The envA Permeability/Cell Division Gene of Escherichia coli Encodes the Second Enzyme of Lipid A Biosynthesis

**UDP-3-O-(R-3-HYDROXYMYRISTOYL)-N-ACETYLGLUCOSAMINE DEACETYLASE**

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The envA gene of Escherichia coli has been shown previously to be essential for cell viability (Beall, B. and Lutkenhaus, J. (1987)). Bacteriol. 169, 5408-5415), yet it encodes a protein of unknown function. Extracts of strains harboring the mutant envA allele display 3.5-18-fold reductions in UDP-3-O-acyl-N-acetylglucosamine deacetylase specific activity. The deacetylase is the second enzymatic step of lipid A biosynthesis. The structural gene coding for the deacetylase has not been assigned. In order to determine if the envA gene encodes the deacetylase, envA was cloned into an isopropyl-1-thio-β-D-galactopyranoside-inducible T7-based expression system. Upon induction, a protein of the size of envA was highly overproduced, as judged by SDS-PAGE. Direct deacetylase assays of cell lysates revealed a concomitant ~5,000-fold overproduction of activity. Assays of the purified, overproduced EnvA protein demonstrated a further ~5-fold increase in specific activity. N-terminal amino acid sequencing of the purified protein showed that the first 20 amino acids matched the predicted envA nucleotide sequence. Contaminating species were present at less than 1% of the level of the EnvA protein. Thus, envA is the structural gene for UDP-3-O-acyl-GlcNAc deacetylase. Based on its function in lipid A biosynthesis, we propose the new designation lpxC for this gene.

The outer membrane of Gram-negative bacteria provides a formidable permeability barrier to the entry of large and hydrophobic compounds, primarily because of the unique lipopolysaccharide (LPS)† associated with the outer leaflet of the outer membrane (Nakae, 1986; Nikaido, 1976; Nikaido and Vaara, 1985). Over the years, a large number of mutant strains of Escherichia coli and other Gram-negative bacteria that exhibit defects in this barrier have been isolated and characterized (Weigand and Rothfield, 1976; Roantree et al., 1977; Scudamore et al., 1979; Coleman and Leive, 1979; Angus et al., 1982; Vaara, 1990; Vuorio et al., 1991). For many of these mutants the molecular defect has not yet been determined.

One well studied example of such mutations is envA1, originally described in the late 1960s by Normark and colleagues (1969). The envA1 mutation was obtained in a study of penicillin resistance by screening ethyl methanesulfonate-treated, penicillinase-producing E. coli for increased ampicillin sensitivity and smooth colony morphology. Strains harboring envA1 exhibit a complex phenotype, including permeability to a wide variety of antibiotics and dyes, suggesting that their EDTA-sensitive outer membrane surface layer is defective (Normark, 1970; Normark et al., 1969; Young and Silver, 1991). Mutants bearing envA1 also display a morphological defect in the completion of cell septation, resulting in growth as short chains (Normark et al., 1969; Normark et al., 1971). In addition, there is a slight (~30%) reduction in the content of apparently normal LPS (Grundstrom et al., 1980).

Only the one original allele of the envA locus, the envA1 mutation, has been described (Normark et al., 1969). envA1 has since been used to locate precisely the mutated envA reading frame to minute 2 on the E. coli chromosome at the end of a large gene cluster required for cell division and cell wall biogenesis (Sullivan and Donachie, 1984). The sequence of the envA gene predicts a protein of 34 kDa. The gene itself has been demonstrated to be essential by insertional mutagenesis (Beall and Lutkenhaus, 1987), implying that the envA1 allele encodes a protein that retains some residual activity (Beall and Lutkenhaus, 1987). However, no further hints as to the function of envA have, as yet, been reported (Beall and Lutkenhaus, 1987), and no related sequences of known function from other organisms are available for comparative studies.

