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Exploration into the spatial and temporal mechanisms of bacterial polarity

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The recognition of bacterial asymmetry is not new: the first high-resolution microscopy studies revealed that bacteria come in a multitude of shapes and sometimes carry asymmetrically localized external structures such as flagella on the cell surface. Even so, the idea that bacteria could have an inherent overall polarity, which affects not only their outer appearance but also many of their vital processes, has only recently been appreciated. In this review, we focus on recent advances in our understanding of the molecular mechanisms underlying the establishment of polarized functions and cell polarity in bacteria.

Spatial awareness within the bacterial cell – evidence of cellular asymmetry

Early on, microscopic studies showed clear evidence of cellular asymmetry in bacteria. Examples include the shapes of stalked bacteria, the polar protrusion of mycoplasmas, the polarized growth of *Streptomyces*, the polar or subpolar location of flagella and pili in some bacteria, and the asymmetric division during sporulation of endospore-forming bacteria or during the normal cell cycle of dimorphic prosthecate bacteria. However, until recently, bacterial cell polarity was widely perceived as an oddity of a few bacteria or a property that arises in bacteria undergoing development. Recent improvements in fluorescence microscopy techniques have radically changed this perception by unveiling the surprisingly structured interior of bacterial cells. It is now evident that the localization or activity of numerous proteins is under strict spatial regulation in many types of bacteria (including those of symmetric appearance), often resulting in asymmetric protein distribution and polarized function. Cell polarity embraces many aspects of bacterial life, as suggested by the rapidly growing number and diversity of bacterial processes involving asymmetric localization of protein activity. Examples include proteins involved in translocation, protein secretion, signal transduction, protein degradation, locomotion, adhesion, chemotaxis, development and cell cycle regulation [1–3]. This list is not exhaustive and is expected to grow as more microbiologists examine the subcellular localization of their favorite bacterial proteins.

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How is polarized function achieved?

Polarized functions can be produced through the asymmetric distribution of protein activity, resulting in a cellular gradient of activity or in the confinement of an activity to a discrete cellular location.

One way to obtain asymmetric partitioning of protein activity is to compartmentalize the cell and induce expression of specific genes in only one compartment. This occurs during sporulation of *Bacillus subtilis* when an asymmetric septum is formed to create two cytoplasmic compartments of unequal size. Differential gene expression between the two cellular compartments causes differential protein activity, which ultimately leads to the release of a heat resistant spore. During this complex developmental program, several sporulation proteins localize to distinct subcellular sites such as the septal membrane. Evidence suggests two distinct mechanisms underlying septal localization. SpoIVFB uses a 'diffusion and capture' mechanism in which the protein is uniformly inserted all around the membrane where it can freely diffuse until it is specifically retained in the septal membrane through interactions with stably anchored factors [4,5]. Another sporulation protein, SpoIIQ, uses a 'targeted insertion and capture' mechanism in which the protein appears to be directly inserted into the forespore membrane at the sporulation septum. At the septum, SpoIIQ remains confined by zipper-like interactions with SpoIIIAH, a protein located in the adjacent mother cell membrane [6,7]. The sophisticated mechanisms involved in the complex developmental program of sporulation have been intensely studied and recently reviewed [8–10].

In the absence of cell compartmentalization, polarized functions in bacteria rely on the preferential localization of proteins (or their activity) to a specific cellular location, typically at a cell pole. Most bacteria have two cell poles and although both poles typically originate from a division event, they differ in age. The younger pole (referred to as 'new pole' hereafter) comes from the most recent division whereas the older one ('old pole') derives from an earlier division. How do certain proteins accumulate at one specific pole? Recent studies suggest the existence of several different mechanisms, examples of which are illustrated here.

Unipolar localization of IcsA by targeted insertion

The Gram-negative pathogen *Shigella flexneri* moves in the host cytoplasm and spreads from cell to cell by recruiting

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the actin polymerization machinery of the host cell to form an actin tail at its old cell pole. This polarized actin-based motility relies on the unipolar localization of the outer membrane protein IcsA (VirG), which promotes continuous actin assembly at the outer surface of the bacterial pole. IcsA is inserted specifically into the outer membrane of the pole [11]. Dispersion by membrane diffusion is reduced by constitutive proteolytic cleavage of IcsA by the IcsP/SopA protease all over the membrane, thereby maintaining the highest concentration of IcsA at the pole [12–15]. How membrane insertion of IcsA is targeted to the pole is not fully understood. However, more recently, experiments with green fluorescent protein (GFP) fusions indicate that polar targeting of IcsA occurs before its secretion across the cytoplasmic membrane by the Sec apparatus (Figure 1a) [16,17]. Some of these experiments were performed in *Escherichia coli* expressing the *Shigella* IcsA protein, which recapitulates the polar targeting of IcsA [17]. The *E. coli* SecAYEG translocation complex, which is located around the entire circumference of the cell, is not required for polar targeting of IcsA [16]. This is consistent with observations in *S. flexneri* showing that proper polar localization of IcsA is independent of the presence of the IcsA signal peptide sequence [17]. It has been proposed that IcsA first recognizes a polarly localized molecule or structure (factor X) at the inner leaflet of the cytoplasmic membrane, thereby restricting Sec-mediated secretion of IcsA to the poles [16]. In about half of the cell population, IcsA shows a unipolar localization whereas the remaining cells have a bipolar IcsA localization [17], suggesting that IcsA localization might be coordinated with the cell cycle. In this scenario, the newborn cell inherits factor X at its old pole, thereby secreting IcsA exclusively at that site (Figure 1a). During growth, factor X accumulates at the other pole, resulting in bipolar secretion of IcsA. In this model, subsequent cell division creates two daughter cells with IcsA at their old poles only.

