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asmB, a Suppressor Locus for Assembly-Defective OmpF Mutants of *Escherichia coli*, Is Allelic to *envA* (*lpxC*)

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A novel genetic scheme allowed us to isolate extragenic suppressor mutations that restored mutant OmpF assembly. One group of these mutations, termed *asmB* for assembly suppressor mutation B, permitted mutant OmpF assembly in a non-allele-specific manner. Genetic mapping analyses placed the *asmB* mutations at the 2-min region of the *Escherichia coli* K-12 chromosome. Further analyses revealed that the *asmB* mutations map within the *envA* (*lpxC*) gene, which encodes an enzyme needed for the synthesis of the lipid A moiety of lipopolysaccharide (LPS). Nucleotide sequence analysis showed that the *asmB* mutations caused a change from F-50 to S (F50S substitution) (*asmB2* and *asmB3*) or a G210S substitution (*asmB1*) in EnvA. Cells bearing the *asmB* alleles displayed increased sensitivity to various hydrophobic compounds and detergents, suggesting an alteration within the outer membrane. Direct examination of the LPS showed that its amounts were reduced by the *asmB* mutations, with *asmB1* exerting a greater effect than *asmB2* or *asmB3*. Thus, it appears that the *asmB* mutations achieve mutant OmpF assembly suppression by reducing LPS levels, which in turn may alter membrane fluidity.

Assembly of trimeric outer membrane proteins, such as OmpF, OmpC, PhoE, and LamB, proceeds through a sequential appearance of biochemically and topologically distinct assembly intermediates (3, 30). Although our understanding of how molecules leaving the inner membrane reach the outer membrane and assemble into their final trimeric form is far from clear, a general view of the assembly pathway can be outlined here. The assembly of outer membrane proteins begins with the cleavage of the signal sequence from nascent monomers at the periplasmic face of the inner membrane. These monomers are assumed to be largely devoid of higher-order structures but rapidly fold to acquire some secondary or tertiary structures either within the aqueous environment of periplasm or upon association with the inner surface (facing periplasm) of the outer membrane. These initial folding events may be catalyzed by two recently discovered periplasmic proteins, DsbA and SurA. The DsbA protein was shown to promote disulfide bond formation in vivo (4). The primary sequence of SurA shows significant homology with a cytoplasmic protein possessing a peptidyl prolyl *cis*/*trans* isomerase activity (16, 32). From this homology, it has been speculated that SurA may be a periplasmic peptidyl prolyl *cis*/*trans* isomerase.

It has been hypothesized that the exposure of hydrophobic surfaces of partially folded assembly intermediates may facilitate their interactions with outer membrane components, such as phospholipids and lipopolysaccharide (LPS). These interactions may occur prior to the insertion of assembly intermediates in the outer membrane but most certainly happen once a protein molecule has gained entry into the outer membrane. Interactions with the lipid A moiety of LPS during the early stages of insertion may not be feasible, considering that LPS is localized exclusively in the outer leaflet of the outer membrane (25). During the final stages of assembly, these intermacromolecular interactions are deemed necessary for completion of the assembly process leading to the formation of functional, stable trimers (8, 14, 31, 34).

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In order to identify cellular components involved in the assembly of outer membrane proteins, we have utilized a novel genetic approach to isolate extragenic suppressor mutations of assembly-defective OmpF proteins (20). Null mutations in one such locus, *asmA*, permitted mutant OmpF proteins to assemble into stable trimers. Interestingly, these suppressor mutations also corrected the assembly defect of wild-type OmpF and LamB observed in an LPS-mutant background (14). AsmA was shown to be a minor inner membrane protein (7) whose sequence shows no homology with other known proteins (22). Although the biochemical basis for the assembly suppression seen in *asmA* mutants is not fully understood, it appears that alterations in the LPS component of the outer membrane somehow specifically permit mutant OmpF assembly without affecting the biogenesis of wild-type outer membrane proteins.

The focus of this study was to isolate and characterize additional extragenic suppressor mutations that restore mutant OmpF assembly. In this paper, we provide a detailed account of one such suppressor locus, termed *asmB*, for assembly suppressor mutation B.

