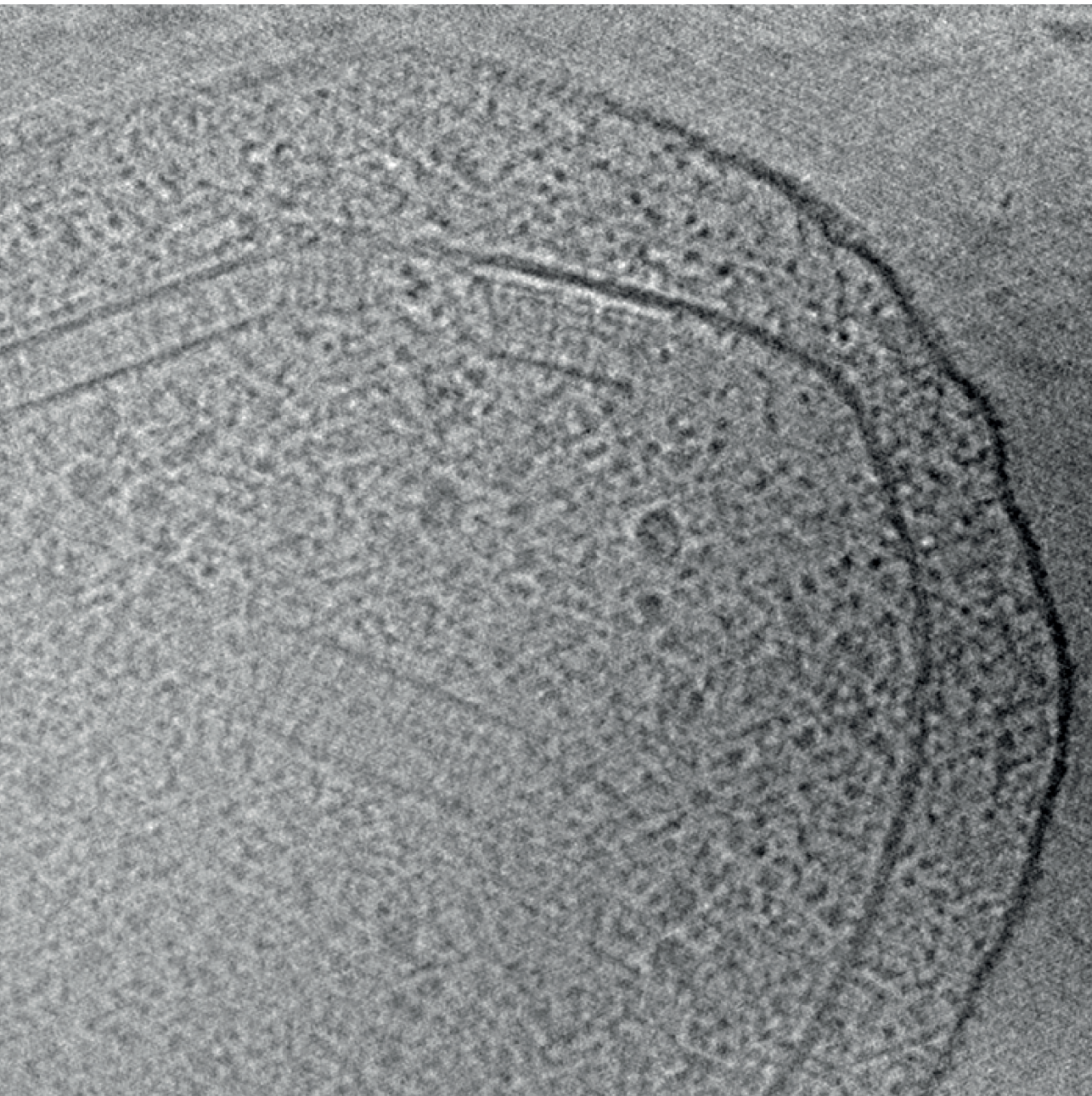
Cryo electron tomography studies of bacterial chemosensory arrays

Yang W.

CHAPTER 2



Diversity in bacteria chemosensory arrays

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Abstract

Chemotaxis is crucial to the survival of bacteria, and the signaling systems associated with it exhibit a high level of evolutionary conservation. The architecture of the chemosensory array and the signal transduction mechanisms have been extensively studied in *Escherichia coli*. More recent studies have revealed a vast diversity of the chemosensory system among bacteria. Unlike *E. coli*, some bacteria assemble more than one chemosensory array and respond to a broader spectrum of environmental and internal stimuli. These chemosensory arrays exhibit a great variability in terms of protein composition, cellular localization and functional variability. Here, we present recent findings that emphasize the extent of diversity in chemosensory arrays and highlight the importance of studying chemosensory arrays in bacteria other than the common model organisms.

Introduction

For a bacterium to move toward a more-favorable location, the cell must constantly sense its surroundings and respond to nutrient and repellent gradients. The ability of microbes to control their motility in response to their chemical environment is called chemotaxis (61). This behavior was first described, and is best understood, in the model organism *Escherichia coli* (2, 6, 62). *E. coli* possesses a single chemosensory pathway that consists of 11 proteins. Within this pathway, four different membrane-bound chemoreceptors (methyl-accepting chemotaxis proteins (MCPs)) and the redox receptor Aer bind directly to a histidine kinase CheA and a coupling protein CheW (also referred to as scaffolding protein or adaptor protein). Together, these proteins assemble into macromolecular complexes known as chemosensory arrays. Chemosensory arrays perceive environmental stimuli and collectively use them to control the phosphorylation level of receiver proteins (CheY and CheB), which mediate motor control and sensory adaptation, respectively (63). Because of their simplicity, chemosensory arrays in *E. coli* have long been the paradigm for understanding the molecular mechanisms of signaling transduction (63, 64) and the architecture of the arrays (43, 44).

