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MEETING REVIEW

Chemotaxis Arrays in *Vibrio* Species and Their Intracellular Positioning by the ParC/ParP System

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ABSTRACT Most motile bacteria are able to bias their movement toward more favorable environments or to escape from obnoxious substances by a process called chemotaxis. Chemotaxis depends on a chemosensory system that is able to sense specific environmental signals and generate a behavioral response. Typically, the signal is transmitted to the bacterial flagellum, ultimately regulating the swimming behavior of individual cells. Chemotaxis is mediated by proteins that assemble into large, highly ordered arrays. It is imperative for successful chemotactic behavior and cellular competitiveness that chemosensory arrays form and localize properly within the cell. Here, we review how chemotaxis arrays form and localize in *Vibrio cholerae* and *Vibrio parahaemolyticus*. We focus on how the ParC/ParP system mediates cell cycle-dependent polar localization of chemotaxis arrays and thus ensures proper cell pole development and array inheritance upon cell division.

KEYWORDS *Vibrio*, chemotaxis, ParC, ParP, cell pole, development, HubP

Many bacterial species are motile and able to explore their surroundings. One of the primary means of motion utilized by bacteria is flagellum-based motility. Flagella are helical protein filaments protruding from the cell body that, when rotated by the flagellar motor complex, propel the bacterium through the surrounding environment. The flagellar motor is positioned in the cell envelope and is able to rotate either clockwise (cw) or counterclockwise (ccw) (reviewed in reference 1). In general, motile bacteria, such as *Escherichia coli*, *Salmonella enterica*, *Shewanella putrefaciens*, *Vibrio cholerae*, and *Vibrio parahaemolyticus*, actively respond to an assortment of stimuli by modulating the direction of rotation of their flagella and thus adjusting their swimming behavior. Because they are so small, bacteria are thought not to be able to measure concentration differences along space, and they have therefore developed a way of sensing external stimuli in a temporal manner (2; reviewed in reference 3). One of the primary means by which motile bacteria sense and respond to changes in their environment is via chemotactic behavior. In particular, chemotaxis enables motile bacteria to recognize changes in local concentrations of chemicals and, via a signaling cascade, to transmit the perceived information to the flagellar motor complex and regulate its rotation. In this way, chemotactic bacteria are able to sense changes in their external milieu and, over time, bias their movement toward favorable conditions and away from toxic compounds (3, 4).

CHEMOTAXIS

In general, external signals are perceived by receptor proteins—the so-called transmembrane methyl-accepting chemotaxis proteins (MCPs) (Fig. 1A). Chemoeffectors bind the MCPs at their periplasmic domain and transmit the binding signal to their cytoplasmic tips, where they interact with the histidine kinase CheA. The interaction between MCPs and CheA is stabilized by the adaptor protein CheW, which also

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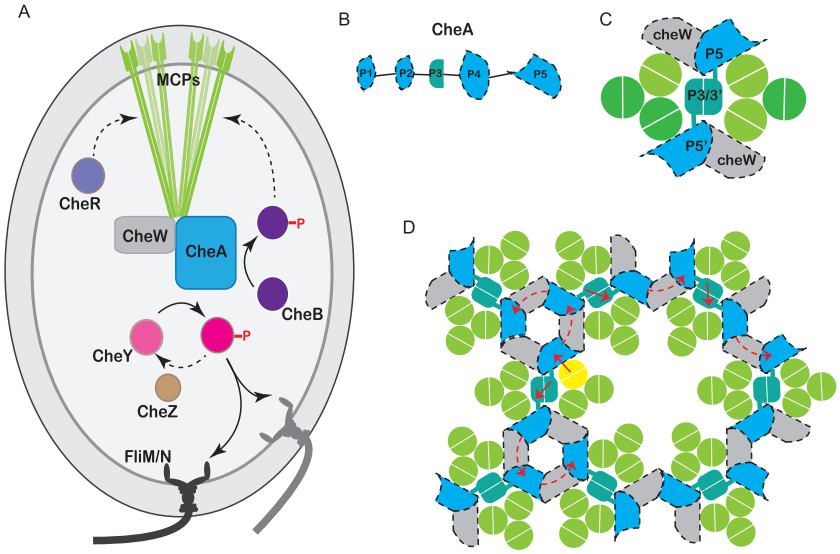

FIG 1 Core component organization and signaling pathway of chemotaxis arrays in *E. coli* cells. (A) Once activated by membrane-bound methyl-accepting chemotaxis proteins (MCPs), CheA transfers a phosphoryl group to CheY and CheB. Phosphorylated CheY diffuses toward the flagellar motors, which lead to the rotation direction switching. CheR and phosphorylated CheB together adjust the methylation state of the MCPs to recalibrate the receptors' sensitivity. (B) Domain architecture of CheA proteins. P1 is the phosphotransfer domain; P2 binds CheY for phosphotransfer from P1; P3 mediates the homodimerization domain; P4 is the kinase domain; and P5 is CheW-like regulatory domain, which binds the signaling tips of MCPs. (C) The chemotaxis core unit. Viewed from receptor toward baseplate, the smallest signaling core unit is formed by two receptor trimers of dimers (shades of green), one CheA homodimer (P5 and dimerization domain P3/3′ annotated; blue and green, respectively) and two CheW proteins (gray). (D) Through the ring structure formed by the CheA subdomain P5 and CheW, signaling cores can assemble into a large lattice of arrays, which facilitates signal cooperativity and amplification. Red arrows, in solid and dashed lines, illustrate the proposed signal-propagating pathways (triggered by a single receptor homodimer, highlighted in yellow) within a single signaling core unit and among arrays, respectively.

contributes to the regulation of CheA kinase activity (3, 5). In the best-understood system in *Escherichia coli*, a decrease in attractant or increase in repellant stimulate autophosphorylation of CheA through the MCPs. Phosphorylated CheA subsequently transfers the phosphoryl group to its cognate response regulator CheY (Fig. 1A). Phosphorylated CheY (CheY-P) is able to diffuse through the cytoplasm and bind the flagellar switch proteins FliM and FliN, which are part of the flagellar motor complex. Binding of phosphorylated CheY to FliM and FliN induces a change in the direction of motor rotation from a ccw to a cw orientation and consequently a change in the direction of bacterial swimming (6–11) (Fig. 1A). The autophosporylation of CheA subsequently activates the methylesterase CheB. Phosphorylated CheB, together with the constitutively active methyltransferase CheR, regulates the methylation state of the MCPs. The level of methylation adjusts receptor output by steadily resetting the kinase activity of the receptor arrays toward the baseline (3, 4). Consequently, CheA activity can be finely tuned through this feedback control, enabling precise adaptation to the cell's immediate environment (Fig. 1A) (reviewed in references 3 and 12).

