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Bacterial chemotaxis coupling protein: Structure, function and diversity

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ABSTRACT

In most signal transduction systems, coupling or scaffold proteins establish crucial connections between receptors and histidine kinases. These connections are important for signal transduction. The bacterial chemotaxis system is a canonical signal transduction system that relies on coupling proteins. The coupling proteins in the chemotaxis system have two architectures: CheW or CheV. In a typical chemotaxis signal transduction system, two CheW coupling protein molecules bridge a histidine kinase CheA dimer and two chemoreceptor (also called as methyl-accepting chemotaxis protein, MCP) trimers of dimers to form a core signaling complex and couple CheA activity to chemoreceptor control. Although CheW is a small cytoplasmic protein, it plays multiple functions in chemotaxis. CheW also builds connections between core signaling complexes, which leads to the formation of large chemosensory arrays that are responsible for collecting and amplifying signals from various chemoreceptors. Another coupling protein, CheV, shares a largely redundant ability with CheW; however, the function of CheV is not identical to that of CheW in chemotaxis. In this article, we summarize the molecular mechanism of chemotaxis in *Escherichia coli* and review the recent advances in the structural details and functions of CheW and CheV. Furthermore, we focus on the diversity of coupling proteins and discuss the relationship among multiple coupling proteins in one organism.

1. Introduction

Chemotaxis offers bacteria the ability to track spatial gradients of chemoeffectors (including attractants and repellents) and to move towards optimal environments (Wadhams and Armitage, 2004; Porter et al., 2011). As a ubiquitous and important characteristic of motile bacteria, chemotaxis is involved in many important biological processes, such as biofilm formation (He and Bauer, 2014; Alexandre, 2015; Mangwani et al., 2016), cell autoaggregation and adhesion (Laganenka et al., 2016; Huang et al., 2017), the establishment of symbiotic association of bacteria with plants (Scharf et al., 2016) and the colonization of hosts by pathogens (Day et al., 2016; Erhardt, 2016; Johnson and Ottemann, 2018). A bacterial chemotaxis study was initiated by Julius Adler in 1960s (Adler, 1966). In the early years, Adler and his colleagues mainly used *Escherichia coli* as the model for chemotaxis studies at the phenotypic level. Over the following three decades, numerous biochemical and molecular studies elucidated the molecular mechanism of chemotaxis signal transduction in *E. coli* (Springer et al., 1977; Parkinson and Houts, 1982; Hess et al., 1988; Maddock and Shapiro, 1993; Sourjik and Berg, 2000). At the same time, chemotaxis in other bacteria has also been studied, such as in *Bacillus subtilis* (Rosario et al., 1994), *Rhodobacter sphaeroides* (Hamblin et al., 1997), and *Agrobacteria tumefaciens* (Wright et al., 1998). After 2000, with the increasing number of completely sequenced bacteria and the identification of chemotaxis proteins in different bacteria, the core components of bacterial chemotaxis systems were identified (Wuichet et al., 2007; Wuichet and Zhulin, 2010). In addition, many auxiliary or alternative components of chemotaxis systems were also constantly identified (Wuichet and Zhulin, 2010; Scharf et al., 2016). The intensive study of the chemotaxis of *E. coli* has made *E. coli* a model organism for chemotaxis studies (Wuichet and Zhulin, 2010; Jones and Armitage, 2015; Micali and Endres, 2016). Although newly identified chemotaxis systems of other bacteria are more complex than that of *E. coli*, the core signaling complex that is formed by the chemoreceptors (because most chemoreceptors can be methylated, they are often called methyl-accepting chemotaxis protein, MCP), histidine kinase CheA and coupling protein CheW is conserved among bacteria (Wuichet and Zhulin, 2010; Bi and Sourjik, 2018).

In eukaryotic and prokaryotic signal transduction systems, coupling or scaffold proteins provide crucial connections for receptors and kinases (Shaw and Filbert, 2009; Good et al., 2011; Kamber et al., 2015; Piñas et al., 2016). These connections are important for the assembly of the signal complex, protein location, and signal transduction (Shaw and Filbert, 2009). The bacterial chemotaxis system is a typical example of a

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prokaryotic signal transduction system that relies on coupling proteins to build connections between chemoreceptors and histidine kinase CheA (Abedrabbo et al., 2017). More importantly, chemotaxis coupling proteins are also responsible for the assembly and function of large chemosensory arrays that consist of thousands of core signaling complexes (Maddock and Shapiro, 1993; Piñas et al., 2016). In this review, we first outline the molecular mechanism of chemotaxis in *E. coli*, describe insights into chemotaxis coupling proteins from structural and functional studies, and finally, discuss the diversity of chemotaxis coupling proteins.

2. Molecular mechanism of bacterial chemotaxis and coupling proteins

To date, the best-studied chemotaxis system is that of *E. coli* (Jones and Armitage, 2015; Parkinson et al., 2015; Colin and Sourjik, 2017; Bi and Sourjik, 2018). In *E. coli*, the chemotaxis system consists of five transmembrane chemoreceptors, Tar, Tsr, Tap, Trg, and Aer, and six core components, CheA, CheW, CheY, CheB, CheZ, and CheR (Fig. 1) (Parkinson et al., 2015). Chemoreceptors are responsible for sensing chemoeffectors (including attractants and repellents), transducing signals to the histidine kinase CheA and modulating CheA activity through the coupling protein CheW (Hess et al., 1988; Typas and Sourjik, 2015; Guo et al., 2017). CheW bridges the chemoreceptors and CheA to form a stable core signaling complex and couples CheA activity to the chemoreceptor control. Ligand-free chemoreceptors activate CheA autophosphorylation several hundred-fold higher than its basal autophosphorylation rate (Nishiyama et al., 2014). Phosphorylated CheA donates its phosphoryl groups to two response regulator proteins, CheY or CheB. Phosphorylated CheY interacts with the flagellar motors and changes the rotational direction of the flagella from counterclockwise (CCW) to clockwise (CW) (Typas and Sourjik, 2015). CCW rotation is the default motor direction and produces forward swimming (“runs”), while CW rotation changes the cell swimming direction randomly and causes cell tumbling. CheZ is a dedicated phosphatase that accelerates the spontaneous dephosphorylation of phosphorylated CheY to terminate the signal (Hess et al., 1988; Typas and Sourjik, 2015). Phosphorylated CheB acts as the methylesterase to remove the methyl groups from some chemoreceptors that can be methylated, which decreases the ability of the MCPs to activate CheA (Springer et al., 1977; Guo et al., 2017). CheR is a constitutively expressed methyltransferase. CheR couples with phosphorylated CheB to reset the activity of CheA and the sensitivity of MCPs to a broad range of environmental stimulus strengths by modulating the methylation state of the MCPs (Colin and Sourjik, 2017). In response to the increase of the attractant concentration or the reduction of the repellent concentration, the conformation of MCPs is changed. CheW conveys the signaling-related conformational changes from the MCPs to CheA, leading to the inhibition of CheA autophosphorylation (Parkinson et al., 2015; Bi and Sourjik, 2018). Next, the transfer of phosphoryl groups from phosphorylated CheA to CheY or CheB is delayed. Because dephosphorylation of phosphorylated CheY is very fast and this process is accelerated by CheZ, the signal that changes the rotational direction of the flagella from CCW to CW is inhibited. *E. coli* cells maintain smooth swimming, leading to chemotaxis. The content of phosphorylated CheB in the cell is also decreased, reducing the demethylation of MCPs. At the same time, CheR still methylates MCPs. The methylation of MCPs strengthens the ability of the MCPs to activate CheA activity, which in turn, increases the phosphorylation of CheY and CheB (Salah Ud-Din and Roujeinikova, 2017). The chemotaxis system of *E. coli* returns to the pre-stimuli state, which results in adaption. The detailed chemotaxis signal transduction pathway in *E. coli* is shown in Fig. 1.