MATERIALS AND METHODS

Media and chemicals. M63 minimal medium and Luria broth (LB) were prepared as previously described (35). The antibiotics tetracycline (20 μg/ml), kanamycin (50 μg/ml), and ampicillin (50 μg/ml) were supplemented as needed. Carbon sources were utilized at final concentrations of 0.4% for glycerol and 0.2% for all other sugars. Maltodextrin was purchased from Pfanstiehl Laboratories, Inc., and was further purified by dialysis as described previously (21). [^{35}S]methionine was obtained from Du Pont-New England Nuclear. The chromogenic indicator for LacZ, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), was purchased from Gold Biotechnology, St. Louis, Mo. Presoaked antibiotic disks were purchased from Difco. All other chemicals were of analytical grade.

Bacterial strains, lambda phages, and genetic techniques. Bacterial strains used in this study are listed in Table 1. P1 transductions and Hfr and F′ conjugational crosses were performed as described by Miller (19) and Silhavy et al. (35). One-step transformations were carried out by a previously described method (11). Kohara lambda clones (13) were propagated on LE392 (6) to a titer of 10^{10} PFU as previously described (18). For lambda transductions, *lamB*$^+$ *leu*::Tn*10 asmB1* recipient strains were grown overnight in LB at 37°C, pelleted, and resuspended in the original volume of 10 mM $MgSO_4$. Resuspended cells (0.2 ml) and the desired phage lysates (0.1 ml) were incubated at a multiplicity of infection of 0.5 at 30°C for 20 min, after which 1 ml of 100 mM sodium

TABLE 1. Bacterial strains

Strain	Relevant characteristics	Source or reference
RAM491	Δ*lamB106 ompF205*(Dex$^+$) Φ(*ompC′-lac*$^+$)*10-15*	20
RAM493	Δ*lamB106 ompF35* Φ(*ompC′-lac*$^+$)*10-15*	20
RAM496	Δ*lamB106 ompF315* Φ(*ompC′-lac*$^+$)*10-15*	20
RAM498	Δ*lamB106 ompF33* Φ(*ompC′-lac*$^+$)*10-15*	20
RAM645	Δ*lamB106 ompF315* Φ(*ompC′-lac*$^+$)*10-15 asmB1*	This study
RAM646	Δ*lamB106 ompF315* Φ(*ompC′-lac*$^+$)*10-15 asmB2*	This study
RAM647	Δ*lamB106 ompF315* Φ(*ompC′-lac*$^+$)*10-15 asmB3*	This study
RAM805	RAM645 *recA*::Kan^r *leu*::Tn*10*; Dex$^+$	This study
RAM663	RAM646 *recA*::Kan^r *leu*::Tn*10*; Rif^r Dex$^+$	This study
RAM664	RAM647 *recA*::Kan^r *leu*::Tn*10*; Rif^r Dex$^+$	This study
RAM665	RAM645 *recA*::Kan^r *leu*::Tn*10*; (F′104 *leu*$^+$ from CGSC4251) Dex$^-$	This study
RAM666	RAM645 *recA*::Kan^r *leu*::Tn*10*; (F′104 *leu*$^+$ from CGSC4251) Dex$^+$	This study
RAM667	RAM663 (F′104 *leu*$^+$ from RAM665)	This study
RAM668	RAM663 (F′104 *leu*$^+$ from RAM666)	This study
RAM669	RAM664 (F′104 *leu*$^+$ from RAM665)	This study
RAM670	RAM664 (F′104 *leu*$^+$ from RAM666)	This study
LE392	*supE44 supF58 hsdR514 galK2 galT22 metB1 trpR55 lacY1*	6
JM109	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ(*lac-proAB*)	37
CGSC4251	Source of F′104	B. Bachmann

citrate–5 mM $CaCl_2$ was added to stop infection. The cells were pelleted and resuspended in 1 ml of the same diluent. Various dilutions were then plated onto either minimal medium lacking leucine or MacConkey medium and incubated overnight at 37°C. Leu$^+$ recombinants were tested for MacConkey sensitivity (Mac^s). When MacConkey-resistant (Mac^r) recombinants were sought directly, only DD8 and K111 produced the desired recombinants.

