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Establishing polar identity in gram-negative rods

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Abstract

In rod shaped bacteria, numerous cellular components are targeted to the cell poles, and such localization is often important for optimal function. In particular, recognition of poles is often linked to division site selection, chromosome segregation, chemotactic signaling, and motility. Recent advances in understanding polarity include identification of a *V. cholerae* protein that mediates polar localization of a chromosome origin and chemotaxis clusters, as well as a downstream protein that contributes solely to localization of chemotaxis proteins. In *C. crescentus*, the molecular mechanisms by which polar determinants and effectors are localized, and the key roles for nucleotide-dependent switches, have been defined. Finally, roles for, and interactions between, factors that mediate environmentally determined polarity in *M. xanthus* have recently been characterized.

Introduction

Despite their overt structural simplicity, bacterial cells maintain extensive spatial organization, and this organization is critical for diverse cellular processes. A pivotal determinant of organization in non-spherical bacteria is cellular polarity, by which we mean the creation, recognition, and operation of cell poles as distinct functional zones. Notably, the two poles within a bacterium often have dissimilar identities based on their history within the cell; polar cellular components are often found specifically at the “new” pole (created by the most recent cell division) or “old” (preexisting) pole, rather than displaying bipolar localization. Polar identity can also be defined functionally, rather than historically, e.g., as in *Myxococcus*, whose “leading” and “lagging” poles are defined based on cellular behavior. In both identification schemes, identity is not fixed; instead, over time a new pole develops into an old pole, and a lagging pole is converted to a leading one. The means by which polar identities are established, and the processes that they control, will be the focus of this review. In particular, we will primarily examine the determinants and consequences of polarity in 3 gram-negative organisms in which it has been deeply investigated: *Vibrio cholerae*, *Caulobacter crescentus*, and *Myxococcus xanthus*.

A wide variety of cellular components/processes have been found to be targeted to bacterial poles, including pili, flagella, chemosensory apparatus, phosphotransferase systems, autotransporters, adhesins, specific RNAs, and chromosome replication and partitioning machinery (reviewed in [1]). Poles can also be the sites of cell growth, via localized synthesis of peptidoglycan, as is seen in some alphaproteobacteria and several types of

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Actinobacteria (e.g., Corynebacteria, Mycobacteria, and Streptomyces) [2] [3]. A biological rationale for polar localization is not always apparent; however, in at least some cases, optimal function is dependent on proper positioning, and several polar determinants have been found to be essential for viability, either singly or in combination (e.g., DivIVA of *Bacillus subtilis*, PopZ/TipN of *C. crescentus*) [4] [5]. Clustering of cellular components at a particular site can make a process more efficient, by limiting the need for diffusion (as is hypothesized for *V. cholerae* chemotaxis); it can also contribute to reliable division of cell components among daughter cells. Furthermore, polar demarcation can permit cellular identification of other domains. For example, processes targeted to the midcells of rod shaped bacteria (in particular, cell division) are often dependent upon proteins that localize to, or oscillate between, cell poles (e.g., [6-8])

A diverse array of factors underlie recognition and/or establishment of polar domains. Ultimately, polar identity is likely to be dependent upon intrinsic features of poles, such as the membrane curvature thought to mediate localization of the *Bacillus* polar determinant DivIVA [9,10]. Poles may also be marked by peptidoglycan with distinct features that ensue from the cell division process and are preserved by the relatively inert nature of polar PG (i.e., a “birth scar”), by distinct lipid content (also associated with membrane curvature), and by proton motive force [11] [1]. However, targeting of proteins to the cell poles (via recognition of these or unknown/additional features) is also central to establishment of polarity. Polar “landmark” proteins can serve to organize multiple polar processes in a single cell, which can enable coupling of distinct processes (e.g., chromosome partitioning and cell division). The protein determinants of polarity are in general better defined than the contributions of intrinsic cellular features, and are consequently highlighted in the discussion below.

