Genetic Interaction Between the *Escherichia coli* AcpT Phosphopantetheinyl Transferase and the YejM Inner Membrane Protein

Nicholas R. De Lay*,[1] and John E. Cronan†,[2]

*Department of Microbiology and †Department of Biochemistry, University of Illinois, Urbana, Illinois 61801

Manuscript received September 11, 2007
Accepted for publication December 31, 2007

ABSTRACT

Strain LH530, a mutant of *Escherichia coli* K-12, was reported by others to show increased outer membrane permeability, temperature-sensitive growth, and reduced synthesis of lipid A. The unmapped mutant gene was found to be suppressed by high-copy-number plasmids carrying the wild-type *acpT* gene, which encodes a protein that catalyzes a post-translational protein modification, the attachment of 4′-phosphopantetheine. We mapped the strain LH530 mutation to a gene of unknown function, *yejM*, known to encode an inner membrane protein. The mutation is a *yejM* nonsense mutation that produces a truncated protein lacking the predicted periplasmic domain. Reconstruction of the mutation gave a strain having the same phenotypes as LH530. In contrast to the nonsense mutants, deletion of the entire *yejM* gene was lethal. Suppression by AcpT overexpression of the *yejM* nonsense mutants encoding the truncated proteins was specific to AcpT. Moreover, AcpT overexpression also suppressed the lethality due to deletion of the entire *yejM* gene and this suppression also did not require that AcpT be enzymatically active. The mechanism whereby overexpression of a specific cytosolic protein bypasses the essentiality of an inner membrane protein is unknown.

THE cell envelopes of gram-negative bacteria consist of an inner membrane, an outer membrane, and a rigid layer of peptidoglycan located between the two membranes. The outer membrane provides a protective barrier that prevents the diffusion of hydrophobic and large hydrophilic antibiotics into the cell and consists of an inner leaflet composed of phospholipids and an outer leaflet composed of lipopolysaccharides. The structure of lipopolysaccharide can be divided into three components: lipid A, the core oligosaccharide, and the O-antigen. The lipid A portion of the lipopolysaccharide anchors the lipopolysaccharide to the outer leaflet of the outer membrane. The synthesis of lipid A in *Escherichia coli* has been well studied and serves as a model system for understanding this process (RAETZ *et al.* 2007). The lipid A molecules synthesized by *E. coli* and other gram-negative bacteria consist of a β-1′-6-linked disaccharide of *N*-acetylglucosamine that is modified by the attachment of fatty acids, phosphates, and additional saccharides (RAETZ *et al.* 2007). Additional covalent modifications to the core lipid A molecule, such as the addition of a palmitate or modification of the 1′- or 4′-phosphates with L-4-aminoarabinose or phosphoethanolamine, occur when the outer membrane is stressed (RAETZ *et al.* 2007).

Mutations have been identified in several lipid A biosynthetic genes that result in reduction of lipid A synthesis and a hypersensitivity to hydrophobic and large hydrophilic antibiotics. These mutations occur in the *lpxA*, *lpxC*, and *lpxD* genes (NORMARK 1970; TSURUOKA *et al.* 1988; GALLOWAY and RAETZ 1990; KLOSER *et al.* 1996; VAARA and NURMINEN 1999). Both *lpxA* and *lpxD* encode enzymes that transfer a fatty acid, β-hydroxymyristate, to nascent lipid A species (CROWELL *et al.* 1986; KELLY *et al.* 1993) whereas *lpxC* encodes an enzyme that deacetylates a precursor of lipid A (YOUNG *et al.* 1995).

HIRVAS *et al.* (1997) isolated another *E. coli* mutant strain, strain LH530, that was hypersensitive to hydrophobic and large hydrophilic antibiotics and showed temperature-sensitive growth. Strain LH530 was reported to be defective in lipid A biosynthesis but the mutant gene was neither mapped nor isolated. However, in their attempts to isolate the responsible gene, these workers identified a gene called ORF195 (or o195), but now called *acpT*, that encodes a phosphopantetheinyl transferase (LAMBALOT *et al.* 1996; FLUGEL *et al.* 2000; DE LAY and CRONAN 2006), which suppressed the temperature-sensitive phenotype of mutant strain LH530. However, strain LH530 carried a wild-type copy of *acpT* and growth at 42° occurred only upon high levels of overexpression (HIRVAS *et al.* 1997). Therefore, growth was due to suppression rather than complementation. Strain LH530 had other phenotypes consistent with a defect in lipid A synthesis; *i.e.*, the strain leaked a periplasmic enzyme, but not a cytoplasmic enzyme, and its outer membrane contained abnormally low levels of the OmpF porin (NURMINEN *et al.* 1997).

We studied this mutation due to our interest in phosphopantetheinyl transferases (FLUGEL *et al.* 2000;

TABLE 1

Strains used in this study

Strain	Relevant features	Source or reference
CAG12177	MG1655 *atoS*::Tn*10*	Singer *et al.* (1989)
DH5α	*recA1 endA1*	Strain collection
HT253	W3110 *pdxJ8*::ΔTn*10*	Strain collection
JM105	F′ *lacI^q proAB/endA* Δ*(lac-proAB)*	Yanisch-Perron *et al.* (1985)
LH530	F′ *lacI^q proAB/endA* Δ*(lac-proAB)* Ts	Hirvas *et al.* (1997)
MC1061	*araD139* Δ*(ara-leu)7696*	Strain collection
Top10F′	F′ *lacI^q* Tn*10* (TetR)/φ80::*lacZ* Δ*M15* Δ*lacX74 recA1 endA1*	Invitrogen (San Diego)
JW0757-1	Δ*araBAD* Δ*lacZ* Δ*bioA746*::kan	CGSC; Baba *et al.* (2006)
JW0014-1	Δ*araBAD* Δ*lacZ* Δ*dnaJ735*::kan	CGSC; Baba *et al.* (2006)
CY1783	Δ*araBAD* Δ*lacZ bioA746*::*lacZY*	This study
CY1817	Δ*araBAD* Δ*lacZ* Δ*dnaJ735*::*lacZY*	This study
NRD142	LH530 *atoS*::Tn*10*	This study
NRD148	JM105 *atoS*::Tn*10* Ts	This study
NRD150	MC1061 Δ*ompC*::*kan*	This study
NRD151	MC1061 Δ*yojI*::*kan*	This study
NRD153	MC1061 Δ*rcsC*::*kan*	This study
NRD159	MC1061 Δ*atoB*::*kan*	This study
NRD161	MC1061 Δ*napB*::*kan*	This study
NRD163	MC1061 Δ*ccmH*::*kan*	This study
NRD169	MC1061 Δ*yejK*::*kan*	This study
NRD178[a]	MC1061 Δ*yejM*::*kan*	This study
NRD183	JM105 Δ*yejM*::*kan* carrying pNRD217	This study
NRD184	MC1061 *yejM569*::*kan*	This study
NRD187	JM105 *yejM569*::*kan*	This study

CGSC, Coli Genetic Stock Center (Yale University).

[a] This strain also carries a chromosomal duplication of the *yejM* region (see text).

De Lay and Cronan 2006). We report the mapping of the mutation responsible for the temperature-sensitive phenotype and other phenotypes of strain LH530. The mutant strain and the *yejM* gene have also been further characterized, including the demonstration that *yejM* is an essential gene. Moreover, we demonstrate that suppression of the temperature-sensitive phenotype of strain LH530 upon overexpression of AcpT is not due to its phosphopantetheinyl transferase activity and that AcpT overexpression also suppresses the lethality of *yejM* null mutants.

MATERIALS AND METHODS

Bacterial strains and plasmids: The bacterial strains used in this study were derivatives of strain *E. coli* K-12. All of these *E. coli* strains are listed in Table 1 except for the Tn*10* insertion strains used for gene mapping, which were previously described. (Singer *et al.* 1989; Nichols *et al.* 1998). All plasmids used in this study are listed in Table 2. The primers used in this study (supplemental Table 1S) were purchased from Integrated DNA Technologies. Fosmid pCC1Fos was obtained from Epicentre. Site-directed mutagenesis was done by the Quickchange PCR method (Stratagene, La Jolla, CA).