Chemotaxis in *Escherichia coli*

Chemotaxis enables *E. coli* to migrate toward attractants and away from repellents. *E. coli* has multiple petrichous flagella powered by flagellar motors in the cytoplasm (65, 66). By default, the flagellar motors rotate counter-clockwise (CCW) and the left-handed helical filaments form a bundle that propels the cell in a more-or-less straight swim (run). When the flagellar motors switch their rotation direction from CCW to clockwise (CW), the flagellar bundle disassembles and the cell tumbles (13). In the presence of a stimulus, runs are lengthened and tumbles occur less frequently (Fig. 1A).

E. coli possesses a single chemotaxis pathway that consists of five different membrane-bound chemoreceptors and six cytoplasmic chemotaxis proteins (Fig. IB) (61). Attractants and repellents bind to the ligand-binding domain of the MCPs either directly or via periplasmic binding proteins (67, 68). With the assistance of the coupling protein CheW, a stimulus is transmitted to the histidine kinase (CheA) (64). Upon a negative stimulus, such as repellents binding to the chemoreceptors, CheA autophosphorylates the response regulator CheY. Phosphorylated CheY (CheY-P) diffuses through the cytoplasm and binds to the flagellar motors to bias their rotation from the default CCW direction to CW, resulting in tumbling. CheY-P is quickly dephosphorylated by the phosphate CheZ, which keeps the overall CheY-P level closely synchronized with the CheA activity. CheA also phosphorylates the methylesterase, CheB, to activate it.

Together with the methyltransferase CheR, CheB reversibly modifies the methylation state of the chemoreceptors in order to maintain an adapted sensitivity (63).

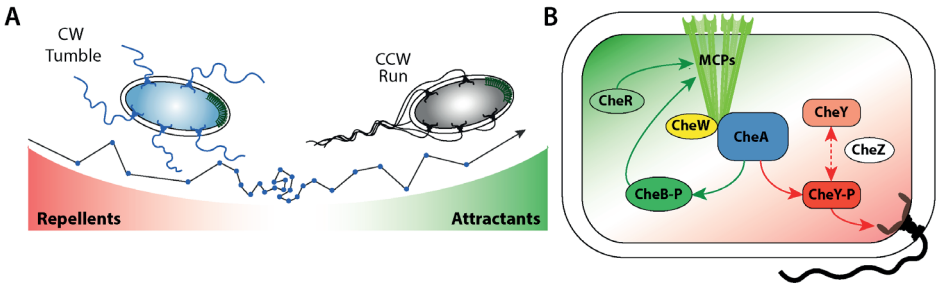

Figure 1. Chemotaxis and chemosensory pathway in *Escherichia coli*. (A) Chemotaxis enables *E. coli* to swim toward favorable environments through a combination of smooth runs when flagellar motors rotate couter-clockwise (black lines) and tumbles when flagellar motor rotate close wise (blue dots). The duration and frequency of the runs and tumbles are regulated by the chemosensory arrays (green patches of pillars at the cell poles) (13-15, 69). (B) Chemosensory pathway mediates both the flagellar motor control (red arrows) and the chemoreceptor sensory adaptation (green arrows).

Chemosensory array architecture in *E. coli*

The chemoreceptor homodimers readily form trimers-of-dimers through the interaction at their cytoplasmic tips (46). To form the signaling core units, receptor trimers bind both the kinase and coupling protein following a strict stoichiometry of 6 receptor trimers-of-dimers: 1 dimeric CheA: 2 monomeric CheWs (Fig. 2A) (46, 94-96). Among the five domains of the kinase CheA, the P5 domain directly binds to the receptor trimers (97-99). The P5 domain topologically resembles two tandem SH3 domains (47). Similarly, CheW is composed of two β-barrels sandwiching a hydrophobic core (100). CheW and P5 bind each other in an alternating order and form pseudo 6-fold symmetric rings that links core units together (Fig. 2B). Within the individual signaling core unit, CheW and P5 establish interface 1 (101); among the signaling core units, CheW and P5 form interface 2 which is crucial for forming the extended array lattice (92).

Through the networks of CheAs and CheWs, the receptor trimers-of-dimers are arranged into a rigid hexagonal pattern with a spacing of ~12 nm. This characteristic hexagonal packing order has become the hallmark for recognizing the arrays and for assessing the structural integrity of the array architecture (Fig. 2C)(51). In the side view, arrays are distinguishable as a continuous layer parallel to the inner membrane (Fig.2D). The chemoreceptors can be seen as pillar-like densities that extend between the baseplate and the membrane. The side view reveals the length of the cytoplasmic fraction of the receptors that co-exist in the arrays and gives important insight into which receptors make up the specific array (51).

