Central Role of the BvgS Receiver as a Phosphorylated Intermediate in a Complex Two-component Phosphorelay*

M. Andrew Uhl‡ and Jeff F. Miller§¶

From the Department of Microbiology and Immunology, School of Medicine, and §Molecular Biology Institute, UCLA, Los Angeles, California 90024

Two-component systems use phosphorylation reactions to regulate stimulus/response pathways. In Bordetella pertussis, a human respiratory pathogen, the infectious cycle of the organism is controlled by the BvgAS two-component system. BvgS has similarities to sensor and response regulator components and is an autophosphorylating kinase that phosphorylates BvgA. BvgA, a response regulator, is a DNA-binding protein that activates virulence gene transcription. Three phosphorylated BvgS domains, the transmitter, receiver, and C terminus, are essential for signal transduction. We now demonstrate that the BvgS transmitter is sufficient for autophosphorylation but is unable to phosphorylate the C terminus or BvgA. The BvgS receiver regulates several phenotypes: dephosphorylation of both the BvgS transmitter and C terminus as well as transfer of a phosphoryl group from the transmitter to the C terminus. Our results indicate that BvgAS signal transduction initiates with autophosphorylation of the transmitter followed by phosphoryltransfer to the receiver. The phosphorylated receiver can donate to the C terminus or to water. The phosphorylated C terminus is then able to transfer the phosphoryl group to BvgA.

Virulence gene activation in Bordetella pertussis is mediated by the BvgAS two-component system (1–5). Two-component systems use phosphorylation cascades to effect adaptive responses and typically contain sensor and response regulator components (6). The sensor protein, which autophosphorylates at a conserved transmitter domain, is the initial site of signal processing. Phosphotransfer from the sensor protein is catalyzed by a conserved receiver domain in the response regulator. Responses such as transcriptional activation and protein-protein interaction are then mediated by the phosphorylated response regulator.

BvgS autophosphorylates with the γ-phosphoryl group of ATP and can phosphorylate BvgA (7). Phosphorylated BvgA has an increased affinity for bvg-activated promoters and is competent in an in vitro transcription assay (8–10). Although these properties of BvgS and BvgA fit well with the paradigm of two-component systems, BvgS employs a mechanism of BvgA phosphorylation that differs from the typical transmitter to receiver phosphoryltransfer reaction. As shown in Fig. 1, BvgS contains three domains that participate in the phosphorylation cascade: the transmitter, receiver, and C terminus (7, 11). The C terminus is specifically responsible for BvgA phosphorylation (11). Mutational studies of BvgS indicated that His-729 of the transmitter is required for autophosphorylation and that Asp-1023 of the receiver is essential for phosphorylation of the C terminus. Hist-1172 of the C terminus is necessary for BvgA phosphorylation. These amino acids are all required in vivo for expression of bvg-activated genes (7, 11, 12).

Although Asp-1023 of the BvgS receiver is essential for the phosphorylation cascade and virulence gene activation, the exact role of the receiver had not been determined (11). The BvgS receiver could act as an autoinhibitory domain, with phosphorylation relieving inhibition of phosphotransfer from the BvgS transmitter to the C terminus. Mutation of Asp-1023, the proposed site of phosphorylation in the receiver, would therefore lock the receiver in an inhibitory state. Alternatively, the BvgS receiver could have a positive role in the phosphorelay, serving as a phosphorylated intermediate between the transmitter and C terminus. In this report, we demonstrate that the BvgS receiver exerts both positive and negative influences on the BvgAS phosphorelay. The BvgS receiver can directly transfer a phosphoryl group to the C terminus, but the receiver also mediates dephosphorylation of BvgS by removing phosphoryl groups from both the transmitter and C terminus.

