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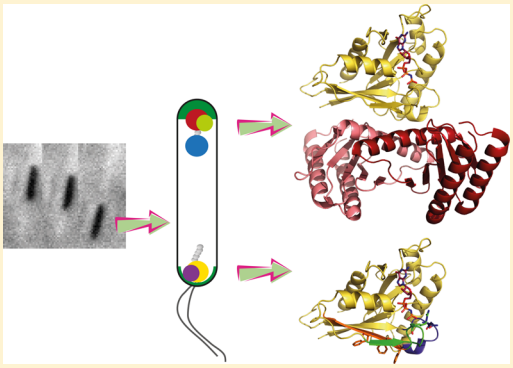
Regulation of Bacterial Cell Polarity by Small GTPases

Daniela Keilberg and Lotte Søgaard-Andersen*

Department of Ecophysiology, Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch Strasse 10, 35043 Marburg, Germany

ABSTRACT: Bacteria are polarized with many proteins localizing dynamically to specific subcellular sites. Two GTPase families have important functions in the regulation of bacterial cell polarity, FlhF homologues and small GTPases of the Ras superfamily. The latter consist of only a G domain and are widespread in bacteria. The rod-shaped *Myxococcus xanthus* cells have two motility systems, one for gliding and one that depends on type IV pili. The function of both systems hinges on proteins that localize asymmetrically to the cell poles. During cellular reversals, these asymmetrically localized proteins are released from their respective poles and then bind to the opposite pole, resulting in an inversion of cell polarity. Here, we review genetic, cell biological, and biochemical analyses that identified two modules containing small Ras-like GTPases that regulate the dynamic polarity of motility proteins. The GTPase SofG interacts directly with the bactofilin cytoskeletal protein BacP to ensure polar localization of type IV pili proteins. In the second module, the small GTPase MglA, its cognate GTPase activating protein (GAP) MglB, and the response regulator RomR localize asymmetrically to the poles and sort dynamically localized motility proteins to the poles. During reversals, MglA, MglB, and RomR switch poles, in that way inducing the relocation of dynamically localized motility proteins. Structural analyses have demonstrated that MglB has a Roadblock/LC7 fold, the central β2 strand in MglA undergoes an unusual screw-type movement upon GTP binding, MglA contains an intrinsic Arg finger required for GTP hydrolysis, and MglA and MglB form an unusual G protein/GAP complex with a 1:2 stoichiometry.



In bacteria as well as in eukaryotes, large GTPases that contain a G domain function in ribosome biogenesis, tRNA modification, translation, and protein secretion.[1] In eukaryotes, small GTPases of the Ras superfamily that consist of only the G domain have well-characterized functions in the regulation of cell polarity, motility, signal transduction, nucleocytoplasmic transport, and vesicular trafficking.[2] In contrast, it is only recently that we have started to appreciate that bacteria also contain GTPases that function in the regulation of the spatial organization of cells. These GTPases belong to two protein families, FlhF homologues and small Ras-like GTPases. The large FlhF proteins are found in a wide variety of bacteria, function in the regulation of flagellar localization, and contain extra domains in addition to the GTPase domain, which belongs to the SIMIBI family of P-loop GTPases.[1,3,4] Many bacteria also contain small GTPases of the Ras superfamily; however, they are not as universally conserved in bacteria as in eukaryotes.[5,6] These GTPases consist of only the G domain and belong to the TRAFAC family of P-loop GTPases.[3] To date, only a few of these small GTPases have been analyzed experimentally in bacteria. CvnD9 is involved in regulating the production of aerial mycelium and antibiotics in *Steptomyces coelicolor*,[7] and MfpB functions in the regulation of DNA gyrase and drug resistance by interacting with the fluoroquinolone resistance protein MfpA in *Mycobacterium smegmatis*.[8] The two best characterized small GTPases in bacteria are MglA and SofG of *Myxococcus xanthus*. Here, we review how MglA and SofG function in the regulation of cell polarity and motility.

G DOMAIN

GTPases of the Ras superfamily share a common structural core, the G domain, which can bind GDP as well as GTP and conduct GTP hydrolysis. The G domain has a molecular mass of approximately 20 kDa and contains four or five highly conserved sequence motifs (G1−G5) that are important for nucleotide binding, nucleotide-dependent conformational changes, and GTP hydrolysis[9,10] (Figure 1A). The G1 motif, also termed the P-loop, contains residues that contact the α- and β-phosphates of GTP and GDP. The G2 motif, also termed switch region I, makes contact with the γ-phosphate in GTP. The G3 motif, also termed switch region II, also makes contact to the γ-phosphate in GTP. The G4 and G5 motifs contact the guanine ring, with G5 being the least conserved motif.