LPS is an essential component of the outer membrane and its associated barrier function (Morrison and Ryan, 1992; Rietschel, 1984). The lipid A component of LPS is the basic building block that comprises most of the outer leaflet (Raetz, 1990, 1993). The biosynthesis of lipid A in E. coli has been described (Raetz, 1990, 1993). It begins with the 3-O-acetylation of UDP-GlcNAc with R-3-hydroxymyristate (Fig. 1), followed by deacetylation and reacylation at the glucosamine ring nitrogen with a second R-3-hydroxymyristate moiety (Raetz, 1990, 1993; Williamson et al., 1991). The genes encoding the UDP-GlcNAc acyltransferases of E. coli have been identified as lpxA and lpxD (firA), respectively (Coleman and Raetz, 1987; Kelly et al., 1993), but the structural gene encoding UDP-3-O-acyl-GlcNAc deacetylase (Anderson et al., 1988) has remained elusive. The deacetylase has been implicated as a point of regulation with respect to the biosynthesis of lipid A (Anderson et al., 1993; Mohan et al., 1994). Identification of the deacetylase structural gene could shed light on the nature of this regulation.

We now demonstrate that extracts of E. coli strains harbor-
cephalosporin. The latter is also the precursor of palmitate residues found in membrane glycerophospholipids. Since the lipid A pathway begins with the acylation at the 3-OH moiety of the glucosamine ring of UDP-GlcNAc (Anderson et al., 1984), the second reaction of the lipid A pathway (the deacetylase) appears to function as the first committed step, and it may be regulated. U, uridine; ACP, acyl carrier protein; PtdEtn, phosphatidylethanolamine.

FIG. 1. Relationship of key precursors of fatty acid, lipid A, and peptidoglycan biosynthesis. Three major cell envelope components arise from two key precursors of E. coli metabolism. UDP-GlcNAc serves as the glucosamine source in both peptidoglycan and lipid A biosynthesis. The lipid A pathway begins with the acylation at the 3-OH moiety of the glucosamine ring of UDP-GlcNAc with R-3-hydroxymyristoyl acyl carrier protein. The latter is also the precursor of palmitate residues found in membrane glycerophospholipids. Since the equilibrium constant for UDP-GlcNAc acylation is unfavorable (Anderson et al., 1993), the second reaction of the lipid A pathway (the deacetylase) appears to function as the first committed step, and it may be regulated. U, uridine; ACP, acyl carrier protein; PtdEtn, phosphatidylethanolamine.
E. coli EnvA Gene Encodes Lipid A Deacetylase

Table I

| Strain          | Relevant genotype | Source or reference |
|-----------------|-------------------|---------------------|
| E. coli strains |                   |                     |
| MB2884          | envA^-             | Merck Culture Collection |
| MB4026          | envA^-             | This study |
| MB5503          | envA^-             | Young and Silver (1991) |
| MB5504          | envA^-             | Young and Silver (1991) |
| BL21 (DE3)      | T7 RNA polymerase lysozyme | Novagen |
| BL21 (DE3)pLysS | T7 RNA polymerase lysozyme/plasmid-encoded T7 lysozyme | Studier et al. (1990) |
| L5583           | envA^+             | Young and Silver (1991) |
| L5584           | envA^-             | Young and Silver (1991) |
| L5822           | BL21 (DE3) envA^- | This study |
| J.B3104         | cdx::Tn10, envA^-  | Life Technologies, Inc. |
| Other strains   |                   |                     |
| MB3548          | Serratia marcescens | Merck Culture Collection |
| MB3125          | Proteus mirabilis  | Merck Culture Collection |
| MB2646          | Enterobacter cloacae | Merck Culture Collection |
| MB4005          | Klebsiella pneumonia | Merck Culture Collection |
| MB3286          | Pseudomonas aeruginosa | Merck Culture Collection |
| Plasmids        |                   |                     |
| pLL24           | Hybrid pBR322-pSC101 replicon | This work |
| pET11a          | T7 expression vector, Ap^- | Studier et al. (1990) |
| pENV15          | 2.5-kilobase EcoRI envA^- fragment in pSC101 replicon | This work |
| pET11a-EnvA     | envA^- clone in pET11a | This work |

*EnvA-linked Tn10 (Young and Silver, 1991).*

polyethyleneimine cellulose plate (PEI-plate, Merck Darmstadt). The plate was then dried, washed in methanol for 10 min, dried again, and developed in 0.2 M guanidinium hydrochloride. The developed plate was dried and analyzed by autoradiography to locate the separated envA^- and product, respectively. The envA^- was dried and analyzed by autoradiography to locate the separated envA^- and product, respectively. The envA^- was dried and analyzed by autoradiography to locate the separated envA^- and product, respectively.

