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Gene Dosage Effects of the Structural Gene for a Lipoprotein of the *Escherichia coli* Outer Membrane

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The gene dosage effects of the structural gene (*lpp*) for the lipoprotein of the *Escherichia coli* outer membrane were examined. A novel F-prime factor containing the *lpp* gene was constructed. The amount of the free-form lipoprotein in the merodiploid strain carrying the F-prime factor was found to be about two times as great as that in the corresponding haploid strain. On the other hand, the amount of the bound-form lipoprotein, which is covalently linked to the peptidoglycan, was not significantly different in the merodiploid strain as compared with the corresponding haploid strain. The present results suggest that the *lpp* gene is expressed constitutively in contrast to another major protein of the *E. coli* outer membrane, tolG protein (protein II*, D. B. Datta et al., J. Bacteriol. **128**:834-841, 1976). The F-prime factor isolated may include a portion of the *E. coli* chromosome (located between 33 and 36 min on the genetic map) that is not covered by any other F-prime factor.

The control mechanisms that govern the gene expression of membrane proteins have been an intriguing phenomena not yet understood. It has been suggested that the synthesis of one of the major proteins in the *Escherichia coli* outer membrane, tolG protein (protein II*), is controlled by a simple feedback mechanism (6). On the other hand, with the vitamin B_{12} receptor in the *E. coli* outer membrane, a distinct gene dosage effect has been observed (4).

Here, we have constructed a novel F-prime factor carrying the structural gene for the lipoprotein (*lpp*) of the outer membrane of *E. coli* and examined the levels of the lipoprotein in a merodiploid strain carrying this F-prime factor. The lipoprotein is the most abundant protein in *E. coli* and is known to exist in two forms: a free form and a bound form that is covalently linked to the peptidoglycan (8, 14). It was found that the amount of the free form was twofold greater in a strain that was merodiploid for the *lpp* gene than in the corresponding haploid strain, whereas the amount of the bound form did not change in the same strain.

MATERIALS AND METHODS

Bacterial strains. The following strains of *E. coli* K-12 were used: KL208 HfrB7 (obtained from B. J. Bachmann) (16); JE5519 F⁻ *aroD man argE lac gal rpsL nalA recA1* (10); JE5507 F⁻ *aroD man argE lac gal rpsL rpsE* (10); JE5525 F⁻ *man lpp-1 pps trp gal rpsL recA1* (15); JE5527 F⁻ *man lpo pps thi his rpsL nalA recA1*; and WM437 F⁻ *leu pro trp dnaI208 his thyA arg met deoB lac gal rpsL sul-1* (obtained from B. J. Bachmann) (3).

Media. Davis minimal medium (7), M9 minimal medium (11), and T broth medium were used. When required, minimal media were supplemented with 40 μg of shikimate and 20 μg each of L-phenylalanine, L-tyrosine, L-arginine, L-histidine, and L-tryptophan per ml.

Growth conditions. For double-label experiments, M9-glucose minimal medium was used. For analysis of the bound-form lipoprotein, M9-glucose minimal medium or T broth was used. In all cases, starting cultures were grown overnight, using selective media as follows: M9-0.2% mannose medium for strains carrying F-prime (*man*⁺), and M9-0.4% glucose medium for F⁻ strains. The starting cultures were diluted 10-fold with T broth or M9-glucose medium, and the mixtures were incubated for three to four generations at 37°C. Segregation of the episome covering the *lpp* gene was prevented by maintaining it constantly on Man⁺ and AroD⁺ selective medium or plate. When the amount of the bound-form lipoprotein was analyzed, the percentage of Man⁻ segregants was determined by plating an appropriate dilution of the culture on eosin-methylene blue-mannose plates.

