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Cooperation of two distinct coupling proteins creates chemosensory network connections

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Although it is appreciated that bacterial chemotaxis systems rely on coupling, also called scaffold, proteins to both connect input receptors with output kinases and build interkinase connections that allow signal amplification, it is not yet clear why many systems use more than one coupling protein. We examined the distinct functions for multiple coupling proteins in the bacterial chemotaxis system of *Helicobacter pylori*, which requires two nonredundant coupling proteins for chemotaxis: CheW and CheV1, a hybrid of a CheW and a phosphorylatable receiver domain. We report that CheV1 and CheW have largely redundant abilities to interact with chemoreceptors and the CheA kinase, and both similarly activated CheA's kinase activity. We discovered, however, that they are not redundant for formation of the higher order chemoreceptor arrays that are known to form via CheA–CheW interactions. In support of this possibility, we found that CheW and CheV1 interact with each other and with CheA independent of the chemoreceptors. Therefore, it seems that some microbes have modified array formation to require CheW and CheV1. Our data suggest that multiple coupling proteins may be used to provide flexibility in the chemoreceptor array formation.

signal transduction | scaffold | chemotaxis | chemoreceptor arrays

Coupling or scaffold proteins provide critical connections between input receptors and output kinases in many types of signal transduction pathways (1–3). These connections confer multiple advantages such as cooperativity, signaling complex assembly, and protein localization (2). Indeed, many cellular signaling systems use multiple coupling proteins to fine tune these advantages, a process that has been well studied in eukaryotic systems (2, 4, 5). Bacterial chemotaxis is an example of a prokaryotic system that relies on coupling proteins of the CheW family to connect chemoreceptors to the CheA kinase and build connections that create multiprotein chemoreceptor arrays. Many bacterial chemotaxis systems possess multiple coupling proteins (6, 7), but the functions and advantages of having more than one coupling protein for these systems are not well understood.

The core bacterial chemotaxis sensory unit is composed of a chemoreceptor, a CheW family coupling protein, and the CheA output kinase (6). The coupling protein allows the chemoreceptors to control the CheA kinase and promotes connections between core units (3, 6–8). Chemotaxis coupling proteins have two basic architectures, CheW or CheV. CheW is a single domain protein with two defined subdomains, and CheV proteins are hybrids that add a C-terminal response regulator-like domain (Rec) to an N-terminal CheW domain (3, 9).

Chemotaxis core sensory units exist in cells as large multiprotein arrays at the poles (8, 10–12). Connections that form these arrays have been elucidated in the single-coupling protein system of *Escherichia coli* and are driven by interactions between CheW and a subdomain of CheA called P5, which is a structural mimic of CheW. These connections are vital for array formation, kinase control, and positive cooperativity that allow small signals to be greatly amplified (11). There are two documented types of CheW–CheA P5 interactions. Interactions at interface 1 occur between CheA P5 subdomain 1 and CheW subdomain 2 and lead to control of CheA kinase activity (3, 8). Interactions at interface 2 occur between CheA P5 subdomain 2 and CheW subdomain 1 and lead to intercomplex connections that build chemoreceptor arrays and positive cooperativity (3, 8). Thus, coupling proteins participate in two types of CheA interactions that are vital for chemotaxis function.

Although these types of CheW–CheA interactions have been elucidated in the single coupling protein *E. coli* system, it is not yet known how and whether these interactions differ in multicoupling protein systems. To date, the best-studied multicoupling protein system is that of *Bacillus subtilis*, a microbe that uses one CheV and one CheW to both perform receptor–kinase coupling in a somewhat functionally redundant manner (13, 14). There is evidence suggesting that some chemoreceptors might operate with particular coupling proteins, displaying greater affinity for one coupling protein than for another (15, 16). CheW and CheV may also have some affinity bias toward different chemoreceptors (17).

The human pathogen *Helicobacter pylori* has a chemotaxis system with four chemoreceptors called Tlps (TlpA, TlpB, TlpC, and TlpD), a CheA kinase, a CheY response regulator, and—relevant to this work—multiple coupling proteins (18, 19). Two of the *H. pylori* coupling proteins, CheW and CheV1, are critical for wild-type chemotaxis, acting in a nonredundant manner. Mutants lacking *cheW* or *cheV1* appear unable to activate the CheA kinase as they swim without changing direction and are either completely (*cheW*) or severely (*cheV1*) compromised in a soft agar chemotaxis assay (9, 20). *H. pylori* also possesses two other CheV-type coupling proteins, but these play only minor roles in chemotaxis (9, 20). Because *H. pylori* CheW and CheV1 were both essential for chemotaxis in a nonredundant manner, we thought it ideal to dissect how these contribute to chemotaxis.