The expression system of Studier (1990). The envA^- was cloned into the pET11a vector by polymerase chain reaction (PCR) amplification using Pyrococcus furiosus DNA polymerase (Stratagene). Restriction sites were introduced on either side of the coding sequence to facilitate cloning. The N-terminal primer consisted of a C/G clamp, an NdeI restriction site overlapping the initiation codon, and the first 26 base pairs of the envA encoding sequence. The sequence of this primer was

5'-CCG GCC GGC GCC GTA TGA ACA AAG GGA CAC TTA AAC G-3'. The C-terminal primer consisted of a G/C clamp, a BamHI restriction site, two terminal codons, and the last 21 base pairs of the envA encoding sequence. The sequence of this primer was 5'-CCG GCC CCT AGG GCC GGA TCC TTA TTA TGC CAG TAC AGC TGA-3'.

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harvested by centrifugation beginning 1.5 h after addition of IPTG. A total of 73.5 g of wet cell paste was resuspended in 365 ml of buffer (10 mM sodium phosphate, pH 7.0, 2 mM DTT), blended to yield a smooth paste, and rapidly frozen in liquid nitrogen for subsequent ligation, as described under “Experimental Procedures.”

The cells were lysed and brought to an ammonium sulfate fraction in two batches. In each case, this was accomplished by thawing the cell paste on ice, followed by addition of Brij 58 to approximately 0.05% (w/v) and incubation in ice water until a viscous mixture was obtained as a result of autolysis mediated by the endogenous T7 lysozyme (encoded by the pLYS plasmid). This mixture was clarified to generate a cell-free extract by centrifugation in a Ti-45 rotor (Beckman) at 40,000 rpm for 60 min. Deacetylase activity was precipitated by the addition of 0.3 g of solid ammonium sulfate/mI of cell-free extract (approximately 50% saturation) over a 45-min period with a further incubation on ice with stirring for 20 min. The ammonium sulfate precipitate was collected by centrifugation in a Sorvall SS-34 rotor at 14,000 rpm for 20 min at 4°C. The ammonium sulfate pellets were resuspended in buffer A (25 mM imidazole, pH 7.0, 20% glycerol, 2 mM DTT) and dialyzed against this buffer at 4°C for 5 h, at which point the conductivity had decreased to a level appropriate for further chromatography. This fraction (40.5 g, 2552 mg of protein) was rapidly frozen in two portions, which were further purified separately on a Q-Sepharose Fast Flow column.

Q-Sepharose Chromatography—All chromatographic steps were carried out at 4°C using a Pharmacia Bio-Pilot system (controller, LCC-500plus; valve, IMV-7; pump, P-6000; detector, Pharmacia LKB UV-1 8.5-cm, 280 nm single wavelength). A column (Pharmacia QX50, 5.0 x 8.5-cm, approximately 167 ml) of Q-Sepharose Fast Flow resin was packed and equilibrated at an 8 ml/min flow rate with buffer A (25 mM imidazole, pH 7.0, 20% glycerol, 2 mM DTT). The dialyzed ammonium sulfate precipitate was diluted 1:10 in buffer A and filtered through a 0.22-

RESULTS

Extracts of E. coli Strains Harboring the envA Allele Are Deficient in UDP-3-O-acyl-GlcNAc Deacetylase Activity—UDP-3-O-(R-3-hydroxymyristoyl)-GlcNAc deacetylase is the second enzyme of lipid A biosynthesis in E. coli (Fig. 1). Previous studies have suggested that this enzyme is subject to regulation (Anderson et al., 1993; Kelly et al., 1993; Mohan et al., 1994). In order to assess the level of deacetylase activity in various bacteria, we assayed extracts prepared from a selection of laboratory strains (Table II). In general, deacetylase-specific activity was of the same order of magnitude among the enteric isolates tested (Table II), and it was also relatively constant within the pH range 5.5–6.5. The deacetylase in extracts of Pseudomonas was unique in that it was not active at pH 5.5, suggesting that the Pseudomonas deacetylase is somewhat different from that of enteric bacteria. Interestingly, E. coli strain MB4926 (envA1) was consistently deficient in deacetylase-specific activity (Table II) compared with other strains of E. coli K-12. Assay of an isogenic pair of E. coli strains differing only at the envA locus revealed an 18-fold reduction in deacetylase activity in the envA1 mutant, MB5504, as compared with the envA+ parent, MB5503 (Table III, Experiment 1). Further, this deficiency was eliminated when the envA1 mutation was complemented in trans with pENV15, a plasmid carrying the wild-type allele in a low copy vector (Table III, Experiment 1). A similarly trans-complementated envA+ chromosomal allele did not produce a measurable increase in expressed cellular deacetylase activity over wild type.

