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The *lytB* Gene of *Escherichia coli* Is Essential and Specifies a Product Needed for Isoprenoid Biosynthesis

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The lytB Gene of Escherichia coli Is Essential and Specifies a Product Needed for Isoprenoid Biosynthesis

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LyBt and GcpE, because they are codistributed with other pathway enzymes, have been predicted to catalyze unknown steps in the nonmevalonate pathway for isoprenoid biosynthesis. We constructed a conditional Escherichia coli lytB mutant and found that LytB is essential for survival and that depletion of LytB results in cell lysis, which is consistent with a role for this protein in isoprenoid biosynthesis. Alcohols which can be converted to pathway intermediates beyond the hypothesized LytB step(s) support limited growth of E. coli lytB mutants. An informatic analysis of protein structure suggested that GcpE is a globular protein of the TIM barrel class and that LytB is also a globular protein. Possible biochemical roles for LytB and GcpE are suggested.

The Escherichia coli K-12 chromosome has about 4,300 genes, approximately one-third of which have unverified or unknown functions (4). In order to extend the annotation of the E. coli genome, we have deleted uncharacterized genes and analyzed the resultant phenotypes. Among our targets was the gene that was called yaaD in EcoMap 9 (3), was later designated slpA (12), and is designated lytB (22) following its functional characterization as a gene that encodes a peptidyl-prolyl cis-trans isomerase of the FKBP family. We show here that our inability to delete fkpB in haploid bacteria can be attributed to the polar effect of deletion of fkpB on expression of the downstream gene lytB.

lytB is highly conserved and has a pattern of distribution similar to that of genes in the nonmevalonate pathway for isoprenoids biosynthesis used by bacteria and plants (7). Isoprenoids are universally required metabolites that in bacteria have roles in processes as diverse as respiration (ubiquinones) and cell wall synthesis (bactoprenols) (23). Recent evidence indicates that the Synechocystis sp. strain PCC 6803 lytB homolog is required for isoprenoid production and is probably essential for survival (7). We found that the E. coli lytB gene is essential for viability and has a phenotype consistent with a role in isoprenoid synthesis.

FkpB is dispensable but LytB is essential for survival in rich medium. In order to delete fkpB, a crossover PCR product was constructed (15) (Fig. 1) and cloned into the PacI site of pNEB193 (New England Biolabs). (Crossover PCR produces a fragment with a central deletion.) A Cmρ cassette was inserted into the central Ecl136II site to obtain pfkpB<->CAT. (In this paper we use the nomenclature suggested by Yu et al. [24], where a<->b means that gene a is replaced by gene b.) P1 phage transduction was used to recover MG1655 mutants in which fkpB had been replaced by CAT, as described previously (8). P1 prepared on CAG18442 (pfkpB<->CAT), in which thr-39::Tn10 (Tetρ) is <1 min to the left of fkpB, was used to transduce MG1655 to Cmpρ with Tetρ as an external linked marker. Of several hundred Tetρ Cmpρ progeny screened, none was Ampρ (i.e., had resolved the duplication resulting from plasmid insertion to replace fkpB), suggesting that fkpB could not be deleted and might be essential.

We modified our procedure to use an external marker, dapB, very closely linked to fkpB (Fig. 1) and introduced compatible, complementing plasmids into both the donor and the recipient so that chromosomal deletants did not lack essential genes. The donor used was MG1655(pfkpB<->CAT, pGB-XY), and the dapB recipient was AT999(pGB-XY). pGB-XY contains the insert from pGM21 (17) (kindly provided by E. Ishiguro) which includes the contiguous ileS, lbp, fkpB, and lytB genes and part of the downstream yaaF gene reconstituted into pGB2 (6), a SpeI pSCI101 replicon. fkpB and lytB are thought to be transcribed from promoters located within ileS (18). Dapρ Cmpρ Ampρ transductants were obtained from the cross, indicating that replacement is possible in the presence of pGB-XY. Replacement of fkpB was verified by Southern blotting, and the close linkage of dapB and Cmpρ was confirmed by further transduction (data not shown). The deletion/replacement was transduced to MG1655(pGB-XY), selecting for Cmpρ, but could not be transferred to plasmid-free recipients.