Ras superfamily GTPases are binary nucleotide-dependent molecular switches that cycle between inactive GDP-bound and active GTP-bound forms[10] (Figure 1B). Generally, only the two switch regions undergo large conformational changes upon GTP binding.[10] To induce a specific response, the GTP-bound form interacts with downstream effectors.[10] These GTPases

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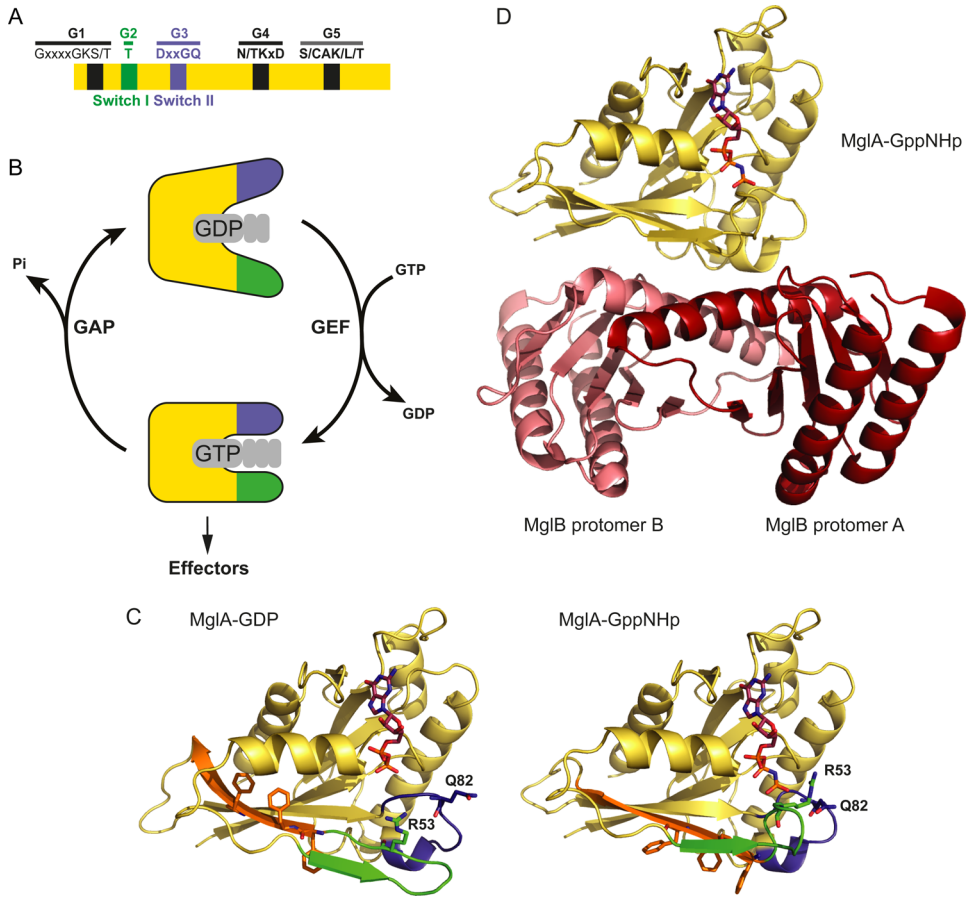

Figure 1. MglA and MglB constitute a cognate GTPase GAP pair. (A) The G domain contains five conserved sequence motifs (G1−G5). Conserved amino acids for each motif are indicated, where x represents any amino acid residue. G1 is also termed the P-loop, and G2 and G3 are termed switch I and switch II, respectively. (B) Canonical GTPase cycle of a G domain protein. Switch regions I and II are color-coded as in panel A. (C) Structure of MglA-GDP and MglA-GppNHp. Notice that the structure of the GDP-bound form was determined in the absence of MglB while the structure of the GppNHp-bound form was determined in the complex with MglB. The central β2 strand with the side chains of the three Phe residues that become surface-exposed in the GppNHp-bound form (orange), switch I with the side chain of Arg53 (green), and switch II with the side chain of Gln82 (blue) are indicated to illustrate the structural differences between the two forms of MglA. (D) Structure of MglA-GppNHp (yellow) bound to the MglB dimer (red and dark red).

bind nucleotides with high affinities and have low intrinsic GTPase activities. Therefore, cycling between the two nucleotide-bound states depends on two types of regulators. Guanine nucleotide exchange factors (GEFs) are positive regulators that facilitate GDP release and GTP binding. GTPase activating proteins (GAPs) are negative regulators that stimulate the low intrinsic GTPase activity by orders of magnitude, in that way converting the active GTP-bound form to the inactive GDP-bound form[11] (Figure 1B).

A PRIMER FOR *M. XANTHUS* MOTILITY

Cells of *M. xanthus* organize into two different cellular patterns depending on their nutritional status. In the presence of nutrients, cells grow and divide, and if placed on a solid surface, they form colonies in which cells spread outward from the center of the colony in a coordinated manner.[12] In the absence of nutrients, growth ceases and cells initiate a developmental program that results in the formation of multicellular spore-filled fruiting bodies. Formation of both cellular patterns crucially depends on cell motility and its regulation. The rod-shaped *M. xanthus* cells can move only when they are placed on a surface, and when they move, they move in the direction of their long axis (Figure 2A). Therefore, a moving cell has a leading pole and a lagging pole. Occasionally, cells stop moving and reverse their direction of movement.[13] Therefore, after a reversal, the old leading pole has become the new lagging cell pole and vice versa (Figure 2A). *M. xanthus* has two motility systems with distinct characteristics. The type IV pili (T4P)-dependent motility system generally depends on cell−cell contact and functions optimally on wet surfaces, while the gliding motility system allows single-cell movement and functions optimally on dry surfaces[14] (Figure 2A).