A priori, these observations could be explained by a regulatory function for the EnvA protein. Alternatively, the EnvA protein could actually be the deacetylase. The deacetylase might be regulated in some manner to maintain a constant level of enzyme activity. The level of expression from each of the envA gene(s) in these complemented strains is not known, and such experiments do not allow us to distinguish between these two possibilities. However, the results do indicate that

FIG. 2. Cloning of the envA gene from E. coli and construction of pET11a-EnvA. Details of the construction strategy and selection and the sequence of primers used for PCR amplification are described under “Experimental Procedures.” For clarity, only restriction sites relevant to the construction are shown. Reading frames encoding resistance to antibiotics are placed as shown and are abbreviated as follows: Amp, ampicillin; Str/Spc, streptomycin/spectinomycin; Tet, tetracycline. The NdeI - BamHI restriction fragment resulting from digestion of pET11a-gyrB was removed prior to subsequent ligation, as described under “Experimental Procedures.”
E. coli **envA** Gene Encodes Lipid A Deacylase

### Table II

| Strain                                      | Extract specific activity \(\text{pmol} \times \text{min}^{-1} \times \text{mg}^{-1}\) |
|---------------------------------------------|----------------------------------------------------------------------------------|
|                                             | pH 5.5                             | pH 6.0                             | pH 6.5                             |
| E. coli (envA\(^+\))                        | 68.1                               | 110                                 | 87.4                               |
| lane 1                                      |                                    |                                    |                                    |
| E. coli (envA\(^-\)) MB4926                  | 10.3                               | 22.9                                | 25.2                               |
| lane 2                                      |                                    |                                    |                                    |
| E. coli (envA\(^-\)) MB2884                  | 131                                | 201                                 | 153                                |
| lane 3                                      |                                    |                                    |                                    |
| S. marcescens MB3548                         | 168                                | 351                                 | 300                                |
| lane 4                                      |                                    |                                    |                                    |
| P. vulgaris MB3125                           | 53.7                               | 76                                  | 49.4                               |
| lane 5                                      |                                    |                                    |                                    |
| E. cloacae MB2646                            | 28.8                               | 40.7                                | 31.5                               |
| lane 6                                      |                                    |                                    |                                    |
| K. pneumonia MB4005                          | 188                                | 230                                 | 150                                |
| lane 7                                      |                                    |                                    |                                    |
| P. aeruginosa MB3286                         | <8                                 | 23                                  | 65.6                               |

\(a\) Assay pH in 40 mM bis-tris chloride.

### Table III

| Strain                                      | Specific activity \(\text{pmol} \times \text{min}^{-1} \times \text{mg}^{-1}\) | Relative |
|---------------------------------------------|-------------------------------------------------------------------------------|----------|
| Exp. 1\(^b\)                                |                                                                             |          |
| MB5503 (envA\(^+\))                         | 64                                                                           | 1.0      |
| MB5504 (envA\(^-\))                         | 3.6                                                                          | 0.056    |
| MB5503/pENV15 (envA\(^-\)/envA\(^+\))       | 65                                                                           | 1.0      |
| MB5504/pENV15 (envA\(^-\)/envA\(^+\))       | 61                                                                           | 0.96     |
| Exp. 2\(^c\)                                |                                                                             |          |
| BL21 (DE3)/pLysS                            | 340                                                                           | 1        |
| BL21 (DE3)/pLysS/pET11a                      | 270                                                                           | 0.79     |
| BL21 (DE3)/pLysS/pET11a-envA                 | 1,790,000                                                                     | 5260     |

\(a\) Two- to threefold variations in deacetylase specific activity are observed with different extracts and different strains of envA\(^+\) E. coli K12. Variation of duplicates of the same extract is approximately 5%.