DNA cloning and sequence determination. To clone wild-type *envA* and various *asmB* alleles from the chromosome, desired DNA fragments were amplified directly from colonies by PCR. For this, two mutagenic oligonucleotides that also created unique *Nco*I and *Xba*I restriction sites at the 5′ and 3′ ends, respectively, of the *envA* coding region were used. PCR-amplified DNA fragments containing the entire *envA* gene were purified with a Wizard PCR Prep DNA purification kit purchased from Promega. Purified DNA fragments were restricted with *Nco*I and *Xba*I and ligated into pTrc99A (Pharmacia Biotech). The ligated mixture was transformed into JM109 (37) with selection for ampicillin-resistant colonies. Plasmids containing the 1.1-kb *envA* DNA obtained from various *asmB* mutants were used for complementation studies (see Results and Discussion). The presence of a mutation(s) within the 1.1-kb coding sequence of EnvA was confirmed by DNA sequencing of the entire *envA* gene. DNA sequencing was performed by the dideoxy method (33) using an Amplicycle sequencing kit purchased from Perkin-Elmer.

Pulse-chase experiments and immunoprecipitation. Pulse-chase experiments to determine the assembly of OmpF trimers were performed essentially as described previously (20, 23), with some modifications. Cells grown overnight in glycerol minimal medium at 37°C were subsequently diluted 1:50 in the same medium and grown at 42°C to mid-log phase. Extraction of [^{35}S]methionine-labeled OmpF trimers was completed as previously described (23). OmpF trimers from cell extracts were immunoprecipitated with OmpF trimer-specific antibodies (20). Immunocomplexes were precipitated with a preparation of formalin-killed *Staphylococcus aureus* cells. The immunocomplexes were washed several times and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) after being heated for 15 min at 60°C to separate heat-stable trimers from heat-labile metastable trimers. The gels were dried and fluorographed at −70°C.

Isolation and examination of cell envelopes. Envelopes from strains grown overnight were prepared by a French press lysis procedure described previously (23). Separation of the inner and outer membranes of whole-cell extracts was achieved by isopycnic sucrose density gradient centrifugation as described previously (29). Samples were electrophoresed on 11% polyacrylamide gels and stained with Coomassie blue as previously described (17). In some cases, 4 M solid urea was added to increase the resolution of protein bands.

LPS and protein assays. LPS from proteinase K-digested whole-cell extracts was examined as described previously (1). The proteolyzed samples were analyzed on 15% polyacrylamide gels, and the LPS bands were visualized by silver staining with a kit purchased from Bio-Rad Laboratories. LPS bands were quantified with a Millipore/Bio Image densitometer. ^{32}P-labeled LPS was analyzed by a previously described method (6a). Total protein concentrations were determined by using a protein assay kit from Bio-Rad Laboratories.

Sensitivity assays. Antibiotic sensitivity assays were performed by seeding LB top agar with strains grown overnight in LB. After solidification, disks were placed on the media and incubated at the appropriate temperature. Efficiency of plaquing (EOP) assays against bacteriophage K20 were performed with glucose minimal medium. Cells grown overnight in glucose minimal medium were incubated with the bacteriophage for 5 min at room temperature prior to being seeded in glucose top agar and incubated at 37°C.

RESULTS AND DISCUSSION

Isolation of extragenic suppressor mutations of *ompF315*. OmpF315 contains two separate amino acid substitutions; one (a change of R-82 to S [R82S substitution]) results in a Dex$^+$ phenotype, and the other substitution (W214E), obtained through amber reversion, causes a temperature-sensitive (*ts*) assembly defect (Dex$^-$ at 42°C) (20). Extragenic suppressors of *ompF315* were obtained by isolating spontaneous Dex$^+$ revertants of the *ts ompF315* allele at the restrictive temperature of 42°C. By this method, a suppressor, *asmA* (assembly suppressor mutation A), which mapped at 45 min on the *Escherichia coli* chromosome (20) was obtained previously. In this study, suppressor mutations that mapped at positions other than 45 min were sought. A total of 12 Dex$^+$ spontaneous revertants obtained from independent cultures were examined. The frequency of Dex$^+$ reversion was estimated to be between 10^{-8} and 10^{-9}. These isolates were tested for the possibility of being bypass suppressors by examining sensitivity to the OmpF-spe-