Polarity in *V. cholerae*

V. cholerae, the cause of the diarrheal disease cholera, has a single polar flagellum, and it was previously shown that the origin of the larger of its two chromosomes (chrI) is tethered to the old pole prior to chromosome replication [12] [13]. Following replication initiation, translocation of one of the duplicated origins to the new pole was observed, and found to depend upon the partitioning proteins ParA1 and ParB1, which interact; however, the means by which these proteins were recruited to the pole was not known.

Recent studies have identified factors underlying the localization of several of *V. cholerae*'s cellular machineries (Fig. 1). Ringgaard et al. reported that chemotactic signaling arrays (methyl-accepting chemotaxis proteins (MCPs; receptors for chemoeffectors) and associated signaling proteins (e.g., CheW, CheY)) display cell-cycle dependent polar localization in *V. cholerae*: in young cells, these proteins are found in a single focus at the old pole, while in older cells a bipolar distribution is evident [14**]. Localization of these chemotaxis proteins was found to be dependent upon ParC, a ParA ATPase homolog that is encoded within the same gene cluster as the associated chemotaxis proteins. In the absence of ParC, a significant subset of cells (>25%) displayed aberrantly localized Che foci or lacked foci altogether, and swimming cells reversed direction (an indication of chemotaxis) ~3x less frequently than did cells that produce ParC. Thus, unlike in *E. coli*, where Che signaling clusters are thought to assemble stochastically [15], in *V. cholerae* the placement of these proteins is spatially and temporally regulated. It seems likely that polar placement of Che proteins in *V. cholerae* facilitates signal transduction between the Che cluster and the organism's polar flagellum, whose rotation it regulates. Such coordination is perhaps less significant in *E. coli*, which produces multiple peritrichous flagella. The bipolar distribution of Che clusters in older cells also facilitates reliable inheritance of Che proteins, by ensuring that each daughter cell has a Che cluster at its old pole at the time of its birth.

A subsequent investigation by Yamaichi et al. identified a pole organizing factor, HubP, that is required for proper localization of ParA1 and ParC, as well as a third ParA family member, FlhG, that contributes to regulation of flagellum production [16**,17]. HubP is a large (~178kd), transmembrane, multidomain protein, with homologs largely among the γ proteobacteriacea. In its absence, ParA1, ParB1, and the *chrI* ori were not recruited to either pole, nor was FlhG, which is either unipolar or bipolar in wt cells, and mutant cells more frequently produced multiple, rather than single flagella. Additionally, a majority of cells contained aberrantly localized ParC foci, which were typically associated with the chemotactic signaling protein CheY, and chemotactic capacity was significantly impaired. Unexpectedly, despite their common ancestry, which might suggest a shared mechanism of action, ParA and FlhG were found to interact directly with HubP, while ParC colocalizes but does not directly interact. Furthermore, control of chemotaxis, chromosome partitioning, and flagella production were each dependent upon different regions of HubP. It is possible that these 3 HubP and ParA family-dependent processes initially relied on a common mechanism for recruitment of proteins to the pole, and that subsequent adaptive changes led to the development of distinct processes.

Although ParA, ParC, and FlhG associate with HubP at the old pole, the localization of these proteins does not fully coincide. In particular, it is noteworthy that HubP is always present at both poles, rather than simply marking the old pole in young cells. Additionally, HubP is detectable at the cell division site following arrival there of FtsZ, which guarantees that it is associated with new poles in newborn cells. HubP contains a putative peptidoglycan-binding domain that may contribute to its positioning/anchoring at the cell pole. To date, no regulatory mechanism has been identified that prevents colocalization of ParC, ParA, or FlhG at the new pole or facilitates their associations at the old pole, and no new pole-specific markers have been identified. Thus, the means by which HubP is licensed to interact with its clients is worthy of further investigation. Additionally, the basis for polar targeting of *V. cholerae* flagella, which is independent of HubP, remains to be identified. Finally, unlike polar determinants in *C. crescentus* (discussed below), the absence of HubP does not impair *V. cholerae*'s recognition of its cell division site or have other deleterious effects upon cell growth. Overall, these observations demonstrate that the polar landmark HubP is necessary for multiple aspects of *V. cholerae* polarity, but suggest that additional polar markers and/or regulatory factors that control interactions among polar makers and their clients are likely to be identified in the future.