We initiated our work analyzing the protein interaction network of CheW and CheV1 as well as their ability to activate and control CheA's kinase function. We found that they had nearly identical abilities in these regards, which did not explain why both were needed. However, when we examined their roles in assembly of the polar chemosensory array, we found that both

Significance

Signal transduction systems are important pathways that organisms use to sense and respond to their environments. Chemotaxis is controlled by a signal transduction system that allows bacteria to coordinate their movement in response to their environment. This response requires proper assembly and localization of large multiprotein chemotaxis complexes, which are built by interactions between coupling proteins. The significance of having multiple types of coupling proteins in a single signal transduction system is poorly understood. Here, we show that multiple coupling proteins allow bacteria to build superprotein interaction networks and localize them to cell poles, a role that is required for optimal chemotaxis.

Author contributions: S.A., J.C., and K.M.O. designed research; S.A., J.C., and K.S.J. performed research; K.D.C. contributed new reagents/analytic tools; S.A. and K.M.O. analyzed data; and S.A. and K.M.O. wrote the paper.

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were required and fulfilled nonredundant roles in these interactions. Our data demonstrate that some microbes use multiple coupling proteins to build the polar chemoreceptor supercluster and thus suggest this aspect of chemotaxis may be fine-tunable by modulating the levels or activities of these coupling proteins.

Results

CheW and CheV1 Both Form Direct Interactions with CheA and Chemoreceptors. To gain an understanding of why *H. pylori* requires two coupling proteins, we first characterized the protein–protein interaction network of *H. pylori* CheV1 and CheW. We identified directly interacting proteins using the bacterial adenylate cyclase two-hybrid (BACTH) system (21). For this approach, CheV1 and CheW were fused to the N or C terminus of T25 fragments, and CheV1, CheW, CheA, CheV2, CheV3, and the chemoreceptors (TlpA, TlpB, and TlpD) were fused to the bait T18 fragments. We found that both CheV1 and CheW displayed interactions with themselves, the other coupling proteins, CheA, and at least one chemoreceptor (Fig. 1 *A* and *C*). We found that only one fusion orientation—with the T25 fragment at the C-terminal end—was functional (Table S1). We thus focused on the functional fusions and quantified all positive interactions using a β-galactosidase assay (Fig. 1). CheV1 and CheW both displayed typical coupling protein interactions with CheA and chemoreceptors. The interaction with CheA was quite strong, yielding β-galactosidase levels that were almost double that of the positive control (Fig. 1). Both proteins also interacted with the TlpA chemoreceptor, with CheV1 showing significant interactions additionally with TlpB and TlpD (Fig. 1). Based on the X-gal plate, CheW did not appear to interact with TlpB and TlpD, as they both appeared similar to the negative control (white colonies) shown in Fig. 1*C* and Table S1. Both CheV1 and CheW were able to interact with one another and with themselves. Lastly, CheV1 interacted with CheV2 and CheV3.