The coupling proteins in bacterial chemotaxis systems have two basic architectures, CheW or CheV (Abedrabbo et al., 2017). CheW is a canonical chemotaxis coupling protein with two subdomains (Griswold and Dahlquist, 2002). CheV is a hybrid protein with an N-terminal CheW-like domain fused to a C-terminal receiver (REC) domain that can be phosphorylated (Alexander et al., 2010). The distribution of *cheW* in the genomes of completely sequenced bacteria and archaea is wider than that of *cheV* (Alexander et al., 2010). However, some Firmicutes species appear to only have a CheV coupling protein, such as *Bacillus cereus*, *Bacillus anthracis*, *Bacillus thuringiensis* and *Listeria spp.*, (Wuichet et al., 2007). Experimental evidence from *Listeria monocytogenes* shows that its chemotaxis system without CheW is functional (Dons et al., 2004), which implies that CheV can completely substitute for CheW in this bacterium. In another genomic analysis of 245 species representative of bacteria and archaea with chemotaxis systems, *cheW* is present in 95% of the genomes that contain at least one chemotaxis gene, while *cheV* is only found in < 40% of these genomes (Wuichet and Zhulin, 2010). In addition, the genomic locations of *cheW* and *cheV* also differ. One *cheW* gene is usually located near a chemotaxis gene cluster that contains a *cheA* gene, whereas *cheV* genes are often distributed in genomic locations that are distinct from these clusters (Wuichet et al., 2007). In ε- proteobacteria, only one *cheV* is located in a

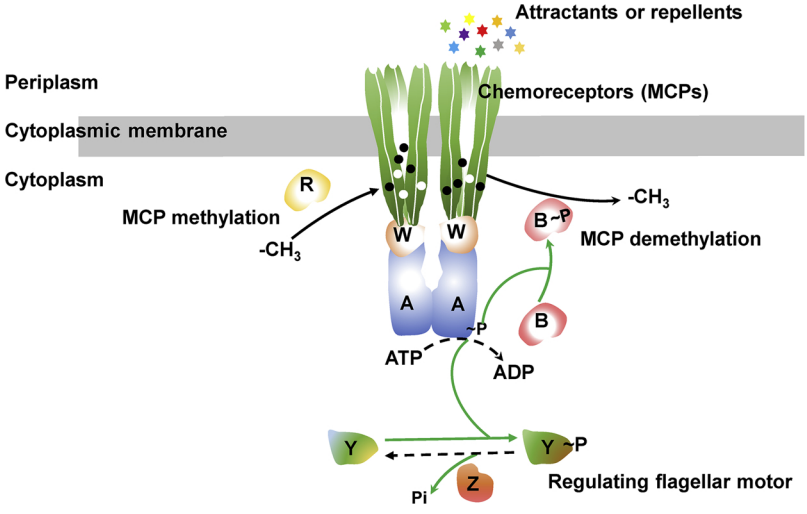

Fig. 1. Chemotaxis signal transduction pathway of *E. coli*. Different types of transmembrane chemoreceptors (also called methyl-accepting chemotaxis proteins, MCP), which can sense different chemoeffectors (including attractants or repellents), organize as dimers and then trimers. Thousands of trimers of dimers cluster at cell poles and build large chemoreceptor arrays. In the arrays, two CheW molecules couple one CheA dimer to two chemoreceptor trimers of dimers to form a chemoreceptor-CheW-CheA ternary signaling complex. In response to the decreasing concentration of attractants, chemoreceptors activate CheA autophosphorylation activity. Phosphorylated CheA transfers its phosphoryl groups to CheY or CheB. Phosphorylated CheY changes the rotational direction of flagella from counterclockwise (CCW) to clockwise (CW). CheZ is a phosphatase that can accelerate the dephosphorylation of phosphorylated CheY and rapidly terminate the signal that changes the rotational direction of flagella. Phosphorylated CheB acts as a dedicated methylesterase to remove the methyl groups from methylated MCPs, while CheR, a dedicated methyltransferase, interacts with MCPs and catalyzes their methylation. Phosphorylated CheB and CheR form a sensory adaption feedback circuit that maintains the methylation level of MCPs. Methylation of MCPs enhances their ability to activate CheA. Small circles on MCPs indicate methylated (black) and unmodified (white) methylation sites. Green arrows indicate the flux of the phosphoryl group. Black arrows indicate the flux of the methyl group. Abbreviations: A, CheA; B, CheB; D, CheD; R, CheR; W, CheW; Y, CheY; Z, CheZ; ~P, phosphoryl group; Pi, inorganic phosphate; -CH_3: methyl group.

