Regulation of cell polarity in bacteria

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Bacteria are polarized cells with many asymmetrically localized proteins that are regulated temporally and spatially. This spatiotemporal dynamics is critical for several fundamental cellular processes including growth, division, cell cycle regulation, chromosome segregation, differentiation, and motility. Therefore, understanding how proteins find their correct location at the right time is crucial for elucidating bacterial cell function. Despite the diversity of proteins displaying spatiotemporal dynamics, general principles for the dynamic regulation of protein localization to the cell poles and the midcell are emerging. These principles include diffusion-capture, self-assembling polymer-forming landmark proteins, nonpolymer forming landmark proteins, matrix-dependent self-organizing ParA/MinD ATPases, and small Ras-like GTPases.

Introduction

In the past 20 years, we have witnessed a fundamental change in our perception of how bacterial cells function. It has long been appreciated that all bacteria sort secreted proteins to their cytoplasmic membrane, the extracellular milieu, and in the case of Gram-negative bacteria also to the periplasm (space between the inner and outer membrane) and the outer membrane. Within a compartment, however, proteins were thought to be homogeneously distributed, giving rise to the notion of bacterial cells as unorganized bags of proteins. We now know that bacterial cells are, in fact, spatially highly organized with many proteins localizing asymmetrically. Although some bacteria contain separate organelles (Diekmann and Pereira-Leal, 2013), the spatial organization of most bacteria occurs in the absence of organelles. Two discoveries launched this paradigm shift: first, the observation that bacterial chemoreceptors and associated signaling proteins localize to the cell poles in two phylogenetically widely separated species (Alley et al., 1992; Maddock and Shapiro, 1993), and second, the finding that the cell division protein FtsZ localizes to the division site (Bi and Lutkenhaus, 1991). These breakthroughs showed that membrane-bound organelles are not a prerequisite for spatial organization of cells. Since then, spatially organized components of bacterial cells have been shown to not only include a large number of proteins, but also the chromosome (Wang et al., 2013), mRNA molecules (Kannaiah and Amster-Choder, 2014), lipids (Mileykovskaya and Dowhan, 2009), and metabolites such as c-di-GMP (Christen et al., 2010) and polyphosphate (Henry and Crosson, 2013). Here, we focus on how proteins become asymmetrically localized within the bacterial cell and how this polarity is regulated and dynamically changes over time. A recent review covers the possible role of localized translation of localized mRNA's for protein localization (Kannaiah and Amster-Choder, 2014) and will not be considered further.

Mechanisms underlying protein localization in bacteria

The number of polarized proteins is growing rapidly and includes proteins involved in growth, division, cell cycle regulation, differentiation, motility, signal transduction, and multi-enzyme complexes (Shapiro et al., 2009; Rudner and Losick, 2010). Thus, cell polarity with the asymmetric localization of proteins touches upon essentially all aspects of cell function. Moreover, protein localization is often highly dynamic and changes over time in response to cell cycle cues or external signals. Typical patterns of protein localization in bacteria include localization to one or both cell poles in rod-shaped cells, to midcell in rod-shaped as well as in spherical cells, along the long axis of rod-shaped cells, to specific structures such as stalks and endospores, and an oscillatory localization (Shapiro et al., 2009; Rudner and Losick, 2010; Lenz and Søgaard-Andersen, 2011; Laloux and Jacobs-Wagner, 2014). Major questions in understanding bacterial cell polarity are how proteins find their correct localization and how this localization may change over time. Three recurring themes, (1) diffusion-capture, (2) matrix-dependent self-organizing ParA/MinD ATPases, and (3) small GTPases, are emerging as important for protein localization.

Diffusion and capture

Diffusion and capture has been proposed to underlie the localization of many proteins in bacteria (Rudner and Losick, 2010). In this mechanism, a protein is synthesized in one location and then diffuses in three dimensions in the case of cytosolic proteins or two dimensions in the case of integral membrane proteins until they recognize and bind to a localized cue (Rudner et al.,

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Abbreviation used in this paper: T4P, type IV pili.

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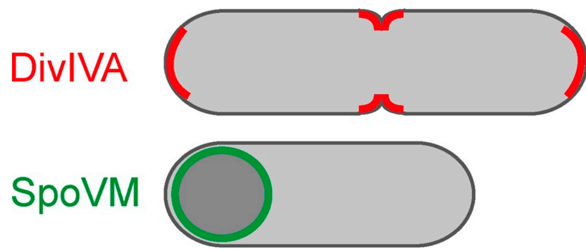

Figure 1. **Curvature as a geometrical cue for protein localization.** DivIVA of *B. subtilis* localizes at sites with negative curvature and SpoVM of *B. subtilis* recognizes the positive curvature of the endospore membrane.

2002; Deich et al., 2004). Over time, this sequence of events results in the localization of the diffusing protein to a particular location determined by the location of the cue. Three distinct types of cues are known: geometrical cues, lipids, and landmark proteins.

Diffusion and capture by geometrical cues. The cytosolic side of the bacterial cytoplasmic membrane is concave and has a negative curvature. However, in rod-shaped cells the membrane is not uniformly concave and the curvature at the cell poles and at the cell division septum is approximately twofold higher than along the lateral sides (Huang and Ramamurthi, 2010). There is evidence that geometrical cues such as positive or negative curvature can dictate the localization of proteins. The DivIVA protein, which is highly conserved in Gram-positive bacteria, localizes at bacterial poles and division sites, as well as at hyphal tips and lateral branches in filamentous Actinobacteria (Fig. 1; and Fig. 2, A and B; Flärdh, 2003; Nguyen et al., 2007; Lenarcic et al., 2009; Ramamurthi and Losick, 2009; Donovan et al., 2012). In *Bacillus subtilis*, the mechanism underlying this localization pattern has been shown to depend on DivIVA directly recognizing membranes of high negative curvature (Lenarcic et al., 2009; Ramamurthi and Losick, 2009). Structural analyses of DivIVA of *B. subtilis* revealed that it is rich in coiled-coil regions (Fig. 2 A) and forms dimers that interact end-to-end and laterally to self-assemble into a two-dimensional polymeric matrix (Stahlberg et al., 2004; Oliva et al., 2010). It is believed that exposed hydrophobic residues and positively charged residues in the N terminus allow the DivIVA assembly to interact with membranes of high negative curvature (Lenarcic et al., 2009; Ramamurthi and Losick, 2009; Oliva et al., 2010).

