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Forum Review

Signaling by the Arc Two-Component System Provides a Link Between the Redox State of the Quinone Pool and Gene Expression

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ABSTRACT

The Arc two-component system is a complex signal transduction system that plays a key role in regulating energy metabolism at the level of transcription in bacteria. This system comprises the ArcB protein, a tripartite membrane-associated sensor kinase, and the ArcA protein, a typical response regulator. Under anoxic growth conditions, ArcB autophosphorylates and transphosphorylates ArcA, which in turn represses or activates the expression of its target operons. Under aerobic conditions, ArcB acts as a phosphatase that catalyzes the dephosphorylation of ArcA-P and thereby releasing its transcriptional regulation. The events for Arc signaling, including signal reception and kinase regulation, signal transmission, amplification, as well as signal output and decay are discussed. *Antioxid. Redox Signal.* 8, 781–795.

INTRODUCTION

LIFE ON EARTH EVOLVED virtually under anaerobic conditions for about 2 billion years that is close to half of the total period of biological evolution (5, 80). For that reason, it is commonly accepted that fermentative modes of metabolism preceded respiratory modes of metabolism and that the highly efficient aerobic respiration process became possible after the emergence of photosynthesis, which generated O_2. Fermentative modes of metabolism achieve redox balance by internal dismutation reactions and generate energy principally by substrate-level phosphorylation. A serious disadvantage is the wasteful sacrifice of some carbon and hydrogen compounds as excretion products. By contrast, respiratory modes of metabolism exploit exogenous electron acceptors (oxidants) and generate energy principally by proton extrusion, with the consequence of enhanced anabolism (125).

To maximize energy generation under different redox conditions, facultative anaerobes, such as *Escherichia coli*, have evolved multiple metabolic pathways, together with complex genetic regulatory networks. Aerobic respiration is preferred because O_2 has the highest oxidizing power ($E'^\circ = +820$ mV) among all utilizable electron acceptors. In *E. coli* the final electron transfer steps in this respiration are mediated in parallel by cytochrome *d* (high O_2 affinity) and cytochrome *o* (high Vmax). When O_2 is unavailable, nitrate ($E'^\circ = +420$ mV) becomes the preferred electron acceptor in anaerobic respiration. If this choice is not available, the cell can resort to even less rewarding compounds, such as fumarate ($E'^\circ = +31$ mV). When no exogenous electron acceptor is accessible, fermentative metabolism becomes the last resort. In aerobic respiratory pathways, ubiquinone ($E'^\circ = +100$ mV) serves as the adapter between electron donor and electron disposal pathways. In anaerobic respiratory pathways, demethylmenaquionone ($E'^\circ = +36$ mV) and menaquinone ($E'^\circ = -74$

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This article is dedicated to the memory of Edmund C.C. Lin.

mV) serve as the adapters for electron transmission. In fermentation, NAD ($E'^{\circ} = -320$ mV) plays the role of the adapter carrier between electron donor and acceptor pathways, resulting in the excretion of compounds such as D-lactate, acetate, formate, succinate, ethanol, CO_2, and H_2 (13, 118).

Setting a mode of energy metabolism at the level of transcriptional control frequently requires the cooperation of several global sensor-regulator proteins. Control of nitrate utilization, which is the preferred anaerobic electron acceptor, and its reduction product nitrite, is modulated by the NarX/L (106) and NarQ/P (89) two-component systems (105). Transcriptional regulation in response to O_2 is mediated by the cytosolic global regulator Fnr (53) and the ArcA/B two component system (39, 42). Under anaerobic growth conditions Fnr activates the expression of genes that encode components of alternative branches of the electron transport chain and it also represses the expression of some genes with aerobic functions. Fnr consists of a carboxy-terminal DNA-binding domain and an amino-terminal sensory domain that contains four essential cysteine residues capable of binding a $[4Fe–4S]^{2+}$ cluster, which functions as a direct oxygen sensor (117). Results from recent gene expression profiling experiments show that nearly two-thirds of the genes whose expression is affected by Fnr are also affected by ArcA (95, 96). The ArcA/B two-component system is a global regulator of gene expression under microaerobic and anaerobic growth conditions, and is the main topic in this review

DISCOVERY OF THE REGULATORY GENES *ARCA* AND *ARCB*

It has been known for many years that the activity levels of numerous enzymes associated with aerobic metabolism are significantly higher in aerobically grown cells than in anaerobic grown cells (30, 31). Similarly, the activity levels of a number of proteins that function in anaerobic electron transport were observed to be higher in anaerobic grown cells (30, 31). The fact that the synthesis of many enzymes is dependent on only one variable, dioxygen tension, gave rise to the speculation that a global control mechanism might exist. With the assumption that such a regulation would likely operate at the transcriptional level, Iuchi and Lin designed an elegant genetic screen to search for mutants that highly expressed operons of aerobic function under anaerobic conditions (42). To do this, they constructed a chromosomal merodiploid harboring both the *sdh*$^+$ (encoding the succinate dehydrogenase complex) and a ϕ(*sdh-lacZ*) operon fusion using a Δ*lac* strain. This strain was grown anaerobically on MacConkey lactose agar for several days, and red papilla growing on the colonies, indicating emergence of mutants with increased expression of the reporter, were collected. The mutants were then purified on the same selective agar and scored for elevated succinate dehydrogenase activity during anaerobiosis. Their approach resulted in the identification of two genes: *arcA*, located at min 0 (10), previously named *dye* gene (11, 12), and *arcB*, located at min 69.5 (39). Because mutations in either gene resulted in elevated anaerobic activity levels of many enzymes involved in aerobic metabolism, they proposed the acronym for aerobic respiration control (*arc*) for this pair of genes.

THE ARC MODULON AND MUTANT PHENOTYPES

Although *arcA* and *arcB* were first identified as trans acting regulatory genes affecting the transcriptional control of the *sdh* operon, subsequent studies revealed that mutations in either *arcA* or *arcB* had pleiotropic effects on expression of many other aerobic function enzymes, including several TCA cycle and fatty acid degradation enzymes, some flavoprotein dehydrogenases, and ubiquinone oxidase (42). The TCA cycle enzymes include citrate synthase, aconitase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, fumarase, and malate dehydrogenase. In addition, the enzyme activities of several dehydrogenases, including L-lactate dehydrogenase, D-lactate dehydrogenase, D-amino acid dehydrogenase, and pyruvate dehydrogenase, involved in reactions supplying carbon precursors to the TCA cycle, were also abnormally elevated. In addition, enzymes in the fatty acid degradation pathway (acyl-CoA dehydrogenase and 3-hydroxyacyl-CoA dehydrogenase), and an enzyme in the glyoxylate shunt (isocitrate lyase) were elevated in the mutants. Also, expression of the *ptsG* encoded enzyme $IICB^{Glc}$, which mediates the first step of glucose metabolism, was recently reported to be regulated by ArcA in response to the redox conditions of growth (47). Because in the *arc* mutants most target operons exhibited increased anaerobic expression, it was proposed that ArcA, in its active state, acts as a repressor. Later on, however, it was shown that in a few cases ArcA also acts as activator. Examples of positive regulation are the control of the *cydAB* operon, encoding cytochrome *d* oxidase, and the *pfl* gene, encoding the pyruvate formate lyase enzyme involved in pyruvate cleavage during fermentative conditions (98). Positive regulation of these two operons is understandable, as their gene products play important roles when cells are deprived of oxygen (16, 40, 97). Over the years, genetic screens led to the identification of about 30 ArcA-controlled operons that are involved in redox metabolism (60, 61). Moreover, biochemical studies suggested a primary DNA sequence motif for ArcA binding, and subsequent sequence alignments generated a consensus sequence, further refined by bioinformatic methods (62, 70). Recently, a couple of comprehensive bioinformatic and array-based approaches predicted that the Arc system recruits some 300 operons that mediate cellular adaptation to anaerobiosis (56, 96), a number by far more extensive than originally thought. In general, the Arc system mediates regulation of operons involved in respiratory metabolism. However, it has become clear that its scope of control extends beyond redox metabolism to include functions such as F plasmid DNA transfer (41, 100, 101), Xer site-specific recombination at *psi* (14), inhibition of chromosomal replication at *oriC* (55), and the SOS response through the inhibition of *uvrA* that encodes the nucleotide excision-repair protein UvrA (78). Also, the expression of the general stress sigma factor σ^s (RpoS) was

recently found to be directly repressed by ArcA (72). Furthermore, the recent microarray-based approaches predict that genes associated with functions such as flagella synthesis and flagellar motor switching (*fliMN* and *fliE*), cell division (*ftsZ*), stress-induced survival (*surA*), and nickel transport (*nikABCDE*) are part of the Arc regulon (56).

The *arcA* gene was previously known and sequenced as the *dye* gene because null mutants were growth sensitive to the redox dyes toluidine blue and methylene blue (22). Later on, it was found that *arcB* null mutants are also dye sensitive (39, 44). Paradoxically, although this phenotype of *arc* mutants was the first to be reported, its molecular basis remains elusive. In recent years, the Arc system has also been implicated in the direct or indirect regulation of virulence in a number of clinically important human pathogens. For instance, this system was recently shown to control resistance to reactive oxygen and nitrogen species in *Salmonella enterica* serovar Enteritidis (58), and to modulate the expression of membrane proteins implicated in serum resistance in *Haemophilus influenzae* (20) and of virulence factors in *Vibrio cholera*e (99).

Subsequent analysis of the *arcA* and *arcB* gene products revealed that they belong to the large family of prokaryotic two-component signal transduction systems (42, 44, 75, 77, 93).

WHAT IS A TWO-COMPONENT SYSTEM?

Almost two decades ago the term "two-component system" was coined to describe a group of regulatory proteins found in bacteria (75, 77, 93). Today, hundreds of these systems have been found in eubacteria, archaea, and some eukaryotes such as plant and fungi (107). The prototypical two-component system comprises two protein components, a sensor kinase and its cognate response regulator, that contain transmitter and receiver domains. Transmitter domains have a conserved kinase core with an invariant histidine residue, whereas receiver domains have an invariant aspartate residue. A typical sensor kinase is anchored to the cytoplasmic membrane with two transmembrane segments that separate a periplasmic signal-sensing (or input) domain from a carboxy-terminal transmitter domain projecting into the cytoplasm, whereas a typical response regulator consists of an N-terminal receiver domain and a C-terminal output domain. Signal reception by the sensor kinase is believed to propagate conformational changes in the protein that stimulate an ATP-dependent autophosphorylation at the conserved His residue in its cytosolic transmitter domain. The phosphokinase then donates the phosphoryl group to the conserved Asp residue in the receiver domain of the cognate response regulator, thereby rendering it functional, in general as a transcriptional regulator. Upon cessation of signaling, both the cognate response regulator and the sensor kinase undergo dephosphorylation, which results in silencing of the system. Many two-component systems, however, are more elaborate. For instance, signal transmission may involve hybrid sensor kinases that contain both transmitter and receiver domains, or additional proteins as phosphorelay components (82, 83, 108). It is noteworthy that most bacterial sensor kinases posses only one transmitter domain, whereas the sensor kinases present in eukaryotic microorganisms or plants are almost exclusively multipartite kinases (126).

