

The ArcB Sensor Kinase of *Escherichia coli*: Genetic Exploration of the Transmembrane Region

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The Arc two-component signal transduction system of *Escherichia coli* regulates the expression of numerous operons in response to respiratory growth conditions. Cellular redox state or proton motive force ($\Delta\bar{\mu}_{\text{H}^+}$) has been proposed to be the signal for the membrane-associated ArcB sensor kinase. This study provided evidence for a short ArcB periplasmic bridge that contains a His47. The dispensability of this amino acid, the only amino acid with a pK in the physiological range, renders the $\Delta\bar{\mu}_{\text{H}^+}$ model unlikely. Furthermore, results from substituting membrane segments of ArcB with counterparts of MalF indicate that the region does not play a stereospecific role in signal reception.

The Arc two-component signal transduction system of *Escherichia coli* regulates the expression of more than 30 operons, depending on the redox conditions of growth (20, 22, 30, 31). The system consists of ArcB, the membrane-bound sensor kinase, and ArcA, the cognate response regulator. ArcB (17, 23, 27, 48) (see Fig. 1A) belongs to the tripartite sensor kinase subfamily (1, 16, 25, 35, 37, 44, 49), is attached to the cytoplasmic membrane by two transmembrane segments (TM1 and TM2) near the N-terminal end (24), and catalyzes a phosphorelay via His292, Asp576, and His717 of ArcB to Asp54 of ArcA (14). The autophosphorylation step is stimulated by effectors, such as D-lactate, pyruvate, and acetate. These metabolites accumulate when exogenous electron acceptors limit respiration during growth (13, 18). Dephosphorylation of ArcA-P occurs by a reverse phosphorelay from the Asp54 of ArcA to the His717 and Asp576 of ArcB. The phosphoryl group is then released as P_i (12).

Most sensor kinases receive their signal from the periplasmic domain, resulting in conformation changes that trigger autophosphorylation. As a sensor kinase, ArcB is unusual in having a short putative periplasmic bridge (24, 29). Relatively little is known about the nature of the signal for ArcB and what role the membrane-associated region plays in signal reception, except that autophosphorylation seems to be activated by excessive reducing equivalents (7, 11, 21, 22, 24, 38). Two studies involving growth of cells at high pH and treatment of cells by protonophores during growth, however, led to the suggestion that ArcB kinase is activated by a decrease in proton motive force (PMF) across the cytoplasmic membrane (2, 5). Here, we confirm the transmembrane topology of ArcB by genetic analysis and probe the function of the membrane region by replacing the chromosomal *arcB*⁺ by a single copy of a mutant allele (Fig. 2; Tables 1 and 2).

Transmembrane topology of ArcB. To test the suggested topology based on hydrophobicity analysis, we constructed four *phoA* protein fusions (32) of *arcB* (Fig. 1B). The PhoA fusions at residues 22 and 102 of ArcB exhibited very low levels of alkaline phosphatase activity. In contrast, the PhoA fusions at residues 41 and 57 of ArcB showed very high levels of the

enzyme activity (Fig. 1B). On the basis of this genetic analysis and a more recent algorithm for determining membrane-spanning regions (40), we suggest that a periplasmic bridge of ArcB is flanked by TM1 delimited by residues 23 to 41 and TM2 delimited by residues 58 to 77.

Testing the periplasmic His47 as a possible PMF sensor. In order for ArcB to sense $\Delta\bar{\mu}_{\text{H}^+}$, at least one amino acid residue on each side of the plasma membrane with pK values within biological range would be required. The only periplasmic candidate would be His47. We therefore tested the phenotypes of His47Gln and His47Arg on the expression of positively controlled $\lambda::\Phi(\text{cydA}'\text{-lacZ})$ or negatively controlled $\lambda::\Phi(\text{lldP}'\text{-lacZ})$ (8, 19). Neither substitution resulted in any significant change in the expression of $\Phi(\text{cydA}'\text{-lacZ})$ or $\Phi(\text{lldP}'\text{-lacZ})$ (data not shown).

Testing the amino acid sequence of membrane regions. To examine the function of various segments of the ArcB membrane region, we replaced them by a corresponding section of MalF (a subunit of maltose permease). MalF was chosen because its periplasmic bridge between the first and second transmembrane segments is also short (45) and because of the lack of any sequence homology with ArcB. In each hybrid construct, a portion of the ArcB N terminus was retained (Fig. 3 and 4). The reason for this measure is that when the cytosolic N-terminal segment of ArcB was replaced by the corresponding segment of MalF, the level of the hybrid protein diminished in the cell extract, as assayed by Western analysis (data not shown).