T4P are dynamic filaments with a length of several micrometers and are among the most widespread bacterial cell surface structures.[15] T4P function not only in motility but also in virulence, biofilm formation, protein secretion, and DNA uptake.[15] T4P-dependent motility depends on three steps: T4P extension, adhesion of the T4P to a surface, and T4P retraction. While T4P extensions do not generate motility, a force exceeding 150 pN per T4P is generated during retractions, pulling a cell forward.[16,17] T4P function depends on 12 proteins, 10 of which form a complex that spans the cell envelope and has components in the cytoplasm, inner membrane (IM), periplasm, and outer membrane (OM)[15,18,19] (Figure 2B, top panel).

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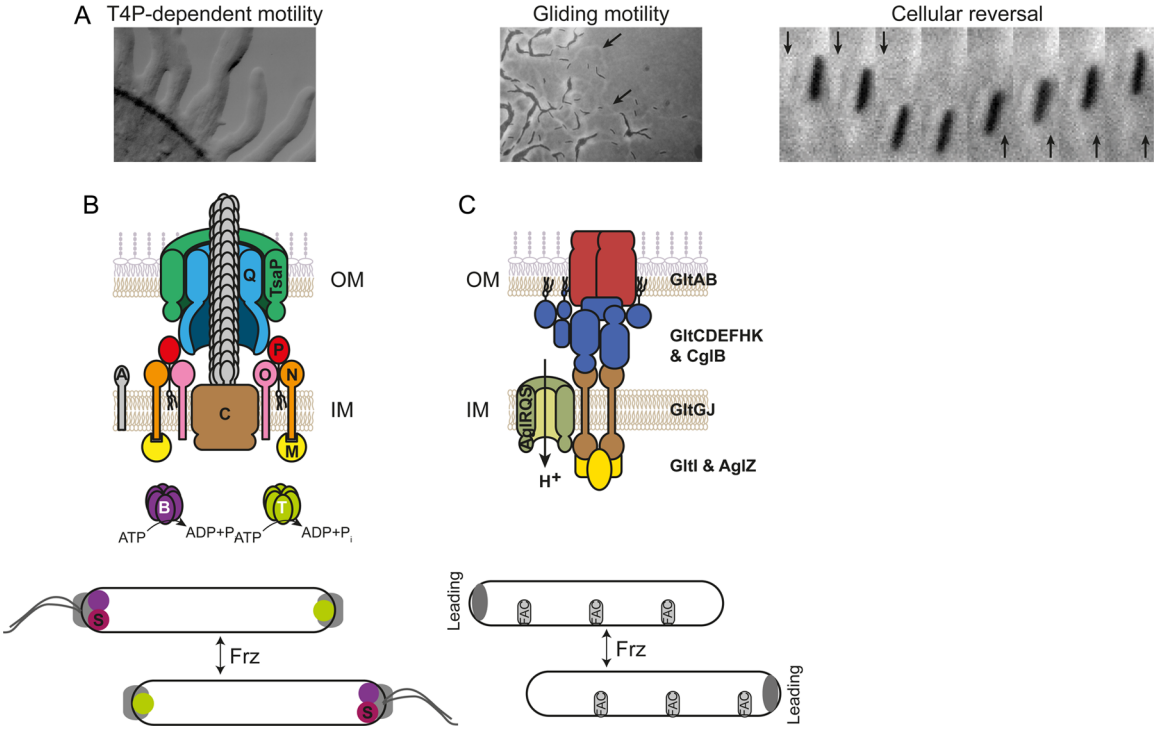