In the case of SpoVM, which is a small protein of 26 amino acids that forms an amphipathic α-helix, the protein recognizes membranes with a positive curvature as they form during sporulation in *B. subtilis* (Fig. 1; Ramamurthi et al., 2009). It is not known how SpoVM recognizes membranes of positive curvature, but it has been suggested that SpoVM molecules directly insert into the membrane (Ramamurthi et al., 2009).

Diffusion and capture by lipids. Certain lipids, especially cardiolipin, have also been suggested to function as spatial cues in bacteria. Cardiolipin-rich domains have been detected at division sites and at the poles of *Escherichia coli* (Mileykovskaya and Dowhan, 2000), *Pseudomonas putida* (Bernal et al., 2007), and *B. subtilis* (Kawai et al., 2004) cells. This localization has been proposed to reflect the shape of a cardiolipin molecule, in which the cross-sectional area of its head group is significantly smaller than that of its hydrophobic tail. The resulting small head/tail ratio would explain how cardiolipin is attracted to membranes of high negative curvature (Huang et al., 2006; Mukhopadhyay et al., 2008; Mileykovskaya and Dowhan, 2009). In *E. coli*, the polar localization of the transporter ProP as well as of the mechanosensitive channel MscC correlates with the cellular cardiolipin content (Romantsov et al., 2010), suggesting that cardiolipin may function in capturing proteins. However, whether this is due to a direct interaction with cardiolipin or due to an interaction with another protein targeted by cardiolipin remains to be shown.

Diffusion and capture by polymer-forming landmark proteins. Probably the best understood mechanisms for protein localization involves cases in which one protein recruits a second protein. These capture proteins are often referred to as landmark proteins and appear to exist in two forms, polymer forming and nonpolymer forming. Polymer-forming landmark proteins are cytoplasmic proteins that self-assemble at cellular locations that are established in a cell cycle–dependent manner, thus providing a straightforward solution for synchronizing cell cycle progression and protein localization and essentially hard-wiring this localization pattern into the cell cycle.

DivIVA is one of the best-studied polymer-forming landmark proteins. As discussed earlier, DivIVA in *B. subtilis* is a membrane-associated protein that self-assembles into a polymeric matrix at membranes of high negative curvature, in this way providing a proteinaceous platform at the cell poles and the cell division septum that can be recognized by other proteins. In growing *B. subtilis* cells, DivIVA preferentially localizes at the cell division septum, forming ring-shaped structures on both sides of the septum and to a lesser degree at the cell poles, where it forms small patches (Eswaramoorthy et al., 2011). Only at the septum, DivIVA directly interacts with and recruits the membrane protein MinJ, which in turn recruits the cell division inhibitory MinCD complex to inhibit additional cell divisions at the division site (Fig. 2 A; Bramkamp et al., 2008; Gregory et al., 2008; Patrick and Kearns, 2008). DivIVA was recently suggested to localize dynamically and relocalize from a previous (old) division site to the new division site (Fig. 2 A; Bach et al., 2014). Similarly, MinC was shown to relocalize from an old to a new division site (Gregory et al., 2008). How this dynamic localization is regulated is not known. Similarly, it is not known how MinJCD interaction with DivIVA is restricted to the division site. In sporulating *B. subtilis,* DivIVA tethers the prespore chromosome to the pole via a direct interaction to the RacA protein that binds to the origin of replication region on the chromosome (Ben-Yehuda et al., 2003, 2005).

In the filamentous Actinobacterium *Streptomyces coelicolor*, DivIVA also spontaneously polymerizes in vitro and has been suggested to form a matrix of short filaments (Wang et al., 2009) at hyphal tips with their high negative curvature (Flärdh, 2010). DivIVA recruits two additional polymer-forming proteins, Scy and FilP, by direct interactions to guide hyphal growth at the tips (Fig. 2 B; Fuchino et al., 2013; Holmes et al., 2013). Scy and FilP are paralogues, predominantly composed of coiled-coil domains, and spontaneously polymerize in vitro (Bagchi et al., 2008; Walshaw et al., 2010). Scy colocalizes with DivIVA

