting assembly-defective OMPs. To increase the likelihood of detecting unassembled OMPs, periplasmic fractions from exponentially grown cultures of wild-type, Δ*degP*, Δ*yfgL*, and Δ*yfgL* Δ*degP* strains were isolated and analyzed by Western blotting using polyclonal antibodies that primarily recognize OmpA and porins (Fig. 5A) or TolC and maltose binding protein (MBP; Fig. 5B). The folding status of OMPs was assessed by examining their proteinase K sensitivity, since assembled and fully folded OMPs are either proteinase K resistant or produce specific cleaved products. Protein samples were treated with proteinase K and then boiled before Western blot analysis.

No soluble OmpA, OmpC, and TolC was detected in the periplasmic fraction of the wild-type strain (Fig. 5). Similarly, no soluble OmpA and OmpC was found in the Δ*degP* strain (Fig. 5A); however, soluble TolC was detectable (Fig. 5B). Interestingly, unlike the wild-type and Δ*degP* strains, both OmpA and OmpC were readily detectable in the Δ*yfgL* strain (Fig. 5A), although there were no signs of soluble TolC (Fig. 5B). When both YfgL and DegP were absent, the levels of periplasmic OmpC and OmpA rose substantially (Fig. 5A), but the amount of TolC remained similar to that seen in the Δ*degP* strain (Fig. 5B). Thus, the absence of YfgL per se produced no effect on TolC. Curiously, extremely low levels of OmpF were detectable in all strains, although this was not always reproducible (see Fig. 6A and B, lanes 1 and 3).

Proteinase K treatment of the periplasmic fraction revealed that OmpC, OmpF, and TolC were folded (Fig. 5). OmpA obtained from the *yfgL* mutant was protected from proteinase K, reflecting its folded status. However, a substantial amount of OmpA obtained from the Δ*yfgL* Δ*degP* strain was proteinase K sensitive (Fig. 5A), indicating the presence of unfolded

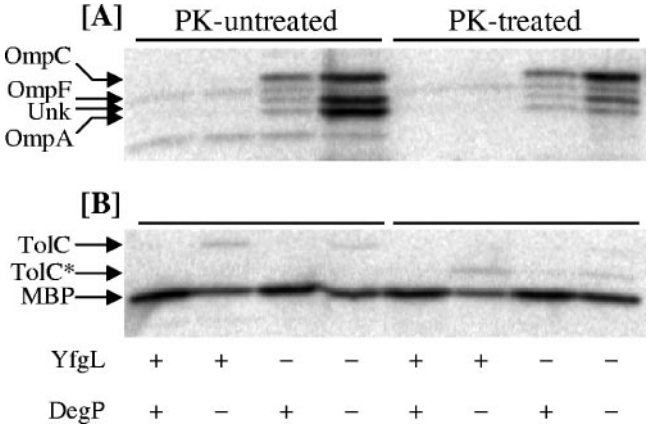

FIG. 5. Western blot analysis of OMPs from periplasmic fractions obtained from various bacterial strains, with relevant protein compositions shown at the bottom of the figure. Bacterial cultures were grown to mid-log phase in LB at 30°C. To avoid casual contamination of OMPs in the periplasmic fraction, samples obtained after periplasmic extraction were centrifuged for 1 h at 100,000 × *g*. Prior to SDS-PAGE, protein samples were treated with proteinase K (PK) or left untreated. After inhibiting the protease activity, protein samples were mixed with SDS sample buffer, boiled for 5 min, and analyzed by SDS-PAGE. Membrane blots were incubated with antibodies that either recognize OmpC, OmpF, OmpA, and an unknown protein band labeled Unk (A) or TolC and MBP (B). TolC* is a characteristic proteinase K-cleaved band generated from assembled TolC.