Polarity in *C. crescentus*

The most extensive knowledge of determinants of polarity has been garnered from *C. crescentus*, a dimorphic bacterium whose lifecycle includes a complex developmental process marked by changes in polar features (extensively reviewed in [18]). *C. crescentus* exists both as mobile, non-replicating, polarly flagellated swarmer cells, and as non-motile, sessile cells in which the flagellum is replaced by a adherence-mediating stalk. Stalked cells develop from swarmer cells following a period of growth, since swarmer cells are smaller at the time of cell division than their stalked sisters. Later, stalked cells give rise to predivisional cells that are both stalked and flagellated, which then divide into one stalked and one flagellated daughter cell (Fig. 2).

Progression of *C. crescentus* through its lifecycle is guided by several polar determinants that enable assembly of polar organelles at the appropriate time and link this development to chromosome replication, segregation, and establishment of the cell division plane. *C. crescentus* new poles are generally marked by TipN, which is essential for placement of flagella at this site in predivisional cells, and is thought to guide localization of CheA (a component of chemotactic signaling arrays) and the histidine kinase PleC as well [19,20].

TipN subsequently relocates (in an FtsZ and Tol-Pal-dependent fashion) to the division plane (site of nascent poles), thereby ensuring its presence as a new pole marker following cell division, and allowing the conversion of the previous new pole into an old pole [19-21]. TipN may also help target pilus assembly proteins (e.g., CpaE) to the new pole in predivisional cells [20]; however, cells lacking TipN still generate pili at the appropriate site [19], suggesting that pilus assembly may be subject to additional constraints. TipN has only a minimal effect on old pole markers: in *tipN* mutants, stalks still form at the old pole, although a small fraction of mutant cells develop bipolar stalks, and old pole markers, such as the histidine kinase PleJ, are properly positioned. TipN overexpression leads to formation of ectopic flagellated poles and branched cells. TipN is found at the tips of these branches, suggesting that, in addition to functioning as a polar anchor like HubP, TipN contains the information sufficient to mediate polar growth. Non-polar HubP foci are not apparent with HubP overexpression and therefore it is not clear whether HubP can also promote ectopic pole formation.

In addition to guiding placement of new pole-specific structures, TipN also controls placement of the cell division plane, due to its interaction with the chromosome partitioning protein ParA [22,23]. Following initiation of chromosome replication in *C. crescentus*, both chromosome origins are initially found at the stalked (old) pole, but one moves to the new pole, guided by ParA, which eventually forms a discrete focus there. Origin segregation results in establishment of a bipolar distribution of ParB, which binds origin-proximal *parS* sequences. ParB also interacts with the essential ATPase MipZ (principally in its monomeric form) and appears to promote its dimerization, which enables MipZ to interact with and be transiently immobilized by chromosomal DNA [8,24**]. In cells with segregated chromosomes, a bipolar gradient of (presumably dimeric) MipZ is generated, with the lowest MipZ concentration near the midcell and the highest near the poles. Accumulation of dimeric MipZ at the new pole displaces FtsZ from this site, where it has resided since the previous division event. Dimeric MipZ also promotes hydrolysis of GTP by FtsZ, and thereby prevents FtsZ polymerization [24**]. Consequently, assembly of the Z ring occurs near the midcell, where MipZ's inhibitory effect is lowest. In cells lacking TipN, ParA dynamics are altered, ParB and MipZ are not efficiently recruited to the new pole, and hence placement of the division site is aberrant, as is the size of the resulting progeny[22].