When TM1 alone or TM1 plus the periplasmic bridge of ArcB was replaced by the counterparts of MalF, the expression of $\Phi(\text{cydA}'\text{-lacZ})$ was increased under both aerobic and anaerobic growth conditions (Fig. 3). As to be expected, the expression of $\Phi(\text{lldP}'\text{-lacZ})$ was partially repressed aerobically. However, because anaerobic repression of $\Phi(\text{lldP}'\text{-lacZ})$ was already severe in the wild-type background, further repression by the chimeric ArcB proteins was not readily discernible. When TM2 was replaced, no significant changes in the expression of either reporter fusion were observed. When TM1, the periplasmic bridge, and TM2 were all replaced by the corresponding MalF region, the protein became inactive as an ArcA kinase (data not shown). However, the lack of kinase activity is difficult to interpret for the following reasons. First, there may be a failure in signal reception. Second, there may be a serious conformational distortion. Third, the protein may fail to

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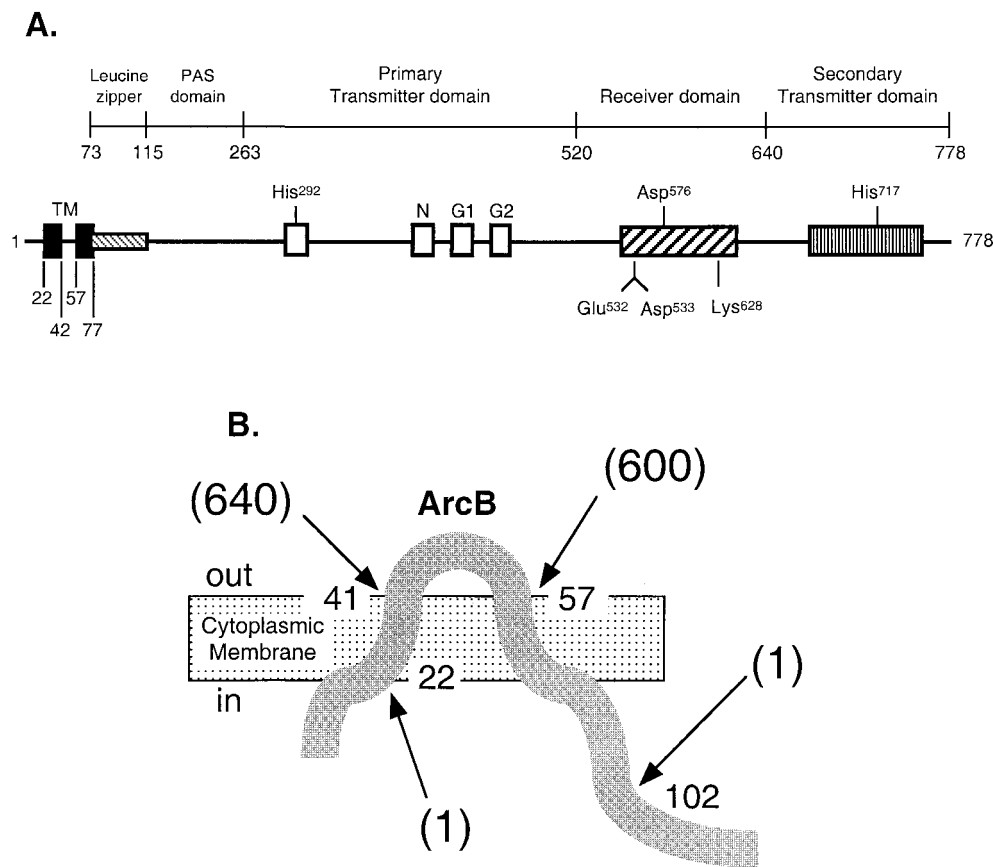


FIG. 1. The ArcB sensor kinase and its transmembrane topology. (A) Schematic representation of ArcB. The putative leucine zipper (12) and the PAS domain (46) are based on amino acid sequence homology. The primary transmitter domain contains the conserved His292 and the catalytic determinants N, G1, and G2. The G1 and G2 sequences typify nucleotide-binding motifs. The receiver domain contains the conserved Asp576, and the secondary transmitter domain contains the conserved His717. (B) Schematic representation of ArcB transmembrane topology. Arrows point to ArcB-PhoA fusions constructed according to a two-step PCR procedure (41). The first PCR amplifications were performed, using plasmid pBB25 (23) as a template and BPH-N and either BPH-22, BPH-41, BPH-57, or BPH-102 as primers (Table 2). Each purified product was used as a primer with BPH-C for the second PCR, using the plasmid pDHB5747 that bears *phoA* (D. Boyd, unpublished data) as a template. The second PCR products were digested with *Bam*HI and *Hind*III and cloned between the corresponding sites of vector pUC18, resulting in pBP22 (Φ [*arcB*¹⁻²²-*phoA*]), pBP41 (Φ [*arcB*¹⁻⁴¹-*phoA*]), pBP57 (Φ [*arcB*¹⁻⁵⁷-*phoA*]), and pBP102 (Φ [*arcB*¹⁻¹⁰²-*phoA*]). Each Φ (*arcB*'-*phoA*) plasmid was transformed into strain DHB4 (Δ *phoA*) and assayed for alkaline phosphatase activity as described previously (6). The alkaline phosphatase activity units are represented by numbers in parentheses and are averages of four experiments, with standard deviations of less than 10%.

dimerize, which is believed to be necessary for signal transmission. It might also be mentioned that when ArcB is liberated from membrane association by removal of the transmembrane domain, the truncated protein becomes constitutively active as an ArcA kinase (data not shown). This is to be expected, since purified ArcB⁷⁸⁻⁷⁷⁸ has been shown to be highly active in vitro as an ArcA kinase and phosphatase (12, 14).