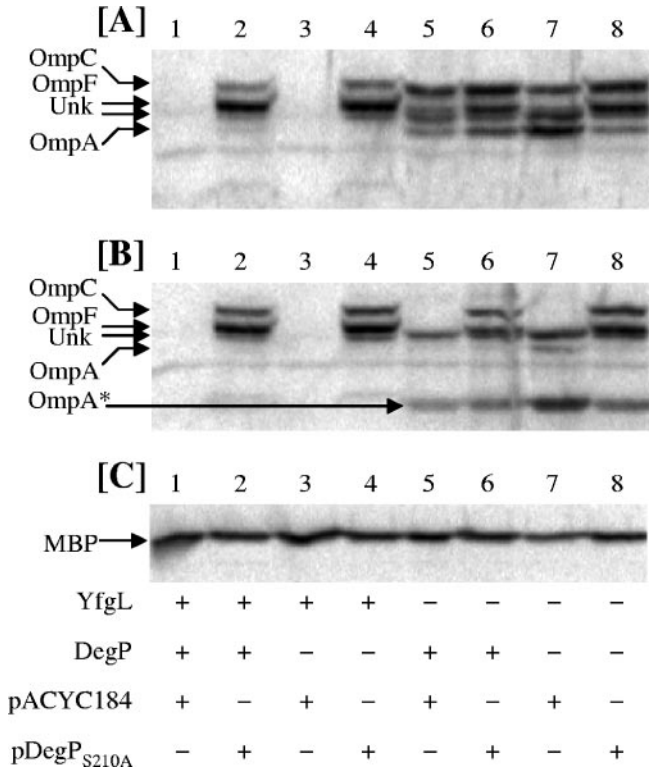

FIG. 6. Effects of DegP$_{S210A}$ overexpression on various OMPs. The relevant characteristics of various strains used are shown at the bottom of the figure. Periplasmic fractions from bacterial cultures, grown to mid-log phase at 30°C in LB, carrying just the vector plasmid (odd-numbered lanes) or a plasmid expressing DegP$_{S210A}$ (even-numbered lanes) were isolated and analyzed by Western blots to detect OmpC, OmpF, OmpA (A and B), and MBP (C). Prior to SDS-PAGE, protein samples were heated (A and C) or were left unheated (B). Expression of DegP$_{S210A}$ was induced by isopropyl-β-D-thiogalactopyranoside (0.4 mM final concentration). OmpA* and Unk refer to folded OmpA and an unknown protein band, respectively.

OmpA. Thus, the proteinase K-sensitive soluble OmpA species represented the unfolded form that accumulated in the absence of YfgL and DegP but was rapidly degraded when DegP was present. Heat sensitivity experiments shown in Fig. 6 further corroborated these findings. It was surprising to find folded OMPs in the periplasmic fraction of Δ*yfgL* and Δ*yfgL* Δ*degP* strains (Fig. 5 and 6), especially OmpC, since it is only weakly affected by the Δ*yfgL* mutation (Fig. 2). Since casual contamination of cells and membrane fragments was diminished by isolating the periplasmic fraction by using a mild extraction procedure and centrifuging the extracts for 1 h at 100,000 × *g* to pellet insoluble material, it appears that the presence of folded OMPs in Δ*yfgL* and Δ*yfgL* Δ*degP* strains indicates a structural aberration of the outer membrane, resulting in the release of small outer membrane fragments containing folded OMPs during the isolation of periplasm. Interestingly, periplasmic fractions obtained from Δ*yfgL* and Δ*yfgL* Δ*degP* strains also showed the presence of LPS (data not shown), further indicating the release of outer membrane fragments rather than just the proteins during the extraction process.

A proteinase K-sensitive protein band, labeled Unk (for "unknown") in Fig. 5 and 6 and migrating just above OmpA, was also present in elevated quantities in the Δ*yfgL* Δ*degP* strain and in detectable amount in the Δ*yfgL* strain, but it was absent in the wild-type and Δ*degP* strains. Although the identity of the band could not be determined, it was neither OmpT nor derived from OmpC or OmpF, since strains lacking one or more of these OMPs still produced the unknown band (data not shown).