EXPERIMENTAL PROCEDURES

Strains and Plasmids—Escherichia coli strain DH5α (13) was utilized for protein overexpression. For quantitation of bvg activity, E. coli strain JFMC3 (MC4100 recA AfaB-lacZYA) was employed (14). Cultures were grown in Luria-Bertani medium (13); when necessary, medium was supplemented with antibiotics at concentrations of 100 μg/ml for ampicillin. β-galactosidase activities were determined with cells permeabilized with SDS/CHCl3, as described (13).

Except for where noted below, construction of all plasmids has been detailed elsewhere (11). pMU714, which contains bvgS with a precise deletion of the regions encoding the receiver and C terminus (BvgSΔRC), was constructed by deleting a 700-base pair BsrGI fragment from pMU677. pMU677 was created by inserting the oligomers DeRC-1 (5′-CTGTACATCTGAC-3′) and DeRC-2 (5′-GTCAGATGTAícGt-3′) into the unique SnaI site of pMU228. pMU228 is identical to pJM26 (14) except that it contains a BsrGI site at the junction of the BvgS transmitter and receiver. Introduction of the mutations that created this site did not affect BvgS function in vivo (data not shown). The vector for overexpression of BvgSARc, pMU540, was created by inserting an NcoI fragment from pMU714 encoding a portion of the BvgS transmitter into pMU100 (11).

The maltose-binding protein-BvgS receiver (MBP-R)3 plasmid, pMU542, was created by taking an NcoI (blunted by treatment with the Klenow fragment of DNA polymerase) and HindIII fragment from pHB50dEic (11) and inserting it into pMalc (New England Biolabs) that

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‡ Supported by National Institutes of Health Predoctoral Training Grant AI07323. Present address: Dept. of Microbiology and Immunology, University of California, San Francisco, 513 Parmassus Ave., San Francisco, CA 94143-0414.

¶ To whom correspondence should be addressed: Dept. of Microbiology and Immunology, UCLA School of Medicine, 10833 Le Conte Ave., Los Angeles, CA 90095-1747. Tel.: 310-206-7926; Fax: 310-206-3865.

1 The abbreviations used are: MBP-R, maltose-binding protein-BvgS receiver; GST, glutathione S-transferase; ATPγS, adenosine 5′-O-(thiotriphosphate).
Role of the BvgS Receiver in the BvgAS Phosphorelay

had been cut with HindIII and EcoRI (blunted with the Klenow fragment of DNA polymerase).

Protein Purification—All protein purification methods were as previously stated for BvgS (the cytoplasmic portion of BvgS; see Fig. 1) and glutathione S-transferase (GST) fusions to BvgS (11). GST-BvgS, GST-BvgSΔRC, GST-BvgSΔC, and MBP-R were purified from insoluble inclusion bodies, dissolved in 6 M guanidine HCl, 50 mM NaHPO4 (pH 7.2), and renatured in 20 mM HEPES (pH 7.4), 50 mM KCl, 10 mM MgCl2, 5 mM dithiothreitol, 50% glycerol. GST-R (the BvgS receiver) and GST-C (the BvgS C terminus) were purified by glutathione-Sepharose affinity chromatography.

For purification of phosphorylated proteins from ATP, phosphorylation reactions were performed as below except that GST-BvgS derivatives were present at 1–3 μM and ATP was at 60 μM. The reaction was stopped after 10 min by the addition of 50 mM EDTA, and 50–100 μl of glutathione-agarose was added. After binding for 10 min on ice, the pellet was washed six times with 5 volumes of phosphate-buffered saline (10 mM buffered phosphate (pH 7.3), 120 mM NaCl, 2.7 mM KCl). Proteins were eluted with 50 mM Tris-HCl (pH 8.0), 10 mM glutathione for 10 min on ice. The yield following purification was typically 25%.

The MBP-R fusion was purified from BvgS and ATP by amylose-agarose affinity chromatography. The purification scheme was identical to glutathione-agarose purification methods except that the washr buffer was 20 mM Tris, pH 7.4, 200 mM KCl, 1 mM EDTA, and the elution buffer was 20 mM Tris, pH 7.4, 100 mM KCl, 1 mM EDTA, and 10 mM maltose. Purification yields of the MBP-R fusion were 10%.