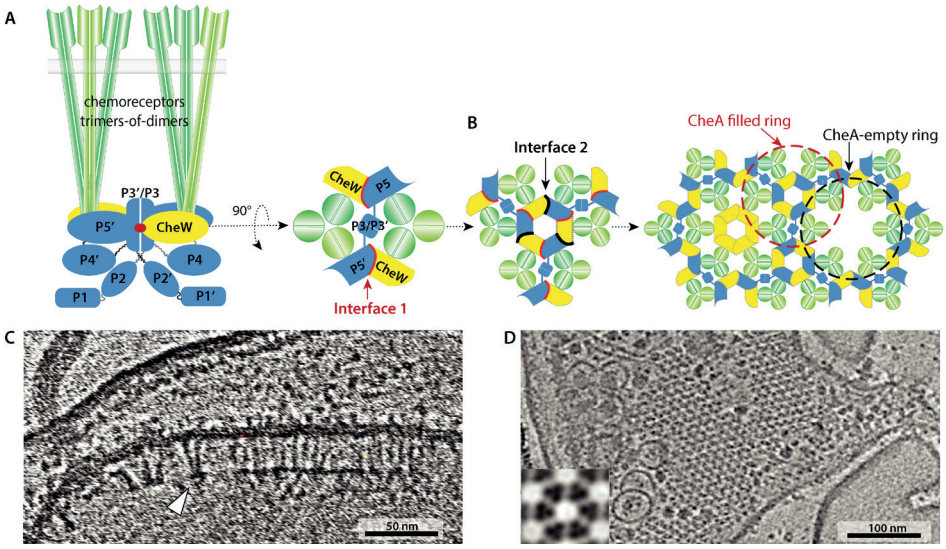

Figure 2. Architecture of chemosensory arrays in *E. coli*. (A) The side view and the top-down view of a signaling core unit is depicted in cartoon. The interface 1 within the core unit is marked with a red line. (B) Repeats of the signaling core units assemble into arrays through interface 2 marked in black lines. Red and black circles in dash lines show CheA filled and CheA-empty rings in baseplate, respectively. (C) Tomographic image of the chemosensory arrays in the side view near a flagellar motor in a lysed *E. coli* cell. The picture is modified from (102). (D) Cryo-electron tomography (cryo-ET) image of chemosensory arrays in the top view in a lysed *E. coli* cell. The insert panel shows subtomogram averaging of the hexagonally packed receptor trimers-of-dimers. The picture is adapted from (58).

Beyond the bacterial chemotaxis paradigm

More than half of all motile bacteria have multiple chemotaxis systems (70). In those bacteria, it appears that at least one chemotaxis pathway is dedicated to the control of the flagella, whereas other chemotaxis pathways may be involved in the regulation of a wide range of programmed cellular events (71). In the past decade, the field of chemotaxis research has broadened its focus to include bacterial species with more-complex chemotaxis systems. These studies have given insight into, among other things, the role of chemotaxis in the infectivity of pathogenic bacteria (71-78). Additionally, we have gained new insights into the adaptability of chemosensory arrays. Bacteria that experience stress conditions and/or transition to a sessile lifestyle remodel their chemotaxis arrays. For example, the expression of specific chemoreceptors are up-regulated in order to respond appropriately to new metabolic needs and environmental conditions (79-83). Moreover, the chemotaxis system in the archaea likely originated via horizontal gene transfer from bacteria and then further adapted to form a unique system that controls the archaellum (55). These studies highlight the great diversity of

chemotaxis systems and the role of chemosensory arrays beyond the paradigm found in *E. coli* (84, 85). However, the physiological relevance of many chemotaxis pathways that diverge from the *E. coli* system remains unclear.

The signaling mechanism of chemosensory arrays in *E. coli* has been described in great detail (61, 63, 64). In this review, we focus on novel aspects of chemosensory arrays that have recently been discovered. There is a great diversity in composition and cellular localization among prokaryotic chemosensory arrays. The co-existence of multiple arrays within a single species raises new questions about the possibility of crosstalk between chemotaxis systems and about how these systems are structurally and functionally separated at the level of chemosensory arrays. We will first describe our current understanding of the array architecture in *E. coli*, followed by an overview of the variability of chemosensory arrays among prokaryotes. We highlight the capability of array structures to tolerate compositional variability and speculate that this ability may facilitate quick adaptation to a board repertoire of sensory inputs.

Chemosensory arrays appear structurally similar despite diverse composition

E. coli has a single chemotaxis pathway with five chemoreceptors that collectively sense the environment and control cellular motility. An "average" bacterial genome contains 14 chemoreceptor genes (86), but this number is highly variable and not proportional to the genome size. Bacteria that periodically encounter stress conditions or need to adapt to multiple or changing ecological niches tend to possess more receptor genes (85). For instance, *Magnetospirillum magnetotacticum*, which is capable of magnetotaxis, has 59 different MCPs (87, 88). A large structural and functional diversity exists among MCPs, especially in the ligand-binding domains that are responsible for sensing attractants and repellents (Fig. 3A). Genomic analysis of known MCP sequences predicts nearly one hundred distinct types of ligand-binding domains that provide an extremely broad sensory spectrum (Fig. 3B) (89, 90). The cytoplasmic signaling domains of the chemoreceptors are less diverse. Based on the number and organization of the seven-residue heptad repeats in the signaling domain, seven different major classes can be distinguished for MCPs (Fig. 3C)(91).

Many bacteria also possess additional cytoplasmic chemotaxis proteins that do not have counterparts in *E. coli*. These bring extra functional complexity to the signaling pathways. Furthermore, more than half of motile bacteria have several chemotaxis gene clusters in their genome, presumably representing separate chemotaxis systems. Based on a classification using a systems-level phylogenomics approach, 19 distinctive chemotaxis systems are currently characterized (70).

