JB Journals.ASM.org

MINIREVIEW

New Functions for the Ancient DedA Membrane Protein Family

William T. Doerrler, Rakesh Sikdar, Sujeet Kumar, Lisa A. Boughner

Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana, USA

The DedA protein family is a highly conserved and ancient family of membrane proteins with representatives in most sequenced genomes, including those of bacteria, archaea, and eukarya. The functions of the DedA family proteins remain obscure. However, recent genetic approaches have revealed important roles for certain bacterial DedA family members in membrane homeostasis. Bacterial DedA family mutants display such intriguing phenotypes as cell division defects, temperature sensitivity, altered membrane lipid composition, elevated envelope-related stress responses, and loss of proton motive force. The DedA family is also essential in at least two species of bacteria: *Borrelia burgdorferi* and *Escherichia coli*. Here, we describe the phylogenetic distribution of the family and summarize recent progress toward understanding the functions of the DedA membrane protein family.

It is predicted that roughly 20 to 25% of polypeptides are integral membrane proteins (1). In contrast, fewer than 1% of all known protein structures in the Protein Data Bank are membrane proteins, and the functions of many are only poorly understood (2, 3). The *Escherichia coli dedA* gene (Ec*dedA*) was given its name in a 1987 publication for its presence in the DNA sequence of a 9.7-kb fragment between *hisT* and *purF* (downstream [of *hisT*] *E. coli* DNA gene A) (4). For clarity, we will refer to the DedA family to describe the protein family and EcDedA or Ec*dedA* to describe specifically the *E. coli* DedA protein or gene. Despite these rather mundane origins, it is now appreciated that the DedA family is a highly conserved protein family represented in most sequenced genomes encoding membrane proteins of unknown function (5). There are virtually thousands of prokaryotic homologs of bacterial DedA proteins currently found in the NCBI protein database, and many sequenced bacterial genomes encode multiple family members (Table 1) (8). However, they remain difficult to classify, as the polypeptides do not resemble known enzymes, transporters, channels, or signaling proteins. While there are examples of multidomain secondary transporters and enzymes in the database containing DedA or "SNARE-associated" domains (9), DedA family proteins are, in most cases, unique polypeptides with no other commonly associated domains. The polypeptides belonging to the DedA family typically contain 4 to 6 predicted transmembrane domains, between 200 and 250 amino acids, and a conserved domain. This DedA domain contains both transmembrane and cytoplasmic domains, as well as a likely amphipathic helix. A strictly conserved amino acid sequence is not present across the entire domain; however, there is at least one universally conserved glycine residue which occurs in or near the potential amphipathic helix (Fig. 1A) and is found in all defined DedA proteins (NCBI Clusters of Orthologous Groups, COG0586; the DedA domain). Here, we summarize recent progress toward understanding the functions of DedA family membrane proteins.

PHYLOGENETIC DISTRIBUTION OF THE DedA FAMILY

To investigate the distribution of the DedA protein family, we scanned a total of 350 sequenced bacterial genomes (10) and 100 sequenced archaeal genomes (11) found in the NCBI database with Protein BLAST using amino acid sequences of all eight *E. coli* DedA proteins. Using a very conservative Protein BLAST score (E value, $\leq 10^{-4}$) as significant, we found that 33 (9.2%) bacterial species and 27 (27%) archaeal species lack a significant DedA homolog (Fig. 1B and C). The largest proportion of bacterial species that lack a significant DedA homolog can be found in the phylum *Tenericutes* (with 13/16 species lacking a clear DedA homolog), followed by the *Thermotogae* (5/5) and *Alphaproteobacteria* (4/46). As for the *Archaea* domain, the largest proportion of species lacking a significant DedA homolog is in the *Euryarchaeota* (14/70) and *Crenarchaeota* (12/24) phyla. However, it is important to note that the majority of sequenced archaeal species fall within the *Euryarchaeota* phylum (11).

The presence of a significant DedA homolog is not consistent among organisms of similar habitats; for example, a significant DedA homolog is present within *Neisseria* spp., *Mycoplasma synoviae*/*M. fermentans*, *Anaplasma phagocytophilum*, and *Ehrlichia chaffeensis* but absent in *Chlamydia*, *Ureaplasma*, and *Neorickettsia sennetsu* (12–14). In fact, a DedA member is not present among any of the sequenced *Chlamydia*/*Chlamydophilia* spp. or *Ureaplasma* spp.; however, *Neisseria* spp. have multiple, and some very significant (E value, $<10^{-100}$) DedA homologs. Interestingly, the majority of *Mycoplasma* spp. and *Rickettsia* spp. do not have any significant DedA members, but DedA homologs are present within the genera, e.g., *Mycoplasma synoviae*/*M. fermentans* and *Rickettsia felis*/*R. bellii* (15, 16). The majority of *Clostridium* spp. (including *C. botulinum*) do have a DedA homolog, whereas *Clostridium thermocellum* does not (17–20). There are many additional genera in which the presence of a DedA homolog is variable, for example, *Psychrobacter*, *Bartonella*, and *Mycobacterium* (21, 22). The significance of the observed distribution of the DedA protein family is as yet unclear.

Another inconsistency in the distribution of DedA homologs is among the reduced genome symbionts and obligate symbionts of various organisms. The DedA family is found in the genomes of several symbionts, including *Wigglesworthia*

Published ahead of print 19 October 2012

Address correspondence to William T. Doerrler, wdoerr@lsu.edu.

doi:10.1128/JB.01006-12

TABLE 1 Numbers of DedA family proteins (amino acid BLAST E value, <0.02) found in sequenced genomes of representative bacterial and archaeal species[a]

Bacterial strain	No. of DedA family homologs
Escherichia coli K-12	8[c]
Salmonella enterica SL480	6
Pseudomonas aeruginosa PAO1	5
Helicobacter pylori J99	2
Vibrio cholerae El Tor N16961	3
Caulobacter crescentus CB15	3
Neisseria meningitidis Z2491	3
Borrelia burgdorferi B31	1[b]
Bacillus subtilis strain 168	6
Bacillus anthracis strain Ames	8
Mycobacterium tuberculosis H37Rv	4
Chlamydia trachomatis D/UW-3/CX	0
Synechocystis sp. strain PCC6803	3
Halobacterium salinarum NRC-1	1

[a] Significant homologs (Protein BLAST E value, <0.02) of *E. coli* YqjA, YghB, DedA, YohD, YabI, YdjZ, YdjX, and YqaA were included in the numbers of proteins displayed in the second column.
[b] The *B. burgdorferi B31*DedA family gene *BB0250* has been demonstrated to be essential (7).
[c] The DedA gene family in *E. coli* K-12 is collectively essential (6). Reproduced from reference 7 and modified with permission. Accession numbers for each listed gene are available (7).

glossinidia (23) and *Buchnera* spp. (24). Some symbionts that lack a DedA homolog are "*Candidatus* Sulcia muelleri," "*Candidatus* Amoebobhilus asiaticus," "*Candidatus* Phytoplasma mali," "*Candidatus* Zinderia insecticola," "*Candidatus* Carsonella ruddii," "*Candidatus* Hodgkinia cicadicola," and "*Candidatus* Tremblaya princeps" (reviewed in reference 25). The possibility exists that this variability of the DedA distribution is related to the genetic makeup and/or physiology of the symbionts' host species (26–28).

As for the archaeal domain, the distribution of DedA members is quite unpredictable; one note of interest is that no sequenced genomes within the *Halobacteriales* or *Sulfolobales* order lack a significant DedA homolog. Also, the reduced genomes of archaeal species "*Candidatus* Parvarchaeum spp." and "*Candidatus* Micrarchaeum acidiphilum" all contain a significant DedA homolog (29). The presence of DedA homologs within several reduced genomes, both bacterial and archaeal, further supports the essentiality of the DedA protein family for species viability. Published phylogenetic trees of the bacterial and archaeal domains were used as a guide for investigating the distribution of DedA members (10, 11).