When all flagellar motors in the peritrichously flagellated bacteria *E. coli* and *Salmonella enterica* rotate in the ccw direction, the flagella combine to form a single helical filament bundle that pushes the cell, resulting in a forward movement—a so-called "run." Upon a reversal in the rotation of one or more motors to a cw direction, the bundle unravels and the flagella separate. This results in a random reorientation of the cell, a so-called "tumble." When the motors switch back to a ccw rotation and the flagellar bundle is formed once again, the cell will move forward in a new random direction (13, 14). In monotrichous bacteria, such as *Shewanella putrefaciens*, *Vibrio cholerae*, and *Vibrio parahaemolyticus*, a ccw rotation of the single polar flagellum pushes the cell forward, while a cw rotation pulls it backward. Reversal from cw to ccw

rotation of the motor is followed by a flick of the flagellum, which induces a random reorientation of the cell before forward movement is initiated—this results in a "run-reverse-flick" swimming behavior (15, 16). The result of both the "run-tumble" (peritrichously flagellated) and the "run-reverse-flick" (monotrichously flagellated) swimming behaviors is a random walk through the environment. However, by actively changing the time period of runs and the frequency of tumbles or reversals, bacteria are able, over time, to bias their movement toward more favorable conditions and to escape from obnoxious environments (3).

STRUCTURE OF CHEMOTAXIS ARRAYS

CheA is comprised of five separate domains (P1 to P5) (Fig. 1B), each with a specific function. P1 contains the substrate histidine for autophosphorylation and acts as the phosphotransfer domain; P2 binds the response regulator CheY (17–19); P3 constitutes the homodimerization domain (20, 21); P4 is the ATP-binding domain; and P5 is similar to CheW (19, 21–27). Both CheW and CheA-P5 interact with the conserved MCP interaction tip (20, 26–28). The MCPs, CheA, and CheW assemble into the ternary core-signaling complex, which is the smallest functional unit—the chemotaxis core unit (Fig. 1C) (29). Multiple core units are arranged into a highly ordered hexagonal array and form a superlattice structure—the chemosensory array (28, 30–34) (Fig. 1D). *E. coli* only contains a single chemotaxis gene cluster that results in a typical membrane-bound chemoreceptor array (30), and the structural architecture of chemoreceptor arrays is well understood in the model organism. Here, the cells typically contain one large polar chemoreceptor array, as well as multiple small arrays that are located along the cell length and around midcell (35–37). In array side views, the arrays can be easily identified as a high electron density layer parallel to the inner membrane (Fig. 2A; membrane-bound array [MA]). This cytoplasmic layer, or baseplate, is the site where CheA and CheW interact with each other and bind to the membrane-distal end of the MCPs. The receptors resemble repetitive, thin, pillar-like densities perpendicular to the base plate, which extend through the inner membrane and display the ligand-binding domains in the periplasm. When the arrays are examined from the top view, the trimers of receptor dimers exhibit characteristic hexagonal packing with a 12-nm center-to-center spacing (Fig. 2B). In *E. coli*, the receptors are networked into this highly ordered lattice by linking hexagons, formed by three core units, together through the dimerization domain of CheA (28, 31). The hexagonal packing of chemoreceptors is universal among bacteria, including in *Vibrio* species, and also in chemotactic archaea (30). While the receptor packing is identical among species, there are differences in the distance between baseplate and inner membrane, which depends on the physical length of the MCP receptors that are present in the arrays (30, 38).

PURPOSE OF CHEMOTAXIS ARRAYS

One of the most remarkable characteristics of the chemotaxis signaling arrays is that the chemotaxis proteins display a strong tendency to assemble into highly ordered macromolecular complexes. This highly ordered packing of the chemotaxis proteins is thought to be critical for correct signal transduction, chemotactic cooperativity, and signal amplification (39, 40). Within arrays, CheA kinase activity is controlled and even greatly enhanced by receptors, and the inhibition of kinase activity is regulated by receptor ligand occupancy (29). Since the cytosolic tips of different chemoreceptors are essentially identical, a mixed population of receptors with the same physical length can assemble into individual signaling complexes and further expand into chemotaxis arrays (41). As a result, the kinases bound in the array are controlled by the collective signal input from the ligand occupancy of multiple MCPs. Furthermore, through allosteric communication across both the CheA dimer interface and the CheA-CheW interface, the signal is thought to propagate among the networks of chemoreceptor arrays (39, 40, 42). This long-range structural dynamic contributes to the amplification of either kinase activation or the kinase-inhibiting effect of CheA (Fig. 1D).

