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Chemosensory signaling controls motility and subcellular polarity in *Myxococcus xanthus*

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Abstract

Myxococcus xanthus is a model system for the study of dynamic protein localization and cell polarity in bacteria. *M. xanthus* cells are motile on solid surfaces enabled by two forms of gliding motility. Motility is controlled by the Che-like Frz pathway, which is essential for fruiting body formation and differentiation. The Frz signal is mediated by a GTPase/GAP protein pair that establishes cell polarity and directs the motility systems. Pilus driven motility at the leading pole of the cell requires dynamic localization of two ATPases and the coordinated production of EPS synthesis. Gliding motility requires dynamic movement of large protein complexes, but the mechanism by which this system generates propulsive force is still an active area of investigation.

Introduction

The spatiotemporal localization of proteins within the prokaryotic cell has a major impact on their function. For example, in *Escherichia coli*, *Caulobacter crescentus* and *Bacillus subtilis*, the localization of proteins that drive the replication and segregation of bacterial chromosomes or that initiate cell division is tightly coordinated with the cell life cycle [1,2]. However, some proteins also change their localization independently of the cell cycle. One striking example of this is the motility proteins of the Gram-negative soil bacterium, *Myxococcus xanthus*.

M. xanthus has a complex life cycle that involves vegetative swarming and predation when nutrients are present and the formation of complex multicellular biofilms (fruiting bodies) and sporulation when nutrients are limited. These activities require the coordinated movement of cells [3]. *M. xanthus* cells do not utilize flagella but use two different motility systems to move on solid surfaces (Fig. 1A) [4]. Social motility (S-motility) is mediated by the extension and retraction of type IV pili localized at the leading cell pole and is predominant when cells move as a group [5]. When cells move individually, gliding is mainly powered by distributed motor complexes (A-motility) [6,7]. The two motility systems are coordinated. Remarkably, cells frequently reverse their direction by inverting

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the cell polarity axis (Fig. 1B). Both motility systems require multiple proteins in distinct, clustered localizations that move from pole to pole when cells invert their polarity. For example, FrzS, a protein required for efficient social motility, is localized primarily in a large cluster associated with the leading cell pole, but oscillates to the lagging cell pole when cells reverse direction [8]. Another example is AglZ, a protein that regulates gliding motility. AglZ forms distributed clusters that assemble at the leading cell pole and appear to migrate towards the lagging cell pole as cells move forward [9]. Strikingly, the dynamic localization of gliding motility proteins responds to a signaling pathway, the *frz* chemosensory system. Frz proteins regulate the frequency of cell reversals and therefore the re-orientation of cell polarity and the gliding motility organelles.

Regulating the frequency of cellular reversals: the *frz* signaling pathway

The *frz* signal transduction pathway is a modified two-component system similar to the *E. coli* chemotaxis (*che*) pathway (Fig. 1C). FrzCD, a methyl accepting chemoreceptor (MCP) activates FrzE, a histidine-kinase, triggering autophosphorylation [10]. The phosphoryl group is subsequently transferred to FrzZ, a dual response regulator that likely serves as an output of the pathway [11]. It has been proposed that FrzZ~P communicates with proteins that determine cell polarity (see below), triggering reversals [12]. Under certain conditions (see below), adaptation of the FrzCD chemoreceptor is achieved by methylation/demethylation catalyzed by a methyltransferase (FrzF) and a methylesterase (FrzG), respectively [13]. Mutations in several components of the Frz pathway can cause either infrequent reversals (hypo-reversing), which results in “frizzy” aggregates under starvation conditions or in very high frequency of reversals (hyper-reversing), which results in compact non-spreading colonies. Both hypo- and hyperreversing mutants cannot aggregate into discrete fruiting bodies, abolishing their ability to differentiate (Fig. 1D).

There are several overlapping theories on the role of the Frz pathway and depending on the conditions, Frz signaling may indeed execute various functions. (1) The first hypothesis, inspired by *E. coli* chemotaxis, suggests that controlling cell reversals is similar to controlling runs and tumbles in flagellated bacteria. According to this model, cells move in a biased random walk and thus controlling reversal bias allows cells to move towards attractants and not enter areas with repellents [14,15]. This hypothesis is consistent with observations of colonies of *M. xanthus*, but chemotactic movement of individual cells remains controversial [16]. (2) An alternative hypothesis is that the Frz pathway allows cells to respond to cell-cell contact. It has been shown that cell contact can trigger cell reversals and that FrzCD receptor clusters realign when cells make contact [17]. Additionally, *M. xanthus* cells respond to contact with prey cells by inducing rapid reversals (predataxis behavior), indicating that tactile sensing may be a major role of the Frz pathway [18]. (3) It has also been proposed that the Frz pathway acts as a pacemaker to promote swarm expansion [19,20]. Recent computational models infer that, due to the dimensions of *M. xanthus* cells and the gliding speed of 0.02 $\mu\text{m/s}$, cell reversals must occur every ~8 min to ensure maximal displacement towards the colony edge [20]. However, these models do not explain how other swarming organisms such as *P. aeruginosa* show colony expansion without cell reversals. In addition, the models can be greatly improved by incorporating

observations that *M. xanthus* cell reversals are reduced in social swarming, yet swarm expansion continues [18,21].