Strain NRD142 was constructed by phage P1*vir* transduction of the Tn*10* of strain CAG12177 into strain LH530 (Miller 1972) followed by screening of the tetracycline-resistant transductants for maintenance of the temperature-sensitive phenotype. Strain NRD148 was generated by transducing the Tn*10* from strain NRD142 into strain JM105 and screening the resulting tetracycline transductants for a strain that had acquired a temperature-sensitive phenotype. The Δ*ompC* strain NRD150 was constructed by λ-Red recombinase-mediated gene replacement (Datsenko and Wanner 2000) using the PCR product generated from the template pKD4 (Datsenko and Wanner 2000) with the OmpCKO For and Rev primers. The strains carrying deletions of *yojI, rcsC, atoB, napB, ccmH, yejM,* and yejK were generated via λ-Red recombinase-mediated gene replacement using the PCR product generated from the template pKD4, using the pair of primers named for the gene (supplemental Table 1S). Strain NRD183 was constructed by transduction of the kanamycin resistance construct from strain NRD178 into strain JM105 carrying plasmid pNRD217. This transduction was performed in the presence of arabinose, and transductants were selected on LB plates containing kanamycin and arabinose. The wild-type *yejM* gene was modified by introducing a UGA termination codon in place of the codon for Y190 by an UGA (opal) codon and by substituting a kanamycin resistance cassette for the *yejM* sequences downstream of the mutated codon by λ-Red-mediated recombination using a PCR product generated from the template plasmid pKD4 using primers YejMKO4 For and YejMKO Rev. Kanamycin-resistant recombinants were selected on LB plates containing kanamycin at 28°. Strain NRD187 was generated by transduction of the kanamycin resistance determinant from strain NRD184 into strain JM105. The *lacZY* transcriptional fusions to *bioA* and *dnaJ* were constructed as described by Ellermeier *et al.* (2002) except that vectors with improved ribosome-binding sites (J. Slauch, personal communication) were used and pCY580 (Cronan 2003) was used

TABLE 2

Plasmids used in this study

Plasmid	Relevant features	Source or reference
pBAD24	Amp[r], *araBAD* promoter-based expression vector, pUC-type *ori*	GUZMAN *et al.* (1995)
pBAD30	Amp[r], *araBAD* promoter-based expression vector, p15a *ori*	GUZMAN *et al.* (1995)
pBAD322	Amp[R], *araBAD* promoter-based expression vector, complete pBR322 origin	CRONAN (2006)
pCR2.1	Amp[r], Kan[r], cloning vector, pUC *ori*	Invitrogen
pKD4	oriRγ, Kan[r] cassette flanked by FRT sites	DATSENKO and WANNER (2000)
pKD46	Amp[R], RepA1019(Ts), λ, γ, β, and *exo* expressed from an *araBAD* promotor	DATSENKO and WANNER (2000)
pQE70-Sfp	Amp[r], pQE70-derived plasmid encoding Sfp with a C-terminal His$_6$ tag	MOFID *et al.* (1999)
pYon113	Amp[r], *acpT* under the control of an *araBAD* promoter	FLUGEL *et al.* (2000)
pNRD28	*acpS* amplified from JM105 using primers AcpSBAD For and Rev and TOPO cloned into pCR2.1	This study
pNRD83	*Eco*RI–*Hin*dIII *acpS* fragment of pNRD28 inserted into the same sites of pBAD322	This study
pNRD192	*Eco*RI–*Xba*I *acpS* fragment of pQE70-Sfp inserted into the same sites of pBAD30	This study
pNRD203	*Eco*RI–*Hin*dIII *acpS acpS* fragment of pNRD83 inserted into the same sites of pBAD24	This study
pNRD204	*Eco*RI–*Xba*I *sfp acpS* fragment of pNRD192 inserted into the same sites of pBAD18	This study
pNRD205	Encodes AcpT D91E. Site-directed mutation in *acpT* in pYon113 was generated using primers AcpTD91E For and Rev.	This study
pNRD206	Encodes AcpT E137S. Site-directed mutation in *acpT* in pYon113 was generated using primers AcpTE137S For and Rev.	This study
pNRD207	Encodes AcpT K141A. Site-directed mutation of *acpT* in pYon113 using primers AcpTK141A For and Rev.	This study
pNRD216	*yejM* amplified from JM105 using primers YejM For and Rev primers and TOPO cloned into pCR2.1	This study
pNRD217	*Nco*I–*Xba*I *yejM* containing fragment from pNRD216 inserted into the same sites of pBAD322	This study

as the source of Flp recombinase in the latter construction. β-Galactosidase activities were determined according to MILLER (1972).

Culture media and growth conditions: Strains were grown in LB liquid medium or on LB agar plates. LB medium was supplemented with final concentrations of glucose (0.2%) or arabinose (0.08%). Antibiotics were used at the following concentrations: 100 mg liter^{-1} ampicillin, 50 mg liter^{-1} kanamycin, and 5 mg liter^{-1} tetracycline.

Gene mapping: The mutation conferring temperature sensitivity to strain LH530 was initially localized on the *E. coli* K-12 chromosome by transduction of strain LH530 with P1*vir* lysates made from a collection of strains harboring Tn*10* insertions spaced around the *E. coli* chromosome (SINGER *et al.* 1989; NICHOLS *et al.* 1998). P1*vir* lysates were made from nearly all of the strains in this collection that have a Tn*10* insertion within the first 56 min of the *E. coli* chromosome. These P1*vir* lysates were used to transduce the Tn*10* insertion from each of these strains into strain LH530. The transductants that acquired the Tn*10* were selected for the ability of these strains to grow at 28° on LB plates containing tetracycline. Between 48 and 102 of tetracycline-resistant transductants obtained from each transduction were screened for the loss of temperature sensitivity by streaking each transductant on two LB plates containing tetracycline followed by incubation of one plate at 28° and the other at 42°. Two Tn*10* insertions cotransduced with the temperature-sensitive mutation of strain LH530. One Tn*10* was inserted into *napA* whereas the other was inserted into *atoS*. To further define the location of the mutation, the cotransduction frequencies of the mutation with a kanamycin

marker replacing *yejK*, *ccmH*, *napB*, *yojI*, *ompC*, *rcsC*, or *atoB* were determined. More than 200 kanamycin-resistant transductants resulting from transduction of strain LH530 with phage P1*vir* lysates grown on strains NRD169, NRD163, NRD161, NRD151, NRD150, NRD153, or NRD159 were screened for temperature sensitivity as described above. Once a very closely linked marker was identified, the gene replaced by that marker, together with ~2500 bp of adjacent genomic DNA from both strain LH530 and the parental strain JM105, was sequenced after amplification by PCR using the YejK, YejL, and YejM primers. Each PCR product was directly sequenced with both primers used for amplification by the Core Sequencing Unit at the Keck Center for Comparative and Functional Genomics at the University of Illinois.