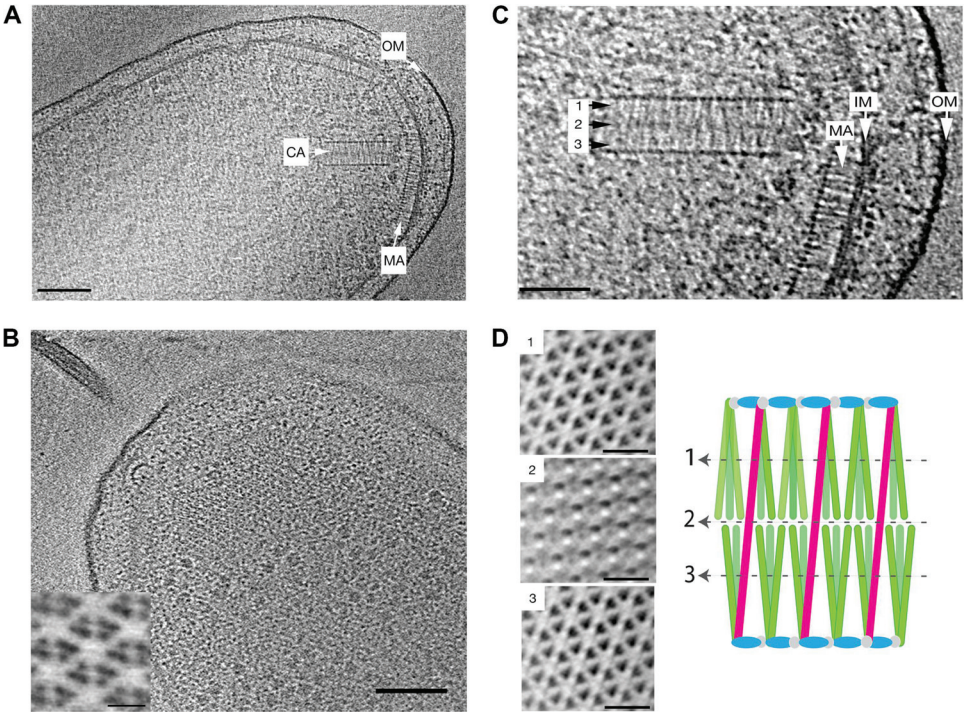

FIG 2 Chemotaxis arrays in *Vibrio* cells. (A) Cluster II membrane-bound arrays (MA) and cluster I cytoplasmic arrays (CA) are both shown in side view. (B) The top view of the cluster II array near the flagellar cell pole and the inset display the hexagonal packing of the receptors. (C) A closeup image of the cytoplasmic array from panel A. The sandwich structure of the cluster II array was formed by two layers of receptor arrays interacting head to head. (D) Sections through the cytoplasmic array coordinate to the numbered positions in panel C and the model proposed based on cryo-electron microscopy (cryo-EM) results. DosM, shown in magenta, is part of the receptor trimers and spans continuously between the two baseplates among other cytoplasmic receptors shown in green. The exact composition of the baseplates, shown in blue and white here, remains unclear for now. CA, cytoplasmic array; MA, membrane-bound array; IM, inner membrane; OM, outer membrane. Bars: panels A and B, 100 nm; panel B inset, 10 nm; panel C, 50 nm; panel D, 20 nm. Panels A, C, and D are adapted from reference 53.

CHEMOTAXIS SYSTEMS AND ARRAYS IN *V. CHOLERAE*

The model organism *E. coli* encodes only one chemotaxis system where both the chemotactic mechanism and structural architecture are well understood. Yet, one or more additional chemotaxis operons are present in the genomes of over half of all chemotactic prokaryotes (43). In *V. cholerae*, there are altogether 22 genes that are annotated as encoding chemotaxis proteins, with another 43 genes encoding possible MCPs scattered throughout the genome (44, 45). Those chemotaxis genes are classified into three distinct clusters, i.e., clusters I, II, and III. Only cluster II proteins have been shown to be essential for chemotactic behavior under traditional cell culturing conditions, and so far, no effect on chemotactic behavior has been observed in cells lacking either cluster I or cluster III (45–47). Furthermore, only cluster II has been found to influence the behavior of *V. cholerae* in the suckling mouse model of infection. In this model, cluster II has been shown to influence the degree and localization of colonization (48–50). Neither cluster I nor III has been shown to influence the behavior of *V. cholerae* under these conditions (49). The cluster II chemotaxis proteins assemble into membrane-bound arrays and are arranged in the typical hexagonal packing with a 12-nm spacing (30).

THE *V. CHOLERAE* CLUSTER III CHEMOTAXIS SYSTEM

Cluster III proteins have been shown to be expressed during the stationary phase and in combination with growth arrest in response to carbon starvation. Furthermore, even though the absence of cluster III does not influence the behavior of *V. cholerae* in the suckling rabbit model, the gene cluster is also expressed under these conditions (51). Expression of cluster III is strictly dependent on the alternative sigma factor RpoS

(51), which is involved in general stress response (52). Furthermore, the CAI-1 quorum-sensing system also plays a role in regulating cluster III expression in stationary phase, as well as in suckling rabbits, but not in response to carbon starvation (51).

THE *V. CHOLERAE* CLUSTER I CHEMOTAXIS SYSTEM

Finally, cluster I proteins form structurally distinct cytoplasmic arrays with a characteristic architecture (53), and expression of cluster I proteins is induced under low-oxygen or extreme nutrient deprivation conditions (54, 55). These proteins assemble into a "sandwich" structure, in which receptor arrays interact through their sensory domain proximal protein interaction tips, between two baseplate layers on the outside (Fig. 2A, cluster arrays [CA]). Both baseplates arrange the receptors into the typical hexagonal packing (Fig. 2C and D, insets 1 and 3) (53). In electron microscopy studies, it was found that the cytoplasmic arrays in *V. cholerae* also contain continuous, pillar-like density spanning the two layers (Fig. 2C and D, inset 2) (53), which is absent in the previously described cytosolic *Rhodobacter sphaeroides* arrays (32). This density has been identified as the unusual chemoreceptor DosM, which contains two signaling domains that point into opposite directions (53). DosM was shown to be necessary for the formation and stabilization of extended cluster I arrays. In addition, DosM is thought to contribute to the rigidity of the array structure. Another difference from organisms that lack a DosM-like receptor, such as, for example, *R. sphaeroides* and *Methanoregula formicicum*, is that in those organisms the arrays exhibit a distinct curvature (32, 53), whereas the *V. cholerae* cluster I arrays are flat. The exact function of the cluster I array is not yet understood; even less is currently known about structure and function of cluster III arrays in *Vibrio*, and current research is underway to begin elucidating the structure of the cluster III array.

INTRACELLULAR LOCALIZATION OF CHEMOTAXIS ARRAYS

In *E. coli*, chemotaxis arrays localize both at the cell poles and along the cell body (35–37). Formation of the signaling arrays in this system is thought to be a stochastic process, in which individual receptors insert randomly in the membrane, where they diffuse freely until they either join existing arrays or nucleate new ones (56). Polar formation of arrays in *E. coli* also depends on the Tol-Pal complex, which is proposed to restrict the mobility of chemoreceptor clusters and ensure their positioning at this site (57). Ultimately, arrays are positioned along the cell length and at the cell poles, which ensures that at cell division, each daughter cell will inherit at least one chemotaxis array.