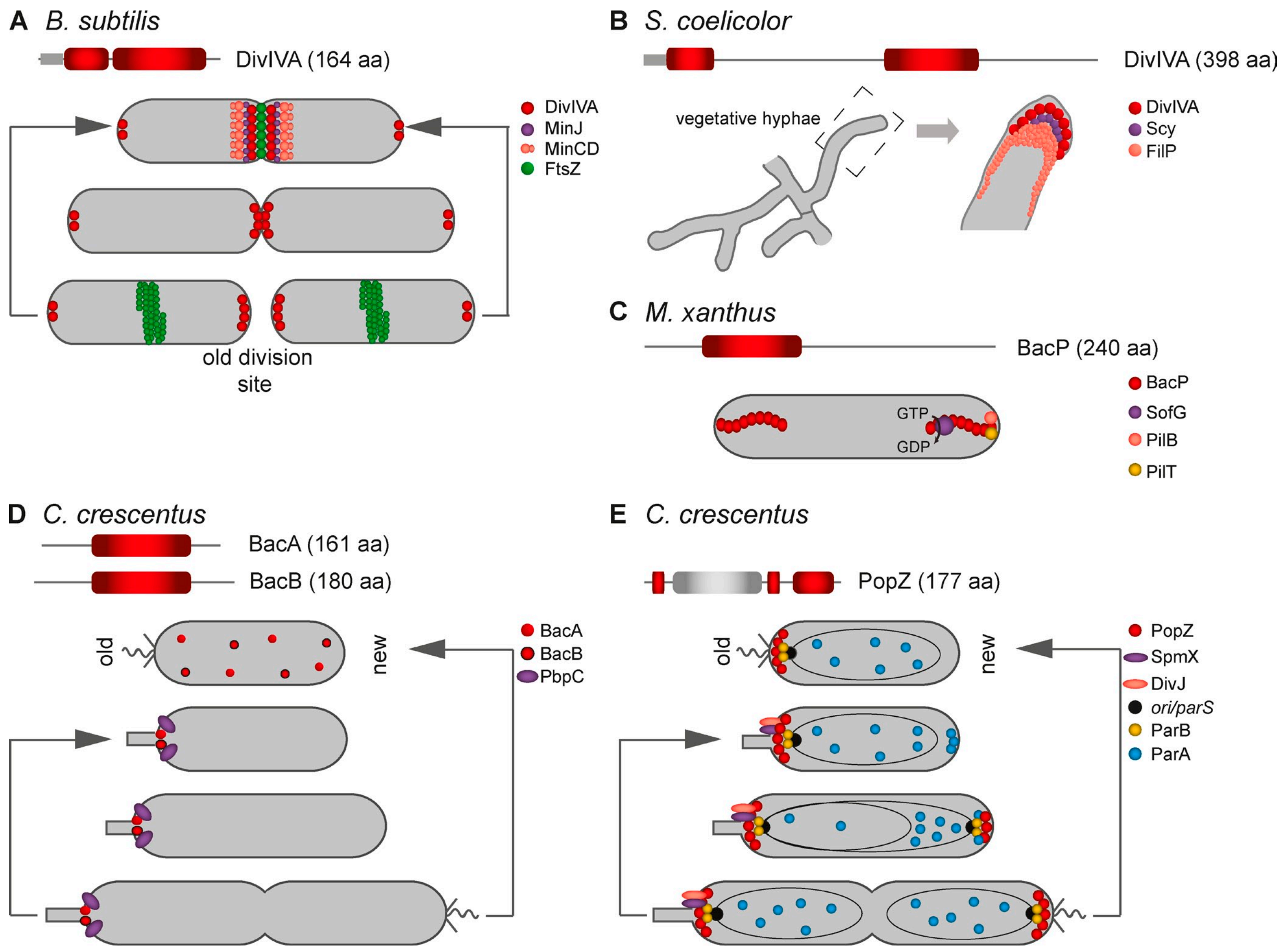

Figure 2. **Polymer-forming landmark proteins.** (A) The domain structure of DivIVA is indicated with the lipid-targeting domain in gray and coiled-coil regions in red. Bottom: localization of DivIVA and associated proteins during a cell cycle. (B) The domain structure of DivIVA of *S. coelicolor* is indicated as in A. Bottom: left diagram illustrates the hyphal growth pattern and the right diagram illustrates the localization of DivIVA, Scy, and FilP at a hyphal tip. (C) The domain structure of BacP with the bactofilin domain in red. Bottom: localization of BacP and associated proteins. GTP hydrolysis by SofG is indicated. Note that SofG is only associated with the BacP landmark at one pole. (D) The domain structures of BacA and BacB with the bactofilin domains in red. Bottom: localization of BacA and BacB and associated protein during the cell cycle and with the old and new poles marked. Flagellum and pili are indicated at the nonstalked pole. (E) The domain structure of PopZ is indicated with α-helical regions in red and a proline-rich domain in gray. Bottom: localization of PopZ and associated proteins during the cell cycle including the ParABS-dependent chromosome segregation process and with the old and new poles marked. Flagellum and pili are indicated at the nonstalked pole. Black ovals represent the chromosome.

at hyphal tips while FilP forms a gradient-like structure extending from the hyphal tips (Fig. 2 B; Fuchino et al., 2013; Holmes et al., 2013). DivIVA together with Scy and FilP have been suggested to form a tip-organizing center or polarizome that recruits proteins involved in hyphal growth (Fuchino et al., 2013; Holmes et al., 2013). Scy and DivIVA also interact directly with two proteins involved in chromosome segregation, the ParA ATPase and ParB (described in detail later), respectively (Donovan et al., 2012; Ditkowski et al., 2013), suggesting that the polarizome may also be involved in chromosome segregation.

Bactofilins are widespread in bacteria and are emerging as important polymer-forming landmark proteins (Kühn et al., 2010). *Myxococcus xanthus* contains four bactofilins and in vitro all four spontaneously polymerize to form filaments (Kühn et al., 2010; Koch et al., 2011; Bulyha et al., 2013). In vivo BacP forms large patches in the two subpolar regions, and one of these patches functions as a landmark for the small GTPase SofG by direct interaction (Bulyha et al., 2013). BacP and SofG in turn are important for the polar localization of the ATPases PilB and PilT involved in type IV pili (T4P)–dependent motility (described later; Fig. 2 C; Bulyha et al., 2013). SofG depends on its GTPase activity for proper function and shuttles back and forth over the BacP matrix, and it is thought that SofG either transports PilB and PilT to the pole or helps to retain them at the pole. The spatial cue for BacP localization is not known. Similarly, it is not known how SofG localization is restricted to one subpolar BacP matrix.

Cells of *Caulobacter crescentus* are highly asymmetric and cell division gives rise to a swarmer cell that has pili, which are filamentous surface structures assembled from the PilA subunit, and a single flagellum at the old pole, as well as a stalked cell with a stalk at the old pole (Fig. 2 D). At the G1-to-S transition, the swarmer cell looses the flagellum and pili and builds a stalk at the same pole. The two bactofilins BacA and BacB in

C. crescentus switch from a diffuse localization pattern in swarmer cells to a focal localization at the stalked pole at the G1-to-S transition and spontaneously polymerizes in vitro (Fig. 2 D; Kühn et al., 2010). The two proteins are thought to copolymerize at the cytoplasmic membrane at the stalked pole, where they recruit the peptidoglycan biosynthetic enzyme PbpC by direct protein–protein interaction (Kühn et al., 2010). It has been speculated that the positively curved membrane emerging at the base of the growing stalk is a spatial cue for BacAB localization (Kühn et al., 2010).