RESULTS

Mapping of the mutation responsible for the temperature-sensitive phenotype of the *E. coli* K-12 strain LH530: HIRVAS *et al.* (1997) previously identified a temperature-sensitive mutant named strain LH530 that had a phenotype similar to that of mutants defective in lipid A synthesis; *i.e.*, the mutant was sensitive to hydrophobic and large hydrophilic antibiotics at permissive temperatures and was reported to have subnormal rates of lipid A synthesis at all temperatures. HIRVAS *et al.* (1997) found that plasmids carrying a gene
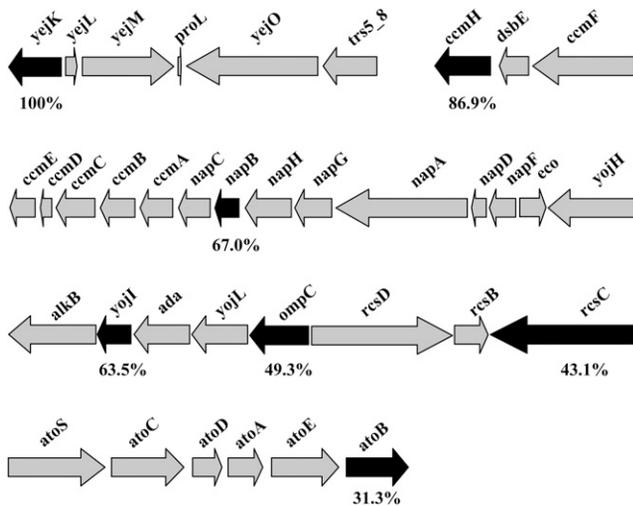
FIGURE 1.—The segment of the *E. coli* chromosome that contains *yejM*. Transductions using strain LH530 as the recipient were performed using P1*vir* lysates made from derivatives of strain MC1061, which had one of the genes denoted by a solid arrow replaced with a kanamycin resistance cassette. Transductants were selected on LB–kanamycin plates incubated at 28° for 2 days. From each cross at least 200 kanamycin-resistant transductants were then screened for temperature-sensitive growth. Below each of the solid arrows is the frequency at which the kanamycin marker replacing the gene of interest cotransduced with the gene that relieved the temperature-sensitive phenotype of strain LH530.
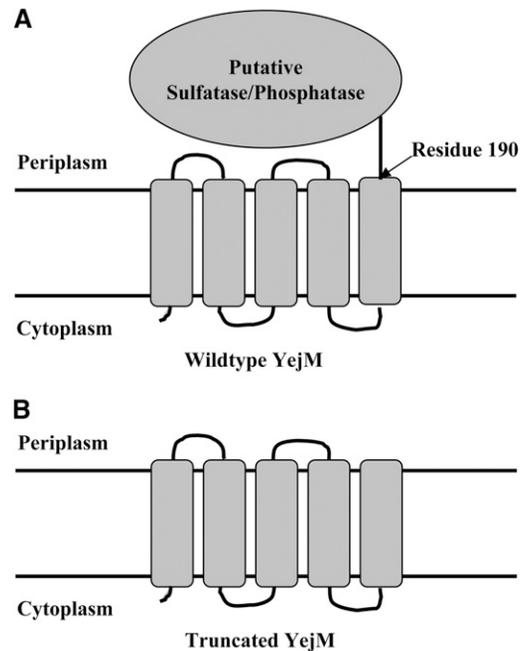

FIGURE 2.—An illustration of the putative structure of the full-length YejM (A) and the truncated form of YejM produced by strain LH530 (B) based on the data and predictions of DALEY *et al.* (2005). YejM is an inner membrane protein that contains five putative membrane-spanning helices and a periplasmic C-terminal domain. The periplasmic domain of YejM is predicted to have sulfatase/phosphatase activity in the UniProtKB/Swiss-Prot database.

named *acpT* (then called ORF195) suppressed the temperature-sensitive phenotype of this mutant strain, but only when the AcpT-encoding plasmids were of high copy number. However, the *acpT* gene of strain LH530 had the same sequence as the parental wild-type strain, indicating suppression rather than complementation. The mutation responsible for the temperature-sensitive phenotype of strain LH530 was not identified or mapped.

We mapped the mutation responsible for the temperature-sensitive phenotype of strain LH530 by transducing strain LH530 with phage P1*vir* lysates of a collection of strains, each of which carried a transposon Tn*10* antibiotic resistance element inserted at a known chromosomal location (SINGER *et al.* 1989; NICHOLS *et al.* 1998). Upon screening the first 56 min of the *E. coli* chromosome, the temperature-sensitive mutation of strain LH530 was found to be ~53 and 29% linked to Tn*10* insertions in *napA* and *atoS*, respectively (Figure 1). To further resolve the location of the mutation, a set of nonessential genes located in this region of the *E. coli* chromosome (*yejK, ccmH, napB, yojI, ompC, rcsC,* and *atoB*) were replaced with kanamycin resistance cassettes via λ-Red recombinase-mediated gene replacement (DATSENKO and WANNER 2000) and the linkage of the mutation with these markers was determined by measuring cotransduction frequencies (Figure 1). The temperature-sensitive mutation of strain LH530 was found to very tightly linked (100%) to the kanamycin

resistance cassette inserted in place of *yejK*. We then amplified the genes *yejK, yejL,* and *yejM* and the intergenic regions between these genes from strain LH530 and its parental strain JM105 and sequenced the PCR products. The sequencing data showed a single difference between the two genomic sequences: a G570A mutation in the *yejM* gene of strain LH530. The G570A mutation resulted in replacement of the UGG codon of tryptophan 190 with a UGA termination codon. YejM has previously been shown to be an inner membrane protein predicted to be composed of a transmembrane domain consisting of five putative membrane-spanning helices and a large periplasmic domain (DALEY *et al.* 2005) (Figure 2). The nonsense mutation would truncate the expressed protein to 190 residues from 586 residues and would cleanly remove the predicted periplasmic domain while leaving the predicted transmembrane domain intact (Figure 2).

We linked the *yejM* mutation of strain LH530 to the Tn*10* insertions within *napA* and *atoS* by transduction and then used these strains as donors to transduce strains JM105 and MG1655 to tetracycline resistance. The resulting transductants were then screened for temperature-sensitive growth. The temperature-sensitivity profile observed in strain NRD187, the derivative of strain JM105 that had acquired the *yejM*(Ts) mutation, was identical to that observed for strain LH530 (data not shown). However, although the *yejM*(Ts)
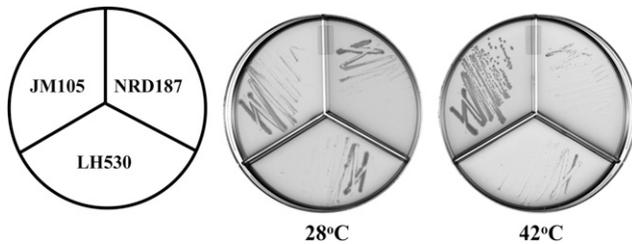
FIGURE 3.—The growth of strains JM105, NRD187, and LH530 on LB plates incubated overnight at either 28° or 42°.
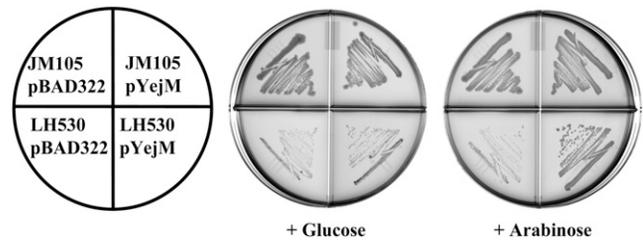

FIGURE 4.—Complementation of the temperature-sensitive strain LH530 by a plasmid-encoded copy of *yejM*. Strain JM105 or the derived temperature-sensitive mutant, strain LH530, carrying either the vector pBAD322 or a plasmid containing a copy of *yejM* under the control of the *araBAD* promoter (pNRD217 called pYejM in the figure) were tested for the ability to grow at 42° on LB plates containing either glucose or arabinose.

derivative of strain MG1655 was clearly temperature sensitive, this strain showed greater growth at 42° than did the strain JM105 derivatives (data not shown). This difference in behavior can be attributed to the fact that strain MG1655 grew significantly faster than strain JM105 and this faster growth accentuated the leakiness (formation of small colonies) shown by strain LH530 upon prolonged incubation at 42°.

Characterization of the *yejM*(Ts) mutation: To confirm that the premature termination codon in the *yejM* gene of strain LH530 was responsible for the temperature-sensitive growth of strain LH530, we used λ-Red recombinase-mediated gene replacement to generate the nonsense (G570A) mutation of strain LH530 in the wild-type gene and to concomitantly replace the *yejM* sequence downstream of the mutation with a kanamycin resistance cassette. This construct was then transduced into strain JM105 to generate strain NRD187. We then compared the abilities of strains JM105, LH530, and NRD187 to grow on LB plates incubated at 28° or 42° overnight. Although all three strains grew well at 28°, only strain JM105 grew at 42° following overnight incubation (Figure 3). However, upon prolonged incubation, strain LH530 formed small colonies at 42° whereas strain NRD187 did not. This difference suggested that in strain LH530 basal readthrough of the UGA nonsense codon (BJORNSSON and ISAKSSON 1993) resulted in low levels of full-length YejM. Strains LH530 and NRD187 had essentially identical lipid A phenotypes (see below) and, as expected from the previous report (HIRVAS *et al.* 1997), both strains were much more sensitive to rifampicin and erythromycin than strain JM105.

