Signal Decay through a Reverse Phosphorelay in the Arc Two-component Signal Transduction System*

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Escherichia coli senses and signals anoxic or low redox conditions in its growth environment by the Arc two-component system. Under those conditions, the tripartite sensor kinase ArcB undergoes autophosphorylation at the expense of ATP and subsequently transphosphorylates its cognate response regulator ArcA through a His → Asp → His → Asp phosphorelay pathway. In this study we used various combinations of wild-type and mutant ArcB domains to analyze *in vitro* the pathway for signal decay. The results indicate that ArcA-P dephosphorylation does not occur by direct hydrolysis but by transfer of the phosphoryl group to the secondary transmitter and subsequently to the receiver domain of ArcB. This reverse phosphorelay involves both the conserved His-717 of the secondary transmitter domain and the conserved Asp-576 of the receiver domain of ArcB but not the conserved His-292 of its primary transmitter domain. This novel pathway for signal decay may generally apply to signal transduction systems with tripartite sensor kinases.

Signal transduction by phosphorylation and dephosphorylation of cellular proteins plays pivotal roles in regulating numerous cellular processes in both prokaryotes and eukaryotes. In prokaryotes, signaling by phosphoryl group transfer reactions depends on two-component systems typically comprising a sensor kinase and its cognate response regulator (1). Upon stimulation, the sensor kinase undergoes ATP-dependent autophosphorylation at a conserved histidine. The kinase then activates its cognate response regulator by transphosphorylating it at a conserved aspartate. Often, the kinase exhibits a third activity that promotes the release of P_i from the phosphoresponse regulator. Such two-component systems have also been found in archaebacteria (2, 3), fungi (4–6), and the plant *Arabidopsis thaliana* (7–9).

The Arc (anoxic redox control, formerly aerobic respiration control) two-component system of *Escherichia coli* consists of ArcB as the membrane-bound sensor kinase and ArcA as its response regulator (Fig. 1). This system regulates the expression of some 30 operons (the Arc modulon) in response to redox conditions of growth (10, 11). The ArcB protein (12, 13) belongs to a subfamily of tripartite hybrid kinases (14); in addition to

the canonical pair of transmembrane segments and the orthodox transmitter domain (H1), there is a central receiver domain (D1) and a secondary C-terminal transmitter domain (H2) (15, 16). This subfamily accounts for about 20% of the currently reported sensor kinases in *E. coli* (17). Other examples of bacterial tripartite kinases include BarA (18), BvgS (19), EvgS (20), LemA (21), RteA (22), and TorS (23).

Results from a previous *in vitro* study on the Arc system indicated that, following ArcB autophosphorylation at the conserved His-292 of H1, the phosphoryl group is successively transferred to the conserved Asp-576 of D1, the conserved His-717 of H2, and the conserved Asp-54 of ArcA in a His → Asp → His → Asp phosphorelay (24). This sequence of signal transmission was previously reported for the Kin/Spo system of *Bacillus subtilis* (25), the Sln1p/Ypd1p/Ssk1p of *Saccharomyces cerevisiae* (26), the BvgS/A of *Bordetella pertussis* (27, 28), and the TorS/R of *E. coli* (29).

So far most studies on these signal transduction pathways have focused on the ATP-dependent autophosphorylation of the sensor kinase and the subsequent phosphotransfer reaction(s) to the response regulator. Considerably less is known about the pathways for signal decay that are equally important in adaptive responses. Here we report experiments designed to analyze the route of ArcA-P dephosphorylation. The results led us to propose a novel pathway for signal decay.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Plasmids, and Oligonucleotides—*E. coli* M15 and plasmids pREP4 and pQE30 were obtained from Qiagen Ltd. Plasmids pQE30ArcB78–778, pQE30ArcB78–661, pQE30ArcB78–520, pQE30ArcB521–778, pQE30ArcB521–661, pQE30ArcB638–778, and pQE30ArcA, used for expression of His-tagged derivatives of ArcB and ArcA, have been described previously (24, 30). To create pQE30ArcA′, a 440-base pair region of DNA containing the coding sequence corresponding to amino acid residues 1–136 of ArcA was amplified by the PCR[1] using primers 5′-CCCGGATCCCATATGCAGACCCCGCACATTCTTATC-3′ and 5′-CCCGGATCCTGCAGTCATTAGCTTTCAACGCTACGACGTTCTTC-3′ with pMW2 (10) as template. The PCR product was digested with *Bam*HI and *Pst*I, and the resulting fragment was cloned between the *Bam*HI and *Pst*I sites of pQE30.