Unlike MipZ, TipN is not essential; cell division is impaired, but not prevented, by its absence. The effect of TipN deficiency is countered in part by the action of PopZ, a multifunctional polar landmark that modulates chromosome segregation and several additional pole-linked processes [5,25]. Notably, mutants lacking both PopZ and TipN are not viable, and overexpression of either can compensate for the absence of the other [5,22]. In swarmer cells, PopZ forms a dense, ribosome-excluding matrix at the old pole, where it colocalizes and interacts with ParB [26]. As these cells elongate, ParB is released from PopZ, flagella and pili are lost, and development of the stalk (another PopZ-dependent process) is initiated. Like ParB and MipZ, a portion of PopZ migrates to the new pole along with the replicated chromosomal origin. Despite their capacity to interact, movement of PopZ and ParB is not interdependent. Instead, accumulation of PopZ at the new pole is likely facilitated by ParA, which promotes formation of a PopZ matrix from cytoplasmic oligomers [27*]. As at the old pole, the PopZ matrix can tether ParB to the new pole, independent of ParA, thereby lessening the need for TipN at this site and permitting TipN's migration to the division plane. PopZ moves more slowly to the new pole in the absence of TipN; however, once arrived it is sufficient for tethering of ParB and downstream division events [22]. PopZ's tethering of the chromosome origin to the new pole ensures that swarmer daughter cells reliably inherit genetic material. In the absence of PopZ, ParB foci are often mislocalized, stalks do not form, and cells filament [5,25].

The synthetic lethal phenotype associated with *tipN popZ* mutants likely reflects (at least in part) a more dramatic failure in the process of chromosome segregation, which is modulated by both gene products [5,22]. It will be interesting to learn what, if any, factors are synthetic lethal with *hubP* in *V. cholerae*; however, given that *V. cholerae*'s *parA1* and *parB1*, unlike *C. crescentus* *parA* and *parB*, are not essential [12] [28], polarity may have a less profound impact on the viability of *V. cholerae*. In general, ParAB-mediated chromosome partitioning appears to be more intricately linked to additional processes in *C. crescentus* than ParA1/B1 mediated *chrI* segregation in *V. cholerae*. In *C. crescentus*, the Par proteins are highly integrated within mechanisms regulating the organism's growth and differentiation cycle, with ParB in particular interacting with and governing the activity of ParA, PopZ, and MipZ, so that many processes can be temporally coupled. In contrast, there is currently no evidence to suggest that *V. cholerae* ParB1 regulates the activity of (or interacts with) any factors other than ParA1. Furthermore, a factor targeting proteins specifically to the new pole, like *C. crescentus*' TipN, has not been described in *V. cholerae*. Since HubP is present at both poles throughout the cell cycle, it is possible that specification of old and new poles in vibrios is determined solely by factors that control HubP's interactions with its clients.

Polarity in *Myxococcus*

In *M. xanthus*, unlike in *V. cholerae* and *C. crescentus*, cell polarity is not an intrinsic attribute of the cell, but instead is generated as a response to the cellular environment. One manifestation is the formation of Type IV pili, which contribute to the motility of this non-flagellated social bacterium on solid surfaces. Type IV pili are present at the leading cell pole, where, following their attachment to neighboring cells or environmental components, they can pull cells forward, in a process known as S motility. When cells reverse polarity (and direction of motion), they dismantle pili at the leading cell pole and generate new ones at the lagging cell pole [29]. This switch is dependent on signaling via the Frz pathway, which is homologous to chemotactic signaling pathways (reviewed in [30]). Deletion of *frz* generally results in a hyporeversing phenotype, while increased phosphorylation of FrzZ, the signaling output, is observed in hyper-reversing mutants [31*]. Thus, in contrast to the systems described above, in *M. xanthus*, chemotactic signaling is the initiator rather than a consequence of cell polarity. A schematic depicting the subcellular positions of the factors that govern *M. xanthus* polarity is presented in Fig. 3.