The presence of an unfolded and soluble species of OmpA in the periplasm of the Δ*yfgL* Δ*degP* strain reflected a defect in OmpA biogenesis. Although we could not experimentally verify it at 37°C, we suspect that higher growth temperatures would exacerbate the OMP assembly defect, leading to the conditional lethal phenotype of the Δ*yfgL* Δ*degP* strain.

Overexpression of DegP$_{S210A}$ reverses Δ*yfgL* Δ*degP* conditional lethality. We asked whether the conditional lethal phenotype of Δ*yfgL* Δ*degP* could be reversed by overexpressing the periplasmic folding factors Skp and SurA and a variant of DegP, DegP$_{S210A}$, which lacks protease activity but maintains its reported chaperone activity (39). Overexpression of Skp and SurA, as confirmed by biochemical and complementation analyses, failed to suppress the Δ*yfgL* Δ*degP* conditional phenotype (Table 2); however, DegP$_{S210A}$ overexpression restored growth of the Δ*yfgL* Δ*degP* strain at both 30°C and 37°C (Table 2).

To gain insight on the mechanism of DegP$_{S210A}$-mediated suppression of the Δ*yfgL* Δ*degP* conditional lethality, we tested whether overexpression of DegP$_{S210A}$ reduces the level of soluble and unassembled OmpA that accumulates in the Δ*yfgL* Δ*degP* strain at 30°C (Fig. 5). Western blots of OMPs from periplasmic fractions produced several interesting observations (Fig. 6). When only the vector plasmid was present (Fig. 6A, odd-numbered lanes), protein profiles in all strains tested were very similar to those seen in Fig. 5A (proteinase K untreated). That is, soluble OMPs were virtually undetectable in wild-type and Δ*degP* strains (Fig. 6A and B, lanes 1 and 3) but were readily detectable in Δ*yfgL* and Δ*yfgL* Δ*degP* strains (Fig. 6A and B, lanes 5 and 7). Again, soluble OmpC from these strains was heat resistant, indicating that it was folded and formed high-molecular-weight complexes with LPS; therefore, it did not migrate at the denatured monomeric position unless samples were boiled prior to SDS-PAGE analysis (Fig. 6A and B, lanes 5 and 7). OmpA was either folded, which is characterized by the faster migrating "heat modifiable" form when samples were unheated (Δ*yfgL* background; Fig. 6B, lane 5), or was comprised of both folded (heat modifiable) and unfolded (heat sensitive; migrating at denatured position without heating) populations (Δ*yfgL* Δ*degP* background; Fig. 6B, lane 7).

When DegP$_{S210A}$ expression was induced (Fig. 6, even-numbered lanes), all strains contained substantial amounts of unassembled, heat-sensitive (denatured without heating) OmpC and OmpF, but not OmpA (Fig. 6B, even-numbered lanes). Soluble and heat-labile OmpC and OmpF, unlike properly assembled OMPs, were completely digested by proteinase K (data not shown), confirming their unassembled state. In contrast to the elevated levels of periplasmic OmpC and OmpF, we noted a reduction in the periplasmic OmpA level when DegP$_{S210A}$ was overexpressed (Fig. 6A and B, lanes 7 and 8). Curiously, this reduction was apparent only in the Δ*yfgL* Δ*degP* background and not in the Δ*yfgL* background (Fig. 6A and B,

lanes 5 and 6). Note that the levels of MBP, a native periplasmic protein, were very similar in all strains (Fig. 6C), indicating that variations in the levels of periplasmic OMPs were not due to different amounts of periplasm being extracted or analyzed. Based on these results, we propose that $DegP_{S210A}$-mediated suppression in part entails sequestering OMPs, particularly OmpC and OmpF, in the periplasm, not improving their assembly. The retention of OMPs in the periplasm may reduce the pool of OMPs from congesting the assembly pathways or competing for the YaeT/YfiO complex, thereby alleviating the loss of the OMP assembly factor YfgL. Interestingly, overexpression of $DegP_{S210A}$ in the Δ*yfgL* Δ*degP* background had just the opposite effect on TolC than that seen for OmpC and OmpF: TolC levels were reduced in the periplasmic fraction with a concomitant increase in the membrane fraction (data not shown). This suggests that TolC may be competing with OmpC and OmpF for the assembly apparatus.