Cell-wall-growth-dependent polarization of surface protein ActA

In a fascinating twist of convergent evolution, the Gram-positive pathogen *Listeria monocytogenes* uses a strategy similar to *Shigella* for intra- and intercellular

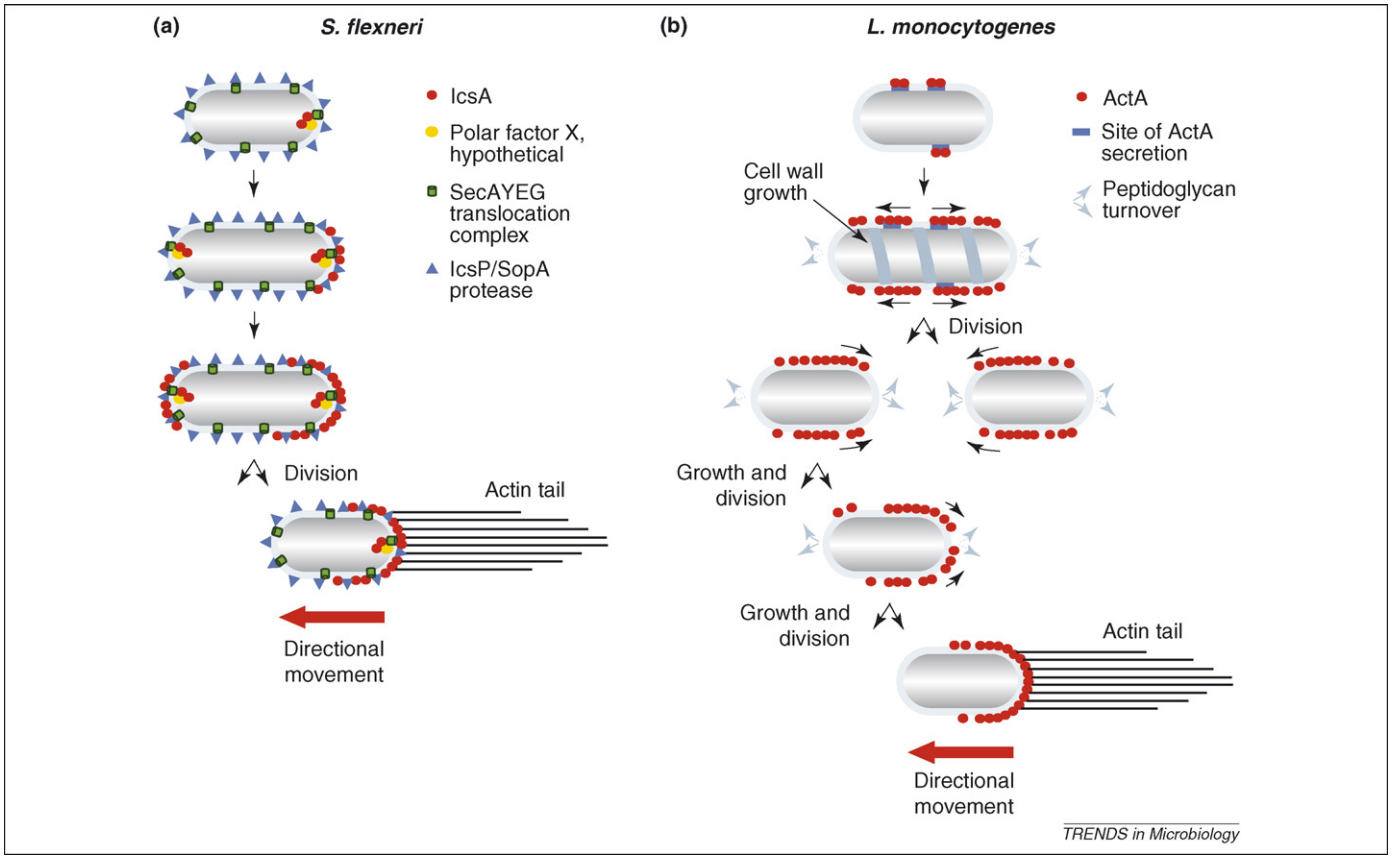

Figure 1. Two different mechanisms for unipolar localization of surface proteins in pathogenic bacteria. **(a)** Model for targeted insertion of outer membrane protein IcsA on the surface of *Shigella flexneri*. Prior to secretion, IcsA interacts with a hypothetical polar factor X, which targets IcsA secretion (mediated by the Sec pathway) to the cell pole (only newly synthesized IcsA is shown; IcsA molecules from the previous cell cycle have been omitted for clarity). Newborn cells inherit factor X at the old pole. During growth, factor X accumulates at the new pole, promoting bipolar IcsA secretion. Following secretion, IcsA diffuses in the outer membrane down the lateral sides of the cell; however, accumulation of IcsA along the cell body is limited by the action of the circumferentially located IcsP/SopA protease. As a result, the highest levels of IcsA are maintained around the poles. In this model, IcsA is secreted at both poles and unipolar IcsA localization results from cell division. Alternatively, IcsA secretion can be targeted exclusively to the old pole (not shown) by unipolarly located factor X. IcsA promotes the polar assembly of host cell actin and thereby directional movement of the bacterium. **(b)** Model for *de novo* polarization of ActA on the surface of *Listeria monocytogenes*. ActA is a surface protein that spans the membrane and the thick cell wall of *L. monocytogenes*. Upon entry of *L. monocytogenes* into a host cell, ActA is synthesized and secreted in a few distinct spots on the lateral sides of the bacterium. ActA passively becomes distributed over the cylindrical cell body as the cell grows by helical insertion of peptidoglycan along its lateral side. During growth and division, cell wall material and associated ActA are slowly pushed towards the poles. ActA accumulates at younger, less inert poles where the rate of peptidoglycan turnover is not as slow as that of older generation poles. Ultimately, after several generations of growth and division, ActA becomes fully polarized at the old pole where it remains trapped because of the slow peptidoglycan turnover at the pole relative to the lateral sides. Polarization of ActA results in the polarized formation of an actin tail necessary for motility in the eukaryotic cytoplasm.

movement in its host [18]. In *L. monocytogenes*, polarization of actin assembly is mediated by the asymmetric localization of the surface protein ActA [19,20]. ActA and IcsA are, however, completely unrelated and their mechanism of polarization is fundamentally different.

High levels of ActA expression are only observed after the bacterium enters a host cell, implying that the pathogen has to secrete and polarize ActA *de novo* before it can start moving [21,22]. Using a fluorescently-tagged ActA construct under inducible expression, Rafelski and Theriot recently showed that ActA is secreted in a few discrete sites along the side of the bacterium [23]. As more protein is secreted, ActA becomes distributed over the surface of the bacterium and becomes polarized only after two to three generations following its synthesis. Because ActA