Phosphorylated FrzZ is targeted to the leading cell pole [31*], as is GTP-bound MglA, a GTPase that is essential for correct localization and activation of motility machinery, and is thought of as a key agent of pole switching [32-34]. The lagging pole is marked by a large cluster of MglB, a GTPase activating protein (GAP) for MglA, which prevents accumulation of MglA at this site, since GDP-bound MglA is diffusely localized. Pole-specific protein accumulation is also observed for the motor ATPases PilB and PilT, which energize pilus extension and pilus retraction, respectively, and thus are key effectors of *M. xanthus* motility [35]. A recent study indicates that the initial targeting of PilB and PilT is not pole specific; instead, both proteins are recruited to the same pole via a process dependent on another small GTPase, SofG, and on BacP, a bactofilin cytoskeletal protein [36*]. BacP localizes in subpolar regions, and SofG associates only transiently with a single cell pole; thus, unlike several polar determinants described above (e.g., PopZ, TipN, HubP), these proteins do not serve as polar landmark or scaffold proteins for downstream factors (i.e., PilB and PilT). Genetic analyses suggest that subsequent sorting of PilT and PilB from a single pole to the correct poles (a key step in establishment of functional polarity) is dependent upon MglA; however, the mechanism for such sorting has not been identified [36*]. MglA is also required for non-pilus based motility (gliding, or "A motility") [34]; its precise role in this process also remains to be defined.

In addition to its regulation by Frz signaling, localization of MglA was recently shown to be dependent upon a response regulator, RomR [37**,38**]. Increased phosphorylation of RomR appears to result in hyperreversal, while the absence of RomR phosphorylation is associated with hyporeversal [39]. It is hypothesized that RomR activity is controlled in a phosphorylation-dependent manner by phosphorylated FrzZ, although the precise regulatory process remains to be characterized [38**]. RomR localizes to both poles, but preferentially to the lagging pole; it interacts with MglA and MglB, and the localization of all three proteins is interdependent. MglB is found at both poles in the *romR* mutant, whereas MglA is distributed throughout the cytoplasm, potentially because RomR directly recruits MglA to the pole. Notably, a *romR* mutant is non-motile, but a *romR mglB* mutant retains motility, suggesting that polar targeting of MglA is not essential for its activity, as long as some polar accumulation of MglA-GTP is possible, and not prevented by MglB's GAP activity [38**]. This is a somewhat unusual finding; it is more common that the activity of pole-targeted polar determinants is dependent on their polar localization. For example, mislocalized TipN (which is observed following overexpression) does not contribute to normal pole development; instead, it induces formation of misshapen (branched) cells with TipN present at sites of aberrant growth [20].

Commonalities and variation in generation of polarity

There is no apparent homology among the proteins that ground establishment of polarity in the organisms described above (e.g., TipN, PopZ, HubP), nor between these proteins and the gram positive polar determinant DivIVA. Thus, it appears that bacterial species have taken a variety of paths toward developing and ensuring polar identities. However, there is notable commonality among the proteins that interact with and/or are guided by these polar anchors. In particular, many species make use of ParA family ATPases, as well as associated proteins that regulate them, to govern the assembly of polar structures. Three ParA family proteins are known to have pole-related roles in *V. cholerae* (ParA1, ParC, and FlhG), and such proteins have critical functions in *C. crescentus* as well (ParA and MipZ, plus their shared regulator, ParB). ParA family proteins have been found to contribute to the subcellular localization of a wide variety of bacterial components (reviewed in [40]), generally via a ATP-dependent switch that allows modulation of their oligomeric state and their affinity for protein and other targets. Interestingly, several GTP-dependent proteins also contribute to establishment of polar processes (e.g., *V. cholerae*'s flagellar determinant FlhF, and *M. xanthus* MglA and SofG) suggesting that the ability to switch between distinct states may be particularly advantageous for establishment of a dynamic system such as polarity.