To create pQE30ArcB521–778, D576A, primers 5′-CCCGGATCCCATATGCCTTTACCGGCGCTGAATGTGC-3′ and 5′-CCCGGATCCATGCATCGCGCACCCCGGTCTAGCC-3′ were used in the PCR with pBB35 (12) as template. The PCR product was digested with *Bam*HI and *Nsi*I and cloned between the *Bam*HI and *Pst*I sites of pQE30.

To create pQE30ArcB521–778, H717Q the mutagenic primer 5′-GGCATGTTGAGGAAGGACAGAAAATTAAAGGTGCGGCG-3′ and primer 5′-CCCGGATCCCATGCATCGCGCACCCCGGTCTAGCC-3′ were used in the PCR with pBB25 (12) as template. The product of this reaction was purified and used as a megaprimer for PCR in combination with primer 5′-CCCGGATCCCATATGCCTTTACCGGCGCTGAATGTGC-3′ and pBB25 as template. Finally, the product of the second PCR reaction was digested with *Bam*HI and *Nsi*I and cloned between the *Bam*HI and *Pst*I sites of pQE30.

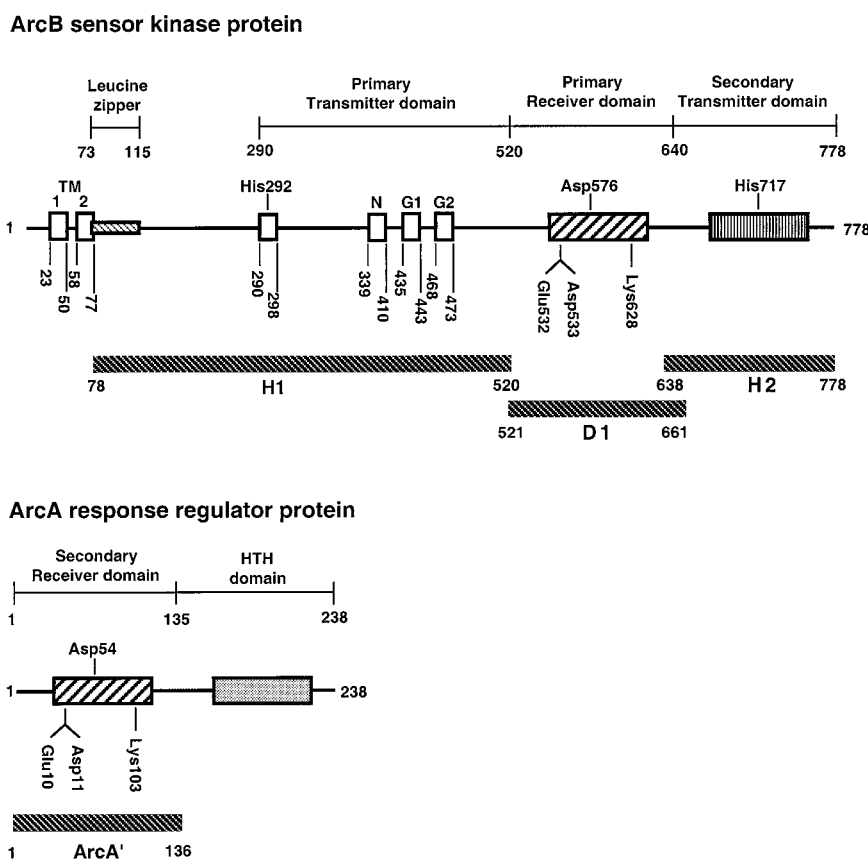All DNA fragments cloned from PCR-amplified material were veri-

[1] The abbreviations used are: PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; AMP-PMP, adenylyl-imidodiphosphate; ATPγS, adenosine 5′-*O*-[thiotriphosphate].

ArcB sensor kinase protein

ArcA response regulator protein

FIG. 1. **Schematic representations of protein domains of the Arc two-component system.** *Top,* the ArcB sensor kinase protein. Two N-terminal transmembrane segments (*TM*) were predicted on the basis of a hydrophobicity plot (61) and supported by ArcB-PhoA fusion analyses (O. Kwon and D. Georgellis, unpublished data.) (62). Residues 73–115 contain a putative leucine zipper motif (63). *H1* (the orthodox transmitter domain) is shown with the conserved His-292 (12) and the catalytic determinants *N*, *G1*, and *G2*. The last two determinants resemble nucleotide-binding motifs (64). *D1* (the receiver domain) is shown with the conserved Asp-576 (12), and *H2* (the secondary transmitter domain) is shown with the conserved His-717 (15). The *hatched bars* indicate the lengths of the individual H1, D1, and H2 modules used in the study. Not illustrated are H1-D1 comprising residues 78–661, and D1-H2 comprising residues 521–778. *Bottom,* the ArcA response regulator protein. The N-terminal receiver domain contains the conserved Asp-54, and the C-terminal domain contains the helix-turn-helix (*HTH*) motif (65). The *hatched bar* indicates the length of ArcA'.

fied by sequencing at Micro Core Facility of the Department of Microbiology and Molecular Genetics, Harvard Medical School.