ARCHITECTURE OF THE ARC COMPONENTS

The arcA gene and its product

As mentioned earlier, the *arcA* gene was first reported as the *dye* gene (22). Its expression, as monitored by a ϕ(*arcA-lacZ*) reporter fusion, was shown to increase about fourfold during anaerobiosis, an effect directly dependent on Fnr (15). DNA sequence analysis of *arcA* indicated that the gene encodes a 29 kDa polypeptide of 238 amino acids (Fig. 1). Comparison with other bacterial proteins revealed that *arcA* encodes a typical response regulator consisting of a helix–turn–helix DNA binding domain at the carboxy terminus and a receiver domain at the amino-terminus (42). In the receiver domain, Glu10, Asp11 and Asp54 correspond to the three conserved amino acid residues that form an acidic pocket in the crystal structure of the CheY protein, which comprises a single receiver domain (121), whereas Lys103 corresponds to an invariant lysine residue found in the carboxy-terminus of the receiver domain in all response regulator proteins. The conserved Asp54 has been demonstrated to be the phosphorylation site. It has been suggested that in its inactive state ArcA exists as a dimer and forms an octamer upon phosphorylation, with a 1:1 ratio of unphosphorylated to phosphorylated protein (46). Recently the crystal structure of the ArcA N-terminal receiver domain was solved (113). In this study it was proposed that the DNA-binding domain of ArcA also participates in oligomerization. This was supported by the fact that while the unphosphorylated receiver domain of ArcA exists as a monomer in solution, intact ArcA has some tendency for dimerization. Moreover, the observation that the DNA-binding domain associates into oligomers larger than tetramers, led to the suggestion that dimerization of full-length ArcA may at least in part be mediated by the DNA-binding domain and that the unphosphorylated regulatory domain may inhibit higher order oligomerization (113).

The arcB *gene and its product*

An early study using a Φ(*arcB-lacZ*) reporter fusion indicated that expression of ArcB is constitutive, and that there is no regulation of *arcB* at the transcriptional level (43). DNA sequence analysis of *arcB* revealed the identity of the second member of this two-component system (Fig. 1); the deduced protein contains 778 amino acids (88 kDa), and is a transmembrane protein as predicted by hydrophobic profile analysis (44). It has now been demonstrated that ArcB is membrane-associated by two transmembrane segments (TM). TM1 corresponding to residues 23 to 41 and TM2 corresponding to residues 58 to 77, separate a periplasmic bridge of about 16 amino acid residues (44, 52). A linker region, containing a putative leucine-zipper (25) and a PAS domain (112), connects TM2 with the catalytic domains of the sensor. ArcB possesses three cytosolic catalytic domains: a primary transmitter domain (H1) at the N-terminus with a conserved

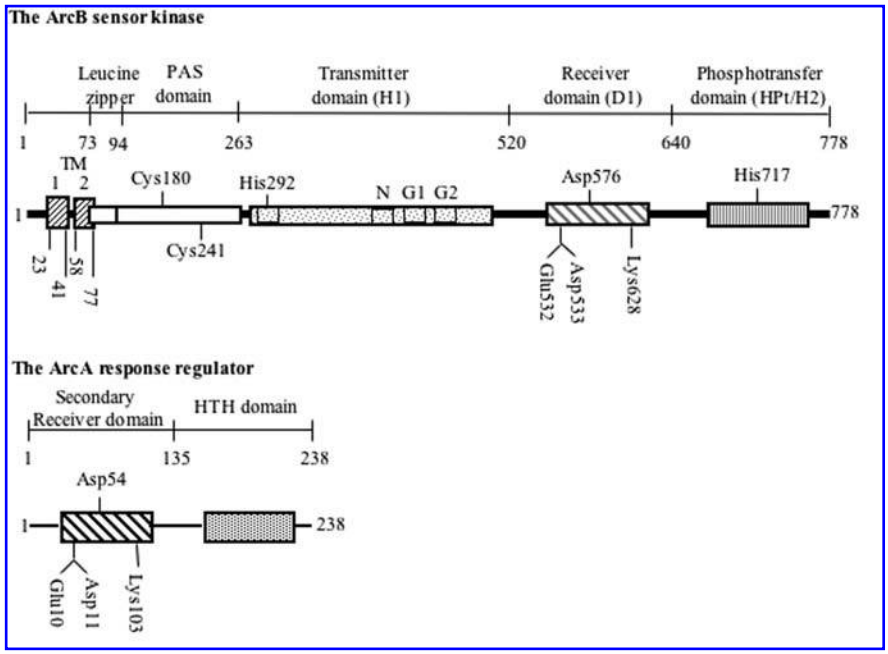

FIG. 1. Schematic representation of domain composition in ArcB and ArcA. ArcB is attached to the plasma membrane by TM1 corresponding to residues 23 to 41 and TM2 corresponding to residues 58 to 77. The linker region contains a putative leucine-zipper and a PAS domain. Depicted, in the linker region, are also the cysteine residues 180 and 241. The primary transmitter domain (H1) contains the conserved His292 and the catalytic determinants N, G1, and G2. The G1 and G2 sequences typify nucleotide-binding motifs. The receiver domain (D1) contains the conserved Asp576, and the histidine phosphotransfer domain (Hpt/H2) contains the conserved His717. ArcA is shown with its N-terminal receiver domain containing the conserved Asp54 and its C-terminal helix–turn–helix (HTH) domain. Adapted from Ref. (25) with some modifications.

His residue at position 292, a central receiver domain (D1) with a conserved Asp residue at position 576, and a secondary transmitter domain, or histidine-containing phosphotransfer domain, at the C-terminus (H2/HPt) with a conserved His residue at position 717 (37, 43). Thus, ArcB belongs to the subclass of hybrid sensor kinases that also includes BarA, BvgS, EvgS, LemA (GacS), RteA, and TorS (3, 35, 73, 102, 104, 119).

STRUCTURE FUNCTION RELATIONSHIPS

The transmembrane domain

Most sensor kinases possess a periplasmic domain of substantial size (approximately 150 amino acid residues) flanked by two transmembrane segments, believed to be involved in signal reception (108). In contrast, the periplasmic sequence of ArcB is extremely small, having only 16 amino acid residues, raising the question of whether this domain participates directly in the reception of the signal. An early study involving treatment of cells with protonophores during growth, led to the suggestion that ArcB is activated by a decrease in proton motive force (PMF) across the cytoplasmic membrane (8). For ArcB to sense the proton motive force, at least one amino acid residue on each side of the plasma membrane with pKa values within biological range would be required. Indeed, the short periplasmic bridge of ArcB contains a His residue at position 47. However, substitution of His47 with either Gln or Arg did not influence the *in vivo* activity of ArcB, rendering the PMF model unlikely (52). In the same study, Kwon and co-workers created a series of mutants in which the transmembrane segments and/or the periplasmic sequence of ArcB were replaced with the corresponding fragments of MalF, a subunit of maltose permease. MalF also possesses a short periplasmic bridge but lacks any sequence homology with ArcB. Surprisingly, all mutants were without significant impairment of the signal transduction process. Therefore, it was concluded that the transmembrane domain of ArcB does not participate directly in the reception of the signal but rather serves as an anchor to keep the protein close to the source of the signal. It was also proposed that tethering ArcB to the plasma membrane might promote the interaction between the cytoplasmic-portion of the sensor protein and an electron transport element(s) (52).

The leucine zipper

The transmembrane domain of ArcB is immediately followed by an intriguing stretch of amino acids, which appears to have a feature characteristic of the well-documented leucine zipper motif (1, 10). The diagnostic feature of this motif is an amphipathic helix with hydrophobic residues clustered on one face and hydrophilic residues on the opposite face, and a leucine residue at the first position in each of four contiguous heptad-repeats (LeuX6LeuX6LeuX6Leu). It is clear from previous studies that such a motif is involved in homo- or heterodimer formation through interaction of the helices from two monomers, via their parallel hydrophobic faces, to give a coiled coil dimeric structure. In general, leucine zippers are found in DNA-binding regulatory proteins (1, 10), but are also present in membrane proteins that do not bind to DNA (54, 131). Computer-aided analysis of the secondary structure of ArcB revealed that the proposed protein section fulfills the characteristics of this well-documented motif, having the conserved leucine residues at positions 73, 80, 87, and 94 (25). The functionality of this structure in ArcB was explored by replacing each of the three Leu residues (at positions 80, 87, and 94) to Ala. Substitution of Leu80 and Leu94 did not alter the ability of ArcB signaling *in vivo* nor its phosphorylating activity *in vitro*, whereas changing Leu87 to Ala resulted in an *arcB* null phenotype *in vivo*

but conservation of the activity *in vitro* (67). Nevertheless, the authors concluded that Leu87 may be somehow implicated in signal-propagation, but they discarded the possibility that ArcB contains a functional leucine-zipper in strict sense.

The PAS domain

ArcB was proposed to contain a PAS domain in its linker, that is the region connecting the transmembrane to the catalytic domains (135). PAS is the acronym formed from the names of the proteins in which imperfect repeat sequences were first recognized: the Drosophila period clock protein (PER), vertebrate aryl hydrocarbon receptor nuclear translocator (ARNT), and Drosophila single-minded protein (SIM) (74). The PAS motif has now been shown to be present in a broad family of proteins from all kingdoms of life, *Bacteria*, *Archaea*, and *Eucarya* (88, 135). In prokaryotes, many PAS-containing proteins are sensor kinases, and it has been demonstrated that they bind various small molecules such as heme, flavin, and a 4-hydroxycinnamyl chromophore to sense molecular oxygen, redox potential, and light, respectively (112). Subsequently, it was suggested that the PAS domain in ArcB is required for sensing the redox conditions (67). This was based on the characterization of several *E. coli* ArcB mutant proteins. It was shown that a deletion spanning the putative PAS domain of ArcB abrogated its ability to repress the *sdh* operon anaerobically. Moreover, one out of four tested substitutions of conserved amino acid residues within the PAS domain (the replacement of Asn181 with Ala) affected *in vivo* signaling by ArcB (67). However, this mutant protein was also inactive *in vitro*, making it difficult to determine whether the *in vivo* defect is at the level of signal reception or is due to a negative effect on the catalytic activity of ArcB.

In this context, it is important to point out that BLAST analyses have identified ArcB homologues in different bacterial species including *Salmonella typhimurium*, *Vibrio fischeri*, *Yersinia pestis*, *Erwinia carotovora*, and *Haemophilus influenzae* (64). Interestingly, the ArcB homologue of *H. influenzae* lacks almost the entire linker region, corresponding to amino acid residues 93 to 271 of *E. coli* ArcB (64), which includes the PAS domain. Despite this, the *H. influenzae* ArcB protein was able to complement *E. coli* strains containing *arcB* null mutations, being capable to mediate responses similar to those of the *E. coli* ArcB protein, under a range of redox conditions (28, 64). Therefore, these studies concluded that the PAS-domain itself is nonessential for signaling, at least in the *H. influenzae* protein, and it was proposed that the PAS domain in other ArcB proteins could play a sensory role that is critical only for cells that must adapt to a broader range of environmental conditions, such as growth outside of a host, but is superfluous for an obligate parasite such as *H. influenzae* (28, 64).