In Vitro Phosphorylation Assays—Phosphorylation assays were essentially as described before (11). Except where otherwise indicated in the figure legends, BvgA and BvgS wild-type or mutant derivatives (GST-BvgS, GST-BvgSΔRC, and GST-BvgSΔC) were present at 0.3 μM. GST-R and GST-C were present at 1 μM. The reaction buffer contained 50 mM HEPES (pH 7.2), 50 mM KCl, 10 mM MgCl2, and 30 μM ATP (specific activity, 0.30 Ci/mmol). Reactions were terminated by the addition of 4 x sample buffer (0.32 mM Tris (pH 6.8), 40% glycerol, 0.1 mM EDTA (pH 8.0), 8% SDS, 0.4 mM dithiothreitol) and heated to 55 °C for 5 min. Samples were separated by SDS-polyacrylamide gel electrophoresis and transferred to an Immobilon membrane (Millipore Corp.). In the case of samples that were analyzed by thin layer chromatography, reactions were terminated by the addition of 50 mM EDTA.

Thin Layer Chromatography—As described by Hess et al. (15), thin layer chromatography was utilized for resolving phosphorylated proteins from ATP and inorganic phosphate. Reactions were normalized to standards by cpm and then spotted onto a polyethyleneimine-cellulose plate (Brinkmann) and resolved with 0.8 M LiCl and 0.8 M acetic acid. The polyethyleneimine-cellulose plate was dried and wrapped in polyvinyl chloride before autoradiography.

RESULTS

Receiver-mediated Dephosphorylation of BvgS—Multiple BvgS domains are involved in the BvgAS phosphorylation cascade (Fig. 1; Refs. 7 and 11). While the phenotypes of mutations indicated a requirement for the BvgS receiver in vivo, the contribution of this domain to the phosphorelay was unknown (7, 11, 12). We speculated that the receiver might mediate dephosphorylation of BvgS, since the receiver had been shown to dephosphorylate the isolated C terminus (11). We tested the role of the receiver in dephosphorylation by using BvgS and BvgS with an asparagin substituted for the conserved aspartic acid in the receiver, Asp-1023 (‘BvgS D1023N). The proteins were phosphorylated and purified from ATP, and the stability of the phosphoryl group was measured by incubation in buffer containing Mg2+ (see “Experimental Procedures”) with other additions noted below. Samples were taken at various time points in the figure and analyzed by SDSPolyacrylamide gel electrophoresis, transferred to a membrane, and autoradiographed. The graph represents densitometry tracing of the autoradiograms. Open circles, BvgS; filled circles, BvgS + 3 mM unlabeled ATP; open squares, BvgS D1023N; filled squares, BvgS D1023N + 3 mM unlabeled ATP. This figure represents the results of a single experiment, which was repeated with similar results. B and C, thin layer chromatography analysis of purified phosphorylated GST-BvgS and GST-BvgS D1023N. B, GST-BvgS was phosphorylated and purified from ATP as in Fig. 2. GST-BvgS (1 μM) was incubated with buffer, and samples were taken at various time points. Reactions were stopped with EDTA, spotted on a polyethyleneimine-cellulose plate, and resolved with 0.8 M LiCl, 0.8 M acetic acid (15). Labeled inorganic phosphate (Pi) was included as a standard. Phospho-BvgS remained at the origin, and inorganic phosphate migrated with an Rf value of approximately 0.8. C is identical to B except that the reactions were performed with GST-BvgS D1023N.