TABLE 2. Antibiotic sensitivities and EOP conferred by various *asmB* alleles in strains expressing OmpF315

Relevant genotype	Zone of inhibition (mm)[a]				EOP[b]
	E	N	R	A	
asmB$^+$	8	8	8	20	0.0017
asmB1	17	14	19	21	0.0043
asmB2	10	10	12	20	0.22
asmB3	10	10	11	21	0.18

[a] Zones of inhibition were measured as diameters; the disk size was 7 mm. E, N, R, and A, disks containing erythromycin (15 μg), novobiocin (30 μg), rifampin (5 μg), and ampicillin (20 μg), respectively.

[b] EOP of parental *ompF205* was taken as 1, and mutant EOP values were normalized accordingly.

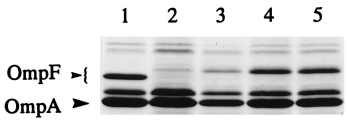

FIG. 1. SDS-PAGE analysis of envelopes prepared from strains carrying various *asmB* alleles grown in glucose minimal medium at 37°C. Lane 1, OmpF205 (non-assembly-defective OmpF); lane 2, OmpF315 (*ts* OmpF); lane 3, OmpF315 *asmB1*; lane 4, OmpF315 *asmB2*; lane 5, OmpF315 *asmB3*. The positions of OmpF and OmpA are shown. Note that OmpF205 migrates faster than OmpF315 (20).

cific bacteriophage K20. At the nonpermissive temperature, the *ts* parent *ompF315* is K20 resistant ($K20^r$) because of the lack of OmpF in the outer membrane. If growth on maltodextrins at the nonpermissive temperature is due to the presence of OmpF, then isolates should become K20 sensitive ($K20^s$). Of the 12 independent Dex^+ isolates, 11 were found to be $K20^s$, and they were further analyzed.

Genetic mapping of extragenic suppressors. The spontaneous Dex^+ $K20^s$ isolates were initially checked to see if they mapped at *asmA* by P1 transductions with known linked markers, including *his*::Tn*10*, *non*::Tn*10*, and *cps*::Tn*10* (20). Of the 11 Dex^+ $K20^s$ revertants, the suppressor mutation in 7 isolates mapped near 45 min, suggesting that these mutations are likely to be alleles of *asmA*. The remaining four were then checked to see if they mapped within *ompF* by P1 transductions using a linked *pyrD* genetic marker (20). The results showed that these mutations did not map at *ompF*. These four suppressor mutations were further mapped by using Hfr conjugational crosses, which placed them between 96 and 6 min on the *E. coli* chromosome. Two-factor P1 transductional mapping was then carried out using a strain with auxotrophic markers in this area. This mapping revealed that these suppressor mutations were linked to the *leu* operon located at 2 min; a previously characterized *leu*::Tn*10* insertion showed a cotransducible linkage of 50% with the suppressor mutations. These suppressor mutations are now referred to as *asmB1*, *-2*, *-3*, and *-4*. Strains carrying the *asmB4* allele showed unusual growth defects and were not analyzed further.

Phenotypic characterization and dominance test. We consistently noticed certain phenotypic differences among strains carrying different *asmB* alleles. To quantitate these differences, we first examined the EOP against K20 in strains carrying different *asmB* alleles. Strains carrying the *asmB1* allele showed a much lower EOP than those bearing the *asmB2* or *asmB3* allele (Table 2). These differences correlated when we examined envelopes prepared from strains grown in the same medium (glucose minimal medium) that was used for the EOP experiments (Fig. 1). All three of the *asmB* alleles conferred cold sensitivity at 25°C on minimal medium with either glucose or maltodextrin as a carbon source. Interestingly, only the *asmB1* allele exhibited Mac^s. When antibiotic sensitivity was examined, strains carrying the *asmB1* allele showed a dramatic increase in sensitivity to only hydrophobic (erythromycin, novobiocin, and rifampin) and not hydrophilic (ampicillin) antibiotics (Table 2). Strains carrying the other two *asmB* alleles also showed an increase in sensitivity but not as dramatic as that observed for strains carrying *asmB1*. These results suggest that mutations in *asmB* not only suppress mutant OmpF assembly, but also alter other envelope-related functions.

