Supporting Information

An Alternative Mechanism for UDP-diacylglucosamine Hydrolysis during Bacterial Lipid A Biosynthesis

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Supporting Table 1. Relevant primers for this study.

Name	Purpose	Primer sequence (5' to 3' orientation)
EcH_FW	To amplify *E. coli lpxH*, confers a 5' *ndeI* restriction site	GCTGATTCATTTTCCGTTTTCAGGA
EcH_RV	To amplify *E. coli lpxH*, confers a 3' *bamH1* restriction site	GAGCTCGAATTCGGATCCTGAAAAC
CcI_FW	To amplify *C. crescentus lpxI*, confers a 5' *ndeI* restriction site	CTGAATTCATATGCGTAAGCTTGG
CcI_RV	To amplify *C. crescentus lpxI*, confers a 3' *bamH1* restriction site	GTCAATTGGATCCTCACGGCCGCT
KanFlank_FW	To amplify a Kan^R cassette from pET28b, while adding a ribosome binding site 7 bases 5' to the start codon. Note that 5' to the engineered ribosome binding site, this primer is complementary to the 41 base pairs immediately flanking *lpxH* on the *E. coli* chromosome.	GAAGACGTTATCATTGAAAGCGTGACCGTTAGCGAGTAATCAG GAGATATACAATGAGCCATATTCAACGGGAAAC
KanFlank_RV	To amplify a Kan^R cassette from pET28b, while adding a 3', 49 base pair overhang complementary to the region of the *E. coli* chromosome immediately 3' to the stop codon of *lpxH*.	GCAAGGAAAACGGTTGCGTGGCTGTGAAATCAGCAAAGTTGC GGGTTTTTTAGAAAAACTCATCGAGCATC
Kan_FW	Complementary to a region of the *E. coli* chromosome 100 base pairs 5' to *lpxH*	GTGTTTGCTGAAGTGGTTGACGGCAT
Kan_RV	Complementary to a region of the *E. coli* chromosome 100 base pairs 3' to *lpxH*	CCCAGTCGCTTTTGGACCCCATCACG
pBAD_FW	Complementary to the pBAD promoter	ATGCCATAGCATTTTTATCC
pBAD_RV	Complementary to the pBAD terminator	GATTTAATCTGTATCAGG

Supporting Table 2.   Distribution of *E. coli* LpxH and *C. crescentus* LpxI orthologues among bacteria with completely sequenced genomes. Those that contain *E. coli* LpxC orthologues are presumed to synthesize lipid A. The sequences were obtained from (http://www.ncbi.nlm.nih.gov/sutils/genom_tree.cgi).

	E. coli LpxC Orthologues	*E. coli* LpxH1 Orthologues	*C. crescentus* LpxI Orthologues
Proteobacteria			
Alpha	89/92	2/92	71/92
Beta	54/54	54/54	0/54
Gamma	132/132	132/132	0/132
Epsilon	16/16	16/16	0/16
Delta	29/29	11/29	18/29
Others	1/1	0/1	1/1
Bacteroides	31/33	32/33	0/33
Chlamydiae	7/7	0/7	0/7
Spirochaetales	5/17	0/17	4/17
Actinobacteria	0/75	0/75	0/75
Cyanobacteria	26/26	0/26	0/26
Mycoplasma	0/21	0/21	0/21
Firmicutes	5/103	0/103	4/103
Others	35/53	2/53	22/53

Supporting Fig. 1. Expression and *in vitro* UDP-diacylglucosamine pyrophosphatase activity of the CC1910 gene product. Panel A. A 12% SDS-PAGE analysis of cell extracts. MW denotes the molecular weight markers, VC_21b and CcI_21b denote *E. coli* C41(DE3) cells expressing either the pET21b vector or the induced pET21b hybrid plasmid harboring CC1910, respectively. Each lane contains 20 μg of protein. Panel B. A qualitative silica TLC analysis for UDP-2,3,-diacylglucosamine hydrolase activity in cell-free extracts. Lane 1, 0.6 mg/ml lysate from strain VC_21b; Lanes 2-5, from left to right, 0.6, 0.06, 0.006, or 0.0006 mg/ml of lysate from induced CcI_21b. The assay duration was 30 minutes. Lipid substrate and product were detected after TLC by charring with 10% sulfuric acid in ethanol. NE: no enzyme.
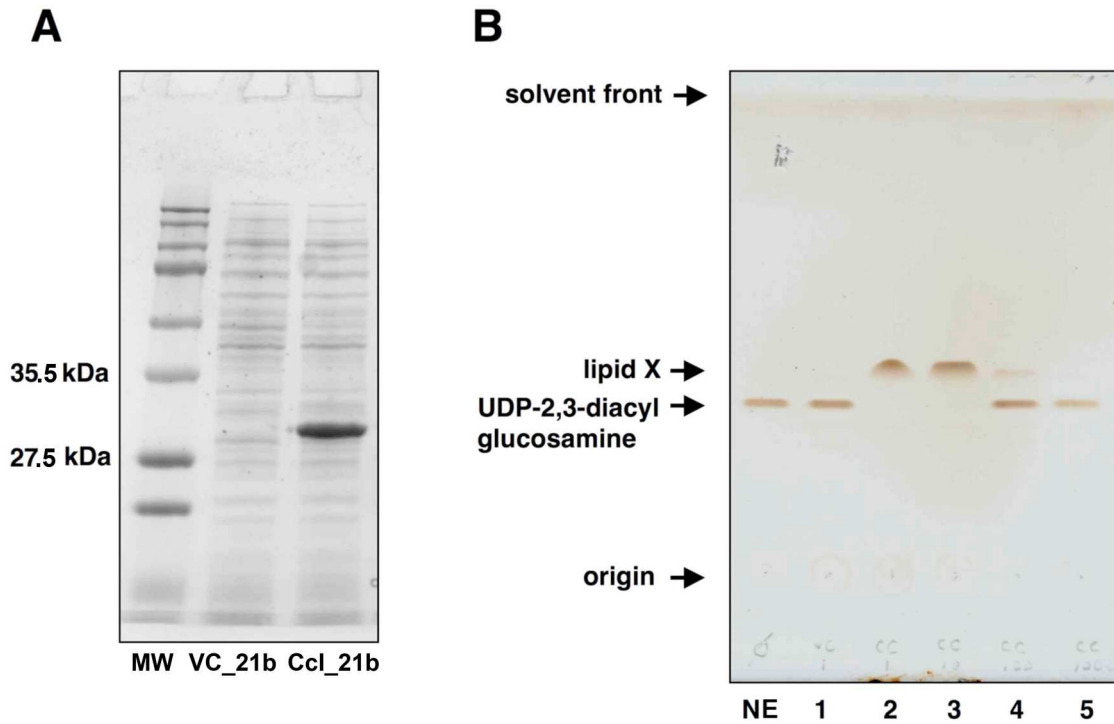
Supporting Fig. 2. Expression of *C. crescentus lpxI* permits deletion of *lpxH* in *E. coli*. The *lpxH* gene could be replaced with a *kan* cassette by linear recombination in *E. coli* DY330 (*19*), provided that a covering plasmid expressing either EcLpxH or CcLpxI was also present, as described in the methods section. In both cases, kanamycin resistant transformants were observed (middle and right plates), whereas in the vector control (left plate) no kanamycin resistant colonies were obtained. Deletion of the *lpxH* gene in kanamycin resistant transformants was confirmed by PCR and DNA sequencing.

DY330/pBAD30   DY330ΔH pBAD30 with *E. coli lpxH*   DY330ΔH pBAD30 with *C. crescentus lpxI*