spans the thick, cross-linked peptidoglycan, its diffusion is restricted by the growth and turnover of the cell wall. Experiments in *L. monocytogenes* tracking peptidoglycan insertion suggest a model in which spreading and polarization of ActA is based on a passive mechanism dependent on the mode of peptidoglycan growth [23]. In this model (Figure 1b), helically patterned peptidoglycan growth during cell elongation pushes older peptidoglycan and associated ActA toward the poles. As poles age, the rate of polar peptidoglycan turnover decreases. Continued division yields daughter cells with ActA concentrated at their oldest poles.

Control of protein localization by post-translational modification and regulated proteolysis

In the dimorphic aquatic bacterium *Caulobacter crescentus*, cell polarity is readily evident by the presence of polar appendages (stalk, flagellum and pili) and the obligatory asymmetric division that yields a sessile 'stalked cell' and a motile 'swarmer cell' with distinct fates [24]. The stalked cell immediately initiates DNA replication and cell division whereas its swarmer cell sibling, after a motile phase, must first differentiate into a stalked cell before initiating a new cycle of division. In *C. crescentus*, many regulatory and structural proteins exhibit asymmetric, and often dynamic, localization during the cell cycle. The spatio-temporal regulation of these proteins is complex and has been the subject of recent reviews [24–26]. Because of space constraints, we will only briefly discuss the polarization of the predivisional cell that leads to differential cell fate expression between the two daughter cells.

At the predivisional cell stage, the *C. crescentus* cell shows a dynamic bipolar localization of the single-domain response regulator DivK, with rapid cytoplasmic diffusion of DivK molecules between the two poles [27] (Figure 2a). Polar localization of DivK depends on its phosphorylation and the exchange of DivK molecules between the two polar pools is powered by the antagonistic activities of the DivJ kinase, which phosphorylates DivK at the stalked pole (creating DivK∼P), and the PleC phosphatase, which dephosphorylates DivK∼P at the opposite flagellated pole [27,28] (Figure 2a). Thereby, DivK and DivK∼P shuttle between the two poles as long as there is a cytoplasmic connection between DivJ and PleC [27]. Completion of cytokinesis effectively compartmentalizes the cell [29], physically separating DivJ and PleC activities and resulting in differential levels of DivK and DivK∼P between the swarmer and stalked cell compartments. One model (Figure 2a) proposes that, because DivK∼P promotes the proteolysis of the master transcriptional regulator CtrA (which functions as a cell fate determinant) [30], the low level of DivK∼P in the swarmer cell compartment results in stabilization of CtrA, allowing initiation of the swarmer cell fate program [27,31]. Conversely, high levels of DivK∼P in the stalked cell compartment contributes to CtrA degradation and thereby initiation of the stalked cell fate. Consistent with this model, shortly after cytokinesis, cytoplasmic CtrA in the stalked cell compartment is recruited to the stalked pole where it is proteolyzed by the ClpXP protease complex [32,33] (Figure 2a). This event is dependent on DivK and RcdA, a factor that mediates the interaction between ClpXP and CtrA [33,34] (Figure 2a).