The transmitter and receiver catalytic domains

As mentioned earlier, ArcB is a hybrid sensor kinase possessing three cytosolic catalytic domains: an N-terminal transmitter domain (H1), a central receiver domain (D1), and a C-terminal phosphotransfer or secondary transmitter domain (HPt/H2) (37, 43). As all histidine kinases, ArcB is identified by the unique fingerprint sequences called H, N, G1, and G2 boxes (83, 109), located in the primary transmitter domain. The H box contains an invariant histidine residue at position 292, which is the autophosphorylation site, whereas the following N, G1, and G2 boxes define the nucleotide-binding cavity. The central receiver domain, about 120 amino acid residues in length, is homologous to the ArcA receiver domain. Glu532, Asp533, and Asp576 correspond to the three conserved amino acid residues that are expected to substitute the canonical acidic pocket, and Lys628 corresponds to the invariant downstream residue. The conserved aspartate residue at position 576 has been demonstrated to be the phospho-accepting site in D1. Finally, the histidine-containing phosphotransfer domain was identified as a stretch of approximately 125 amino acid residues containing a histidine residue at position 717 that participates in phosphoryl-group transfer reactions (37). X-ray structure determination revealed that this domain forms a four-helix bundle motif, in which His717 is located on a solvent-exposed helical face (48). It is of interest to note that this fold is also shared by the P1 domain of *E. coli* CheA, the *B. subtilis* SpoOB, and the *S. cerevisae* YPD1, although less than 10% sequence identity is shared between them (6, 120, 129).

Notably, each of the three ArcB domains has evolved sufficient autonomy to fold correctly into catalytically active units, with or without covalent linkage of the contiguous domains (29). This fortunate fact proved to be instrumental for subsequent *in vitro* studies addressing various aspects of the Arc signal transduction mechanisms.

SIGNAL TRANSMISSION: THE HIS–ASP–HIS–ASP PHOSPHORELAY

In contrast to the operation mode of the prototypical system, the Arc system follows a four-step reaction cascade (Fig. 2), also known as phosphorelay (29, 50). It is thought that anoxic conditions cause a shift in the conformation of ArcB that favors activation of its kinase activity. The active kinase then undergoes autophosphorylation at His292 by accepting the γ-phosphate of ATP (43). Both *in vitro* and *in vivo* complementation studies of CheA, NtrB, and VirA showed that autofosphorylation occurs intermolecularly between the subunits of a homodimer, since the γ-phosphoryl group of ATP bound by the conserved kinase site of one subunit must be transferred intermolecularly to the conserved His residue in the other subunit (9, 76, 81, 110, 127, 133). By analogy autophosphorylation of ArcB is also expected to proceed through an intermolecular reaction within an ArcB homodimer. The phosphoryl group from His292 is then transferred to Asp576, which in turn is able to direct it reversibly to His717 or irreversibly to H_2O (29). Subsequently the phosphoryl group from His717 is transferred to Asp54 of ArcA (29, 50). Interestingly, both *in vitro* and *in vivo* experiments indicated that His292 also serves as a phosphoryl group donor for ArcA, albeit less effectively than His717 (29, 43, 44, 115). In addition, it was proposed, on the basis of growth of *arcB* null strains bearing plasmids with different *arcB* mutant alleles, that the nature of the carbon source regulates the ArcA phosphorylating activity of His292 and that the respira-

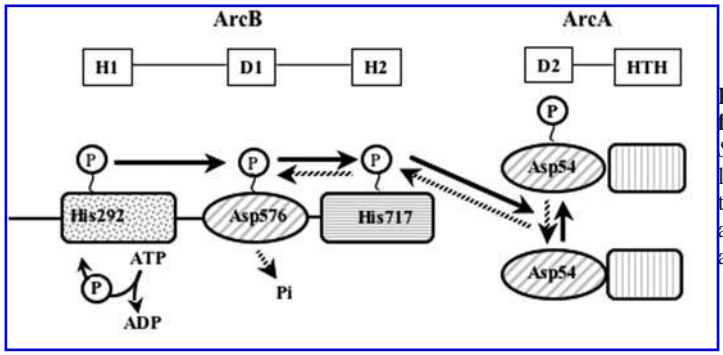

FIG. 2. Schematic representation of phosphotransfer reactions in the Arc two-component system. *Solid arrows* indicate the signal transmission pathway leading to ArcA-P formation. *Hatched arrows* indicate the signal decay pathway leading to ArcA and Pi. The amino acid residues involved in the phosphotransfer reactions are in **bold**.

tory state regulates the donor activity of His717 (66). However, possible misleading results caused by *in vitro* artifacts or gene dosage effects and different degrees of catabolite repression during the utilization of various carbon sources were not discussed. Indeed, a subsequent *in vivo* study based on chromosomal *arcB* mutant alleles, demonstrated that the sole route of phosphotransfer from ArcB to ArcA is the phosphorelay that involves His292, Asp576, and His717 of the sensor kinase, thus ruling out the hypothesis of the dual-signaling property of ArcB (50). In agreement, a more extensive study of BvgA/S and EvgA/S, two highly related systems that also participate in a multistep phosphorelay, demonstrated that the specificity of response regulator phosphorylation is mediated by the secondary, rather than the primary, transmitter domain (87).

SIGNAL DECAY: THE REVERSE PHOSPHORELAY

Upon cessation of signaling, both the cognate response regulator and the sensor kinase undergo dephosphorylation that results in silencing of the system. Several modes of signal decay seem to have evolved for the two-component systems. Phospho-response regulators may undergo spontaneous hydrolysis. The rate of hydrolysis of the inherently labile mixed anhydride phospho–aspartyl bond may be intrinsically set by the protein structure, since the half-life of phosphoresponse regulators varies from seconds to hours (128). Dephosphorylation of a phosphoresponse regulator can also be catalyzed by its cognate sensor kinase (17, 33, 36, 57, 90, 122). In more complex systems, separate specific phosphatases may be involved in the process of signal decay (34, 85, 86). Consequently, after a shift from anoxic to oxidizing conditions rapid dephosphorylation of both ArcB-P and ArcA-P occurs, resulting in release of the Arc dependent transcriptional control (84). ArcB-P dephosphorylates, most likely, by the intrinsic lability of the phospho–aspartyl bond in D1 (29, 38). Also, the phosphohistidine phosphatase, SixA, which has been reported to specifically dephosphorylate His717-P of ArcB (79), may contribute to ArcB-P dephosphorylation. On the other hand, since the *in vitro* half-life of the phospho–aspartyl bond of ArcA-P is relatively long, exceeding 1 h (25), spontaneous hydrolysis of ArcA-P can be considered as an insignificant factor in signal decay. Instead, it has been shown that before the phosphoryl group is hydrolyzed, it needs to be transferred from ArcA-P to H2 at the conserved His717 and subsequently to the conserved Asp576 of D1 (Fig. 2). It is noteworthy that H1, the classical transmitter domain, plays no apparent role in the dephosphorylation process of ArcA-P. This mode of signal decay was demonstrated by *in vitro* studies (25), and also supported by *in vivo* studies involving plasmid-borne wild-type or mutant ArcB domains (84). Nevertheless, the possibility of additional dephosphorylation pathways catalyzed by unknown proteins has not been excluded. Dephosphorylation by a reverse phosphorelay has also been demonstrated for the TorS/R system (2), and the LuxN–LuxU/LuxO system of *Vibrio harveyi*, in which LuxN is a hybrid sensor kinase consisting of a primary transmitter domain and a receiver domain, while the phosphotransfer domain is located in a different protein, LuxU (23). These additional examples reinforce the idea that dephosphorylation of phosphoresponse regulators partnered with a tripartite kinase is, in general, likely to follow a similar Asp–His–Asp dephosphorylation pathway.

CROSS TALK, CROSS REGULATION, ACETYL-P, AND CYTOSOLIC EFFECTORS

Cross talk and cross regulation

A number of sensor kinases has been shown to catalyze the *in vitro* transphosphorylation of noncognate response regulators, a reaction often referred to as cross talk. For instance, CheA can phosphorylate OmpR (36), and EnvZ can phosphorylate NtrC (36). The sensor PhoR of *E. coli* can even phosphorylate the response regulator VanR of *Enterococcus faecium* (33). Cross regulation, in contrast to cross talk, pertains to the phosphorylation of a response regulator by a noncognate kinase under physiological conditions (124). For instance, the maintenance of a basal level PhoB-P by the noncognate sensor kinase CreC in a strain lacking the cognate sensor PhoR has been cited as a possible example of cross regulation (123). The Arc system has also been proposed to be involved in both cross talk and cross regulation. For instance, the phosphotranfer domain was reported to be an efficient *in vitro* phosphodonor, not only for its cognate response regulator ArcA, but also for the chemotaxis protein CheY, and the osmoregulator OmpR (37, 115, 130). Also, a deletion of *arcB* resulted in altered chemotactic behavior of *E. coli* under microaerobic conditions (48, 130). However, a recent

in vitro study where the proteins of all two-component systems were examined for cross talk demonstrated that ArcB is not able to transphosphorylate CheY or OmpR (132). On the other hand, the same study demonstrated that ArcB does transphosphorylate the orphan noncognate response regulator RssB. Indeed, a recent study provided genetic and biochemical evidence that ArcB is able to directly phosphorylate RssB, and thereby stimulate σ^s proteolysis (72). Yet another study suggested that His717 received the phosphoryl group from an unknown sensor kinase (37). However, when the chromosomal *arcB* allele was replaced by either a full-length *arcB* mutated at H292 and D576 or when the phosphotranfer domain (*arcB*$^{638–778}$) was expressed from a low-copy-number plasmid in a Δ*arcB* background, an *arcB*-null phenotype was found. In contrast, when the same plasmid (expressing *arcB*$^{638–778}$) was tested in an *arcB*$^{1–661}$ background (lacking the phosphotransfer domain), an *arcB*+ phenotype was obtained, indicating that the phosphorylation of ArcA via His717 depended solely on the presence of His292 and Asp576 (50). These apparent contradicting observations emphasize the need of extra caution when results from *in vitro* experiments using purified proteins, and *in vivo* complementation experiments using plasmid-borne alleles are interpreted.

Acetyl-P

ArcA has been found to catalyze its own phosphorylation, *in vitro* and *in vivo*, at the expense of the high-energy phosphate compound acetyl phosphate (62, 84), like most other response regulators (18, 19, 21, 59, 92). In all cases, the *in vivo* evidence comes from studies using mutant strains lacking the sensor kinase. Also, it is of interest to note that the residue of phosphorylation is the very same conserved Asp that serves as the phosphoryl group acceptor in the transphosphorylation reaction catalyzed by the sensor kinase. It has been suggested that acetyl-P contributes to maintain and/or augment the basal level of many response regulators in the phosphorylated state. But this pivotal intermediate, containing carbon, phosphorus, and a high energy bond, may also signal global metabolic conditions (69). Such a hypothesis is attractive in view of the finding that acetyl-P levels in the cell can vary from <0.04 m*M* (mid-exponential growth on glycerol) to 1.2 m*M* (mid-exponential growth on pyruvate) (68). However, this ArcA phosphorylation route does not seem to be significant under physiological conditions, as it has been clearly demonstrated that the phosphatase activity of ArcB will nullify the acetyl-phosphate-dependent phosphorylation of ArcA. Therefore, the relative contribution of acetyl-P might be significantly overestimated in the absence of the sensor kinase.