PopZ in *Caulobacter crescentus* has functional similarity to DivIVA but the two proteins are not homologues. PopZ is restricted to the α-proteobacteria, is a cytoplasmic protein, and self-assembles into a polymeric matrix in chromosome-free regions at the cell poles (Bowman et al., 2008; Ebersbach et al., 2008). Here it recruits other proteins including ParA, ParB, and cell cycle regulators important for the G1-to-S transition (Bowman et al., 2008, 2010; Ebersbach et al., 2008; Schofield et al., 2010; Ptacin et al., 2014). PopZ is rich in α-helical regions but is not thought to form coiled-coils (Fig. 2 E). In vitro PopZ spontaneously assembles into a branched matrix (Bowman et al., 2008, 2010; Ebersbach et al., 2008). After cell division, PopZ localizes at the old pole and anchors ParB on the centromere-like sequence *parS*, which is located close to the replication origin, to this pole (Bowman et al., 2008; Ebersbach et al., 2008). After initiation of DNA replication, a second PopZ matrix is formed at the opposite pole, giving rise to a bipolar pattern. As replication proceeds, one of the ParB–*parS* complexes is transferred to the new pole by the ParA ATPase, where it is captured by the newly formed PopZ cluster (Fig. 2 E). Upon cell division, the unipolar localization pattern with PopZ at the old pole is reestablished. The cell cycle–dependent assembly of the new PopZ cluster is thought to be nucleated by the ParA ATPase as it accumulates at the new pole during chromosome segregation (Fig. 2 E; Laloux and Jacobs-Wagner, 2013). Thus, the cell cycle–dependent assembly of a new polar PopZ cluster is tied in with the initiation of chromosome replication and segregation. PopZ is important for recruiting the integral membrane protein SpmX protein to the old pole at the G1-to-S transition (Fig. 2 E; Bowman et al., 2010). SpmX in turn recruits the cell cycle regulator DivJ (Radhakrishnan et al., 2008). DivJ localization causes its activation as a kinase and this activation stimulates cell cycle progression (Tsokos and Laub, 2012). Thus, PopZ has essential functions in chromosome segregation and cell cycle regulation. Whether the recruitment of SpmX by PopZ and DivJ by SpmX involve direct interactions is currently not known.

It is interesting to note that different species use different polymer-forming landmarks for similar tasks, i.e., DivIVA and PopZ both anchor ParB–*parS* for chromosome segregation, and similar polymer-forming landmarks for different tasks, i.e., bactofilins recruit PbpC for peptidoglycan synthesis in *C. crescentus* and SofG for T4P-dependent motility in *M. xanthus*. Whether these differences are due to differences in cell morphology or natural habitats is not known, but the differences suggest that landmark proteins have evolved to comply with the specific needs of individual bacterial species.

Diffusion and capture by nonpolymer-forming landmark proteins. Whereas polymer-forming landmark proteins are cytoplasmic, the second type of landmark proteins are integral membrane proteins and often contain a periplasmic peptidoglycan-binding domain. So far, no evidence has been provided that these landmark proteins assemble to form polymeric structures. Therefore, we refer to these proteins as nonpolymer-forming landmark proteins. Nonpolymer-forming landmark proteins also localize in a cell cycle–dependent manner and have emerged as an important concept for understanding the cell cycle–dependent localization of other proteins.

As described earlier, cell division in *C. crescentus* gives rise to two asymmetric cells, i.e., the swarmer with pili and a flagellum at the old pole and the stalked with a stalk at the old pole (Fig. 2 D). The cell cycle–regulated synthesis and removal of these polarly localized structures have provided a rich playground for the identification of landmark proteins important for their proper localization. The landmark protein TipN is essential for the proper placement of the flagellum (Huitema et al., 2006; Lam et al., 2006). TipN has two transmembrane regions in the N-terminal region and a large C-terminal coiled-coil domain (Fig. 3 A). TipN homologues are present in other α-proteobacteria. TipN localizes to the new pole in both daughter cells after division and relocalizes to the cell division site in the late predivisional cell (Fig. 3 A). Therefore, both daughter cells have TipN at the new pole after division. TipN recruits the polytopic membrane protein TipF, which is a positive regulator of flagella assembly and a receptor of the second messenger c-di-GMP at the G1-to-S transition in response to an increase in cellular levels of c-di-GMP (Fig. 3 A; Davis et al., 2013). TipF, in turn, recruits PflI, a bitopic membrane protein that is also required for proper flagellum placement as well as proteins of the flagella basal body (Obuchowski and Jacobs-Wagner, 2008; Davis et al., 2013). In total, this set of interactions is proposed to result in the formation of a "flagellar organizational center" at the new pole of a stalked cell (Davis et al., 2013). The stalked as well as the swarmer cell inherits TipN at the new pole; however, in swarmer cells the c-di-GMP level is low, thus hindering TipF binding to TipN, whereas the c-di-GMP level is high in stalked cells, resulting in the recruitment of TipF and PflI to the new pole in stalked cells (Davis et al., 2013). The cell division proteins FtsZ and FtsI are important for localization of TipN to the cell division site in predivisional cells (Huitema et al., 2006; Lam et al., 2006); however, it is not known if these proteins interact directly with TipN. Similarly, it is not known what cues the release and relocation of TipN in predivisional cells. TipN also interacts with ParA at the new pole and helps to maintain directionality of the segregating ParB–*parS* complex (Ptacin et al., 2010; Schofield et al., 2010).