Purification of His$_6$-tagged Proteins, Isolation of His$_6$-ArcA$^{1-136}$-P, and Dephosphorylation Assays—Expression and purification of the His$_6$-tagged proteins were described previously (24). Phosphorylation of His$_6$-ArcA$^{1-136}$ (~0.4 nmol) was carried out at 25 °C in 20 μl of buffer A (33 mM HEPES at pH 7.5, 50 mM KCl, 5 mM MgCl$_2$, 1 mM dithiothreitol, 0.1 mM EDTA, and 10% glycerol) containing His$_6$-ArcB$^{78-778}$ (~50 pmol) and 40 μM [γ-^{32}P]ATP (specific activity 2 Ci/mmol, NEN Life Science Products). After 10 min, the reaction was terminated by addition of 100 μl of buffer B (50 mM Tris-HCl at pH 7.0, 150 mM KCl, 5 mM EDTA, and 3% Triton X-100). His$_6$-ArcB$^{78-778}$ was separated from the reaction mixture by ultrafiltration, using a Nanosep 30K device (Pallfiltron). The filtrate, containing His$_6$-ArcA$^{1-136}$-P was passed through a Nanosep 10K device, and the retained material was washed 4 times with 500 μl of buffer B and once with 500 μl of buffer A to remove small molecules such as ATP and P$_i$. Finally, the retained material containing His$_6$-ArcA$^{1-136}$-P (essentially free of His$_6$-ArcB$^{78-778}$, nonhydrolyzed ATP and P$_i$) was diluted to a final volume of 200 μl with buffer A. Dephosphorylation reactions were carried out at 25 °C in mixtures of 35 μl of buffer A, containing ~50 pmol of His$_6$-ArcA$^{1-136}$-P and ~5 pmol of ArcB$^{78-778}$ or its subdomain(s). At various time points, a 5-μl sample was withdrawn, mixed with 5 μl of SDS sample buffer, and kept on ice. One μl of each sample was analyzed for P$_i$ by TLC in 2 N formic acid and 0.5 M LiCl for 45 min, using polyethyleneimine cellulose plates (Aldrich). The rest of the sample (9 μl) was analyzed for the proteins by SDS-PAGE on 15% polyacrylamide gels. The radioactivity of proteins or P$_i$ resolved on SDS gels or TLC plates was determined qualitatively by autoradiography with X-Omat AR (Kodak) and quantitatively by a PhosphorImager (Molecular Dynamics).

RESULTS

Effect of ArcB on the Dephosphorylation of ArcA-P—To test whether ArcB catalyzes the dephosphorylation of ArcA-P, we carried out an exploratory experiment in which His$_6$-ArcB$^{78-778}$ (hereafter referred to as H1-D1-H2) and His$_6$-ArcA (hereafter referred to as ArcA) were incubated with [γ-^{32}P]ATP.