In contrast to *E. coli*, other organisms, such as *Caulobacter crescentus* (58), *R. sphaeroides* (59), *Pseudomonas aeruginosa* (60, 61), *S. putrefaciens* (62), and *Vibrio* species (50, 63), contain mechanisms to specifically localize the chemosensory arrays to the bacterial cell poles. In the following section, we will focus on how chemotaxis arrays are actively positioned at the cell poles by the ParC/ParP system in *V. cholerae* and *V. parahaemolyticus*.

POLAR LOCALIZATION OF CHEMOTAXIS SIGNALING ARRAYS IN *VIBRIO* SPECIES BY THE ParC/ParP SYSTEM

In *V. parahaemolyticus* swimmer cells (63–65) and in *V. cholerae* (50, 66), the chemotaxis signaling arrays are exclusively found at the cell poles, and their localization is dependent on the ParC/ParP system. In *V. cholerae*, only cluster II arrays are positioned by the ParC/ParP system, while cluster I and III arrays, as discussed below, localize independently of ParC/ParP. In recently divided cells, the chemotaxis array is located unipolarly to the old flagellated cell pole (Fig. 3A, yellow arrowheads). As the cell cycle progresses, a second array is formed and localizes at the opposite cell pole, resulting in a bipolar distribution of signaling arrays (Fig. 3A, green arrowheads) (50, 63). As a consequence, each daughter cell will inherit an array at their respective old flagellated pole upon completion of cell division. Positioning of the arrays to the cell pole is regulated by the ParA-like ATPase ParC and its partner protein ParP, both of which function as cell pole determinants. Both *parC* and *parP* genes are part of the

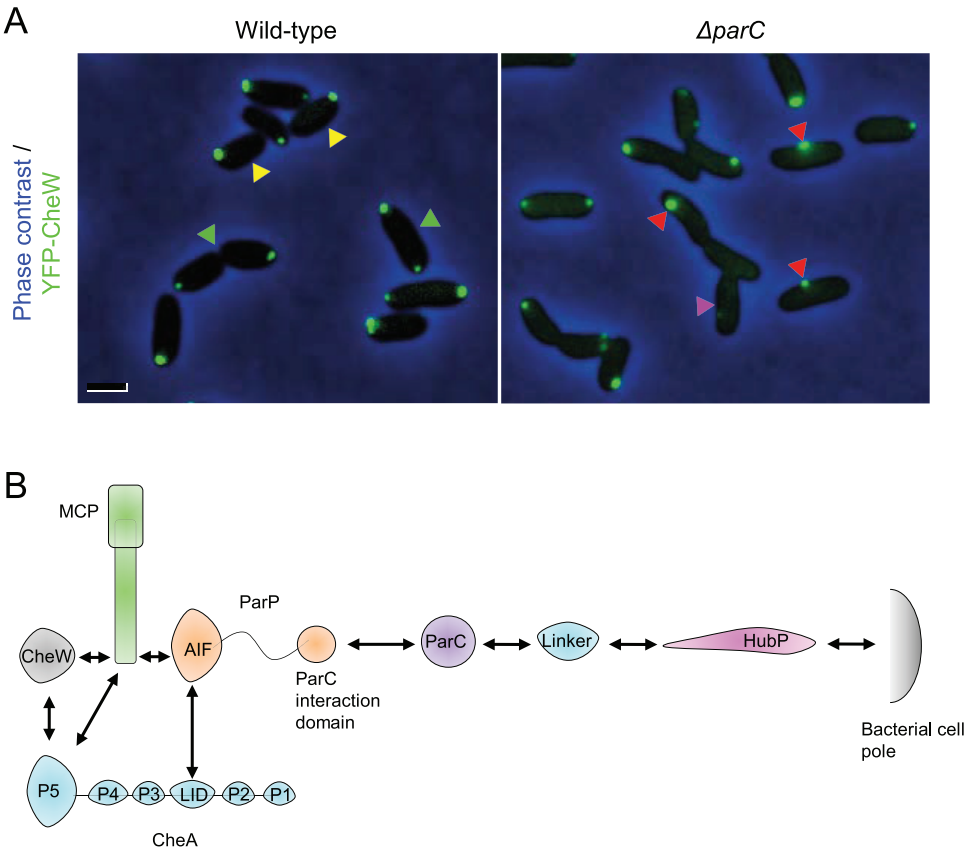

FIG 3 Polar localization of chemotaxis arrays in *Vibrio* cells. (A) Fluorescence microscopy showing the intracellular localization of chemotaxis arrays in *Vibrio parahaemolyticus*, using YFP-CheW as a marker for positioning. YFP-CheW localization is shown in wild-type and Δ*parC* strain backgrounds. Arrowheads indicate the following localizations of YFP-CheW clusters: yellow, unipolar localization; green, bipolar; red, random nonpolar; purple, cells with no YFP-CheW clusters. Images are adapted from reference 63. (B) Schematic showing the interaction network centered on ParP responsible for polar localization of chemotaxis arrays.

chemotaxis gene operon, and *parP* is always positioned immediately downstream of *parC*. In the absence of either ParC or ParP, the cells lose their pole identity and are thus unable to distinguish between their old and new poles (50). As such, there is no preference for the old pole early in the cell cycle, and arrays can form at the new nonflagellated cell pole without an array already being positioned at the old pole (50). Accordingly, an array forms but localizes at a random position along the cell length (Fig. 3A, red arrowheads) (50, 63). As a result, in the absence of either ParC or ParP, bipolar array localization is not established prior to cell division, and, consequently, only one daughter cell inherits an array upon cell division (Fig. 3A, purple arrowhead). Even though the other daughter cell starts out without any chemotaxis array, a new array is, however, formed with a short delay after cell division, again at a random position along the cell length (50).

ParC and ParP themselves display a localization pattern similar to that of chemotaxis arrays: unipolarly at the old flagellated cell pole in young newborn cells, bipolarly later in the cell cycle, and evenly divided to daughter cells (50, 63). Double-labeling fluorescence microscopy in *V. parahaemolyticus* showed that localization of ParC and ParP to the new pole precedes that of the chemotaxis protein CheW (63). Recruitment of ParP to the cell pole is dependent on ParC, and in its absence, ParP forms nonpolar clusters that colocalize with chemotaxis arrays (63, 66).