Figure 2. Motility in *M. xanthus* depends on proteins that localize dynamically to the cell poles. (A) *M. xanthus* has two motility systems. T4P-dependent motility is contact-dependent; the image illustrates the flares formed by groups of cells at the edge of a colony on 0.5% agar. Gliding motility allows single-cell movement; the image illustrates single cells at the edge of a colony on 1.5% agar, and arrows point to slime trails. *M. xanthus* cells occasionally reverse. Images from a time-lapse recording with 30 s between the frames of a single cell that reverses between 90 and 120 s; arrows indicate the direction of cell movement. The cell reverses after frame 4. (B) T4P-dependent motility depends on proteins localized in a polar fashion. The top panel shows a model of the T4P machinery. Protein nomenclature indicated by single letters represents the Pil nomenclature. T4P are composed of the PilA pilin subunit and are assembled at the base, with PilA being incorporated from an IM reservoir; during retractions, PilA subunits are removed from the base and reinserted into the IM.[57] In the OM, the PilQ secretin forms a channel that serves as a conduit for T4P,[58] and TsaP likely anchors the secretin to the peptidoglycan.[19] PilC in the IM has been suggested to function as an assembly platform for T4P.[59,60] The PilM, PilN, PilO, and PilP proteins form a complex that connects from the cytoplasm to PilQ in the OM.[18,61−68] The ATPases PilB and PilT provide the energy for T4P extension and retraction, respectively.[69−71] It is not known how PilB and PilT interact with the base of the machinery. The bottom panel shows the localization of T4P proteins during motility and after Frz-induced reversals. The seven proteins (PilQ, TsaP, PilM, PilN, PilO, PilP, and PilC) are thought to form preassembled complexes (gray) at both poles; FrzS (S) and PilB are primarily at the leading pole where T4P are present, and PilT is primarily at the lagging pole. PilB and PilT are color-coded as in the top panel. During a reversal, these three proteins switch poles. (C) Gliding motility depends on proteins localized in a polar fashion and FAC. The top panel shows a model of FAC. The localization of gliding motility proteins to the cytoplasm, IM, periplasm, and OM is based on experimental evidence and bioinformatic analyses.[30,31,72] The color code indicates subcellular localization. No direct interactions between proteins in a FAC have been demonstrated. The motor of the gliding motility machinery is AglQRS that depends on the proton motive force.[31,34] AglR is a homologue of MotA, TolQ, and ExbB, and AglQ and AglS are homologues of MotB, TolR, and ExbD.[31,34] The bottom panel shows the localization of gliding motility proteins during motility and Frz-induced reversals. AglZ, GltD, GltF, and AglQ form clusters (gray) at the leading cell pole where FAC are thought to be assembled. These four proteins also localize to FAC and presumably do so with other gliding motility proteins. During a reversal, the proteins localized in a polar fashion switch poles.

In *M. xanthus*, T4P assemble only at the leading cell pole, and during a reversal, the pole at which T4P assemble switches[20−22] (Figure 2B, bottom panel). To elucidate the mechanism underlying this switch in polarity of T4P during a cellular reversal, the localization of proteins required for T4P function has been determined.[18,23,24] These analyses showed that T4P proteins can be divided into two classes. One class includes seven proteins that localize to both cell poles and remain at the two poles during a reversal (Figure 2B, bottom panel). The second class is composed of the two ATPases PilB and PilT. PilB stimulates T4P extension and primarily localizes to the leading cell pole. PilT stimulates retractions and primarily localizes to the lagging cell pole and only occasionally accumulates at the leading cell pole (Figure 2B, bottom panel). Importantly, during a reversal, PilB and PilT dissociate from their respective poles, relocate between the two poles, and then associate with the new leading and lagging pole, respectively (Figure 2B, bottom panel). Therefore, the mechanism underlying a switch in T4P polarity depends on PilB and PilT switching polarity, and depending on the localization pattern of PilB and PilT, an *M. xanthus* cell has T4P at one pole or the other. T4P-dependent motility in *M. xanthus* also depends on exopolysaccharides (EPS).[25] EPS function in T4P-dependent motility by stimulating T4P retractions.[26] The FrzS protein has been identified as one of many proteins required for EPS accumulation.[27] FrzS localizes in an asymmetric pattern with a large cluster at the leading pole.[21] Like PilB and PilT, FrzS is dynamically localized and switches localization during a reversal, with the large cluster relocating from the old leading pole to the new leading cell pole[21] (Figure 2B, bottom panel).

While T4P-dependent motility is relatively well understood, the mechanism underlying gliding motility in *M. xanthus*

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remains poorly understood. On the basis of early studies, it was proposed that gliding motility is powered by polar slime secretion that would push a cell forward[28] (Figure 2A). However, recent studies suggest that slime is secreted in a manner that is independent of cell movements.[29] In the current model, gliding motility depends on protein complexes that are distributed along the cell body. This model emerged from the analysis of the subcellular localization of several gliding motility proteins, including AglZ.[30−32] These proteins each localize with a large cluster at the leading cell pole and in smaller clusters along the cell body. Intriguingly, these small clusters remain at fixed positions with respect to the substratum in moving cells similar to focal adhesion complexes (FACs) in eukaryotic cells. Therefore, these fixed clusters in *M. xanthus* cells are often termed FAC. Time-lapse microscopy of cells containing fluorescently tagged gliding motility proteins suggests that FACs are assembled at the leading cell pole and disassembled at the lagging cell pole.[30−32] During a reversal, the polar clusters of motility proteins at the old leading cell pole relocate to the new leading cell pole. Two models have been proposed for the composition of a FAC. In one model, the proteins required for gliding interact to build a large multiprotein complex that includes proteins in the cytoplasm, the periplasm, and the inner and outer membrane[30,33] (Figure 2C, top panel). In this model, proteins on the cell surface are hypothesized to interact with the substratum to generate traction. In the second model, the protein complexes span from the cytoplasm over the IM to the periplasm and translocate in a helical trajectory; protein complexes are hypothesized to stall upon contact with a surface (giving the visual impression of a nonmoving complex), resulting in the distortion of the OM and the generation of drag force.[34,35] Regardless, both models depend on the cell being able to differentiate between the leading and the lagging cell pole to guarantee that the gliding motility complexes are assembled only at the leading pole and disassembled at the lagging pole. Moreover, during a reversal, the poles for assembly and disassembly switch.