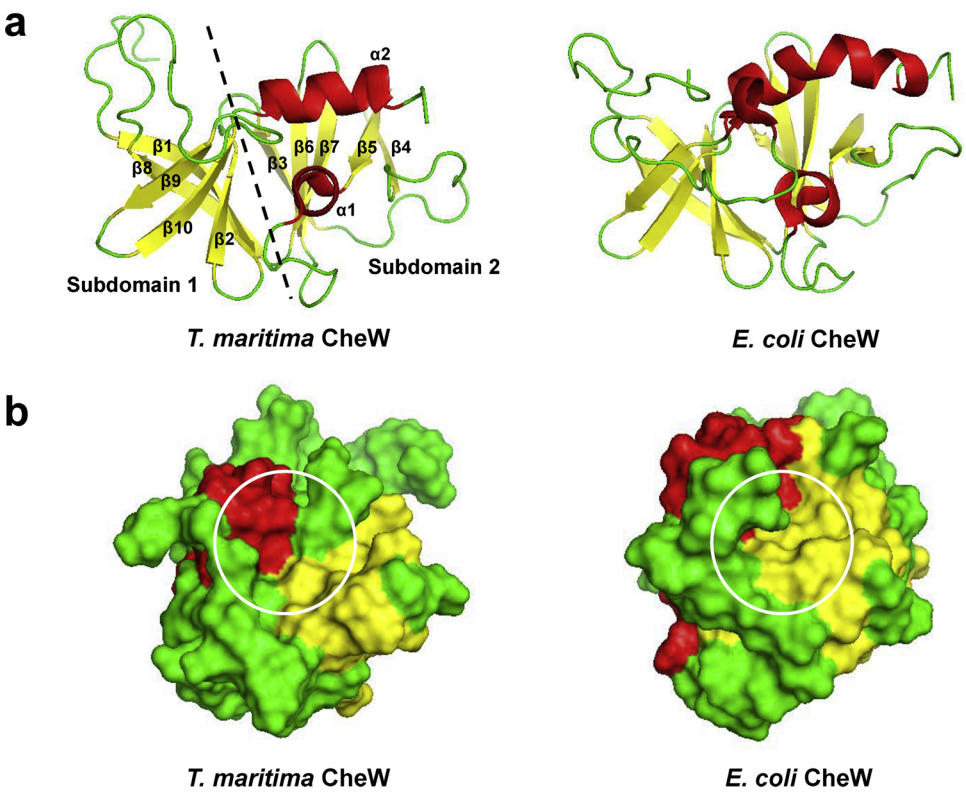

Fig. 2. Structure of CheW.
a, The solution structure of *T. maritima* CheW (1K0S) (left panel) and *E. coli* CheW (2HO9) (right panel). The dashed line is the boundary of two subdomains. All α-helixes (red) and β-sheets (yellow) are annotated in the figure. b, CheA P5-binding pocket (white circle) of *T. maritima* CheW (left panel) and *E. coli* CheW (right panel). The figure was generated using the program PyMOL.

predicted operon that contains *cheA* and *cheW* (Alexander et al., 2010). Therefore, CheW is the core component of the chemotaxis system.

3. Structural features of coupling proteins

Although CheW and CheV have been studied for decades, only the structure of CheW has been solved for an isolated protein and a complex associated with fragments of CheA and MCP from *E. coli* and some thermophilic bacteria (Griswold et al., 2002; Park et al., 2006; Li et al., 2007; Briegel et al., 2012; Li et al., 2013; Falke and Piasta, 2014). Hence, in this section, we will only describe the structure of CheW. Most of the structural information of CheW is obtained from thermophilic bacteria, such as *Thermotoga maritima*. *T. maritima* CheW (PDB: 1K0S) is composed of two five-stranded β-sheet domains, designated as subdomain 1 and subdomain 2 (Fig. 2a). Subdomain 1 is formed by the β1, β2, and β8–β10 strands, while subdomain 2 is formed by the β3–β7 strands (Griswold et al., 2002; Griswold and Dahlquist, 2002). *T. maritima* CheW also contains two α-helices. α-helix 1 is located in the loop that connects the β5 and β6 strands, and α-helix 2 is located at the C-terminus of the CheW protein (Griswold et al., 2002). The solution structure of the well-studied *E. coli* CheW (PDB: 2HO9) is highly similar to that of the *T. maritima* CheW, but has some differences (Li et al., 2007). For example, the C-terminal helix of *E. coli* CheW, which slightly kinks in the middle, is considerably longer than that of *T. maritima* CheW (Li et al., 2007). This longer C-terminal helix of *E. coli* CheW appears to shrink the active binding pocket (white cycle) with CheA (Fig. 2b) and might affect the binding affinity between CheW and CheA.

E. coli CheW has been subjected to several extensive mutational studies, and a number of residues have been identified that have been implicated in the interactions of CheW with CheA and MCP or in the formation of protein structure (Liu and Parkinson, 1991; Boukhvalova et al., 2002a, 2002b; Alexandre and Zhulin, 2003; Piñas et al., 2016). All residues are labeled with different symbols in the alignment of CheWs from different bacteria (Fig. 3). Except for two *T. maritima* CheW proteins, all of the other CheWs that are listed in Fig. 3 have been verified to be involved in chemotaxis. Most of these residues distribute in β3-β5, β6-β9, and the loops between these β sheets. Only three residues are absolutely conserved. These amino acids are Gly57, Arg62, and Gly63 as numbered residues in *E. coli* CheW. Gly57 is vital for chemotaxis of *E. coli*. Mutation of Gly57 eliminates CheW binding to CheA and results in a null phenotype (Boukhvalova et al., 2002b). Arg62 and Gly63 are located in the loop between β4 and β5. Both are involved in the interaction between CheW and MCP. Mutation of these two residues also eliminate the function of CheW (Liu and Parkinson, 1991; Boukhvalova et al., 2002a). Hence, these three conserved residues are important for protein-protein interactions and the structure of the protein. Interestingly, several key residues that are predicted to be located on the protein interacting interfaces are not conserved among different CheWs, which indicates that the solution structures of these CheWs might be variable. The local conformational variations of these CheWs might directly affect protein-protein interactions and the efficiency of signal transduction. For better visualization, all of the important residues that are mentioned above are marked by different colors and are displayed in the solution structure of *E. coli* CheW (Fig. 4). The residues (red and green) that are related to the CheW interaction with CheA are mainly distributed in interface 1 and interface 2. The residues (blue) that affect the CheW interaction with MCP are located on a surface that is adjacent to interface 1, and this surface is designated as interface 3. In addition, the residues that are labeled with yellow are required for CheW binding to CheA and MCP. The interactions that occur in these three interfaces have been extensively studied using cryoelectron tomography, cross-linking assays, and nuclear magnetic resonance (Liu et al., 2012; Vu et al., 2012; Li et al., 2013; Natale et al., 2013; Piasta et al., 2013; Pedetta et al., 2014; Piñas et al., 2016; Briegel and Jensen, 2017).