In regard to the distribution of the DedA protein family, there may be subtle differences between organisms that lack a DedA homolog, which has allowed for a select few to counteract the necessity of DedA proteins. For example, it is possible that other proteins have taken over their role or that symbiotic relationships enable this selective genotypic evolution. Regardless, these species have found a way to exist without the DedA protein family. It is important to note, however, that the identification of significant DedA homologs using a strict BLAST score cutoff (E value, $\leq 10^{-4}$) may have overlooked DedA family members with lower sequence identity to the *E. coli* DedA proteins.

MUTATIONS IN *E. COLI yghB* AND *yqjA* ARE SYNTHETICALLY LETHAL AT ELEVATED TEMPERATURES AND LEAD TO DEFECTS IN CELL DIVISION, ELEVATED STRESS, AND MEMBRANE DEFECTS

The *E. coli* genome encodes eight predicted members of the DedA family (*yqjA*, *yghB*, *yabI*, *yohD*, Ec*dedA*, *ydjX*, *ydjZ*, and *yqaA*). Our interest in the DedA family was initiated by the observation that simultaneous deletion of *yqjA* and *yghB* from *E. coli* results in a strain (named BC202) that is temperature sensitive for growth and displays striking defects in cell division (Fig. 2A and B) (5, 30). YghB and YqjA are proteins of 219 and 220 amino acids, respectively, displaying 61% amino acid identity to each other and possessing likely four membrane-spanning domains with cytoplasmic N and C termini (Fig. 3). The other six *E. coli* homologs display roughly 25 to 30% amino acid identity with each other and YghB/YqjA.

The *E. coli* mutant BC202 (Δ*yqjA* Δ*yghB*) displays several intriguing phenotypes that reflect important functions for the DedA family. As mentioned, BC202 grows at 30°C but not 42°C (Fig. 2A) and does not complete cell division, forming chains of cells (5). We have demonstrated that the periplasmic amidases AmiA and AmiC are not exported to the periplasm in BC202 and that this is responsible for the cell division defect (Fig. 2B) (30). These amidases are normally exported across the inner membrane via the twin arginine transport (Tat) pathway in *E. coli* (31), a Sec-independent protein export pathway found in many bacteria and also present in archaea and plants (32–35). AmiA and AmiC are required for normal cell division and envelope integrity (36), and Δ*tat* mutants display cell division defects due to loss of amidase export (31, 37). However, *E. coli* Δ*tat* (T. Palmer, personal communication) and Δ*ami* mutants are not temperature sensitive for growth, unlike BC202. Therefore, the temperature sensitivity of BC202 is independent of the cell division phenotype. BC202 is also not hypersensitive to antibiotics or detergents (5), likely signifying the presence of an intact outer membrane, unlike the situation with Δ*tat* mutants (37, 38) and mutants lacking periplasmic amidases (36). Interestingly, while the *Borrelia* Δ*bb0250* strain (described below) also exhibits cell division defects (Fig. 2D), the *B. burgdorferi* genome does not encode a functional twin arginine pathway or any predicted Tat substrates (39), indicating that the DedA family is involved in functions independent of the Tat pathway.

A number of extracytoplasmic stress response pathways are activated in BC202 under permissive growth conditions (see the accompanying paper by Sikdar et al [40]). *E. coli* responds to extracytoplasmic stress by activating one or more of several well-known stress response pathways such as the σ^E, Cpx, Psp, Bae, and Rcs pathways, which help the cells detect and combat alterations in their cell envelope when challenged with conditions that compromise envelope integrity (41). The Cpx and σ^E pathways are activated in response to disruptions in the folding of envelope proteins and loss of outer membrane integrity, and they have partially overlapping regulons (41–44). The Psp (phage shock protein) response is activated by perturbations in the integrity of the inner membrane by conditions that result in dissipation of the proton motive force (PMF) and/or change the physiological redox state of the cell (extreme heat shock, exposure to ethanol, ionophores, and pIV secretin stress) (45–47). The Bae stress response is induced by toxic compounds such as indole and ethanol (41, 48,

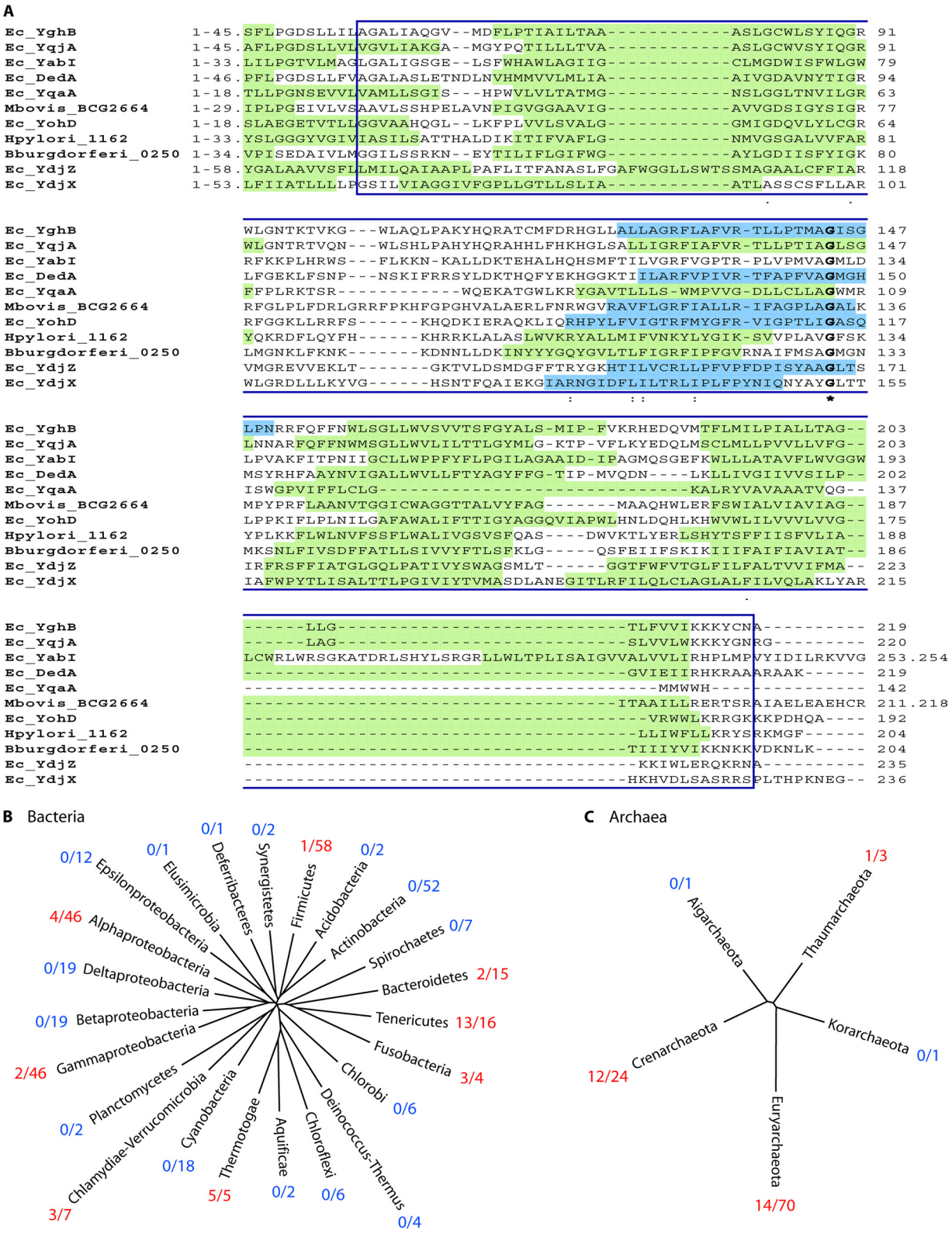


FIG 1 Phylogenetic analysis of the DedA protein family. (A) Alignment of *E. coli* DedA proteins and homologs found in *Borrelia burgdorferi*, *Mycobacterium bovis*, and *Helicobacter pylori* from the NCBI database to illustrate the DedA domain, COG0586 (boxed in region). Predicted transmembrane (TM) domains are highlighted in green, and partial TM regions, possibly amphipathic helixes, are highlighted in blue. The singularly conserved amino acid residue of the DedA domain (glycine) is in bold. Of interest, the only conserved glycine residue is in or near the amphipathic helix for all aligned members. The TM prediction software used was TMHMM (84). (B and C) Bacterial (B) and archaeal (C) domain representative trees are shown. Numbers in red demonstrate the proportion of species lacking a significant DedA homolog (Protein BLAST E value, $\leq 10^{-4}$) in each bacterial (B) or archaeal (C) phylum. Otherwise, all species of each phylum contain at least one significant DedA homolog (numbers in blue). Phylogenetic trees were constructed with MEGA (85), using a single 16S rRNA sequence from a representative species of each phylum. Previously published phylogenetic trees for both *Bacteria* (350 species) (10) and *Archaea* (100 species) (11) were used as a basis for phylogenetic analyses, though additional species were investigated; presented values are solely from published trees. Significant DedA homologs are found within the *Thermotogae* phylum although not in the five completed genomes analyzed.