Decay of BvgS in the Presence of ATP—When phosphorylated BvgS and BvgS D1023N were incubated with excess ATP in pulse-chase reactions, the phosphorylated proteins had similar rates of decay (7). However, in the absence of excess ATP,
'BvgS D1023N have vastly different decay rates (Fig. 2). These results suggested that the presence of excess ATP may influence the rate of dephosphorylation of 'BvgS. We purified phosphorylated 'BvgS and 'BvgS D1023N, added excess ATP, and measured phosphorylation as a function of time following ATP addition. The decay rate of the phosphoryl group on 'BvgS was not measurably affected (Fig. 2, closed circles). However, 'BvgS D1023N dephosphorylated at a significantly increased rate (~7-fold) in the presence of excess ATP, with a 

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t_{1/2} \approx 2.5 \text{ min} \text{ (Fig. 2, closed squares).} 
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Nearly identical results were seen upon the addition of ADP or ATPγS to 'BvgS D1023N (data not shown).

If binding of ATP or ADP to 'BvgS D1023N stimulates a phosphatase activity of the transmitter, then generation of inorganic phosphate should accompany dephosphorylation. Alternatively, dephosphorylation of 'BvgS could be due to donation of the phosphoryl group to ADP, forming ATP. Reversibility of transmitter phosphorylation has been demonstrated for the NRII (NtrB) sensor protein (16). As shown in Fig. 3, dephosphorylation of 'BvgS D1023N in the presence of unlabeled ATP, ATPγS, or ADP is coincident with formation of labeled ATP. Negligible amounts of inorganic phosphate were detected. Formation of labeled ATP after incubation of phospho-'BvgS D1023N with ATP and ATPγS is most likely due to the presence of ADP, which is either generated by 'BvgS-mediated hydrolysis of ATP or is present as a contaminant in the ATP or ATPγS preparation. We conclude that hydrolysis of the γ-phosphoryl group of ATP by 'BvgS to form phospho-'BvgS and ADP can be reversed but is detectable only in the absence of the receiver function.

**Dephosphorylation of Individual Domains by the BvgS Receiver.—**The BvgS transmitter and C terminus are both potential targets for dephosphorylation by the receiver. To examine the interaction of the receiver with these domains in more detail, we determined the rates of receiver-mediated dephosphorylation of the isolated transmitter and C terminus. The transmitter and C terminus, purified as fusions to glutathione S-transferase, were relatively stable in the absence of the receiver (t1/2 of 10 and 15 min, respectively; Fig. 4). Both proteins were rapidly dephosphorylated in the presence of a 10-fold molar excess of the BvgS receiver. However, the C terminus was dephosphorylated at faster rate (t1/2 = 6 s) than the transmitter (t1/2 = 30 s). As measured by affinity chromatography and thin layer chromatography, dephosphorylation of the transmitter and C terminus by the receiver is due to transfer of the phosphoryl group to the receiver. The receiver phosphoryl group then undergoes autohydrolysis, generating inorganic phosphate (data not shown).

**Fig. 2. Mechanism of GST-BvgS D1023N dephosphorylation.** Purified phosphorylated GST-BvgS D1023N was incubated with 1 mM ATP, 1 mM ADP, or 1 mM ATPγS for 7 or 20 min. The reaction was stopped with EDTA, spotted on a polyethyleneimine-cellulose plate, and resolved as detailed under "Experimental Procedures." Labeled ATP (Rv of 0.24) and inorganic phosphate (P1, Rv of 0.8) were included as standards.

**Fig. 3.** Rates of dephosphorylation of the transmitter and C terminus by the receiver. Reactions were performed and analyzed essentially as in Fig. 2. The BvgS transmitter (GST-T, 3 μM) or the BvgS C terminus (GST-C, 3 μM) was phosphorylated and purified from ATP with the exception that 'BvgS (1 μM) was utilized to phosphorylate GST-C. The purified proteins (0.3 μM) were incubated in buffer either with or without the addition of 3 μM BvgS receiver (GST-R). The graph is a densitometry analysis of an autoradiogram of samples separated by SDS-polyacrylamide gel electrophoresis and transferred to a membrane. Open circles, GST-T; filled circles, GST-T + GST-R; open squares, GST-C; filled squares, GST-C + GST-R. Depicted in this figure are results of a single experiment, which was repeated with similar results.