Despite the compositional diversity of arrays discussed above, a hexagonal packing order of chemoreceptors in the chemosensory array is conserved across all chemotactic prokaryotes examined thus far (51, 55). These analyses strongly suggest that the chemosensory array is an evolutionarily optimized structure with unique advantages to facilitate the cellular responses. The array serves as a platform to accommodate inputs from a variety of receptors simultaneously that ensures effective communication with the cytoplasmic components of the chemotaxis pathway. Furthermore, the conserved array architecture is thought to provide the structural basis for chemotactic cooperativity and signal amplification via allosteric interactions within the arrays (92, 93).

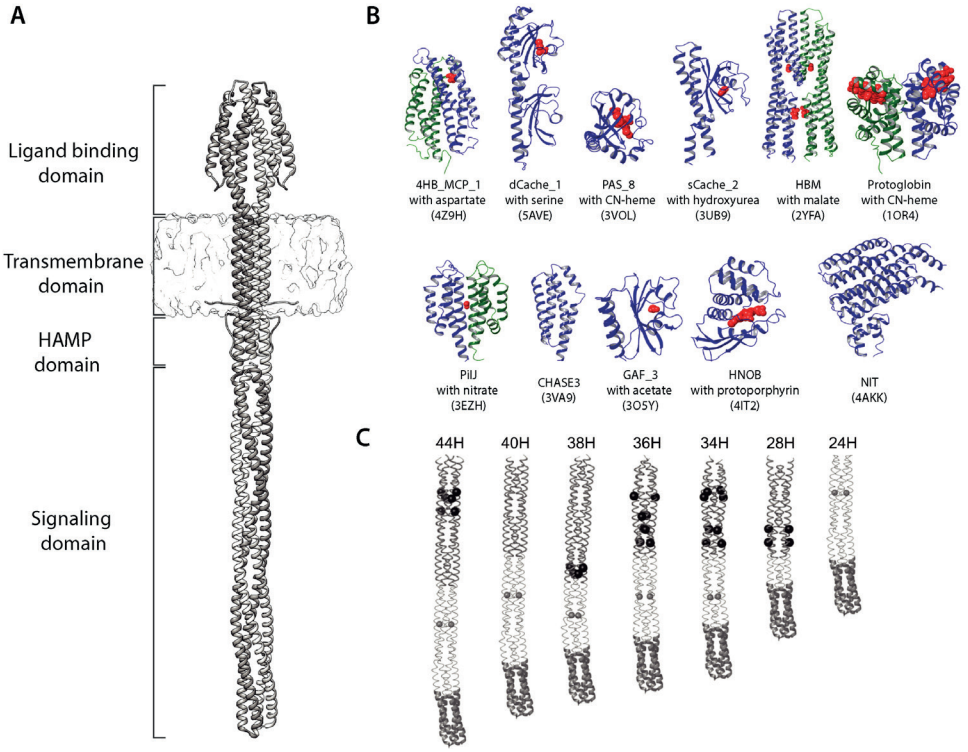

Figure 3. Structural diversity of chemoreceptors. (A) Model of the homodimer of Tsr serine receptor in *E. coli* with different functional modules marked. (B) The structural diversity of the ligand-binding domains. Ligands are colored in red. Ligand –binding domains known to be dimeric are depicted in green and blue for the different chains. Panel B is adapted from (89) with permission. (C) Models of different signaling domain classes. Within the signaling domain, the sensory adaptation subdomain, flexible bundle subdomain and the hairpin sub domain are colored in light gray, white and dark gray, respectively. Methylation sites are highlighted with black spheres to show the different patterns of methylation for chemoreceptor classes displaying different heptad numbers. Gray spheres show the conserved glycine residues located in the center of flexible bundle. Figure is adapted from (91).

Alternative architectures of array assembly

The molecular arrangement of the chemosensory arrays is best understood in *E. coli* [9,10]. Assembly of chemosensory arrays depends on the stringent regulation of the stoichiometric ratio of receptors, kinases and coupling proteins. Overexpression of one component can lead to the formation of alternative protein complexes (58). For example, when there are no baseplate components present, the cytoplasmic tips of the receptors trimers can associate with each other and result in "zippers," or micelle-like structures (Fig. 4A-B). Together with the coupling protein and kinase, truncated chemoreceptors lacking transmembrane regions are capable of forming an array "sandwich" *in vitro* in the presence of crowding agents (Fig. 4C)(58, 103).

This "sandwich" structure is very similar to the entirely cytosolic chemosensory arrays observed in *Vibrio cholerae*, *Rhodobacter sphaeroides* and the archaeon *Methanoregula formicica* (55, 105). Approximately 14% of all chemoreceptors are predicted to be cytoplasmic (106). The native structure of cytoplasmic arrays observed so far show that two hexagonal lattices of receptor trimers-of-dimers interact head-to-head and sandwich between two CheA : CheW baseplates (Fig. 4D). The cytoplasmic arrays in *V. cholerae* display significant rigidity and are nearly flat, unlike membrane-bound arrays that follow the curvature of the inner membrane, or the flexible cytoplasmic arrays found in *R. sphaeroides* and *M. formicica*. This rigidity is likely due to the presence of the protein DosM that connects the baseplates to stabilize the arrays (104).