We performed complementation experiments to assess whether or not expression of a wild-type copy of *yejM* either from its native promoter on a cosmid vector or from an *araBAD* promoter on a multicopy plasmid allowed growth of strain LH530 at 42°. A fragment of DNA that contained *yejM*, together with the upstream and downstream genes *yejL* and *proL*, respectively, was amplified from strain JM105. This genome segment was then inserted into the single-copy F-factor-based cosmid pCC1Fos. The presence of this construct in strain LH530 allowed growth at the nonpermissive temperature (data not shown). We then placed the *yejM* gene under the *araBAD* promoter of pBAD322 and tested the ability

of the resulting plasmid, pNRD217, to complement growth of strain LH530 at 42° on plates containing either glucose or arabinose. Strain LH530 containing pNRD217 grew on the LB–arabinose-containing plates, but did not grow on the plates containing glucose (which represses expression from the *araBAD* promoter) (Figure 4). The introduction of plasmid pNRD217 into strain NRD187 similarly allowed growth at 42° on LB plates containing arabinose but not on those containing glucose (data not shown). The inability to recover a plasmid-encoding *yejM* by complementation of the mutation of LH530 indicates that the clone bank screened by HIRVAS *et al.* (1997) lacked a *yejM* plasmid presumably due to a bias in construction of the bank.

The *yejM*(Ts) mutation is suppressed by overexpression of active and inactive forms of AcpT, but not by high-level expression of two other phosphopantetheinyl transferases, AcpS and Sfp: HIRVAS *et al.* (1997) have previously shown that the temperature-sensitive phenotype of strain LH530 is suppressed by high-copy-number plasmids carrying *acpT*, which encodes a phosphopantetheinyl transferase that plays a minor role in *E. coli* K-12 lipid metabolism (DE LAY and CRONAN 2006). Phosphopantetheinyl transferases are enzymes that transfer the 4′-phosphopantetheinyl moiety of CoA to a conserved serine residue of a carrier protein such as the acyl carrier proteins of lipid synthesis (LAMBALOT *et al.* 1996). Attachment of this prosthetic group is mandatory because the thiol group of the phosphopantetheinyl moiety carries the intermediates of fatty acid biosynthesis. We tested the ability of three phosphopantetheinyl transferases, AcpT, AcpS, and Sfp, to suppress the temperature-sensitive phenotype of LH530 upon overexpression. *E. coli* AcpS is a trimeric phosphopantetheinyl transferase that catalyzes the modification of acyl carrier proteins of fatty acid biosynthesis (FLUGEL *et al.* 2000) whereas Sfp is a monomeric *Bacillus subtilis* enzyme that catalyzes phosphopantetheinylation of carrier proteins involved in secondary metabolic processes such as nonribosomal peptide and polyketide syntheses (LAMBALOT *et al.* 1996; MOOTZ *et al.* 2001). As previously
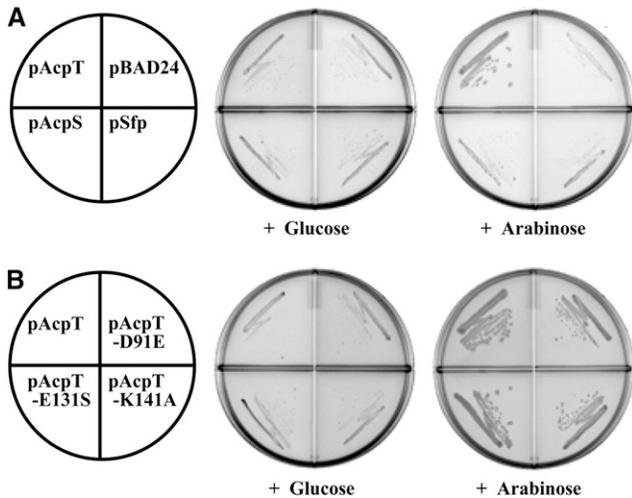
FIGURE 5.—Suppression analyses of strain LH530 by plasmids encoding either diverse phosphopantetheinyl transferases or site-directed mutants of the AcpT phosphopantetheinyl transferase. (A) Derivatives of strain LH530 carrying the vector pBAD24 or a plasmid that expressed *sfp, acpS,* or *acpT* under the control of an *araBAD* promoter (pNRD204, pNRD83, and pYon113, respectively) were tested for the ability to grow at 42° on LB plates containing glucose or the inducer, arabinose. (B) The ability of wild-type AcpT or of several site-directed mutants of AcpT that eliminate phosphopantetheinyl transferase activity *in vivo* (Table 2) was tested for the ability to suppress the defective growth of strain LH530 at 42° on LB plates containing either glucose or arabinose.

reported by HIRVAS *et al.* (1997), high-level overexpression of AcpT suppressed the temperature-sensitive phenotype of strain LH530 (Figure 5) whereas low levels of overexpression did not (data not shown). However, neither overexpression of AcpS nor high-level expression of Sfp suppressed the temperature-sensitive phenotype of strain LH530 (Figure 5). Similar results were obtained for strain NRD187, which carries the *in vitro*-constructed *yejM* nonsense allele (data not shown).

The lack of suppression of the temperature-sensitive phenotype of strain LH530 by high-level expression of AcpS or Sfp suggested that the phosphopantetheinyl transferase activity of AcpT was not required for suppression. Thus, we made amino acid substitutions for three key AcpT residues (D91E, E137S, and K141A) conserved among phosphopantetheinyl transferases (LAMBALOT *et al.* 1996) since they are involved in binding of the CoA substrate by Sfp and AcpS (REUTER *et al.* 1999; PARRIS *et al.* 2000; MOFID *et al.* 2004). The AcpT amino acid substitutions were substitutions previously shown to eliminate Sfp phosphopantetheinyl transferase activity (MOFID *et al.* 2004) and were constructed in the pBAD24 vector. Since, in agreement with a prior report (LAMBALOT *et al.* 1996), we found that, upon overexpression, AcpT was inactive and largely insoluble, we assayed the activities of these proteins by the abilities of the plasmids to support growth of a strain carrying a conditional mutation in *acpS* (FLUGEL *et al.*

2000; DE LAY and CRONAN 2006). Upon high-level expression, all of the mutant *acpT* alleles lacked the ability to functionally replace AcpS whereas the wild-type allele showed the expected strong complementation (FLUGEL *et al.* 2000; DE LAY and CRONAN 2006) (data not shown). However, the plasmids encoding the wild-type and mutant AcpT proteins all suppressed the temperature-sensitive phenotype of strain LH530 (Figure 5). Therefore, suppression of *yejM* nonsense mutations did not require that AcpT be enzymatically active.