Central to this regulation is the localization of PleC and DivJ at opposite poles. It is not clear how DivJ localizes to the stalked pole, but it does not require the kinase activity of DivJ [35]. PleC is recruited to the new pole by PodJ, a protein synthesized in the early predivisional cell stage [36,37]. What mediates the polar localization of PodJ after its synthesis is not well understood. PodJ is a membrane-anchored protein with a cytoplasmic region required for its polar localization, and a periplasmic region involved in polar targeting of PleC [38]. Following division, PodJ has to be cleared from the flagellated pole of the swarmer daughter cell as the identity of this pole changes from new to old after cell division. Interestingly, this clearing step involves regulated intramembrane proteolysis (Rip), which is triggered by the completion of cytokinesis sensed by the DivJ–PleC–DivK pathway [39,40].

Besides DivK, two other response regulators, PleD and CpdR, have been shown to regulate their polar localization in response to their phosphorylation status. In its phosphorylated form, PleD, a protein involved in polar development, localizes to the old pole of stalked cells [41]. In the case of CpdR, dephosphorylated CpdR localizes to the stalked pole after cytokinesis where it promotes the polar localization of the ClpXP–RcdA complex for targeted degradation of CtrA [34] (Figure 2a).

Polarization of cell movement using an oscillating protein

Myxococcus xanthus provides a fascinating example of a bacterium in which dynamic protein localization has a role in the regulation of a polarized function. This rod-shaped bacterium moves by gliding on solid surfaces. This process is driven by two different engines, the A- and S-engines, which function in a cooperative way to move the cell in one direction. Components of the two engines appear to be present at both cell poles at all times, but each engine is only fully assembled and active at one pole. The S-engine is active at the leading pole of the cell where it helps to pull the cell forward by retracting type IV pili. At the rear of the cell, the A-engine is thought to develop a pushing force by secreting slime through several ring-shaped nozzles [42] (Figure 2b). When myxobacteria move, the direction of movement changes many times during the lifetime of the cell. Reversal is based on inactivation of the engines at one pole followed by their activation at the opposite pole