Effectors

An advantage of the elaborate structure and complex phosphotransfer mechanism in tripartite sensor kinases may be the availability of different check points that could allow various inputs to be integrated in a multilevel control mechanism that fine-tunes their signaling activity. ArcB may represent an example for such a mechanism, as an early study suggested that the presence of the fermentative metabolites D-lactate, acetate, and pyruvate enhance the *in vitro* level of net ArcB phosphorylation, and impede the rate of Pi release from the protein (38). Because the intracellular levels of these compounds are elevated during anoxia, it was suggested that they could serve as input signals by inhibiting the autophosphatase activity of ArcB (38).

A more recent study with modular ArcB units, however, failed to confirm any inhibiting effect of these metabolites on the various steps of the dephosphorylation pathway (26). On the other hand, the same study confirmed that D-lactate has an enhancing effect on the *in vitro* rate of ArcB autophosphorylation. Although the reaction mechanism by which D-lactate modulates the activity of ArcB remains unknown, it is worth mentioning that the ArcB receiver domain (D1), even when it is catalytically inactive (e.g., Asp576 mutated to Ala) is indispensable for D-lactate to exert its effect *in vitro* (26). For that reason, it was suggested that either the effector binding-site is located in D1 or the structural presence of D1 enables the effector to bind to the primary transmitter domain of ArcB (H1). A subsequent study provided further *in vivo* evidence that D-lactate plays a physiological role as an allosteric effector that is able to accelerate, but not to activate the kinase activity of ArcB (91). It was therefore proposed that effector binding on ArcB might cause a conformational change that results in increasing the Vmax of the autophosphorylation reaction (91). Most importantly, these results ruled out the proposal that D-lactate might serve as the actual signal through which ArcB senses anaerobic environments *in vivo* (38).

IDENTIFICATION OF THE SIGNAL

It has been postulated that ArcB becomes progressively activated as a kinase during transition from aerobic to anaerobic growth (114). Consequently the concentration of phosphorylated ArcA increases, resulting in activation or repression of its target operons. Although it is apparent that the level of enzymes controlled by the Arc system varies depending on the oxygen availability to the cell, two lines of evidence demonstrated that molecular oxygen is not the direct signal or stimulus for ArcB. First, it was shown that deletion of *cyoABCDE* (encoding the low oxygen affinity terminal oxidase), *cydAB* (encoding the high oxygen affinity terminal oxidase) or both, strongly repressed a ϕ(*cyo-lacZ*) reporter fusion (*cyo* is repressed by ArcA-P) (40), indicating that the Arc system is operative in the presence of O_2. Second, during anaerobic respiratory growth, the ArcA-P-dependent repression of the ϕ(*sdh-lacZ*) reporter (*sdh* is repressed by ArcA-P) was lifted by supplementation of an electron acceptor, such as nitrate or fumarate. Interestingly, the degree of derepression was proportional to the oxidizing power (midpoint potential) of the supplemented electron acceptor (42). Therefore, it was suggested that ArcB indirectly senses O_2 tension by monitoring the level of a metabolite that can exist physiologically in either an oxidized or reduced form, and the membrane-located quinone electron carriers were proposed as possible candidates (42). *E. coli* can synthesize three different quinones, ubiquinone (Q), menaquinone (MK), and demethylmenaquinone (DMK), which function as adapters between various electron-donating and electron-accepting enzyme

complexes (32, 71, 116). Ubiquinone is predominantly used during aerobic respiration and during anaerobic respiration with nitrate as the electron acceptor, while menaquinone and demethylmenaquinone serve as the adaptors for anaerobic respiration (24). Indeed, a recent study demonstrated that the quinones do signal the redox conditions to ArcB (27). The surprising finding, however, was that the quinones in their reduced form did not convey a kinase activating signal but in their oxidized form did convey a kinase inhibiting signal (Fig. 3). This was demonstrated *in vitro*, by testing the effect of the soluble analogs ubiquinone-0 (Q0) and menadione (MK3) on the autophosphorylation rate of the purified ArcB cytoplasmic portion. In this regard, it has to be mentioned that the use of the soluble portion of ArcB was prompted by the previous discovery that the transmembrane domain of this sensor does not participate in signal reception (52). *In vivo* support for the involvement of quinones in Arc signaling was provided by the observation that a ϕ(*cyd–lacZ*) reporter (*cydAB* is activated by ArcA-P) in an *ubiCA* mutant that is blocked in the synthesis of the Q precursor 3-octaprenyl-4-hydroxybenzoate (103) was activated during aerobic growth (27). This finding can readily explain the earlier observation that in a mutant lacking both the cytochrome *bo* and cytochrome *bd* terminal oxidases, the Arc system is operative during aerobiosis (40). In such a mutant, the pool of Q should be trapped in its reduced form despite the oxidizing growth conditions. Because reduced quinones are unable to inhibit the kinase activity of ArcB, the level of ArcA-P should rise and accordingly alter the expression of the target operons. It is important to mention that Q and MK account for 60% and 3% of the total aerobic quinones and the corresponding values when cells are grown anaerobically on fumarate are 10% and 74%, respectively. The rest of the quinone pool is made up by demethylmenaquinone (DMK), whose effect on the activity of ArcB has not yet been tested. These facts together with the finding that the half-maximal inhibition of ArcB phosphorylation by Q0 occurred at about 5 μ*M*, whereas that by MK3 occurred at about 50 μ*M* (27) provide an explanation for the observation that during anaerobic respiratory growth ArcB is a partially active kinase and that its level of activity correlates with the midpoint potential of the alternative electron acceptor (28, 42). Sensing the quinone pool is not restricted to the ArcB sensor, as the unorthodox histidine kinases BvgS and EvgS of *Bordetella pertussis* and *E. coli*, respectively, were shown to be responsive to oxidized ubiquinone-0 (Q-0) *in vitro* (7). Finally, it is worth mentioning that studies directed towards the discovery of the signal may have been hampered by the fact that the ArcB sensor kinase is not activated but rather silenced by the actual signal. Therefore, searches for an activating signal, which has been at least our pursuit, were condemned to be fruitless.

SOLVING THE MECHANISM FOR ARCB SILENCING

Recently, the mechanism for quinone-dependent silencing of the ArcB kinase was clarified (63). Various observations in our laboratory provided a number of indications regarding this mechanism. First, the purified ArcB$^{78–778}$, lacking the transmembrane domain, exhibited a kinase activity that was inhibited by Q0, even when purified under denaturing conditions. Thus, no prosthetic groups could be present in the preparation of ArcB under denaturing conditions, indicating that they are not necessary for the regulation of the kinase (B. Franco, unpublished data). Second, the kinase activity of this protein was inhibited not only by Q0 but also by chloramine T. Because the chemical structures of chloramine T and Q0

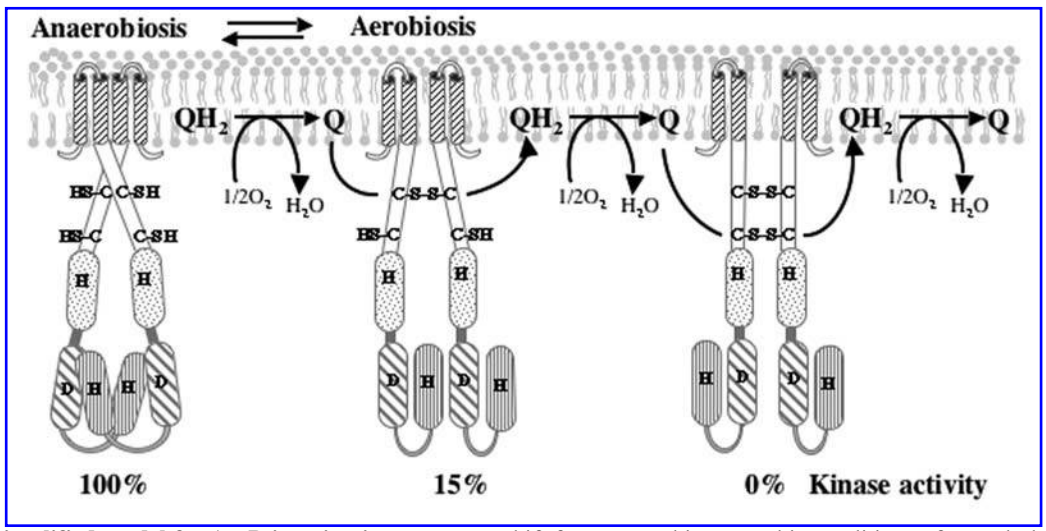

FIG. 3. A simplified model for ArcB inactivation. Upon a shift from anaerobic to aerobic conditions of growth the quinone pool shifts to its oxidized state. This allows the electron transfer from the Cys180 of ArcB to quinones leading to the formation of an intermolecular disulfide-bond between the Cys180 of two monomers, which results in a significant reduction of the kinase activity of ArcB. As the electrons rapidly flow towards O_2 via cytochrome *bd* or *bo* oxidase, the quinone pool maintains its oxidized state and induces the formation of a second disulfide bond between the two Cys241, resulting in the complete silencing of the ArcB kinase activity. Adapted from Ref. (63).

are different, and chloramine T specifically oxidizes cysteine and methionine residues of proteins, it was postulated that ArcB is sensitive to oxidation rather than to the allosteric binding of Q0 (63). Third, the presence of the membrane-permeating reductants dithiothreitol (DTT) and ß-mercaptoethanol in the culture medium of aerobic grown cells did activate ArcB, whereas glutathione (GSH), a reducing agent not able to permeate the plasma membrane because of its lipophobic nature, did not. Because all the above and taking into account that ArcB possesses a cytoplasm-located signal reception-site (52), and it has two unique cysteine-residues (Cys180 and Cys241) in the linker region, it was postulated that ArcB might respond to changes in redox conditions through a thiol redox mechanism (63). Cysteine residues are uniquely suited to sensing a range of redox signals because the thiol side-chain can be oxidized to several different redox states, many of which are readily reversible. Indeed, both *in vivo* and *in vitro* experiments demonstrated that both cysteine residues are able to undergo oxidation and to form intermolecular disulfide bonds between two ArcB monomers. Also it was shown that oxidation of Cys180 contributes to ~85% of ArcB kinase inhibition, whereas oxidation of Cys241 contributes to only ~15%. Therefore, it was concluded that regulation of the ArcB kinase activity requires Cys180, and to a lesser extent Cys241 (Fig. 3). The evolution of such a mechanism involving intermolecular disulfide bond formation would require coadaptation of a signal receptor protein to function as a dimer to promote the proximity of the cysteine residues. A possibility remains that the putative leucine zipper is in fact functional and serves as a dimerization domain.