Diploid analysis was carried out to assess the nature of the *asmB* alleles. For this, F′104, which carries the 2-min wild-type region of the chromosome, was utilized. Dominant *asmB* suppressor alleles are expected to result in a Dex^+ phenotype in the presence of a wild-type *asmB* allele, whereas a recessive nature would yield a Dex^- phenotype. Initially, the results of this *asmB*$^+$/*asmB1*, *asmB2*, or *asmB3* complementation analysis were mixed, yielding both Dex^+ and Dex^- exconjugants. When the F′ from Dex^- exconjugants was reintroduced into strains carrying various *asmB* suppressor alleles, only Dex^- exconjugants were obtained. On the other hand, recycling of the F′ from Dex^+ exconjugants yielded only Dex^+ colonies. This suggests that in these Dex^+ exconjugants, the F′ may have acquired mutational alterations. The subsequent stability of F′104 in new strain backgrounds suggests that the original source of F′104 may have contained a mixed population of this plasmid. We believe that the mutant *asmB* alleles are recessive to wild-type *asmB*, at least with respect to the Dex phenotype, since there is no other way in a *recA* background that Dex^- exconjugants can be obtained. Interestingly, when the antibiotic sensitivity of these exconjugants was examined, the results showed intermediate zones of inhibition, indicating a codominant nature of the mutant *asmB* alleles with respect to antibiotic sensitivity.

***asmB* suppressor mutations are alleles of *envA* (*lpxC*).** Since only a general location of the *asmB* mutations had been determined, Kohara lambda clones encompassing the 2-min region were used to pinpoint their position in the chromosome (see Materials and Methods for details). The results showed that wild-type *asmB* DNA is present in a region that encompasses five known open reading frames, including FtsZ, EnvA (LpxC), OrfX, SecA, and MutT, and several minor predicted reading frames are located in the area of overlap (Fig. 2) (2, 39).

Three observations raised the possibility that the *asmB* mutations are allelic to *envA* (*lpxC*). First, both the *asmB1* and the *envA1* mutations conferred sensitivity to a variety of noxious compounds (see above and references 26 to 28). Second, recent reports established a precise biochemical role for EnvA (LpxC) in the synthesis of the lipid A moiety of LPS (38), which is known to influence the assembly of outer membrane proteins (8, 14, 31, 34). Finally, the other four open reading frames have not been implicated in the assembly of outer

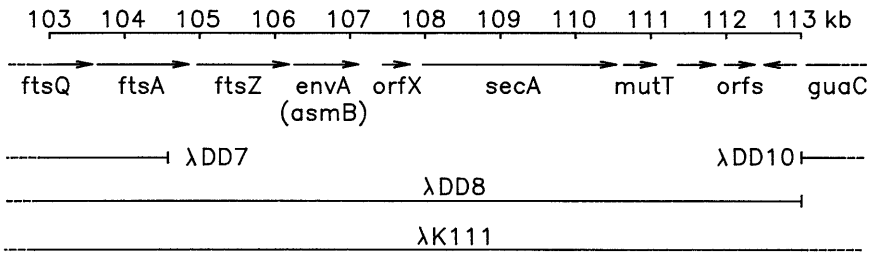

FIG. 2. Physical map (in kilobases) of the *E. coli* 2-min region and its alignment to lambda clones used to map the *asmB1* mutation. The corresponding open reading frames (orfs), both known and predicted, are also shown.