An N-terminally truncated ArcB was used because the removal of the two transmembrane segments facilitated the pu-
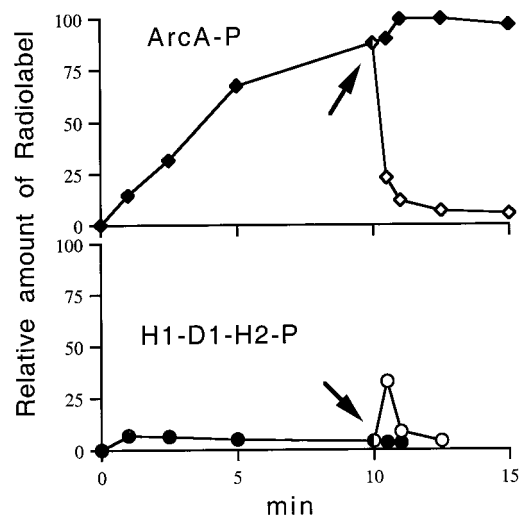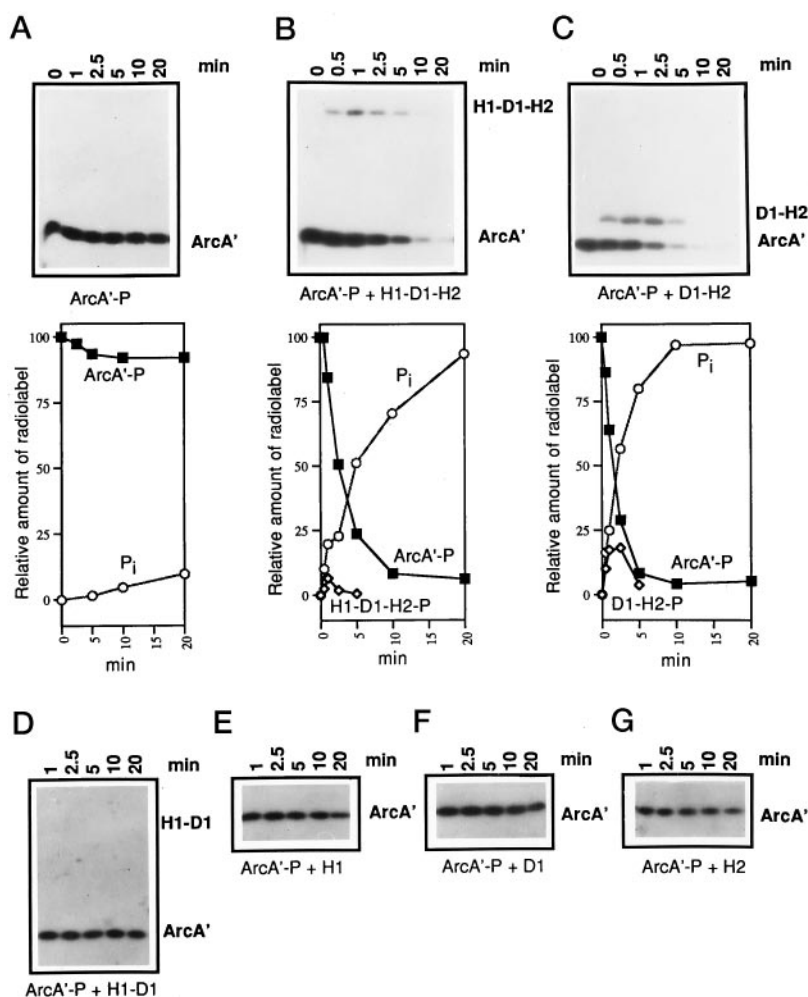

FIG. 2. **Acceleration of ArcA-P dephosphorylation by ArcB.** ArcA (100 pmol) and H1-D1-H2 (10 pmol) were incubated with [γ-^{32}P]ATP (50 μmol) in a total reaction volume of 100 μl. After 10 min, the reaction mixture was divided into two equal portions (indicated by *arrows*): one served as control, and the other received 25 pmol of H1-D1-H2 (0.5 μl). At the indicated time intervals, 5 μl of the reaction mixtures were withdrawn for analysis of ArcA-P (*top panel*) and H1-D1-H2-P (*bottom panel*). *Closed diamonds*, ArcA-P in the control reaction; *open diamonds*, ArcA-P in the reaction fortified with H1-D1-H2; *closed circles*, H1-D1-H2-P in the control reaction mixture; *open circles*, H1-D1-H2-P in the reaction mixture fortified with H1-D1-H2.

rification of the protein which is constitutively active as an autokinase (24, 31). After 10 min of incubation, the reaction mixture was divided into 2 aliquots as follows: one served as the control and the other was fortified with additional H1-D1-H2 (Fig. 2). In the control reaction, the amount of ArcA-P

Signal Decay in the Arc Two-component System

FIG. 3. **ArcA′-P dephosphorylating activity of ArcB and its subdomains.** Purified ArcA′-P (50 pmol) was incubated with different ArcB domains (5 pmol) in a 35-μl reaction mixture. At the indicated time points, a 5-μl sample was withdrawn for SDS-PAGE and TLC analysis (see "Materials and Methods"). Polypeptides present in the reaction are indicated *below* each autoradiogram. The position of each polypeptide in the gel is indicated on the *right side* of each panel. *A*, ArcA′-P alone; *B*, ArcA′-P with H1-D1-H2; *C*, ArcA′-P with D1-H2; *D*, ArcA′-P with H1-D1; *E*, ArcA′-P with H1; *F*, ArcA′-P with D1; and *G*, ArcA′-P with H2. When useful, the normalized rates of phosphoryl group transfer and P_i release are presented graphically *below* the autoradiogram. *Filled squares*, ArcA′-P; *open circles,* P_i; and *open diamonds*, H1-D1-H2-P in the case of reaction B or D1-H2-P in the case of reaction C.

continued to increase until it reached a steady state. By contrast, in the reaction mixture supplemented with H1-D1-H2, the amount of ArcA-P decreased rapidly. Furthermore, the level of phosphorylated H1-D1-H2 in the second reaction mixture increased transiently. This increase may reflect either the autophosphorylation of the added protein or its transphosphorylation by ArcA-P. The radioactivity lost from ArcA greatly exceeded that acquired by H1-D1-H2. The difference is essentially accounted for by the P_i liberated (not shown). The results suggest that ArcB catalyzes both the phosphorylation of ArcA and the dephosphorylation of ArcA-P.