Cells lacking ParC, ParP, or both proteins have decreased chemotactic ability and altered swimming behavior, reversing their swimming direction much less frequently than wild-type cells. Therefore, proper positioning of arrays by the ParC/ParP system at

the cell poles and the passing on of an array to each daughter cell are critical for proper chemotactic behavior (50, 63).

HubP, A POLAR LANDMARK PROTEIN, DIRECTS ParC TO THE CELL POLE

In *V. cholerae*, localization of ParC to the cell pole depends on the polar landmark protein HubP (67). HubP is conserved in *Vibrio* species and anchors at least three ParA-like ATPases, ParA1, ParC, and FlhG, to the bacterial cell poles. It thereby facilitates proper polar localization of the origin of chromosome 1 (*ori1*) via ParA1, the chemotactic machinery via ParC, and the flagellum via FlhG (67). In the absence of HubP, ParC, and consequently also chemotaxis arrays, is no longer recruited to the cell poles (67). HubP also directs the chromosome segregation protein ParA1 to the cell poles (67), where ParA1 in turn recruits *ori1* via its interactions with its partner protein ParB1 that binds specific sequences (*parS1*) in the *ori1* region (68–70). In newborn cells, ParA1/*ori1* is positioned at the old cell pole by HubP. Then, later in the cell cycle, chromosome replication is initiated, and ParA1 segregates the origin (ParB1/*ori1*) to the new cell pole, where it is anchored via HubP, resulting in one *ori1* tethered to both cell poles. It was shown in *V. cholerae* that recruitment of ParC to the new pole is coordinated with the cell cycle via HubP, and that it only occurs after segregation of *ori1*/ParB1 from the old pole to the new pole has been completed (50). The spatiotemporal localization of HubP was first reported in *V. cholerae* by ectopic expression of HubP-yellow fluorescent protein (YFP). Here, HubP was reported as always being bipolarly localized and recruited to the site of division before cell division is completed (67). In a recent study, however, HubP-superfolder green fluorescent protein (sfGFP) was expressed at its native locus and under the control of its native promoter. Here, HubP only localized to the cell poles in a cell cycle-dependent manner, similar to that of ParC (71). First, HubP localizes unipolarly at the old pole of recently divided cells, and then it is recruited to the opposite new cell pole, where it gradually accumulates as the cell cycle progresses (71). Thus, development of the new cell pole occurs in a hierarchal order, is cell cycle dependent, and is coordinated with chromosome segregation. No direct interaction has been observed between HubP and ParC, and the two proteins do not exactly colocalize at the cell pole, postulating the existence of a so far uncharacterized intermediary factor bridging HubP and ParC (Fig. 3B) (67). Similarly, in *S. putrefaciens*, the polar localization of chemotaxis proteins is dependent on HubP. In its absence, chemotaxis proteins are no longer recruited to the cell poles, but instead localize randomly along the cell (62). *S. putrefaciens* also encodes a ParC/ParP system, though it remains to be analyzed if ParC also directs arrays to the cell poles in this organism.

A DIFFUSION-AND-CAPTURE MECHANISM DRIVES CELL CYCLE-DEPENDENT LOCALIZATION OF ParC

Photoactivation fluorescence microscopy using ParC-PAmCherry revealed that recruitment of ParC to the new pole is the result of redistribution of ParC molecules from the old pole to the new pole (50). Additionally, fluorescence recovery after photobleaching (FRAP) experiments showed that ParC undergoes a continuous cycle between the cell pole and the cytoplasm, and that early in the cell cycle this exchange only occurs at the old pole (50). Based on these localization studies, a diffusion-and-capture model (50, 72) for ParC localization dynamics was proposed. Early in the cell cycle, ParC is recruited to HubP at the old cell pole via a HubP-dependent anchor that at this point in the cell cycle is exclusively found at this site. A continuous exchange of ParC between the cell pole and the cytoplasm ensures that there is a constant pool of cytosolic ParC at any given time. Then, later in the cell cycle, HubP localizes to the new cell pole, making the HubP-dependent ParC anchor available at this site, too. ParC molecules from the cytoplasmic pool can then be captured at both poles. In consequence, a redistribution of ParC from the old to the new cell pole occurs. Eventually, equilibrium is reached, resulting in an equal distribution of ParC accumulating at both poles (in addition to the continuous cytoplasmic pool) (50).

The mechanism underlying ParC's cycle between its localization to the cell pole and

the cytoplasm has not been characterized yet. However, it is at least partially regulated by the ability of ParC to bind and hydrolyze ATP (50, 63). ParC belongs to the superfamily of Walker-type ATPases, and ParCs from both *V. cholerae* and *V. parahaemolyticus* possess weak intrinsic ATPase activity *in vitro* (50, 63). Interestingly, amino acid substitutions that are predicted to block ATP binding or block ATP hydrolysis result in nonfunctional ParC variants. Such cells are defective in recruitment of ParP and chemotaxis arrays to the cell poles (50, 63). A ParC variant that is unable to bind ATP is diffusely localized in the cytoplasm, and a ParC that binds ATP but is defective for hydrolysis localizes to the cell pole in a unipolar and bipolar manner similar to that of wild-type ParC (50, 63). Thus, the ParC-mediated recruitment of chemotaxis arrays to the cell pole is an active process that requires ATP binding and hydrolysis. It is possible that the ParC ATP cycle secures a constant turnover of ParC at the cell pole, which ensures the presence of a constant cytoplasmic pool of ParC, needed for its immediate recruitment to the new cell pole once its polar anchor develops at this site.

INTERACTION BETWEEN ParP AND ParC IS REQUIRED FOR PROPER LOCALIZATION OF CHEMOTAXIS ARRAYS

ParP has multiple interaction partners and specifically interacts with ParC, the histidine kinase CheA, and MCPs (Fig. 3B) (63, 66). ParP's interaction with ParC is mediated by its extreme N terminus. In particular, eight amino acids near the N terminus of ParP are highly conserved among ParP proteins, and a single amino acid substitution within this region in ParP of *V. parahaemolyticus* (ParPY16A), resulted in a ParP variant defective for interaction with ParC but not for interaction with CheA (63). Interaction between ParC and ParP is required for ParC-mediated polar localization of ParP and chemotaxis arrays; when interaction between ParP and ParC is disrupted, ParP and chemotaxis arrays form nonpolar clusters that colocalize. This localization pattern is similar to that of a strain that is lacking ParC (63).