The absence of major OMPs can reverse Δ*yfgL* Δ*degP* conditional lethality. Experiments described above suggested that the conditional lethal phenotype of the Δ*yfgL* Δ*degP* strain can be reversed by sequestering unassembled OMPs in the periplasm, thus preventing them from entering the normal assembly pathway. If so, the growth defect of Δ*yfgL* Δ*degP* may also be reversed in strains lacking one or more major OMPs, i.e., OmpC, OmpF, and OmpA. The temperature sensitivity of the Δ*yfgL* Δ*degP* strain lacking various OMPs was evaluated by testing its ability to form single colonies on LBA medium at 30°C and 37°C (Table 2). At 30°C the absence of a single major OMP significantly improved the growth of the Δ*yfgL* Δ*degP* strain; however, at 37°C the strains grew in the first two or three streaks but failed to form single colonies (data not shown). Interestingly, the simultaneous absence of two OMPs, OmpF and OmpA or OmpC and OmpA, substantially improved growth, so much so that the Δ*yfgL* Δ*degP* strain lacking two OMPs showed growth patterns identical to those of the Δ*degP* strain (Table 2). These results support the hypothesis that a compromised assembly process or machinery in the Δ*yfgL* Δ*degP* strain results in the conditional lethal phenotype, which can be relieved by preventing major OMPs from entering the assembly pathway.

DISCUSSION

An outer membrane protein complex comprised of a nonlipoprotein, YaeT, and three lipoproteins, YfiO, NlpB, and YfgL, was recently identified through coimmunoprecipitation with YfgL (47). Of the three lipoproteins, only YfiO is essential (32), and like YaeT, it is required for the biogenesis of all model OMPs examined so far, including OmpA, OmpC, OmpF, LamB, and TolC (25). Whereas the absence of NlpB imposes no ill effect on OMP biogenesis (32, 47), results presented here and those previously published (32, 36) showed that the absence of YfgL significantly interferes with the biogenesis of OmpF and LamB, moderately affects OmpA, and has little or no effect on OmpC. In contrast to these OMPs, the effect of the *yfgL* mutation on TolC biogenesis was remarkably different, as significantly higher TolC levels were present in the *yfgL* mutant than in the $yfgL^+$ strain. Such differential effects of the *yfgL* mutation on OMP biogenesis suggest that in the absence of YfgL, the function of the YfiO-YaeT core complex is not affected in a general way, because if this were not the case, biogenesis of all nonlipoprotein OMPs, including TolC, would have been affected. This also suggests that the assembly requirements and pathways of various β- and α/β-barrel OMPs are diversified through the step involving YfgL and then converge at the step involving YfiO-YaeT, which likely catalyzes the final step of OMP assembly and membrane insertion.

Our data show that the increase in the TolC levels in *yfgL* mutants is due to changes in the postsynthesis step, which may involve YaeT/YfiO-mediated insertion in the outer membrane. TolC levels are also elevated when synthesis of several major OMPs is halted by mutations in their structural genes (Fig. 3). These observations suggest that TolC and major OMPs may be in competition for the YaeT/YfiO complex. Compensatory adjustments among OMPs are routinely observed; for example, mutations affecting the structural gene for a major OMP often result in an increase in the level of the remaining OMPs such that the overall OMP levels remain fairly constant (24). This suggests that even the major OMPs might compete with each other for YaeT/YfiO-mediated insertion in the outer membrane.