yejM is an essential gene, but its essentiality is suppressed by AcpT overexpression: The temperature-sensitive phenotype of strain LH530, which was due to the production of a truncated form of YejM lacking the C-terminal periplasmic domain, raised the question of the effects of a deletion of the entire *yejM* gene. Repeated attempts to delete *yejM* from strain MC1061 by λ-Red recombinase-mediated gene replacement were unsuccessful despite our ready success in deleting other genes by this method. We eventually isolated a single *yejM* deletion candidate, strain NRD178. We confirmed the presence of the *yejM* deletion in this strain using PCR primers that yielded a product only if the *yejM* deletion was present. However, the difficulty that we experienced in construction of this strain suggested that this *yejM* deletion strain might have a second characteristic that allowed viability of the deletion strain. We therefore transduced the *yejM* deletion into a derivative of strain JM105 that carried a second plasmid-borne copy of *yejM* under control of the *araBAD* promoter. Transduction was performed in the presence of arabinose and the transduction mixtures were plated on LB plates containing arabinose and kanamycin. Six transductants were tested for the ability to grow on LB plates containing glucose (where expression of plasmid-borne *yejM* was repressed) or arabinose (where expression of plasmid-borne *yejM* was induced). All six transductants grew on LB–arabinose plates but not on LB–glucose plates (Figure 6). Similar results were obtained when this experiment was performed with an MG1655 Δ*yejM* strain carrying pNRD217, the *yejM* plasmid (data not shown). Therefore, transduction seemed to have resolved the Δ*yejM* allele from another character present in the original strain, NRD178. Further analysis of the genome of the original *yejM* deletion strain NRD178, using PCR primers that generated products of different lengths depending upon the presence or absence of a wild-type copy of *yejM*, demonstrated that the original *yejM* deletion strain, strain NRD178, contained a duplication of the region of the genome that includes *yejM*. The duplicated region contained a copy of wild-type *yejM* plus a copy of the Δ*yejM* allele (data not shown). We attribute the chromosomal arrangement of strain NRD178 to λ-Red-mediated recombination into one copy of a preexisting chromosomal duplication of the *yejM* region present in the cell undergoing the recombination event. Such spontaneous duplications of bacterial chromosomes are well docu-
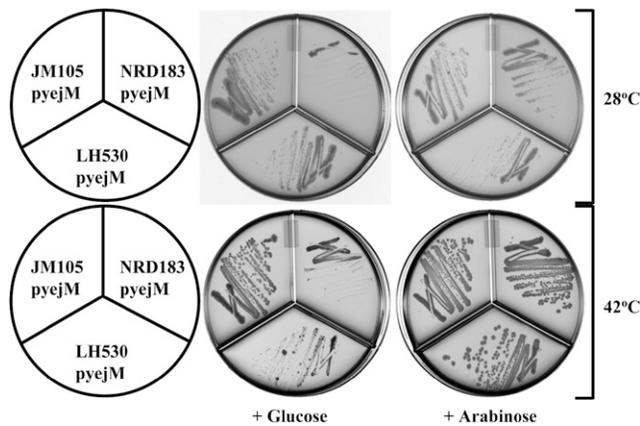
FIGURE 6.—Determination of the essentiality of *yejM*. Strains JM105, LH530, and NRD183, a derivative of strain JM105 containing a deletion of *yejM* that carries plasmid pNRD217 encoding *yejM* (called p*yejM* above) under the control of the *araBAD* promoter, were tested for the ability to grow overnight at 28° or 42° on LB plates containing either arabinose or glucose.
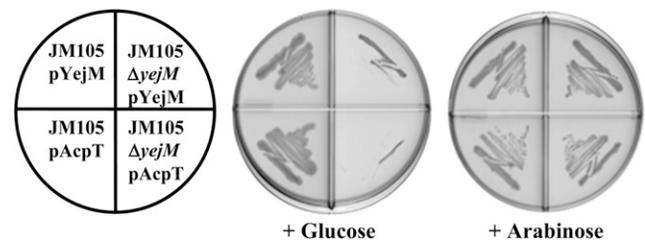

FIGURE 7.—Suppression of the lethality of a *yejM* deletion strain by overexpression of AcpT. The *yejM* deletion of strain NRD178 was transduced via phage P1*vir* into strain JM105 containing a plasmid that expressed either *yejM* (pNRD217) or *acpT* (pYon113) from the arabinose-inducible *araBAD* promoter. Transductants that acquired the *yejM* deletion were selected on LB–kanamycin–arabinose plates. A transductant from each cross, together with the parental strains, was streaked onto plates of LB–glucose or LB–arabinose that were incubated overnight at 30°.

mented (ROTH *et al.* 1996). Moreover, an F-factor-based cosmid generated from strain NRD178 genomic DNA contained a genome fragment that spanned the region between *fruA* and *ccmF* (including a wild-type copy of *yejM*) but lacked the kanamycin-marked Δ*yejM* deletion. This cosmid also complemented the Δ*yejM* deletion strain NRD186 (data not shown). This suggests that this duplicated region could include all of the genes between *fruA* and *ccmF*, consistent with the sizes of many documented duplications (ROTH *et al.* 1996).

To further test whether or not *yejM* is essential, a Δ*yejM* deletion was constructed by a λ-Red recombinase-mediated gene replacement in strain DY330 (YU *et al.* 2000) carrying the *yejM* plasmid pNRD217. Two of the recombinants obtained were shown to contain the *yejM* deletion by PCR and both recombinants (one of which is shown in Figure 6) grew only upon induction of the *yejM* expression from the plasmid.

Given that *yejM* is essential for growth, whereas the protein lacking the periplasmic domain allows growth at low-growth temperatures, the question was raised of whether or not AcpT overexpression would suppress the lethal phenotype of strains carrying a total deletion of the *yejM* gene. We found that the Δ*yejM* deletion mutation was readily transduced into a derivative of JM105 that carried a multicopy plasmid with *acpT* expression under an inducible (*araBAD*) promoter. The strain grew under inducing conditions but not under conditions that repressed expression of the plasmid-encoded *acpT* (Figure 7). The observed growth was very similar to that given by induction of a plasmid-borne copy of *yejM* and thus AcpT overproduction fully suppressed the effects of this otherwise lethal mutation. As reported above for suppression of the temperature sensitivity of the strain expressing the truncated YejM, suppression of the *yejM*

null mutation did not require AcpT enzymatic activity. When the *yejM* null mutation was transduced into a derivative of JM105 expressing the inactive E137S AcpT encoded by plasmid pNRD206, growth proceeded as seen with wild-type AcpT (Figure 7). The stain expressing the E137S AcpT grew well in the presence of arabinose, but failed to grow when expression was blocked by the absence of arabinose (in either the presence or the absence of glucose) (data not shown).

Does AcpT overexpression induce the heat-shock response? One possibility for suppression of *yejM* mutations by AcpT overexpression is that overexpression triggers a stress response that induces synthesis of a protein that replaces (or bypasses) YejM function. One stress response known to be induced by high-level expression of insoluble proteins such as AcpT is the heat-shock response. Hence, we have asked if the heat-shock regulon is induced upon AcpT overexpression by assaying expression of the classical heat-shock protein, DnaJ, by use of a chromosomal *dnaJ-lacZY* fusion construct. Construction of the fusion eliminated DnaJ function, but since DnaJ is functionally replaced by either of two other cochaperone proteins, DjlA and CbpA (GUR *et al.* 2004), DnaJ mutants behave normally in regards to heat shock. Use of this fusion provides an assay for DnaK expression since it is encoded by the promoter-proximal gene in the *dnaK-dnaJ* operon (COWING *et al.* 1985; BARDWELL *et al.* 1986).

Following construction of the *dnaJ-lacZY* fusion strain CY1817, the behavior of the strain was tested in a classical 30°–42° temperature shift. The strain showed the expected rapid increase in *dnaK-dnaJ* expression (assayed by β-galactosidase activity) soon after a temperature shift that reached a plateau about threefold greater than the 30° expression level ~30 min after shift (Figure 8A) as expected from the increases in DnaK levels seen in the same temperature-shift regimen (GROSS 1996). As a control, a *lacZY* fusion to a housekeeping biosynthetic gene, *bioA*, was assayed in parallel. In contrast to the *dnaJ*

fusion, the *bioA::lacZY* fusion strain showed no effect of temperature; the β-galactosidase activity was constant (Figure 8A).