The PodJ protein also functions in pole morphogenesis by serving as a landmark for proteins involved in pili assembly, including the ParA-like ATPase CpaE and several cell cycle regulators, including the membrane protein DivL (Viollier et al., 2002a,b; Lawler et al., 2006; Curtis et al., 2012). PodJ homologues are present in most other α-proteobacteria (Lawler et al., 2006). PodJ exists in two forms: the full-length $PodJ_L$ and $PodJ_S$, which is generated by proteolytic cleavage of $PodJ_L$ during

cell division, and both localize to the flagellated pole. During the G1-to-S transition $PodJ_S$ is degraded and then resynthesized in predivisional cells. $PodJ_L$ comprises a single transmembrane domain, a C-terminal peptidoglycan-binding domain in the periplasm, and a cytoplasmic part containing coiled-coil domains, whereas $PodJ_S$ lacks the periplasmic and transmembrane domain (Fig. 3 B; Viollier et al., 2002a,b; Lawler et al., 2006; Curtis et al., 2012). Genetic dissection of PodJ suggests that the cytoplasmic part is sufficient for polar localization (Lawler et al., 2006), whereas the cytoplasmic as well as the periplasmic domain are required for recruiting the different proteins that depend on PodJ for proper localization (Lawler et al., 2006; Curtis et al., 2012). Among the different proteins that depend on PodJ for localization, only the integral membrane protein DivL has been shown to directly interact with PodJ (Curtis et al., 2012). It is currently not known how PodJ becomes polarly localized.

HubP is a polarly localized nonpolymer-forming landmark protein in *Vibrio cholerae* and serves important functions in recruiting other polarly localized proteins (Yamaichi et al., 2012). HubP has an N-terminal LysM peptidoglycan-binding domain, a single transmembrane region, and a C-terminal region rich in repeat structures (Fig. 3 C). The protein is conserved among *Vibrio* species and less well-conserved in other γ-proteobacteria. HubP localizes to both poles and the periplasmic domain, including the peptidoglycan-binding LysM, is required and sufficient for polar localization, suggesting that an interaction between HubP and polar peptidoglycan contributes to targeting and/or retention of HubP at a pole. Immediately after cell division, HubP is bipolarly localized and begins to accumulate at midcell before cell division (Fig. 3 C). Interestingly, FRAP experiments demonstrate that the polar HubP clusters are dynamic with an exchange of HubP protein between the poles. Thus, recruitment of HubP to a pole not only occurs during cell division. Polarly localized HubP interacts directly with the ParA1 ATPase, which is involved in segregation of chromosome 1 (Fogel and Waldor, 2006), and the ParA ATPase FlhG, which regulates flagellar assembly together with the GTPase FlhF (Fig. 3 C; Correa et al., 2005; Yamaichi et al., 2012). HubP also recruits—but does not directly interact with—the ParA ATPase ParC, which is required for the polar recruitment of chemotaxis proteins (Fig. 3 C; Ringgaard et al., 2011; Yamaichi et al., 2012). ParC, in turn, recruits the ParP protein to the pole by direct interaction, and ParP as well as ParC interact with the CheA kinase, in that way stimulating the formation of a large complex of chemotaxis proteins at the pole (Ringgaard et al., 2014). Interestingly, although HubP is present at both poles and at midcell before division, ParA1, FlhG, and ParC do not interact with HubP at both poles at all times. For example, in newborn cells ParC is at the old pole, and only becomes bipolar as a cell progresses through the cell cycle (Ringgaard et al., 2011). However, it is not known what prevents ParC, ParA1, and FlhG from interacting with HubP at both poles.

A major question concerns how nonpolymer-forming landmark proteins become correctly localized in the first place. It is interesting that TipN, PodJ, and HubP are all integral membrane proteins, and two (PodJ and HubP) have predicted peptidoglycan-binding domains, suggesting that binding to the

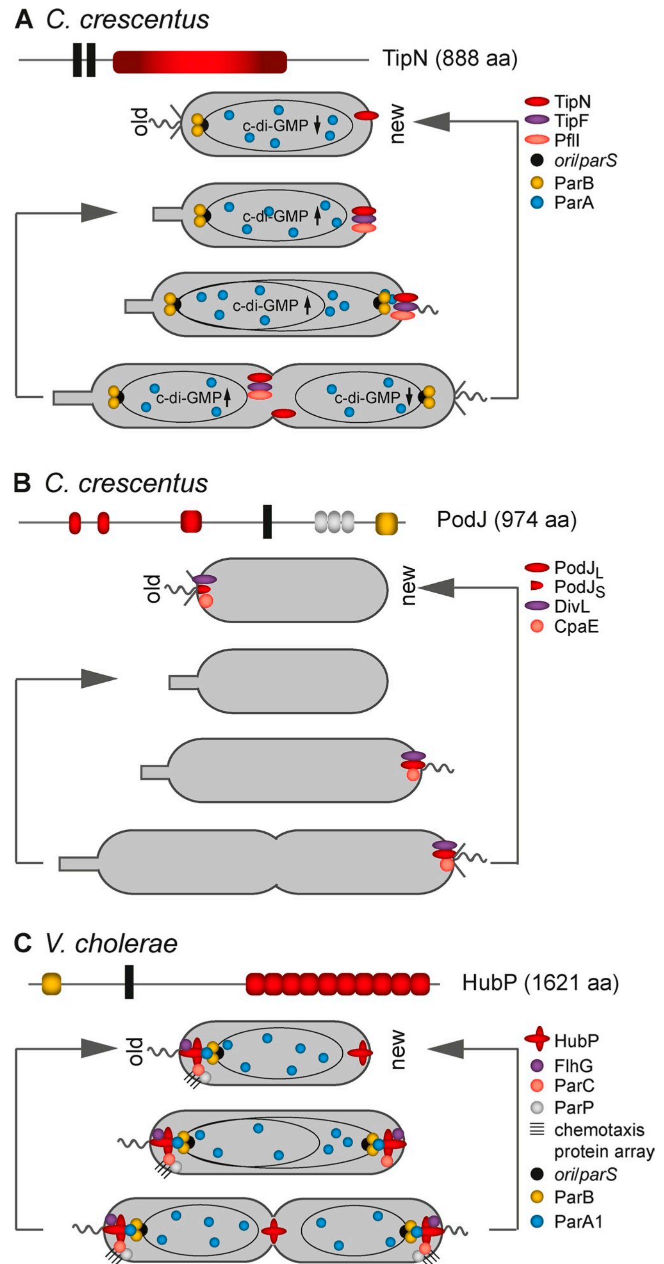

Figure 3. **Nonpolymer-forming landmark proteins.** (A) The domain structure of TipN is indicated with transmembrane domains in black and a coiled-coil region in red. Bottom: localization of TipN and associated proteins during the cell cycle including the ParABS-dependent chromosome segregation process and with the old and new poles marked. c-di-GMP levels in different cell types are indicated as high (upward arrow) and low (downward arrow). Flagellum and pili are indicated at the nonstalked pole. (B) The domain structure of PodJ is indicated with coiled-coil regions in red, a transmembrane domain in black, three TPR domains in gray, and the peptidoglycan binding muramidase domain in yellow. Bottom: localization of PodJ and associated proteins during a cell cycle. Flagellum and pili are indicated at the nonstalked pole. (C) The domain structure of HubP is indicated with the LysM peptidoglycan-binding domain in yellow, a transmembrane domain in black, and the repeat rich region in red. Bottom: localization of HubP and associated proteins during a cell cycle including the chromosome segregation process, the flagellum, and the array of chemotaxis proteins.