At the present time we do not understand why *yfgL* mutation affects various OMPs differently, but it may be in part due to an OMP's dependence on specific folding requirements, regulatory mechanisms, or expression levels. For example, TolC, which is not adversely affected by the Δ*yfgL* mutation, has a unique α/β-barrel structure (20) and folds independently of factors that influence the folding of OmpA, OmpC, OmpF, and LamB (47). Similarly, expression levels can influence the extent of the Δ*yfgL* effect on a particular OMP. The dramatic effect of Δ*yfgL* on OmpF levels (a roughly 70% drop; Fig. 2) under conditions when OmpF is weakly expressed relative to OmpC is significantly reduced (a mere 20% drop) when OmpF is strongly expressed in the absence of OmpC (data not shown). It is worth mentioning that different laboratories have reported somewhat different results on the effect of the *yfgL* null mutation on OMP levels, particularly those of OmpC and OmpA. For example, Rolhion et al. (35) and our work here (Fig. 2) showed little or no reduction in OmpC levels, whereas data reported by Onufryk et al. indicated an almost 50% drop of OmpC in the *yfgL* mutant background (32). For OmpA, our data and those from Onufryk et al. (32) and Ruiz et al. (36) showed a modest reduction, but Rolhion et al. reported no reduction (35). We suspect that when the effect is small or modest, as is the case for OmpC and OmpA, factors such as expression levels, growth conditions, media composition, genetic background, and membrane extraction methods can influence the OMP levels such that it is inconclusive as to whether an OMP is affected or not by the *yfgL* mutation.

The data from this study showed that the OMP biogenesis defect in the Δ*yfgL* strain is not due to a decrease in LPS biogenesis. In fact, the level of LPS in the outer membrane of the Δ*yfgL* strain is significantly greater than that in the $yfgL^+$ strain. Interestingly, expression of the LPS synthesis genes, among others, is under the control of σ^E (13, 33), a transcription factor that specifically responds to envelope stress (2, 14). Since the σ^E level is shown to increase in the *yfgL* mutant (32), this could in turn elevate LPS synthesis and levels, as we have shown here. Unlike the Δ*yfgL* strain, there were no changes in the LPS level when YaeT was depleted from the cell, despite

the fact that OMP levels were severely reduced (16, 47) and σ^E levels were presumably elevated. One reason for this could be that unlike the deletion of *yfgL*, depletion of YaeT may affect the assembly of the Imp protein, which is thought to be involved in translocating LPS to the outer membrane (6).

We found that the absence of both YfgL and DegP causes conditional lethality at 37°C, and the double knockout mutant grows poorly at 30°C but nevertheless forms homogeneous colonies. We have shown the accumulation of unfolded OmpA in a Δ*yfgL* Δ*degP* mutant grown at 30°C. The lethality of the Δ*yfgL* Δ*degP* strain at 37°C is likely due to the toxic buildup of misfolded OMPs, which presumably also occurs in Δ*degP* cells grown on rich media at temperatures above 40°C (23, 40). A buildup of misfolded OMPs in the absence of DegP may block normal functioning of the YaeT complex. The conditional lethal phenotype of Δ*yfgL* Δ*degP* could not be reversed when Skp or SurA was overexpressed, indicating little or no functional overlap between YfgL and Skp or SurA. Interestingly, expression of a protease-deficient DegP variant, $DegP_{S210A}$, could rescue Δ*yfgL* Δ*degP*-mediated lethality. Our results showed that this was not due to the restoration of normal OMP biogenesis but presumably due to $DegP_{S210A}$-mediated sequestering of OMPs in the periplasm. Because OmpF and OmpC assembled normally in the $yfgL^+$ strain and yet were sequestered in the periplasm, we think that $DegP_{S210A}$ does not specifically target misfolded OMPs. However, we do think that $DegP_{S210A}$ has some selectivity, because unlike OmpC and OmpF, OmpA and TolC were not sequestered in the periplasm when $DegP_{S210A}$ was expressed. Instead, soluble OmpA and TolC levels were reduced when $DegP_{S210A}$ was overexpressed. The retention and sequestration of some OMPs in the periplasm would not only alleviate the need for their degradation but also lessen the load on a YaeT-YfiO complex devoid of YfgL for OMP assembly. Consistent with this hypothesis, we found that the absence of at least two major OMPs, OmpA and OmpC or OmpA and OmpF, also alleviated the conditional lethal phenotype of Δ*yfgL* Δ*degP*. We have previously reported a similar $DegP_{S210A}$-mediated reversal mechanism of lethal phenotypes when assembly-defective OmpF (28) and OmpC (10) were expressed in the absence of DegP. In particular, we showed a direct interaction between a mutant OmpC protein and $DegP_{S210A}$ in the periplasm (10).

