The ArcB Leucine Zipper Domain Is Required for Proper ArcB Signaling

Luis Alberto Nuñez Oreza¹, Adrián F. Alvarez¹, Imilla I. Arias-Olguín², Alfredo Torres Larios³, Dimitris Georgellis¹*

¹Departamento de Genética Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México, D.F., México, ²Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, México, D.F., México, ³Departamento de Bioquímica y Biología Estructural, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México, D.F., México

Abstract

The Arc two-component system modulates the expression of numerous genes in response to respiratory growth conditions. This system comprises ArcA as the response regulator and ArcB as the sensor kinase. ArcB is a tripartite histidine kinase whose activity is regulated by the oxidation of two cytosol-located redox-active cysteine residues that participate in intermolecular disulfide bond formation. Here, we report that the ArcB protein segment covering residues 70–121, fulfills the molecular characteristics of a leucine zipper containing coiled coil structure. Also, mutational analyses of this segment reveal three different phenotypic effects to be distributed along the coiled coil structure of ArcB, demonstrating that this motif is essential for proper ArcB signaling.

Introduction

The Arc (anoxic redox control) two-component system plays an important role in the complex transcriptional regulatory network that allows facultative anaerobic bacteria, such as Escherichia coli, to sense changes in respiratory growth conditions and adapt their gene expression accordingly [1–3]. This system consists of ArcB as the sensor kinase and ArcA as the response regulator [4,5]. The ArcB protein belongs to a subfamily of tripartite hybrid kinases; in addition to the canonical pair of transmembrane segments and the orthodox transmitter domain (H1), it possesses a central receiver motif (a leucine zipper), and that its structural integrity is required for proper regulation of ArcB activity.

Characteristics of a Leucine Zipper Coiled Coil Fold that Fulfills the Theoretical Sequence Analysis of the ArcB Linker Region Suggests a putative leucine zipper-like motif in the linker region of ArcB [7], and the fact that leucine zipper motifs have been implicated in homo- and hetero-dimer formation of various proteins, prompted us to hypothesize that this motif may also act as a dimerization domain for the ArcB protein. It has to be noted that in a previous study addressing this question, it was concluded that this putative leucine zipper is not a functional motif [23]. However, the results presented here indicate that the putative leucine zipper motif in the linker region of ArcB [7] fulfills the molecular characteristics of a bona fide leucine zipper, and that its structural integrity is required for proper regulation of ArcB activity.

Results

Sequence Analysis of the ArcB Linker Region Suggests a Coiled Coil Fold that Fulfills the Theoretical Characteristics of a Leucine Zipper

The transmembrane domain of ArcB is immediately followed by a stretch of amino acids, which appears to have a feature characteristic of the well-documented leucine zipper motif [24].
The diagnostic feature of this motif has been defined as 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 (LX6LX6LX6LX6). In each of these coiled coil heptad-repeats that are denoted ‘abcdefg’, positions ‘a’ and ‘d’ are occupied by non-polar residues, having the leucine in position ‘d’, whereas positions ‘e’ and ‘g’ are occupied by polar residues that are solvent-exposed [25]. It is clear from previous studies that such a motif is involved in homo- or hetero-dimer formation through interaction of the helices from two monomers, via their parallel hydrophobic faces, to give a coiled coil dimeric structure [26].

Computer-aided analysis of the secondary structure of ArcB, using the program 2zip [27], revealed that the proposed protein section fulfills the characteristics of this well-documented motif, having the conserved leucine residues at position 73, 80, 87, and 94 (Fig. 1B). Moreover, the COILS/PCOILS prediction method [28] suggested that the coiled coil structure continues to amino acid 150 with a significant score (Fig. 1B), having the leucines 108 and 115 in the same face of the helix as the ones of the upstream leucine zipper. In Fig. 1C it is presented a structural model of the dimeric coiled coil (PDB code 3he5). The positions of leucine residues 73, 80, 87, 94, 108 and 115 are indicated in yellow and the position of Leu 102, which was used as an experimental control, is shown in blue. doi:10.1371/journal.pone.0038187.g001

The Integrity of the Leucine-zipper is Required for Proper in vivo ArcB Signaling

The functionality of the predicted leucine zipper motif in ArcB was explored by constructing a series of low copy number plasmids (pMX 734–748) expressing ArcB proteins in which each of the four leucine residues, or combinations of them, were substituted to valine. Valine was chosen because it provides the most moderate...
amino acid substitution, producing a weakness of the coiled coil bundle without disrupting the helicity of this region of the protein.