The *dnaJ-lacZY* fusion strain CY1817 was then transformed with plasmids encoding the wild type, the E137S mutant AcpT, or the empty vector. Cultures of these strains were grown 30°, protein expression was induced with 0.2% arabinose, and β-galactosidase activities were measured. The low-growth temperature was chosen to decrease heat-shock protein expression levels and thereby increase the sensitivity of the assay. All three cultures showed a constant level of expression over a 90-min time course (Figure 8B), indicating no induction of the heat-shock response. A possible caveat to this experiment is that the time course may be too brief to allow induction of the heat-shock regulon, although induction of the heat-shock response upon expression of other insoluble proteins was seen during roughly comparable time intervals (LESLEY *et al.* 2002; SMITH 2007). However, each protein seems to be a different case and thus we asked how long induction of AcpT overexpression takes

to bypass loss of YejM. Strain NRD183, which contains a complete deletion of *yejM* and a plasmid encoding AcpT under arabinose control, was grown in the presence of arabinose at 30° and the cells of early log phase cultures were recovered by centrifugation. The cells were then washed with and suspended in medium lacking arabinose. This culture was then divided into halves. One-half received 0.2% arabinose, and the second received water. These cultures were then incubated at 30° and their growth was followed by turbidity. When growth of the culture lacking arabinose began to slow due to depletion of AcpT, the culture was split and one-half was supplemented with arabinose. The time required before growth of the arabinose-supplemented culture, accelerated relative to the culture lacking arabinose, was taken as a measure of the time required for accumulation of AcpT levels sufficient to bypass the *yejM* deletion. Acceleration of growth by the addition of arabinose was seen within 36 min of inducer addition (Figure 8C) and thus the time course chosen to test induction of the heat-shock response was deemed appropriate.
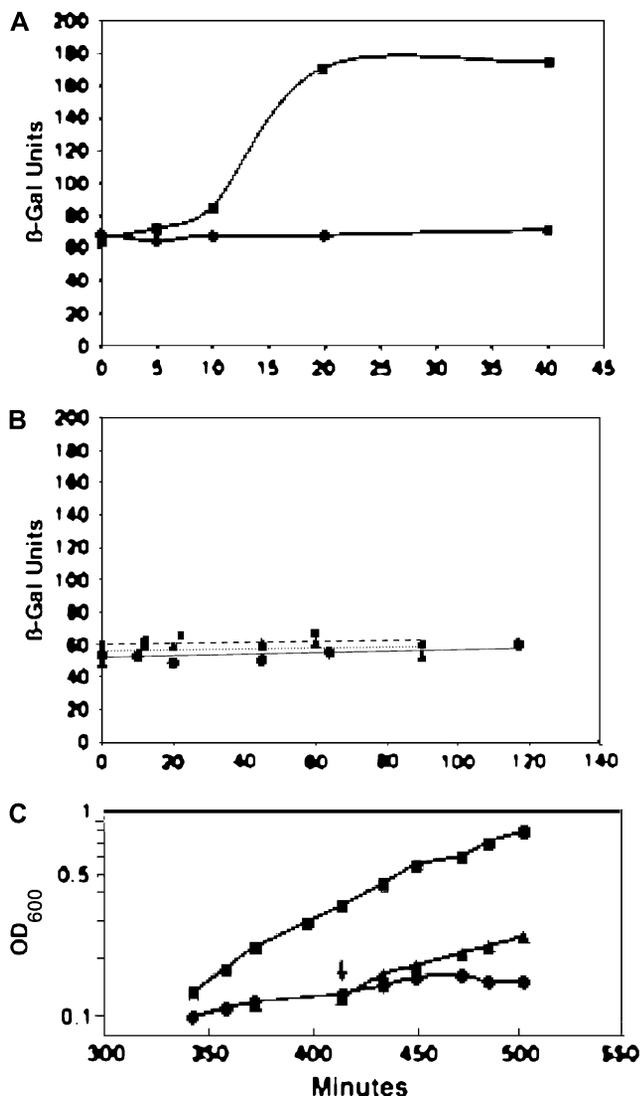

FIGURE 8.—Effects of heat shock and AcpT overproduction on *dnaJ* induction. (A) Behavior of the *dnaJ::lacZY* fusion in the heat-shock response. Cultures of the *dnaJ::lacZY* fusion strain CY1817 (squares) or the *bioA::lacZY* fusion strain CY1783 (circles) growing exponentially in early log phase at 30° were shifted to 42° at time zero and samples were taken for β-galactosidase assays at the times given. β-Galactosidase activities are given in Miller units (MILLER 1972). (B) Derivatives of strain CY1817 carrying plasmids encoding either the wild-type (pYon13) or mutant (pNRD207) AcpT proteins or the empty vector (pBAD24) were grown at 30° in LB–ampicillin. Exponentially growing cultures then received arabinose to a final concentration of 0.2% to induce AcpT overexpression and samples were taken for β-galactosidase assays at the times given. Triangles, vector control; squares, pYon13; and circles, pNRD207. β-Galactosidase activities are given in Miller units and the ordinate scale is identical to that of A. (C) The cells of a culture of strain NRD183 grown in LB–ampicillin medium containing 0.2% arabinose at 30° were harvested by centrifugation, washed, and resuspended in LB medium. The culture was then diluted to an OD_{600} of 0.05 and split in half. One culture received 0.2% arabinose whereas the other half remained unsupplemented. The cultures were then grown overnight at 30°. The washed cells of both cultures were recovered as before and resuspended at an OD_{600} of 0.1 in the same medium in which they had been grown. The turbidities of the cultures during growth at 30° were monitored with periodic dilution to keep the cultures in exponential growth until the growth of the culture lacking arabinose began to slow (at ~5 hr after dilution of the overnight cultures). Half of this culture was then added to a prewarmed flask containing sufficient arabinose to give a final concentration of 0.2%. The growth of the three cultures was then monitored. Squares denote the culture grown with arabinose throughout the experiment; circles denote the culture lacking arabinose throughout the experiment; triangles denote the culture supplemented with arabinose following growth in the absence of arabinose.

DISCUSSION

YejM is an inner membrane protein predicted to have five membrane-spanning helices and a large C-terminal periplasmic domain (Figure 2) (DALEY *et al.* 2005) that shares sequence similarities with sulfatases and phosphatases (Swiss Protein database). If the topological prediction is accurate, then the G205A *yejM* mutant allele of strain LH530 would encode a YejM protein containing the complete transmembrane domain, but lacking the entire C-terminal periplasmic domain. We constructed the *yejM* mutation *in vitro* and the resulting strain was temperature sensitive. Moreover, the original and constructed *yejM* G205A mutant strains had similar phenotypes with respect to lipid A biosynthesis and hypersensitivity to antibiotics. Chromatographic and mass spectral analyses (C. MICHAEL REYNOLDS, personal communication) showed that both strains (LH530 and NRD187) have essentially normal lipid A compositions in that the bulk of the lipid A produced by these strains and their parent strain JM105 was the hexa-acylated lipid A 1,4′-*bis*-phosphate species normally found in *E. coli* (RAETZ *et al.* 2007). However, the two mutant strains both synthesized small but discernible levels of a hepta-acylated lipid A species that contains an additional palmitoyl group (C. MICHAEL REYNOLDS, personal communication). We believe that the presence of this lipid A species in the mutant strains is a secondary response to problems in maintaining outer membrane integrity (RAETZ *et al.* 2007). Previous studies have shown that addition of compounds that affect outer membrane integrity, such as cationic peptides, result in the synthesis of palmitoylated lipid A in *Salmonella enterica* serovar Typhimurium (ZHOU *et al.* 1999). An LpxM-deficient mutant strain of *E. coli* O157:H7 has also been reported to accumulate this lipid A species (RAETZ *et al.* 2007).

Although the nonsense mutations resulted in a conditionally lethal phenotype, deletion of the entire *yejM* gene was unconditionally lethal. Together, these results suggest that YejM has two functions. The function carried out by the N-terminal transmembrane domain is essential at all growth temperatures whereas the function of the C-terminal periplasmic domain is dispensable at low-growth temperatures. Both functions may be important for the same cellular process. During our work BABA *et al.* (2006) reported *yejM* to be an essential gene in that they were unable to construct a strain carrying a complete deletion of *yejM* by λ-Red recombinase-mediated gene replacement. In contrast, two groups reported that *yejM* is not an essential gene on the basis of transposon mutagenesis (GERDES *et al.* 2003; KANG *et al.* 2004). However, in these reports all six of the reported transposon insertions were located downstream of the nonsense mutation of strain LH530 such that the N-terminal transmembrane domain would be translated. These mutant strains would produce YejM proteins at least 60 residues longer than that produced by strain LH530. Hence, the nature of

transposon mutagenesis essentially precluded detection of *yejM*. Growth of the strains carrying the *yejM* transposon insertion alleles was assayed only at 30° or 37° (GERDES *et al.* 2003; KANG *et al.* 2004), temperatures at which strains LH530 and NRD187 grow reasonably well. It thus remains unclear whether or not the strains carrying *yejM* transposon insertion alleles are temperature sensitive for growth.