Regarding the effect of *yfgL* mutation on OMP levels, it is important to mention that the paper by Rhodius et al. also showed that in contrast to increased LPS gene expression, elevated levels of σ^E had an inverse effect on the expression of the major OMP genes, including *ompA*, *ompC*, and *ompF* (33). Since σ^E activity is also upregulated in the absence of YfgL (32), it is likely that the effect of the *yfgL* mutation on OMP levels may in part be due to an effect on their expression rather than just their assembly. The data presented in this paper showed a distinct OmpA biogenesis defect in the *yfgL* mutant and abnormal release of mostly folded OMPs in the soluble periplasmic fraction obtained by the mild extraction method (4). Thus, a decrease in OMP levels cannot be entirely due to reduced OMP synthesis; rather, we think that in *yfgL* mutants, both synthesis and assembly of OMPs are affected. Interestingly, it has been reported that compared to the $yfgL^+$ strain, the *yfgL* mutant shows a decreased release of outer membrane vesicles (35). These effects of the *yfgL* mutation, together with our finding of the release of outer membrane fragments during periplasmic extraction, point to an aberrant envelope structure.

ACKNOWLEDGMENTS

We are grateful to Phu Vuong for critically reading the manuscript and performing the experiment described in Fig. 3. We thank the reviewers for several insightful comments.

This work was supported by a grant from the National Institute of General Medical Sciences (R01-GM048167). Emily S. Charlson was partly supported by the School of Life Sciences Undergraduate Research Fellowship.