To ascertain that each ArcB-MalF hybrid protein remains membrane associated, we performed Western blot analysis on cytosolic and membrane fractions of the cells. In all cases, the hybrid proteins were found to be associated with the cytoplasmic membrane (Fig. 4).

Discussion and conclusion. Most sensor kinases have a periplasmic domain of substantial size flanked by two TM segments for sensing signals (10, 15, 26, 33, 34, 37, 47). For many sensor kinases, however, the true signal and its input site on the protein remain unknown. According to the PMF sensing model by ArcB (2, 5), anaerobic growth diminishes the energy yield, thereby diminishing the $\Delta\bar{\mu}_{H^+}$, and activates the kinase. Results of His47 replacement experiments deprive this model of an obvious mechanism. It might be recalled that PMF was suggested as the signal primarily on the basis of protono-

phore effects on target gene expression. The validity of the conclusion, however, is compromised by the severe growth inhibition. Also, from a theoretical point of view, $\Delta\bar{\mu}_{H^+}$ seems not to be ideal as a signal, since its level is likely to be homeostatically controlled by the F_0F_1 -ATPase. Moreover, even during aerobic growth, the energy source may become limiting. The resultant drop in PMF would repress the tricarboxylic acid cycle and the electron transport system in a situation when derepression would help to enhance the substrate-scavenging power of the starving cell.

The lack of evidence for the PMF model redirected our focus on the redox model and the possible functional importance of the membrane-associated portion of ArcB for signal reception. Three kinds of mechanisms may be envisaged. First, a redox-signaling element generated within the lipid bilayer may stereospecifically interact with a transmembrane or periplasmic segment of ArcB. In such a case, a drastic change in amino acid sequence should disrupt signal recognition. Second, the transmembrane region may simply serve as a mechanical anchor to ensure proximity of the rest of ArcB to the cytoplasmic membrane for signal reception. Third, one or both of the TM segments may play an entirely novel and unus-