The consequences of ArcB modifications were then analyzed by monitoring the in vivo levels of phosphorylated ArcA, as indicated by the expression of the positively controlled cydA⁺⁻⁻lacZ and negatively controlled lldP⁻⁻⁻⁻lacZ target operons. To this end, the generated plasmids were transformed into the ΔarcB strains ECL5004 and ECL5012, carrying a λΦ(cydA⁺⁻⁻lacZ) operon fusion and a λΦ(lldP⁻⁻⁻⁻lacZ) operon fusion, respectively [34]. All plasmid born arcB alleles produced wild-type levels of ArcB (Fig. 2B), as judged by Western blot analysis of the cell extracts with polyclonal antiserum raised against purified His₆-ArcB₇₈–₅₂₀ (Fig. 2B), indicating that the L to V substitutions do not affect the stability of the mutant proteins.

The transformants were grown aerobically or anaerobically in buffered Luria-Bertani broth (LB) to an OD₆₀₀ of 0.5, and their β-galactosidase activity was determined (Fig. 2A). It was found that substitution of L₇₃V resulted in a 2-fold lower lldP⁻⁻⁻⁻lacZ expression and a 2.8-fold higher cyd-lacZ expression under aerobic growth, indicating a partially constitutive active ArcB. Likewise, substitution L₈₀V, having a more drastic effect, showed an almost 3-fold lower lldP⁻⁻⁻⁻lacZ expression and 3.5-fold higher cyd-lacZ expression. Moreover, this aerobic activity was more profound in the double L₇₃V-L₈₀V substitution, showing a 7-fold lower lldP⁻⁻⁻⁻lacZ expression and a 4.3-fold higher cyd-lacZ expression. However, no significant difference was observed on the anaerobic β-galactosidase activities, indicating that these ArcB mutants are still subjected to redox regulation. On the other hand, substituting L₇₃ or L₉₄ to V, and all combination mutants involving either of L₇₃V or L₉₄V resulted in an arcB null phenotype (Fig. 2, and data not shown). It has to be mentioned that in a previous study it was reported that substitution of L₉₄ to A, in contrast to the L₉₄ to V mutant, resulted in a wild type ArcB activity [23]. In an attempt to confirm this result, the effect of substituting either of L₇₃, L₉₀ and L₉₄ to A on the activity of ArcB was tested. All L to A mutants had the same effect as the L to V mutants on the activity of ArcB, except the L₉₄ to A, which in accordance to the previous report, resulted in a wild type ArcB activity (data not shown).

As mentioned earlier, L₁₀₉ and L₁₁₅ are predicted to be in the same face of the helix as the ones of the upstream leucine zipper. To test whether these leucine residues are of importance for the activity/regulation of ArcB, they were substituted to valine and their aerobic and anaerobic reporter expression was monitored (Fig. 2). It was found that the L₁₀₉V mutation rendered ArcB inactive, whereas the L₁₁₅V mutation exhibited lower anaerobic ArcB activity. Finally, substitution of L₁₀₂ to V was used as a control, because it was predicted to not be in the same face of the

Figure 2. Effect of mutations in the leucine zipper of ArcB on the expressions of λΦ(cydA⁺⁻⁻lacZ) and λΦ(lldP⁻⁻⁻⁻lacZ) operon fusions. A) Strains ECL5012 [λΦ(lldP⁻⁻⁻⁻lacZ)] and ECL5004 [λΦ(cydA⁺⁻⁻lacZ)] carrying low copy plasmids that harbor the arcB mutant variants were grown aerobically (solid bars) or anaerobically (empty bars) in Luria-Bertani broth containing 0.1 M MOPS (morpholinepropanesulfonic acid; pH 7.4) and 20 mM D-xylose. In the case of the λΦ(lldP⁻⁻⁻⁻lacZ)-bearing strains the growth medium was supplemented with 20 mM L-lactate as an inducer. At mid-exponential growth phase (OD₆₀₀ = 0.5) the cells were harvested and the β-Galactosidase activity was assayed and expressed in Miller units. The data are averages from three independent experiments and the standard deviations are indicated. (B) Equal number of bacteria of the above aerobic cultures were analyzed by Western blot analysis, using ArcB polyclonal antibodies as previously described [34].
helix as the ones of the upstream leucine zipper. As expected this mutant exhibited almost wild type ArcB activity.