REFERENCES

1. **Abe, S., T. Okutsu, H. Nakajima, N. Kakuda, I. Ohtsu, and R. Aono.** 2003. n-Hexane sensitivity of *Escherichia coli* due to low expression of *imp*/*ostA* encoding an 87 kDa minor protein associated with the outer membrane. Microbiology **149:**1265–1273.
2. **Alba, B. M., and C. A. Gross.** 2004. Regulation of the *Escherichia coli* σ^E-dependent envelope stress response. Mol. Microbiol. **52:**613–616.
3. **Ames, G. F.-L., E. N. Spudich, and H. Nikaido.** 1974. Protein composition of the outer membrane of *Salmonella typhimurium*: effect of lipopolysaccharide mutations. J. Bacteriol. **117:**406–416.
4. **Arié, J.-P., N. Sassoon, and J.-M. Betton.** 2001. Chaperone function of FkpA, a heat shock prolyl isomerase, in the periplasm of *Escherichia coli*. Mol. Microbiol. **39:**199–210.
5. **Bolla, J.-M., C. Lazdunski, and J.-M. Pages.** 1988. The assembly of the major outer membrane protein OmpF of *Escherichia coli* depends on lipid synthesis. EMBO J. **11:**3595–3599.
6. **Bos, M. P., B. Tefsen, J. Geurtsen, and J. Tommassen.** 2004. Identification of an outer membrane protein required for the transport of lipopolysaccharide to the bacterial cell surface. Proc. Natl. Acad. Sci. USA **101:**9417–9422.
7. **Bouvier, J., A. P. Pugsley, and P. Stragier.** 1991. A gene for a new lipoprotein in the *dapA-purC* interval of the *Escherichia coli* chromosome. J. Bacteriol. **173:**5523–5531.
8. **Braun, M., and T. J. Silhavy.** 2002. Imp/OstA is required for cell envelope biogenesis in *Escherichia coli*. Mol. Microbiol. **45:**1289–1302.
9. **Casadaban, M. J.** 1976. Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J. Mol. Biol. **104:**541–555.
10. **Castillo Keller, M., and R. Misra.** 2003. Protease-deficient DegP suppresses lethal effects of a mutant OmpC protein by its capture. J. Bacteriol. **185:**148–154.
11. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134:**1141–1156.
12. **Danese, P. N., and T. Silhavy.** 1998. Targeting and assembly of periplasmic and outer-membrane proteins in *Escherichia coli*. Annu. Rev. Genet. **32:**59–94.
13. **Dartigalongue, C., D. Missiakas, and S. Raina.** 2001. Characterization of the *Escherichia coli* σ^E regulon. J. Biol. Chem. **276:**20866–20875.
14. **Daritigalongue, C., H. Loferer, and S. Raina.** 2001. EcfE, a new essential inner membrane protease: its role in the regulation of heat shock response in *Escherichia coli*. EMBO J. **20:**5908–5918.
15. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA **97:**6640–6645.
16. **Doerrler, W. T., and C. R. H. Raetz.** 2005. Loss of outer membrane proteins without inhibition of lipid export in an *Escherichia coli* YaeT mutant. J. Biol. Chem. **280:**27679–27687.
17. **Eggert, U. S., N. Ruiz, B. V. Falcone, A. A. Branstrom, R. C. Goldman, T. J. Silhavy, and D. Kahne.** 2001. Genetic basis for activity differences between vancomycin and glycolipid derivatives of vancomycin. Science **294:**361–364.
18. **Fralick, J. A.** 1996. Evidence that TolC is required for functioning of the Mar/AcrAB efflux pump of *Escherichia coli*. J. Bacteriol. **178:**5803–5805.
19. **Guzman, L. M., D. Belin, M. J. Carson, and J. Beckwith.** 1995. Tight regulation, modulation, and high-level expression by vectors containing the P_{BAD} promoter. J. Bacteriol. **177:**4121–4130.
20. **Koronakis, V., A. Sharff, E. Koronakis, B. Luisi, and C. Hughes.** 2000. Crystal structure of the bacterial membrane protein TolC central to multidrug efflux and protein export. Nature **405:**914–919.
21. **Laird, M. W., A. Kloser, and R. Misra.** 1994. Assembly of LamB and OmpF in deep rough lipopolysaccharide mutants of *Escherichia coli* K-12. J. Bacteriol. **176:**2259–2264.
22. **Lazar, S. W., and R. Kolter.** 1996. SurA assists the folding of *Escherichia coli* outer membrane proteins. J. Bacteriol. **178:**1770–1773.
23. **Lipinska, B., O. Fayet, L. Baird, and C. Georgopoulos.** 1989. Identification, characterization, and mapping of the *Escherichia coli htrA* gene, whose product is essential for bacterial growth only at elevated temperatures. J. Bacteriol. **171:**1574–1584.