4. Functions of coupling proteins

In bacterial chemotaxis systems, the coupling proteins not only bridge CheA and chemoreceptors but also couple CheA to chemoreceptor control (Porter et al., 2011; Parkinson et al., 2015; Abedrabbo et al., 2017). Moreover, the coupling proteins are vital for the assembly

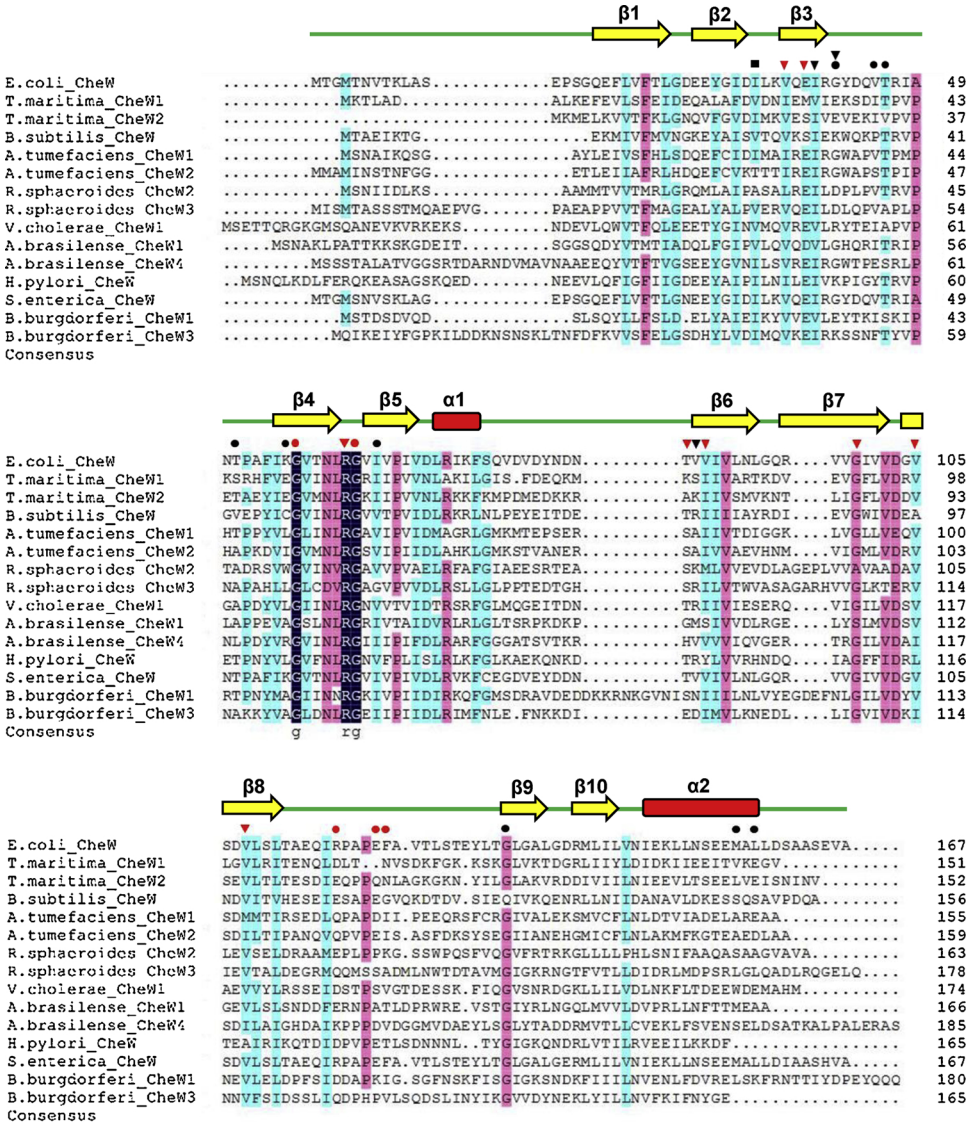

Fig. 3. Amino acid sequence comparison of CheW from different bacteria.
Alignment of fifteen CheW polypeptide sequences from *Escherichia coli* (NP_416401.1), *Thermotoga maritima* (NP_228510.1, NP_228527.1), *Bacillus subtilis* (NP_389526.1), *Agrobacterium tumefaciens* (NP_355040.2; NP_355554.2), *Rhodobacter sphaeroides* (YP_351629.1, YP_351630.1), *Vibrio cholera* (WP_001881777.1), *Azospirillum brasilense* (WP_014198311.1, WP_035675900.1), *Helicobacter pylori* (NP_207189.1), *Salmonella enterica* (WP_000147295.1), and *Borreliella burgdorferi* (NP_212699.1; NP_212804.1) (GenBank accession numbers in parentheses). Secondary structure elements of *E. coli* CheW are shown upon the sequences. Bars denote α-helices, arrows denote β-sheets, and thin lines denote coiled structures. The numbers in the right indicate the position of the amino acid. All key residues that are identified in *E. coli* CheW are labeled using different symbols (including inverted triangle, circle, and square). The inverted triangles represent the residues that are identified to be involved in the CheW/MCP interactions in *E. coli* CheW; the circles represent the residues that are identified to be involved in the CheW/CheA interactions in *E. coli* CheW; the squares represent functionally important residues in the *E. coli* CheW sequence (Liu and Parkinson, 1989; Boukhvalova et al., 2002a, 2002b; Alexandre and Zhulin, 2003; Piñas et al., 2016). The residues labeled with red symbols denote that the mutation of these residues eliminate CheW function completely, but mutation of the other residues that are labeled with black symbols only partly affect CheA binding or MCP binding. Sequences were aligned using DNAman 8.