\(b\) Performed at pH 6.5.

\(c\) Performed at pH 5.5.

The validated pET11a-EnvA construct was transformed into BL21(DE3)/pLysS. Cell-free extracts made from small cultures of BL21(DE3)/pLysS/pET11a-EnvA after 2-h induction with 1 mM IPTG were examined by SDS-PAGE. They showed strong overexpression of an approximately 34-kDa polypeptide (Fig. 3, lane 10), identical in size to the protein predicted from the inferred amino acid sequence of the envA gene. Further, this band was unique to these extracts and was not seen in induced extracts of either BL21(DE3)/pLysS (host) or BL21(DE3)/pLysS/pET11a (vector) (Fig. 3, lanes 4 and 7, respectively).

The soluble extracts of these induced host, vector, and pET11a-EnvA-bearing strains were assayed for UDP-3-O-acyl-GlcNAc deacylase activity under standard conditions (Table III, Experiment 2). The extract made from cells harboring the pET11a-EnvA clone was found to overexpress greatly the deacylase activity, consistent with massive induction of the envA gene product. These results indicate that deacylase activity parallels the apparent level of envA gene expression but formally does not rule out the alternative possibility that the expressed envA gene product directs expression of a similarly sized protein, which itself is the deacylase.

Purification of the envA Gene Product—In order to identify positively the overexpressed protein found in induced BL21(DE3)/pLysS/pET11a-EnvA cells as the EnvA polypeptide and to prove its function as UDP-3-O-acyl-GlcNAc deacylase, we isolated this protein from an induced, large scale preparation as described under “Experimental Procedures.” From a 15-liter culture of BL21(DE3)/pLysS/pET11a-EnvA cells, grown and induced as described under “Experimental Procedures,” we obtained 73.5 g of cell paste. This material was conveniently lysed with a single freeze-thaw step and the subsequent addition of 0.05% (w/v) Brij detergent. The presence of T7 lysozyme in these cells, which aids in suppressing the expression of cloned protein during growth prior to induction, acts to autolyze the peptidoglycan layer upon freeze-thawing. Analysis of the high speed supernatant fraction by SDS-PAGE and deacylase assay revealed the presence of a high level of a 34-kDa protein and of a correspondingly massive amount of enzymatic activity (Fig. 4, lane 1, and Table IV). We found that enzyme held at this stage of purification was unstable with a decay half-life of approximately 40 h (stability studies data not shown). This instability was essentially eliminated by the next step.

![Fig. 3](http://www.jbc.org/)

**Fig. 3.** Overexpression of the envA\(^+\) gene product under control of the T7 promoter. Portions of whole culture (lanes 2, 3, 5, 6, 8, and 9) and soluble extract derived from these cultures (lanes 4, 7, and 10) were prepared for SDS-PAGE analysis, as described under “Experimental Procedures.” Lanes 1 and 11, protein molecular weight standards: phosphorylase b (97,400); bovine serum albumin (66,200); ovalbumin (45,000); carbonic anhydrase (31,000); soybean trypsin inhibitor (21,500); trypsin (14,400). Lane 2, strain BL21(DE3)/pLysS (host) without IPTG induction. Lane 3, strain BL21(DE3)/pLysS after IPTG induction. Lane 4, soluble extract derived from lane 3. Lane 5, strain BL21(DE3)/pLysS/pET11a (host/vector) without induction. Lane 6, BL21(DE3)/pLysS/pET11a after IPTG induction. Lane 7, soluble extract derived from lane 6. Lane 8, BL21(DE3)/pLysS/pET11a-EnvA (host/vector with envA insert), without IPTG induction. Lane 9, BL21(DE3)/pLysS/pET11a-EnvA after IPTG induction. Lane 10, soluble fraction derived from lane 9.