inert peptidoglycan formed at a septum (de Pedro et al., 1997) may have a function in polar localization and/or polar retention. TipN, PodJ, and HubP only have a limited phylogenetic distribution,

suggesting that different species have evolved different solutions for the polar localization of proteins. This may not be that surprising given that their protein partners or "client proteins" are very different. It is also interesting to note that TipN, PodJ, and HubP are dispensable for growth, whereas a *B. subtilis divIVA* mutant is viable but grows more slowly than the wild type, DivIVA is essential in *S. coelicolor*, and a *C. crescentus popZ* mutant is viable but also grows more slowly than the wild type. On the other hand, bactofilins are dispensable for growth in *C. crescentus* as well as in *M. xanthus*. Although only a few landmark proteins have been characterized, these comparisons raise the question of whether polymer-forming landmark proteins are at the heart of the circuits that spatially regulate essential cellular processes, whereas nonpolymer-forming landmarks spatially regulate more peripheral processes.

Protein localization by matrix-dependent, self-organizing ParA/MinD ATPases

Proteins of the ParA/MinD superfamily of P-loop ATPases have emerged as ubiquitous and versatile players for spatially organizing bacterial cells. These proteins function in plasmid and chromosome segregation, positioning of the cell division site, positioning of motility structures, and large cytoplasmic and polar protein complexes (Lutkenhaus, 2012; Vecchiarelli et al., 2012). ParA/MinD proteins localize other proteins by two different mechanisms (Lutkenhaus, 2012). In the landmark mechanism, a ParA protein serves as an adaptor between a landmark protein and a protein to be localized as described for MinD interacting with MinJ in *B. subtilis*, ParA interacting with TipN in *C. crescentus*, and ParA1, FlhG, and ParC interacting with HubP in *V. cholerae*. The second mechanism depends on the ability of Par/MinD proteins to self-organize on a matrix. Among the latter proteins, ParA proteins use the chromosome and MinD proteins the cytoplasmic membrane as a matrix.

The matrix-dependent self-organizing ParA/MinD ATPases are nucleotide-dependent molecular switches, and their interaction with the matrix depends on the nucleotide-bound state (Lutkenhaus, 2012): Generally, ATP binding occurs spontaneously and results in dimer formation. ParA/MinD proteins have a low intrinsic ATPase activity and ATP hydrolysis is often stimulated by a partner protein, causing the formation of the monomeric ADP-bound form followed spontaneously by the formation of the monomeric apo-form. Importantly, the ATP-bound dimer can interact with a matrix whereas the monomeric forms cannot. The interaction between ParA/MinD proteins and their cognate matrices allows the formation of a variety of different localization patterns.

The best-studied ParA proteins are involved in chromosome and plasmid segregation, and the analysis of these systems has been instrumental for understanding how these proteins may function in protein localization (Gerdes et al., 2010; Lutkenhaus, 2012; Vecchiarelli et al., 2012). The plasmid and chromosome segregation systems consist of two additional components: *parS*, a centromere-like site on the plasmid or chromosome, and a ParB protein that binds to *parS* sequences and also interacts with the ATP-bound ParA dimer to stimulate its ATPase activity. ParA binds nonspecifically to the chromosome in the ATP-bound dimer form, whereas monomeric ParA is diffusely localized. Plasmid-associated *parABS* systems distribute plasmids regularly over the nucleoid, whereas chromosome-associated *parABS* systems segregate the chromosomal replication origin to the polar regions and depends on additional landmark proteins for this polar positioning, i.e., PopZ, TipN, and HubP (compare Fig. 2 E with Fig. 3, A and C). Although there is general agreement that DNA binding by the ParA dimer and ParB stimulation of ParA ATPase activity with the concomitant release of ParA from the DNA is important, two different models exist to describe how this results in self-organization of the ParA protein on the chromosome.

In the filament-pulling model (Gerdes et al., 2010), ParA polymerizes into a DNA-bound filament upon ATP binding. Upon encountering a ParB–*parS* complex, ATPase activity is stimulated, and the filament begins to disassemble with the release of monomeric ParA that is diffusely localized. In this model, the ParB–*parS* complex moves in the wake of a depolymerizing ParA filament. Upon ATP binding, ParA reassociates nonspecifically with the DNA away from the ParB–*parS* complex and forms a filament. Eventually this ParA filament makes contact with the *parS*–ParB complex and the cycle is repeated. These interactions result in ParA continuously oscillating over the nucleoid, resulting in the distribution of plasmids over the chromosome. In the diffusion-ratchet model (Vecchiarelli et al., 2012, 2013, 2014; Hwang et al., 2013), ParA dimers independently bind the chromosome. The ParB–*parS* complex interacts with DNA-bound ParA, resulting in ParA being chased off the DNA by ParB–*parS*. Subsequently, the ParB–*parS* complex reassociates with a different DNA-bound ParA, causing the plasmid to relocate on the chromosome. Because the released ParA does not immediately rebind to the chromosome, a ParA gradient is formed around the ParB–*parS* complex, resulting in the biased diffusion of the ParB–*parS* complex on the chromosome.