THE C-TERMINAL AIF DOMAIN OF ParP MEDIATES ITS INTERACTION WITH CheA AND MCPs

The N-terminal ParC interaction domain of ParP is followed by a long variable region with a high content of proline residues, suggesting a high flexibility (63, 66). This proline-rich region links the ParC interaction domain of ParP to its C-terminal array integration and formation domain (AIF domain). This AIF domain is similar to both CheW (*V. cholerae*, 15% identity; *V. parahaemolyticus*, 18% identity) and the P5 domain of the CheA protein (*V. cholerae*, 15% identity; *V. parahaemolyticus*, 10% identity) (66) and mediates the interaction of ParP with both CheA and MCPs (Fig. 3B) (66). Both CheW and CheA-P5 are composed of two subdomains, subdomains 1 and 2. The junction between the subdomains consists of branched hydrophobic residues that mediate their interaction with the MCP interaction tip (20, 26–28). Based on the homology between the AIF domain and CheW/CheA-P5, the structure of the AIF domain is predicted to be comparable to that of CheW/CheA-P5 (66). ParP relies on residues analogous to those of CheW and CheA-P5 for its interaction with MCPs. Amino acid substitutions of the predicted corresponding hydrophobic residues in ParP-AIF disrupt the interaction between ParP and MCPs. However, abolishing ParP's interaction with MCPs does not prevent its interaction with CheA or ParC. The AIF domain of ParP interacts with the same residues within the conserved MCP interaction tip that also mediate interactions between MCPs, CheW, and the P5 domain of CheA. This suggests that ParP competes with either CheW, CheA-P5, or both, for MCP binding as a central part of the chemotaxis core and thus structurally integrates into the chemotaxis signaling arrays (66).

In *V. cholerae*, ParP interacts with a specific region within CheA called the localization and inheritance domain (LID). The LID is positioned between the P2 and P3 domains of CheA and is found only in CheA proteins with an associated ParC/ParP-system (63). Interaction between ParP and CheA is mediated by the AIF domain ParP (Fig. 3B), and one highly conserved tryptophan residue in ParP is required for this interaction (*V.*

cholerae, W338; *V. parahaemolyticus*, W305). An amino acid substitution of this tryptophan to alanine abolishes the interaction between ParP and CheA (63, 66) but not with the MCPs. Interaction between ParP and CheA is required for proper ParP function, since the disruption of this interaction in *V. parahaemolyticus* results in defective recruitment of chemotaxis arrays to the cell pole. However, in *V. cholerae* the phenotype is not as severe as that observed in *V. parahaemolyticus* (66), suggesting that the importance of ParP-CheA interaction might vary between bacteria. Alternatively, the severity of the disruption in interaction differs between the two bacteria. The residues mediating the ParP-AIF interaction with CheA and MCPs are predicted to be positioned on opposite sides of the AIF domain. Thus, ParP-AIF appears to contain distinct binding interfaces that orchestrate the interactions with both MCPs and CheA (66).

The association of ParP with the chemotaxis arrays relies only on its interactions with CheA and MCPs and can be facilitated by either interaction (66). When the interaction of ParP with both MCPs and CheA is simultaneously disrupted, it results in a nonfunctional ParP variant that is no longer able to associate with chemotaxis arrays and mediate their polar localization (66).

INTEGRATION OF ParP WITHIN CHEMOTAXIS ARRAYS STIMULATES THEIR FORMATION

In a combination of fluorescence microscopy experiments and cryo-electron tomography analysis, it was shown that, in addition to its function in mediating polar localization of signaling arrays, ParP also stimulates array formation (66). In *V. cholerae*, proper array formation requires the presence of either ParP or CheA, which are individually adequate for the proper stimulation of chemotaxis array assembly. In contrast, the formation of arrays is severely compromised in the absence of both ParP and CheA. ParP stimulates array formation via its interactions with MCPs and CheA, and the resulting integration of its AIF domain into the signaling arrays. Arrays form at almost wild-type levels in the absence of CheA alone, which suggests that ParP is able to fully replace CheA within the core unit in the formation of arrays. In contrast, CheW appears to only have a slight effect on array formation, and it requires the presence of either ParP or CheA (66).

It is still not clear how, precisely, the AIF domain of ParP fits within the overall array structure. In the general model of array structure, a chemotaxis core unit consists of two trimers of MCP dimers, two CheW proteins, and two CheA proteins that are dimerized through their P3 domain, thereby contributing to array stability (Fig. 4, scenario 1) (27–29, 33, 73). In the proposed model, ParP-AIF replaces CheA-P5 and binds the MCP interaction tip within the chemotaxis core unit. In the case of only one CheA being replaced, ParP is tethered there through its interaction with the MCP and via binding to the LID of the remaining CheA of the core unit (Fig. 4, scenario 2). Interaction between ParP-AIF and CheA-LID stabilizes the core unit and substitutes for the loss of CheA-P3 dimerization due to the absence of one CheA protein (66). ParP has been shown to stabilize chemotaxis arrays in *V. parahaemolyticus* by preventing the dissociation of CheA from the arrays and by directly stimulating CheA clustering (63). ParP is likely able to substitute for CheA within the core units, as arrays are still able to form at almost wild-type levels in the absence of CheA alone but not in the absence of both CheA and ParP (66). In this scenario, the core unit consists only of CheW, ParP, and MCPs, and it is possibly stabilized by ParP dimerization (Fig. 4, scenario 3), as has been shown for ParP of *V. parahaemolyticus* (63). In both scenarios 2 and 3, one would expect an interaction between ParP-AIF and CheW similar to that observed for CheA-P5 and CheW in scenario 1. However, no such interaction between ParP-AIF and CheW has been reported yet, and further studies are needed to address whether such an interaction takes place.