Double-label experiments. *E. coli* strains JE5519 F⁻ and JE5519/F506 *aroD*⁺ *lpp*⁺ *man*⁺ were grown in M9-glucose minimal medium at 37°C. M9 medium was supplemented with 40 μg of shikimate, 20 μg of L-phenylalanine, 20 μg of L-tyrosine, and 20 μg of L-arginine per ml for JE5519 F⁻ and with only 20 μg of L-arginine per ml for JE5519/F506. At about 2×10^8 cells/ml, 5 μCi of L-[^{14}C]arginine (312 mCi/mmol; Schwarz/Mann) and 25 μCi of L-[^{3}H]arginine (28.7

Ci/mmol; New England Nuclear Corp.) were added to 15 ml of the cultures of JE5519 F⁻ and JE5519/F506, respectively. The cultures were incubated for another generation time, and the two cultures were then combined. The preparation of membrane fractions and sodium dodecyl sulfate-gel electrophoresis was carried out as described previously (13). Gel electrophoresis was performed with use of internal molecular-weight standards made of fluorescent proteins (12).

Amount of the bound-form lipoprotein. To measure the content of the bound-form lipoprotein, the peptidoglycan was prepared from the envelope fraction of exponentially growing cells, using 4% sodium dodecyl sulfate as described by Braun and Sieglin (5). The purified peptidoglycan was hydrolyzed in 6 N HCl for 16 h at 105°C, and amino acid analysis was carried out with a JEOL JLC-6AH amino acid analyzer. The amount of the bound form (B) was expressed as a relative molar ratio of the bound form to the peptide unit of the peptidoglycan as follows:

$$(B) = \frac{(Asp)}{14} \Big/ \left((Glu) - \frac{5 \times (Asp)}{14}\right)$$

where (Asp) and (Glu) represent the contents of aspartic acid and glutamic acid obtained from the amino acid analysis of the peptidoglycan preparations. In each case, the amount of the free-form lipoprotein was analyzed by sodium dodecyl sulfate-slab gel electrophoresis according to the method of Anderson et al. (1). Gels were scanned with a Joyce-Loebl 3CS microdensitometer. For each culture, the level of Man⁻ segregants was monitored on eosin-methylene blue–mannose plates.

RESULTS

Isolation of an F-prime factor carrying the *lpp* gene. We have shown that the structural gene for the lipoprotein (*lpp*) is located at 36.5 min on the *E. coli* chromosomal map (15, 18). Of the existing F-prime factors, only F500, recently isolated by Novel and Novel (17), carries this portion of the *E. coli* chromosome. However, they reported that strain F500 was strongly mucoid and difficult to grow. Therefore we attempted to isolate a new F-prime factor carrying the *lpp* gene as follows. The donor strain, KL208 (HfrB7), and the recipient strain, JE5519 F⁻, were grown in T broth at 37°C. A 9-ml amount of T broth was added to a mixture of 0.5 ml from each of the two cultures. The final mixture was incubated at 37°C overnight, without shaking. After the incubation, the cells were collected by centrifugation and washed with M9 minimal medium containing no supplements. The cells were then plated onto selective medium plates containing streptomycin (100 μg/ml) and mannose as a sole carbon source but lacking shikimate, phenylalanine, and tyrosine. The colonies that appeared on these selective plates are considered to be F⁺ conjugants (Man⁺ AroD⁺ and *rpsL*). All 20 conjugants isolated were able to transfer both the Man⁺ and AroD⁺ phenotypes to a recipient, JE5507. This indicates that all conjugants have F-prime factors carrying both *man*⁺ and *aroD*⁺ genes.

We next examined whether these conjugants carried the *lpp* gene as follows. F-prime factors from these conjugants were transferred to another recipient, JE5527. Newly formed conjugants were first selected on selective medium plates containing mannose as a sole carbon source and lacking arginine but containing histidine. Since the recipient. JE5527, lacks the lipoprotein gene (mutation referred to as *lpo*) (10), the existence of the *lpp*⁺ gene on the F-prime factors can be easily detected by analyzing membrane proteins of these newly formed conjugants.

It was found that only two membrane preparations had the lipoprotein band in sodium dodecyl sulfate-slab gel electrophoresis. This indicates that these two F-prime factors contain the *lpp*⁺ gene.