Interestingly, *in vivo* and *in vitro* analyses indicated that molecular oxygen and hydrogen peroxide (H_2O_2) do not promote disulfide bond formation in ArcB (63), despite that their E'° values, +816 mV for O_2 and +295 mV for H_2O_2, are much higher than the E'° values of Q0 (+162 mV) and MK3 (± 0 mV). This, in turn, suggests that the specificity of the reaction is provided at the level of interaction between ArcB and the oxidizing molecule, whose structure seems to be of central importance for the reaction. Recently several quinone analogues covering a broad range of redox potential values were tested for their effect on the [γ^{32}P]ATP-dependent phosphorylation of ArcB (unpublished results of our laboratory). The molecules tested included chloramine T (E'° = +900 mV), and the quinone analogues Q0 (E'° = +162 mV), junglone (E'° = +30 mV), MK3 (E'° = ± 0 mV), plumbagine (E'° = −39 mV) and lawsone (E'° = −152 mV). We observed that chloramine T, Q0, and junglone were very effective inhibitors of the kinase activity of ArcB, whereas MK3 was less effective in inhibiting ArcB phosphorylation. In contrast, plumbagine and lawsone were without effect. Therefore, it is important to point out that the aromatic ring is the only common feature in the molecular structure of all inhibiting molecules. While chloramine T and Q0 consist of only one aromatic ring, junglone and MK3, whose structure is more reminiscent to plumbagine and lawsone, consist of two. Therefore, it appears that the redox potential of the inhibiting molecule is of chief importance, and also that the quinone binding-pocket of ArcB is able to accommodate both these molecular structures.

An interesting question is how do the membrane-embedded quinones communicate with the two cytosol-located cysteine residues? Although, no answer has been provided so far, it is of interest to note that quinones have been shown to efficiently oxidize the periplasmic located cysteine residues of DsbB, a membrane-bound protein that is a central component in thiol oxidation reactions (4). Also, these thiol-based redox sensing mechanisms raise the intriguing question of how a stable disulfide can form in the reducing environment of the cytoplasm. Presumably, ArcB is not in redox equilibrium with the rest of the cytoplasm. Such a mechanism would not be unique for ArcB, as it has been demonstrated that RegB kinase inhibition during aerobiosis involves an intermolecular disulfide bond formation that renders an inactive RegB tetramer (111). Also, a number of cytoplasmic proteins that are regulated by reversible disulfide bond formation are known, including the transcriptional factors Yap1p in *Saccharomyces cerevisiae*, OxyR and the chaperone Hsp33 in *Escherichia coli*, and the CtrJ in *Rhodobacter capsulatus* (45, 49, 65, 134).

Inhibition of the kinase activity of ArcB through disulfide bond formation could also provide a logical explanation to the previous finding that regulation of the catalytic activity of ArcB is set by rotational movements that alter the orientation of the cytosolic portion of one monomer relative to the other in the homodimer (51). This conclusion was based on the signaling activity of various Tar–ArcB chimeras in which the transmembrane domain of the Tar sensor protein is fused to the cytosolic portion of the ArcB sensor kinase. The transmembrane domain of Tar forms a stable dimer in the cytoplasmic membrane (94), and therefore the relative orientation between the two cytosolic ArcB monomers in a dimer was altered by fusing a set of Tar transmembrane domains with progressively extended C-terminal region to a constant cytosolic portion of ArcB. Since the rigid helical structure of the second transmembrane segment of Tar protrudes into the cytoplasm, each extra residue of Tar added at the Tar–ArcB junction rotates the attached ArcB kinase by approximately 100°. The reported Tar–ArcB chimeras presented three different phenotypes: a redox-regulated, a constitutively active, and a catalytically inactive ArcB kinase. The relative orientation of the kinase moieties in the redox regulated chimera, most likely represent that of the wild-type ArcB. In this conformation it is expected that Cys180 and Cys241 are located on the same side on the structure of the monomer and that they face the ones of the other monomer. A 100° rotation of the ArcB monomers relative to each other will place the cysteines almost at the opposite sides, rendering disulfide bond formation practically impossible, and therefore resulting in a constitutively active kinase. Rotation with additional 100° results in a catalytically inactive chimera, even though no disulfide bond formation is expected to be able to occur. A possible explanation may be that this conformation distorts the structure of the dimer in a way that one or more steps of the phosphorelay from ArcB to ArcA is blocked.

It is of interest to point out that both Cys180 and Cys241 are conserved in the ArcB homologues of different bacterial species except for the one of *H. influenzae*, which lacks almost the entire linker region, and therefore the two conserved cysteine residues (28, 64). However, in contrast to most other homologues that possess only two cysteine residues, ArcB of *H. influenzae* possesses five cysteine residues that are located

at positions 37, 268, 472, 574, and 596 of the protein. More detailed characterization of the *H. influenzae* ArcB sensor kinase might help to determine additional functional roles of the linker region, present in *E. coli* ArcB but not in *H. influenzae* ArcB. Also, the *H. influenzae* ArcB may respond to a signal other than the oxidized forms of the quinones and/or it might represent a different mechanism for redox regulation.

CONCLUDING REMARKS

Recent advances have contributed significantly to understanding several aspects of the ArcA/B signal transduction, and a more complete picture of this system starts to emerge. It is now known that the ArcB sensor kinase is kept inactive during aerobiosis through oxidation of two cytosol-located redox-active cysteine residues that participate in intermolecular disulfide bond formation (63), a reaction in which the quinones provide the source of oxidative power (27). However, under these conditions, ArcB is active as a specific ArcA-P phosphatase, in a reaction that occurs via an Asp54 → His717 → Asp576 → Pi phosphorelay (25, 84). Hence, this specific ArcA-P phosphatase activity should eliminate the effect of any inappropriate cross reactivity between ArcA and a noncognate sensor kinase, and also nullify the acetyl-phosphate dependent autophosphorylation of ArcA. During anaerobiosis, the inhibitory signals are absent and ArcB autophosphorylates at the expense of ATP, a reaction that has been shown to be enhanced by certain anaerobic metabolites such as D-lactate, acetate, and pyruvate (26, 38, 91), and transphosphotylates its cognate response regulator ArcA via a His292 → Asp576 → His717 → Asp54 phosphorelay (29, 50). Phosphorylated ArcA is thereby activated as a transcriptional regulator of some 300 target operons (42, 56, 96). Despite the progress made, we are still far from understanding many key aspects of this signal transduction pathway. For example, it is curious that the conserved Asp576 and His717 residues participate in both the forward and reverse phosphorelay. Thus, it would be useful to find out the implements that control the direction of the phosphoryl group transfers and/or influence the individual transfer rates with regard to signal transmission and signal decay. Furthermore, a pivotal link in the pathways for Arc signaling that also remains elusive is how do the disulfide bonds become reduced for ArcB reactivation upon entry to anaerobiosis? Another challenge will be to solve the crystal structure of ArcB as this will provide valuable information not only for the steps of phosphoryl group transfer between the various modules of ArcB but most importantly for the mode of communication of the cytosol-located cysteine residues of the sensor with the membrane embedded quinone electron carriers. These studies could provide further mechanistic insight into the operation of this fascinating signal transduction system that might also facilitate the understanding of other signal transduction systems involving tripartite sensor kinases.

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ABBREVIATIONS

Arc, anoxic redox control; DMK, demethylmenaquinone; DTT, dithiothreitol; E′°, standard reduction potential; GSH, glutathione; MK3, menadione; MK, menaquinone; NAD, nicotinamide adenine dinucleotide; PAS, acronym of period clock protein (PER)–aryl hydrocarbon receptor nuclear translocator (ARNT)–single-minded protein (SIM); PMF, proton motive force; TCA, tricarboxylic acid cycle; TM, transmembrane; Q, ubiquinone; Q0, ubiquinone-0.

REFERENCES

1. Abel T and Maniatis T. Gene regulation. Action of leucine zippers. *Nature* 341: 24–25, 1989.
2. Ansaldi M, Jourlin-Castelli C, Lepelletier M, Theraulaz L, and Mejean V. Rapid dephosphorylation of the TorR response regulator by the TorS unorthodox sensor in *Escherichia coli*. *J Bacteriol* 183: 2691–2695, 2001.
3. Arico B, Miller JF, Roy C, Stibitz S, Monack D, Falkow S, Gross R, and Rappuoli R. Sequences required for expression of *Bordetella pertussis* virulence factors share homology with prokaryotic signal transduction proteins. *Proc Natl Acad Sci USA* 86: 6671–6675, 1989.
4. Bader M, Muse W, Ballou DP, Gassner C, and Bardwell JC. Oxidative protein folding is driven by the electron transport system. *Cell* 98: 217–227, 1999.
5. Barnabas J, Schwartz RM, and Dayhoff MO. Evolution of major metabolic innovations in the precambrian. *Orig Life* 12: 81–91, 1982.
6. Bilwes AM, Alex LA, Crane BR, and Simon MI. Structure of CheA, a signal-transducing histidine kinase. *Cell* 96: 131–141, 1999.
7. Bock A and Gross R. The unorthodox histidine kinases BvgS and EvgS are responsive to the oxidation status of a quinone electron carrier. *Eur J Biochem* 269: 3479–3484, 2002.
8. Bogachev AV, Murtazina RA, and Skulachev VP. Cytochrome d induction in *Escherichia coli* growing under unfavorable conditions. *FEBS Lett* 336: 75–78, 1993.
9. Brencic A, Xia Q, and Winans SC. VirA of *Agrobacterium tumefaciens* is an intradimer transphosphorylase and can actively block vir gene expression in the absence of phenolic signals. *Mol Microbiol* 52: 1349–1362, 2004.
10. Buckland R and Wild F. Leucine zipper motif extends. *Nature* 338: 547, 1989.
11. Buxton RS and Drury LS. Identification of the dye gene product, mutational loss of which alters envelope protein composition and also affects sex factor F expression in *Escherichia coli* K-12. *Mol Gen Genet* 194: 241–247, 1984
12. Buxton RS, Drury LS, and Curtis CA. Dye sensitivity correlated with envelope protein changes in dye (sfrA) mutants of *Escherichia coli* K12 defective in the expression of the sex factor F. *J Gen Microbiol* 129: 3363–3370, 1983.
13. Böck A and Sawers G. Fermentation. In: *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Bi-*