24. **Lugtenberg, B., R. Peters, H. Bernheimer, and W. Berendsen.** 1976. Influence of cultural conditions and mutations on the composition of the outer membrane proteins of *Escherichia coli*. Mol. Gen. Genet. **147:**251–262.
25. **Malinverni, J. C., J. Werner, S. Kim, J. G. Sklar, D. Kahne, R. Misra, and T. J. Silhavy.** 2006. YfiO stabilizes the YaeT complex and is essential for outer membrane protein assembly in *E. coli*. Mol. Microbiol. **61:**151–164.
26. **Misra, R.** 1993. OmpF assembly mutants of *Escherichia coli* K-12: isolation, characterization, and suppressor analysis. J. Bacteriol. **175:**5049–5056.
27. **Misra, R., and S. A. Benson.** 1989. A novel mutation, cog, which results in the production of a new porin protein (OmpG) of *Escherichia coli* K-12. J. Bacteriol. **171:**4105–4111.
28. **Misra, R., M. Castillo Keller, and M. Deng.** 2000. Overexpression of protease-deficient $DegP_{S210A}$ rescues the lethal phenotype of *Escherichia coli* OmpF assembly mutants in a *degP* background. J. Bacteriol. **182:**4882–4888.
29. **Misra, R., A. Peterson, T. Ferenci, and T. J. Silhavy.** 1991. A genetic approach for analyzing the pathway of LamB assembly into the outer membrane of *Escherichia coli*. J. Biol. Chem. **266:**13592–13597.
30. **Miyadai, H., K. Tanaka-Masuda, S. Matsuyama, and H. Tokuda.** 2004. Effects of lipoprotein overproduction on the induction of DegP (HtrA) involved in quality control in the *Escherichia coli* periplasm. J. Biol. Chem. **279:**39807–39813.
31. **Nikaido, H., and M. Vaara.** 1985. Molecular basis of bacterial outer membrane permeability. Microbiol. Rev. **49:**1–32.
32. **Onufryk, C., C. Marie-Laure, F. C. Fang, and C. A. Gross.** 2005. Characterization of the six lipoproteins in the σ^E regulon. J. Bacteriol. **187:**4552–4561.
33. **Rhodius, V. A., W. C. Suh, G. Nonaka, J. West, and C. A. Gross.** 2006. Conserved and variable functions of the σ^E stress response in related genomes. PLoS Biol. **4:**43–59.
34. **Ried, G., I. Hindennach, and U. Henning.** 1990. Role of lipopolysaccharide in assembly of *Escherichia coli* outer membrane proteins OmpA, OmpC, and OmpF. J. Bacteriol. **172:**6048–6053.
35. **Rolhion, N., N. Barnich, L. Claret, and A. Darfeuille-Michaud.** 2005. Strong decrease in invasive ability and outer membrane vesicle release in Crohn's disease-associated adherent-invasive *Escherichia coli* strain LF82 with the *yfgL* gene deleted. J. Bacteriol. **187:**2286–2296.
36. **Ruiz, N., B. Falcone, D. Kahne, and T. J. Silhavy.** 2005. Chemical conditionality: a genetic strategy to probe organelle assembly. Cell **21:**307–317.
37. **Sampson, B. A., R. Misra, and S. A. Benson.** 1989. Identification and characterization of a new gene of *Escherichia coli* K-12 involved in outer membrane permeability. Genetics **122:**491–501.
38. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
39. **Spiess, C., A. Beil, and M. Ehrmann.** 1999. A temperature-dependent switch from chaperone to protease in a widely conserved heat shock protein. Cell **97:**339–347.
40. **Strauch, K. L., K. Johnson, and J. Beckwith.** 1989. Characterization of *degP*, a gene required for proteolysis in the cell envelope and essential for growth of *Escherichia coli* at high temperature. J. Bacteriol. **171:**2689–2696.
41. **Thanassi, D. G., E. T. Saulino, and S. J. Hultgren.** 1998. The chaperone/usher pathway: a major terminal branch of the general secretory pathway. Curr. Opin. Microbiol. **1:**223–231.
42. **Tokuda, H., and S. Matsuyama.** 2004. Sorting of lipoproteins to the outer membrane in *E. coli*. Biochim. Biophys. Acta **1693:**5–13.
43. **Veenendaal, A. K., C. van der Does, and A. J. Driessen.** 2004. The protein-conducting channel SecYEG. Biochim. Biophys. Acta **1694:**81–95.
44. **Voulhoux, R., M. P. Bos, J. Geurtsen, M. Mols, and J. Tommassen.** 2003. Role of a highly conserved bacterial protein in outer membrane protein assembly. Science **299:**262–265.
45. **Werner, J., A. M. Augustus, and R. Misra.** 2003. Assembly of TolC, a structurally unique and multifunctional outer membrane protein of *Escherichia coli* K-12. J. Bacteriol. **185:**6540–6547.
46. **Werner, J., and R. Misra.** 2005. YaeT (Omp85) affects the assembly of lipid-dependent and lipid-independent outer membrane proteins of *Escherichia coli*. Mol. Microbiol. **57:**1450–1459.
47. **Wu, T., J. Malinverni, N. Ruiz, S. Kim, T. J. Silhavy, and D. Kahne.** 2005. Identification of a multicomponent complex required for outer membrane biogenesis in *Escherichia coli*. Cell **121:**235–245.