Establishing cell polarity: MglA and MglB localize to opposite cell poles

M. xanthus cells move on a solid surface in the direction of their long axis with distinct cell polarity; when cells reverse, the polarity axis is inverted and the motility motors re-orient their mode of action [22]. Two recent studies revealed that cell polarity depends on the asymmetric polar localization of MglA and MglB [**23,**24]. MglA, a Ras-like GTPase first characterized by Patricia Hartzell [25,26], localizes to the leading cell pole and migrates to the opposite pole when cells reverse (Fig. 2A). In the absence of MglA, cells appear non-motile, although some rapid oscillatory movements have been observed with no net cell migration [27]. The polar localization of MglA depends on its nucleotide binding state, which is determined by MglB, a GTP hydrolysis activating protein (GAP) (Fig. 2B). MglB, recruited to the lagging cell pole by an unknown mechanism (Fig. 2A), promotes MglA GTPase activity, preventing MglA accumulation at the lagging pole (for a recent review see [28]). The molecular basis of the nucleotide dependent MglA/MglB switch was characterized by the analysis of functional point mutations in MglA and MglB [29,30]. Mutations in residues that effect the GTPase cycle of MglA or its interaction with MglB result in motility defects and aberrant localization of the proteins. Furthermore, Miertzschke *et al.* analyzed the structures of MglA and MglB homologues from *Thermus thermophilus* and observed an unusual conformational transition upon GTP hydrolysis, suggesting a new type of catalytic mechanism for MglA-type Ras-GTPases [30].

In two recent studies, the response regulator protein RomR was identified as an additional determinant of cell polarity [31,32]. RomR was shown to directly interact with both MglA and MglB, forming complexes at the leading and lagging pole, respectively. The unipolar localization of MglA depends on the formation of the MglB-RomR complex, and vice versa. Therefore, cell polarity in *M. xanthus* is established by the concerted action of at least three proteins that are linked in a mutually-dependent circuit.

In the absence of the MglA/MglB polarity axis, key regulators of gliding motility like FrzS, AglZ and also RomR [33] show aberrant localization and loss of cell motility [**23,**24]. However, it is unclear how MglA and MglB participate in the recruitment of motility proteins, although a direct interaction between MglA and AglZ and FrzS proteins has been demonstrated [34].

The dynamic localization of MglA and MglB depends on the *frz* signaling pathway [**23,**24]. In *frz* hyperreversing or hyporeversing mutants, MglA and MglB localize to the correct cell poles, but reverse their positions frequently or infrequently, respectively. Possibly, *frz* signaling either modulates MglA GTPase activity directly or assists in recruiting MglB to the cell pole. Based on genetic analysis, the response regulator RomR was proposed to interface between the *frz* signaling system and the MglA/B polarity module [31,32]. However, the relation of RomR and *frz* signaling components, as well as the role of RomR phosphorylation, requires further investigation.

Social motility: a tangle of two extracellular polymers

S-motility is mediated by Type IV pili (TFP) that extend from the leading cell pole (Fig. 3A). TFP play a critical role in surface attachment, migration and biofilm formation in numerous bacterial species including *Pseudomonas aeruginosa*, *Neisseria gonorrhea*, and *Vibrio cholerae*. TFP utilize ATP as an energy source to power both polymerization and extension of the pilus as well as depolymerization/retraction [35]. The *M. xanthus* pilus generates a force of ~150 pN, even more force than the *N. gonorrhea* TFP at ~110 pN [36]. Most Pil proteins, as for example the cytoplasmic membrane protein PilC or the outer membrane channel PilQ, show a bipolar localization, but at any given time only the leading pole is active in assembling functional pili [5]. The leading (active) pole is characterized by higher abundance of PilB, the ATPase required for extension of TFP, and intermittent bursts of PilT, the ATPase primarily responsible for retraction [37].

To function, S-motility also requires exopolysaccharides (EPS). While the specific composition of EPS in *M. xanthus* is unknown, EPS synthesis is dependent on a 37 gene cluster (MXAN7415-MXAN7451). Both TFP and EPS are synthesized from monomers at the cytoplasmic membrane (Fig. 3B). Thus, transport and synthesis are linked. For example, *pilA* mutants express very low levels of EPS, suggesting that pili may serve as sensors in the pathway that regulates EPS production [*38]. *frzS* mutants are also defective in EPS production [*39]. FrzS localization has been shown to be dependent on signals from the Frz pathway (Fig. 3D), but it is not known which component of the Frz pathway interacts with FrzS [8]. Social motility in *frzS* and *epsZ* (encodes a glycosyl transferase channel) mutants are both rescued by the extracellular addition of EPS (Fig. 3C) [*39]. There is ample evidence to indicate that EPS can serve as an anchor for TFP retraction [40,41] but may not be essential as an anchor [42,43]. This indicates that the binding substrate for the pilus can be a variety of surfaces.