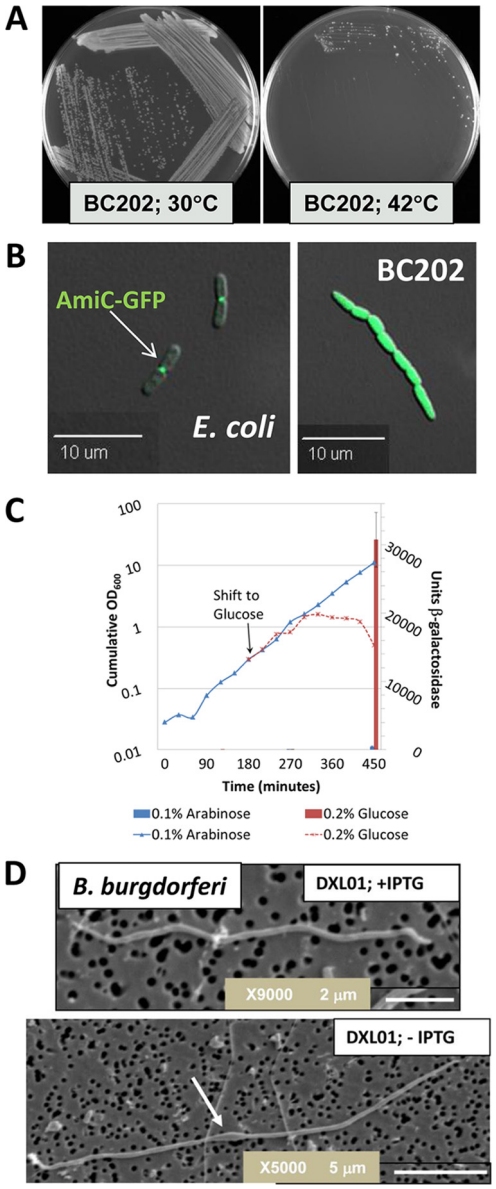


FIG 2 Characteristics of DedA family mutants. (A) BC202 (Δ*yghB*::Kan^r Δ*yqjA*::Tet^r) grows at 30°C but not at 42°C (5). The Δ*yghB* and Δ*yqjA* single mutants grow at all temperatures (not shown) (5, 62). (B) Cell division defects of BC202 are caused by failure to export periplasmic amidases via the twin arginine transport pathway. W3110 (left) and BC202 (right) transformed with plasmid pTB28 expressing AmiC-green fluorescent protein (GFP) fusion protein (31) were grown at 30°C and visualized with differential interference contrast (DIC) and fluorescence microscopy. The bright fluorescence observed in the right panel is due to cytoplasmic accumulation of AmiC-GFP (30). Expression of wild-type *yghB* from a plasmid restores wild-type appearance and AmiC export to BC202 (not shown) (30). (C) The *E. coli* DedA family is collectively essential at all temperatures. BAL801 (W3110, Δ*ydjXYZ*::*cam* Δ*yabI772* Δ*EcdedA726* Δ*yohD762* Δ*yqjA785* Δ*yqaA770* Δ*yghB781*::*kan* pBAD-*EcdedA*) fails to grow when EcDedA expression from a plasmid is repressed by growth in glucose (6). (D) The sole DedA family member of *Borrelia burgdorferi* is essential (not shown), and depletion of the protein causes cell division defects (7). *B. burgdorferi* DXL-01 (Δ*bb0250*, pWTD0250 expressing *bb0250* behind a borrelia-optimized *lac* promoter [86]) was cultured with either 1 mM (top) or 0 mM (bottom) IPTG (isopropyl-β-D-thiogalactopyranoside) for 4 to 5 days and visualized by scanning electron microscopy. Depletion of BB0250 causes membrane bulging (arrow) and cell division defects. Images A to D are reproduced with permission (5–7, 30).

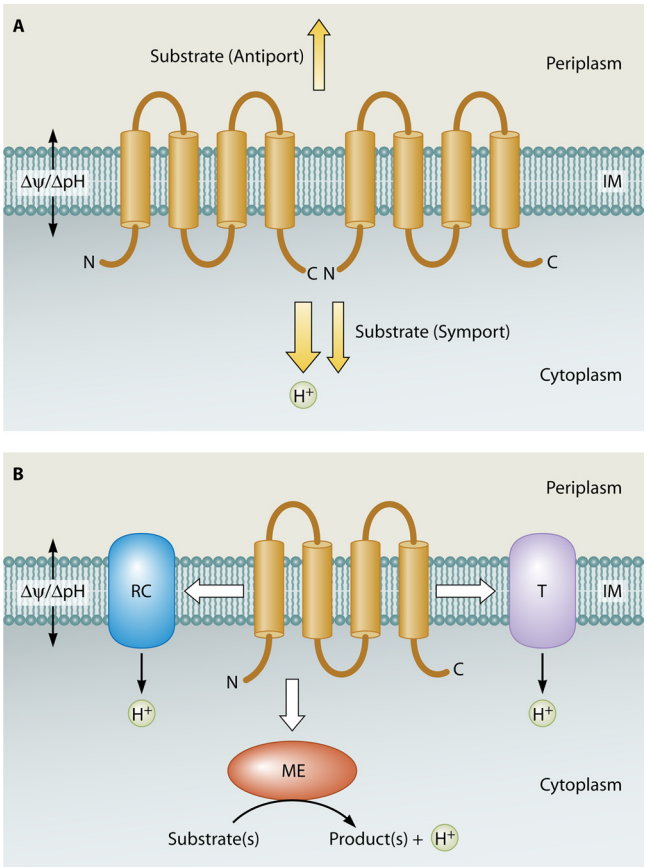


FIG 3 Potential physiological roles of *E. coli* YqjA and YghB. (A) Formation of YqjA homodimer as proposed for LeuT family members (8) transports protons into the cell coupled with symport or antiport of an uncharacterized substrate. The proposed function of YqjA here is similar to that of the Na^+/K^+-H^+ antiporter MdfA (61, 74) with a significant role in pH/PMF homeostasis. Heterodimerization with YghB or an unknown partner is also a possibility here. (B) An alternative model demonstrating an indirect role for YqjA in pH/PMF homeostasis. Regulation of activity or functional modulation of RC (respiratory complexes), ME (metabolic enzymes), or certain classes of transporters (T) such as MdfA, all of which participate in the pH/PMF homeostasis mechanism in *E. coli*. The topological model of YqjA, comprising 4-transmembrane helices with cytoplasmic N and C termini, is derived from previously published data (87), SOSUI topological prediction software (88), and unpublished observations. IM, inner membrane.

49). Finally, the Rcs pathway is activated by stresses that affect envelope composition (50–53) or the integrity of the peptidoglycan layer (54).