ology, Neidhardt FC, Ingraham JL, Lin EC, Low KB, Magasanik B, Schaechter M, and Umbarger HE (eds.). Washington, DC: ASM Press, 1996, pp. 262–282.
14. Colloms SD, Alen C, and Sherratt DJ. The ArcA/ArcB two-component regulatory system of *Escherichia coli* is essential for Xer site-specific recombination at psi. *Mol Microbiol* 28: 521–530, 1998.
15. Compan I and Touati D. Anaerobic activation of arcA transcription in *Escherichia coli*: roles of Fnr and ArcA. *Mol Microbiol* 11: 955–964, 1994.
16. Cotter PA, Chepuri V, Gennis RB, and Gunsalus RP. Cytochrome o (cyoABCDE) and d (cydAB) oxidase gene expression in *Escherichia coli* is regulated by oxygen, pH, and the fnr gene product. *J Bacteriol* 172: 6333–6338, 1990.
17. Dahl MK, Msadek T, Kunst F, and Rapoport G. The phosphorylation state of the DegU response regulator acts as a molecular switch allowing either degradative enzyme synthesis or expression of genetic competence in *Bacillus subtilis*. *J Biol Chem* 267: 14509–14514, 1992.
18. Dailey FE and Berg HC. Change in direction of flagellar rotation in *Escherichia coli* mediated by acetate kinase. *J Bacteriol* 175: 3236–3239, 1993.
19. Danese PN, Snyder WB, Cosma CL, Davis LJ, and Silhavy TJ. The Cpx two-component signal transduction pathway of *Escherichia coli* regulates transcription of the gene specifying the stress-inducible periplasmic protease, DegP. *Genes Dev* 9: 387–398, 1995.
20. De Souza-Hart JA, Blackstock W, Di Modugno V, Holland IB, and Kok M. Two-component systems in *Haemophilus influenzae*: a regulatory role for ArcA in serum resistance. *Infect Immun* 71: 163–172, 2003.
21. Deretic V, Leveau JH, Mohr CD, and Hibler NS. *In vitro* phosphorylation of AlgR, a regulator of mucoidy in *Pseudomonas aeruginosa*, by a histidine protein kinase and effects of small phospho-donor molecules. *Mol Microbiol* 6: 2761–2657, 1992.
22. Drury LS and Buxton RS. DNA sequence analysis of the dye gene of *Escherichia coli* reveals amino acid homology between the dye and OmpR proteins. *J Biol Chem* 260: 4236–4242, 1985.
23. Freeman JA, Lilley BN, and Bassler BL. A genetic analysis of the functions of LuxN: a two-component hybrid sensor kinase that regulates quorum sensing in *Vibrio harveyi*. *Mol Microbiol* 35: 139–149, 2000.
24. Gennis RB and Stewart V. Respiration. In: *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, Neidhardt FC, Ingraham JL, Lin EC, Low KB, Magasanik B, Schaechter M and Umbarger HE (eds.). Washington, D.C.: ASM Press, 1996, pp. 217–261.
25. Georgellis D, Kwon O, De Wulf P, and Lin EC. Signal decay through a reverse phosphorelay in the Arc two-component signal transduction system. *J Biol Chem* 273: 32864–32869, 1998.
26. Georgellis D, Kwon O, and Lin EC. Amplification of signaling activity of the arc two-component system of *Escherichia coli* by anaerobic metabolites. An *in vitro* study with different protein modules. *J Biol Chem* 274: 35950–35954, 1999.
27. Georgellis D, Kwon O, and Lin EC. Quinones as the redox signal for the arc two-component system of bacteria. *Science* 292: 2314–2316, 2001.
28. Georgellis D, Kwon O, Lin EC, Wong SM, and Akerley BJ. Redox signal transduction by the ArcB sensor kinase of *Haemophilus influenzae* lacking the PAS domain. *J Bacteriol* 183: 7206–7212, 2001.
29. Georgellis D, Lynch AS, and Lin EC. *In vitro* phosphorylation study of the arc two-component signal transduction system of *Escherichia coli*. *J Bacteriol* 179: 5429–5435, 1997.
30. Gray CT, Wimpenny JW, Hughes DE, and Mossman MR. Regulation of metabolism in facultative bacteria. I. Structural and functional changes in *Escherichia coli* associated with shifts between the aerobic and anaerobic states. *Biochim Biophys Acta* 117: 22–32, 1966.
31. Gray CT, Wimpenny JW, and Mossman MR. Regulation of metabolism in facultative bacteria. II. Effects of aerobiosis, anaerobiosis and nutrition on the formation of Krebs cycle enzymes in *Escherichia coli*. *Biochim Biophys Acta* 117: 33–41, 1966.
32. Guest JR and Shaw DJ. Molecular cloning of menaquinone biosynthetic genes of *Escherichia coli* K12. *Mol Gen Genet* 181: 379–383, 1981.
33. Haldimann A, Fisher SL, Daniels LL, Walsh CT, and Wanner BL. Transcriptional regulation of the *Enterococcus faecium* BM4147 vancomycin resistance gene cluster by the VanS–VanR two-component regulatory system in *Escherichia coli* K-12. *J Bacteriol* 179: 5903–5913, 1997.
34. Hess JF, Oosawa K, Kaplan N, and Simon MI. Phosphorylation of three proteins in the signaling pathway of bacterial chemotaxis. *Cell* 53: 79–87, 1988.
35. Hrabak EM and Willis DK. The lemA gene required for pathogenicity of *Pseudomonas syringae* pv. syringae on bean is a member of a family of two-component regulators. *J Bacteriol* 174: 3011–3020, 1992.
36. Igo MM, Ninfa AJ, Stock JB, and Silhavy TJ. Phosphorylation and dephosphorylation of a bacterial transcriptional activator by a transmembrane receptor. *Genes Dev* 3: 1725–1734, 1989.
37. Ishige K, Nagasawa S, Tokishita S, and Mizuno T. A novel device of bacterial signal transducers. *EMBO J* 13: 5195–5202, 1994.
38. Iuchi S. Phosphorylation/dephosphorylation of the receiver module at the conserved aspartate residue controls transphosphorylation activity of histidine kinase in sensor protein ArcB of *Escherichia coli*. *J Biol Chem* 268: 23972–23980, 1993.
39. Iuchi S, Cameron DC, and Lin EC. A second global regulator gene (arcB) mediating repression of enzymes in aerobic pathways of *Escherichia coli*. *J Bacteriol* 171: 868–873, 1989.
40. Iuchi S, Chepuri V, Fu HA, Gennis RB, and Lin EC. Requirement for terminal cytochromes in generation of the aerobic signal for the arc regulatory system in *Escherichia coli*: study utilizing deletions and lac fusions of cyo and cyd. *J Bacteriol* 172: 6020–6025, 1990.
41. Iuchi S, Furlong D, and Lin EC. Differentiation of arcA, arcB, and cpxA mutant phenotypes of *Escherichia coli* by sex pilus formation and enzyme regulation. *J Bacteriol* 171: 2889–2893, 1989.
42. Iuchi S and Lin EC. arcA (dye), a global regulatory gene in *Escherichia coli* mediating repression of enzymes in aerobic pathways. *Proc Natl Acad Sci USA* 85: 1888–1892, 1988.

43. Iuchi S and Lin EC. Mutational analysis of signal transduction by ArcB, a membrane sensor protein responsible for anaerobic repression of operons involved in the central aerobic pathways in *Escherichia coli*. *J Bacteriol* 174: 3972–3980, 1992.
44. Iuchi S, Matsuda Z, Fujiwara T, and Lin EC. The arcB gene of *Escherichia coli* encodes a sensor-regulator protein for anaerobic repression of the arc modulon. *Mol Microbiol* 4: 715–727, 1990.
45. Jakob U, Muse W, Eser M, and Bardwell JC. Chaperone activity with a redox switch. *Cell* 96: 341–352, 1999.
46. Jeon Y, Lee YS, Han JS, Kim JB, and Hwang DS. Multimerization of phosphorylated and non-phosphorylated ArcA is necessary for the response regulator function of the Arc two-component signal transduction system. *J Biol Chem* 276: 40873–40879, 2001.
47. Jeong JY, Kim YJ, Cho N, Shin D, Nam TW, Ryu S, and Seok YJ. Expression of ptsG encoding the major glucose transporter is regulated by ArcA in *Escherichia coli*. *J Biol Chem* 279: 38513–38518, 2004.
48. Kato M, Mizuno T, Shimizu T, and Hakoshima T. Insights into multistep phosphorelay from the crystal structure of the C-terminal HPt domain of ArcB. *Cell* 88: 717–723, 1997.
49. Kuge S, Arita M, Murayama A, Maeta K, Izawa S, Inoue Y, and Nomoto A. Regulation of the yeast Yap1p nuclear export signal is mediated by redox signal-induced reversible disulfide bond formation. *Mol Cell Biol* 21: 6139–6150, 2001.
50. Kwon O, Georgellis D, and Lin EC. Phosphorelay as the sole physiological route of signal transmission by the arc two-component system of *Escherichia coli. J Bacteriol* 182: 3858–3862, 2000.
51. Kwon O, Georgellis D, and Lin EC. Rotational on-off switching of a hybrid membrane sensor kinase Tar-ArcB in *Escherichia coli*. *J Biol Chem* 278: 13192–13195, 2003.
52. Kwon O, Georgellis D, Lynch AS, Boyd D, and Lin EC. The ArcB sensor kinase of *Escherichia coli*: genetic exploration of the transmembrane region. *J Bacteriol* 182: 2960–2966, 2000.
53. Lambden PR and Guest JR. Mutants of *Escherichia coli* K12 unable to use fumarate as an anaerobic electron acceptor. *J Gen Microbiol* 97: 145–160, 1976.
54. Lau PC, Wang Y, Patel A, Labbe D, Bergeron H, Brousseau R, Konishi Y, and Rawlings M. A bacterial basic region leucine zipper histidine kinase regulating toluene degradation. *Proc Natl Acad Sci USA* 94: 1453–1458, 1997.
55. Lee YS, Han JS, Jeon Y, and Hwang DS. The arc two-component signal transduction system inhibits *in vitro Escherichia coli* chromosomal initiation. *J Biol Chem* 276: 9917–9923, 2001.
56. Liu X and De Wulf P. Probing the ArcA-P modulon of *Escherichia coli* by whole genome transcriptional analysis and sequence recognition profiling. *J Biol Chem* 279: 12588–12597, 2004.
57. Lois AF, Weinstein M, Ditta GS, and Helinski DR. Autophosphorylation and phosphatase activities of the oxygen-sensing protein FixL of *Rhizobium* meliloti are coordinately regulated by oxygen. *J Biol Chem* 268: 4370–4375, 1993.
58. Lu S, Killoran PB, Fang FC, and Riley LW. The global regulator ArcA controls resistance to reactive nitrogen and oxygen intermediates in *Salmonella enterica serovar Enteritidis*. *Infect Immun* 70: 451–461, 2002.
59. Lukat GS, McCleary WR, Stock AM, and Stock JB. Phosphorylation of bacterial response regulator proteins by low molecular weight phospho-donors. *Proc Natl Acad Sci USA* 89: 718–722, 1992.
60. Lynch AS and Lin EC. Responses to molecular oxygen. In: *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, Neidhardt FC, Ingraham JL, Lin EC, Low KB, Magasanik B, Schaechter M and Umbarger HE (eds.) Washington, DC: ASM Press, 1996, pp. 1526–1538.
61. Lynch AS and Lin EC. Regulation of aerobic and anaerobic metabolism by the Arc system. In: *Regulation of Gene Expression in Escherichia coli*, Lin EC, and Lynch AS (eds.). Austin: Chapman and Hall, 1996, pp. 361–381.
62. Lynch AS and Lin EC. Transcriptional control mediated by the ArcA two-component response regulator protein of *Escherichia coli*: characterization of DNA binding at target promoters. *J Bacteriol* 178: 6238–6249, 1996.
63. Malpica R, Franco B, Rodriguez C, Kwon O, and Georgellis D. Identification of a quinone-sensitive redox switch in the ArcB sensor kinase. *Proc Natl Acad Sci USA* 101: 13318–13323, 2004.
64. Manukhov IV, Bertsova YV, Trofimov DY, Bogachev AV, and Skulachev VP. Analysis of HI0220 protein from *Haemophilus influenzae*, a novel structural and functional analog of ArcB protein from *Escherichia coli*. *Biochemistry (Mosc)* 65: 1321–1326, 2000.
65. Masuda S, Dong C, Swem D, Setterdahl AT, Knaff DB, and Bauer CE. Repression of photosynthesis gene expression by formation of a disulfide bond in CrtJ. *Proc Natl Acad Sci USA* 99: 7078–7083, 2002.
66. Matsushika A and Mizuno T. A dual-signaling mechanism mediated by the ArcB hybrid sensor kinase containing the histidine-containing phosphotransfer domain in *Escherichia coli*. *J Bacteriol* 180: 3973–3977, 1998.
67. Matsushika A and Mizuno T. Characterization of three putative sub-domains in the signal-input domain of the ArcB hybrid sensor in *Escherichia coli*. *J Biochem (Tokyo)* 127: 855–860, 2000.
68. McCleary WR and Stock JB. Acetyl phosphate and the activation of two-component response regulators. *J Biol Chem* 269: 31567–31572, 1994.
69. McCleary WR, Stock JB, and Ninfa AJ. Is acetyl phosphate a global signal in *Escherichia coli*? *J Bacteriol* 175: 2793–2798, 1993.
70. McGuire AM, De Wulf P, Church GM, and Lin EC. A weight matrix for binding recognition by the redox-response regulator ArcA-P of *Escherichia coli*. *Mol Microbiol* 32: 219–221, 1999.
71. Meganathan R. Biosynthesis of the isoprenoid quinones menaquinone (vitamin K2) and Ubiquinone (coenzyme Q). In: *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, Neidhardt FC, Ingraham JL,