Suppression of the growth defects of strains lacking either the YejM periplasmic domain or the entire protein by overexpression of the cytosolic AcpT phosphopantetheinyl transferase presents a conundrum. How can a mutation resulting in a defective outer membrane be suppressed by overexpression of an enzyme that post-translationally modifies a cytosolic carrier protein? We have shown that the phosphopantetheinyl transferase activity of AcpT is not involved in *yejM* mutant suppression and thus that AcpT overexpression must act indirectly. One possible explanation based on the properties of AcpT and the nature of the suppression phenomena is that suppression might involve a stress response. In agreement with the results of LAMBALOT *et al.* (1996), we found that AcpT accumulates as an insoluble aggregate upon even fairly modest overexpression conditions (data not shown). Aggregation may be due to the fact that AcpT has been evolutionarily isolated from its substrates (DE LAY and CRONAN 2006) and thus lacks substrates to aid proper protein folding. Moreover, fairly high levels of AcpT overexpression are required for full suppression of the temperature-sensitive phenotype of strain LH530; low levels of overexpression fail to suppress (HIRVAS *et al.* 1997) (data not shown). If misfolding is involved in suppression of YejM mutations, it seems specific to AcpT overexpression since three *acpT* clones were the only clones isolated from a clone bank of 8000 wild-type *E. coli* DNA fragments in a high-copy-number vector that allowed growth of strain LH530 at 42° (HIRVAS *et al.* 1997). We also tested the specificity of AcpT overexpression on the temperature-sensitive phenotype of strain LH530 by overexpression of AcpH, another *E. coli* protein involved in ACP metabolism that aggregates under all of the many overexpression conditions tested (THOMAS and CRONAN 2005; THOMAS *et al.* 2007). Overexpression of AcpH using the same vector employed in Figure 7 failed to allow growth of strain LH530 at 42° (data not shown). Consistent with the observed and inferred specificity of suppression, AcpT overexpression failed to induce the heat-shock regulon. Induction of the heat-shock regulon by misfolded proteins is frequently observed (OH and LIAO 2000; LESLEY *et al.* 2002; SMITH 2007), and thus if induction of heat shock played a major role in the AcpT overproduction suppression phenomenon, a large and diverse set of the Hirvas clones (instead of only *acpT* clones) would have suppressed the *yejM* mutation.

Another possible explanation (suggested by a reviewer) is that overexpressed AcpT might specifically

bind another normally more abundant protein(s). This protein (or proteins) might then become trapped in AcpT aggregates, thus depleting the cells of the functional AcpT-binding protein(s). The weakness in these and other possible explanations that we have considered is that they fail to provide a plausible mechanism whereby overexpression of a normally cytosolic protein allows growth of *E. coli* cells that lack an essential membrane protein.

The function of YejM remains obscure. Somehow loss of the periplasmic domain of this inner membrane protein results in an increased permeability of the outer membrane that is exacerbated by increased growth temperature. The reported phenotype of reduced lipid A biosynthesis in strain LH530 (HIRVAS *et al.* 1997; VAARA and NURMINEN 1999) is complicated by the possibility of translocation of phospholipids into the outer leaflet of the outer membrane known to occur in strains that carry mutations affecting the organization of lipopolysaccharide on the cell surface (WU *et al.* 2006). Hence, since lipid A synthesis was assayed using phospholipid synthesis as a standard, the results previously reported could be at least partially due to increased phospholipid synthesis rather than to decreased lipid A biosynthesis. Indeed, the accumulation of phospholipids in the outer leaflet is thought to render cells sensitive to otherwise impermeable antibiotics (RAETZ *et al.* 2007), a phenotype of strain LH530. Increased sensitivity to such antibiotics is also seen in lipid A mutants. However, all of the known lipid A mutants have marked alterations of the compositions of their lipid A species whereas the *yejM* mutant strains have essentially normal lipid A compositions (C. MICHAEL REYNOLDS, personal communication).

LITERATURE CITED

BABA, T., T. ARA, M. HASEGAWA, Y. TAKAI, Y. OKUMURA *et al.*, 2006 Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol. Syst. Biol. **2:** 2006.0008.

BARDWELL, J. C., K. TILLY, E. CRAIG, J. KING, M. ZYLICZ *et al.*, 1986 The nucleotide sequence of the *Escherichia coli* K12 dnaJ+ gene. A gene that encodes a heat shock protein. J. Biol. Chem. **261:** 1782–1785.

BJORNSSON, A., and L. A. ISAKSSON, 1993 UGA codon context which spans three codons: reversal by ms2i6A37 in tRNA, mutation in *rpsD*(S4) or streptomycin. J. Mol. Biol. **232:** 1017–1029.

COWING, D. W., J. C. BARDWELL, E. A. CRAIG, C. WOOLFORD, R. W. HENDRIX *et al.*, 1985 Consensus sequence for *Escherichia coli* heat shock gene promoters. Proc. Natl. Acad. Sci. USA **82:** 2679–2683.

CRONAN, J. E., 2003 Cosmid-based system for transient expression and absolute off-to-on transcriptional control of Escherichia coli genes. J. Bacteriol. **185:** 6522–6529.

CRONAN, J. E., 2006 A family of arabinose-inducible *Escherichia coli* expression vectors having pBR322 copy control. Plasmid **55:** 152–157.

CROWELL, D. N., M. S. ANDERSON and C. R. RAETZ, 1986 Molecular cloning of the genes for lipid A disaccharide synthase and UDP-N-acetylglucosamine acyltransferase in *Escherichia coli.* J. Bacteriol. **168:** 152–159.

DALEY, D. O., M. RAPP, E. GRANSETH, K. MELEN, D. DREW *et al.*, 2005 Global topology analysis of the *Escherichia coli* inner membrane proteome. Science **308:** 1321–1323.

DATSENKO, K. A., and B. L. WANNER, 2000 One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA **97:** 6640–6645.

DE LAY, N. R., and J. E. CRONAN, 2006 A genome rearrangement has orphaned the Escherichia coli K-12 AcpT phosphopantetheinyl transferase from its cognate *Escherichia coli* O157:H7 substrates. Mol. Microbiol. **61:** 232–242.

ELLERMEIER, C. D., A. JANAKIRAMAN and J. M. SLAUCH, 2002 Construction of targeted single copy lac fusions using lambda Red and FLP-mediated site-specific recombination in bacteria. Gene **290:** 153–161.

FLUGEL, R. S., Y. HWANGBO, R. H. LAMBALOT, J. E. CRONAN, JR. and C. T. WALSH, 2000 Holo-(acyl carrier protein) synthase and phosphopantetheinyl transfer in *Escherichia coli.* J. Biol. Chem. **275:** 959–968.

GALLOWAY, S. M., and C. R. RAETZ, 1990 A mutant of *Escherichia coli* defective in the first step of endotoxin biosynthesis. J. Biol. Chem. **265:** 6394–6402.

GERDES, S. Y., M. D. SCHOLLE, J. W. CAMPBELL, G. BALAZSI, E. RAVASZ *et al.*, 2003 Experimental determination and system level analysis of essential genes in *Escherichia coli* MG1655. J. Bacteriol. **185:** 5673–5684.