We observed that the Cpx, Psp, Rcs, and Bae stress responses are induced in BC202 under permissive growth conditions while σ^{32} (controlling the cytoplasmic heat shock response) and σ^E are not significantly induced (40). This nonspecific induction of multiple envelope stress responses is reminiscent of a general loss of envelope integrity when challenged with certain stresses such as growth in 5% ethanol (41). These findings demonstrate the critical importance of certain DedA family members in proper envelope function of *E. coli*.

It has been previously demonstrated that dissipation of the PMF results in induction of the Psp stress response (45, 55). Efficient Tat-mediated export of substrates also relies on optimal PMF (56, 57). The PMF in *E. coli* is comprised of two compo-

nents—the transmembrane electrical membrane potential difference, $\Delta\psi$ ($= \psi_{in} - \psi_{out}$), with ψ_{in} (electrical potential inside) being more negative than ψ_{out} (electrical potential outside), and the transmembrane pH difference, ΔpH ($= pH_{in} - pH_{out}$), with pH_{in} (intracellular pH) being more alkaline than pH_{out} (extracellular pH) under normal growth conditions. We found that BC202 exhibits a significant loss of membrane potential compared to parent strain W3110 using the membrane-permeating dye JC-1, consistent with the observed activation of the Psp pathway in BC202 (40). BC202 (as well as the single Δ*yqjA* mutant [58]) cannot survive at elevated pH, but temperature sensitivity of BC202 is rescued when it is grown at a lower pH (pH 6.0) or when the *mdfA* (*cmr*) gene is overexpressed (40). MdfA is a member of the major facilitator superfamily involved in drug efflux and is an Na^+/K^+-H^+ antiporter (59). It is likely that BC202 is unable to maintain the PMF and requires protons outside the cell to be exchanged with sodium/potassium ions. The single Δ*mdfA* mutant is also sensitive to mild alkaline pH and grows poorly at neutral pH (60). MdfA is unique in that it is capable of transporting diverse substrates across the membrane using both components of the electrochemical gradient ($\Delta\Psi$ and ΔpH) for electrogenic transport of neutral compounds, while using only ΔpH for electroneutral transport of cationic compounds (61). These observations collectively suggest that YqjA/YghB may play a more general role in membrane protein function or quality control and may be necessary for the homeostasis of the PMF. Whether YqjA/YghB are true transporters or are required for sensing and/or maintaining the PMF is not yet clear (see below and Fig. 3).

DedA FAMILY GENES ARE COLLECTIVELY OR INDIVIDUALLY ESSENTIAL IN BACTERIA

As stated above, the *E. coli* genome contains eight members of the DedA family. Each of these DedA homologs (*yqjA*, *yghB*, *yabI*, *yohD*, Ec*dedA*, *ydjX*, *ydjZ*, and *yqaA*) is individually nonessential, as the single gene knockouts have been made and are available from the Keio collection (62). The study of the essentiality of the DedA family in *E. coli* was stymied due to this high level of genetic redundancy. However, we have recently succeeded in creating a number of strains with deletions of all members of this family (BAL800 series) (6). Each BAL800 strain requires expression of a DedA family member in *trans* from a hybrid plasmid. Growth of these strains is dependent upon the presence of an inducing agent (in this case, arabinose) in the growth media. Growth in the presence of glucose results in cell lysis (Fig. 2C). Further analysis of the BAL800 series mutants promises to provide a greater understanding of the essential functions of the DedA membrane protein family.

The genome of *Borrelia burgdorferi*, the cause of Lyme disease, possesses only a single DedA family gene, annotated *bb0250* (Table 1) (63). In order to investigate the essentiality of the DedA family and to expand our knowledge of DedA family function, we created a *B. burgdorferi* Δ*bb0250* knockout. Strikingly, *bb0250* is essential in its host organism and depletion of BB0250 protein, expressed behind an inducible promoter, results in cell death preceded by defects in cell division (Fig. 2D) (7). In other words, the *Borrelia* mutant phenotypes resemble those of BC202, with the exception being that *bb0250* is essential at all temperatures. In addition, cloned *bb0250* can fully complement the growth and cell division phenotypes of BC202 even though BB0250 displays only ~19% amino acid identity to *E. coli* YqjA (and less to YghB) (7). These results demonstrate conservation of function of DedA family proteins found in two distantly related species of Gram-negative bacteria.

Four of the eight *E. coli* DedA family genes can restore normal growth and cell division to BC202 when expressed from a plasmid (*yqjA*, *yghB*, *yabI*, and *yohD*), while four cannot (*ydjX*, *ydjZ*, Ec*dedA*, and *yqaA*). We have categorized the eight *E. coli* DedA family genes as C (complements BC202) or NC (does not complement BC202). Interestingly, the plasmid required for isolation of BAL800 mutants can harbor a gene from either the C group (i.e., *yqjA*) or the NC group (i.e., Ec*dedA*). In regard to cell division and temperature sensitivity, the phenotype of the BAL800 family mutants depends upon whether the cloned DedA family gene belongs to the C or NC group. When *yqjA* (C group) is expressed from the inducible promoter in such a strain, the cell division defects and temperature sensitivity are corrected. When Ec*dedA* (NC group) is expressed from the inducible promoter, the mutant still exhibits cell division defects and is temperature sensitive for growth but is viable at 30°C as long as an inducing agent is supplied. Thus, we can create a BAL800 series mutant just as easily if a gene is expressed belonging to the C group as from the NC group (though not all possible mutants have been isolated to date). These results suggest that all *E. coli* DedA family genes share a function that is required for survival and is independent of the cell division defects and temperature sensitivity of BC202 (6). Why are there so many DedA proteins in *E. coli* and other organisms (Table 1)? We cannot answer this question at present, but we may speculate that we are witnessing protein evolution in progress, with certain members duplicated and acquiring new functions (i.e., C group) while still retaining older ancestral functions.

DedA PROTEINS AS POTENTIAL DRUG TARGETS AND ROLES IN VIRULENCE

Recent reports suggest that members of the DedA family may represent potential drug targets and may play roles in virulence in some species. The genomes of most *Mycobacterium* species encode multiple DedA proteins (Table 1), and one DedA homolog (BCG2664) from *M. bovis* is possibly the target for the antibiotic halicyclamine A, as *bcg2664* confers resistance to this drug when overexpressed in *M. smegmatis* (64). Halicyclamine A was first isolated from the marine sponge *Haliclona* sp. and was originally thought to inhibit IMP dehydrogenase (IMPDH), but this turned out not to be the case (65, 66). There exists a possibility that halicyclamine A and/or derivatives of this drug may act as general inhibitors of the DedA membrane protein family. Alternatively, DedA proteins may promote the efflux of antibiotics when overexpressed in these species.

Cationic peptides are important components of the host innate immune system. DedA family proteins appear to be required for resistance to cationic peptides in both *Salmonella enterica* and *Neisseria meningitidis*. In *Salmonella*, YqjA is regulated by PhoP and required for resistance to protamine and the alpha helical cationic peptide magainin 2 (but not polymyxin) (67). Often, resistance to cationic peptides requires covalent modifications to lipid A (68), but the *Salmonella* Δ*yqjA* strain exhibits a wild-type lipid A profile (67). Therefore, it is not yet clear what role YqjA plays in cationic peptide resistance in *Salmonella*. Similarly, a *Neisseria* NMB1052 (*dedA*) mutant was found to be hypersensitive to polymyxin (69). *Neisseria* uses a combination of lipopolysaccharide (LPS) modification, efflux pumps, and type IV pilin

secretion to resist effects of cationic peptides (69, 70). Again, the role of DedA genes in promoting resistance to cationic peptides remains unclear, but the similarity of these two mutants in their cationic peptide sensitivity is intriguing.

Type III secretion is used by a number of Gram-negative pathogens to deliver effector proteins to host cells (71, 72). A screen for *Yersinia pestis* insertion mutants defective in type III secretion identified the DedA family gene *ctgA* (formerly *y0447*, encoding a polypeptide most closely related to *E. coli* YabI). This mutant, termed CHI 1345, was found to have impaired secretion of Yops and attenuated virulence in a mouse infection model (73). While likely not playing a direct role in this protein secretion pathway, CtgA may be required to maintain specific membrane properties that are required for the efficient assembly and/or operation of the type III secretion system (see next section).