Lin EC, Low KB, Magasanik B, Schaechter M and Umbarger HE (eds.). Washington, D.C.: ASM Press, 1996, pp. 642–656.
72. Mika F and Hengge R. A two-component phosphotransfer network involving ArcB, ArcA and RssB coordinates synthesis and proteolysis of sigmaS in *E. coli*. *Genes Dev* 19: 2770–2781, 2005.
73. Nagasawa S, Tokishita S, Aiba H, and Mizuno T. A novel sensor-regulator protein that belongs to the homologous family of signal-transduction proteins involved in adaptive responses in *Escherichia coli*. *Mol Microbiol* 6: 799–807, 1992.
74. Nambu JR, Lewis JO, Wharton KA, Jr., and Crews ST. The Drosophila single-minded gene encodes a helix–loop–helix protein that acts as a master regulator of CNS midline development. *Cell* 67: 1157–1167, 1991.
75. Ninfa AJ and Magasanik B. Covalent modification of the glnG product, NRI, by the glnL product, NRII, regulates the transcription of the glnALG operon in *Escherichia coli*. *Proc Natl Acad Sci USA* 83: 5909–5913, 1986.
76. Ninfa EG, Atkinson MR, Kamberov ES, and Ninfa AJ. Mechanism of autophosphorylation of *Escherichia coli* nitrogen regulator II (NRII or NtrB): trans-phosphorylation between subunits. *J Bacteriol* 175: 7024–7032, 1993.
77. Nixon BT, Ronson CW, and Ausubel FM. Two-component regulatory systems responsive to environmental stimuli share strongly conserved domains with the nitrogen assimilation regulatory genes ntrB and ntrC. *Proc Natl Acad Sci USA* 83: 7850–7854, 1986.
78. Ogasawara H, Teramoto J, Yamamoto S, Hirao K, Yamamoto K, Ishihama A, and Utsumi R. Negative regulation of DNA repair gene (uvrA) expression by ArcA/ArcB two-component system in *Escherichia coli*. *FEMS Microbiol Lett* 251: 243–249, 2005.
79. Ogino T, Matsubara M, Kato N, Nakamura Y, and Mizuno T. An *Escherichia coli* protein that exhibits phosphohistidine phosphatase activity towards the HPt domain of the ArcB sensor involved in the multistep His-Asp phosphorelay. *Mol Microbiol* 27: 573–585, 1998.
80. Papagiannis MD. Life-related aspects of stellar evolution. *Orig Life* 14: 43–50, 1984.
81. Park H, Saha SK, and Inouye M. Two-domain reconstitution of a functional protein histidine kinase. *Proc Natl Acad Sci USA* 95: 6728–6732, 1998.
82. Parkinson JS. Signal transduction schemes of bacteria. *Cell* 73: 857–871, 1993.
83. Parkinson JS and Kofoid EC. Communication modules in bacterial signaling proteins. *Annu Rev Genet* 26: 71–112, 1992.
84. Pena-Sandoval GR, Kwon O, and Georgellis D. Requirement of the receiver and phosphotransfer domains of ArcB for efficient dephosphorylation of phosphorylated ArcA *in vivo*. *J Bacteriol* 187: 3267–3272, 2005.
85. Perego M. A peptide export-import control circuit modulating bacterial development regulates protein phosphatases of the phosphorelay. *Proc Natl Acad Sci USA* 94: 8612–8617, 1997.
86. Perego M, Hanstein C, Welsh KM, Djavakhishvili T, Glaser P, and Hoch JA. Multiple protein-aspartate phosphatases provide a mechanism for the integration of diverse signals in the control of development in *B. subtilis*. *Cell* 79: 1047–1055, 1994.
87. Perraud AL, Kimmel B, Weiss V, and Gross R. Specificity of the BvgAS and EvgAS phosphorelay is mediated by the C-terminal HPt domains of the sensor proteins. *Mol Microbiol* 27: 875–887, 1998.
88. Ponting CP and Aravind L. PAS: a multifunctional domain family comes to light. *Curr Biol* 7: R674–677, 1997.
89. Rabin RS, Collins LA, and Stewart V. *In vivo* requirement of integration host factor for nar (nitrate reductase) operon expression in *Escherichia coli* K-12. *Proc Natl Acad Sci USA* 89: 8701–8705, 1992.
90. Raivio TL and Silhavy TJ. Transduction of envelope stress in *Escherichia coli* by the Cpx two-component system. *J Bacteriol* 179: 7724–7733, 1997.
91. Rodriguez C, Kwon O, and Georgellis D. Effect of D-lactate on the physiological activity of the ArcB sensor kinase in *Escherichia coli*. *J Bacteriol* 186: 2085–2090, 2004.
92. Roggiani M and Dubnau D. ComA, a phosphorylated response regulator protein of *Bacillus subtilis*, binds to the promoter region of srfA. *J Bacteriol* 175: 3182–3187, 1993.
93. Ronson CW, Nixon BT, and Ausubel FM. Conserved domains in bacterial regulatory proteins that respond to environmental stimuli. *Cell* 49: 579–581, 1987.
94. Russo AF and Koshland DE, Jr. Separation of signal transduction and adaptation functions of the aspartate receptor in bacterial sensing. *Science* 220: 1016–1020, 1983.
95. Salmon K, Hung SP, Mekjian K, Baldi P, Hatfield GW, and Gunsalus RP. Global gene expression profiling in *Escherichia coli* K12. The effects of oxygen availability and FNR. *J Biol Chem* 278: 29837–29855, 2003.
96. Salmon KA, Hung SP, Steffen NR, Krupp R, Baldi P, Hatfield GW, and Gunsalus RP. Global gene expression profiling in *Escherichia coli* K12: effects of oxygen availability and ArcA. *J Biol Chem* 280: 15084–15096, 2005.
97. Sawers G. Specific transcriptional requirements for positive regulation of the anaerobically inducible pfl operon by ArcA and FNR. *Mol Microbiol* 10: 737–747, 1993.
98. Sawers G and Suppmann B. Anaerobic induction of pyruvate formate-lyase gene expression is mediated by the ArcA and FNR proteins. *J Bacteriol* 174: 3474–3478, 1992.
99. Sengupta N, Paul K, and Chowdhury R. The global regulator ArcA modulates expression of virulence factors in *Vibrio cholerae*. *Infect Immun* 71: 5583–5589, 2003.
100. Silverman PM, Rother S, and Gaudin H. Arc and Sfr functions of the *Escherichia coli* K-12 arcA gene product are genetically and physiologically separable. *J Bacteriol* 173: 5648–5652, 1991.
101. Silverman PM, Wickersham E, and Harris R. Regulation of the F plasmid traY promoter in *Escherichia coli* by host and plasmid factors. *J Mol Biol* 218: 119–128, 1991.
102. Simon G, Mejean V, Jourlin C, Chippaux M, and Pascal MC. The torR gene of *Escherichia coli* encodes a response regulator protein involved in the expression of the

trimethylamine N-oxide reductase genes. *J Bacteriol* 176: 5601–5606, 1994.
103. Soballe B and Poole RK. Requirement for ubiquinone downstream of cytochrome(s) b in the oxygen-terminated respiratory chains of *Escherichia coli* K-12 revealed using a null mutant allele of ubiCA. *Microbiology* 144 (Pt 2): 361–373, 1998.
104. Stevens AM, Sanders JM, Shoemaker NB, and Salyers AA. Genes involved in production of plasmidlike forms by a Bacteroides conjugal chromosomal element share amino acid homology with two-component regulatory systems. *J Bacteriol* 174: 2935–2942, 1992.
105. Stewart V. Biochemical Society Special Lecture. Nitrate- and nitrite-responsive sensors NarX and NarQ of proteobacteria. *Biochem Soc Trans* 31: 1–10, 2003.
106. Stewart V, Parales J Jr., and Merkel SM. Structure of genes narL and narX of the nar (nitrate reductase) locus in *Escherichia coli* K-12. *J Bacteriol* 171: 2229–2234, 1989.
107. Stock AM, Robinson VL, and Goudreau PN. Two-component signal transduction. *Annu Rev Biochem* 69: 183–215, 2000.
108. Stock JB, Surette MG, Levit M and Park P. Two-component Signal Transduction Systems: Structure-Function Relationships and Mechanisms of Catalysis. In: *Two-component Signal Transduction*, Hoch JA, and Silhavy TJ (eds.). Washington D.C.: ASM press, 1995, pp. 25–51.
109. Swanson RV, Alex LA, and Simon MI. Histidine and aspartate phosphorylation: two-component systems and the limits of homology. *Trends Biochem Sci* 19: 485–490, 1994.
110. Swanson RV, Bourret RB, and Simon MI. Intermolecular complementation of the kinase activity of CheA. *Mol Microbiol* 8: 435–441, 1993.
111. Swem LR, Kraft BJ, Swem DL, Setterdahl AT, Masuda S, Knaff DB, Zaleski JM, and Bauer CE. Signal transduction by the global regulator RegB is mediated by a redox-active cysteine. *EMBO J* 22: 4699–4708, 2003.
112. Taylor BL and Zhulin IB. PAS domains: internal sensors of oxygen, redox potential, and light. *Microbiol Mol Biol Rev* 63: 479–506, 1999.
113. Toro-Roman A, Mack TR, and Stock AM. Structural analysis and solution studies of the activated regulatory domain of the response regulator ArcA: a symmetric dimer mediated by the alpha4-beta5-alpha5 face. *J Mol Biol* 349: 11–26, 2005.
114. Tseng CP, Albrecht J, and Gunsalus RP. Effect of microaerophilic cell growth conditions on expression of the aerobic (cyoABCDE and cydAB) and anaerobic (narGHJI, frdABCD, and dmsABC) respiratory pathway genes in *Escherichia coli*. *J Bacteriol* 178: 1094–1098, 1996.
115. Tsuzuki M, Ishige K, and Mizuno T. Phosphotransfer circuitry of the putative multi-signal transducer, ArcB, of *Escherichia coli*: *in vitro* studies with mutants. *Mol Microbiol* 18: 953–962, 1995.
116. Unden G. Differential roles for menaquinone and demethylmenaquinone in anaerobic electron transport of *E. coli* and their fnr-independent expression. *Arch Microbiol* 150: 499–503, 1988.
117. Unden G, Achebach S, Holighaus G, Tran HG, Wackwitz B, and Zeuner Y. Control of FNR function of *Escherichia coli* by O2 and reducing conditions. *J Mol Microbiol Biotechnol* 4: 263–268, 2002.
118. Unden G and Bongaerts J. Alternative respiratory pathways of *Escherichia coli*: energetics and transcriptional regulation in response to electron acceptors. *Biochim Biophys Acta* 1320: 217–234, 1997.
119. Utsumi R, Katayama S, Taniguchi M, Horie T, Ikeda M, Igaki S, Nakagawa H, Miwa A, Tanabe H, and Noda M. Newly identified genes involved in the signal transduction of *Escherichia coli* K-12. *Gene* 140: 73–77, 1994.
120. Varughese KI, Madhusudan, Zhou XZ, Whiteley JM, and Hoch JA. Formation of a novel four-helix bundle and molecular recognition sites by dimerization of a response regulator phosphotransferase. *Mol Cell* 2: 485–493, 1998.
121. Volz K. Structural conservation in the CheY superfamily. *Biochemistry* 32: 11741–11753, 1993.
122. Walker MS and DeMoss JA. Phosphorylation and dephosphorylation catalyzed *in vitro* by purified components of the nitrate sensing system, NarX and NarL. *J Biol Chem* 268: 8391–8393, 1993.
123. Wanner BL, Jiang W, Kim, SK, Yamagata S, Haldimann A and Daniels LL. Are the mutliple signal transduction pathways of the Pho regulon due to cross talk or cross regulation? In: *Regulation of gene expression in Escherichia coli*, Lin EC and Lynch AS (eds.). Austin: R. G.: Landes Company, 1996, pp. 297–315.
124. Wanner BL and Wilmes-Riesenberg MR. Involvement of phosphotransacetylase, acetate kinase, and acetyl phosphate synthesis in control of the phosphate regulon in *Escherichia coli*. *J Bacteriol* 174: 2124–2130, 1992.
125. Wilson TH and Lin EC. Evolution of membrane bioenergetics. *J Supramol Struct* 13: 421–446, 1980.
126. Wolanin PM, Thomason PA, and Stock JB. Histidine protein kinases: key signal transducers outside the animal kingdom. *Genome Biol* 3: 3013.1–3013.8, 2002.
127. Wolfe AJ and Stewart RC. The short form of the CheA protein restores kinase activity and chemotactic ability to kinase-deficient mutants. *Proc Natl Acad Sci USA* 90: 1518–1522, 1993.
128. Wright GD, Holman TR, and Walsh CT. Purification and characterization of VanR and the cytosolic domain of VanS: a two-component regulatory system required for vancomycin resistance in *Enterococcus faecium* BM4147. *Biochemistry* 32: 5057–5063, 1993.
129. Xu Q and West AH. Conservation of structure and function among histidine-containing phosphotransfer (HPt) domains as revealed by the crystal structure of YPD1. *J Mol Biol* 292: 1039–1050, 1999.
130. Yaku H, Kato M, Hakoshima T, Tsuzuki M, and Mizuno T. Interaction between the CheY response regulator and the histidine-containing phosphotransfer (HPt) domain of the ArcB sensory kinase in *Escherichia coli*. *FEBS Lett* 408: 337–340, 1997.