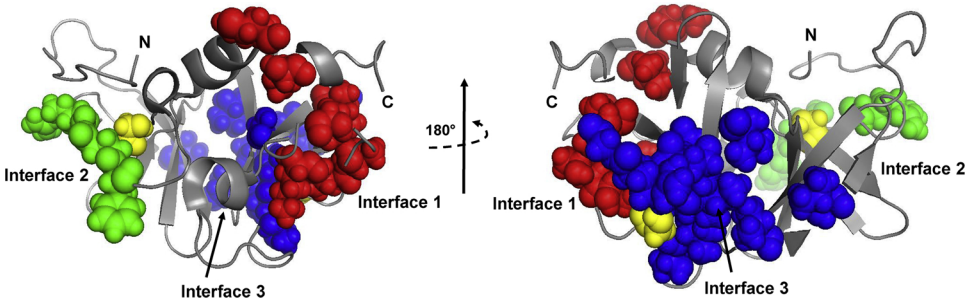

Fig. 4. Interfaces of *E. coli* CheW interacting with CheA and MCP.
All residues that are involved in the interaction of CheW with CheA and MCP are mapped onto the structure of *E. coli* CheW. The residues that affect the binding of CheA are depicted in space-filling mode in red and green. The residues that affect the binding of MCP are depicted in space-filling mode in blue. The remaining two residues that affect the binding of CheA and MCP are depicted in yellow. They are mainly distributed in three interfaces. The interaction of CheW and CheA occurs in interface 1 (consisting of red residues) and interface 2 (consisting of green residues), and the interaction of CheW and MCP occurs in interface 3 (consisting of blue residues). The figure was generated using the program PyMOL.

and function of large chemoreceptor arrays (Briegel et al., 2014a; Parkinson et al., 2015; Piñas et al., 2016; Abedrabbo et al., 2017). Therefore, the coupling proteins are not only scaffolds of protein complexes but are also adaptors of chemotaxis signal transduction. In this section, we will discuss the functions of CheW and CheV in detail.

4.1. Functions of CheW

CheW is the core component of the chemotaxis system. Mutations of CheW proteins cause aberrant swimming patterns and nonchemotactic phenotypes (Parkinson and Houts, 1982; Martin et al., 2001; Zhang et al., 2012). The assembly of the chemoreceptor-CheW-CheA complex is disrupted or abrogated in these *cheW*-deletion mutants (Maddock and Shapiro, 1993; Martin et al., 2001; Zhang et al., 2012; Abedrabbo et al., 2017; Huang et al., 2018). In recent years, the interactions of CheW with CheA and chemoreceptors have been well elucidated in the *E. coli* chemotaxis system, which has a single coupling protein (Briegel et al., 2012; Liu et al., 2012; Vu et al., 2012; Pedetta et al., 2014; Piñas et al.,

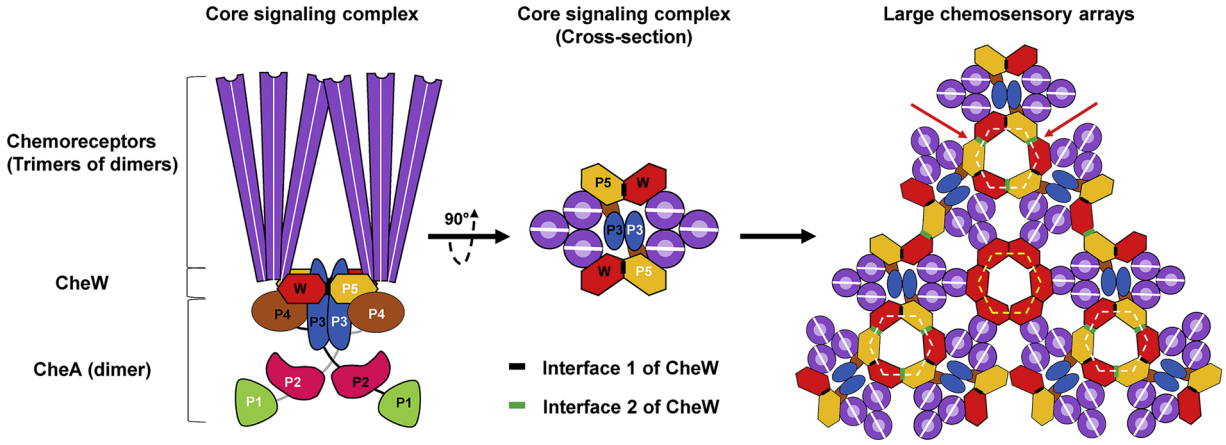

Fig. 5. Formation of a large chemosensory array in *E. coli*.
Two CheW molecules couple one CheA dimer to two chemoreceptor trimers of dimers to form a core signaling complex of the large chemosensory array. CheA has five structural domains with distinct functions, which are designated P1 to P5 (Nishiyama et al., 2014). P1 contains the autophosphorylation site, His-48; P2 possesses high-affinity docking surface for binding CheY and CheB; P3 is the main determinant for the dimerization of CheA; P4 is the ATP-binding domain that catalyzes autophosphorylation of P1 in another CheA of the homodimeric CheA and closely interacts with both P3 and P5 domains (Cassidy et al., 2015; Piñas et al., 2018); P5 is the regulatory domain that interacts with CheW. In the core signaling complex, subdomain 2 of CheW interacts with subdomain 1 of CheA P5 domain at interface 1 (black bar), which is vital for the regulation of CheA kinase activity by the chemoreceptor. Both the P5 domain of CheA and CheW directly interact with the chemoreceptor trimer of dimers. To distinguish between the domains of the two CheA molecules in the dimer, the fonts in five domains from the two CheA molecules are colored black and white, respectively. The interaction between CheW subdomain 1 and CheA P5 subdomain 2 at interface 2 (green bar) results in the production of a lattice of hexagonally packed chemoreceptor trimers of dimers networked by P5/CheW rings (white dashed hexagon) (Piñas et al., 2016). The P5/CheW ring is the foundation of the assembly of a large chemosensory array. In addition, in the extended array, a six-member CheW ring (yellow dashed hexagon) that couples neighboring chemoreceptor trimers of dimers has also been confirmed recently (Liu et al., 2012; Cassidy et al., 2015). Abbreviations: W: CheW; P1: P1 domain of CheA: P2: P2 domain of CheA; P3: P3 domain of CheA; P4: P4 domain of CheA; P5: P5 domain of CheA.