Multiple coupling proteins can coexist in the baseplate

As stated previously, chemotaxis proteins, including components that are directly integrated into the chemosensory arrays, exhibit a great diversity among bacteria (107). In addition to CheW, the protein CheV is encoded in 60% of all chemotactic prokaryotes (70). CheV is a chimeric protein consisting of a CheW-like adaptor domain and CheY-like response regulator domain. CheV can integrate into the baseplate and interact directly with receptors and CheA (108). Additionally, CheV can be phosphorylated by CheA, a process that is necessary for sensory adaption in *Bacillus subtilis* (109). A recent study reveals that genomes that contain CheV typically encode about four times more MCPs than genomes without CheVs (110). CheV has been proposed to co-evolve with a subpopulation of MCPs in order to preserve the protein interaction interfaces that are crucial for the incorporation and function of these MCPs in the arrays. This hypothesis was experimentally confirmed in *Campylobacter jejuni* (111).

In *V. cholerae*, the protein ParP also integrates into the baseplate to promote array formation and localization (Fig. 5A)(112). ParP contains a SH3-like domain with a similar topological architecture as the P5 domain of CheA and CheW, which enables it to integrate into the baseplate. In addition to promoting array formation, ParP also

facilitates array localization near the flagellar pole with the assistance of the ParC protein (113). This control of array positioning may allow a tighter control of the single polar motor of *V. cholerae*, which may be particularly important for bacteria that rely on a highly localized population of CheY molecules for chemotaxis (114). Therefore, certain components of the baseplate can actively regulate array positioning in addition to being part of the baseplate scaffold.

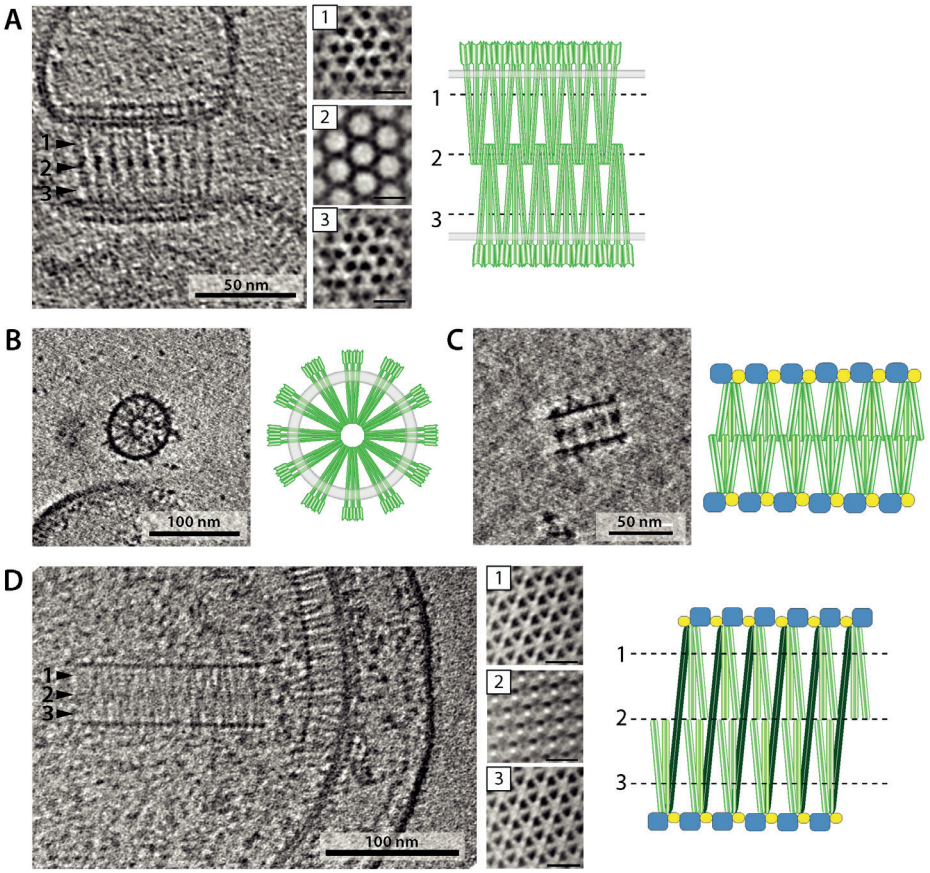

Figure 4. Chemoreceptor arrays in vitro and alternative chemosensory arrays *in vitro* and *in vivo*. In the absent of other chemotaxis proteins, membrane-bound chemoreceptors form "zipper" like arrays (A) or micelle-like structures (B) observed in cryo-ET images. Cryo-ET images are adapted from (58). (C) In vitro, truncated Tar receptors form "sandwich" structures consisting two layers of arrays flanked by CheA and CheW. Image adapted from (103). (D) The native cytoplasmic chemosensroy arrays in a *V. cholerae* cell shown in tomographic image. Cryo-ET image is adapted from (104). Insets 1-3, in both panel A and C, show the subtomogram averaging results corresponding to the cross sections of the arrays indicated in the tomographic image and the cartoon. The scale bars for insets 1-3 in panel A are 10 nm; the scale bars for insets 1-3 in panel D are 20 nm. For all cartoon illustrations, receptors are colored in green; kinases are in blue; the coupling proteins are in yellow and the membrane are depicted in gray. The DosM receptor in panel D is highlighted in dark green.