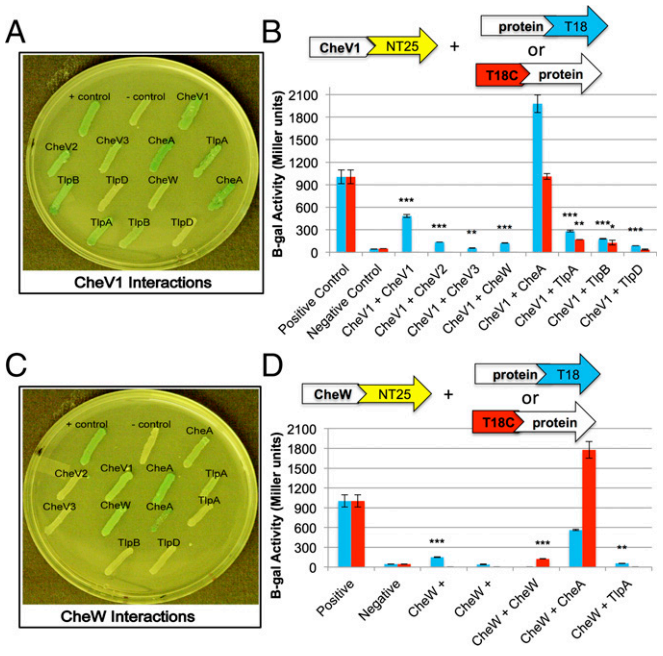

Fig. 1. BACTH analysis of CheV1 and CheW interactions. *H. pylori* (*A*) CheV1 and (*B*) CheW were fused to the *N* or C termini of the T25 fragments and tested for interaction with CheV1, CheV2, CheV3, CheW, CheA, TlpA, TlpB, or TlpD, fused to the *N* or C termini of the T18 fragments in *E. coli cya*$^{-}$ BTH101. Positive (+) control, pKT25-zip and pUT18C-zip; negative (–) control, CheV1 or CheW plasmids cotransformed with empty T18 plasmids. (*A* and *C*) Representative LB X-gal and IPTG plates ($n = 3$). (*B* and *D*) β-galactosidase levels from positive interactions. $n = 3$ and error bars indicate SDs. Strains were compared with a negative control using unpaired *t* test (*$P = 0.0127$, **$P < 0.001$, ***$P < 0.0001$).

The BACTH protein interactions were verified using coimmunoprecipitation with purified proteins. Consistent with the BACTH, both CheW and CheV1 interacted with CheA and *H. pylori*'s cytoplasmic chemoreceptor, TlpD (Fig. 2 *A* and *B*). It is interesting to note that CheW did interact with the TlpD chemoreceptor in the coimmunoprecipitation experiments but not in the BACTH experiments. Based on these data, it seems possible that the BACTH cloning could have affected CheW's structure and thus its ability to interact with TlpD. All *H. pylori* chemoreceptors have highly similar signaling regions that conserve residues that interact with coupling proteins in other systems (Fig. S1). Because of the high degree of similarity and the fact that TlpD is soluble in its full-length form, we used TlpD to characterize chemoreceptor–coupling protein interactions. It is possible, however, that CheW and CheV1 interact with the other chemoreceptors with varying strength or preference (7, 17). Finally, we saw that both CheV1 and CheW interacted with each other (Fig. 2*C*). Thus, the BACTH and coimmunoprecipitation results suggest that the protein interaction networks of both CheV1 and CheW are largely similar (Fig. 2*D*). Both interact directly with CheA, chemoreceptors, each other, and themselves. CheV1 may have several additional interactions with other coupling proteins, but these were not pursued (Fig. 2*D*).

Both CheW and CheV1 Possess Receptor–CheA Coupling. We next tested whether CheW and CheV1 could each allosterically function to activate CheA autophosphorylation and to couple CheA to a chemoreceptor. These studies were done using an in vitro CheA phosphorylation assay similar to one used in previous studies (22, 23). In this assay, purified CheA ± coupling protein and receptor are incubated in vitro with radioactive [γ^{-32}P]-ATP and the amount of phosphorylated CheA determined using phosphorimaging of SDS PAGE gels (Fig. 3 *A* and *D*).

We first examined how addition of CheW or CheV1 to purified CheA protein would allosterically affect CheA phosphorylation, by measuring total phosphorylated CheA (Fig. 3*B*). CheV1 or CheW protein each significantly increased the CheA activity by 1.8- or 2.8-fold, respectively (Fig. 3*C*). These differences were not changed upon addition of a greater amount of CheW or CheV1 protein, suggesting the CheA was largely saturated. These results suggest that both CheV1 and CheW cause CheA to be more active, with CheW triggering greater activation. Previous work has shown that CheA activation is maximal with coupling proteins (24, 25).

We then examined CheV1 and CheW for each one's ability to couple CheA to a chemoreceptor. In other systems, CheA becomes substantially more active when coupled to a chemoreceptor (26, 27). We thus incubated purified CheA with TlpD and either CheV1 or CheW (Fig. 3*D*). CheA phosphorylation was significantly increased with the addition of the chemoreceptor TlpD alone by twofold (Fig. 3*F*). The addition of CheV1 or CheW to the chemoreceptor–TlpD reaction significantly increased CheA phosphorylation by threefold or 4.8-fold, respectively, compared with CheA alone (Fig. 3*F*). Although both coupling proteins significantly increased CheA phosphorylation, CheW was marginally better able to activate CheA than CheV1 (P value= 0.0186). Overall, these data suggest that both CheV1 and CheW can connect chemoreceptors to CheA and lead to its activation, with CheW having a greater activity in this respect.

We then determined how a combination of both CheV1 and CheW coupling proteins would affect CheA phosphorylation. Addition of both CheV1 and CheW resulted in CheA activation that was in between that of CheV1 and CheW (Fig. 3). This outcome was true whether there was a chemoreceptor or not. This finding suggests that both proteins act independently on CheA but do not appear to synergize.