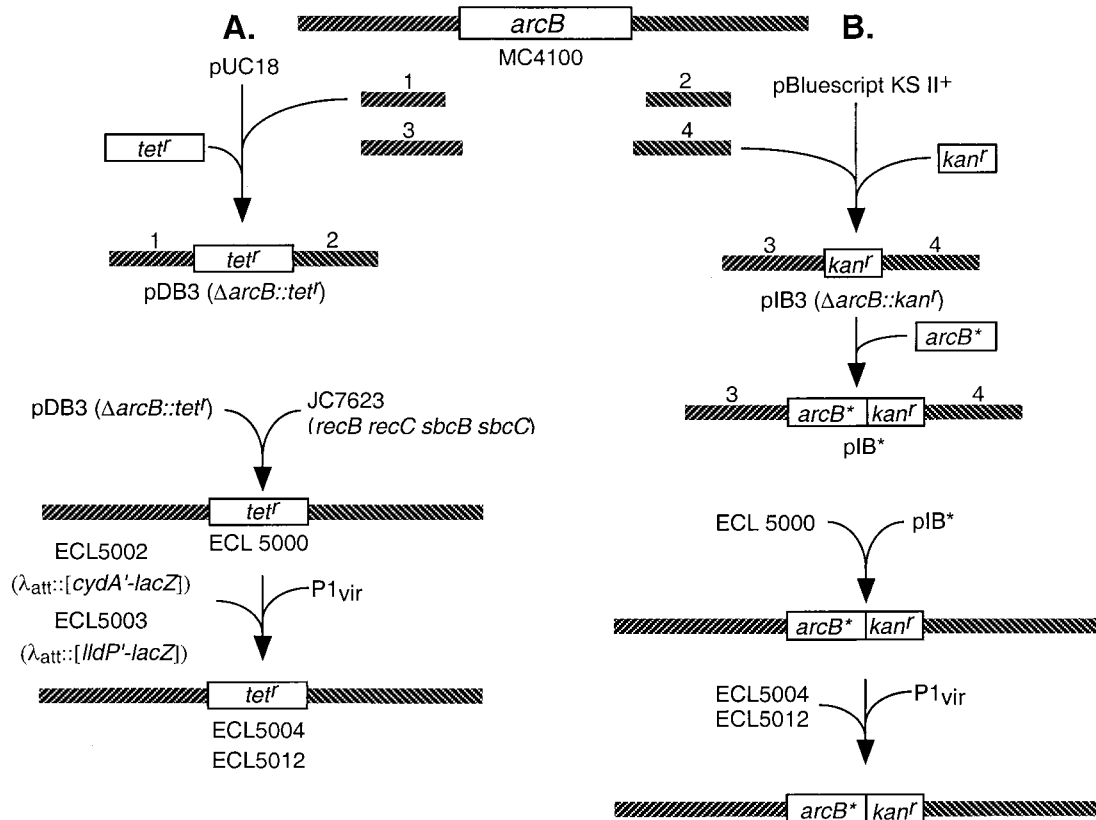


FIG. 2. Construction of strains. (A) To construct the $\Delta arcB::Tet^f$ strain, the 5'- and 3'-flanking DNA fragments of *arcB* (fragments 1 and 2) were prepared by PCR, using chromosomal DNA from strain MC4100 as template and, respectively, the primer pairs DAB-5N/DAB-5C and DAB-3N/DAB-3C (Table 2). The products were cloned into pUC18. A *Tet^f* cassette isolated from pNK81 (50) was then inserted between the two *arcB*-flanking fragments, generating pDB3. This plasmid was transformed into strain JC7623 (36) to create $\Delta arcB::Tet^f$ strain (ECL5000) by homologous recombination. The *arcB::Tet^f* allele was then P1 transduced into strain ECL5002 or ECL5003, respectively, resulting in ECL5004 and ECL5012. (B) To introduce the modified *arcB* sequence into the $\Delta arcB::Tet^f$ strain, the 5'- and 3'-flanking DNA fragments of *arcB* (fragments 3 and 4) were prepared by PCR, using chromosomal DNA from strain MC4100 as template and, respectively, the primer pairs IAB-5N/IAB-5C and IAB-3N/IAB-3C (Table 2). The products were cloned into pBluescript KS II(+). A *Kan^f* cassette, isolated from pUC4-KIXX (3), was then inserted between the two *arcB*-flanking fragments, generating pIB3. The 5'-flanking fragment includes the *arcB* promoter, ribosome-binding site, and introduced *NdeI* site which included the initiation codon of *arcB* followed by a *HindIII* site. Modified *arcB* sequences (*arcB**) were cloned into the pIB3 between the *NdeI* site and *HindIII* site, generating pIB*. This plasmid was transformed into strain ECL5000 to replace *arcB::Tet^f* allele with *arcB*::Kan^f* by homologous recombination. Recombinants were selected by the *Tet^r Kan^r Amp^s* phenotype and confirmed by the PCR. The *arcB*::Kan^f* was then P1 transduced into strains ECL5004 or ECL5012. Not illustrated is the construction of the reporter fusions. To construct the $\Phi(cydA'-lacZ)$ operon fusion, a 1.0-kb *BamHI-EcoRI* fragment of plasmid pBTKScyd1 (31) was ligated into *BamHI-EcoRI*-digested *lacZ* operon fusion vector pRS528 (42), resulting in pCAZ1. To generate the $\Phi(ldp'-lacZ)$ operon fusion, a 3.6-kb *PstI-BamHI* fragment of plasmid pLCT2 (9) was subcloned into pBluescript SK(-) (Stratagene), resulting in pLLD2. A 0.8-kb *EcoRI-BglII* fragment of pLLD2 was then ligated into the *EcoRI-BamHI*-digested *lacZ* operon fusion vector pRS415 (42), resulting in pLPZ1. The $\Phi(cydA'-lacZ)$ and $\Phi(ldp'-lacZ)$ were then transferred to the λ transducing phage λ RS45 (42), yielding, respectively, λ CAZ1 and λ LPZ1. Lysates with high titers of λ CAZ1 and λ LPZ1 were used to lysogenize strain MC4100, and single lysogens were selected (28), yielding, respectively, strains ECL5001 and ECL5003. The $\Delta fur::Tn9$ (*Cm^r*) allele of strain JRG1728 (43) was P1 transduced into strain ECL5001 (yielding strain ECL5002) in order to avoid the transcriptional repression of $\Phi(cydA'-lacZ)$ by Fnr (8).

TABLE 1. *E. coli* K-12 strains, bacteriophages, and plasmids used in this study^a