In the plasmid systems, the ParABS system distributes plasmids over the chromosome and the plasmids can be considered cargo of the ParA ATPase. Interestingly, in *Rhodobacter sphaeroides* a cytoplasmic cluster of chemotaxis proteins are positioned on the chromosome by the ParA homologue PpfA (Roberts et al., 2012). PpfA associates with the chromosome and the cluster of chemotaxis proteins in the ATP-bound form. This association with the chemotaxis proteins depends on TlpT. Similarly to ParB, TlpT has dual functions: it functions as a bridge between PpfA and the chemotaxis proteins, and it is an activator of PpfA ATPase activity. It is though that the nucleotide-dependent repeated associations between PpfA and the chromosome and the chemotaxis proteins result in the positioning of the chemotaxis protein cluster over the middle of the chromosome early in the cell cycle and later, after the cluster by an unknown mechanism has split into two, causes the distribution to the two daughter chromosomes (Fig. 4 A).

In bacteria, cell division generally initiates with assembly of FtsZ into a ring-like structure, the Z-ring, at the division site (Lutkenhaus, 2012). Two systems, the oscillating MinCDE system in *E. coli* and the gradient forming ParB/MipZ system in *C. crescentus*, which regulate Z-ring positioning, are

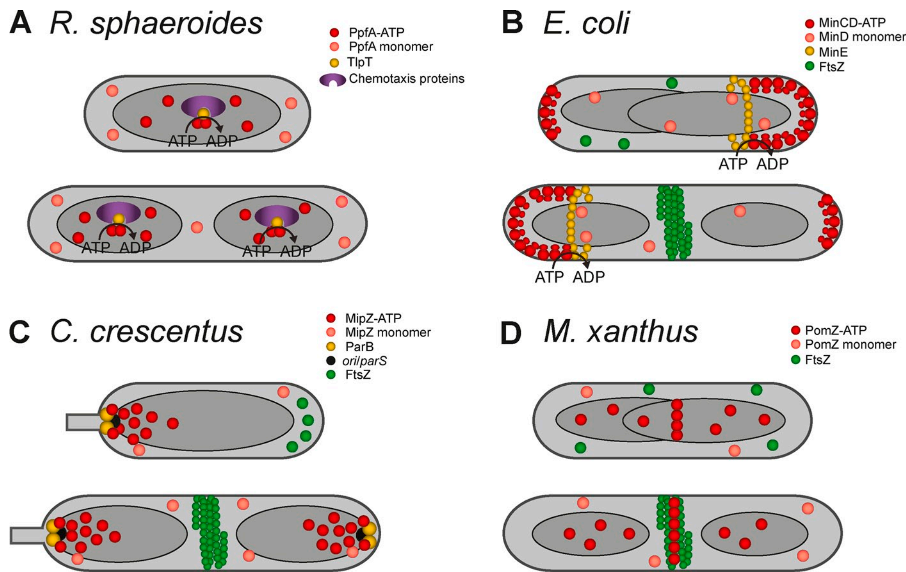

Figure 4. **Protein localization by matrix-dependent, self-organizing ParA/MinD ATPases.** (A) PpfA localization in *R. sphaeroides* and its association with the chromosome (dark gray) and the cluster of chemotaxis proteins. TlpT-induced ATP hydrolysis by PpfA is indicated. (B) Localization of the MinCDE proteins in *E. coli*. MinE-induced ATP hydrolysis by MinD is indicated. The level of MinC is lowest at midcell where the Z-ring is formed. Chromosomes are indicated in dark gray. (C) Localization of MipZ in *C. crescentus*. A MipZ gradient is formed over the chromosome (dark gray) and with the lowest MipZ concentration at midcell where the Z-ring is formed. (D) PomZ localization in *M. xanthus*. PomZ localizes at midcell before and independently of FtsZ. Chromosomes are indicated in dark gray. The PomZ monomer is shown to not bind to the chromosome; however, this has not been experimentally verified.

particularly well-studied and depend on matrix-dependent, self-organizing ATPases.

In *E. coli* positioning of the Z-ring is negatively regulated by the MinCDE system (Fig. 4 B; de Boer et al., 1989). This Min system consists of three proteins, MinC, MinD, and MinE. MinC is the inhibitor of Z-ring formation (Dajkovic et al., 2008; Shen and Lutkenhaus, 2010). In the presence of ATP, MinD dimerizes, binds MinC, and also binds to the cytoplasmic membrane (de Boer et al., 1991; Lackner et al., 2003). MinE, in turn, interacts with membrane-bound MinD and stimulates MinD ATPase activity and, therefore, the release of MinD-ADP from the membrane (Raskin and de Boer, 1997; Hu and Lutkenhaus, 2001). Subsequently, released MinD replaces ADP for ATP and rebinds to the membrane. These repeated interactions between MinD and MinE result in the formation of an oscillatory behavior of all three proteins in which the Min proteins oscillate from pole to pole with a period of 1–2 min (Hu and Lutkenhaus, 1999; Raskin and de Boer, 1999a,b; Fu et al., 2001; Hale et al., 2001). In this system, the time-averaged concentration of MinC is highest at the cell poles and lowest at midcell, resulting in inhibition of Z-ring formation at the cell poles and no inhibition at midcell (Meinhardt and de Boer, 2001). Thus, FtsZ polymerizes and localizes at midcell, not because it is recruited to midcell but because the midcell region is the only region where the MinC concentration is sufficiently low to permit polymerization.

C. crescentus uses a gradient of an FtsZ-inhibitory protein to regulate the position of the Z-ring. In this case, the inhibitor is the ParA homologue MipZ (Fig. 4 C; Thanbichler and Shapiro, 2006). After cell division, MipZ localizes to the old pole by interacting with the ParB–*parS* complex. After initiation of replication, one of the ParB–*parS* complexes segregate to the opposite cell pole, resulting in bipolar localization of ParB–*parS* and, therefore, also bipolar localization of MipZ. The interaction of MipZ with ParB–*parS* stimulates the ATP-dependent dimerization of MipZ, whereas ATP hydrolysis by MipZ is spontaneous. Because MipZ dimers bind nonspecifically to chromosomal DNA, this leads to the formation of a bipolar gradient of MipZ that extends from the two ParB–*parS* complexes over the chromosome and with a trough at midcell allowing Z-ring formation at this position (Thanbichler and Shapiro, 2006; Kiekebusch et al., 2012). Thus, similarly to FtsZ in *E. coli*, FtsZ in *C. crescentus* is not directly recruited to midcell but localizes there because this is the region where the MipZ concentration is sufficiently low to allow FtsZ polymerization.