Cellular reversals are induced by the Frz chemosensory system, and mutants that are unable to regulate the reversal frequency are unable to form spreading colonies and fruiting bodies.[13] The Frz system is homologous to the chemosensory systems regulating chemotaxis in many bacteria.[36] Of note, phosphotransfer from the kinase FrzE to the output response regulator FrzZ is required to induce reversals.[37,38] Recent localization studies revealed that FrzZ is recruited to the leading cell pole upon phosphorylation and switches to the opposite pole during a reversal.[39] Signaling by the Frz system ultimately causes the relocation of the dynamically localized polar motility proteins during a reversal. Recent evidence suggests that the missing link between the Frz system and the dynamically localized motility proteins is a module consisting of the small GTPase MglA, its partner GAP MglB, and the response regulator RomR.

MGLA IS A RAS-LIKE GTPASE AND MGLB ITS COGNATE GAP

The *mglA* gene in *M. xanthus* was identified more than 30 years ago and is essential for T4P-dependent motility and gliding motility.[40] MglA is a 22 kDa protein, and like other small Ras-like GTPases, MglA consists of only the G domain. On the basis of sequence comparisons, Leipe et al. defined six subfamilies of Ras-like GTPases, and MglA is the founding member of the sixth subfamily of these proteins.[3] MglA contains motifs G1−G4 required for GDP and GTP binding,[41] and the major difference from other Ras-like GTPases is the absence of the highly conserved Asp in G3[42] (Figure 1A).

For the investigation of MglA and its cognate GAP MglB, the homologous proteins from *Thermus thermophilus* have proven to be useful. MglA and MglB of *M. xanthus* and *T. thermophilus* are 81% and 52% similar and 62% and 28% identical, respectively. While the function of MglA and MglB in *T. thermophilus* is unknown, it is interesting to note that *T. thermophilus* has T4P[43] but not homologues of the gliding motility proteins. Biochemical analyses revealed that MglA of *M. xanthus*[44−46] and its homologue from *T. thermophilus*[42,47] have a low intrinsic GTPase activity. Moreover, MglA of *T. thermophilus* binds GDP and GTP with nanomolar affinities. Also, for MglA of *M. xanthus* as well as of *T. thermophilus*, it was demonstrated that their partner MglB protein, which is encoded in an operon with MglA in both organisms, functions as a GAP.[42,45,47] The high binding affinities of MglA of *T. thermophilus* for GDP suggest that this MglA, and implicitly also MglA of *M. xanthus*, would depend on a GEF for the exchange of GDP for GTP; however, so far, no GEF has been identified.

To understand how MglB stimulates MglA GTPase activity, the two *T. thermophilus* proteins were studied by X-ray crystallography separately as well as complexed.[42] These analyses demonstrated that MglA in the GDP-bound form displays a typical G domain fold (Figure 1C) and that MglB forms a dimer with each monomer having a Roadblock/LC7 fold. While crystals of MglA alone in the active GTP or GppNHp-bound forms were not obtained, crystals of a complex consisting of MglA-GppNHp and MglB were obtained. Notably, this complex is composed of one MglA molecule and an MglB dimer (Figure 1D), while other G protein/GAP complexes have a 1:1 stoichiometry.[42] Moreover, MglA contacts the two MglB molecules asymmetrically in this complex. Compared to the MglA-GDP complex, MglA in the MglA-GppNHp/MglB complex has a significantly different conformation, suggesting that MglA undergoes dramatic conformational changes upon GTP binding (Figure 1C). Specifically, this proposed GDP-dependent to GTP-dependent conformational change would involve a screw-type back-to-front movement of the central β2 strand (termed the β2 screw[42]) toward the nucleotide. This conformational change includes the back-to-front movement of the β2 strand as well as a 180° rotation and is distinct from the interswitch toggle of the β2 and β3 strands of Arf and Arl proteins that occurs in a front-to-back manner without a rotation upon GTP binding.[48] In MglA, the repositioning of the β2 strand has two important consequences. First, it results in the exposure of three Phe residues on the surface of MglA and allows these residues to make contact with MglB (Figure 1C). Second, it causes a shift of Arg53 into a position where it contacts the γ-phosphate (Figure 1C). Finally, switch region II also changes conformation upon GTP binding, resulting in the positioning of the conserved catalytic residue Gln82, which corresponds to the catalytic residue Gln61 in Ras,[10] close to the γ-phosphate (Figure 1C). It should be emphasized that in the absence of a structure of MglA-GppNHp without MglB, the possibility that some of these structural changes are induced or possibly stabilized by binding to MglB cannot be excluded.

