New Functions for the Ancient DedA Membrane Protein Family

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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 (EcDedA) 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 EcDedA 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/Chlamydomphilia spp. or Ureaplasma spp.; however, Neisseria spp. have multiplets, 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
bbers is quite unpredictable; one note of interest is that no se-
tification of significant DedA homologs using a strict BLAST
that other proteins have taken over their role or that symbiotic
DedA homolog, which has allowed for a select few to counter-
there may be subtle differences between organisms that lack a
DedA homolog are “Amoebobhilus asiaticus,” “
Candidatus
Parvarchaeum spp.” (reviewed in reference 25). The
C. princeps” (26–28).
Tremblaya princeps” (26–28).

distribution of DedA members (10, 11).
and archaeal domains were used as a guide for investigating the
species viability. Published phylogenetic trees of the bacterial
sequences within the Halobacteriales or Sulfolobales or-
der lack a significant DedA homolog. Also, the reduced
related to the genetic makeup and/or physiology of the symbi-
onts’ host species (26–28).
As for the archaeal domain, the distribution of DedA mem-
ers is quite unpredictable; one note of interest is that no se-
quenched genomes within the Halobacteriales or Sulfolobales or-
der lack a significant DedA homolog. Also, the reduced
ancestry of the DedA family protein for species viability. Published phylogenetic trees of the bacterial
further supports the essentiality of the DedA protein family
for 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 counter-
act 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 iden-
tification of significant DedA homologs using a strict BLAST
score cutoff (E value, ≤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, EcdelaA, 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 pos-
sessing likely four membrane-spanning domains with cytoplas-
imic 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 (ΔyqjA ΔyghB) displays several in-
triguing 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 ami-
dases are normally exported across the inner membrane via the twin arginine transport (Tat) pathway in E. coli (31), a Sec-inde-
pendent 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
Δtat mutants display cell division defects due to loss of amidase
export (31, 37). However, E. coli Δtat (T. Palmer, personal com-
munication) and Δami 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 Δtat mutants (37, 38) and mutants lacking periplasmic ami-
dases (36). Interestingly, while the Borrelia Δhbo250 strain (de-
scribed 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 path-
way.

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 ex-
tracytoplasmic stress by activating one or more of several well-
known stress response pathways such as the σ52. Cpx, Psp, Bae, and
Rcs pathways, which help the cells detect and combat alterations
in their cell envelope when challenged with conditions that com-
promise envelope integrity (41). The Cpx and σ32 pathways are
activated in response to disruptions in the folding of envelope
proteins and loss of outer membrane integrity, and they have par-
tially overlapping regulons (41–44). The Psp (phage shock pro-
tein) response is activated by perturbations in the integrity of the
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, iono-

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

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>No. of DedA family homologs</th>
</tr>
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<tbody>
<tr>
<td>Escherichia coli K-12</td>
<td>8</td>
</tr>
<tr>
<td>Salmonella enterica SL480</td>
<td>6</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa PA01</td>
<td>5</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>9</td>
</tr>
<tr>
<td>Vibrio cholerae El Tor N16961</td>
<td>3</td>
</tr>
<tr>
<td>Caulobacter crescentus CB15</td>
<td>3</td>
</tr>
<tr>
<td>Neisseria meningitidis Z2491</td>
<td>3</td>
</tr>
<tr>
<td>Borrelia burgdorferi B31</td>
<td>1</td>
</tr>
<tr>
<td>Bacillus subtilis strain 168</td>
<td>6</td>
</tr>
<tr>
<td>Bacillus anthracis strain Ames</td>
<td>8</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis H37Rv</td>
<td>4</td>
</tr>
<tr>
<td>Chlamydia trachomatis D/UW-3/CX</td>
<td>0</td>
</tr>
<tr>
<td>Synechocystis sp. strain PCC6803</td>
<td>3</td>
</tr>
<tr>
<td>Halobacterium salinarum NRC-1</td>
<td>1</td>
</tr>
</tbody>
</table>

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 B31DedA family gene BB0250 has been demonstrated to be essential (7). 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).

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glossinid (23) and Buschera spp. (24). Some symbionts that
lack a DedA homolog are “Candidatus Sulcia muelleri,” “
Candidatus Amoebobhilus asiaticus,” “Candidatus Pytho-
plasma mali,” “Candidatus Zinderia insecticola,” “Candidatus Carso-
nella ruddii,” “Candidatus Hodgkinia cicadica,” and “Candi-
datus 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 symbi-
onts’ host species (26–28).

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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, /H11349/4) 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 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, /H11349/4) 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.
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 \( \Delta yghB \) and \( \Delta yqjA \) 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-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).

The \( E. coli \) DedA family is collectively essential at all temperatures. BAL801 (W3110, \( \Delta ydhXYZ::cam \Delta ecdedA726 \Delta yohD762 \Delta yqja785 \Delta yqja770 \Delta yghb781::kan \) 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 \( B. burgdorferi \) is essential (not shown), and depletion of the protein causes cell division defects (7). \( B. burgdorferi \) DXL-01 (\( \Delta bb0250 \), pWTD0250 expressing bb0250 behind a borrelia-optimized lac promoter [86]) was cultured with either 1 mM (top) or 0 mM (bottom) IPTG (isopropyl-\( \beta \)-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 2 Characteristics of DedA family mutants. (A) BC202 (\( \Delta yghb::Kan^r \Delta yqja::Tet^r \)) grows at 30°C but not at 42°C (5). The \( \Delta yghb \) and \( \Delta yqja \) 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-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, \( \Delta ydhXYZ::cam \Delta yohb772 \Delta ecdedA726 \Delta yohb772 \Delta qja785 \Delta qja770 \Delta yghb781::kan \) 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 \( B. burgdorferi \) is essential (not shown), and depletion of the protein causes cell division defects (7). \( B. burgdorferi \) DXL-01 (\( \Delta bb0250 \), pWTD0250 expressing bb0250 behind a borrelia-optimized lac promoter [86]) was cultured with either 1 mM (top) or 0 mM (bottom) IPTG (isopropyl-\( \beta \)-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).

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 \( \sigma^{52} \) (controlling the cytoplasmic heat shock response) and \( \sigma^S \) 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, \( \Delta \Psi = (\psi_{\text{m}} - \psi_{\text{out}}) \), with \( \psi_{\text{m}} \) (electrical potential inside) being more negative than \( \psi_{\text{out}} \) (electrical potential outside), and the transmembrane pH difference, \( \Delta pH = (pH_{\text{m}} - pH_{\text{out}}) \), with \( pH_{\text{m}} \) (intracellular pH) being more alkaline than \( pH_{\text{out}} \) (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 Fsp pathway in BC202 (40). BC202 (as well as the single \( yqjA \) 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\(^+\) antipporter (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 \( \Delta mdfA \) 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 \( \Delta pH \)) for electrogenic transport of neutral compounds, while using only \( \Delta pH \) 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 (\( yqjA, yghB, yabI, yohD, EcededA, ydjX, ydjZ, \) 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* \( \Delta bb0250 \) 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 (\( ydjX, ydjZ, EcededA, \) 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., *EcededA*). 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 *EcededA* (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 (BGC2664) from *M. bovis* is possibly the target for the antibiotic halicinonamide A, as *bgc2664* confers resistance to this drug when overexpressed in *M. smegmatis* (64). Halicinonamide A was first isolated from the marine sponge *Halichondra* 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 halicinonamide 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* *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, \( \Delta \phi \), 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 \( \Delta \phi \) 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 solutesodium 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 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 *ecM*), 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 Mos1 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 \( \Delta \text{bus-19} \) 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 yDxJ. 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.

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