Future prospects

A great deal of progress has been made in identification of proteins required for establishment of bacterial polarity and dissection of the processes by which it is regulated. Far less is understood about the cellular features that guide localization of these protein determinants, and further investigation of this topic is warranted. Additionally, it will be interesting to investigate the extent to which structures are organized within the poles and whether such organization (if present) has functional consequences. Advances in microscopy over the last decade have enabled increasingly fine scale mapping of polar (and other) bacterial features, and it seems likely that emerging technologies will continue to enhance our view of this terrain.

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Highlights

HubP tethers chromosome origins and chemotactic signaling arrays to *V. cholerae* poles.

HubP and ParC ensure reliable inheritance of chemotactic signaling arrays.

Aggregation of PopZ at new poles in *C. crescentus* is coupled to accumulation of ParA.

A nucleotide and dimerization-dependent MipZ gradient guides *C. crescentus* division.

Small GTPases, response regulators, and motor ATPases underlie *M. xanthus* polarity.

Fig. 1.

Development of polar identity in *V. cholerae*.

V. cholerae's old and new poles are both marked by the transmembrane protein HubP, which interacts either directly or indirectly with 3 ParA family ATPases: ParA, ParC, and FlhG. These partners colocalize with HubP at the old pole in young cells, then adopt a largely bipolar distribution as cells age. Localization of HubP at the division plane probably ensures its presence at the new pole of newborn cells. HubP's association with ParA1 enables polar localization of the *chrI* origin-binding protein ParB1, and thereby tethers the *chrI* ori(s) to the pole(s). HubP-dependent localization of ParC promotes polar localization and reliable inheritance of chemotaxis clusters. ParA1/ParB1 typically arrive at the new pole prior to ParC; however, movement of ParC is not dependent upon ParAB1 (Ringgaard). In the absence of HubP-mediated polar localization of FlhG, *V. cholerae* are prone to production of multiple, rather than single, flagella. Additionally, several HubP clients adopt a diffuse, rather than focal, distribution (shown as small dots).

Fig. 2.

Development of polar identity in *C. crescentus*.

In flagellated swarmer cells, PopZ initially tethers ParB and the associated chromosome origin (via *parS*) to the old cell pole. During pole maturation, ParB is released from the PopZ matrix, the flagellum is ejected, DNA replication is initiated, and PopZ recruits additional stalked pole-specific factors. The replicated chromosome origin and associated ParB migrate across the cell, guided by ParA, until they reach the new pole. TipN, a marker of the new pole and ParA-interacting protein, promotes ParA's directional movement. Accumulation of ParA at the new pole induces formation of a PopZ matrix at this site, which serves to further tether ParB and the origin. The bipolar distribution of ParB induces bipolar distribution of another ParB-interacting protein, the FtsZ inhibitor MipZ. MipZ displaces FtsZ from the new pole, and FtsZ subsequently polymerizes near the midcell, which is the site of lowest MipZ concentration. TipN is recruited to the midcell in an FtsZ and Tol-Pal dependent fashion prior to cell division. A flagellum is also generated at the new pole prior to division, so that division yields one stalked cell and one flagellated swarmer cell.

Fig. 3.

Localization of polarity determinants linked to type IV pilus based motility (“S motility”) in *M. xanthus*.

External stimuli trigger phosphorylation of FrzZ, (via the Frz chemosensory pathway), which localizes to the leading pole. The response regulator RomR, which lies downstream in the signaling pathway, localizes to both poles, but preferentially to the lagging pole. It interacts with MglA and MglB, and the localization of all 3 proteins is interdependent. Pilus based motility is controlled by the motor ATPases PilB and PilT, which are preferentially found at the leading at lagging poles, respectively. Non-specific polar targeting of these proteins requires bactofillin (BacP) and SofG, and sorting of ATPases to the proper poles is mediated by MglAGTP, which is always present at the leading pole. Upon recognition of appropriate stimuli, the distribution of pole-specific proteins is reversed, resulting in a changed direction of movement.