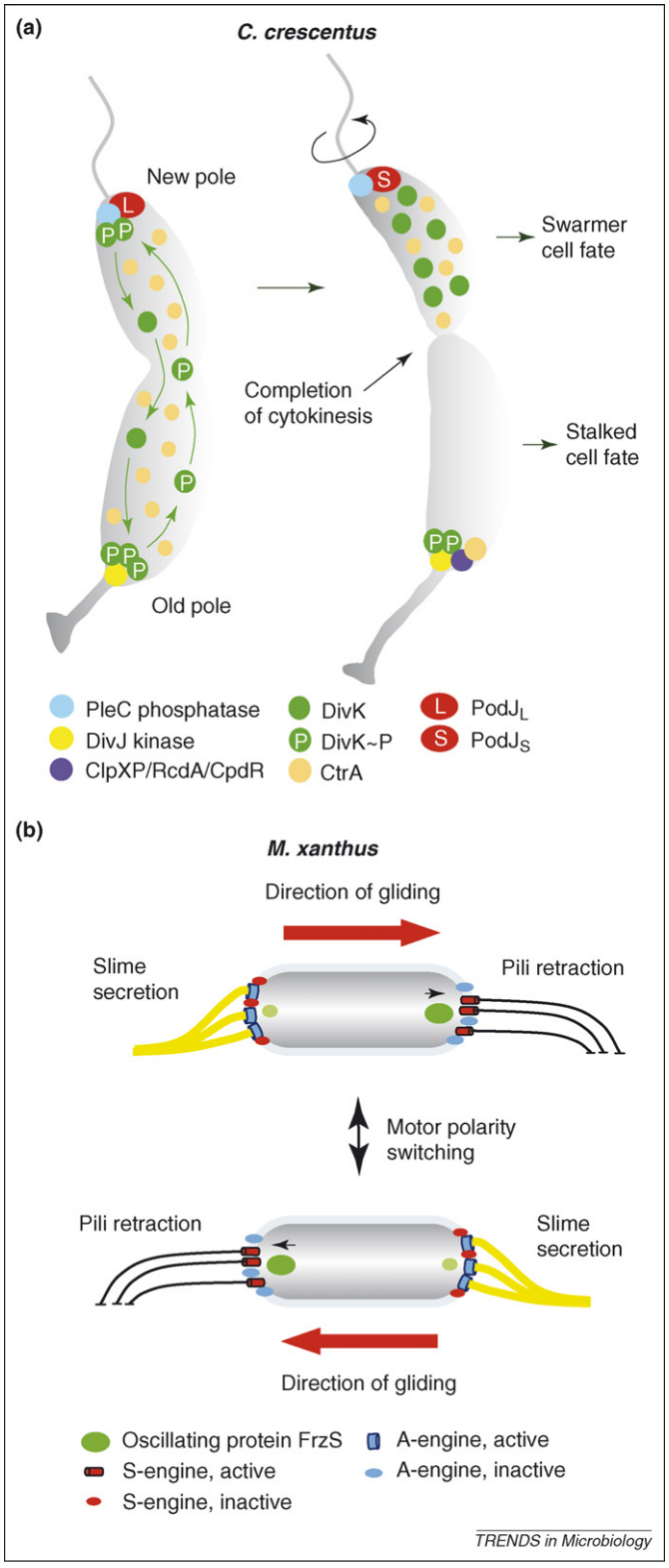

Figure 2. Control of polarized activity by dynamic protein localization in *Caulobacter crescentus* and *Myxococcus xanthus*. **(a)** Model showing how dynamic localization of the single-domain response regulator DivK leads to differential cell fate expression in the daughter cells of the dimorphic bacterium *C. crescentus* in response to completion of cytokinesis. DivK controls the targeted degradation of the cell fate determinant CtrA. In the predivisional cell, a dynamic localization pattern of DivK is established in which DivK and phosphorylated (active) DivK (DivK~P) rapidly shuttle between the two cell poles. At the stalked pole, phosphorylation of DivK by the DivJ kinase promotes localization of DivK~P to the opposite, flagellated pole. Dephosphorylation of DivK~P by the phosphatase PleC at the flagellated pole releases DivK, which diffuses back to DivJ, repeating the cycle. Polar localization of PleC requires the presence of full length PodJ ($PodJ_L$). Completion of cytokinesis sets up a communication barrier that segregates PleC and DivJ activities, disrupting the pole-to-pole shuttling of DivK and DivK~P. This results in low level of DivK~P diffusively distributed in the cytoplasm of the swarmer cell compartment, allowing stabilization of CtrA and swarmer cell fate expression. In the stalked cell compartment, a high level of active DivK~P remains at the DivJ stalked pole, thereby promoting degradation of CtrA by the ClpXP protease, which forms a complex with RcdA at the stalked pole. ClpX and RcdA also accumulate at the site of constriction in the predivisional cell (not shown) where they presumably perform another, unrelated proteolytic event [32]. Polar localization of ClpXP requires the response regulator CpdR in its unphosphorylated form. Clearance of CtrA activity results in expression of stalked cell fate. Completion of cytokinesis also triggers degradation of PodJ in a sequence of regulated proteolytic events that starts with the formation of a shorter form of PodJ (shown as $PodJ_S$). **(b)** Model for the regulation of motor polarity switching in gliding cells of *M. xanthus* by an oscillating protein FrzS. Gliding motility in *M. xanthus* is mediated by the coordinated action of two polar engines. At the leading pole, the S-engine pulls the cell forward by retracting type IV pili. At the opposite pole, the A-engine pushes the cell forward by slime extrusion. Even though each engine is fully assembled and active only at one pole at a time, components of both engines appear to be present at both poles at all times. Accordingly, reversal of the gliding direction of the cell involves inactivation of both engines at one pole and reactivation at the opposite pole. Control of polarity switching in *M. xanthus* involves the oscillating protein FrzS, which moves from pole to pole. Movement of FrzS is correlated with reversal of gliding in such a way that FrzS is always present at the leading pole with the active S-engine.

[43,44]. The frequency of motility switching is controlled by a chemosensory pathway consisting of proteins encoded by the *frz* operon. Strains carrying mutations in these genes show an altered frequency of motility reversals [42]. Recently, localization studies of FrzS revealed that this protein accumulates primarily at the leading, piliated pole with a weaker localization at the trailing pole [45]. Localization of FrzS is dynamic as the protein oscillates between poles. Importantly, relocalization of FrzS from one pole to the other correlates with reversal of motility. This correlation is also found in cells in which the reversal frequency is either increased or decreased by introducing mutations in other Frz proteins [45]. These data suggest a model (Figure 2b) in which oscillation of FrzS has an important regulatory role in controlling the site of pili extrusion and thereby the polarization and directional motility of *M. xanthus* [45].

Establishment of cell polarity – telling poles apart

In eukaryotes, the process of cell polarization has been extensively studied and follows a prototypical hierarchy of events. First, a spatial cue on the cell surface specifies the orientation of the cell polarity axis [46,47]. This cue can be either extrinsic (e.g. pheromone gradients established during mating in yeast or cell-to-cell contacts in epithelial tissues) or intrinsic (e.g. bud scar in yeast) [46,47]. Once established, the spatial cue is subsequently 'marked' by so-called landmark proteins followed by inward propagation of the polarity signal through a signaling pathway involving the specific activation of small GTP-binding proteins and cognate effectors [46,47]. Can bacteria use a similar strategy for cell polarization? If so, what are the spatial cues and landmark proteins, and how is this spatial information translated into cell polarization?