Efforts to isolate ArcA-P for direct assays of its dephosphorylation were unsuccessful due to its tendency to aggregate during the purification process. Since a previous study showed that the response regulator NtrC, possessing only its N-terminal receiver domain, retained the activity as a substrate of the cognate sensor kinase (32), we used a similar approach to bypass our problem. His$_6$-ArcA$^{1-136}$ (hereafter referred to as ArcA′), containing the receiver domain but lacking the HTH (DNA binding) domain, was prepared. The purified ArcA′ was indeed found to be an effective substrate for phosphorylation when incubated with H1-D1-H2 in the presence of [γ-^{32}P]ATP. The product, ArcA′-P, was separated from H1-D1-H2, ATP, and P_i and used as substrate for testing the phosphatase activity of ArcB or its subdomains. We used H1-D1-H2 and its modular derivatives to test the ArcA-P dephosphorylating activities, since in a previous study on signal transmission, these polypeptides were found to be catalytically active (24).

Spontaneous and Catalytic Dephosphorylation of ArcA′-P— ArcA′-P was first tested for the intrinsic stability of its phos-

pho-aspartyl bond. When incubated in buffer A (pH 7.5) at 25 °C, the half-life of the phospho-protein was found to exceed 1 h (Fig. 3*A*). Incubation of ArcA′-P with purified H1-D1-H2 resulted in a rapid release of P_i with concomitant loss of radiolabel from ArcA′-P. During the course of this reaction, there was a slight transient rise of labeled H1-D1-H2 followed by a gradual decay, suggesting that the phosphoryl group was transferred from ArcA′-P to H1-D1-H2 and subsequently to H_2O (Fig. 3*B*).

Attempts were then made to locate the dephosphorylating activity in H1-D1-H2 by the use of its various modular derivatives. The possibility of the phosphatase activity residing in H1 (His$_6$-ArcB$^{78-521}$) was first tested by incubating this domain with ArcA′-P. No dephosphorylating activity was detected (Fig. 3*E*). H1-D1 (His$_6$-ArcB$^{78-661}$) also had no effect on the stability of ArcA′-P (Fig. 3*D*). Moreover, neither H1 nor H1-D1 acquired the phosphoryl group from ArcA′-P at a detectable level. By contrast, D1-H2 (His$_6$-ArcB$^{521-778}$) catalyzed the hydrolysis of ArcA′-P at a rate similar to that by H1-D1-H2 and became transiently labeled itself (Fig. 3*C*). When D1 (His$_6$-ArcB$^{521-661}$) or H2 (His$_6$-ArcB$^{638-778}$) was tested separately, no ArcA-P dephosphorylating activity was observed (Fig. 3, *F* and *G*). A mixture of the two proteins, however, did (Fig. 4*A*). The rate was equivalent to that catalyzed by the covalently linked D1-H2 (compare with Fig. 3*C*). Thus D1 and H2 each provides an essential catalytic function for the ArcA′-P dephosphorylation process. Because of the similarity in molecular weights of D1, H2, and ArcA′, this experiment could not reveal whether or not D1 or H2 acquired the phosphoryl group from ArcA′-P.