PUTATIVE FUNCTIONS FOR DedA FAMILY MEMBERS

Based upon the mutant phenotypes described above, we can hypothesize potential functions of DedA family members. In *E. coli*, YqjA and YghB are together required for several cellular functions, all involving inner membrane proteins or protein complexes. For example, BC202 is defective in cell division due to inefficient function of the Tat pathway (30) and has an altered membrane phospholipid composition possibly due to an inefficiency in certain lipid synthesis pathways (5). The altered membrane composition is a property shared with the *B. burgdorferi* DedA family mutant (7). In addition, BC202 cannot survive at elevated pH (pH 8.8), but temperature sensitivity and cell division are rescued when it is grown at a lower pH (pH 6.0) or when the *mdfA* gene encoding an Na^+/K^+-H^+ antiporter is overexpressed (40). These observations collectively suggest that these DedA family proteins may play a more general role in membrane protein function or quality control and may play a role in maintenance of the PMF.

The membrane potential, $\Delta\psi$, of BC202 is significantly lower than that of the wild type, resulting in induction of the Psp stress response under permissive growth conditions (40). It is possible that under permissive growth conditions, the PMF homeostasis mechanism is inefficient in BC202 while under nonpermissive conditions the PMF falls below the minimum necessary threshold, leading to cell death. The Na^+/K^+-H^+ antiporter MdfA participates in PMF homeostasis by catalyzing the import of protons into the cytoplasm coupled with the export of monovalent cations (60). The resulting influx of protons lowers the cytoplasmic pH and necessitates bacterial adaptation and survival during exposure to alkaline conditions. Similarly, growth of *E. coli* in media of low pH reinforces the PMF by providing an enhanced ΔpH component and promotes the influx of protons into the cytoplasm (74). As the pH homeostasis mechanism is physiologically linked to the PMF homeostasis mechanism (74), it is probable that the intracellular pH of BC202 is altered (likely more alkaline than normal). This also explains why conditions promoting proton influx in BC202 alleviate temperature sensitivity and cell division defects.

An analysis of protein evolutionary relationships using a novel software program called AlignMe revealed that bacterial DedA family proteins may share structural motifs with the LeuT protein superfamily (8). LeuT is a bacterial homolog of the neurotransmitter sodium symporter (75) and vSGLT of the solute:sodium symporter family. These protein families share certain structural similarities, including two sets of five transmembrane helices that share a pseudo 2-fold axis of symmetry along the plane of the membrane (76). It is important to note that the evolutionary relationship of the DedA and LeuT families is derived not from amino acid content but from hydrophobicity profiles and therefore would not turn up in a simple BLAST search. The data from the study by Khafizov et al. also suggest that DedA family proteins may adopt dual topologies in the membrane (8).

Since DedA family proteins may share structural motifs with LeuT-type transporters (8), it is possible that *E. coli* YqjA and YghB form homo- and/or heterodimers and participate in PMF homeostasis in a manner similar to that of MdfA. This would require these functional complexes to display a proton symporter or antiporter activity (Fig. 3A). This is also consistent with a proposed role for a DedA family protein in the uptake of selenite in *Ralstonia metallidurans* (77) and the occurrence of DedA domains in secondary transporters of the tripartite ATP-independent periplasmic transporter (TRAP-T) family (9). A second possibility is that these proteins regulate the function/activity of a crucial component(s) of the PMF homeostasis mechanism—such as respiratory complexes, metabolic enzymes, or distinct Na^+/K^+-H^+ transporters like MdfA (Fig. 3B) (74). This model also derives support from the regulation of *yqjA* by the CpxAR two-component system. YqjA is an important member of the Cpx regulon in *E. coli* (58, 78), and deletion of either *cpxR* or *yqjA* renders *E. coli* sensitive to alkaline pH, demonstrating the necessity of YqjA for *E. coli* to adapt and survive under alkaline conditions (58). *yqjA* is also in an operon with *mzrA* (previously known as *yqjB* or *ecfM*), encoding a protein that links the Cpx pathway to the EnvZ/OmpR two-component system (79). (Remarkably, while *yghB* is not part of the Cpx regulon, its transcription is strongly induced by the quorum-sensing molecule autoinducer-2 [AI-2] [80].)

EUKARYOTIC DedA GENES

While this review focuses upon the functions of the bacterial DedA family proteins, some information on the roles DedA proteins play in multicellular organisms is available. A BLAST search reveals the presence of hundreds of DedA family homologs in eukaryotes (most closely related to *E. coli* YdjX or YdjZ). Many DedA genes, even in bacteria, are annotated "SNARE-associated Golgi protein." This is in reference to the reported physical association of protein Tvp38 (an *E. coli* YdjX homolog) from *Saccharomyces cerevisiae* with T-SNARE found in purified Tgl2-containing (late) Golgi compartments (81). It was hypothesized that Tvp38, and other unknown proteins isolated in this proteomic approach, may be involved in maintenance of late Golgi/endosomal compartments. The functional significance of this interaction remains to be elucidated, but it clearly suggests that DedA family members play some role in the eukaryotic secretory pathway.

An interesting screen for *Caenorhabditis elegans* mutants (generated by MosI mutagenesis) resistant to the bacterial pathogen *Microbacterium nematophilum* revealed the involvement of a DedA family member named Bus-19 (bacterially unswollen-19; with similarity to *E. coli* YdjX) (82). *M. nematophilum* is able to colonize the rectum of susceptible worms and induce an inflammatory response that requires the extracellular signal-regulated kinase/mitogen-activated protein (ERK/MAP) kinase pathway (83). The Δ*bus-19* worms were resistant to infection because the bacteria were unable to adhere to the surface of the rectum, perhaps due to loss of a nematode cell surface receptor. Bus-19 also contains a putative endoplasmic reticulum (ER) localization signal. Again, this study supports the notion that DedA family pro-

teins may play a role in proper functioning of the eukaryotic secretory pathway. Vertebrates, including mice and humans, also harbor DedA family homologs. The *Mus musculus* and *Homo sapiens* gene products are annotated Tmem41A, and they share about 25% amino acid identity with *E. coli* YdjX. Whether the functions of eukaryotic DedA family members are similar to their functions in prokaryotes is one of the more interesting questions in regard to this ancient family and, sadly, cannot be answered at this time.

CONCLUSIONS

Genetics and biochemistry, coupled with insight provided from proteomics and genome sequencing projects, have provided a glimpse into the wide distribution of and important functions carried out by members of the highly conserved DedA membrane protein family. In *E. coli*, some DedA family members appear to be required to maintain the membrane proton motive force. Others may play different roles in bacterial physiology. These functions have important implications, not just for elucidation of a potential drug target but for the insight that may be provided into the process of protein evolution.

ACKNOWLEDGMENTS

We thank Patrick Lane for valuable assistance in preparation of figures and anonymous reviewers for helpful comments. We thank Aaron Smith for critical reading of the manuscript. We thank Holger Kneuper and Tracy Palmer for sharing unpublished observations regarding growth of Δ*tat* mutants.

Financial support has been provided by the National Science Foundation (MCB-0841853, to W.T.D.).