CheV1 and CheW Independently Promote CheA–Chemoreceptor Interactions in Vivo. Our results above showed that CheV1 and CheW have overlapping interaction networks and both activate

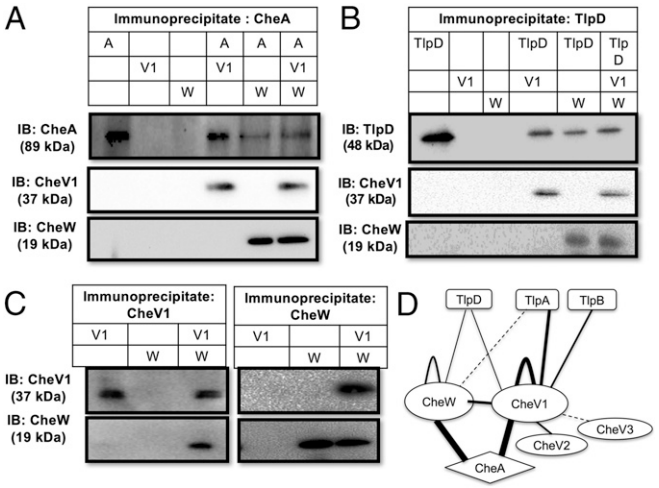

Fig. 2. Coimmunoprecipitation with purified proteins confirm that CheV1 and CheW interact with CheA, TlpD, and each other. (*A–C*) Mixtures of purified CheA (A), TlpD, CheV1 (V1), and/or CheW (W) were preincubated and immunoprecipitated as indicated along the top, followed by detection of specific proteins as indicated at the left. Each gel is representative of three immunoprecipitations ($n = 3$). (*D*) Model of the CheV1 and CheW protein interaction network identified by BACTH and coimmunoprecipitation, with line thickness denoting interaction strength.

CheA, although to somewhat different levels. Given their similarities, one would predict that either could function in chemotaxis, but this is not the case (9, 20). We therefore hypothesized that CheV1 and CheW have in vivo activities in addition to protein–protein interactions and kinase activation. We thus sought to gain more insight into these putative in vivo roles. Our first step was to analyze CheW and CheV1's roles in promoting the formation of the chemoreceptor–CheA complex. Both transmembrane and cytoplasmic chemoreceptors are associated with the membrane and retain CheA, which is normally cytoplasmic, at the membrane via coupling protein interactions (28–31). We therefore measured the amount of CheA associated with the membrane as an indicator of overall complex formation. We isolated membrane and cytoplasmic fractions from multiple strains using high-speed centrifugation and membrane washing as previously described (28, 30). Equal amounts of total protein from each fraction were separated by SDS/PAGE, followed by Western blotting (Fig. S2*A*). Control blots confirmed that the membrane and cytoplasmic fractions were substantially free of cross-contamination (Fig. S2*B*) (30). We then quantified and compared the amount of CheA found in the membrane relative to the cytoplasm (Fig. 4*A*). In wild type, the membrane had twice as much CheA as the cytoplasm. In a mutant lacking all chemoreceptors, CheA was almost fully cytoplasmic, consistent with the idea that CheA membrane interactions occur via chemoreceptors. CheA at the membrane was partially but significantly decreased in strains lacking either *cheV1* or *cheW*, compared with wild type, and more substantially decreased when both proteins were lacking (Fig. 4*B*). These findings suggest that CheV1 and CheW each promote CheA interactions with the chemoreceptor complex in vivo. Furthermore, our data show they function in an additive way, suggesting each acts independent of the other.

CheV1 and CheW Alter Each Other's Interaction with CheA and Chemoreceptors in Vivo. The membrane fractionation results suggested that CheV1 and CheW each promote CheA–chemoreceptor interactions. We next confirmed these interactions and examined what proteins were critical for forming them. To this end CheA, CheV1, or CheW were immunoprecipitated from whole cell lysates and examined for the presence of the other proteins.

In wild-type cells, all three proteins interacted with each other, with each being able to immunoprecipitate the other two, although the CheA bands were quite weak in the *cheV1* and *cheW* mutants (Fig. 5 *B–D*). Similar results were obtained in immunoprecipitation experiments with mutants lacking all chemoreceptors (Fig. 5*B*). This outcome suggested that CheA forms complexes with each coupling protein independent of chemoreceptors.

We also found evidence that CheV1 and CheW interacted with each other in wild-type *H. pylori* and in mutants lacking CheA or all chemoreceptors. These experiments were somewhat challenging, as the expression of CheW in the Δ*cheA* background was substantially lessened (Fig. 5 *C* and *D*). These experiments are consistent with the idea that CheA, CheW, and CheV1 all form independent interactions.