The Integrity of the Leucine-zipper is Required for Proper in vitro ArcB Activity

It has been previously shown that the kinase activity of ArcB is inhibited by ubiquinone-0 (Q0, a soluble analog of ubiquinone-8) [20]. We, therefore, tested the effect of Q0 on the activity of the wild type ArcB, and the mutant ArcB<sup>L80V</sup> and ArcB<sup>L87V</sup> proteins in isolated membrane vesicles containing high amounts of either the three ArcB variant proteins. ArcB<sup>L80V</sup> and ArcB<sup>L87V</sup> were selected as representatives for mutants with increased or no ArcB activity, respectively.

SDS-PAGE analysis of the membrane fractions followed by Coomassie Blue staining revealed that ArcB was the major component in the membrane preparations (data not shown). Subsequently, 1 µg of total protein (membrane vesicles), carrying either the wild type or the mutant ArcB proteins, were incubated with [γ-<sup>32</sup>P]ATP in the presence of dithiothreitol (DTT) or Q0, as described previously [20]. In agreement with the previous report using a truncated form of ArcB (ArcB<sup>78–778</sup>), the full length ArcB protein was rapidly phosphorylated in the presence of DTT, and its phosphorylation was inhibited in the presence of Q0 (Fig. 3), indicating that the over-expressed protein in the membrane fractions preserves its activity and regulation. However, although the net-phosphorylation kinetics of the ArcB<sup>L80V</sup> mutant were similar to the one of the wild type ArcB in the presence of DTT, the ArcB<sup>L87V</sup> mutant was significantly more resistant to Q0-dependent inhibition than the wild type ArcB protein (Fig. 3). Thus, in agreement with the in vivo results, the L80V substitution renders the protein partially insensitive to Q0. On the other hand, no phosphorylation was observed for the ArcB<sup>L87V</sup> mutant (Fig. 3), indicating that the L87V mutation renders the protein inactive.

The ArcB Mutants Exhibit a Dominant Negative Phenotype

Because the above described in vivo and in vitro analyses of the leucine-zipper ArcB mutants revealed either a semi-constitutive ArcB kinase (L73V and L80V) or an inactive ArcB kinase (L87V and L94V), we asked whether these phenotypes are dominant in a wild-type strain. To this end, the arcB<sup>+</sup> strain ECL5003 was transformed with the mutant ArcB expressing plasmids, and the expression of the λ<sup>B</sup>(cydA'-lac<sup>Z</sup>) operon fusion was monitored. The transformants were grown aerobically or anaerobically in buffered Luria-Bertani broth (LB) to an OD<sub>600</sub> of ~0.5, and their β-galactosidase activities were determined (Fig. 4A). It was found that the arcB<sup>+</sup> strain carrying either the L67V or the L94V ArcB mutant expressing plasmids failed to activate cydA'-lac<sup>Z</sup> expression under anaerobic conditions (Fig. 4A). Also, expression of the plasmid borne L108V and the L115V ArcB mutants in an arcB<sup>+</sup> strain resulted, respectively, in a 2.2-fold and a 1.5-fold reduction of cydA'-lac<sup>Z</sup> expression under anaerobic conditions (Fig. 4A).

On the other hand, the substitution L73V and L80V that exhibit a semi-constitutive ArcB activity, and also the substitution L102V, which was used as a control, had no effect on the cydA'-lac<sup>Z</sup> expression under the above mentioned conditions (Fig. 4A). It thus appears that the L to V substitutions that result to either lower or no ArcB kinase activity, cause a dominant negative phenotype.

The Dominant Negative Phenotype of the Leucine Zipper Mutants is Not Due to the Phosphatase Activity of ArcB