REFERENCES

1. **Elofsson A, von Heijne G.** 2007. Membrane protein structure: prediction vs reality. Annu. Rev. Biochem. **76**:125–140.
2. **Rose PW, Beran B, Bi C, Bluhm WF, Dimitropoulos D, Goodsell DS, Prlic A, Quesada M, Quinn GB, Westbrook JD, Young J, Yukich B, Zardecki C, Berman HM, Bourne PE.** 2011. The RCSB Protein Data Bank: redesigned web site and web services. Nucleic Acids Res. **39**:D392–D401.
3. **White SH.** 2009. Biophysical dissection of membrane proteins. Nature **459**:344–346.
4. **Nonet ML, Marvel CC, Tolan DR.** 1987. The hisT-purF region of the Escherichia coli K-12 chromosome. Identification of additional genes of the hisT and purF operons. J. Biol. Chem. **262**:12209–12217.
5. **Thompkins K, Chattopadhyay B, Xiao Y, Henk MC, Doerrler WT.** 2008. Temperature sensitivity and cell division defects in an Escherichia coli strain with mutations in yghB and yqjA, encoding related and conserved inner membrane proteins. J. Bacteriol. **190**:4489–4500.
6. **Boughner LA, Doerrler WT.** 2012. Multiple deletions reveal the essentiality of the DedA membrane protein family in Escherichia coli. Microbiology **158**:1162–1171.
7. **Liang FT, Xu Q, Sikdar R, Xiao Y, Cox JS, Doerrler WT.** 2010. BB0250 of Borrelia burgdorferi is a conserved and essential inner membrane protein required for cell division. J. Bacteriol. **192**:6105–6115.
8. **Khafizov K, Staritzbichler R, Stamm M, Forrest LR.** 2010. A study of the evolution of inverted-topology repeats from LeuT-fold transporters using AlignMe. Biochemistry **49**:10702–10713.
9. **Barabote RD, Tamang DG, Abeywardena SN, Fallah NS, Fu JY, Lio JK, Mirhosseini P, Pezeshk R, Podell S, Salampessy ML, Thever MD, Saier MH, Jr.** 2006. Extra domains in secondary transport carriers and channel proteins. Biochim. Biophys. Acta **1758**:1557–1579.
10. **Wu DY, Hugenholtz P, Mavromatis K, Pukall R, Dalin E, Ivanova NN, Kunin V, Goodwin L, Wu M, Tindall BJ, Hooper SD, Pati A, Lykidis A, Spring S, Anderson IJ, D'Haeseleer P, Zemla A, Singer M, Lapidus A, Nolan M, Copeland A, Han C, Chen F, Cheng JF, Lucas S, Kerfeld C, Lang E, Gronow S, Chain P, Bruce D, Rubin EM, Kyrpides NC, Klenk HP, Eisen JA.** 2009. A phylogeny-driven genomic encyclopaedia of Bacteria and Archaea. Nature **462**:1056–1060.
11. **Brochier-Armanet C, Forterre P, Gribaldo S.** 2011. Phylogeny and evolution of the Archaea: one hundred genomes later. Curr. Opin. Microbiol. **14**:274–281.
12. **Detels R, Green AM, Klausner JD, Katzenstein D, Gaydos C, Handsfield HH, Pequegnat W, Mayer K, Hartwell TD, Quinn TC.** 2011. The incidence and correlates of symptomatic and asymptomatic Chlamydia trachomatis and Neisseria gonorrhoeae infections in selected populations in five countries. Sex. Transm. Dis. **38**:503–509.
13. **Hotopp JCD, Lin MQ, Madupu R, Crabtree J, Angiuoli SV, Eisen J, Seshadri R, Ren QH, Wu M, Utterback TR, Smith S, Lewis M, Khouri H, Zhang CB, Niu H, Lin Q, Ohashi N, Zhi N, Nelson W, Brinkac LM, Dodson RJ, Rosovitz MJ, Sundaram J, Daugherty SC, Davidsen T, Durkin AS, Gwinn M, Haft DH, Selengut JD, Sullivan SA, Zafar N, Zhou LW, Benahmed F, Forberger H, Halpin R, Mulligan S, Robinson J, White O, Rikihisa Y, Tettelin H.** 2006. Comparative genomics of emerging human ehrlichiosis agents. PLoS Genet. **2**(2):e21.doi:10.1371/journal.pgen.0020021.
14. **Larsen B, Hwang J.** 2010. Mycoplasma, ureaplasma, and adverse pregnancy outcomes: a fresh look. Infect. Dis. Obstet. Gynecol. **2010**:521921. doi:10.1155/2010/521921.
15. **Kowalczewska M, Vellaiswamy M, Nappez C, Vincentelli R, Scola BL, Raoult D.** 2012. Protein candidates for the serodiagnosis of rickettsioses. FEMS Immunol. Med. Microbiol. **64**:130–133.
16. **Sprong H, Wielinga PR, Fonville M, Reusken C, Brandenburg AH, Borgsteede F, Gaasenbeek C, van der Giessen JWB.** 2009. Ixodes ricinus ticks are reservoir hosts for Rickettsia helvetica and potentially carry flea-borne Rickettsia species. Parasit. Vectors **2**:41.
17. **Nerandzic MM, Cadnum JL, Eckart KE, Donskey CJ.** 2012. Evaluation of a hand-held far-ultraviolet radiation device for decontamination of Clostridium difficile and other healthcare-associated pathogens. BMC Infect. Dis. **12**:120.
18. **Shao XJ, Raman B, Zhu MJ, Mielenz JR, Brown SD, Guss AM, Lynd LR.** 2011. Mutant selection and phenotypic and genetic characterization of ethanol-tolerant strains of Clostridium thermocellum. Appl. Microbiol. Biotechnol. **92**:641–652.
19. **Siddiqui AR, Bernstein JM.** 2010. Chronic wound infection: facts and controversies. Clin. Dermatol. **28**:519–526.
20. **Zhang W, Ma J, Zang C, Song Y, Liu P.** 2011. The fur transcription regulator and fur-regulated genes in Clostridium botulinum A ATCC 3502. J. Biomed. Biotechnol. **2011**:934756. doi:10.1155/2011/934756.
21. **Liu QY, Eremeeva ME, Li DM.** 2012. Bartonella and Bartonella infections in China: from the clinic to the laboratory. Comp. Immunol. Microbiol. Infect. Dis. **35**:93–102.
22. **Wirth SE, Ayala-del-Rio HL, Cole JA, Kohlerschmidt DJ, Musser KA, Sepulveda-Torres LD, Thompson LM, Wolfgang WJ.** 2012. Psychrobacter sanguinis sp nov., recovered from four clinical specimens over a 4-year period. Int. J. Syst. Evol. Microbiol. **62**:49–54.
23. **Akman L, Yamashita A, Watanabe H, Oshima K, Shiba T, Hattori M, Aksoy S.** 2002. Genome sequence of the endocellular obligate symbiont of tsetse flies, Wigglesworthia glossinidia. Nat. Genet. **32**:402–407.
24. **Shigenobu S, Watanabe H, Hattori M, Sakaki Y, Ishikawa H.** 2000. Genome sequence of the endocellular bacterial symbiont of aphids Buchnera sp. APS. Nature **407**:81–86.
25. **McCutcheon JP, Moran NA.** 2012. Extreme genome reduction in symbiotic bacteria. Nat. Rev. Microbiol. **10**:13–26.
26. **McCutcheon JP, Moran NA.** 2010. Functional convergence in reduced genomes of bacterial symbionts spanning 200 My of evolution. Genome Biol. Evol. **2**:708–718.
27. **Moran NA, Tran P, Gerardo NM.** 2005. Symbiosis and insect diversification: an ancient symbiont of sap-feeding insects from the bacterial phylum Bacteroidetes. Appl. Environ. Microbiol. **71**:8802–8810.
28. **Penz T, Horn M, Schmitz-Esser S.** 2010. The genome of the amoeba symbiont "Candidatus Amoebophilus asiaticus" reveals common mechanisms for host cell interaction among amoeba-associated bacteria. Virulence **1**:541–545.
29. **Baker BJ, Comolli LR, Dick GJ, Hauser LJ, Hyatt D, Dill BD, Land ML, VerBerkmoes NC, Hettich RL, Banfield JF.** 2010. Enigmatic, ultrasmall, uncultivated Archaea. Proc. Natl. Acad. Sci. U. S. A. **107**:8806–8811.
30. **Sikdar R, Doerrler WT.** 2010. Inefficient Tat-dependent export of periplasmic amidases in an *Escherichia coli* strain with mutations in two DedA family genes. J. Bacteriol. **192**:807–818.