Strain, phage, or plasmid	Relevant characteristic(s) or genotype	Reference or source
Strains		
MC4100	F ⁻ <i>araD139</i> Δ (<i>argF-lac</i>) <i>U169 rpsL150 relA1 flbB5301 deoC ptsF25 rbsR</i>	42
JM109	<i>recA1 endA1 gyrA96 thi hsdR17 supE44 relA1</i> Δ (<i>lac-proAB</i>)/F' <i>traD36 proAB⁺ lacI^q lacZ</i> Δ M15	Promega
JRG1728	Δ <i>fnr::Tn9</i> (Cm ^r)	43
DHB4	<i>araD139</i> Δ (<i>ara-leu</i>)7697 Δ <i>lacX74</i> Δ <i>phoA</i> (PvuII) <i>phoR</i> Δ <i>malF3 galE galK thi rpsL</i> /F' <i>lacI^q pro</i>	6
JC7623	<i>recB21 recC22 sbcB15 sbcC201</i>	36
ECL5000	JC7623 but Δ <i>arcB::Tet^r</i>	This study
ECL5001	MC4100 but Φ (<i>cydA'</i> - <i>lacZ</i>)	This study
ECL5002	MC4100 but Φ (<i>lldP'</i> - <i>lacZ</i>)	This study
ECL5003	MC4100 but Δ <i>fnr::Tn9</i> (Cm ^r) Φ (<i>cydA'</i> - <i>lacZ</i>)	This study
ECL5004	Δ <i>arcB::Tet^r</i> Φ (<i>cydA'</i> - <i>lacZ</i>) Δ <i>fnr::Tn9</i> (Cm ^r)	This study
ECL5005	Δ <i>arcB::Kan^r</i> Φ (<i>cydA'</i> - <i>lacZ</i>) Δ <i>fnr::Tn9</i> (Cm ^r)	This study
ECL5006	<i>arcB⁺::Kan^r</i> Φ (<i>cydA'</i> - <i>lacZ</i>) Δ <i>fnr::Tn9</i> (Cm ^r)	This study
ECL5007	<i>arcB^{H47Q}::Kan^r</i> Φ (<i>cydA'</i> - <i>lacZ</i>) Δ <i>fnr::Tn9</i> (Cm ^r)	This study
ECL5008	<i>arcB^{H47R}::Kan^r</i> Φ (<i>cydA'</i> - <i>lacZ</i>) Δ <i>fnr::Tn9</i> (Cm ^r)	This study
ECL5009	Φ (<i>arcB^{I-22}-malF¹⁷⁻³⁵-arcB⁴²⁻⁷⁷⁸</i>)::Kan ^r Φ (<i>cydA'</i> - <i>lacZ</i>) Δ <i>fnr::Tn9</i> (Cm ^r)	This study
ECL5010	Φ (<i>arcB^{I-57}-malF⁴⁰⁻⁵⁸-arcB⁷⁸⁻⁷⁷⁸</i>)::Kan ^r Φ (<i>cydA'</i> - <i>lacZ</i>) Δ <i>fnr::Tn9</i> (Cm ^r)	This study
ECL5011	Φ (<i>arcB^{I-22}-malF¹⁷⁻³⁹-arcB⁵⁸⁻⁷⁷⁸</i>)::Kan ^r Φ (<i>cydA'</i> - <i>lacZ</i>) Δ <i>fnr::Tn9</i> (Cm ^r)	This study
ECL5012	Δ <i>arcB::Tet^r</i> Φ (<i>lldP'</i> - <i>lacZ</i>)	This study
ECL5013	Δ <i>arcB::Kan^r</i> Φ (<i>lldP'</i> - <i>lacZ</i>)	This study
ECL5014	<i>arcB⁺::Kan^r</i> Φ (<i>lldP'</i> - <i>lacZ</i>)	This study
ECL5015	<i>arcB^{H47Q}::Kan^r</i> Φ (<i>lldP'</i> - <i>lacZ</i>)	This study
ECL5016	<i>arcB^{H47R}::Kan^r</i> Φ (<i>lldP'</i> - <i>lacZ</i>)	This study
ECL5017	Φ (<i>arcB^{I-22}-malF¹⁷⁻³⁵-arcB⁴²⁻⁷⁷⁸</i>)::Kan ^r Φ (<i>lldP'</i> - <i>lacZ</i>)	This study
ECL5018	Φ (<i>arcB^{I-57}-malF⁴⁰⁻⁵⁸-arcB⁷⁸⁻⁷⁷⁸</i>)::Kan ^r Φ (<i>lldP'</i> - <i>lacZ</i>)	This study
ECL5019	Φ (<i>arcB^{I-22}-malF¹⁷⁻³⁹-arcB⁵⁸⁻⁷⁷⁸</i>)::Kan ^r Φ (<i>lldP'</i> - <i>lacZ</i>)	This study
Phages		
λ RS45	' <i>bla lacZ lacY⁺</i>	42
λ LPZ1	Φ (<i>lldP'</i> - <i>lacZ⁺</i>) <i>lacY⁺</i>	This study
λ CAZ1	Φ (<i>cydA'</i> - <i>lacZ⁺</i>) <i>lacY⁺</i>	This study
P1vir		Laboratory stock
Plasmids		
pUC18	Cloning vector	Stratagene
pBluescript SK(-)	Cloning vector	Stratagene
pBluescript KS II(+)	Cloning vector	Stratagene
pNK81	Tet ^r	50
pUC4-KIXX	Kan ^r	3
pBB25	<i>arcB⁺</i>	23
pDHB5747	' <i>phoA</i>	D. Boyd
pDHB32	<i>malF⁺</i>	6
pBP22	Φ (<i>arcB^{I-22}-'phoA</i>)	This study
pBP41	Φ (<i>arcB^{I-41}-'phoA</i>)	This study
pBP57	Φ (<i>arcB^{I-57}-'phoA</i>)	This study
pBP102	Φ (<i>arcB^{I-102}-'phoA</i>)	This study
pLCT2	<i>lldPRD⁺</i>	9
pLLD2	<i>lldPR⁺</i>	This study
pRS415	<i>lacZ⁺ lacY⁺ bla⁺</i>	42
pLPZ1	Φ (<i>lldP'</i> - <i>lacZ</i>)	This study
pBTKScyd1	<i>cydA'</i>	31
pRS528	<i>lacZ⁺ lacY⁺ bla⁺</i>	42
pCAZ1	Φ (<i>cydA'</i> - <i>lacZ</i>)	This study
pDB3	Δ <i>arcB::Tet^r</i>	This study
pIB3	Δ <i>arcB::Kan^r</i>	This study
pQE30ArcB ⁷⁸⁻⁷⁷⁸	His ₆ -ArcB ⁷⁸⁻⁷⁷⁸	14
pABW	<i>arcB⁺</i> in pBluescript KS II(+)	This study
pABS	<i>arcB⁷⁸⁻⁷⁷⁸</i> in pBluescript KS II(+)	This study
pIBW	<i>arcB⁺::Kan^r</i> in pIB3	This study
pIBHQ	<i>arcB^{H47Q}::Kan^r</i> in pIB3	This study
pIBHR	<i>arcB^{H47R}::Kan^r</i> in pIB3	This study
pIBM1	Φ (<i>arcB^{I-22}-malF¹⁷⁻³⁵-arcB⁴²⁻⁷⁷⁸</i>)::Kan ^r in pIB3	This study
pIBM2	Φ (<i>arcB^{I-57}-malF⁴⁰⁻⁵⁸-arcB⁷⁸⁻⁷⁷⁸</i>)::Kan ^r in pIB3	This study
pIBM3	Φ (<i>arcB^{I-22}-malF¹⁷⁻³⁹-arcB⁵⁸⁻⁷⁷⁸</i>)::Kan ^r in pIB3	This study