GROSS, C. A., 1996 Function and regulation of heat shock proteins, pp. 1382–1399 in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, edited by F. C. NEIDHARDT, III, J. L. INGRAHAM, E. C. C. LIN, K. B. LOW, B. MAGASANIK, W. S. REZNIKOFF, M. RILEY, M. SCHAECHTER and H. E. UMBARGER. American Society for Microbiology, Washington, DC.

GUR, E., D. BIRAN, N. SHECHTER, P. GENEVAUX, C. GEORGOPOULOS *et al.*, 2004 The *Escherichia coli* DjlA and CbpA proteins can substitute for DnaJ in DnaK-mediated protein disaggregation. J. Bacteriol. **186:** 7236–7242.

GUZMAN, L. M., D. BELIN, M. J. CARSON and J. BECKWITH, 1995 Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. J. Bacteriol. **177:** 4121–4130.

HIRVAS, L., M. NURMINEN, I. M. HELANDER, R. VUORIO and M. VAARA, 1997 The lipid A biosynthesis deficiency of the *Escherichia coli* antibiotic-supersensitive mutant LH530 is suppressed by a novel locus, ORF195. Microbiology **143:** 73–81.

KANG, Y., T. DURFEE, J. D. GLASNER, Y. QIU, D. FRISCH *et al.*, 2004 Systematic mutagenesis of the *Escherichia coli* genome. J. Bacteriol. **186:** 4921–4930.

KELLY, T. M., S. A. STACHULA, C. R. RAETZ and M. S. ANDERSON, 1993 The *firA* gene of *Escherichia coli* encodes UDP-3-O-(R-3-hydroxymyristoyl)-glucosamine N-acyltransferase. The third step of endotoxin biosynthesis. J. Biol. Chem. **268:** 19866–19874.

KLOSER, A. W., M. W. LAIRD and R. MISRA, 1996 *asmB*, a suppressor locus for assembly-defective OmpF mutations of *Escherichia coli*, is allelic to *envA* (*lpxC*). J. Bacteriol. **178:** 5138–5143.

LAMBALOT, R. H., A. M. GEHRING, R. S. FLUGEL, P. ZUBER, M. LACELLE *et al.*, 1996 A new enzyme superfamily: the phosphopantetheinyl transferases. Chem. Biol. **3:** 923–936.

LESLEY, S. A., J. GRAZIANO, C. Y. CHO, M. W. KNUTH and H. E. KLOCK, 2002 Gene expression response to misfolded protein as a screen for soluble recombinant protein. Protein Eng. **15:** 153–160.

MILLER, J. H., 1972 *Experiments in Molecular Genetics.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

MOFID, M. R., M. A. MARAHIEL, R. FICNER and K. REUTER, 1999 Crystallization and preliminary crystallographic studies of Sfp: a phosphopantetheinyl transferase of modular peptide synthetases. Acta Crystallogr. D Biol. Crystallogr. **55:** 1098–1100.

MOFID, M. R., R. FINKING, L. O. ESSEN and M. A. MARAHIEL, 2004 Structure-based mutational analysis of the 4'-phosphopantetheinyl transferases Sfp from *Bacillus subtilis*: carrier protein recognition and reaction mechanism. Biochemistry **43:** 4128–4136.

MOOTZ, H. D., R. FINKING and M. A. MARAHIEL, 2001 4'-Phosphopantetheine transfer in primary and secondary metabolism of *Bacillus subtilis*. J. Biol. Chem. **276:** 37289–37298.

NICHOLS, B. P., O. SHAFIQ and V. MEINERS, 1998 Sequence analysis of Tn*10* insertion sites in a collection of *Escherichia coli* strains used for genetic mapping and strain construction. J. Bacteriol. **180:** 6408–6411.

NORMARK, S., 1970 Genetics of a chain-forming mutant of *Escherichia coli*: transduction and dominance of the *envA* gene mediating increased penetration to some antibacterial agents. Genet. Res. **16:** 63–78.

NURMINEN, M., L. HIRVAS and M. VAARA, 1997 The outer membrane of lipid A-deficient *Escherichia coli* mutant LH530 has reduced levels of OmpF and leaks periplasmic enzymes. Microbiology **143:** 1533–1537.

OH, M. K., and J. C. LIAO, 2000 DNA microarray detection of metabolic responses to protein overproduction in *Escherichia coli*. Metab. Eng. **2:** 201–209.

PARRIS, K. D., L. LIN, A. TAM, R. MATHEW, J. HIXON *et al.*, 2000 Crystal structures of substrate binding to *Bacillus subtilis* holo-(acyl carrier protein) synthase reveal a novel trimeric arrangement of molecules resulting in three active sites. Structure **8:** 883–895.

RAETZ, C. R., C. M. REYNOLDS, M. S. TRENT and R. E. BISHOP, 2007 Lipid A modification systems in gram-negative bacteria. Annu. Rev. Biochem. **76:** 295–329.

REUTER, K., M. R. MOFID, M. A. MARAHIEL and R. FICNER, 1999 Crystal structure of the surfactin synthetase-activating enzyme Sfp: a prototype of the 4′-phosphopantetheinyl transferase superfamily. EMBO J. **18:** 6823–6831.

ROTH, J. R., N. BENSON, T. GALITSKI, K. HAACK, J. G. LAWRENCE *et al.*, 1996 Rearrangements of the bacterial chromosome: formation and applications, pp. 2256–2276 in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, edited by F. C. NEIDHARDT, III, J. L. INGRAHAM, E. C. C. LIN, K. B. LOW, B. MAGASANIK, W. S. REZNIKOFF, M. RILEY, M. SCHAECHTER and H. E. UMBARGER. American Society for Microbiology, Washington, DC.

SINGER, M., T. A. BAKER, G. SCHNITZLER, S. M. DEISCHEL, M. GOEL *et al.*, 1989 A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. **53:** 1–24.

SMITH, H. E., 2007 The transcriptional response of *Escherichia coli* to recombinant protein insolubility. J. Struct. Funct. Genomics **8:** 27–35.

THOMAS, J., and J. E. CRONAN, 2005 The enigmatic acyl carrier protein phosphodiesterase of *Escherichia coli*: genetic and enzymological characterization. J. Biol. Chem. **280:** 34675–34683.

THOMAS, J., D. J. RIGDEN and J. E. CRONAN, 2007 Acyl carrier protein phosphodiesterase (AcpH) of *Escherichia coli* is a non-canonical member of the HD phosphatase/phosphodiesterase family. Biochemistry **46:** 129–136.

TSURUOKA, T., M. ITO, S. TOMIOKA, A. HIRATA and M. MATSUHASHI, 1988 Thermosensitive *omsA* mutation of *Escherichia coli* that causes thermoregulated release of periplasmic proteins. J. Bacteriol. **170:** 5229–5235.

VAARA, M., and M. NURMINEN, 1999 Outer membrane permeability barrier in *Escherichia coli* mutants that are defective in the late acyltransferases of lipid A biosynthesis. Antimicrob. Agents Chemother. **43:** 1459–1462.

WU, T., A. C. MCCANDLISH, L. S. GRONENBERG, S. S. CHNG, T. J. SILHAVY *et al.*, 2006 Identification of a protein complex that assembles lipopolysaccharide in the outer membrane of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **103:** 11754–11759.

YANISCH-PERRON, C., J. VIEIRA and J. MESSING, 1985 Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:** 103–119.

YOUNG, K., L. L. SILVER, D. BRAMHILL, P. CAMERON, S. S. EVELAND *et al.*, 1995 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. J. Biol. Chem. **270:** 30384–30391.

YU, D., H. M. ELLIS, E. C. LEE, N. A. JENKINS, N. G. COPELAND *et al.*, 2000 An efficient recombination system for chromosome engineering in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **97:** 5978–5983.

ZHOU, Z., S. LIN, R. J. COTTER and C. R. RAETZ, 1999 Lipid A modifications characteristic of *Salmonella typhimurium* are induced by NH_4VO_3 in *Escherichia coli* K12. Detection of 4-amino-4-deoxy-L-arabinose, phosphoethanolamine and palmitate. J. Biol. Chem. **274:** 18503–18514.

Communicating editor: M. HAMPSEY