A two- to threefold increase in deacetylase specific activity was observed with different extracts and different strains of envA\(^+\) E. coli K12. Variation of duplicates of the same extract is approximately 5%.
The protein band and deacetylase activity co-precipitated in high yield in a 50% ammonium sulfate fraction (Fig. 4, lane 2, and Table IV). The latter was dialyzed, filtered, and processed by anion ion exchange chromatography. The 34-kDa protein band and deacetylase activity were both recovered at approximately 110 mM potassium chloride (Fig. 4, lane 3, and Fig. 5). The isolated protein was judged to be >98% pure by SDS-PAGE (Fig. 4, lane 3).

A sample of similarly purified material was analyzed by Edman degradation in order to demonstrate that this isolated protein was indeed the expression product of the envA gene. The sequence obtained, Met-Ile-Lys-Gln-Arg-Thr-Leu-Lys-Arg-Ile-Val-Gln-Ala-Thr-Gly-Val-Gly-Leu-His, was exactly that predicted for the first 19 amino acids of the envA reading frame (Beall and Lutkenhaus, 1987). Further, the molar yield of phenylthiohydantoin-derivative products was consistent with the absence of measurable contaminating proteins, thus confirming the EnvA protein itself was the source of the comigrating deacetylase activity. We propose the designation LpxC for this gene.

### DISCUSSION

Early studies of strains bearing the envA1 mutation indicated a slight reduction of lipopolysaccharide content compared with wild-type, possibly accounting for the antibiotic hypersensitivity associated with envA1 (Wolf-Watz and Normark, 1976; Grundstrom et al., 1980). We (Young and Silver, 1991) postulated that, in envA1 bearing mutants, increased entry of hydrophobic antibiotics might be due to the lowered LPS content but that increased entry of hydrophilic antibiotics and exit of periplasmic proteins could be due to breakage and rejoining of the outer membrane during inefficient separation at cell division. A number of pleiotropic mutants of E. coli and Salmonella exhibit similar "leaky" phenotypes with accompanying septal morphological defects (Weigand and Rothfield, 1976).

Wolf-Watz and Normark (1976) found that N-acetylmuramyl-L-alanine amidase activity, possibly involved in peptidoglycan remodeling at the septum, was 6-fold decreased in an envA1 strain. Since a 20-fold decrease of this amidase caused an antibiotic phenotype similar to that associated with envA1 and the finding that UDP-3-O-acyl-GlcNAc deacetylase activity is decreased in envA1-bearing strains suggested that the envA gene product might also play a role in lipid A biosynthesis.

**Fig. 4.** Analysis by SDS-PAGE of fractions obtained during purification of the EnvA protein. Samples from each step of purification were boiled for 5 min in SDS-PAGE buffer and analyzed by SDS-PAGE. Lane 1, protein molecular weight standards; phosphorylase b (97,400); bovine serum albumin (66,200); ovalbumin (45,000); carbonic anhydrase (31,000); soybean trypsin inhibitor (21,500); lysozyme (14,400). Lane 2, induced, soluble crude extract. Lane 3, dialyzed and filtered ammonium sulfate precipitate. Lane 3, Q-Sepharose chromatography peak fraction number 50.

**Fig. 5.** Co-chromatography of 34-kDa band and deacetylase activity. Equal volumes of representative fractions from the Q-Sepharose column were boiled for 5 min in SDS-PAGE buffer and analyzed by SDS-PAGE. Lane L, column load (lane 2, Fig. 4). Lane W, Q-Sepharose wash. Lanes 2–20, column fractions as indicated. Lane S, protein molecular weight standards; phosphorylase b (97,400); bovine serum albumin (66,200); ovalbumin (45,000); carbonic anhydrase (31,000); soybean trypsin inhibitor (21,500); and lysozyme (14,400).

**TABLE IV**

| Step                | Total protein (mg) | Total units (pmol) | Specific activity (pmol min⁻¹ mg⁻¹) | Yield (%) | Purification (fold) |
|---------------------|--------------------|--------------------|-------------------------------------|-----------|--------------------|
| Crude extract       | 5375               | 3.30 × 10⁶         | 6.14 × 10⁵                           | 100       | 1                  |
| (NH₄)₂SO₄,c         | 3898               | 3.24 × 10⁶         | 8.31 × 10⁵                           | 98        | 1.35               |
| Q-Sepharose FPLC    | 3816               | 3.11 × 10⁶         |                                    | 94        |                    |
| Peak                | 814                | 2.41 × 10⁶         | 2.96 × 10⁶                           | 73        | 4.82               |