The above results led us to consider the possibility that the inactive ArcB variants could be trapped in a kinase-/phosphatase<sup>+</sup> state, counteracting the kinase activity of the chromosomally expressed wild type ArcB. To test this possibility, we generated ArcB-independent ArcA-P in vivo, and examined whether the ArcB mutant variants were able to dephosphorylate ArcA-P [18]. To this end, the ECL5004 strain (arcB<sup>+</sup>, cydA'-lac<sup>Z</sup>) harboring the low copy number plasmids expressing either the wild type ArcB or the various ArcB mutants, was grown aerobically in defined minimal medium supplemented with 20 mM pyruvate as the sole carbon and energy source, to an OD<sub>600</sub> of ~0.5, and the β-galactosidase activity was determined. Growth on pyruvate was chosen because it has been shown that under this condition the intracellular concentration of acetyl-phosphate is an order of magnitude higher than in cells grown aerobically on glycerol as the sole carbon and energy source [35], and because in the absence of their cognate sensor kinase, many response regulators undergo in vivo autophosphorylation at the expense of acetyl-phosphate [36–39]. In agreement with a previous report [18], it was found that the cydA'-lac<sup>Z</sup> reporter was suitably expressed in the ΔarcB strain, indicating a deficiency in ArcA-P dephosphorylating activity, whereas reporter expression was 6-fold lower in the strain carrying the wild type ArcB expressing plasmid (pMXN732), indicating specific ArcA-P dephosphorylation by ArcB under aerobic growth conditions (Fig. 4B). Moreover, it was found that the L73V and L80V semi-constitutive ArcB mutants exhibited almost the same level of reporter expression as the ΔarcB strain, indicating a complete loss of phosphatase activity. On the other hand, the L87V and L94V ArcB mutants resulted in approximately 1.4-fold lower reporter expression than the one observed in the ΔarcB mutant strain, and ~4.5-fold higher reporter expression than the wild type ArcB, indicating not only loss of kinase activity (Fig. 2) but also a severe deficiency in their phosphatase activity under aerobic growth. Thus, the possibility of an ArcB conformation trapped in a kinase-/phosphatase<sup>+</sup> state can be discarded. Instead, formation of heterodimers between chromosomally expressed wild type ArcB and plasmid borne inactive ArcB proteins, could provide a possible explanation for the above result. Finally, the L102V substitution resulted in reporter expression levels similar to those of the wild type ArcB, whereas L108V and L115V mutations resulted to a slight lower reporter expression than the wild type ArcB, indicating that these two latter mutants might have higher phosphatase activity than the wild type ArcB.

**Figure 3. Effect of DTT and ubiquinone-0 on the rate of ArcB net-phosphorylation.** Membrane vesicles (1 µg) containing high amounts of wild type ArcB<sup>778–778</sup> (circles) or the mutant ArcB variants (ArcB<sup>L80V</sup> (squares) and ArcB<sup>L87V</sup> (diamonds)) were incubated at room temperature with 50 µM [γ-<sup>32</sup>P]ATP in the presence of 5 mM DTT (open symbols) or 250 µM Q0 (closed symbols) in a 20 µl reaction mixture. At the indicated time intervals a 5 µl sample was withdrawn for SDS-PAGE analysis. Left panel: autoradiograms of the gels. Right panel: net increase of ArcB-P with time, as quantitated with a PhosphorImager. doi:10.1371/journal.pone.0038187.g003
Coiled coils are common structural motifs, resulting from the packing of two to five α-helices, one wrapped around the other into a left-handed helix, to form a supercoil [40]. Each helix consists of multiple copies of a heptad-repeating unit (denoted abcdEfg), containing a similar configuration of residues [41]. Coiled coil motifs are often found in sensor kinases and in many cases they have been shown to play a significant role in the signaling mechanisms. For example, the HAMP domain, which is present in ~31% of the sensor kinases, is usually located immediately after the transmembrane region and is of main importance for signal transmission [42]. Another common functional coiled coil motif in sensor kinases is the signaling helix (S-helix), which was suggested to function as a switch that prevents constitutive activation of downstream signaling domains [43].

Leucine zippers constitute a subtype of coiled coil structures, in which the amino acid leucine is predominant at the “d” position of the heptad repeat [24]. Although leucine zippers have been best characterized in DNA binding proteins, they also exist in many other signaling proteins [30–33]. Recently, it was reported that the leucine zipper of the cell-cycle regulated Nek2 kinase is important for its dimerization and activation [44].