2016, 2018). In *E. coli*, chemoreceptor molecules are homodimers. Three chemoreceptor homodimers form a trimer of dimers. Two CheW coupling proteins couple a CheA kinase dimer to two chemoreceptor trimers of dimers to form a stable core signaling complex (Fig. 5) (Li and Hazelbauer, 2011; Jones and Armitage, 2015; Alvarado et al., 2017). Important interaction interfaces in the core signaling complexes include the interface between the chemoreceptor homodimers, the interface of the chemoreceptor trimers with either the P5 domain of CheA or CheW, and the interface between the P5 domain of CheA and CheW. The interactions between the chemoreceptor homodimers at their cytoplasmic, membrane-distal tips lead to the formation of trimers of dimers (Kim et al., 1999; Studdert and Parkinson, 2004). The P5 domain and CheW have homologous structures composed of two subdomains. Both the P5 domain and CheW can interact with the chemoreceptor (Vu et al., 2012; Piñas et al., 2018). The interaction between the P5 domain and the chemoreceptor *in vivo* is strictly dependent on CheW (Piñas et al., 2018). The control of CheA kinase activity by the chemoreceptor also requires CheW to interact with the chemoreceptor (Boukhvalova et al., 2002b). A recent study has suggested that this control is conducted by only one chemoreceptor dimer of each trimer of dimers (Li and Hazelbauer, 2014). Hence, the CheW-chemoreceptor interface was proposed to be the major route for conformational changes between CheA and the chemoreceptor during signaling transmission (Pedetta et al., 2014; Piñas et al., 2018).

CheW interacts with the P5 domain at two distinct interfaces: interface 1 and interface 2. In the core signaling complex, subdomain 2 of CheW interacts with the P5 subdomain 1 at interface 1. Although the P5 domain of CheA directly interacts with the chemoreceptor, CheA is unable to establish stable interactions with the chemoreceptors in the absence of CheW, which indicates that the interaction between subdomain 2 of CheW and the P5 subdomain 1 is vital for the assembly of the core signaling complex (Gegner et al., 1992; Maddock and Shapiro, 1993; Abedrabbo et al., 2017; Piñas et al., 2018). The linkage of two chemoreceptor trimers within one core signaling complex is also dependent on the CheA-CheW interaction at interface 1 (Briegel et al., 2012, 2014b). Furthermore, this interaction leads to the control of CheA activity by chemoreceptor (Liu et al., 2012; Piñas et al., 2016). Some CheW proteins that are mutated in interface 1 cannot form an activated core signaling complex with CheA and chemoreceptor (Boukhvalova et al., 2002b). At interface 2, the interaction of CheW subdomain 1 and P5 subdomain 2 results in the formation of a large chemosensory array and positive cooperativity that allows small signals to be greatly amplified (Liu et al., 2012; Piñas et al., 2016). CheW proteins mutated in interface 2 weaken the connections between core signaling complexes, leading to more loosely packed chemoreceptor structures (Piñas et al., 2016). The interaction that occurs at interface 2 primarily links three core signaling complexes to form a hexagonal ring of chemoreceptor trimers of dimers. At the center of the hexagonal ring is a P5/CheW ring that contains three CheWs and three P5 domains of CheAs (Fig. 5). With the extension of the array, many hexagonal rings of chemoreceptor trimers are networked by several P5/CheW rings (Briegel et al., 2012; Briegel and Jensen, 2017). P5/CheW rings are the foundation of the arrays. Additionally, another feature named six-membered CheW rings in the array model have been recently confirmed (Liu et al., 2012; Cassidy et al., 2015). The CheW rings are analogous to the P5/CheW rings but do not directly interact with CheW or CheA in the core signaling complexes (Liu et al., 2012; Piñas et al., 2016). They are apparently formed through subdomain 1 and subdomain 2 interactions between the CheW molecules (Piñas et al., 2016). Such structure formed by six-membered CheW might provide additional interactions that link adjacent chemoreceptor trimers of dimers and reinforce the large chemosensory arrays (Cassidy et al., 2015).

4.2. Functions of CheV

CheV was first described in *B. subtilis* (Rosario et al., 1994). It acts as an alternative coupling protein and shares largely redundant ability with CheW but not identical in chemotaxis (Karatan et al., 2001; Abedrabbo et al., 2017). In all present experimental studies on the coupling proteins, CheV usually accompanies CheW (Rosario et al., 1994; Pittman et al., 2001; Wang et al., 2006; Lowenthal et al., 2009; Abedrabbo et al., 2017). Except for the case of *B. subtilis*, in which

CheW and CheV play nearly equal roles in chemotaxis signal transduction, CheV always plays an auxiliary role. Nonetheless, in *Salmonella enterica* or in *Helicobacter pylori,* the effect of *cheW*-deletion on chemotaxis is more severe than that of *cheV*-deletion (Rosario et al., 1994; Wang et al., 2006; Lowenthal et al., 2009). As for the protein itself, the main difference between CheV and CheW is that CheV possesses an auxiliary REC domain linking to its CheW domain. In *B. subtilis*, the CheW domain of CheV can act as the sole coupling protein to confer the *cheW-cheV* double-mutant near-normal chemotaxis; however, the strains that contain the CheW domain of CheV cannot adapt well to chemotactic stimulus after attractant stimulation (Rosario et al., 1994). However, the strains that contain wild-type CheW as the sole coupling protein showed normal adaptions, which suggests that the functions of the CheW domain of CheV and wild-type CheW are not identical (Karatan et al., 2001). The REC domain of CheV is required for resetting the chemotaxis system to its pre-stimuli state (Rosario et al., 1994; Karatan et al., 2001). The REC domain is homologous to proteins that are phosphorylated on a conserved aspartate (Rosario et al., 1994; Alexander et al., 2010). CheA is a likely candidate kinase to phosphorylate CheV in bacteria. In *H. pylori* and *B. subtilis*, CheV catalyzed the dephosphorylation of CheA (Karatan et al., 2001; Jimenez-Pearson et al., 2005), which implies that the REC domain might be involved in the dephosphorylation of CheA. Current views suggested that the REC domain might act as a phosphate sink to obtain the extra phosphoryl groups from the CheA P1 domain and, finally, lead to the normalization of the overall CheY-P concentration downstream of the chemotaxis system (Rosario et al., 1994; Alexander et al., 2010; Ortega and Zhulin, 2016). Unfortunately, few studies focused on the functions of CheV in recent years. The role of the REC domain in adaption and the precise interactions of CheV with CheA and chemoreceptors are still not well understood. Recently, one study suggested that CheV and CheW share the same binding spot on the chemoreceptor structure (Ortega and Zhulin, 2016). Another study showed that *H. pylori* CheV1 independently promotes CheA–chemoreceptor interactions *in vivo*, as well as CheW (Abedrabbo et al., 2017). Anyway, the function of CheV still needs further investigation.