^a Luria-Bertani broth and agar (17 g/liter) were used for routine growth. When used, ampicillin, tetracycline, kanamycin, and chloramphenicol were provided at final concentrations of 50, 12, 40, and 20 μ g/ml, respectively.

TABLE 2. Oligonucleotides used in this study^a

Primer	Sequence ^b
BPH-N	5'-CCCGGATCCGGATGCGGTGCTGGATCTGC-3' <i>Bam</i> HI
BPH-22	5'-CGCTACTTGTGTATAAGAGTCCGGGCGCACCAGACCTAACTTCATC-3'
BPH-41	5'-CGCTACTTGTGTATAAGAGTCCGGGCGCCATTTGTACCACAATGG-3'
BPH-57	5'-CGCTACTTGTGTATAAGAGTCCGGGACGAATAACATCAATGCTTTTCGACC-3'
BPH-102	5'-CGCTACTTGTGTATAAGAGTCCGGCAAATCGCGCTCGCGCATCTCC-3'
BPH-C	5'-TCAGCAAGCTTTCGCCCCGTGATCTGCC-3' <i>Hind</i> III
DAB-3N	5'-TCGGTTCGACAGATCTCTGCGCCAACACCAGGG-3' <i>Sal</i> I <i>Bgl</i> II
DAB-3C	5'-CCACTGCAGGTCGCCAAATTCGG-3' <i>Pst</i> I
DAB-5N	5'-TGCAGCTCCCTGCCTTGAAGTGC-3' <i>Sac</i> I
DAB-5C	5'-GTCAGATCTCCCCTCAACGACCTACTCCG-3' <i>Bgl</i> II
IAB-5N	5'-AGCGATATCGAACTGACGACAAAACCAGC-3' <i>Eco</i> RV
IAB-5C	5'-AGCAAGCTTCATATGGAATTTCCTCACGACAACC-3' <i>Hind</i> III <i>Nde</i> I
IAB-3N	5'-ACTGTCGACCCGGGTGCGGAATACTGC-3' <i>Sal</i> I <i>Sma</i> I
IAB-3C	5'-TCACTCGAGGATCCCCAGCTACGCCCATCCC-3' <i>Xho</i> I <i>Bam</i> HI
B5NDE	5'-CCCGATCCCATATGAAGCAAATTCGTCTGCTGGCGC-3' <i>Bam</i> HI <i>Nde</i> I
B3NRU	5'-GTAATGTCGCGACCAAAGCCCATCAAACCG-3'
BH47Q	5'-GCGTAACCATGGTGTCTGCAGGGTCAGGTGCGAAAGCATTGATG-3'
BH47R	5'-ATGGCGGTAACCATGGTGTCTCCGCGGTCCAGGTGCGAAAGCATTG-3'
BMF-22	5'-CCGAGCAGACCTAGCACTGACCAAGCGCACCAGACCTAACTTCATC-3'
BMF-35	5'-GCAGCACCATGGTTACGTACATTAACAACAAGGTAACC-3'
BMF-39	5'-CAGCAAACCAAAGAAGATAGATTCCTTGTGCGTACATTAACAAC-3'
BMF-40	5'-GCGTGGTAATGGCGAACAGGTAACGAATAACATCAATGC-3'
BMF-58	5'-GTCGTGACTCTCCAGTTGCTCGCGGAAAATATACAGCCCCGC-3'