The fact that the regulation of ArcB signaling involves intermolecular disulfide-bonds formation between two ArcB proteins, suggests that the evolution of such a mechanism would require adaptation of the protein to function as a dimer to promote the proximity of the two cysteine residues. A suitable candidate to promote such a dimer-formation in the linker region of ArcB appears to be the leucine zipper. Accordingly, substitution to valine of any of the leucine residues in this coiled coil motif produced a mutant phenotype. Interestingly, substitution of the
first two leucine residues, L73 and L80, resulted in a semi-
constitutive ArcB kinase with concomitant loss of its ArcA-P
deprophosphorylating activity. Thus, the weakness of the hydropho-
bic helix-helix interaction by the L73V and L80V substitutions
could affect the downstream conformation in such a manner that
it partially traps the protein in the kinase conformation, which
is reminiscent to the effect of mutating the interaction region of the
E. coli chemoreceptors Tar and Tsr [45]. On the other hand,
alterations of the second half of the leucine zipper, L87 and L94,
resulted in an inactive ArcB protein, not only as kinase but also as
a phosphatase. Finally, a third phenotypical group is formed by
L108 and L115. While the kinase activity of ArcB was totally and
partially lost by the L108 and L115 substitutions to valine, the
phosphatase activity of these mutants was not affected. Even
though it is difficult to directly determine the effects of a given
“knob” truncation on helix packing stability, the three different
phenotypical effects are distributed along the coiled coil structure
of ArcB, from the second TM region to the reldx active cysteine
residues.

Noteworthy, in a previous study, based on in vivo and in vitro
experiments, it was suggested that the leucine zipper of ArcB is not
a functional motif. This conclusion was based on the fact that the
L94A substitution was without effect, a result confirmed in our
study (data not shown), but overlooking the fact that a L80A
substitution showed a semi-constitutive kinase activity and a L87A
substitution resulted in an arcB null phenotype [23]. Although the
reasons of the difference between the L94V and L94A substitution
are not clear, the higher capability of alanines in comparison to
valines to form an α-helix could provide a suitable explanation
[46]. Interestingly, the L to V substitutions, resulting in null or
lower ArcB kinase activity, were shown to exhibit a dominant
phenotype when expressed in an arcB+ strain. Such an effect could
occur if the ArcB mutants were trapped in a kinase or phospha-
tase conformation, which appears to be the case of the ArcB180V
and ArcB115V mutants, thereby counteracting the kinase activity
of the chromosomal encoded ArcB protein. On the other hand,
heterodimer formation between the chromosomal encoded ArcB
protein and the inactive ArcB variants, could provide a possible
explanation for the dominant negative phenotype of the ArcB180V
and ArcB115V mutants.

Taken together, our experimental results clearly indicate that
the structural integrity of not only the leucine zipper but also the
overall coiled coil fold of ArcB is essential for the correct
orientation of the monomers within a dimer, and thereby required
for proper ArcB signaling.

Materials and Methods

Bacterial Strains, Plasmids and Oligonucleotides

Escherichia coli strains and plasmids used in this study are listed in
Table 1. Plasmid pMX712 was constructed by cloning the BamHI-
HindIII fragment from plasmid pIBW [34], which carries the arcB
promoter, the arcB ribosome binding site, an introduced NdeI site
that includes the initiation codon of arcB, and the arcB ORF and
stop codon, into pBlueScript II KS+. Plasmids pMX734-737 and
pMX520-530 were constructed by site-directed mutagenesis of
plasmids pMX712, substituting either of leucine 73, 80, 87, 94,
pMX528-530 were constructed by site-directed mutagenesis of
phenotype when expressed in an
strain. Such an effect could
in vivo phosphorylation activity of the ArcB variants, ArcB-independent ArcA-P
was generated by growing cells in a defined minimal medium [1 mM
KH2PO4, 40 mM KCl, 54 mM NaCl, 20 mM (NH4)2SO4, 1 μM
FeSO4, 0.3 mM MgSO4, 1 μM ZnCl2, 10 μM CaCl2, and 0.1 M
MOPS, at a final pH of 7.4] supplemented with 20 mM pyruvate
as described previously [9].