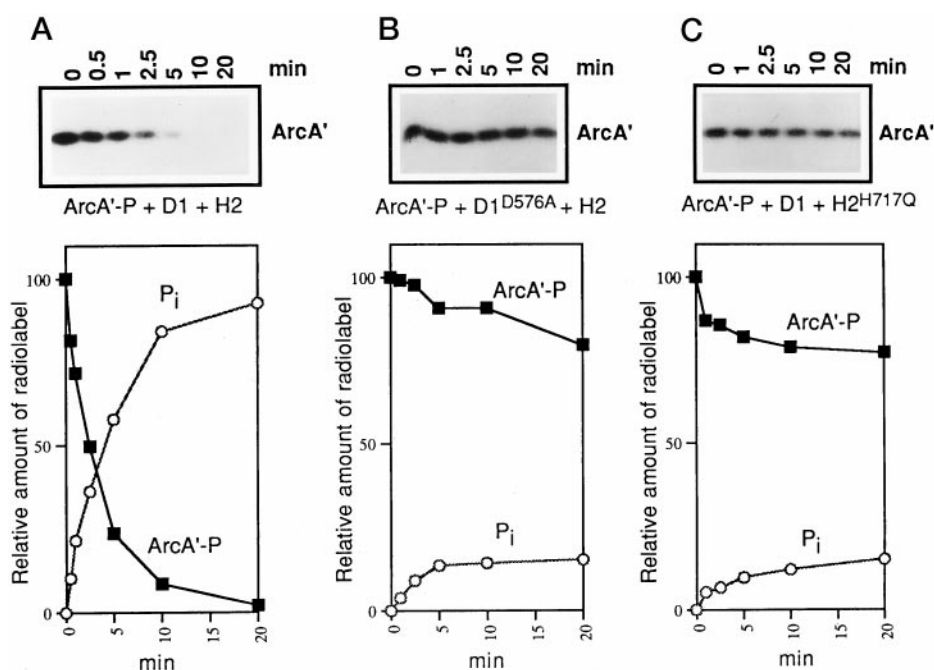*Dephosphorylation of ArcA′-P by a Reverse Phosphore-*

FIG. 4. **The roles of Asp-576 and His-717 of ArcB in ArcA′-P dephosphorylation.** Purified ArcA′-P (50 pmol) was incubated with D1 and H2 or the mutant peptides in a 35-μl reaction mixture. At the indicated time points, a 5-μl sample was withdrawn for SDS-PAGE and TLC analysis and subsequent quantitation by the PhosphorImager (see "Materials and Methods"). *A*, ArcA′-P incubated with D1 and H2; *B*, ArcA′-P incubated with D1^{D576A} and H2; *C*, ArcA′-P incubated with D1 and H2^{H717Q}. *Top,* autoradiogram of the protein band in the gel. *Bottom,* the rate of ArcA′-P dephosphorylation graphically presented as relative amount of labeled protein or P_i *versus* time. *Filled squares*, ArcA′-P; *open circles*, P_i.

lay—To probe whether both His-717 of H2 and Asp-576 of D1 are critical in the concerted process of ArcA′-P dephosphorylation, a mixture of D1^{D576A} and H2 or a mixture of D1 and H2^{H717Q} was tested. Neither exhibited any significant phosphatase activity (Fig. 4, *B* and *C*). To answer the question of whether the order of phospho-transfer was from ArcA′-P to H2 and then to D1, mutant D1-H2 proteins were used, since they could be separated from ArcA′-P. When ArcA′-P was incubated with D1^{D576A}-H2, the phosphoryl group was transferred to the mutant protein, and the radiolabel lost from ArcA′-P was accounted for by the radiolabel gained by D1^{D576A}-H2. By contrast, when ArcA′-P was incubated with D1-H2^{H717Q}, the mutant protein did not receive any phosphoryl group. As expected, neither mutant protein catalyzed the release of P_i (Fig. 5). It thus appears that ArcA′-P dephosphorylation proceeds in the reverse direction of the His → Asp → His → Asp phosphorelay (24), except that H1 is dispensable.

Possible Effectors in the Signal Decay Pathway—It has been reported that ATP or its nonhydrolyzable analogs accelerate the dephosphorylation of the response regulators by their orthodox cognate sensors, namely NtrB (32, 33), EnvZ (34, 35), and DegS (36). We therefore tested the effects of ATP, ADP, AMP-PMP, ATPγS, and GTP on the dephosphorylation of ArcA′-P by H1-D1-H2. No significant effect was observed (data not shown). In a previous study on ArcB$^{128-778}$, physiological concentrations (mM range) of D-lactate, acetate, or pyruvate were found to accelerate the rate of autophosphorylation, heighten the peak phosphorylation level, and slightly retard the rate of P_i loss from the kinase protein. Because the intracellular levels of these compounds were known to be elevated during anoxia, it was suggested that they serve as input signals by retarding the autophosphatase activity of ArcB (37). When we tested the influence of those metabolites on the ArcA′-P dephosphorylating activity of H1-D1-H2 and D1-H2, none of them showed any significant effect on either the rate of ArcA′-P decay or P_i release (data not shown). Preliminary experiments

indicated that these effectors act on the kinase phosphorelay pathway.[2]

DISCUSSION

Several modes for signal decay seem to have evolved for the two-component systems. The rate of dephosphorylation of a response regulator appears to be controlled by the inherent lability of the mixed anhydride phospho-aspartyl bond and/or a phosphatase-like activity embodied in the cognate sensor kinase or another protein. The rate of spontaneous hydrolysis may be intrinsically set by the protein structure, since the half-life of the phospho-response regulators vary from seconds to hours (Ref. 38 and references therein).