^a Oligonucleotides were synthesized by either Oligos Etc. or Integrated DNA Technologies Inc. PCRs were carried out by using the TaqPlus Precision PCR system (Stratagene). Sequence verification of PCR-amplified DNA was performed at Micro Core Facility of the Department of Microbiology and Molecular Genetics, Harvard Medical School.

^b Restriction enzymes whose sites were introduced for subsequent cloning are indicated.

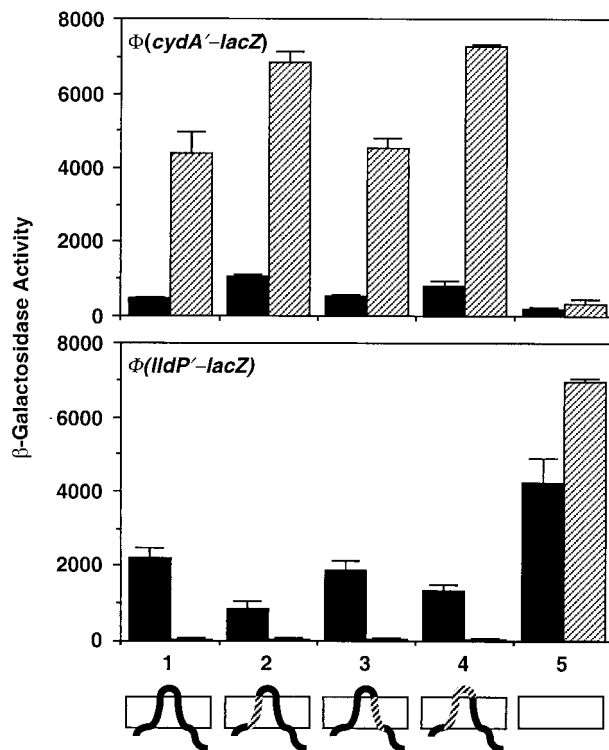


FIG. 3. Effects of substituting segments of ArcB with MalF on the expressions of $\Phi(cydA'-lacZ)$ and $\Phi(lldP'-lacZ)$. To construct $\Phi(arcB^{1-22}\text{-malF}^{17-35}\text{-arcB}^{42-778})$, PCR was performed with pIBW as a template and BPH-N and BMF-22 as primers. The purified PCR product and BMF-35 were used as primers for the second PCR with pDHB32 (6) as a template. The PCR product and B3NRU were used as primers for a third PCR with the pABW as a template. The product was digested with *Nde*I and *Nru*I and cloned between the corresponding sites of the pIBW, resulting in pIBM1. To construct $\Phi(arcB^{1-57}\text{-malF}^{40-58}\text{-arcB}^{78-778})$, PCR was performed with pIBW as a template and BPH-N and BMF-40 as primers. The purified PCR product and BMF-58 were used as primers for the second PCR with pDHB32 as a template. The PCR product and B3NRU were used as primers for a third PCR with the pABW as a template. The product was digested with *Nde*I and *Nru*I and cloned between the corresponding sites of the pIBW, resulting in pIBM2. To construct a $\Phi(arcB^{1-22}\text{-malF}^{17-39}\text{-arcB}^{58-778})$, PCR was performed with pIBW as a template and BPH-N and BMF-22 as primers. The purified PCR product and BMF-39 were used as primers for the second PCR with pDHB32 as a template. The PCR product and B3NRU were used as primers for a third PCR with pABW as a template. The product was digested with *Nde*I and *Nru*I and cloned between the corresponding sites of the pIBW, resulting in pIBM3. Plasmids pIBM1, pIBM2, and pIBM3 were used to integrate the modified *arcB* sequences into the chromosome of the reporter-bearing strains by the gene replacement techniques, as described in the legend of Fig. 2. For the β -galactosidase activity assay, the $\Phi(cydA'-lacZ)$ -bearing strains were cultured in buffered Luria-Bertani broth containing 0.1 M MOPS (morpholinepropanesulfonic acid) (pH 7.4) and 20 mM D-xylose. For the growth of $\Phi(lldP'-lacZ)$ -bearing strains, the above medium was supplemented with 20 mM L-lactate as an inducer (9). The data are averages of four experiments and the standard deviations are indicated. The different alleles of *arcB* are shown as follows: 1, *arcB*⁺; 2, $\Phi(arcB^{1-22}\text{-malF}^{17-35}\text{-arcB}^{42-778})$; 3, $\Phi(arcB^{1-57}\text{-malF}^{40-58}\text{-arcB}^{78-778})$; 4, $\Phi(arcB^{1-22}\text{-malF}^{17-39}\text{-arcB}^{58-778})$; 5, $\Delta arcB$. The topology of the chimeric proteins is illustrated at the bottom: solid segments represent ArcB sequences, and hatched segments represent MalF sequences. Solid bars, aerobically grown cells; hatched bars, anaerobically grown cells.