Compositional variability in the baseplate

The variety of baseplate components raises the interesting question of how bacteria utilize the diverse coupling proteins. For example, *V. cholerae* is capable of increasing CheV abundance in the baseplate to compensate for the absence of CheA in the F6 arrays (102). Fluorescence microscopy revealed that, under standard growth conditions, only the CheV2 protein encoded within the F6 gene cluster form localized foci at the cell pole. However, in the absence of CheA, CheV1 and CheV4 also form foci at the cell pole (Fig. 5B), demonstrating that the baseplate composition is adaptable. CheV3 remains diffuse in the cytoplasmic regardless of the abundance of CheA. This could mean that CheV3 interacts specifically with a particular MCP that is not expressed under the conditions tested (110) and is therefore not incorporated into the baseplate. This variability in structure is likely explained by the presence of SH3-like domains that differ in the coupling proteins, which allows for the dynamic swapping of the coupling proteins, allowing the incorporation of their specific MCPs. Thus, by adjusting the baseplate elements, bacteria can rapidly regulate the sensory functions of the chemosensory arrays to better suit the current environment.

Different baseplate stoichiometry

The stoichiometry of array components has been determined in *E. coli* (94). The baseplate has a ratio of monomeric CheA and monomeric CheW that fluctuates between 1: 1 and 1: 2. The latter ratio is in agreement with the extreme scenario where all CheA-empty rings are fully occupied with CheW hexamers. An increased abundance of CheW hexamers can be promoted *in vitro* by addition of excess CheW (45). In comparison to other bacteria, *E. coli* appears to have an unusually high ratio of CheA in the baseplate. In *B. subtilis*, the ratio of the baseplate components for CheA : CheW : CheV is 1:1:3 (115). In the F6 arrays of *V. cholerae*, there is even fewer CheA, with a CheA : CheW : CheV stoichiometry of 1:7:2 (102). Additionally, in *V. cholerae*, a small number of ParP molecules also integrate into the baseplate, suggesting that the baseplate consists of more coupling proteins than kinases. In *B. subtilis*, the ratio between receptor dimers and dimeric CheA was determined as 23 to 1, which is about four times higher than the ratio in *E. coli*, where the ratio is 6 to 1. Thus, in *B. subtilis* and *V. cholerae*, many receptors in the array bind solely to the coupling proteins.

The low abundance of kinase in the arrays of some species raises the question of whether the concept of core unit consisting of two receptor trimers-of-dimers, one CheA dimer, and two CheWs is applicable for those bacteria. In order to include a variety of baseplate components, Alvarado *et al.* proposed a variety of possible core unit schemes for the F6 arrays in *V. cholerae* (112). Alternatively, the core unit may be specific to *E. coli* arrays because of its high kinase abundance, whereas in most arrays receptor trimers-of-dimers are capable of integrating into the arrays as long as the

appropriate coupling protein is attached to its hairpin tips.