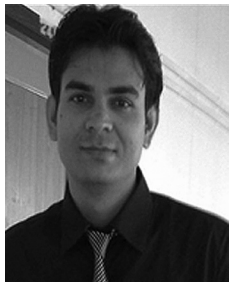
31. **Bernhardt TG, de Boer PA.** 2003. The Escherichia coli amidase AmiC is a periplasmic septal ring component exported via the twin-arginine transport pathway. Mol. Microbiol. **48**:1171–1182.
32. **Berks BC, Palmer T, Sargent F.** 2005. Protein targeting by the bacterial twin-arginine translocation (Tat) pathway. Curr. Opin. Microbiol. **8**:174–181.
33. **Berks BC, Sargent F, Palmer T.** 2000. The Tat protein export pathway. Mol. Microbiol. **35**:260–274.
34. **De Buck E, Lammertyn E, Anne J.** 2008. The importance of the twin-arginine translocation pathway for bacterial virulence. Trends Microbiol. **16**:442–453.
35. **Lee PA, Tullman-Ercek D, Georgiou G.** 2006. The bacterial twin-arginine translocation pathway. Annu. Rev. Microbiol. **60**:373–395.
36. **Heidrich C, Ursinus A, Berger J, Schwarz H, Holtje JV.** 2002. Effects of multiple deletions of murein hydrolases on viability, septum cleavage, and sensitivity to large toxic molecules in Escherichia coli. J. Bacteriol. **184**:6093–6099.
37. **Stanley NR, Findlay K, Berks BC, Palmer T.** 2001. Escherichia coli strains blocked in Tat-dependent protein export exhibit pleiotropic defects in the cell envelope. J. Bacteriol. **183**:139–144.
38. **Ize B, Stanley NR, Buchanan G, Palmer T.** 2003. Role of the Escherichia coli Tat pathway in outer membrane integrity. Mol. Microbiol. **48**:1183–1193.
39. **Dilks K, Rose RW, Hartmann E, Pohlschroder M.** 2003. Prokaryotic utilization of the twin-arginine translocation pathway: a genomic survey. J. Bacteriol. **185**:1478–1483.
40. **Sikdar R, Simmons AR, Doerrler WT.** 2013. Multiple envelope stress response pathways are activated in an *Escherichia coli* strain with mutations in two members of the DedA membrane protein family. J. Bacteriol. **195**:12–24.
41. **Bury-Mone S, Nomane Y, Reymond N, Barbet R, Jacquet E, Imbeaud S, Jacq A, Bouloc P.** 2009. Global analysis of extracytoplasmic stress signaling in Escherichia coli. PLoS Genet. 5:e1000651. doi:10.1371/journal.pgen.1000651.
42. **Connolly L, De Las Penas A, Alba BM, Gross CA.** 1997. The response to extracytoplasmic stress in Escherichia coli is controlled by partially overlapping pathways. Genes Dev. **11**:2012–2021.
43. **Raivio TL, Silhavy TJ.** 1999. The sigmaE and Cpx regulatory pathways: overlapping but distinct envelope stress responses. Curr. Opin. Microbiol. **2**:159–165.
44. **Vogt SL, Raivio TL.** 2012. Just scratching the surface: an expanding view of the Cpx envelope stress response. FEMS Microbiol. Lett. **326**:2–11.
45. **Engl C, Beek AT, Bekker M, de Mattos JT, Jovanovic G, Buck M.** 2011. Dissipation of proton motive force is not sufficient to induce the phage shock protein response in Escherichia coli. Curr. Microbiol. **62**:1374–1385.
46. **Joly N, Engl C, Jovanovic G, Huvet M, Toni T, Sheng X, Stumpf MP, Buck M.** 2010. Managing membrane stress: the phage shock protein (Psp) response, from molecular mechanisms to physiology. FEMS Microbiol. Rev. **34**:797–827.
47. **Jovanovic G, Lloyd LJ, Stumpf MP, Mayhew AJ, Buck M.** 2006. Induction and function of the phage shock protein extracytoplasmic stress response in Escherichia coli. J. Biol. Chem. **281**:21147–21161.
48. **Hirakawa H, Inazumi Y, Masaki T, Hirata T, Yamaguchi A.** 2005. Indole induces the expression of multidrug exporter genes in Escherichia coli. Mol. Microbiol. **55**:1113–1126.
49. **Nishino K, Honda T, Yamaguchi A.** 2005. Genome-wide analyses of Escherichia coli gene expression responsive to the BaeSR two-component regulatory system. J. Bacteriol. **187**:1763–1772.
50. **Ebel W, Vaughn GJ, Peters HK, III, Trempy JE.** 1997. Inactivation of mdoH leads to increased expression of colanic acid capsular polysaccharide in Escherichia coli. J. Bacteriol. **179**:6858–6861.
51. **Majdalani N, Gottesman S.** 2005. The Rcs phosphorelay: a complex signal transduction system. Annu. Rev. Microbiol. **59**:379–405.
52. **Parker CT, Kloser AW, Schnaitman CA, Stein MA, Gottesman S, Gibson BW.** 1992. Role of the rfaG and rfaP genes in determining the lipopolysaccharide core structure and cell surface properties of Escherichia coli K-12. J. Bacteriol. **174**:2525–2538.
53. **Shiba Y, Yokoyama Y, Aono Y, Kiuchi T, Kusaka J, Matsumoto K, Hara H.** 2004. Activation of the Rcs signal transduction system is responsible for the thermosensitive growth defect of an Escherichia coli mutant lacking phosphatidylglycerol and cardiolipin. J. Bacteriol. **186**:6526–6535.
54. **Laubacher ME, Ades SE.** 2008. The Rcs phosphorelay is a cell envelope stress response activated by peptidoglycan stress and contributes to intrinsic antibiotic resistance. J. Bacteriol. **190**:2065–2074.
55. **Jovanovic G, Engl C, Mayhew AJ, Burrows PC, Buck M.** 2010. Properties of the phage-shock-protein (Psp) regulatory complex that govern signal transduction and induction of the Psp response in Escherichia coli. Microbiology **156**:2920–2932.
56. **Alder NN, Theg SM.** 2003. Energetics of protein transport across biological membranes. a study of the thylakoid DeltapH-dependent/cpTat pathway. Cell **112**:231–242.
57. **Bageshwar UK, Musser SM.** 2007. Two electrical potential-dependent steps are required for transport by the Escherichia coli Tat machinery. J. Cell Biol. **179**:87–99.
58. **Price NL, Raivio TL.** 2009. Characterization of the Cpx regulon in Escherichia coli strain MC4100. J. Bacteriol. **191**:1798–1815.
59. **Krulwich TA, Lewinson O, Padan E, Bibi E.** 2005. Do physiological roles foster persistence of drug/multidrug-efflux transporters? A case study. Nat. Rev. Microbiol. **3**:566–572.
60. **Lewinson O, Padan E, Bibi E.** 2004. Alkalitolerance: a biological function for a multidrug transporter in pH homeostasis. Proc. Natl. Acad. Sci. U. S. A. **101**:14073–14078.
61. **Lewinson O, Adler J, Poelarends GJ, Mazurkiewicz P, Driessen AJ, Bibi E.** 2003. The Escherichia coli multidrug transporter MdfA catalyzes both electrogenic and electroneutral transport reactions. Proc. Natl. Acad. Sci. U. S. A. **100**:1667–1672.
62. **Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H.** 2006. Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol. Syst. Biol. **2**:2006 0008. doi:10.1038/msb4100050.
63. **Fraser CM, Casjens S, Huang WM, Sutton GG, Clayton R, Lathigra R, White O, Ketchum KA, Dodson R, Hickey EK, Gwinn M, Dougherty B, Tomb JF, Fleischmann RD, Richardson D, Peterson J, Kerlavage AR, Quackenbush J, Salzberg S, Hanson M, van Vugt R, Palmer N, Adams MD, Gocayne J, Weidman J, Utterback T, Watthey L, McDonald L, Artiach P, Bowman C, Garland S, Fuji C, Cotton MD, Horst K, Roberts K, Hatch B, Smith HO, Venter JC.** 1997. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. Nature **390**:580–586.
64. **Arai M, Liu L, Fujimoto T, Setiawan A, Kobayashi M.** 2011. DedA protein relates to action-mechanism of halicyclamine A, a marine spongean macrocyclic alkaloid, as an anti-dormant mycobacterial substance. Mar. Drugs **9**:984–993.
65. **Arai M, Sobou M, Vilcheze C, Baughn A, Hashizume H, Pruksakorn P, Ishida S, Matsumoto M, Jacobs WR, Jr., Kobayashi M.** 2008. Halicyclamine A, a marine spongean alkaloid as a lead for anti-tuberculosis agent. Bioorg. Med. Chem. **16**:6732–6736.
66. **Jaspars M, Pasupathy V, Crews P.** 1994. A tetracyclic diamine alkaloid, halicyclamine A, from the marine sponge *Haliclona* sp. J. Org. Chem. **59**:3253–3255.
67. **Shi Y, Cromie MJ, Hsu FF, Turk J, Groisman EA.** 2004. PhoP-regulated Salmonella resistance to the antimicrobial peptides magainin 2 and polymyxin B. Mol. Microbiol. **53**:229–241.
68. **Raetz CR, Reynolds CM, Trent MS, Bishop RE.** 2007. Lipid A modification systems in gram-negative bacteria. Annu. Rev. Biochem. **76**:295–329.
69. **Tzeng YL, Ambrose KD, Zughaier S, Zhou X, Miller YK, Shafer WM, Stephens DS.** 2005. Cationic antimicrobial peptide resistance in Neisseria meningitidis. J. Bacteriol. **187**:5387–5396.
70. **Lewis LA, Choudhury B, Balthazar JT, Martin LE, Ram S, Rice PA, Stephens DS, Carlson R, Shafer WM.** 2009. Phosphoethanolamine substitution of lipid A and resistance of Neisseria gonorrhoeae to cationic antimicrobial peptides and complement-mediated killing by normal human serum. Infect. Immun. **77**:1112–1120.
71. **Cornelis GR.** 2006. The type III secretion injectisome. Nat. Rev. Microbiol. **4**:811–825.
72. **Galan JE, Wolf-Watz H.** 2006. Protein delivery into eukaryotic cells by type III secretion machines. Nature **444**:567–573.
73. **Houppert AS, Kwiatkowski E, Glass EM, DeBord KL, Merritt PM, Schneewind O, Marketon MM.** 2012. Identification of chromosomal genes in Yersinia pestis that influence type III secretion and delivery of Yops into target cells. PLoS One **7**:e34039. doi:10.1371/journal.pone.0034039.
74. **Krulwich TA, Sachs G, Padan E.** 2011. Molecular aspects of bacterial pH sensing and homeostasis. Nat. Rev. Microbiol. **9**:330–343.
75. **Yamashita A, Singh SK, Kawate T, Jin Y, Gouaux E.** 2005. Crystal