Terminus—We had previously determined that phosphorylation of the C terminus occurred by a phosphotransfer reaction, but we could not distinguish whether the transmitter or receiver served as a phosphodonor. To determine which domains of BvgS are required or sufficient for particular steps of the phosphorelay, we precisely deleted specific BvgS domains and assayed the resulting mutant proteins. As shown in Fig. 5, 'BvgS was able to autophosphorylate, transphosphorylate the BvgS C terminus (+GST-C), and phosphorylate BvgA (+BvgA). 'BvgS wild type was also slightly dephosphorylated by the addition of the BvgS receiver in trans (+GST-R), and faint labeling of the receiver was detected. 'BvgS with deletion of the receiver and C terminus (ΔRC) retained autophosphorylation ability (Fig. 5) and was dephosphorylated by the BvgS receiver (+GST-R). However, 'BvgSΔRC was deficient for BvgA phosphorylation and transphosphorylation of the C terminus (Fig. 5, +BvgA and +GST-C). From these results, we conclude that the transmitter is not sufficient for phosphorylation of the BvgS C terminus. The inability of the transmitter to phosphorylate BvgA indicates that the only relevant BvgS domain identified thus far for BvgA phosphorylation is the C terminus (11).

An exact deletion of the BvgS C-terminal domain ('BvgSΔC) retained autophosphorylation ability (Fig. 5). While 'BvgSΔC was able to transphosphorylate the C terminus (+GST-C), it was unable to detectably phosphorylate BvgA (+BvgA). The in vitro phenotypes of 'BvgSΔRC and 'BvgSΔC indicate that the transmitter is sufficient for autophosphorylation, while the receiver is required for transphosphorylation of the C terminus. It is interesting to note that 'BvgSΔC and 'BvgS were not efficiently dephosphorylated by the addition of the receiver in trans, whereas 'BvgSΔRC was (Fig. 5, +GST-R lanes). The presence of a receiver in cis appears to decrease the efficiency of receiver-mediated dephosphorylation in trans, and we hypothesize that this is due to either stearic effects or a more efficient interaction of the receiver with the transmitter and C terminus in cis. Coincident with their inability to phosphorylate BvgA, BvgSΔRC and BvgSΔC were unable to activate an fhaB::lacZYA fusion in vivo, while wild type BvgS could direct high levels of expression of this fusion (Table I).

The results of Fig. 5 suggested that the BvgS receiver di-
Fig. 5. Effects of deletions of the BvgS receiver and C terminus in vitro. BvgS wild type (GST- BvgS; 98 kDa), BvgS with a deletion of the receiver and C terminus (GST-BvgSaRC; 74 kDa), or BvgS with a deletion of the C terminus (GST-BvgSaRC; 86 kDa) were assayed for their ability to autophosphorylate, to transfer to the BvgS C terminus (+GST-C) or BvgA (+BvgA), or to be dephosphorylated by the BvgS receptor (+GST-R). 0.3 μM of GST-BvgS or mutant derivatives was incubated in phosphorylation reactions with 30 μM ATP (specific activity, 0.3 Ci/mmol). When added, GST-C (34 kDa) and GST-R (34 kDa) were present at 1 μM, and BvgA (29 kDa) was present at 0.5 μM. EDTA and SDS were added to terminate the reactions, which were then separated by SDS-polyacrylamide gel electrophoresis, transferred to a membrane, and autoradiographed overnight.