Extrinsic spatial cues

It is well known that bacteria are able to sense and respond to various signals from their surrounding environment. The question is whether such signals can influence the

polarity of the cells and thereby function as extrinsic polarity cues. One example in which this seems to be the case is the C-signal that regulates the coordinated motor polarity switching in gliding *M. xanthus* cells [48]. As described earlier, motility in *M. xanthus* relies on the coordinated activity of the S- and A-engines, which are active at opposite cell poles. The frequency at which the cell changes direction, referred to as reversal frequency, is controlled by a regulatory network that responds to the level of C-signaling [49,50]. C-signaling is produced by cell–cell contact through the interaction of a surface-associated protein, the C-factor, from one cell with its putative surface receptor from the neighboring cell (Figure 3a).

Intrinsic spatial cues

Because many proteins are located at the poles of bacterial cells, one obvious possibility is that the poles themselves contain information that can function as intrinsic spatial cues. Relative to other cellular regions of the cell envelope, bacterial poles are characterized by their concave shape, a layer of peptidoglycan that is less metabolically active [51,52], and a different phospholipid composition [53,54]. Furthermore, protein diffusion in the outer membrane is more constrained at the poles [23,51,52]. These characteristics might be exploited by the cell to distinguish polar regions from others.

How bacterial cells tell the new and old poles apart could rely on the age difference between these two poles. A recent study in which poles were tracked over several generations suggests that the polar peptidoglycan becomes progressively more inert as it ages [23]. The cell might be able to recognize the difference in peptidoglycan metabolic activity between the new and old poles. Because the poles of most bacteria are created by cell division, it is also possible that the new pole contains molecular information left over from the last division event. Consistent with this hypothesis, MacAlister *et al.* reported the observation of a zone of attachment between the membrane and the peptidoglycan at the site of division in plasmolyzed cells of *Salmonella typhimurium* (Figure 3b). This zone was present at the leading edge of the septum. After cell division, the site of adhesion between the membrane and the peptidoglycan remained at the new pole of the two daughter cells, much like a birth scar [55]. However, in the case of IcsA, positional information does not require cell division [3]. The apparent variety of mechanisms involved in polar protein localization suggests that there might be more than one way for the cell to identify its poles.

Landmark proteins involved in cell polarization

If bacteria use internal and external spatial cues to set up their polarity, how is this spatial information recognized