The structure of the MglA-GDP-AlF_4/MglB transition state mimic is only slightly different from that of the MglA-GppNHp/MglB complex. However, these minor conforma-

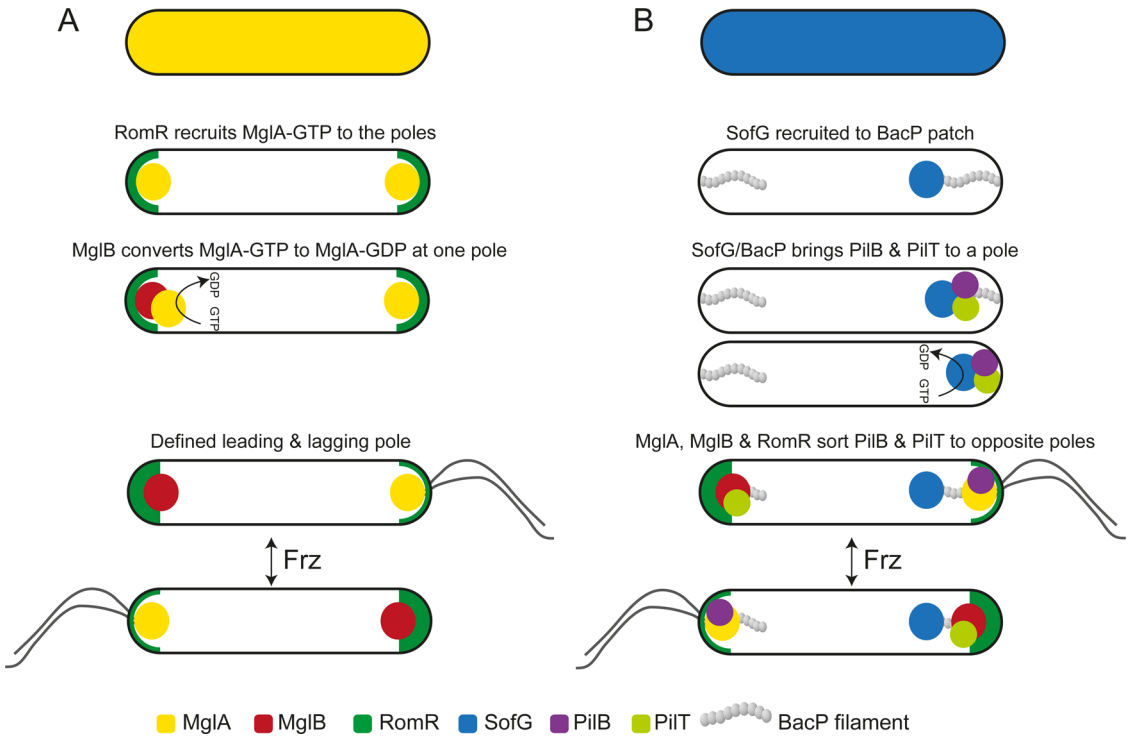

Figure 3. Small GTPases in *M. xanthus* polarity. (A) RomR, MglA, and MglB interact to define the leading and lagging cell poles. Schematics illustrate how RomR recruits MglA-GTP to the poles and how MglB sets up MglA-GTP asymmetry by stimulating GTP hydrolysis by MglA at one pole. During a Frz-induced reversal, RomR, MglA, and MglB are released from the poles and relocate between the poles, resulting in an inversion of polarity. (B) Dynamic polar localization of PilB and PilT is established by three hierarchically organized modules. SofG is recruited to one of the BacP patches; SofG and BacP bring about localization of PilB and PilT at the same pole by a mechanism that depends on GTP hydrolysis by SofG, and MglA, MglB, and RomR sort PilB and PilT to opposite poles. During a reversal, RomR, MglA, MglB, PilB, and PilT are released from the poles and relocate between the poles, resulting in an inversion of polarity. Note that SofG remains associated with the same BacP patch during a reversal. For the sake of simplicity, only a single BacP filament is shown in each subpolar region.

tional changes bring Arg53 and Gln82 closer to the γ-phosphate. MglB does not provide part of the active site and does not directly participate in catalysis but rather helps to properly orient the catalytic machinery in MglA. This is in stark contrast to many eukaryotic G protein/GAP complexes in which the GAP completes the catalytic site by inserting a residue (typically an Arg residue termed the Arg finger) into the active site.[11,49] Biochemical analyses of *T. thermophilus* MglA have confirmed that variants with either an Arg53Ala or a Gln82Ala substitution, which corresponds to the oncogenic GTP-locked $Ras^{Gln61Ala}$ variant,[10] are still able to bind GTP; however, their level of GTP hydrolysis is strongly reduced in the absence and presence of MglB and, thus, also locked in the GTP-bound form.[31] Also, a $MglA^{Gly21Val}$ variant with a substitution in the P-loop corresponding to the oncogenic GTP-locked $Ras^{Gly12Val}$ variant[10] is locked in the GTP-bound active state, and the intrinsic activity and MglB-stimulated GTPase activity are dramatically reduced.[47] Finally, the $MglA^{Thr26/27Asn}$ variant, which corresponds to the inactive empty or GDP-locked variant $Ras^{Ser17Asn}$,[10] has a strongly reduced affinity for GTP.[47] Genetic analyses of the corresponding *M. xanthus* MglA variants demonstrated that the GTP-locked forms are constitutively active whereas the $MglA^{Thr26/27Asn}$ variant is inactive.[45,47] Thus, *in vitro* as well as *in vivo* Ras and MglA behave similarly, and a major difference between the two proteins is that MglA contains an intrinsic Arg finger while the catalytic site in Ras is completed by an extrinsic Arg finger supplied by the GAP.