Because fkpB is cotranscribed with the downstream lytB gene, it was not clear whether fkpB or lytB or both are essential. To determine this, we transduced the fkpB replacement to MG1655 with extrachromosomal copies of both fkpB and lytB on pSP47, of only fkpB on SP47ΔlytB, or of only lytB on pBAD-L (pBAD18 [9] in which the lytB coding sequence was cloned under the control of the arabinose operon promoter, P araB). The chromosomal DNA in these plasmids is shown in Fig. 1. Table 1 shows that fkpB is dispensable but LytB is not. The growth rate of MG1655 fkpB<->CAT (pBAD-L) on Luria-Bertani (LB) medium containing arabinose was identical to that of MG1655 (data not shown). Microscopic observation showed that cells were normal in appearance. We concluded that fkpB is not needed for normal growth under these conditions.
To study LytB function further, we constructed a conditionally expressing system. Crossover PCR was used to construct a 1-kb fragment in which the sequence flanking lytB was joined. The fragment was cloned into the SmaI/Sall sites of pKO3 (15), and a Kan^r cassette was cloned into the central NotI site created during amplification. The resulting plasmid, pflytB/H11021/H11022KAN, was transformed into MG1655(pBAD-L) grown with arabinose, and replacements were isolated as described previously (15). A representative construct, strain MG/H9004Ly, was unable to form colonies under conditions in which PBAD is inactive (arabinose absent, glucose present), confirming that LytB is an essential protein.

![Diagram](image.png)

**FIG. 1.** The 0.5-min region of the *E. coli* chromosome. The genes referred to in this paper are drawn to scale, and the directions of transcription are indicated by the arrowheads. Promoters predicted from the sequence (3) are indicated by P. Chromosomal restriction sites used in plasmid construction are shown (Ps, PstI; X, XbaI; RV, EcoRV; S, SnaBI). pSP47ΔlytB was constructed from pSP47 by deletion; the inserts for the remaining plasmids, other than pGB-XY, were constructed by using PCR amplification. The following primers were used to construct deletion plasmid pfkpB<>CAT: For (5’AAATTCGCGTATTATTACGATTTCCACGAAGTG) and Fr (5’CCGATGGACCGCTCCGGGTCACCGATGCGTCTGTACAGATTCAGACATGCAGG). The following primers were used to construct deletion plasmid pflytB<>KAN: For (5’ATTGCTGCGAAATCGTCGACCG) and Fr (5’ACGCTACGCGGCCGCTACACGGTTGTCATTAGCAGCCTAAGTTATGCG). The DNA between the primer pairs is absent from the chromosomes of deletion strains.

![Graph](image.png)

**FIG. 2.** Growth of MG/H9004Ly after depletion of LytB. Cultures grown in LB medium containing 0.2% arabinose and 50 μg of ampicillin per ml were diluted and grown in the same medium for 3.5 generations. At the times indicated by the arrows cultures were diluted into medium with arabinose or glucose (0.2%). Cultures were maintained in the exponential phase at all times. Large decreases in optical density at 600 nm (O.D. 600) indicate times of dilution. (A) Growth with arabinose or glucose; (B) cultures contain glucose and each of the alcohols indicated at a concentration of 10 mM.

### TABLE 1. Successful transduction of fkpB<>CAT requires lytB expression

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Arabinose</th>
<th>No. of Cmp^r transductants</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSP47^a</td>
<td>NA^c</td>
<td>200</td>
</tr>
<tr>
<td>pSP47ΔL^b</td>
<td>NA</td>
<td>2^d</td>
</tr>
<tr>
<td>pBAD18</td>
<td>+</td>
<td>6^d</td>
</tr>
<tr>
<td>pBAD18</td>
<td>+</td>
<td>3^d</td>
</tr>
<tr>
<td>pBAD-L</td>
<td>+</td>
<td>1^d</td>
</tr>
<tr>
<td>pBAD-L</td>
<td>+</td>
<td>223</td>
</tr>
</tbody>
</table>

^a pSP47 (a gift from E. Ishiguro) is pET30-c in which an XbaI-EcoRV fragment of the pOM21 (17) insert was cloned. pSP47 lacks all DNA upstream of the final 20 bp of *lp* (which contains a putative σ70 promoter).

^b pSP47ΔL was made by deleting all DNA between the SmaI site 240 bp downstream from the *lytB* start and the EcoRV site at the end of the region cloned in pSP47.

^c NA, not applicable.