5. Co-existence of multiple coupling proteins in one organism

A mass of phylogenomics analysis and experimental studies suggested that many additional chemotaxis components in conjugation with the core components make up more complex chemotaxis systems in microbes (Wuichet and Zhulin, 2010; Walukiewicz et al., 2014; Moon et al., 2016; Ortega and Zhulin, 2016; Scharf et al., 2016; Bi and Sourjik, 2018). The additional chemotaxis components include certain phosphatases, hybrid proteins, homologs of some core components, and regulatory proteins that are involved in adaption. For example, *E. coli* has a single coupling protein CheW, but multiple coupling proteins are common in many other bacteria and archaea (Alexander et al., 2010; Buchan et al., 2010). The chemotaxis systems of these microbes are more complex than that of *E.coli*. Many of them even have multiple chemotaxis systems (Wuichet and Zhulin, 2010; Sampedro et al., 2015; Mukherjee et al., 2016; Scharf et al., 2016; Gasperotti et al., 2018).

5.1. Multiple coupling-protein-encoding genes exist in different ways

There is increasing evidence that different coupling proteins incorporate into different chemotaxis signal transduction pathways in one organism (Porter et al., 2008; Zhang et al., 2012; Mukherjee et al., 2016). For instance, *Azospirillum brasilense*, a diazotrophic motile soil bacterium, is capable of colonizing the rhizosphere of cereals (Bible et al., 2012). There are five *cheW* homologs that are distributed in four distinct chemotaxis operons of *A. brasilense*: *cheW1* in *che1*; *cheW2* in *che2*; *cheW3a* and *cheW3b* in *che3*; and *cheW4* in *che4* (Mukherjee et al., 2016). Che1 is responsible for regulating transient increases in swimming speed in response to attractants, but it plays a minor role in chemotaxis and root surface colonization (Bible et al., 2012). In contrast, Che4 is essential for all chemotaxis responses in *A. brasilense* and for competitive wheat root surface colonization (Mukherjee et al., 2016). Another example is found in *R. sphaeroides*, a metabolically diverse purple, non-sulfur bacterium (Martin et al., 2001). *R. sphaeroides* has four homologous *cheW* genes that are organized in three chemotaxis operons (*che* Op): $cheW_1$ in *che* Op_1, $cheW_2$ and $cheW_3$ in *che* Op_2, $cheW_4$ in *che* Op_3 (Porter et al., 2008). Under laboratory conditions, only *che* Op_2 and *che* Op_3 are involved in chemotaxis of *R. sphaeroides* (Porter et al., 2002). The encoded products of those genes in the *che* Op_2, in conjugation with transmembrane chemoreceptors, form polar chemotaxis clusters, while the encoded products of the genes in *che* Op_3 establish cytoplasmic chemotaxis clusters with cytoplasmic chemoreceptors (Wadhams et al., 2003; Porter et al., 2008). Interestingly, the polar and cytoplasmic chemotaxis clusters were thought to sense extracellular and intracellular signals, respectively (Porter et al., 2008). Hence, we believe that one important reason for the emergence of multiple coupling proteins in one organism is to help the organism to assemble multiple chemotaxis systems that are involved in different environmental stimuli. This flexible strategy can provide organisms with survival advantages and strengthen their adaptation to multiple environments.

Intriguingly, in most completely sequenced bacteria and archaea with one or more chemotaxis systems, the number of coupling proteins (including CheW and CheV) is more than that of CheA (Alexander et al., 2010), which indicates that multiple coupling proteins may be incorporated in one chemotaxis signal transduction pathway. This special but widespread situation has been identified in several bacteria with one or more chemotaxis systems. Based on previous studies, multiple coupling proteins are mainly divided into three cases. (i) Only one of the multiple coupling proteins is essential for chemotaxis, and the others are dispensable or conditionally affect chemotactic responses. For example, *Vibrio cholerae* has three CheWs, but only CheW-1 participates in the chemotaxis signal transduction (Butler et al., 2006). Another example is CheW2 and CheW3 in *R. sphaeroides*. Under photoheterotrophic condition, CheW2 and CheW3 are required for the normal localization of McpG and for normal chemotactic response. Under aerobic condition, CheW2 is the only coupling protein required for chemotaxis (Martin et al., 2001). (ii) Two coupling proteins coexist in one chemotaxis signal transduction pathway and act in a redundant manner. Deleting any one of two coupling proteins will attenuate the bacterial chemotactic response but not abolish it, such as CheW and CheV in *B. subtilis* (Rosario et al., 1994), $CheW_1$ and $CheW_2$ in *A. tumefaciens* (Huang et al., 2018). In this case, one coupling protein can partially substitute for another coupling protein, but the optimal chemotactic responses of bacteria require the cooperation of two coupling proteins. (iii) Two of multiple coupling proteins coexist in one chemotaxis signal transduction pathway and act in a non-redundant manner. Deleting any one of two coupling protein will abrogate the chemotactic response, such as CheW1 and CheW3 in *Borrelia burgdorferi* (Zhang et al., 2012) and CheW and CheV1 in *H. pylori* (Abedrabbo et al., 2017). In other words, any of these coupling proteins in chemotaxis is unique and irreplaceable.

5.2. Regulation and cooperation of multiple coupling proteins in one chemotaxis system

Until now, the differences of multiple coupling proteins in one chemotaxis system are still not well understood. There is no unequivocal result to explain the reason for having more than one coupling proteins in one chemotaxis system. In our opinion, gene expression levels, protein affinity, and the cooperation of multiple coupling proteins might be different in these bacteria that have multiple coupling proteins in one chemotaxis signal transduction pathway, and these differences might directly affect chemotaxis.

5.2.1. The expressions of multiple coupling proteins affect chemotaxis

Generally, the genes that encode the core components of chemotaxis systems often appear in bacterial genomes in the form of operons or clusters (Wuichet and Zhulin, 2010). However, it is very common in these bacteria with multiple coupling proteins that some coupling-protein-encoding genes distribute in other unlinked loci and not in the chemotaxis operon or cluster (Zhang et al., 2012; Huang et al., 2018). The transcription and translation of these genes are out of sync with the others in the chemotaxis operon. The expression levels of multiple coupling proteins might be different. Appropriate expression levels of coupling proteins is important for chemotaxis. In *E. coli*, the high expression level of CheW disrupts chemoreceptor signaling arrays, which leads to the inhibition of chemotactic response (Liu and Parkinson, 1989; Cardozo et al., 2010). Excess $CheW_1$ or $CheW_2$ also impairs *A. tumefaciens* chemotaxis (Huang et al., 2018). It is still unknown whether excess CheV affects chemotaxis. Since all identified CheV proteins share largely redundant functions with CheW, we speculate that overexpression of CheV may also exert similar negative effects on chemotaxis.