Integration of ParP into arrays has various effects. It modifies its own and both CheA and ParC subcellular localization dynamics and thereby prevents the dissociation of ParP itself, CheA, and ParC from the cell pole, hereby promoting their polar retention. When the interactions of ParP with CheA and MCPs are disrupted simultaneously, ParP and ParC are retained much less efficiently at the cell pole, and it was shown that the

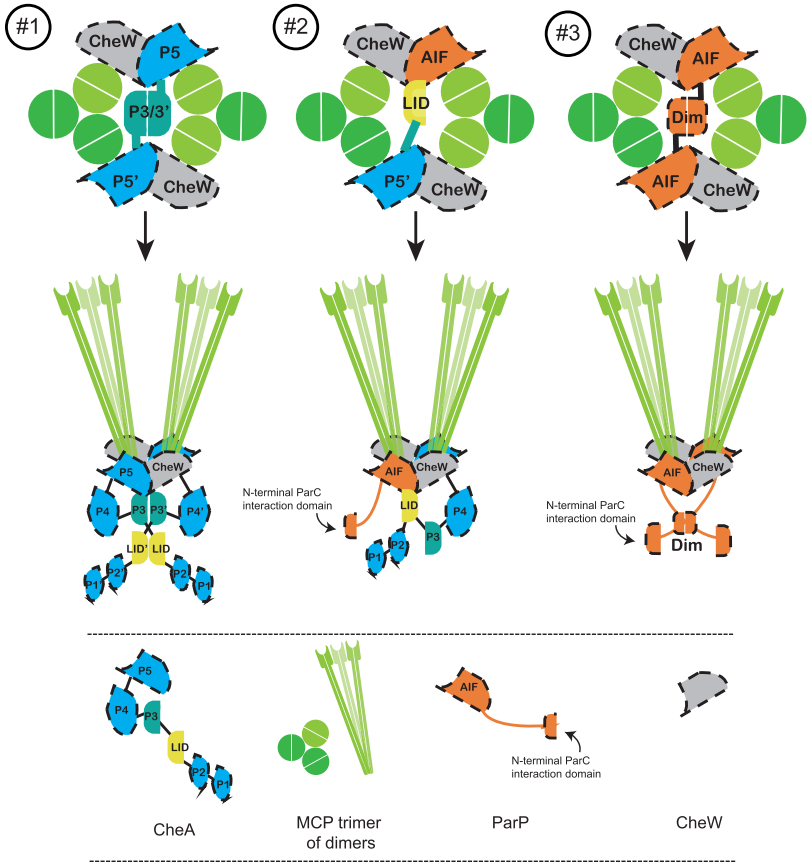

FIG 4 Model of the structure of the chemotaxis core unit. Schematic model of the composition of chemotaxis core units and the interactions of ParP-AIF with MCPs and CheA-LID that drive array formation. The model is discussed in detail in the text. "Dim" indicates ParP dimerization via its N-terminal flexible region, which links its N-terminal ParC interaction domain and the C-terminal AIF domain.

release of ParP from the pole into the cytoplasm occurs at a higher rate (63, 66). The effect of ParP on array stabilization is separable from its involvement in array localization, because in *V. parahaemolyticus*, ParP prevents the dissociation of CheA from chemotaxis arrays in the absence of ParC (63). Thus, ParP consists of two functionally distinct domains, namely, (i) the N-terminal ParC interaction domain, which is responsible for directing ParP to the cell pole, and (ii) the C-terminal AIF domain, which is responsible for integration of ParP into the chemotaxis core unit and therefore for the stimulation of array formation. The linkage of the two domains enables ParP to couple the formation of signaling arrays to their intracellular localization and ultimately results in the targeted formation of chemotaxis arrays at the cell pole (66). Hereby, ParC primarily administers array localization by mediating the polar localization of ParP, and ParP in turn functions to stimulate array formation at this site. Thus, the ParC/ParP-system provides a diffusion-and-capture mechanism, wherein chemotaxis complexes diffuse freely in the membrane. When they come in contact with ParP at the cell pole, ParP, as a part of the chemotaxis core unit, stimulates array formation at this site, prevents the dissociation of captured proteins, and consequently drives array assembly and sequestration at the cell pole (50, 63, 66).

SUMMARY MODEL OF CELL POLE DEVELOPMENT IN *VIBRIO* SPECIES

Altogether, the results summarized here have led to the following understanding of cell pole development in *Vibrio* species, particularly in regard to the localization and formation of chemotaxis arrays (Fig. 5). In recently divided cells, HubP is localized to the old flagellated cell pole, where it recruits ParA1 and ParC. ParA1 in turn recruits