One of the two strains carrying the F-prime factors was designated as JE5519/F506. To avoid segregation of the F-prime factor, the strain was usually kept in selective culture medium or on a selective culture plate containing mannose as a sole carbon source, and RecA phenotype of the host was monitered by UV sensitivity. Strain JE5519/F506 was able to transfer the *pps* gene (ability to use lactic acid as a sole carbon source) to strain JE5527 as the recipient, but was not able to transfer the *trp* gene to JE5525 as the recipient or the *dnaI* gene to WM437 as the recipient. Presence of F factor in strain WM437 was checked by isolating f2 phage (male specific)-sensitive colonies and retransferring the F-prime factor to the recipient, JE5507 (transfer of Man⁺ and aroD⁺ markers to the recipient).

Amount of the free-form lipoprotein. [^{3}H]arginine-labeled envelope fraction from JE5519/F506 was mixed with [^{14}C]arginine-labeled envelope fraction from JE5519. The mixture was subjected to sodium dodecyl sulfate-gel electrophoresis.

Peaks of ^{3}H and ^{14}C radioactivities were almost superimposable on each other (Fig. 1). The only significant difference in the ratios of ^{3}H to ^{14}C in the individual peaks was in the lipoprotein peak, which ran close to internal molecular-weight standard f. The ratio of ^{3}H to ^{14}C in the lipoprotein peak was about twice as great as in the other peaks. Therefore the amount of the free-form lipoprotein increased about twofold in the F-prime strain (JE5519/F506) without any significant effects on the amounts of the other membrane proteins.

Amount of the bound-form lipoprotein. It is of great interest whether the amount of

the bound-form lipoprotein also increases as does the amount of the free form in the merodiploid strain. The quantity of the bound form was examined for JE5519/F506 under different growth conditions (Table 1). The amount of free-form lipoprotein in the merodiploid strain was examined by sodium dodecyl sulfate-slab gel electrophoresis and was found to be at least twofold greater in the merodiploid strain than in the haploid strain (data not shown). On the other hand, the amount of bound-form lipoprotein in the merodiploid strain did not differ significantly from the haploid strain under any of the growth conditions. The average ratio of the amount of the bound form in the merodiploid strain to that in the haploid strain from Table 1 was calculated to be 1.08 ± 0.15, clearly indicating that there was no gene dosage effect of the lipoprotein gene on the amount of the bound-form lipoprotein. The values of 1/(B) in Table 1 express the number of peptidoglycan units on the basis of glutamic acid content per lipoprotein molecule bound to the peptidoglycan. In both the haploid and merodiploid strains, one molecule of the lipoprotein was bound to every 13 to 16 peptidoglycan repeating units, depending upon the growth conditions. This value is slightly higher than the value 10 obtained by Braun and Sieglin (5).

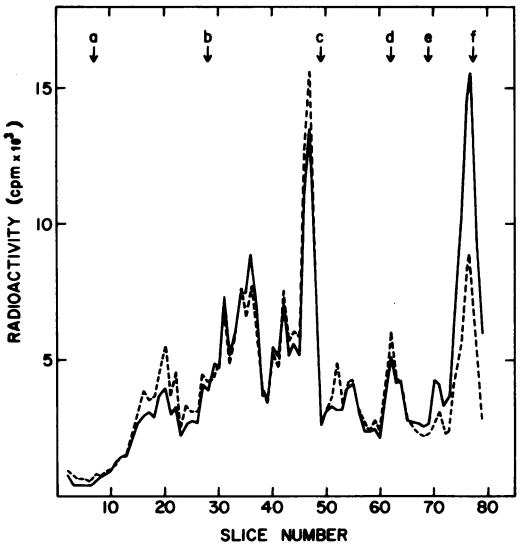

FIG. 1. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the membrane fractions of a merodiploid (JE5519/F506) and a haploid (JE5519 F⁻) strain for the lipoprotein gene, lpp. Double-label experiments were carried out as described in Materials and Methods. Symbols: (——) Membrane fraction from E. coli JE5519/F506 labeled with [³H]arginine; (-----) membrane fraction from JE5519 F⁻ labeled with [¹⁴C]arginine. Arrows with letters indicate the positions of the internal molecular-weight standards: (a) dimer; (b) monomer of 5-dimethylaminonaphthalene-1-sulfonyl (DANS) bovine serum albumin: (c) dimer; (d) monomer of DANS-egg white lysozyme; (e) cytochrome c; (f) DANS-insulin (12).*