In wild-type bacterial cells, different coupling-protein-encoding genes are controlled by different promoters, which implies that these genes might be regulated by different regulatory elements. In *A. tumefaciens*, the ratio of two CheW proteins varies in different growth phases, which implies that the promoters of two *cheW* genes may be regulated by different transcription factors (Huang et al., 2018). A previous study had suggested that the *H. pylori cheV1* gene was under the control of sigma-28, meanwhile the other *cheV* and chemotaxis genes were under the control of the housekeeping sigma factor (Sharma et al., 2010). As a result, verifying the expression of multiple coupling proteins is a good approach that may help us to gain insight into the difference of multiple coupling proteins in one chemotaxis signal transduction pathway and the dynamic regulation of chemotaxis systems.

5.2.2. The different affinities of multiple coupling proteins with CheA and chemoreceptors might affect chemotaxis

In *A. tumefaciens*, even under the same cellular stoichiometry, $CheW_1$ and $CheW_2$ still have different abilities to recover the chemotactic response of the mutant lacking coupling protein. The affinities of two CheWs with CheA and the chemoreceptors appear to be an important reason (Huang et al., 2018). If the affinities of two coupling proteins with CheA and chemoreceptors are different, the chemosensory arrays assembled by any single coupling protein might be less efficient than a canonical protein. Moreover, some chemoreceptors might interact with particular coupling proteins, which suggests that different coupling protein may be responsible for coupling CheA to different chemoreceptors (Parrish et al., 2007; Hartley-Tassell et al., 2010). CheW and CheV might also have an affinity bias towards different chemoreceptors (Ortega and Zhulin, 2016). Hence, it is possible that different coupling proteins may identify special chemoreceptors in some chemotaxis systems. Generally, bacteria use different chemoreceptors to sense different environmental signals. The environmental signals sensed by chemoreceptors are various and mainly include attractants, repellents, antagonists, and other chemical effectors (Bi and Lai, 2015). In nature, attractants can further be divided into two types: primary nutrients and pure signal molecules (Bi and Lai, 2015). Many pure signal molecules that are released by bacteria or hosts play an important role in modulating the physiological activity and pathogenicity of bacteria, such as interspecies quorum signal autoinducer 2 of bacteria (Pereira et al., 2013; Laganenka et al., 2016) and plant-released agrobacterial virulence gene inducer acetosyringone (Yang et al., 2015; Guo et al., 2017). If this case is true, different coupling proteins should have the ability to distinguish these different signals from different chemoreceptors and modulate different biological processes of bacteria.

5.2.3. The cooperation of multiple coupling proteins in the chemosensory unit might affect chemotaxis

The core signaling complex in the large chemosensory arrays of *E. coli* contains two chemoreceptor trimers of dimers, two CheW molecules, and one CheA dimer. Each chemoreceptor trimer is linked to one subunit of the dimer CheA molecule by one CheW molecule (Liu et al., 2012; Piñas et al., 2016). In other bacteria with multiple coupling proteins in one chemotaxis signal transduction pathway, the combination mode of the coupling proteins in the functional unit might be different. As we have mentioned above, two distinct coupling proteins that coexist in one chemotaxis signal transduction pathway can act in a redundant or non-redundant manner. Therefore, we propose that the combination mode of these coupling proteins in the core signaling complex may have two cases: (i) any of two distinct coupling protein molecules can form the core signaling complex with CheA and chemoreceptors. Two coupling protein molecules in the complex may be the same or not. This case can be found in the chemotaxis system in which multiple coupling proteins act in a redundant manner. Deleting one of these coupling proteins impaired chemotaxis but does not abrogate it, which implies that they cannot completely substitute each other. Recently, this case has been identified in *A. tumefaciens* through observing the localization of eGFP-CheA in different *cheW*-deletion mutants (Huang et al., 2018). (ii) None of two distinct coupling proteins can form the core signaling complex with CheA and chemoreceptors alone. They are more likely to coexist in one core signaling complex. This case appears in the chemotaxis system in which multiple coupling proteins act in a non-redundant manner. Deficiency of any coupling protein leads to no chemotactic phenotype, which implies that the core signaling complex is non-functional. It was suggested that all coupling proteins are required for the core signaling complex to form a robust spatial conformation and to conduct signal transduction. For example, in *H. pylori*, loss of CheW, CheV1, or both abrogates the formation of the chemoreceptor-CheA complex at the cell poles (Abedrabbo et al., 2017). Deletion of CheW1 or CheW3 in *B. burgdorferi* also affects chemoreceptor assembly at the cell poles (Zhang et al., 2012).

6. Perspectives for the study of coupling proteins

Coupling proteins have been identified in several bacteria. As the core component of chemotaxis system, the coupling protein CheW mainly has three functions: holding the histidine kinase CheA and chemoreceptors together to form ternary signaling complexes, enabling chemoreceptors to modulate CheA activity, and promoting the connection between the core signaling complexes. Another coupling protein, CheV, also has similar functions (Rosario et al., 1994; Abedrabbo et al., 2017). The functions of the coupling proteins appear to be relatively clear; however, some questions remain to be answered, such as the role of the REC domain of CheV and CheR-like domain of some CheW in chemotaxis. Due to the fusion of these additional domains to the CheW domain in some coupling proteins, it is possible that these unusual coupling proteins might interact with other known or unknown proteins to perform new functions in chemotaxis or in other processes. In addition, a large number of bacterial chemotaxis systems possess multiple coupling proteins; however, the authentic difference of the function of these coupling proteins in these chemotaxis systems is still known. Recently, one study suggested that multiple coupling proteins may conduct more flexible and complex chemotaxis systems that are distinguishable from the canonical chemotaxis system (Abedrabbo et al., 2017), which is meaningful to allow bacteria to deal with constantly changing environmental stimuli. Further studies on multiple coupling proteins in one chemotaxis system will not only expand our knowledge of bacterial chemotaxis systems but also help us to understand the mechanism of chemotactic behaviors in different microbes.

Conflict of interests

The authors declare no conflict of interest.

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