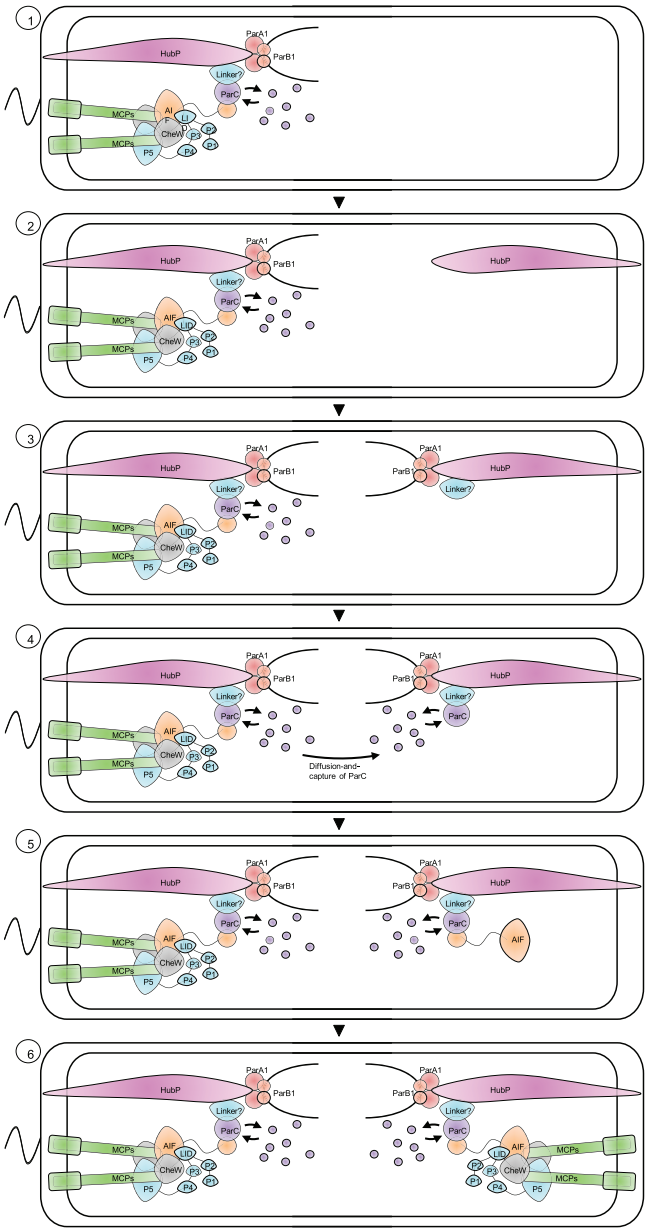

FIG 5 Hierarchical cell pole development in *Vibrio* cells. Schematic showing the cell pole development in *V. cholerae* and *V. parahaemolyticus*. The model is described in the text.

ParB1/*ori1* to the cell pole. ParC is recruited to HubP by a thus far uncharacterized protein that links HubP and ParC. ParC undergoes a continuous cycle of capture and release at the pole, which ensures a constant pool of cytosolic ParC and polarly localized ParC. ParC localized to the pole in turn recruits ParP via ParP's N-terminal domain. At the pole, ParP, via its C-terminal AIF domain, captures and sequesters chemotaxis proteins, thereby driving localized array formation at the cell pole (Fig. 5, step 1). As the cell cycle progresses, HubP is recruited to the new cell pole (Fig. 5, step 2), where it again recruits ParA1. Subsequently, ParA1 recruits ParB1/*ori1* to the new cell pole after chromosome segregation has been initiated. After completion of chromosome segregation, HubP positions the ParC anchor at the new cell pole (Fig. 5, step 3). Following the development of a ParC anchor at the new pole, ParC molecules released from the old pole are able to diffuse in the cytoplasm and are captured at both poles. This results in redistribution of ParC from the old to the new

cell pole and an equal distribution of ParC between both poles (Fig. 5, step 4). ParC positioned at the new pole recruits ParP to this site (Fig. 5, step 5). Once positioned at the new pole, ParP drives the formation of a new chemotaxis signaling array at this site through its interactions with MCPs and CheA as a part of the chemotaxis core unit, ultimately resulting in a bipolar localization of chemotaxis arrays and in the development of the new cell pole into a functional old pole after completion of cell division (Fig. 5, step 6).

ParC-INDEPENDENT LOCALIZATION OF SIGNALING ARRAYS

Yet, even in *Vibrio* species, not all types of chemotaxis arrays depend on the ParC/ParP system for their intracellular localization. In *V. cholerae*, chemotaxis proteins from cluster III localize to the cell pole independently of ParC/ParP and do not colocalize with cluster II arrays (51). Cluster I arrays also localize independently of ParC to the polar region of the cell (53) and form along the length of the cell during hypoxia conditions (55). Depending on its environment, *V. parahaemolyticus* differentiates into two distinct cell types—swimmer and swarmer cells, which are specialized for growth in liquid surroundings and on solid surfaces, respectively. Swarmer cells are often highly elongated and produce a multitude of lateral flagella that are required for swarmer behavior, while swimmer cells possess a single polar flagellum (74–77). Signaling arrays not only localize to the cell poles in swarmer cells but also form along the length of the cell (64, 65, 78). Localization to the cell poles is strictly dependent on ParC; however, formation and localization of lateral arrays are ParC independent and likely are determined by stochastic assembly of chemotaxis proteins in a manner similar to that observed for *E. coli* arrays (64). It is not known how the ParC/ParP system can distinguish between chemotaxis proteins and direct only specific types of arrays to the cell pole. Since CheAs from cluster I and III in *V. cholerae* both lack the LID region, it is possible that ParP is not able to form core units with CheAs from these clusters. It is also possible that differences among MCPs between the clusters only allow ParP to stimulate formation of arrays with a specific MCP or MCP/CheA composition. However, further studies are needed to answer these open questions.

CONCLUDING REMARKS

Understanding the cell cycle-dependent spatiotemporal positioning of chemotaxis arrays will add to our knowledge of the complexity with which bacteria are internally organized. In particular, cells can combine sensing of external cues with the positioning of internal structures, the development of the cell pole, and ultimately the inheritance of macromolecular apparatuses (the chemotaxis arrays)—all in order to ensure the survival of themselves and their offspring in a changing environment. Phylogenetic analysis revealed that ParC forms its own clade of ParA-like ATPases, distinct from that of ParA proteins involved in chromosome segregation, plasmid segregation, and positioning of cytosolic signaling arrays in *R. sphaeroides* (50). Furthermore, ParC associated with ParP homologs was found encoded within the chemotaxis operons of many polarly flagellated *Gammaproteobacteria*, hence suggesting that these bacterial species also rely on the ParC/ParP system for polar targeting of their chemotaxis signaling arrays (50, 63).

Chemotaxis array structure and function have been extensively studied in *E. coli* in the past, due to the elegant simplicity of this system. However, the structure and function of chemotaxis arrays in species that possess multiple chemotaxis clusters are less well understood, and the variance of spatial organization and structural architecture of different chemotaxis arrays is not yet well characterized. In addition, little is known about the functionality of different chemotaxis arrays and the potential environmental or cell cycle-dependent associations among different clusters. In order to reveal the spatiotemporal positioning of the chemotaxis arrays through the *Vibrio* life cycle, fluorescence microscopy and photobleaching time-lapse microscopy are routinely used. Furthermore, cryo-electron microscopy can be

employed for the direct visualization of the arrays in native state. In addition, electron cryotomography can provide a unique advantage to confirm the correct packing in the intact array and reveal a possible structural variance in different chemotaxis arrays.

Vibrio species are equipped with far more auxiliary chemotaxis components than *E. coli*, which allows a greater diversity in the structure of chemoreceptor arrays and a higher adaptability to their living environments. Better understanding of the chemotaxis arrays in *Vibrio* species could yield new and unanticipated insights into the molecular mechanism of chemoreceptor array formation and the chemotactic signaling process.

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