Each of the five eukaryotic subfamilies of small Ras-like GTPases has its own specific GEFs and GAPs, and these proteins are generally large multidomain proteins.[11] On the other hand, MglB is a small 17 kDa protein and does not share homology with eukaryotic GAPs. Even though Roadblock/LC7 domain proteins are present in eukaryotes,[5] these proteins have so far not been implicated in the regulation of Ras-like GTPases. As mentioned, MglA is likely to depend on a GEF for the exchange of GDP for GTP. Homology-based searches in *M. xanthus* have not revealed proteins with homology to eukaryotic GEFs (unpublished).

MGLA, MGLB, AND ROMR INTERACT TO DEFINE THE LEADING AND LAGGING CELL POLES

How then does MglA stimulate motility in *M. xanthus*? Using a combination of genetics and cell biology approaches, major strides have been made in recent years to address this question. Between reversals, i.e., when a cell is moving, MglA in its active GTP-bound form localizes at the leading cell pole, inactive MglA-GDP localizes diffusely throughout cells, and MglB is localized at the lagging pole.[45,47] MglA interacts directly with the RomR response regulator, and MglA-GTP is targeted to the pole by RomR, which is also required for the full function of the two motility systems.[6,50] MglB also interacts directly with RomR.[6,50] Consistently, RomR localizes asymmetrically with a large cluster at the lagging cell pole and a small cluster at the leading cell pole.[6,50,51] Finally, localization studies revealed that all three proteins are mutually dependent for localization.[6,50] In particular, RomR and MglA-GTP are symmetrically localized at

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both cell poles in the absence of MglB. Therefore, MglB is absolutely required to establish the asymmetry of MglA-GTP (Figure 3A). In the current model, MglB establishes this asymmetry by converting MglA-GTP into the GDP-bound form at the lagging cell pole. Currently, it is not clear how MglB asymmetry with MglB binding to only one pole is accomplished. In total, RomR, MglA, and MglB have been proposed to constitute a polarity module that helps to define the leading and lagging cell poles, with the MglA-GTP/RomR complex at the leading pole and the MglB/RomR complex at the lagging pole (Figure 3A).

During cellular reversals, MglA-GTP, MglB, and RomR are released from their respective poles and then switch poles. Recently, it was shown that RomR acts downstream of FrzZ, the output response regulator of the Frz system, and upstream of MglA and MglB during reversals, suggesting that RomR acts at the interface between the Frz chemosensory system and MglA and MglB during reversals.[6,51] Importantly, if RomR is locked in a form that likely mimics the phosphorylated form, cells hyperreverse whereas they essentially stop reversing if RomR cannot be phosphorylated.[6,51] On the basis of these findings, it has been proposed that RomR would become phosphorylated in response to Frz signaling, and that, in turn, causes the release and relocation of RomR, MglA, and MglB between the two poles, resulting ultimately in an inversion of the leading−lagging polarity axis (Figure 3A). The phosphotransfer between FrzZ and RomR does not seem to be direct, and how RomR becomes phosphorylated remains unknown.

MGLA ESTABLISHES THE CORRECT POLARITY OF DYNAMICALLY LOCALIZED MOTILITY PROTEINS

MglA in combination with its cognate GAP, MglB, and RomR stimulate motility by setting up the correct localization of dynamically localized proteins. Specifically, in the absence of MglA, the PilB and PilT ATPases, which normally localize to opposite cell poles, localize to the same pole.[52] Similarly, FrzS localizes in a more asymmetric pattern in the absence of MglA compared to wild-type cells.[46] FrzS interacts with MglA in pull-down experiments using cell extracts;[46] however, it is not known if this interaction is direct or nucleotide-dependent. On the basis of these findings, it has been suggested that MglA sorts PilB and PilT to opposite poles and that MglA is not required for polar localization per se of PilB and PilT[52] (Figure 3B). In the case of FrzS, it has been suggested that MglA brings about its polar localization.[46]

In the gliding motility system, MglA is required for polar localization of AglZ as well as for the formation of FAC.[45,46] AglZ interacts directly with MglA in pull-down experiments;[46] however, it is not known if this interaction is nucleotide-dependent. On the basis of these findings, MglA-GTP has been proposed to bring about the polar localization of AglZ and to stimulate the formation of FAC at the leading pole.[45]

In total, in current models, MglA stimulates T4P-dependent motility by setting up the correct polarity of PilB and PilT at opposite cell poles and by recruiting FrzS to the leading cell pole. In the gliding motility systems, MglA is thought to recruit AglZ to the leading cell pole and stimulate the formation of FAC at this pole. Except for the suggested direct interaction between FrzS and AglZ that could be involved in recruiting these two proteins to the leading cell pole, it is not known how MglA brings about the correct localization of PilB and PilT. Similarly, it is not known how MglA stimulates FAC formation.