We next examined whether the interactions of CheV1 or CheW with CheA were dependent on the other coupling protein. A strain lacking CheW resulted in less CheV1 immunoprecipitated with CheA compared with the wild-type strain (Fig. 5*B*). A strain lacking CheV1 also showed reduced levels of CheW pulled down from CheA immunoprecipitation, compared with wild type (Fig. 5*B*). Deletion of either *cheV1* or *cheW* did not affect the expression of the other (Fig. 5*A*), consistent with the fact that both are in separate operons and under distinct transcriptional control (7). Taken together, these results and the CheA membrane

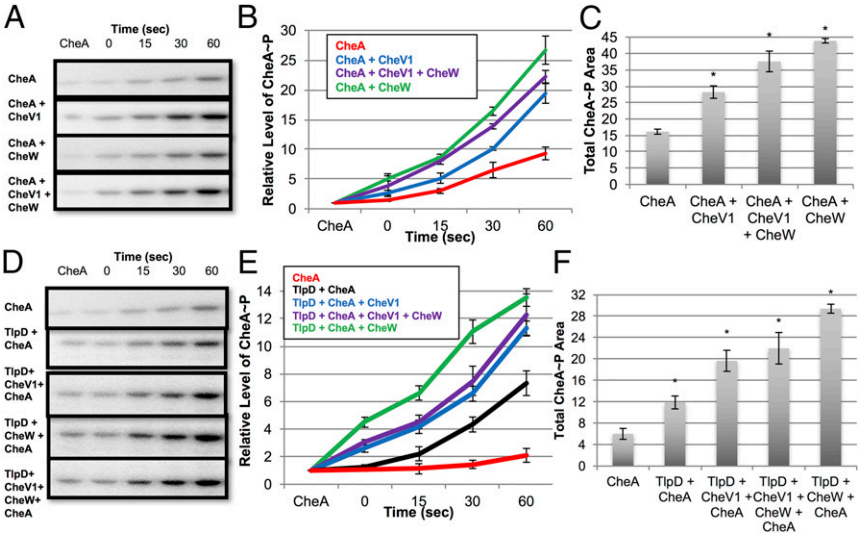

Fig. 3. CheV1 and CheW activate CheA phosphorylation on their own and with the addition of the chemoreceptor TlpD. Equimolar mixtures of purified proteins as indicated (2 μM of each) were incubated with [γ-^{32}P]-ATP and phosphorylated CheA detected using SDS/PAGE. (*A* and *D*) Representative blots. (*B* and *E*) The level of phosphorylated CheA was compared with phosphorylated CheA at time 0 (*C* and *F*) The amount of CheA was calculated using the area under the curve with the definite integral as opposed to the rate, as the rate was not linear. $n = 3$, with error bars representing SD. *$P < 0.001$ determined by an unpaired *t* test for all compared with CheA only.

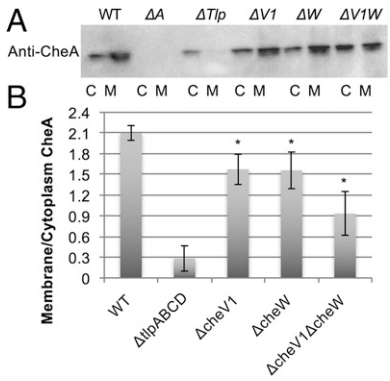

Fig. 4. CheV1 and CheW are necessary to retain CheA at the cell membrane. (*A*) Western blots from cytoplasmic "C" and membrane "M" fractions of indicated strains probed with anti-CheA. (*B*) Quantification of the amount of CheA found in membrane fractions relative to CheA amount found in cytoplasmic fractions. Each image is representative of three independent cultures ($n = 3$). *$P < 0.01$ determined by an unpaired Student's t test for strains compared with WT. Error bars represent the SD. *Δtlp*, strain lacking all chemoreceptors; *ΔA, ΔcheA; ΔV1, ΔcheV1; ΔW, ΔcheW; ΔV1W, ΔcheV1 ΔcheW*.

association experiments suggest that CheV1, CheW, and CheA all interact in vivo in a chemoreceptor-independent manner. Furthermore, CheV1 and CheW interact with each other and enhance the interaction of the other with CheA.