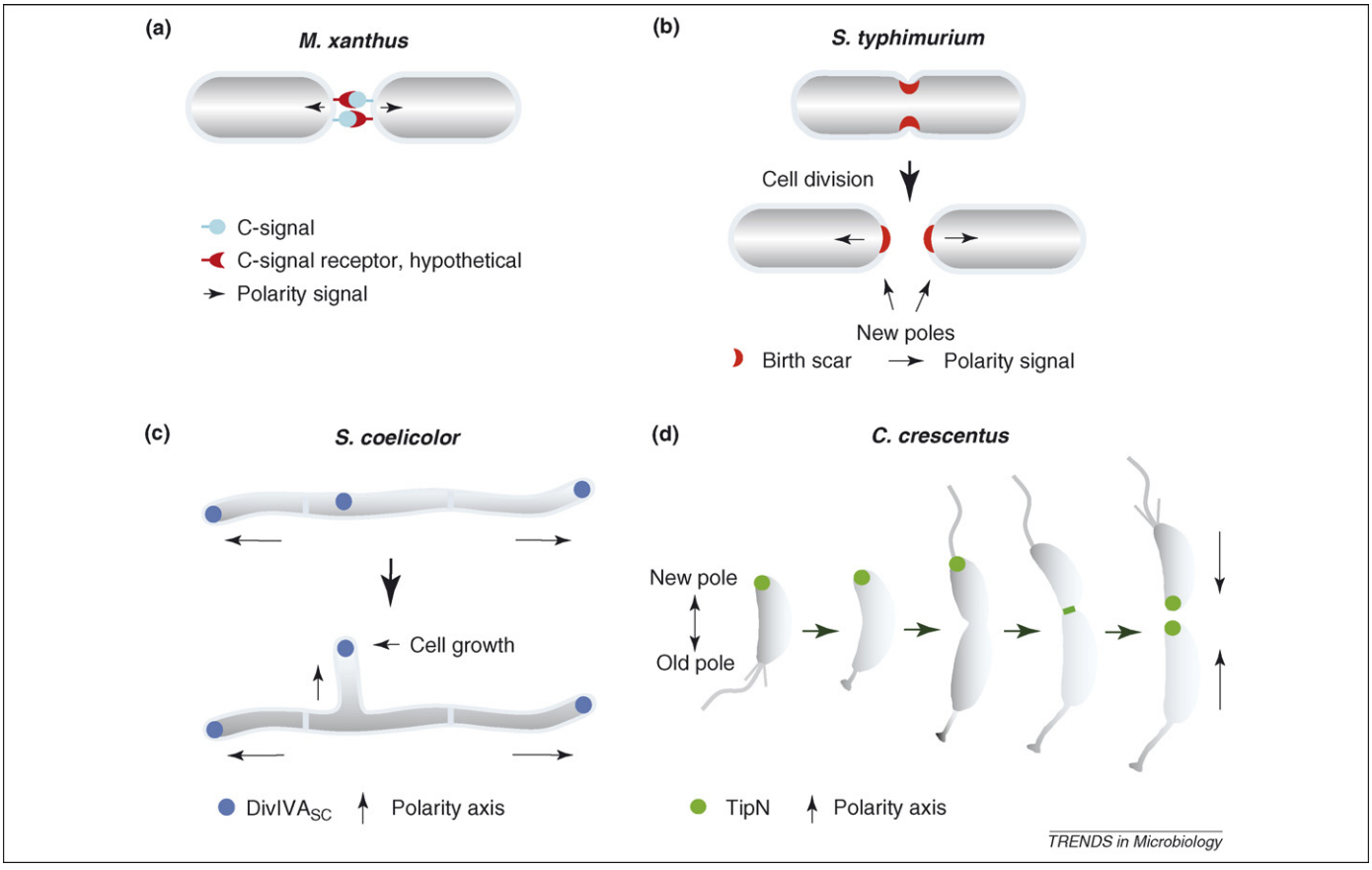

Figure 3. Spatial cues and landmarks involved in cell polarity in bacteria. **(a)** Cell-to-cell contact in *Myxococcus xanthus* promotes interaction of surface-exposed C-signal protein (putative extrinsic polarity signal) with hypothetical receptors on neighboring cells. The C-signal is propagated into the cell where it controls the polarized activity of the gliding motility engines (not shown). **(b)** In *Salmonella typhimurium*, a zone of membrane-peptidoglycan adhesion is observed at the leading edge of the growing septum of a dividing cell. After division, remnants of the membrane-peptidoglycan attachment site leave a birth scar at the new pole of the two daughter cells. **(c)** Localization of *Streptomyces coelicolor* $DivIVA_{SC}$ to the tip of growing hyphae. After cell septation, a new site of growth is established by branching of the sub-apical cell. $DivIVA_{SC}$ is the first protein known to localize to the nascent branch site. **(d)** Landmark protein TipN marks the new pole in *Caulobacter crescentus*. In the late predivisional cell, TipN moves to the future site of division. Cell division results in newborn cells, each inheriting TipN at the new pole.

and transduced by the cell? Studies in *Streptomyces coelicolor* and *C. crescentus* have identified proteins that might have a function similar to eukaryotic landmark proteins [56–58].

Similar to filamentous fungi, *S. coelicolor* grows by branching and extension of hyphal tips into the substrate where they form a mycelial network. This mode of polarized growth requires the *S. coelicolor* $DivIVA_{SC}$ protein [58], which, unlike its *B. subtilis* homolog [59,60], does not seem to affect cell division and chromosome localization. Moderate depletion of $DivIVA_{SC}$ in *S. coelicolor* results in abnormal hyphal shape and apical branching. Overproduction of DivIVA leads to cell swelling and a hyperbranching phenotype [58]. These phenotypes are consistent with $DivIVA_{SC}$ having a key role in polarized growth. The localization of $DivIVA_{SC}$ at the tip of growing hyphae and nascent branching sites suggests that $DivIVA_{SC}$ might function as a landmark protein to establish new sites of polarized growth (Figure 3c).

As mentioned earlier, *C. crescentus* shows several morphological manifestations of cell polarity (e.g. the presence of polar organelles and the polarization of the division plane, which yields daughter cells of different size). Recent evidence suggests that *C. crescentus* uses a landmark/birthmark protein (named TipN), which is inherited from the preceding cell cycle to set up the correct polarity of the daughter cells after division [56,57]. TipN, which is a membrane-bound coiled-coil rich protein, is found at the new pole during most of the cell cycle. At the predivisional cell stage, TipN is released from the pole, followed by a cell division-dependent relocalization of TipN to the site of constriction [56,57] (Figure 3d). Thereby, cell division yields newborn cells with TipN marking each new pole. Deletion of *tipN* results in several polarity defects including mispolarization of the division plane, misplacement of the polar flagellum, and mislocalization of several molecular polarity markers [56,57]. Overexpression of *tipN* leads to formation of aberrant TipN foci along the lateral site of the cell. These foci are sufficient to create new axes of polarity, resulting in branching and formation of new cell poles fully competent for polar protein localization and flagellum assembly [56].

How the cell decodes the positional information provided by TipN and $DivIVA_{SC}$ is not known. A clue could come from the observation that TipN is important for proper subcellular organization of the actin homolog, MreB [56]. The importance of this observation awaits further study. However, it is tempting to speculate that bacteria might have evolved polarity mechanisms that resemble those found in eukaryotic cells, in which signaling pathways link landmark proteins to the cytoskeleton. Major players in these eukaryotic signaling pathways are small GTPases [61]. Bacteria also encode several small GTPases, although these are not closely related to their eukaryotic counterparts [62]. An exciting exception is MglA, a Ras-like GTPase with an important role in motility switching in *M. xanthus* [63,64]. Most other small bacterial GTPases, however, seem to be involved in processes associated with ribosome biogenesis [62]. It is conceivable that bacteria use other types of signaling pathways to propagate the polarity signal into the cell interior. Alternatively, landmark proteins might interact directly with the cytoskeleton, a possibility that still remains to be experimentally addressed.