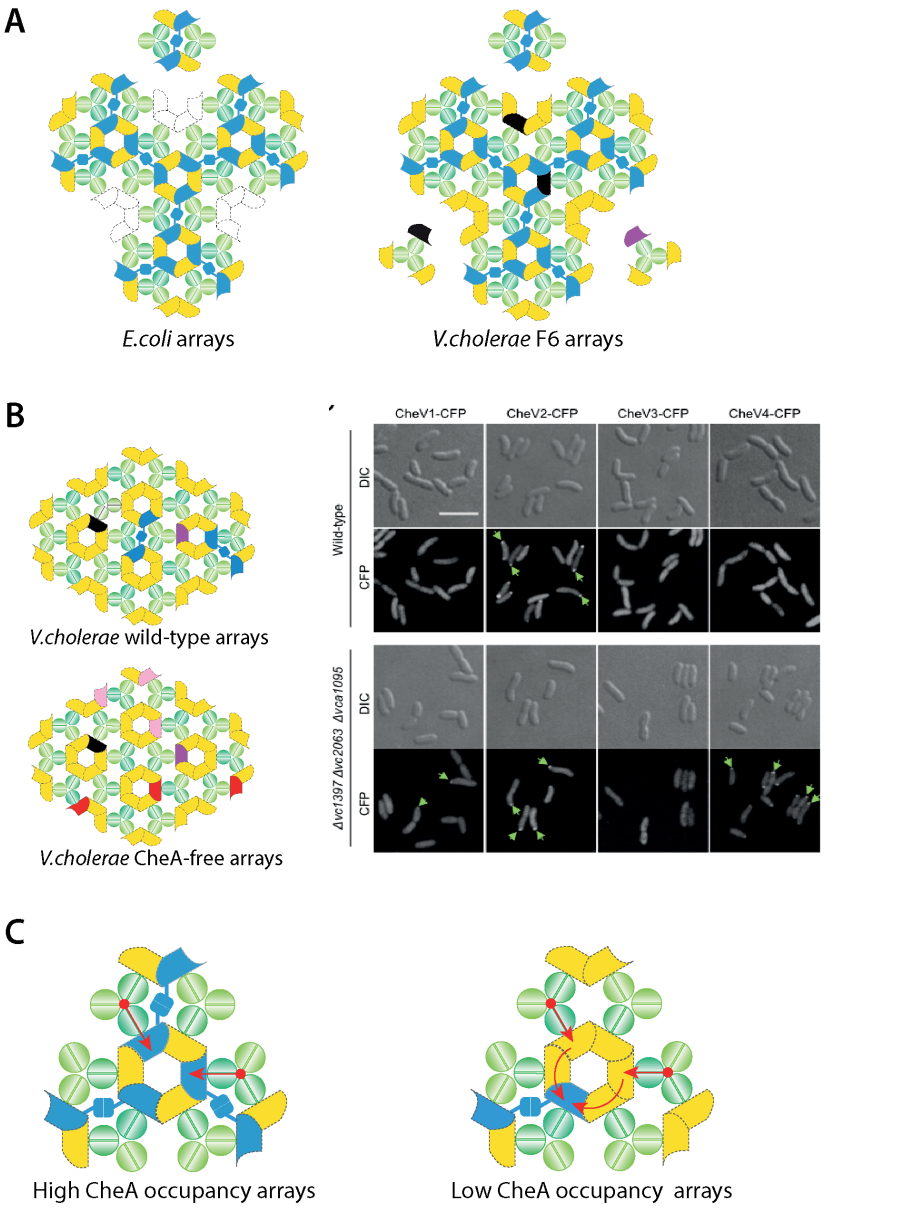

Figure 5. The baseplate variability of chemosensory arrays. (A) Comparison between chemosensory arrays in *E. coli* arrays (left) and the F6 arrays in *V. cholerae* (right). In V.cholerae, both ParP (black) and CheV (magenta) can directly integrate into the baseplate bringing in receptor trimer-of-dimers. (B) Cartoons (left) illustrates that more CheV proteins intergrate into the F6 arrays in *V. cholerae* when CheA is absent. Fluorescence microscopy images (right) show that in wild-type arrays (top 2 rows) only CFP-tagged CheV2 foci were observed at the cell poles. The foci of CFP-tagged CheV1 and CheV4 emerged at cell poles only when CheA

is absent (bottom 2 rows). The image is adapted from (102). CheV1, CheV2 and CheV4 are colored in red, magenta and pink, respectively, for the cartoons. (C) Arrays with low CheA occupancy (right) potentially encourage a higher cooperativity compared to arrays with a high CheA occupancy (left). Red dots represent receptors sensing a stimulus; red arrows indicate the possible direction of signal propagation in the baseplate.

A higher cooperativity among the receptors may also be required for species with a low abundance of kinase in chemosensory arrays. In *E. coli*, every CheA is directly in contact with a receptor trimers-of-dimers. In contrast, a single CheA in *V. cholerae* is assigned to an average of 6 receptor trimers-of-dimers, based on the stoichiometry data (102). Therefore, in *V. cholerae*, the signaling state of the receptors is likely to propagate horizontally through one or multiple multiple interfaces 2 before it can reach the nearest CheA (Fig. 5C). As interface 2 is considered to facilitate signal amplification and cooperativity, a long-range horizontal signaling propagation is potentially crucial for integration of diverse signaling inputs from a collection of various receptors and the proper kinase control. In arrays with a low kinase abundance and a high diversity in chemoreceptors, a higher signaling cooperativety is expected to facilitate the large repertoire of sensory inputs.

Chemoreceptor clustering depends on receptor length

The arrays undergo stochastic self-assembly and spontaneously nucleate new clusters that tend to collide and fuse with the cluster already localized at the cell pole (116). This often results in large polar chemosensory arrays and multiple small lateral clusters (117). However, not all receptors are expected to cluster into a single array. For membrane-bound arrays, the physical length of receptors predicts whether receptors can incorporate into the same chemotaxis array (51, 91). The intracellular length of chemoreceptors is influenced by the presence of several components, including the number of heptad repeats in the signaling domain, the number of the HAMP domains, the presence or absence of the PAS domains, and additional linker regions (118-120).
In *E. coli*, all five chemoreceptors share a 36H signaling domain and possess the same physical length, and they indeed cluster together into a single array. Experiments have shown that the wild-type and artificially shortened receptors in *E. coli* cluster into separate arrays in a length-dependent manner and function separately (121). In *V. cholerae* and other bacteria, polarly positioned but distinctively separate arrays exist naturally, as shown in the tomographic image in Figure 6 (122). The short and long membrane-associated arrays are assigned as F6 and F7 arrays, respectively. The F6 arrays contain 40H receptors whose ligand-binding domains can be recognized in the periplasm. In contrast, the F7 arrays do not have discernible periplasmic domains, consistent with the absence of periplasmic and transmembrane regions in the known F7 chemoreceptors. How the F7 chemoreceptor array is anchored to the membrane is

unknown. The F7 arrays also contain distinctive additional density layers between the baseplate and the inner membrane, which is proposed to correlate with the presence of PAS and HAMP domains.

The requirement that receptors that coexist in single arrays must be of the same length appears to be stricter in the case of membrane-bound arrays compared to the cytoplasmic arrays. One special case is the cytoplasmic array in *V. cholerae* (104, 105). In this case, the DosM receptor (44H) forms arrays together with other, much shorter cytoplasmic receptors (20H). This unusual DosM receptor contains 2 signaling domains and interacts with both base plates in the array to serve as a structural scaffold.

What is the advantage of multiple arrays?

The explanation for why bacteria presenting multiple membrane-bound arrays at the same cell pole remains elusive. The spatial isolation of the two arrays suggests that the chemosensory pathways benefit from segregation of the receptors by preventing possible crosstalk. However, crosstalk among different pathways is reported in the bacterium *Comanonas testosterone* (123). Here, the kinase of the motility control pathway can directly phosphorylate not only the response regulator within the same pathway, but also the response regulator from the pathway that regulates biofilm formation. Similarly, crosstalk between pathways has also been proposed in other bacteria, and it might be common in bacteria that are capable of multiple forms of motility (124-127).