ArcB-enriched Inverted Vesicles Preparation and Phosphorylation Assays

Strain ECL5012 carrying pMX517, pMX520 or pMX521, was
grown in 250 ml of ampicillin containing LB medium at 37°C
until an OD600 of 0.5. Then, expression of ArcB was induced by
addition of L-arabinose to a final concentration of 0.13 mM. Cells
were harvested 4 hours after induction, resuspended in 6 ml of ice-
cold MOPS buffer (50 mM K-MOPS, pH 7.0, 5 mM MgSO4,
and 100 mM KCl), and broken by passing through a French Press.
The cell lysate was cleared by centrifugation at 10,000 g for 15
min, and the ArcB embedded into inner membranes was
centrifuged at 32,500 g for 40 min at 4°C. Finally, the membranes
were solubilized in 500 μl of MOPS buffer containing 30%
glycerol, and stored at −20°C. Phosphorylation assays were
conducted at room temperature in the presence of 40 mM
[γ-32P]ATP (specific activity, 2 Ci mmol−1; New England
Nuclear), 33 mM HEPES (pH 7.5), 50 mM KCl, 5 mM MgCl2,
0.1 mM EDTA, and 10% glycerol. 1 μg of the total protein
(membrane vesicles), carrying either the wild type or the mutant
ArcB proteins, was used in phosphorylation assays in the presence
of 5 mM dihydrothioleitol (DTT) or 250 mM Q0, as described
previously [48].
The ArcB Leucine Zipper Is a Functional Domain

| Table 1. Escherichia coli strains and plasmids used in this study are listed. |
|-----------------------------|-----------------------------|
| **Strain**                  | **Relevant characteristics** | **Source**         |
| ECL5004                    | ΔarcB::Tet' lphcylA-lacZ Δnfr::Tn9(Cm')| [34] |
| ECL5012                    | ΔarcB::Tet' lph(Δp-lacZ) | [34] |
| ECL5003                    | arcB' lphcylA-lacZ Δnfr::Tn9(Cm')| [34] |

Plasmids

- pBluescript SK II Cloning vector, Amp’ (+) Stratagene
- pEXT22 Low copy number vector, Kan’ [47]
- pMX712 arcB’ in pBluescript KS II (+) This study
- pMX715 arcB’ΔOV in pBluescript KS II (+) This study
- pMX716 arcB’ΔOV in pBluescript KS II (+) This study
- pMX732 arcB’ in pEXT22 This study
- pMX734 arcB’ΔTTS in pEXT22 This study
- pMX735 arcB’ΔOV in pEXT22 This study
- pMX736 arcB’ΔOV in pEXT22 This study
- pMX737 arcB’ΔOV in pEXT22 This study
- pMX528 arcB’ΔTTS in pEXT22 This study
- pMX529 arcB’ΔOV in pEXT22 This study
- pMX530 arcB’ΔTTS in pEXT22 This study
- pMX738 arcB’ΔTTSΔOV in pEXT22 This study
- pMX739 arcB’ΔTTSΔOV in pEXT22 This study
- pMX741 arcB’ΔTTSΔOV in pEXT22 This study
- pMX744 arcB’ΔTTSΔOV in pEXT22 This study
- pMX200 arcB’ΔS71–778 under control of ara promoter, Amp’ | [18] |
- pMX201 arcB’ΔS71–778 under control of ara promoter, Amp’ | [18] |
- pMX202 arcB’ΔS71–778 under control of ara promoter, Amp’ | [18] |
- pMX203 arcB’ΔS71–778 under control of ara promoter, Amp’ | [18] |

References

1. Georgellis D, Kwon O, Lin EC (1999) Amplification of signaling activity of the ArcB sensor kinase in Escherichia coli. J Biol Chem 274: 35950–35954.
2. Kwon O, Georgellis D, Lin EC (2000) Phosphorylation study of the ArcB sensor kinase of Escherichia coli autophosphorylates by an intramolecular reaction. J Bacteriol 182: 1735–1739.
3. Rodriguez C, Kwon O, Georgellis D (2004) Effect of D-lactate on the physiological activity of the ArcB sensor kinase in Escherichia coli. J Bacteriol 186: 2083–2090.
4. Georgellis D, Kwon O, Lin EC (1999) Amplification of signaling activity of the ArcB sensor kinase of Escherichia coli autophosphorylates by an intramolecular reaction. J Bacteriol 182: 35950–35954.
5. Kwon O, Georgellis D, Lin EC (2000) Phosphorylation study of the ArcB sensor kinase of Escherichia coli autophosphorylates by an intramolecular reaction. J Bacteriol 182: 35950–35954.
6. Georgellis D, Kwon O, De Wolf P, Lin EC (1998) Signal decay through a reverse phosphorylation in the Arc two-component signal transduction system. J Biol Chem 273: 32964–32969.