Examples of catalyzed dephosphorylation of phospho-response regulators include those that are sensor kinase-dependent, such as the EnvZ/OmpR (35, 39), NarX/NarL (40, 41), and CpxA/R (42) systems of *E. coli*; the DegS/U system of *B. subtilis* (36, 43); the VanS/R system of *Enterococcus faecium* (44), and the FixL/J system of *Rhizobium meliloti* (45). Mutational analyses have shown that the conserved histidine of the sensor kinases EnvZ (46, 47), CpxA (42), and VanS (38) is not essential for the dephosphorylating activity.

Another class of phospho-response regulator dephosphorylations involve a third protein. Examples include the Ntr and Che systems of *E. coli* and the Kin/Spo phosphorelay system of *B. subtilis*. The dephosphorylation of NtrC-P by NtrB is controlled by the protein P_{II} (33, 48), whereas the dephosphorylation of CheY-P is accelerated by CheZ (49–51). In the Kin/Spo system (52), the dephosphorylation of SpoOA-P (the terminal receiver domain) is catalyzed by SpoOE (53), and the dephosphorylation of SpoOF-P (the intermediate receiver domain) is catalyzed by a family of Rap (response regulator aspartate phosphatases) proteins (54–56). From the mechanistic point of view, the dephosphorylation by sensor kinases or other proteins may in-

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[2] D. Georgellis, unpublished data.

A

ArcA'-P + D1^{D576A}-H2

B

ArcA'-P + D1-H2^{H717Q}

FIG. 5. **Effects of D1^{D576A}-H2 and D1-H2^{H717Q} on the ArcA'-P dephosphorylation.** Purified ArcA'-P (50 pmol) was incubated with D1^{D576A}-H2 or D1-H2^{H717Q} (5 pmol) in a 35-μl reaction mixture. At the indicated time points, a 5-μl sample was withdrawn for SDS-PAGE and TLC analysis and subsequent quantitation by the PhosphorImager (see "Materials and Methods"). *A*, ArcA'-P incubated with D1^{D576A}-H2; *B*, ArcA'-P incubated with D1-H2^{H717Q}. *Top panel* shows the autoradiogram of the protein bands. *Bottom panel* shows the rate of ArcA'-P dephosphorylation graphically presented as relative amount of labeled protein *versus* time. *Filled squares*, ArcA'-P; *open diamonds*, D1^{D576A}-H2-P or D1-H2^{H717Q}-P.
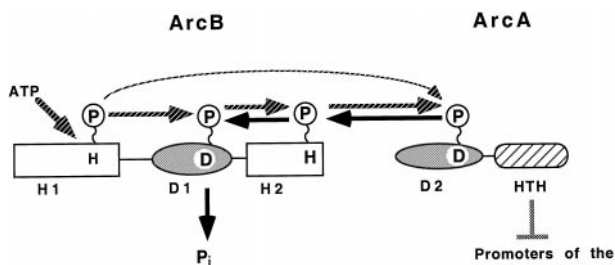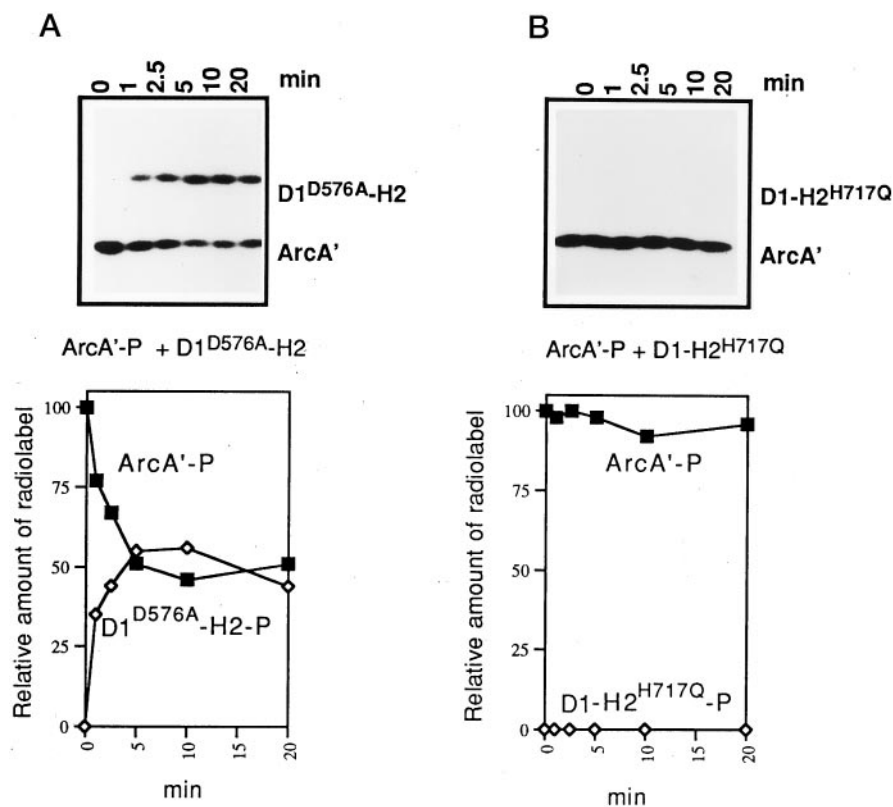