TABLE 3. *asmB* (*envA*) mutations affecting outer membrane properties and protein assembly

Chromosomal allele	MacConkey phenotype[a]	Nucleotide change	Amino acid substitution
envA$^+$ (*lpxC*$^+$/*asmB*$^+$)	R		
envA1[b]	S	c55t	H19Y
asmB1	S	g628a	G210S
asmB2	R	t149c	F50S
asmB3	R	t149c	F50S

[a] R, resistant; S, sensitive.
[b] Previously identified mutation (5, 26).

membrane proteins. Thus, we proceeded to clone the *envA* (*lpxC*) gene from *asmB*$^+$ and various *asmB* mutant strains. The resulting clones of *envA* (*lpxC*) lacked indigenous transcription-translation signals, so *envA* (*lpxC*) expression was dependent on an isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible plasmid promoter. The presence of these plasmid clones retarded colony growth even under noninducing conditions, presumably because of leaky expression of *envA*. Expression of EnvA above physiological levels is known to be detrimental to cellular growth (36).

The wild-type *envA* (*lpxC*) clone obtained from an *asmB*$^+$ strain complemented the Mac^s phenotype of the *asmB1* allele. Similarly, *envA* (*lpxC*) clones obtained from mutants carrying the *asmB2* or *asmB3* allele also complemented the Mac^s phenotype. This was the case because, as mentioned above, *asmB2* and *asmB3* do not confer Mac^s. In contrast to these results, an *envA* (*lpxC*) clone obtained from a mutant carrying the *asmB1* allele failed to complement the Mac^s phenotype of a mutant carrying the chromosomal *asmB1* allele. These results strongly suggested the possibility that at least the *asmB1* mutation resided within the *envA* (*lpxC*) gene. Direct proof of this was obtained by DNA sequence analysis of the entire *envA* (*lpxC*) gene (Table 3). This analysis showed that the *asmB1* allele contained a point mutation resulting in a G210S substitution in the EnvA (LpxC) protein. The *asmB2* and *asmB3* alleles also carried a lesion within the *envA* (*lpxC*) gene, and in both cases, a point mutation resulted in an F50S substitution in the EnvA (LpxC) protein. The only other known viable *envA* mutant contained an H19Y substitution (5). These results unambiguously showed that the *asmB* suppressors are indeed alleles of the *envA* (*lpxC*) gene.

Examination of OmpF in various *asmB* backgrounds. To examine whether the presence of *asmB* suppressor alleles restored OmpF levels, envelopes prepared from strains grown at 42°C were analyzed by SDS-PAGE (Fig. 3A). As expected, strains carrying the wild-type *asmB*$^+$ allele produced no detectable levels of OmpF. However, in the presence of any one of the mutant *asmB* alleles, a significant level of OmpF was observed. As a control, strains carrying a previously characterized extragenic suppressor allele of *ompF315*, *asmA1*, were included. The *asmB* alleles were able to suppress *ompF315* to an extent similar to that of *asmA1*. We also included different *ompF* alleles, *ompF33* and *ompF35*, to examine their suppression by various *asmB* alleles. These *ompF* alleles contained point mutations that resulted in Y231Q and W214Q substitutions within OmpF, respectively. These experiments showed that similarly to *asmA1*, *asmB* mutations displayed no allele specificity towards different *ts ompF* alleles (Fig. 3B and C). The higher level of OmpF present in *ompF35* and *ompF33* strains is readily explained by the fact that the two alleles do not confer as strong an assembly defect as *ompF315*.

The level of stable OmpF trimers produced in the presence

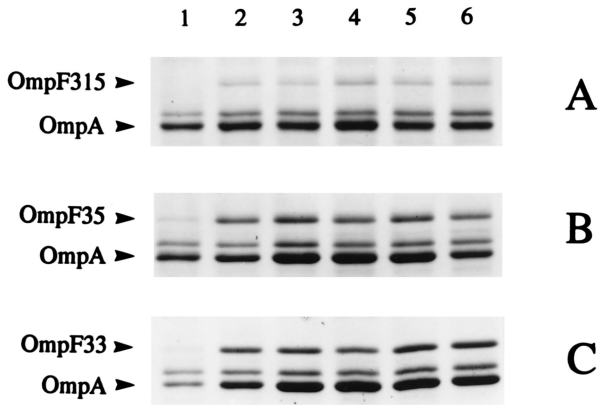

FIG. 3. SDS-PAGE analysis of envelopes prepared from various *asm* strains with different OmpF *ts* assembly-defective alleles. Strains were grown at 42°C on glycerol minimal medium. Lane 1, *asmA*$^+$ *asmB*$^+$; lanes 2 and 4, *asmA1*; lanes 3, 5, and 6, *asmB1*, *asmB2*, and *asmB3*, respectively. (A) OmpF315; (B) OmpF35; and (C) OmpF33.