Given the similarities in the interactions between CheV1 and CheW, we revisited whether addition of each protein would affect the interaction of the other with purified CheA protein in this same assy. We mixed CheA or chemoreceptor with a combination of CheW and CheV1. These samples were then immunoprecipitated with anti-CheA or antichemoreceptor antibodies and then compared using Western blotting to samples that had only CheW or CheV1. Using this approach, we found that neither CheW nor CheV1 changed the amount of the other pulled down in vitro (Fig. 2 *A* and *B*). These results suggest that CheW and CheV1 do not substantially affect the binding of the other and therefore may have distinct and independent binding interactions. Ultimately the combination of our in vivo and in vitro interaction experiments suggests that both coupling proteins work to enhance chemoreceptor signaling complex interaction.

Loss of CheV1, CheW, or Both Abrogates Chemoreceptor–CheA Complex Formation at Cell Poles. The above experiments showed that CheW and CheV1 interact directly and with CheA. CheW is known to interact with another CheW-like domain in the form of CheA's P5 domain (3). These interactions build the multiprotein chemoreceptor–CheA arrays at the cell pole and promote signal amplification (32, 33). Given that CheV1 has a CheW domain, we thus explored the role of CheW and CheV1 in the building of the *H. pylori* chemoreceptor array. We used immunofluorescence on whole cells, with all proteins expressed from the native loci in native forms. The polar chemotaxis complex was detected using anti-CheA, antichemoreceptors (Tlps), or anti-CheW. All antibodies detected a discrete locus at one or both cell poles in the wild-type strain, as reported previously (Fig. 6*A*) (30, 31, 34). Mutants lacking the chemoreceptors lost polar localization of the other complex members, consistent with the idea that these proteins are critical to build the chemotaxis arrays (Fig. 6*C*), as reported previously (30, 34). Published data have previously shown that CheV1 localizes to cell poles in wild type and loses this localization in mutants lacking chemoreceptors (30, 34), identical to the behavior of CheW shown here. *cheA*, *cheV1*, or *cheW* mutants also were unable to build a polar signaling complex (Fig. 6 *D–F*). In other words, none of these proteins was redundant with any other. These results suggest that polar chemosensory array creation requires two coupling proteins, CheV1 and CheW, in addition to CheA.

Discussion

Coupling proteins are key components of many types of signal transduction systems, providing functions beyond simply holding proteins together. Here, we analyzed the roles of two different coupling proteins, CheW and CheV, in a single chemotaxis system. Altogether, our results suggest that some microbes use two coupling proteins to build a robust chemosensory complex.

The results presented here show that CheW and CheV1 are both required to create the functional polar chemosensory array. Mutants that lack either CheW, CheV1, or both have chemotaxis proteins that appear in punctate, nonpolar structures (Fig. 6) and have a severe decrease in retaining CheA at the membrane (Fig. 4). Because both *cheW* and *cheV1* mutants are nonchemotactic, we surmise that these nonpolar chemotaxis units are not functional. This finding sheds light on why *cheV1* and *cheW* mutants are nonchemotactic, whereas both proteins are capable of typical coupling protein functions as we show here. Large chemosensory arrays are critical for chemotaxis because they allow the high positive cooperativity (Hill coefficients of 15–20), a property that underlies the high sensitivity and ability to amplify the chemotaxis system's response to ligands (3, 8, 35). Indeed, isolated core chemoreceptor–CheW–CheA signaling complexes are able to regulate CheA but with little positive cooperativity (36). Our studies suggest that *cheW* and *cheV1* mutant arrays are defective because they lack the positive cooperativity necessary for wild-type chemotaxis.

We envision several mechanisms that might underlie the ability of CheV1 or CheW to form functional polar chemosensory arrays. Cryo-electron tomography revealed the existence of CheW rings symbolic of CheW–CheW interactions in *E. coli* chemoreceptor array structures (37). We suggest a similar chemotaxis array in *H. pylori* in which CheW–CheV1 rings form the core of the complex. Recent work has shown that in *E. coli*, which does not contain CheV, CheW interacts with other CheW and CheA molecules to form large chemosensory arrays that are critical for cooperativity of signal response (3, 8). Piñas et al.