THE SMALL SOFG GTPASE BRINGS ABOUT POLAR LOCALIZATION OF PILB AND PILT

The two motor ATPases, PilB and PilT, in the T4P system localize to opposite cell poles while a cell is moving forward,[24] and they are sorted to opposite poles by MglA.[52] However, MglA is not required for polar localization per se of PilB and PilT, only for sorting the two ATPases to opposite cell poles.[52] Similarly, it was recently shown that PilB and PilT are localized in a polar fashion in a manner independent of all other T4P proteins,[18] raising the question of how PilB and PilT become localized to the poles. On the basis of the observation that two or more small GTPases often function in parallel or in the same pathway to regulate motility or polarity in eukaryotes,[53,54] Bulyha et al.[52] hypothesized that polar localization of PilB and PilT could involve an additional small GTPase. Indeed, this candidate approach proved to be successful with the identification of the small GTPase SofG being important for the polar localization of PilB and PilT. SofG is 34% identical and 50% similar to MglA, consists of only the G domain, and with the G1−G4 signature motifs involved in nucleotide binding, GTP-induced conformational changes, and conserved GTP hydrolysis. Like MglA, SofG lacks the highly conserved Asp in G3 and contains an intrinsic Arg finger that is important for GTP hydrolysis *in vitro* and function *in vivo*;[52] however, a GAP or a GEF has not been identified.

SofG directly interacts with BacP, a bactofilin cytoskeletal protein, and BacP is also required for polar localization of PilB and PilT.[52] Bactofilins are cytoskeletal proteins that are widely conserved in bacteria and polymerize spontaneously *in vitro* in the absence of cofactors.[55] Localization studies revealed that BacP forms two subpolar patches, while SofG localizes in one subpolar cluster and associates directly with one of the two BacP patches (Figure 3B). The SofG cluster is highly dynamic, shuttling back and forth between the subpolar localization and the cell pole over a BacP patch. A mutant variant of SofG with a substitution of the intrinsic Arg finger with Ala showed a reduced level of GTP hydrolysis *in vitro*, did not display the shuttling dynamics seen for the WT protein, and was not able to bring about polar localization of PilB and PilT. Thus, the GTPase cycle of SofG is essential for SofG dynamics and function, suggesting that the shuttling of SofG on BacP promotes the polar localization of PilB and PilT. In total, these findings lead to a model in which the dynamic localization of PilB and PilT to opposite cell poles is regulated in a cascade-like manner: SofG associates with one of the subpolar BacP patches forming a subpolar cluster that shuttles to the pole to establish polar localization of PilB and PilT at the same pole (Figure 3B). Following the SofG/BacP-dependent localization of PilB and PilT to the same pole, a second event follows in which MglA sorts PilB and PilT to opposite poles to set up their correct polar localization, in this way allowing T4P-dependent motility. Thus, the two small GTPases, SofG and MglA, function in a cascadelike manner to regulate PilB and PilT polarity. During reversals, the Frz chemosensory system causes the inversion of the leading−lagging polarity axis by inducing the relocation of MglA, MglB, and RomR. Thus, three regulatory systems function sequentially to regulate the dynamic localization of PilB and PilT.

OUTSTANDING QUESTIONS

It is now generally appreciated that bacterial cells are spatially highly organized with many proteins localizing dynamically to

specific subcellular sites.[56] This dynamic localization is often regulated by cell cycle cues, while the dynamic localization of motility proteins and motility regulators in *M. xanthus* is independent of cell cycle. In the past few years, major progress has been made in understanding how MglA and SofG regulate the polarity of motility proteins in *M. xanthus*. However, major gaps still need to be filled. One gap concerns the regulation of MglA and SofG, and it will be an exciting challenge to search for and possibly identify GEFs for MglA and SofG and a GAP for SofG. Also, future research will have to address how the asymmetry of RomR, MglA, and MglB is established, how these three proteins are released and relocate between poles during a reversal, and how they connect to the Frz system. Finally, the mechanistic details of how MglA establishes correct polarity of the downstream motility proteins remain to be determined. To address this question, effector proteins of MglA will have to be identified. Beyond *M. xanthus*, MglA, MglB, and RomR homologues co-occur in many genomes,[6] suggesting that these three proteins may also be involved in regulating cell polarity in other organisms. Although we are only beginning to understand how small GTPases function in bacterial cell polarity, it is already clear that they are as important in bacteria as they are in eukaryotes.

AUTHOR INFORMATION

Corresponding Author

*E-mail: sogaard@mpi-marburg.mpg.de. Telephone: +49-(0) 6421-178201. Fax: +49-(0)6421-178209.

Notes

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