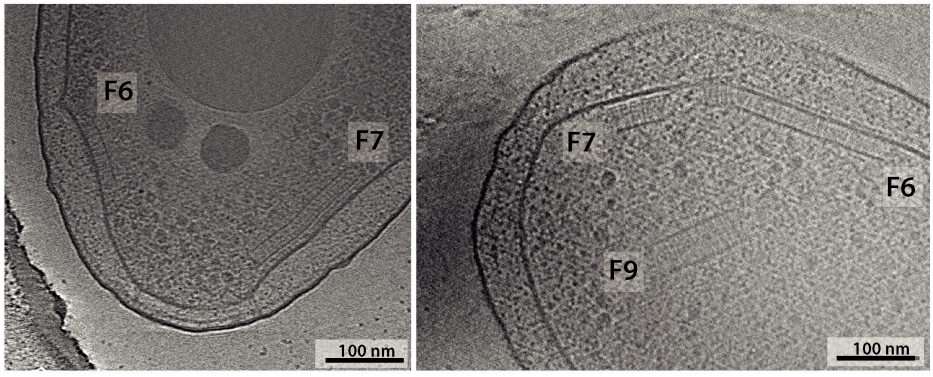

Figure 6. Co-existence of different chemosensory arrays shown in tomographic images of *P. aeruginosa* (left) and *V. cholerae* (right). The F6, F7 and F9 represent different chemotaxis systems classified on the basis of evolutionary history (70). Images are adapted from (122).

Understanding the functional specificity of chemosensory arrays for bacteria with multiple chemotaxis pathways is particularly challenging (128). Even assigning receptors to a certain pathway is not trivial. The difficulty is that the receptors and

cytoplasmic components of the same pathways are not always localized within the same operon. *P. aeruginosa* has five chemotaxis gene clusters comprising four different pathways. However, 22 of the 26 receptors encoded in the genome are located outside of the five gene clusters. Shown in a recent study, genetic analysis of the MCP-CheA, MCP-CheW binding regions identified the pathway-specific motifs of the receptors, which assisted the assignment of all receptors to the four pathways with known function (129, 130). For *Myxococcus xanthus*, the receptor phylogenetic distribution, genomic organization and subcellular localization analysis in combine revealed the distribution of 21 chemoreceptors in 8 chemotaxis pathways (127).

Conclusions and future perspectives

The physiological relevance of chemotaxis pathways depends on the specificities of the receptors displayed in the chemosensory arrays. However, our current understanding of what each chemoreceptor can sense is still rather limited. We find a vast diversity of MCPs in sequenced genomes, but the lack of a structure-function correlation in the ligand-binding domains prevents a reliable prediction of what a specific receptor may be able to sense (89). The growing insight into the immense diversity of bacterial chemotaxis presages the challenges ahead to determine the functional specificity of receptors, chemosensory arrays and chemotaxis pathways.

A fascinating characteristic of the chemosensory arrays is that, despite its highly conserved architecture, it has an incredibly high tolerance to its compositional variability. The increasing number of studies reporting a variety of chemosensory arrays found in various bacteria is truly exciting. However, the correlation between the architecture of the arrays and the stage in the life cycle of the cell or the presence of particular environmental cues is still elusive in most cases. Understanding and appreciating the variability of chemosensory arrays require two things: 1) case studies that illustrate how chemosensory in a single model organism functions; and 2) general cross-species studies that can reveal generally applicable trends.

To gain new insights into the diversity of chemotaxis systems and their function, a combination of approaches will be necessary. For instance, we need to study chemotaxis under culture conditions that mimic the native environment or that reflect specific environmental niches instead of standard laboratory cultures. This need is highlighted by a recent study in which proteomic analysis was combined with cryo-electron tomography (cryo-ET) to study viable but non-culturable *V. cholerae* found in natural water samples (83). This study revealed distinctive changes in receptor composition of the F6 array, and the presence of the additional chemotaxis system (F9) in the non-

culturable state. Cryo-ET provides a direct visualization of the chemosensory arrays *in situ* in three dimensions. It is a powerful tool that can give insight into the native packing order of the receptors and even the overall architecture of the arrays at the molecular level (60, 131). However, this method is limited by the absence of detectable labels to identify proteins of interest. This limitation can be overcome by the use of fluorescence light microscopy for protein-specific targeting correlated (52) , which can detect the localization and identification of the chemosensory arrays (105). In addition, electron microscopy can also provide valuable high-resolution structural information about either isolated array components or recombinant sensory complexes (132). Another powerful tool to study chemosensory arrays is fluorescence microscopy. This method is widely used for studying the clustering of chemotaxis proteins and their dynamics *in vivo* (102, 112). With an ever-improving resolution, for example using photoactivated localization microscopy (PALM) or stochastic optical reconstruction microscopy (STORM), fluorescence microscopy can potentially reveal the clustering of chemotaxis proteins and array segregation at the macromolecular level in living cells (133-137).

Besides imaging techniques, genomics-driven bioinformatics studies have greatly contributed to the tremendous progress in unraveling the diversity of chemotaxis pathways and their evolutionary plasticity. The classification scheme based on evolutionary history of signal transduction systems established by Wuichet and Zhulin has set up 19 classes for assigning chemotaxis clusters in unstudied species (70). Evolutionary genomics studies can also focus on common features of chemotaxis components and predict their function based on combination of phylogenetic profiling and comparative protein sequence analysis (110). Within a single species, comparative sequences analysis has revealed the specificity of the conservative motif of receptor/kinase and receptor/coupling protein interfaces and has assigned the receptors to different chemotaxis pathways (129). Undoubtedly, with more genomes sequenced, bioinformatics analysis will continue to provide valuable insights to direct experiments to test various hypotheses that are potentially applicable to a broader set of subjects.