of the *asmB* suppressor alleles was determined by pulse-chase experiments using OmpF trimer-specific antibodies. The mutant *asmB* alleles were able to restore stable trimer formation, in contrast to the control strain bearing the wild-type *asmB* allele (Fig. 4). This showed that mutant *asmB* alleles suppress the mutant OmpF assembly defect by permitting the conversion of metastable trimers to stable trimers. Thus, like the *asmA* suppressors, mutations in *asmB* acted at the level of assembly and not synthesis.

Outer membrane characteristics. In an *envA1* background, the amount of LPS was reported to be somewhat smaller (about 30%) than that present in the parental strain (10). Since the *asmB1* allele of *envA* confers phenotypes similar to those conferred by *envA1*, it was of interest to examine LPS in the *asmB1* background. Proteinase K-digested envelope samples were analyzed by SDS-PAGE, and LPS bands were visualized by silver staining (1). Although there were no apparent qualitative differences in LPS noted, the amount of LPS isolated from the *asmB1* mutant was consistently smaller (26%) than that present in an *asmB*$^+$ strain (Fig. 5). The reduction in LPS was also confirmed by examining ^{32}P-labeled LPS molecules, although in this case the reduction was around 15% (data not

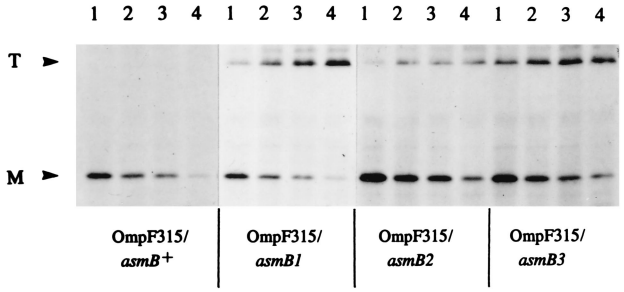

FIG. 4. OmpF trimer assays of *asmB* strains. Cells were grown in glycerol minimal medium at the nonpermissive temperature of 42°C. At mid-log-phase growth, cells were labeled for 20 s with [^{35}S]methionine and chased with an excess of nonradioactive methionine. Chase samples were removed after 1, 5, 10, and 30 min (lanes 1 to 4, respectively). The cells were lysed, and OmpF was immunoprecipitated with OmpF trimer-specific antibodies. Immunoprecipitates were heated to 60°C prior to SDS-PAGE analysis. The gels were dried and autoradiographed at −70°C. The positions of OmpF315 trimers (T) and denatured monomers (M) are shown. Relevant genetic backgrounds are indicated.

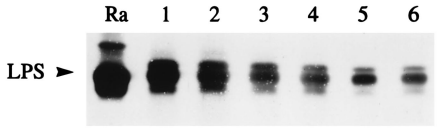

FIG. 5. Examination of LPS levels from *asmB*$^+$ (odd-numbered lanes) and *asmB1* (even-numbered lanes) strains. LPS was visualized from 5.0 (lanes 1 and 2), 2.5 (lanes 3 and 4), and 1.0 (lanes 5 and 6) μg of total envelope protein. Ra, purified Ra-type LPS (0.25 μg) from *E. coli.*

shown). A relatively small reduction in the LPS level was seen in the *asmB3* background by silver staining. It has also been reported that the presence of the *envA1* allele reduces the buoyant density of the outer membrane (10). We tested this for a mutant carrying the stronger *asmB1* allele. Envelopes were analyzed through isopycnic sucrose gradients to separate the inner and outer membranes (data not shown). The outer membranes from *asmB*$^+$ and *asmB1* strains peaked in fractions corresponding to densities of 1.2324 and 1.2351, respectively. The inner membranes peaked at densities of 1.1612 (*asmB*$^+$) and 1.1688 (*asmB1*). These results show that unlike *envA1*, the *asmB1* allele of *envA* does not cause any significant change in the buoyant density of the outer membrane.