Role of the cytoskeleton in cell polarity

A connection between the actin homolog MreB and cell polarity was first described in *C. crescentus*, in which depletion or overproduction of MreB results in mislocalization of several polarity markers [65]. Most intriguing was the observation that upon replenishment of MreB after depletion, the polarity markers regain polar localization, but often at the wrong pole [65]. Studies in *E. coli* have shown that polar localization of the chemoreceptor Tar, *S. flexneri* IcsA and *Vibrio cholerae* EpsM is impaired in Δ*mreB* cells [66,67]. MreB could thus be a general cell polarity determinant.

How MreB affects cell polarity is not clear. One difficulty comes from the fact that MreB has multiple functions and has an essential role in rod shape determination such that MreB depletion results in gradual cell rounding [68]. The loss of wild-type geometry in MreB-depleted cells complicates data interpretation. The discovery of A22, a drug that rapidly disrupts MreB localization and function in Gram-negative bacteria [69,70], will facilitate future analyses of MreB function in cell polarization.

MreB forms pole-to-pole helical structures, presumably underneath the cytoplasmic membrane [65,71–74]. Time-lapse experiments of GFP-MreB in *B. subtilis* revealed a highly dynamic localization pattern in which movement of MreB filaments often seemed to be directed towards poles [75]. This opens the possibility that dynamic polymerization of MreB could provide the force required for delivery of proteins to specific polar locations. This mechanism would be similar to the way the bacterial actin-like protein ParM carries out plasmid segregation in *E. coli* [76,77]. By analogy to eukaryotic mechanisms, MreB filaments could alternatively serve as tracks for unknown motor proteins transporting molecules towards polar regions.

Recently, a single molecule study in live *C. crescentus* cells showed that MreB, like actin, undergoes directional treadmilling of monomers within filaments [78]. The inherent polarity of individual MreB polymers, in which monomers are added at one end and released at the other end, might be important for cell polarization and protein localization. However, calculations of average filament lengths revealed that most filaments are considerably shorter than the length of the cell, indicating that MreB is unlikely to form a continuous pole-to-pole polymer; rather, the data indicated that an individual MreB 'helix' consists of bundles of short filaments [78]. Even though single MreB filaments are polarized in nature, the MreB structure as a whole does not display any polarity with respect to the two cell poles [78]. This seems to argue against a direct role of MreB in polar trafficking of molecules. However, the possibility remains that cells contain more than one helical structure and that filaments within individual structures could be oriented in the same direction relative to the cell poles [78].

Given its role in cell morphogenesis, MreB might mediate cell polarity in a more indirect way, for instance, by spatially regulating peptidoglycan synthesis [79]. In

Box 1. Future questions

At this early stage of our understanding of bacterial cell polarity, there are far more questions than there are answers. Some important questions, among others, are:

- How are proteins retained at specific subcellular positions such as the cell poles?
- What is the chemical nature of the spatial cues involved in setting up cell polarity?
- How is spatial information transduced inside the cell?
- What is the exact role of the MreB cytoskeleton in bacterial cell polarity?
- What is the role of the peptidoglycan cell wall in cell polarization?
- How is cell polarity maintained and transmitted to the progeny?

round *E. coli* cells lacking MreB the localization of IcsA and EpsM is abnormal [67]. However, these two unrelated proteins often co-localize at multiple subcellular sites, which led to the proposal that these sites correspond to regions with inert peptidoglycan providing polar information [67]. A connection between cell polarity, MreB and the peptidoglycan is supported by the observations that *C. crescentus* cells recovering from depletion of MreB or RodA (a protein involved in cell wall elongation) or from pre-treatment with amdinocillin (whose presumed target is the peptidoglycan cross-linking enzyme, PBP2) often form ectopic poles [80].

Apart from MreB and its homologs, other polymer-forming proteins could be involved in some form of cell polarization. MinD is an attractive candidate because its pole-to-pole oscillation, which is independent of MreB, defines an axis of reference for positioning the division plane in *E. coli* and other bacteria [66].

Concluding remarks

Bacteria are polarized organisms that exploit their cellular asymmetries to enrich their physiology and adaptability. Many bacterial processes depend on polarly located proteins and the list is rapidly expanding. This fact, barely imaginable a decade ago, has revolutionized the way microbiologists perceive and study bacteria. The field of bacterial cell polarity is still young, however. The development of cellular asymmetry, the nature of the spatial cues, and the propagation of spatial information inside the cell remain poorly understood (Box 1). Clues seem to lie within the cytoskeleton and within the chemical and dynamic properties of the polar regions. The recent surge of interest in bacterial cell biology among scientists across fields holds the promise of new and exciting discoveries from these tiny, yet surprisingly sophisticated, creatures.

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