* Yield back-calculated for processing of entire sample in several batches.
* This is an ammonium sulfate precipitate which has been dialyzed, filtered, and diluted as described under "Experimental Procedures."
* Fast protein liquid chromatography.
When assayed in the range of pH 5.5–6.5, we found that crude extracts made from a number of common enterobacterial strains possess deacetylase specific activities that are within an order of magnitude of each other (Table II). However, extracts of envA1-bearing strains of E. coli K12 were 3.5–18-fold less active than closely related constructs (Tables II and III). The depression of deacetylase activity in strains harboring the envA1 mutation was especially apparent in an isogenic pair of E. coli strains (Table III). The deficit in deacetylase activity could be corrected specifically, although not increased above normal levels, by complementation in trans with the low copy vector pENV15 (envA+).

Forced expression of the envA+ reading frame using the T7 system of Studier (1987) resulted in massive overexpression of deacetylase activity in broken cell preparations (Table III). The demonstration that essentially all the deacetylase activity in broken cell preparations (Table III) was contributed by the EnvA protein verified that the envA locus is indeed the structural gene encoding UDP-3-O-acyl-GlcNAc deacetylase. We propose the designation lpxC to replace envA, given its function in lipopolysaccharide biosynthesis.

In the lipid A pathway, the deacetylase functions between two acyltransferases, encoded by the lpxA and lpxD (fimA) genes, respectively (Fig. 1). LpxA and LpxD map to a macromolecular synthesis operon at minute 4, containing genes involved in DNA, phospholipid, lipid A, and outer membrane protein biosynthesis (Fig. 1) (Kelly et al., 1993; Raetz, 1993). In contrast, the lpxC (envA) gene near minute 2 resides at the 3′ end of a large cluster of murein and cell division genes, the regulation of which is complex and not fully understood (Donachie, 1993; Errington, 1993). LpxC (envA) appears to have its own promoter (Sullivan and Donachie, 1994), but transcripts arising from upstream promoters could also contribute to deacetylase expression under some conditions. Whether the up-regulation of the deacetylase (Anderson et al., 1993) under conditions of limited lipid A biosynthesis occurs at the level of transcription or by some other mechanism remains to be established. The association of LpxC with genes involved in peptidoglycan biosynthesis and cell division may reflect the operation of a novel, global regulatory network for envelope assembly.

Genetic interruption of the early steps in LPS biosynthesis is bactericidal (Galloway and Raetz, 1993; Kelly et al., 1993). Mutants in these genes must be studied as conditional lethals (Raetz, 1990; Raetz, 1993). The previous demonstration by Beall and Lutkenhaus (Beall and Lutkenhaus, 1987) of lethality associated with insertional inactivation of the envA reading frame is consistent with these observations. We have confirmed that extracts of the envA1-bearing point mutant do indeed possess residual enzymatic function (Tables II and III), as postulated by Beall and Lutkenhaus (1987). The question of why the depletion of lipid A deacetylase activity has such pleiotropic effects on cell morphology and outer membrane permeability requires further examination.

The apparent instability of the lpxC/envA+ gene, when introduced into E. coli on medium or high copy number plasmids (Sullivan and Donachie, 1984), also requires further investigation. Overexpression of the deacetylase might consume too much R-3-hydroxymyristoyl acyl carrier protein (Fig. 1) resulting in depletion of membrane glycerophospholipids.

The lpxC (envA) gene bears no significant relationship to other reading frames in GenBankTM. It contains no structural motifs that would suggest to what family of amidasase (i.e. metallo, serine, or cysteine) it belongs. Mechanistic studies will be facilitated by the availability of large amounts of deacetylase. Cloning of the lpxC gene from other bacterial species, like Pseudomonas, would also serve to identify critical amino acid residues and might shed light on the mechanism of the deacetylase.

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The envA Permeability/Cell Division Gene of Escherichia coli Encodes the Second Enzyme of Lipid A Biosynthesis: UDP-3-O-(R-3-HYDROXYMYRISTOYL)-N-ACETYLGUCOSAMINE DEACETYLASE

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