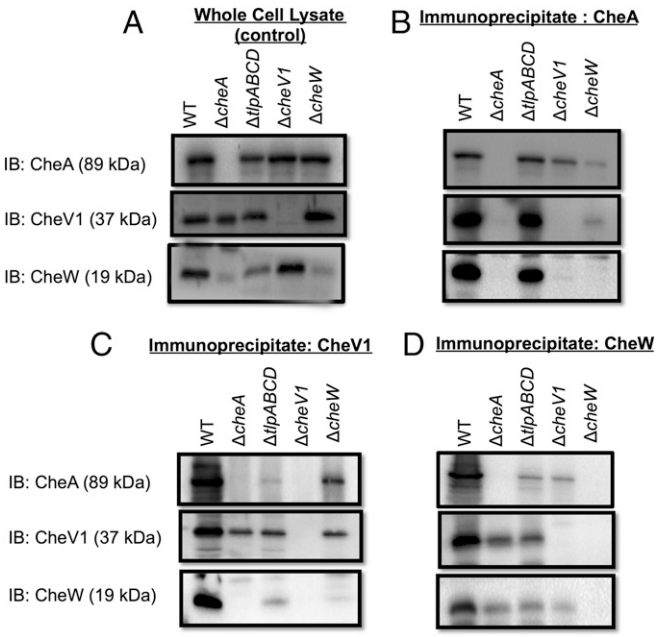

Fig. 5. CheV1 and CheW interact between themselves and with CheA in whole cell lysates. (*A*) CheA, CheV1, and CheW were detected by immunoblotting (IB) from whole-cell protein extracts of wild-type, *ΔcheA*, *ΔtlpABCD*, *ΔcheV1*, and *ΔcheW H. pylori* strains. Identical amounts of whole cell extracts were immunoprecipitated (IP) using anti-CheV1, anti-CheW, and anti-CheA antibodies and probed by IB using the respective antibody (*B–D*). Each gel is representative of three immunoprecipitations ($n = 3$).

defined two CheW–CheA interaction faces: interface 1, which promotes CheW–CheA interactions required for kinase control, and interface 2, which creates the interactions that connect chemosensory arrays (3). Our work suggests that some microbes have altered CheW such that the array-forming function is split between two coupling proteins. Indeed, the residues that form interface 2 are conserved among enteric CheW but not among CheW as a whole (7, 17). Other support comes from models that suggested complexes lacking CheA and having only CheW—so-called CheW-only linkers—create high cooperativity in signal sensing (38). In *Borrelia burgdorferi*, two distinct CheW proteins are necessary for chemotaxis and formation of the chemosensory arrays (39). These two CheW, like CheV1 and CheW in *H. pylori*, are under distinct transcriptional control (7, 39–41). We speculate that bacteria that use two coupling proteins may thus modulate the expression of each to alter the cooperativity behavior of the chemoreceptor–CheA cluster array.

We report here that CheV1 and CheW possess largely overlapping but not identical interactions. Although their interaction strengths with various chemoreceptors may vary, as suggested for other systems (17), we envision that both proteins still interact with all chemoreceptors due to conserved residues in their signaling domains. TlpA and TlpB are integral membrane proteins in *H. pylori*, and due to the challenging nature of their purification, we were only able to test their interaction with CheV1 and CheW using the BACTH assay. However, it is possible that multiple coupling proteins in a single organism do provide some chemoreceptor specificity, as seen in *Campylobacter jejuni*, where the aspartate chemoreceptor prefers CheV over CheW (16, 42).