Interplay between lipid A and phospholipid biosynthesis pathways. The EnvA protein has been examined in some detail primarily by a group led by Chris Raetz (38). Their studies have shown that the EnvA (LpxC) protein is the UDP-3-*O*-acyl-GlcNAc deacetylase, which is required during the second step of lipid A biosynthesis. Only a single mutant allele of *envA*, *envA1*, has been isolated prior to this study (5, 26). Cells bearing *envA1* display a pleiotropic phenotype (26, 28), including reduced levels of LPS (10), and possess up to 18-fold-lower deacetylase activity (38). Compared with the *envA1* mutation, the *asmB* alleles of *envA* cause less severe phenotypic alterations and modest reductions in LPS levels. Because of the unstable phenotype of *envA1*, it was not possible to assess the ability of *envA1* to suppress the mutant OmpF assembly defect.

Biochemical assays revealed that strains carrying various mutant *asmB* alleles possessed reduced deacetylase activities (data not shown). These reductions may allow the following scenario to develop. A decrease in deacetylase activity may stimulate the phospholipid biosynthetic pathway by diverging the pool of (3*R*)-hydroxyacyl acyl carrier proteins (ACPs), common precursors of phosphatidylethanolamine and lipid A, more towards the synthesis of the former. Thus, suppression of mutant OmpF assembly may be achieved by increasing the phospholipid content of the outer membrane. Results obtained from several genetic studies corroborate such an intricate interplay between the two pathways (10, 24). For example, suppressor mutations of *envA1*, called *sefA*, that are now known to map within *fabZ*, which codes for a (3*R*)-hydroxymyristoyl-ACP dehydrase (24), have been isolated (10). It has been shown that these suppressor mutations decrease the dehydrase activity so that the elevated level of UDP-3-*O*-(3*R*)-hydroxymyristoyl-GlcNAc may become available for the synthesis of lipid A. Similarly, suppressor mutations of a *ts* allele of *lpxA*, *lpxA2*, that also map in the *fabZ* gene have been isolated. The LpxA protein has the UDP-*N*-acetylglucosamine acyltransferase activity needed to carry out the first obligatory step in the formation of lipid A (9). In our laboratory, we have obtained Mac^r revertants of cells carrying the *asmB1* allele. The suppressor mutations were termed *sabA*, for suppressor of *asmB* mutation A (12). These mutations, besides restoring the outer membrane permeability properties, revert the assembly suppression phenotype of *asmB* and map near 4 min on the *E. coli* chromosome (15), the same location designated for the *fabZ* (*sefA*) mutations. Since we have yet to pinpoint the location of the *sabA* mutations, their relationship with *sefA* cannot be established at present. However, given the phenotypic and genetic similarities between *envA1* and *asmB1*, it is likely that the suppressor mutations *sefA* and *sabA* may define the same gene, *fabZ.*

A working model for mutant OmpF assembly suppression. The *asmB* alleles of *envA* reduce the levels of LPS without altering its structure. Moreover, in a wild-type OmpF background, a reduction in LPS by the *asmB* mutations did not result in any significant increase in outer membrane protein levels. Thus, a specific increase in assembly-defective mutant OmpF proteins rather than a general increase in all outer membrane proteins was caused by the presence of the *asmB* suppressor alleles. Given these results, we do not favor the notion that assembly suppression is achieved by some kind of simple compensatory adjustments involving LPS and proteins. Instead, we favor the possibility that changes in the outer membrane environment, possibly alterations in its fluidity, resulting from higher protein-to-LPS or phospholipid-to-LPS ratios in the *asmB* mutants, somehow permit a larger pool of mutant OmpF metastable trimers to be properly assembled into stable trimers. Our current efforts are directed at further understanding the biochemical basis of this suppression.

While it has been known for some time that LPS plays an important positive role in the assembly of outer membrane proteins (8, 14, 31, 34), the molecular nature and biochemical basis of such a relationship remain biological enigmas. We have only begun to appreciate the complexity of this process, and to this end, genetic analyses such as that presented here may provide important clues in understanding the biogenesis of the *E. coli* outer membrane.

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