DISCUSSION

The present data clearly demonstrate the gene dosage effect of the *lpp* gene, the structural gene for the lipoprotein of the *E. coli* outer membrane. This is in contrast to the case of the other major outer membrane protein, tolG protein (protein II*), where gene dosage does not result in an increased amount of the protein (6). If outer membrane proteins are produced constitutively, the amount of an outer membrane protein is expected to be doubled in a strain that is diploid for the outer membrane protein. This appears to be the case for the free-form lipoprotein as shown in the present paper. On the other hand, in the case of the tolG protein there seems to be a regulatory mechanism to control the production of the protein.

It has been shown that the envelope of *E. coli* cells starved of histidine can accommodate the free-form lipoprotein in amounts as high as two to three times the amount of the free-form lipoprotein of the normally growing cells (8). Therefore, it is not surprising that the amount of the free-form lipoprotein increased about twofold in the strain merodiploid for the *lpp* gene. On the other hand, the bound-form lipoprotein did not show the gene dosage effect in the mer-

TABLE 1. *Amounts of bound-form lipoprotein in strains haploid and diploid for the lpp gene*

Strain	Genotype	Growth condition[a]	Amt of bound form (B)[b]	1/(B)
JE5519 F⁻	*lpp*⁺	T broth	0.073	13.7
		M-9 medium	0.064	15.7
JE5519/F506	*lpp*⁺/F'*lpp*⁺	T broth[c]	0.068 (0.93)	14.7
		M-9 medium[d]	0.079 (1.23)	12.7

[a] Cells were harvested in exponential stage in all experiments.

[b] Calculated from the equation given in Materials and Methods. Values in parentheses express the ratios of the amount of the bound form in the F-prime strain to that in the F⁻ strain for the individual experiments.

[c] Ninety-two percent were F-prime strains.

[d] Ninety-five percent were F-prime strains.

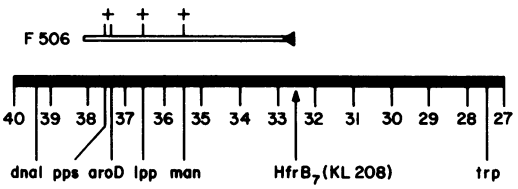

FIG. 2. *Chromosomal map of E. coli K-12 (2) from min 27 to min 40, showing the genes covered by the F-prime factor F506. The arrowhead indicates the origin and direction of chromosome transfer by strain KL208 HfrB7 (14), from which the F-prime F506 (the top line) was derived. (+) Markers tested, present on F-prime F506.*

odiploid strain. There may be no additional sites in the peptidoglycan to which the lipoprotein can bind. Alternately, the enzyme required for the conversion reaction of the bound form may be limited.

The present F-prime factor was isolated from strain KL208 HfrB7, in which the F factor is inserted at near 33 min on the *E. coli* K-12 chromosome map (16). This F-prime factor (F506) was found to carry *man*$^+$ (36 min; 2), *lpp*$^+$ (36.5 min; 15, 18), *aroD*$^+$ (37 min; 2), and *pps*$^+$ (37 min; 2) but not *dnaI*$^+$ (39 min; 2) or *trp*$^+$ (27 min; 2). Therefore the F-prime factor appears to include a portion of the *E. coli* chromosome at least from 33 min to 37 min on the genetic map, as shown in Fig. 2. Part of this portion has not been reported to be covered by any other F-prime factor so far isolated. Since this new F-prime factor may contain part of the longest silent region of the *E. coli* chromosome near the termination site of DNA replication (2), it would be a useful tool for studying the mechanism of the termination of DNA replication. Currently we are attempting to isolate the F-prime factor DNA in order to estimate its size.

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