In *M. xanthus,* which lacks Min and MipZ/ParB systems, the ParA protein PomZ was recently shown to be important for Z-ring formation at midcell (Treuner-Lange et al., 2013). In contrast to the negative regulators of Z-ring formation, PomZ localizes to midcell before and independently of FtsZ, suggesting that PomZ is part of a system that positively regulates Z-ring formation and recruits FtsZ directly to midcell (Fig. 4 D). The mechanism by which PomZ recognizes midcell is currently not understood; however, the localization to midcell depends on ATPase activity.

Protein localization by small GTPases

The localization of landmark proteins and their "client" proteins at the cell poles and FtsZ at midcell is cell cycle regulated. Recently, a system that regulates the dynamic localization of proteins independently of the cell cycle has been uncovered in the rod-shaped cells of *M. xanthus*. *M. xanthus* moves on surfaces in the direction of their long axis using two motility systems, one of which depends on T4P (Zhang et al., 2012a). T4P only localizes to the leading cell pole. In response to signaling by the Frz chemosensory system, and independently of the cell cycle, cells reverse their direction of movement, and during a reversal the pole at which T4P assemble switches. Among the 12 proteins required for T4P function, eight—by an unknown mechanism—localize to both cell poles (Nudleman et al., 2006; Bulyha et al., 2009; Friedrich et al., 2014), whereas the PilB and the PilT ATPases primarily localize to the leading and lagging poles, respectively. During a reversal, PilB and PilT are released from their respective poles and then rebind to the opposite poles. Thus, over time PilB and PilT essentially oscillate

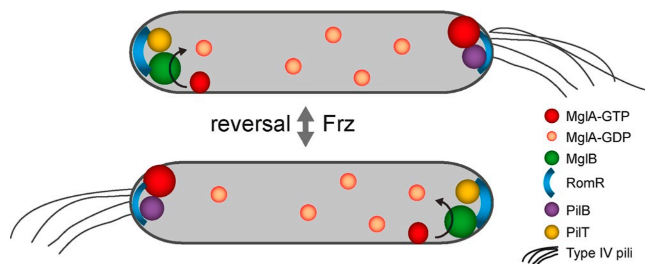

Figure 5. **Protein localization by a small GTPase.** Localization of MglA, MglB, and RomR in *M. xanthus* together with the T4P ATPases PilB and PilT before and after a Frz-induced cellular reversal. MglB-induced GTP hydrolysis by MglA at the lagging pole is indicated by the bent arrow.

irregularly between the cell poles in parallel with cellular reversals (Fig. 5).

Recent evidence suggests that the dynamic localization of PilB and PilT depends on two small Ras-like GTPases, MglA and SofG, and BacP, a polymer-forming polar landmark protein of the bactofilin family. MglA is a nucleotide-dependent molecular switch with MglA/GTP representing the active and MglA/GDP the inactive form (Leonardy et al., 2010; Zhang et al., 2010; Miertzschke et al., 2011). MglB is the cognate GTPase-activating protein (GAP) of MglA. MglA-GTP is recruited to the leading cell pole by the RomR response regulator, which may function as a landmark protein for MglA-GTP, whereas MglA-GDP is distributed uniformly throughout cells (Fig. 5). MglB localizes to the lagging cell pole together with RomR and excludes MglA-GTP from this pole by converting MglA-GTP to MglA-GDP and, thus, sets up MglA-GTP asymmetry (Keilberg et al., 2012; Zhang et al., 2012b). During a reversal, MglA, MglB, and RomR are released from the poles and switch polarity, and this release may depend on phosphorylation of RomR (Keilberg et al., 2012). MglA stimulates T4P-dependent motility by setting up the proper localization of PilB and PilT at opposite cell poles (Bulyha et al., 2013). How MglA executes this function is currently unknown. Therefore, during a reversal, when MglA switch poles, PilB and PilT also switch poles (Bulyha et al., 2013). Recently, it was shown that in order for MglA to sort PilB and PilT to opposite poles, the small GTPase SofG together with BacP first bring about the polar localization of PilB and PilT to the same pole. Subsequently, MglA sorts PilB and PilT to opposite poles (Fig. 2 E; Bulyha et al., 2013).

Concluding remarks

We have reviewed examples of dynamic protein localization in bacteria. A major theme in the dynamic polar localization of proteins is the existence of cues, such as geometry, lipids, or landmark proteins, to recruit proteins to the poles. The participation of matrix-dependent self-organizing ParA/MinD ATPases is a recurring theme in the positioning of the cell division site to midcell. Finally, small Ras-like GTPases are emerging as important in the cell cycle–independent regulation of dynamic protein localization to the cell poles. Overall, perhaps the most important theme is that much remains to be learned about how proteins become localized. With a few notable exceptions, the localization mechanisms of landmark proteins are not known. Though landmark proteins are a common spatial cue for protein localization, ultimately these proteins themselves must be localized by some mechanism. If the peptidoglycan-binding domains found in some nonpolymer-forming landmark proteins are responsible for their polar localization, a prediction would be that the peptidoglycan at the poles is different from that along the lateral sides. An interesting future challenge will be to address such differences. The overlapping localization of several landmark proteins as observed at the flagellated pole of predivisional *C. crescentus* cells with the localization of PopZ, $PodJ_L$, and TipN suggests that new discoveries of landmark proteins in other bacterial organisms are ahead of us. Moreover, only little is known about how landmark proteins recruit other proteins. Clearly, studying dynamic protein localization that occurs at membranes poses many challenges. It is our hope that the themes, connections, and questions identified here will stimulate further progress on the most important challenges facing a better understanding of dynamic protein localization in bacteria.

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