structure of a bacterial homologue of Na+/Cl– dependent neurotransmitter transporters. Nature **437**:215–223.
76. **Faham S, Watanabe A, Besserer GM, Cascio D, Specht A, Hirayama BA, Wright EM, Abramson J.** 2008. The crystal structure of a sodium galactose transporter reveals mechanistic insights into Na+/sugar symport. Science **321**:810–814.
77. **Ledgham F, Quest B, Vallaeys T, Mergeay M, Coves J.** 2005. A probable link between the DedA protein and resistance to selenite. Res. Microbiol. **156**:367–374.
78. **Yamamoto K, Ishihama A.** 2006. Characterization of copper-inducible promoters regulated by CpxA/CpxR in Escherichia coli. Biosci. Biotechnol. Biochem. **70**:1688–1695.
79. **Gerken H, Charlson ES, Cicirelli EM, Kenney LJ, Misra R.** 2009. MzrA: a novel modulator of the EnvZ/OmpR two-component regulon. Mol. Microbiol. **72**:1408–1422.
80. **DeLisa MP, Wu CF, Wang L, Valdes JJ, Bentley WE.** 2001. DNA microarray-based identification of genes controlled by autoinducer 2-stimulated quorum sensing in Escherichia coli. J. Bacteriol. **183**:5239–5247.
81. **Inadome H, Noda Y, Kamimura Y, Adachi H, Yoda K.** 2007. Tvp38, Tvp23, Tvp18 and Tvp15: novel membrane proteins in the Tlg2-containing Golgi/endosome compartments of Saccharomyces cerevisiae. Exp. Cell Res. **313**:688–697.
82. **Yook K, Hodgkin J.** 2007. MosI mutagenesis reveals a diversity of mechanisms affecting response of Caenorhabditis elegans to the bacterial pathogen Microbacterium nematophilum. Genetics **175**:681–697.
83. **Nicholas HR, Hodgkin J.** 2004. The ERK MAP kinase cascade mediates tail swelling and a protective response to rectal infection in C. elegans. Curr. Biol. **14**:1256–1261.
84. **Krogh A, Larsson B, von Heijne G, Sonnhammer EL.** 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J. Mol. Biol. **305**:567–580.
85. **Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S.** 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. **28**:2731–2739.
86. **Blevins JS, Revel AT, Smith AH, Bachlani GN, Norgard MV.** 2007. Adaptation of a luciferase gene reporter and lac expression system to Borrelia burgdorferi. Appl. Environ. Microbiol. **73**:1501–1513.
87. **Daley DO, Rapp M, Granseth E, Melen K, Drew D, von Heijne G.** 2005. Global topology analysis of the Escherichia coli inner membrane proteome. Science **308**:1321–1323.
88. **Hirokawa T, Boon-Chieng S, Mitaku S.** 1998. SOSUI: classification and secondary structure prediction system for membrane proteins. Bioinformatics **14**:378–379.

William T. Doerrler is an Associate Professor in the Department of Biological Sciences at LSU. He received his B.S. from Stevens Institute of Technology in 1987 and spent time as a lab technician in the labs of Kenneth O. Lloyd (Sloan-Kettering) and Carl Grunfeld (UCSF) before working on his Ph.D. thesis in the area of glycobiology in the lab of Mark A. Lehrman (University of Texas Southwestern Medical Center). He carried out postdoctoral work in the lab of the late Christian R. H. Raetz at Duke University before arriving at LSU in 2004. In addition to being a father and husband, he has been interested in the DedA family and LSU Athletics in recent years.

Lisa A. Boughner is a doctoral candidate in William Doerrler's lab at Louisiana State University. Lisa obtained a B.Sc. in Honours Biological Sciences with Thesis from the University of Windsor, Windsor, Ontario, followed by working as a field and laboratory assistant at the Greenhouse and Processing Crops Research Center in Harrow, Ontario, Canada. Lisa joined LSU in 2007, and her doctoral research consists of investigating the essential role of the *E. coli* DedA protein family. In her "spare" time, Lisa enjoys playing both the classical and bass guitar, as well as traveling.

Rakesh Sikdar recently was awarded his Ph.D. degree and carried out his graduate work with William Doerrler at LSU. He received his B.Tech (Hons.) in Biotechnology and Biochemical Engineering at the Indian Institute of Technology, Kharagpur, in West Bengal, India, before arriving at LSU in 2007. His research in *Escherichia coli* seeks to discover the roles of the proteins YqjA and YghB belonging to the DedA protein family. When not actively pursuing his prominent academic and research interests, he is a hobbyist photographer and indulges in extensive travelling and adventure hiking across the country.



Sujeet Kumar received a B.Tech degree in Biotechnology at Vellore Institute of Technology University, Vellore, India, in 2011. Thereafter, he joined the Biological Sciences Department at LSU. Currently, he is a doctoral candidate working with William Doerrler and investigating the roles of essential DedA protein family members in *Escherichia coli*.