β-Galactosidase Activity Assay

The ΔphcylA-lacZ bearing strains were grown in Luria-Bertani broth containing 0.1 M MOPS (morpholinepropanesulfonic acid; pH 7.4) and 20 mM D-xylene. The ΔphcylA-lacZ-bearing strains were grown in the above medium supplemented with 20 mM L-lactate as an inducer. For aerobic growth, cells were cultured in 10 ml of medium in 250-ml baffled flasks at 37°C with shaking (300 rpm). For anaerobic growth, cells were cultured in a screw-capped tube filled with medium up to the rim at 37°C and stirred by a magnet. β-Galactosidase activity was assayed with exponential growing cultures and expressed in Miller units as described previously [49].

Western Blot Analysis

Aerobically grown cultures were harvested by centrifugation during mid-exponential growth. The cell pellet was resuspended in 5X SDS sample buffer and separated by SDS-PAGE (10% polyacrylamide gel), and the proteins were transferred to a Hybond-ECL filter (Amersham Biosciences). 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 (1% milk in TTBS) for 1 h at room temperature. ArcB polyclonal antibodies, raised against His-ArcB776–780 [12], 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 IgG antibody conjugated to horseradish peroxidase and the ECL detection system (Amersham Biosciences).

Structural Model of the ArcB Coiled Coil Linker Region

The structural model was inferred using the results, with the highest normalized Z-score, provided by the I-TASSER server (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) [50]. The predicted model is based on the monomeric helix (covering amino acid residues 70–89) derived from the solution structure of the ArcB transmembrane domain (PDB code 2ksd), and using the original coordinates of the dimeric coiled coil motif from PDB code 3he5 as a template.

Acknowledgments

We thank Claudia Rodriguez and Miriam Vazquez-Acevedo for technical assistance, and the Unidad de Biología Molecular from the Instituto de Fisiología Celular, Universidad Nacional Autónoma de México for oligonucleotide synthesis and sequencing.

Author Contributions

Conceived and designed the experiments: DG. Performed the experiments: LANO AFA. Analyzed the data: AFA DG. Contributed reagents/materials/analysis tools: ATL IIAO. Wrote the paper: AFA DG. Computer-aided analysis and modeling of the ArcB coiled coil: ATL IIAO.
The ArcB Leucine Zipper Is a Functional Domain

16. Lynch AS, Lin EC (1996) 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.

17. Alvarez AF, Malpica R, Couteras M, Escamilla E, Georgellis D (2010) Cytochrome b but not cytochrome a rescue the toluidine blue growth sensitivity of arc mutants of Escherichia coli. J Bacteriol 192: 391–399.

18. Peña-Sandoval GR, Kwon O, Georgellis D (2005) Requirement of the receiver and phosphotransfer domains of ArcB for efficient dephosphorylation of phosphorylated ArcA in vivo. J Bacteriol 187: 3267–3272.

19. Kwon O, Georgellis D, Lin EC (2003) Rotational on-off switching of a hybrid membrane sensor kinase Tar-ArcB in Escherichia coli. J Biol Chem 278: 13192–13199.

20. Georgellis D, Kwon O, Lin EC (2003) Quinones as the redox signal for the Arc two-component system of bacteria. Science 292: 2314–2316.

21. Malpica R, Franco R, Rodriguez C, Kwon O, Georgellis D (2004) Identification of a quinone-sensitive redox switch in the ArcB sensor kinase. Proc Natl Acad Sci U S A 101: 13310–13315.

22. Tomomori C, Tanaka T, Dutta R, Park H, Saha SK, et al. (1999) Solution structure of the homodimeric core domain of Escherichia coli histidine kinase EnvZ. Nature structural biology 6: 729–734.

23. Matsushika A, Mizuno T (2000) Characterization of three putative sub-domains in the signal-input domain of the ArcB hybrid sensor in Escherichia coli. J Biochem 127: 855–860.

24. Landshulz WH, Johnson PF, McKnight SL (1988) The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. Science 240: 1759–1764.

25. Mason JM, Arndt KM (2004) Coiled coil domains: stability, specificity, and biological implications. Chemphyschem 5: 170–176.

26. Vinson C, Acharya A, Taparovski EJ (2006) Deciphering B-ZIP transcription factor interactions in vitro and in vivo. Biochim Biophys Acta 1759: 4–12.

27. Bormberg-Bauer E, Rivals E, Vingron M (1998) Computational approaches to the identification of a quinone-sensitive redox switch in the ArcB sensor kinase. FEBS Lett 417: 409–413.