Table I

In vivo activities of bvgS and mutant derivatives

| Plasmid name | bvgS allele | β-Galactosidase activity |
|--------------|-------------|-------------------------|
| pDM29        | Wild type   | 12,000 ± 1,000          |
| pMU714       | ΔRC         | 9.3 ± 0.3               |
| pHB50delC    | ΔC          | 10.1 ± 0.3              |
| pBR322       | Vector      | 10.9 ± 0.7              |

directly mediated the phosphotransfer reaction to the C terminus. This was seemingly contradicted by the fact that we had not been able to detect significant phosphorylation of the BvgS receiver in vitro (7). During experiments in which we were attempting to reconstruct the phosphorylation cascade, we noticed that we could readily purify phosphorylated receiver by affinity chromatography. When the phosphorylated receiver was subjected to SDS-polyacrylamide gel electrophoresis immediately following purification (Fig. 6, lane 1), phosphorylation of the receiver could no longer be detected. This same phenomenon was also observed if the phosphorylated receiver was incubated in buffer containing MgCl2 prior to electrophoresis (lane 2). Despite the apparent lability of the phosphorylated receiver to SDS-polyacrylamide gel electrophoresis, we reasoned that transfer of the phosphoryl group from the receiver to the C terminus should be readily detectable, since the phosphorylated C terminus is stable to SDS-polyacrylamide gel electrophoresis (11). When the C terminus was incubated with the purified phosphorylated BvgS receiver, the C terminus was now phosphorylated (lane 3), demonstrating that the receiver can serve as a phosphodonor for the C terminus. In contrast, when the BvgS transmitter was incubated with the phosphorylated receiver, no transfer to the transmitter was detected (BvgSaRC, lane 4), suggesting that the phosphotransfer reaction between the transmitter and the receiver is not readily reversible.

The instability of the phosphorylated BvgS receiver to SDS-polyacrylamide gel electrophoresis distinguishes it from the phosphorylated receiver, no transfer to the transmitter was detected (BvgSaRC, lane 4), suggesting that the phosphotransfer reaction between the transmitter and the receiver is not readily reversible.

Fig. 6. Transfer of the phosphoryl group from the BvgS receiver to the BvgS C terminus. In this experiment, the BvgS receiver was purified as a fusion to maltose-binding protein (MBP-R) to clearly distinguish it from the BvgS C terminus (GST-C) by SDS-polyacrylamide gel electrophoresis. MBP-R (1 μM) was phosphorylated by 0.5 μM BvgS and 60 μM ATP (specific activity, 0.3 Ci/mmol) and then purified by amylose agarose affinity chromatography (see “Experimental Procedures”). Lane 1, 0.2 μM MBP-R added to SDS-EDTA immediately following purification; lane 2, 0.2 μM MBP-R incubated in buffer for 1 min; lane 3, 0.2 μM MBP-R with the addition of 2 μM GST-C (BvgS C terminus); lane 4, 0.2 μM MBP-R with the addition of 2 μM GST- BvgSaRC (BvgS transmitter). Products were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. The apparent lack of phosphorylation of MBP-R is due to an extreme lability of the phosphoryl group on the BvgS receiver to the alkaline pH values (pH of 8.5 and above) obtained during SDS-polyacrylamide gel electrophoresis.

BvgA receiver and many other receivers. Because the BvgS receiver dephosphorylates in the presence of SDS and urea and in the absence of added Mg2+ (data not shown), it is unlikely that extensive secondary structure is required for this phenotype. We suspected that an amino acid adjacent to the proposed site of phosphorylation in the BvgS receiver may be partially responsible for dephosphorylation during SDS-polyacrylamide gel electrophoresis. Interestingly, in a comparison of amino acid similarity among 79 receiver domains (17), a negatively charged amino acid is never found adjacent to the aspartic acid corresponding to Asp-1023, and BvgS is the only receiver that contains a cysteine adjacent to the conserved aspartic acid. When the receiver is incubated for extended periods in the presence of 0.1% SDS, buffers with pH values of 8.5 or above result in dephosphorylation to background levels, which indicates that electrophoresis per se is not required for dephosphorylation (data not shown). This sensitivity of the phosphorylated BvgS receiver to pH values above 8.5 corresponds with the pH value of the –SH side group of cysteine of 8.3 (18). We propose that the presence of a negatively charged amino acid adjacent to the site of phosphorylation in the BvgS receiver is destabilizing and that the pH value of 9.5 of the Tris/glycine front during SDS-polyacrylamide gel electrophoresis (19) is the reason for the apparent instability of the receiver in vitro. Despite the extreme base lability of the BvgS receiver, we have been able to clearly define two roles for this domain: dephosphorylation of BvgS and phosphotransfer to the C terminus.