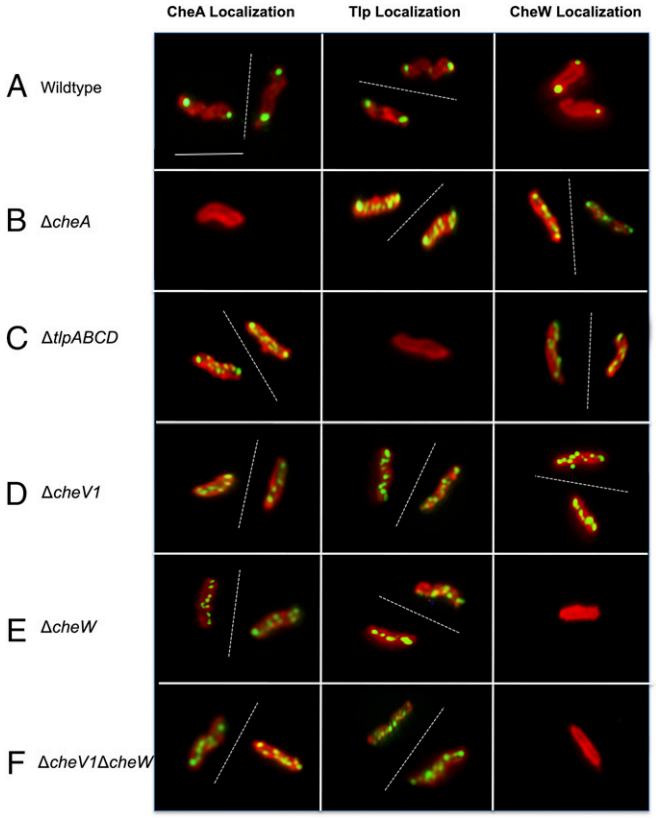

Fig. 6. *cheV1* and *cheW* mutants are defective in localizing CheA and chemoreceptors to polar chemosensory clusters. CheA, the chemoreceptors (Tlp), and CheW were visualized in *H. pylori* G27 and its isogenic mutants using immunofluorescence (*A*–*F*). All proteins were expressed from their native loci and are marked with green, whereas *H. pylori* cells were visualized with anti-*H. pylori* antibodies and red secondary antibodies. Dashed lines indicate bacteria from different fields of view. (Scale bar, 4 μm.)

Furthermore, our localization results suggest that both proteins are important to build large sensory arrays, but we do not yet know the structural and protein compositional difference between the small patches and large polar superarrays.

Coupling proteins play an important role in CheA kinase activity. Our results revealed that both CheV1 and CheW activate CheA and couple it to a chemoreceptor. CheW, however, had a greater ability to activate CheA than CheV1. This observation is consistent with the data presented by Ortega and Zhulin that CheV proteins limit CheA kinase output and thus can function with chemoreceptors that have a high intrinsic ability to activate CheA (17). These authors proposed that this limitation is due to CheV acting as a phosphate sink, a protein that can be phosphorylated by CheA to direct phosphates away from other targets (17). We did not detect phosphorylated CheV1 in our CheA kinase assay, a finding also reported by others, and so do not have direct support for this aspect of the model (22, 23). Another possibility, consistent with our data, is that CheV1 itself is less able to activate CheA via aspects of its structure. Our results may reflect the nature of the chemoreceptor–CheA complex as demonstrated in *B. subtilis*, where CheV increased CheA kinase activity depending on the methylation nature of chemoreceptors, which is reflective of ligand binding (43). CheV was also able to decrease CheA activity in *B. subtilis* when its coupling domain was mutated, leaving only its receiver domain active; this suggests an important interplay between CheV's domains influencing its ultimate function (14). The idea that coupling proteins have different abilities to enhance or inhibit signal transduction is also seen in eukaryotic scaffold systems (44). We suggest that although both CheV1 and CheW appear to have similar functions, CheW may play a more critical role for CheA kinase activation than CheV1. Future research on overexpressing CheW in a *cheV1* mutant may provide valuable knowledge on whether a greater amount of either CheW or CheV1 can make up for the loss of the other. These experiments are challenging, however, due to limited *H. pylori* genetic tools and the fact that overexpression of CheW, at least in *E. coli*, inhibits chemotaxis apparently by binding to chemoreceptors and CheA individually (45, 46).

CheV1 may function to connect the chemoreceptor complex to additional proteins and may be able to interact with more proteins than CheW, including CheV2 and CheV3. It is also possible that CheV1 or CheW have additional interactions beyond chemotaxis proteins, similar to the ParP protein from *Vibrio parahaemolyticus* (47). ParP, like CheV1, is a hybrid protein with a CheW-like domain. Like CheV1, ParP mutants have decreased soft agar migration and are more smooth swimming than wild type (9, 19, 40). ParP interacts with CheA and a membrane protein called ParC, and stabilizes chemotaxis complexes at the cell pole by preventing CheA dissociation (47). A common theme is the use of the CheW domain as a connection point for protein–protein interactions either between arrays or with other proteins.

In sum, our work suggests that division between two coupling proteins in a signaling complex may provide several advantages to bacteria similar to the advantages conferred by scaffolding proteins in mammalian cell signaling. The combination of two coupling proteins results in proper formation of the chemoreceptor–CheA chemotaxis complex and large polar arrays, localization to the cell membrane of the complex components, and finally stimulation of CheA kinase activity leading to optimal chemotaxis in *H. pylori*. Our results thus highlight the important functions of multiple coupling proteins in signal transduction systems in helping organisms efficiently respond to dynamic environments by rewiring key interactions among signal transduction proteins.

Materials and Methods

Detailed materials and methods are provided in *SI Materials and Methods*, as are all *H. pylori* and *E. coli* strains (Table S2) and primers (Table S3). *H. pylori* strains were grown under microaerobic conditions on either Columbia horse blood agar or *Brucella* broth with FBS. All antibodies were created to purified *H. pylori* proteins. Immunoprecipitations from whole cell extract and immunofluorescence were analyzed on *H. pylori* with natively expressed proteins.

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