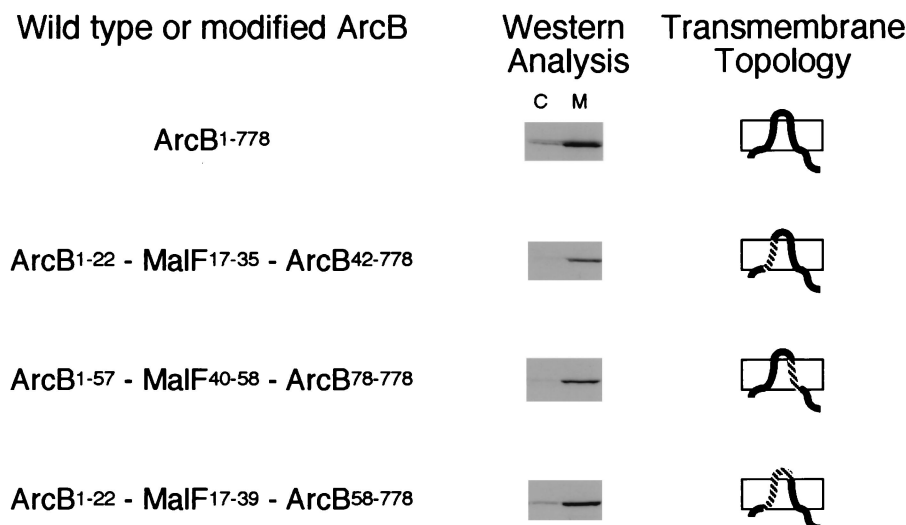


FIG. 4. Membrane association of the ArcB-MalF hybrid proteins. Cultures grown aerobically in Luria-Bertani broth were harvested during mid-exponential growth. The cells were washed with buffer S (50 mM Na-phosphate [pH 7.8], 300 mM NaCl, and 1 mM EDTA) by centrifugation. The cell pellet was resuspended in 3 ml of the same buffer and disrupted by sonication. Cell debris was removed by centrifugation for 10 min at $4,000 \times g$. The supernatant fluid was again centrifuged for 45 min at $35,000 \times g$ to separate the cytosolic (C) and the membrane (M) fractions. The resultant supernatant fluid containing the soluble proteins was collected. The remaining pellet was resuspended in 0.5 ml of buffer M (20 mM HEPES [pH 7.5], 50 mM KCl, 1 mM EDTA, and 50% glycerol). Samples of cytosolic and membrane (containing 10 μ g of protein) fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12% polyacrylamide gel) and the proteins were transferred to a Hybond-ECL filter (Amersham). The filter was equilibrated in TTBS buffer (25 mM Tris, 150 mM NaCl, and 0.05% Tween-20) for 10 min and incubated in blocking buffer (0.5% bovine serum albumin in TTBS) for 1 h at 37°C. ArcB polyclonal antibodies raised against His₆-ArcB⁷⁸⁻⁵²⁰ were added at a dilution of 1:10,000 to the filter and incubated for 1 h at room temperature. The bound antibody was detected by using anti-rabbit immunoglobulin G antibody conjugated to horseradish peroxidase and the ECL detection system (Amersham). The topology of the chimeric proteins is depicted: solid segments represent ArcB sequences, and hatched segments represent MalF sequences.

pected role in signal sensing. Results from the TM replacement experiments would favor the second or third model. An example of the second model is the Aer protein, which acts as a sensor for aerotaxis. In that case, anchorage of the protein to cytoplasmic membrane by the two TM segments is thought to allow the bound flavin adenine dinucleotide (FAD) to detect the redox state of the electron transport chains (4, 39). There is no evidence, however, that a cytosolic domain of ArcB binds to FAD. First, although everted vesicles containing ArcB catalyzed the phosphorylation of ArcA, the addition of FAD did not stimulate the reaction (18). Second, unlike the case of Aer (4), extracts of cells containing abundant ArcB (specified by a multicopy plasmid) did not exhibit a detectable absorption spectrum that is characteristic of flavins (O. Kwon, unpublished data). Eventual identification of the true signal and the characterization of its mode of reception will likely require a combined biochemical, physiological, and genetic approach and the development of rigorous *in vitro* assays. In the meantime, the results of our structural probing revealed an unexpected degree of robustness of apparent ArcB function to wholesale substitutions in the transmembrane region.

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