DISCUSSION

The BvgAS phosphorylation cascade can be modeled as a stepwise process involving an initial autophosphorylation event followed by three phosphotransfer reactions (Fig. 7). In our model, signal inputs are relayed through the membrane to the transmitter. The transmitter then autophosphorylates at His-729 with the γ-phosphate of ATP to form phospho-BvgS and ADP. This reaction is reversible in vitro, but whether the reversibility is significant in vivo is not known. His-729 donates the phosphoryl group to His-1172 of the BvgS C terminus. The C terminus can then transfer back to the BvgS receiver. The C terminus can also transfer to the BvgA (Fig. 1A) back to the BvgS BvgA then binds to specific DNA repeats upstream of bvg-activated promoters and effects gene regulation (8–10, 20). In all cases, mutations that interrupt the phosphorelay in vitro have a
corresponding defect in virulence gene activation in vivo (7, 11). The BvgS receiver is a pivotal component of the phosphorelay. It appears to act as a biochemical checkpoint, mediating phosphorylation and dephosphorylation of the C terminus as well as phosphorylation of the transmitter. We currently do not know what conditions influence these activities of the receiver or whether auxiliary proteins are involved in regulation of receiver activity such as in chemotaxis in E. coli or sporulation in Bacillus subtilis (21–23).

A new type of two-component regulatory system that utilizes a His → Asp → His → Asp phosphorelay is emerging, as first described in the regulation of sporulation initiation in B. subtilis and recently described for osmoregulation in yeast (24, 25). This subclass contains a receiver that acts as a phosphorylated intermediate (the BvgS receiver) as well as a histidine phosphotransfer domain (the BvgS C terminus), which acts as an intermediate between two receiver domains. These features distinguish this class from traditional two-component systems. Some of these phosphorelay components can be linked in a single protein, as in BvgS, or unlinked as in the B. subtilis sporulation cascade (24). Another putative member of this subclass includes the ArcB/ArcA proteins, which repress genes involved in aerobic metabolism (26–29). There are several other defined sensor proteins besides ArcB and BvgS that have a transmitter/receiver/C terminus architecture; these are expected to follow the same multidomain phosphorylation strategy (see Refs. 28 and 30 for listings). No specific biological theme common to this subclass of two-component systems has yet been described.

We have been characterizing the biochemistry of BvgAS signal transduction to gain a better understanding of the biological interactions of Bordetella with its animal hosts. bvg is the key regulatory locus controlling a transition between two distinct phases of Bordetella. Several operons are either activated or repressed by BvgAS in B. pertussis and Bordetella bronchiseptica (2, 31). In B. bronchiseptica, bvg-activated genes are necessary for colonization of the respiratory tract, while at least one class of bvg-repressed genes interfere with infection (32, 33). Bvg-repressed genes in B. bronchiseptica include the motility operon and genes required for production of a siderophore (34, 35). It has been shown that the Bvg phase is advantageous for survival under nutrient-limiting conditions (33). The involvement of the BvgAS signal transduction system in several aspects of the Bordetella life cycle suggests that BvgAS function is highly pleotropic and coupled to other regulatory pathways. The multiple domains required for BvgAS signal transduction may allow for coordination of several intracellular and extracellular inputs into a central phosphorelay.

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