28. Gruber M, Söding J, Lupas AN (2005) REPPER–repeats and their periodicities in fibrous proteins. Nucleic acids res 33: 239–243.

29. Buckland R, Wild F (1989) Leucine zipper motif extends. Nature 338: 542.

30. Mukai H, Ono Y (1994) A novel protein kinase with leucine zipper-like sequences: its catalytic domain is highly homologous to that of protein kinase C. Biochem Biophys Res Co 199: 897–904.

31. Lau PC, Wang Y, Patel A, Labbé D, Bergeron H, et al. (1997) A bacterial basic region leucine zipper histidine kinase regulating toluene degradation. Proc Natl Acad Sci U S A 94: 1453–1458.

32. Yaku H, Minuno T (1997) The membrane-located osmosensory kinase, EnvZ, that contains a leucine zipper-like motif functions as a dimer in Escherichia coli. FEBS Lett 417: 469–473.

33. Fry AM, Arnaud L, Nigg EA (1999) Activity of the human centrosomal kinase, Nek2, depends on an unusual leucine zipper dimerization motif. J Biol Chem 274: 16304–16310.

34. Kwon O, Georgellis D, Lynch AS, Boyd D, Lin EC (2000) The ArcB sensor kinase of Escherichia coli genetic exploration of the transmembrane region. J Bacteriol 182: 2960–2966.

35. McCleary WR, Stock JB (1994) Acetyl phosphate and the activation of two-component response regulators. J Biol Chem 269: 31567–31572.

36. Lee TY, Makino K, Shinagawa H, Nakata A (1998) Overproduction of acetyl kinase activates the phosphate regulon in the absence of the phaR and phaM functions in Escherichia coli. J Bacteriol 172: 2245–2249.

37. Wanner BL, Wilmes-Riesenberg MR (1992) Involvement of phosphotransacetylase, acetate kinase, and acetyl phosphate synthase in control of the phosphate regulon in Escherichia coli. J Bacteriol 174: 2124–2130.

38. Dieder FE, Berg HC (1993) Change in direction of flagellar rotation in Escherichia coli mediated by acetyl kinase. J Bacteriol 175: 3236–3239.

39. Feng J, Atkinson MR, McCleary W, Stock JB, Wanner BL, et al. (1992) Role of phosphorylated metabolic intermediates in the regulation of glutamine synthetase synthesis in Escherichia coli. J Bacteriol 174: 6061–6070.

40. Crick HFC (1953) The packing of α-helices: simple coiled-coils. Acta Crystallogr 6: 689–697.

41. McLachlan AD, Stewart M (1975) Tropomyosin coiled-coil interactions: evidence for an unstaggered structure. J Mol Biol 98: 293–304.

42. Gao R, Stock AM (2009) Biological insights from structures of two-component proteins. Annu Rev Microbiol 63: 133–154.

43. Anantharaman V, Balaji S, Aravind L (2006) The signaling helix: a common functional theme in diverse signaling proteins. Annu Rev Microbiol 63: 133–154.

44. Gao R, Stock AM (2009) Biological insights from structures of two-component proteins. Annu Rev Microbiol 63: 133–154.

45. Anantharaman V, Balaji S, Aravind L (2006) The signaling helix: a common functional theme in diverse signaling proteins. Annu Rev Microbiol 63: 133–154.

46. Gregoret LM, Sauer RT (1998) Tolerance of a protein helix to multiple alanine substitutions. Folding & design 3: 119–126.

47. Swain KE, Gonzalez MA, Falke JJ (2009) Engineered socket study of signaling through a four-helix bundle: evidence for a yin-yang mechanism in the kinase control module of the aspartate receptor. Biochemistry 48: 9266–9277.

48. Gessot LM, Sauer RT (1986) Tolerance of a protein helix to multiple alanine and valine substitutions. Folding & design 3: 119–126.

49. Dykhooam DM, St Pierre R, Linn T (1996) A set of compatible lac promoter expression vectors. Gene 173: 133–136.

50. Alvarez AF, Georgellis D (2010) In vitro and in vivo analysis of the ArcB/A redox signaling pathway. Method Enzymol 471: 203–229.

51. Miller JH (1972) β-Galactosidase Assay. In: Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor. pp 352–355.

52. Roy A, Kucukural A, Zhang Y (2010) I-TASSER: a unified platform for automated protein structure and function prediction. Nat Protoc 5: 725–730.