FIG. 6. **A model for signal transduction by the Arc system with special focus on the reactions leading to the phosphorylation and dephosphorylation of the response regulator, ArcA.** *Arrows* indicate the directions of phosphoryl group transfer with the thickness of the *arrows* indicating the relative activities of these reactions observed under *in vitro* conditions.

volve either their direct participation in the hydrolytic reaction or their allosteric binding to the cognate phospho-response regulator, resulting in destabilization of the phosphoryl bond (50, 57). Future studies will be required to resolve this issue.

Yet another mode of signal decay emerged from our *in vitro* study on ArcA-P dephosphorylation by the ArcB tripartite kinase: P$_i$ release through a reverse phosphorelay (Fig. 6). The relatively long half-life of ArcA-P (>1 h) seems to render its spontaneous hydrolysis as an insignificant factor in signal decay. Instead, before the phosphoryl group is hydrolyzed, it needs to be transferred from ArcA-P to H2 at the conserved His-717 and subsequently to the conserved Asp-576 of D1. This conclusion is supported by *in vivo* studies involving plasmid-borne wild-type or mutant ArcB domains.[3] However, the possibility of additional dephosphorylation pathways catalyzed by unknown proteins cannot be excluded.

It is noteworthy that H1, the classical transmitter domain, plays no apparent role in the dephosphorylation process of ArcA-P. Despite the previous observation that H1 transphos-

phorylated ArcA at a relatively low rate (24), it is unable to accept a detectable amount of the phosphoryl group from ArcA-P, possibly because of an unfavorable equilibrium constant. It was originally suggested that the primary transmitter of ArcB serves as the direct phosphoryl group donor for ArcA (12) and that the secondary transmitter is involved in cross-talk with a hypothetical response regulator(s) (13, 15). However, subsequent studies showed that *in vitro* H2 is more effective than H1 in transphosphorylating ArcA (24) and that *in vivo* both may serve as phosphoryl group donors, depending on the regulatory inputs (58). A recent study of BvgA/S and EvgA/S demonstrated that the specificity of the phosphorelay is mediated by the secondary, rather than the primary, transmitter domain (28). Recently, a *sixA* (signal inhibitory factor-X) gene was discovered to encode a phosphatase that is active on the His-717-P of ArcB$^{H292L, D576Q}$ but not on the His-273-P of an N-terminally truncated version of EnvZ (59). A specific physiological role of SixA on the Arc signal transduction system, however, remains to be shown.

In the BvgS/A system, the phosphorylated receiver domain of BvgS has been shown to reversibly transphosphorylate the secondary transmitter or to undergo spontaneous hydrolysis (60). Although not demonstrated, the dephosphorylation of BvgA-P is likely to occur through a reverse phosphorelay (Asp → His → Asp), as in the case of ArcA-P. Indeed, such a multi-step dephosphorylation pathway may apply to all two-component systems comprising a tripartite sensor kinase.

An advantage of a multi-step phosphorelay network may be the availability of different rate control points for signaling, as illustrated by the Kin/Spo system (52–56). In the Arc system, it has been suggested that the ArcB autophosphatase activity is regulated at the receiver domain by several fermentative metabolites, such as D-lactate (37). The present study with modular ArcB units, however, failed to confirm any inhibiting effect of D-lactate on the various steps of ArcA-P dephosphorylation. In any event, the receiver domain seems to play a pivotal role in directing the phosphoryl group reversibly to His-717 or ir-

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[3] D. Georgellis and O. Kwon, unpublished data.

reversibly to H_2O. A challenge therefore remains to pinpoint the action of the effectors and to determine whether there are additional elements that influence signal transmission and signal decay.

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