^d The small number of Cmp^r transductants were Amp^r and most likely resulted from insertion and transduction of the complementing plasmid from donor to recipient.
Evidence that LytB is defective in isoprenoid synthesis. Because LytB synthesis in MGΔly cultures could be inhibited by removing arabinose and adding glucose, it was possible to examine the effects of LytB depletion on cells. During depletion, growth continued normally for about 3 h and then slowed; lysis followed at about 4.6 h (Fig. 2A). Examination of the cultures showed that cells were converted to spheroplasts en route to lysis (Fig. 3). This phenotype can be explained since isoprenoids are required to make the bactoprenols which transport peptidoglycan precursors to the periplasm. Cunningham et al. (7) used 3-methyl-3-buten-1-ol (A3) and 3-methyl-2-buten-1-ol (A2), alcohol analogs of 3-methyl-3-buten-1-ol diphosphate (isopentenyl diphosphate [IPP]) and 3-methyl-2-buten-1-ol diphosphate (dimethylallyl diphosphate [DMAPP]) (see pathway in Fig. 4), to support the growth of Synechocystis cells deficient in LytB. We tested these alcohols to see if they were able to replace the requirement for LytB in E. coli. We found that they could not (presumably because they were not efficiently converted to the diphosphorylated derivatives that they would need to replace) during exponential growth in broth. However, they must have been successfully transported into the cell to some extent because they prevented lysis and allowed growth to continue for a period beyond the time when lysis would have occurred (Fig. 2B). The response to A2 was better than the response to A3 and was similar to the response to the two alcohols together. On LB medium plates containing glucose (to repress P_BAD) adding A2 or both alcohols resulted in slow colony formation, most likely because viability was sustained until changed intracellular conditions resulted in P_BAD induction. To show that the alcohols circumvent the lytB mutation rather than prevent lysis generally, we added the alcohols to dapA cells which had been deprived of diaminopimelic acid. The time and rate of lysis of the dapA mutant were not altered.

We also constructed a strain in which a single copy of lytB was present on the chromosome under the control of the P_BAD promoter by using the method and a plasmid kindly provided by Hans Loferer (2). This strain, MG1655 araBCDΔH11021/H11022 lytB, was not able to form colonies in the absence of arabinose on rich or minimal solid media with or without alcohols, showing that the alcohols could not replace LytB activity. Addition of alcohols to broth cultures of this strain delayed lysis (data not shown).

Nonmevalonate pathway and LytB function. The 1-deoxy-d-xylulose-5-phosphate (DOXP) pathway (14) is used in green plants and many bacteria instead of the mevalonate pathway to support growth of Synechocystis cells deficient in LytB. The pathway involves the production of 2C-methyl-D-erythritol-2,4-cyclophosphate (MCP), which is then used to synthesize isoprenoids. The reaction sequence involves several enzymes, including DOXP synthase (Dxs), DOXP reductoisomerase (Dxr), and isopentenyl diphosphate (IPP) synthase (Gpp). The resulting IPP is then used to synthesize bactoprenol, which is essential for peptidoglycan transport.

FIG. 3. Cell lysis in LytB-depleted cultures. Samples were taken from the cultures described in the legend to Fig. 2A at 360 min, fixed, and later photographed. (A) Spheroplasting in LytB-depleted cultures grown with glucose; (B) cells grown with arabinose.

FIG. 4. Known and postulated reactions of the DOXP pathway.
E. coli pathway, is present and active in that although and the isomers IPP and DMAPP. It has been shown (10) that reactions occur in two steps: dehydration followed by enoyl alcohols in a single enzymatic step. Most commonly, these eliminations (reduction of a primary alcohol and reduction of a secondary alcohol) and a ring-opening elimination reaction. Ei- ter IPP or DMAPP could be the product of this reaction, depending on which carbon contributes the hydrogen atom to the elimination. There are no known examples of reduction of alcohols in a single enzymatic step. Most commonly, these reactions occur in two steps: dehydration followed by enoyl reduction by NADPH. These considerations imply either that there are still a number of unrecognized genes in the DOXP pathway or that the alcohol reduction steps occur by non-pathway-specific mechanisms.

The two remaining genes with a pattern of homology that indicates that their functions may be specific to this pathway are lytB and gcpE (7). Our results and those of Cunningham et al. (7) show that lytB is an essential gene in the pathway, gcpE has recently also been shown to be essential (1, 5). Secondary-structure predictions (Fig. 5 and 6) indicate that both proteins are globular, α/β-units of the TIM barrel tertiary structure, inferred from a comparison of the sequences of homologues of GcpE and of the PDB structure 1THF.

generate the isoprenoid precursors IPP and DMAPP. This pathway (Fig. 4) originates with pyruvate and glyceraldehyde-3-phosphate. Almost all of the biochemical steps are now known.

The most recently identified reactions and genes are those that follow the formation of DOXP. The first reaction is simultaneous reduction and isomerization to 2-C-methyl-D-erythritol-4-phosphate, catalyzed by the dxr gene product. This enzyme has been isolated from E. coli and characterized (13). The chemistry of the three following steps has also been established: CTP-dependent cytidylation catalyzed by YgbP (20) and characterized (13). Our results and those of Cunningham et al. (7) show that gcpE is an essential gene in the pathway. gcpE indicates that their functions may be specific to this pathway.
and Salmonella typhimurium: cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.