131. Yaku H and Mizuno T. The membrane-located osmosensory kinase, EnvZ, that contains a leucine zipper-like motif functions as a dimer in *Escherichia coli*. *FEBS Lett* 417: 409–413, 1997.
132. Yamamoto K, Hirao K, Oshima T, Aiba H, Utsumi R, and Ishihama A. Functional characterization *in vitro* of all two-component signal transduction systems from *Escherichia coli*. *J Biol Chem* 280: 1448–1456, 2005.
133. Yang Y and Inouye M. Intermolecular complementation between two defective mutant signal-transducing receptors of *Escherichia coli*. *Proc Natl Acad Sci USA* 88: 11057–11061, 1991.
134. Zheng M, Aslund F, and Storz G. Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science* 279: 1718–1721, 1998.
135. Zhulin IB, Taylor BL, and Dixon R. PAS domain S-boxes in Archaea, Bacteria and sensors for oxygen and redox. *Trends Biochem Sci* 22: 331–333, 1997.

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2. Seulgi Yun, Jong Moon Shin, Oh-Cheol Kim, Young Ryul Jung, Doo-Byoung Oh, Sang Yup Lee, Ohsuk Kwon. 2012. Probing the ArcA regulon in the rumen bacterium Mannheimia succiniciproducens by genome-wide expression profiling. *Journal of Microbiology* **50**:4, 665-672. [CrossRef]
3. Tino Krell, Jesús Lacal, M. Eugenia Guazzaroni, Andreas Busch, Hortencia Silva-Jiménez, Sandy Fillet, José A. Reyes-Darías, Francisco Muñoz-Martínez, Miriam Rico-Jiménez, Cristina García-Fontana, Estrella Duque, Ana Segura, Juan-Luis Ramos. 2012. Responses of Pseudomonas putida to toxic aromatic carbon sources. *Journal of Biotechnology* **160**:1-2, 25-32. [CrossRef]
4. Mariela del Carmen Carrica, Ignacio Fernandez, Marcelo Adrián Martí, Gastón Paris, Fernando Alberto Goldbaum. 2012. The NtrY/X two-component system of Brucella spp. acts as a redox sensor and regulates the expression of nitrogen respiration enzymes. *Molecular Microbiology* **85**:1, 39-50. [CrossRef]
5. Emilio Bueno , Socorro Mesa , Eulogio J. Bedmar , David J. Richardson , Maria J. Delgado . 2012. Bacterial Adaptation of Respiration from Oxic to Microoxic and Anoxic Conditions: Redox Control. *Antioxidants & Redox Signaling* **16**:8, 819-852. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
6. H. Silva-Jimenez, J. L. Ramos, T. Krell. 2012. Construction of a prototype two-component system from the phosphorelay system TodS/TodT. *Protein Engineering Design and Selection* . [CrossRef]
7. Caroline Zaoui, Jörg Overhage, Dagmar Löns, Ariane Zimmermann, Mathias Müsken, Piotr Bielecki, Christian Pustelny, Tanja Becker, Manfred Nimtz, Susanne Häussler. 2012. An orphan sensor kinase controls quinolone signal production via MexT in Pseudomonas aeruginosa. *Molecular Microbiology* no-no. [CrossRef]
8. Hortencia Silva-Jiménez, Cristina García-Fontana, Bilge Hilal Cadirci, María Isabel Ramos-González, Juan Luis Ramos, Tino Krell. 2012. Study of the TmoS/TmoT two-component system: towards the functional characterization of the family of TodS/TodT like systems. *Microbial Biotechnology* no-no. [CrossRef]
9. S. Steinsiek, S. Frixel, S. Stagge, K. Bettenbrock. 2011. Characterization of E. coli MG1655 and frdA and sdhC mutants at various aerobiosis levels. *Journal of Biotechnology* **154**:1, 35-45. [CrossRef]
10. M. Huvet, T. Toni, X. Sheng, T. Thorne, G. Jovanovic, C. Engl, M. Buck, J. W. Pinney, M. P. H. Stumpf. 2011. The Evolution of the Phage Shock Protein Response System: Interplay between Protein Function, Genomic Organization, and System Function. *Molecular Biology and Evolution* **28**:3, 1141-1155. [CrossRef]
11. Pierre Mandin, Susan Gottesman. 2010. Integrating anaerobic/aerobic sensing and the general stress response through the ArcZ small RNA. *The EMBO Journal* **29**:18, 3094-3107. [CrossRef]
12. Nicolas Joly, Christoph Engl, Goran Jovanovic, Maxime Huvet, Tina Toni, Xia Sheng, Michael P.H. Stumpf, Martin Buck. 2010. Managing membrane stress: the phage shock protein (Psp) response, from molecular mechanisms to physiology. *FEMS Microbiology Reviews* no-no. [CrossRef]
13. Sylvain Durand, Gisela Storz. 2010. Reprogramming of anaerobic metabolism by the FnrS small RNA. *Molecular Microbiology* **75**:5, 1215-1231. [CrossRef]
14. T. Ueki, D. R. Lovley. 2010. Genome-wide gene regulation of biosynthesis and energy generation by a novel transcriptional repressor in Geobacter species. *Nucleic Acids Research* **38**:3, 810-821. [CrossRef]
15. Adrián F. Alvarez, Dimitris GeorgellisIn Vitro and In Vivo Analysis of the ArcB/A Redox Signaling Pathway **471**, 205-228. [CrossRef]
16. Regine Hengge. 2009. Proteolysis of #S (RpoS) and the general stress response in Escherichia coli. *Research in Microbiology* **160**:9, 667-676. [CrossRef]
17. Xueqiao Liu, Gabriela R. Peña Sandoval, Barry L. Wanner, Won Seok Jung, Dimitris Georgellis, Ohsuk Kwon. 2009. Evidence against the physiological role of acetyl phosphate in the phosphorylation of the ArcA response regulator in Escherichia coli. *The Journal of Microbiology* **47**:5, 657-662. [CrossRef]
18. Goran Jovanovic, Christoph Engl, Martin Buck. 2009. Physical, functional and conditional interactions between ArcAB and phage shock proteins upon secretin-induced stress in Escherichia coli. *Molecular Microbiology* **74**:1, 16-28. [CrossRef]

19. Birgit E. Scharf, Phillip D. Aldridge, John R. Kirby, Brian R. Crane. 2009. Upward mobility and alternative lifestyles: a report from the 10th biennial meeting on Bacterial Locomotion and Signal Transduction. *Molecular Microbiology* **73**:1, 5-19. [CrossRef]
20. Mark P Brynildsen, James C Liao. 2009. An integrated network approach identifies the isobutanol response network of Escherichia coli. *Molecular Systems Biology* **5**. . [CrossRef]
21. Won Seok Jung, Young Ryul Jung, Doo-Byoung Oh, Hyun Ah Kang, Sang Yup Lee, Maria Chavez-Canales, Dimitris Georgellis, Ohsuk Kwon. 2008. Characterization of the Arc two-component signal transduction system of the capnophilic rumen bacterium Mannheimia succiniciproducens. *FEMS Microbiology Letters* **284**:1, 109-119. [CrossRef]
22. M.Julia Pettinari, Pablo I. Nikel, Jimena A. Ruiz, Beatriz S. Méndez. 2008. ArcA Redox Mutants as a Source of Reduced Bioproducts. *Journal of Molecular Microbiology and Biotechnology* **15**:1, 41-47. [CrossRef]
23. Jon Marles-Wright, Richard J Lewis. 2007. Stress responses of bacteria. *Current Opinion in Structural Biology* **17**:6, 755-760. [CrossRef]
24. Maria S. Fadeeva, Evgenia A. Yakovtseva, Galina A. Belevich, Yulia V. Bertsova, Alexander V. Bogachev. 2007. Regulation of expression of Na+-translocating NADH:quinone oxidoreductase genes in Vibrio harveyi and Klebsiella pneumoniae. *Archives of Microbiology* **188**:4, 341-348. [CrossRef]
25. Koreaki Ito . 2006. Redox Control of Protein Processing: From Electrons to Cells. *Antioxidants & Redox Signaling* **8**:5-6, 729-730. [Citation] [Full Text PDF] [Full Text PDF with Links]