It is curious that *M. xanthus* has evolved a separate chemosensory pathway to regulate EPS production: the Dif pathway. Phenotypically, DifA, DifC and DifE are required for EPS production, whereas DifD-G inhibit EPS production. DifE is an autokinase that works in concert with DifA and DifC. Phosphotransfer from DifE to DifD was observed, as well as subsequent phosphatase activity by DifG on DifD [44]. These results lead to a model where DifD and DifG act as phosphate sinks, regulating the active state of DifE. It still remains to be discovered which component of the Dif pathway interacts with the EPS production pathway. The overall integration of signals from the Frz, Dif and Pil pathways into TFP-driven cell movement requires further study. While there is a clear link in the co-production of TFP and EPS, and a coordination of both motility systems at the leading cell pole, the integration of Frz and Dif signals remains unclear. It is important to note, however, that while isolated cells frequently reverse polarity, cells in groups reverse less frequently [18,21].

A large protein complex powers A-motility

The engine that powers A-motility remained elusive for many years (for recent reviews see [6,7,12]. Initially, the secretion of slime or EPS was considered to be the force driving single

cell motility in *M. xanthus*. However, force for gliding motility seems to be created along the full length of the cell body, which is not consistent with a polar engine [45]. In a recent study, Ducret *et al.* used wet-surface enhanced ellipsometry contrast microscopy to directly image slime secretion [*46] Apparently slime is deposited underneath the cell as a function of time, independent of cell movement. However, polysaccharide slime might provide an optimal substratum for force generation explaining its impact on cell movement in *M. xanthus*.

Recent experiments indicate that A-motility is mediated by a multi-component, membrane spanning protein complex that travels from the leading to the lagging end of cells, pushing them forward. This hypothesis is based on observations that several gliding motility proteins form distributed clusters that assemble at the leading cell pole and remain fixed relative to the substratum while cells moves forward (Fig 4A). [9,47,*48]. Several components of the gliding motility motor complex have been identified by genetic studies and protein interaction analysis, as well as by a phylogenetic search for gene clusters that have co-evolved with known components [47–51]. Energy for gliding motility is supplied by proton motive force (PMF), implicating the TolQ/TolR analogues AglR, AglS and AglQ that couple PMF to energy consuming processes as components of the gliding motility protein complex [47–*49]. Time lapse fluorescence microscopy and fluorescence recovery after photobleaching (FRAP) experiments confirm the dynamic localization the motility protein complexes [*48,*49]. Presumably the bacterial cytoskeleton provides a scaffold for the dynamic localization of the complex, since drugs affecting MreB polymerization also abolish single cell movement [34]. Moreover, direct protein interaction between MreB and AglZ was shown [34], and a dynamic, helical localization pattern was observed for several components of the gliding motility complex [*49].

The specific mechanism for how force is generated by motility motor complexes is still under investigation, with wave propagation through periplasmic proteins rotating along a helical track and focal adhesion sites as the leading theories (Fig. 4B) [6,12]. For the adhesion model, identification of the peptidoglycan and membrane spanning components as well the surface proteins are needed to round out the theory. For the helical rotor, identification of the periplasmic cargo would be helpful. For both models, discreet identification and analysis of the minimal set of motor proteins is required to understand gliding motility.

Future research directions

Myxococcus xanthus provides exceptional opportunities for further investigations into the regulation of protein localization as cells periodically invert their polarity. For example, how does the Frz system contact the MglAB regulators and how do these regulators redirect protein localization? Are additional cytoskeletal proteins involved? Do they interact with MreB? What protein motifs target pole-to-pole transport of proteins during cellular reversals? The answers to these questions will undoubtedly give us important new insights into the cell biology of other bacteria, as these mechanisms are sure to be manifest in many diverse microorganisms.

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Highlights

- Dynamic localization of motility organelles in *M. xanthus* is regulated by Frz signaling
- Cell polarity requires the Ras-like GTPase and GAP proteins MglA and MglB
- Coordinated secretion of EPS and pilin is required for social motility
- Recent studies reveal that gliding motility is powered by distributed motor complexes

Fig. 1. Gliding motility and cellular reversals in *Myxococcus xanthus*

A) Cells at the colony edge swarm outwards (left panel). Groups of cells primarily use S-motility, while single cells move by A-motility (right panel). **B)** Time lapse microscopy (40 sec intervals) of a single cell. At reversals, cells change their direction of movement (indicated by arrows). Note that for clarity a hyperreversing mutant is shown with reversals every 2 min. **C)** The *frz* pathway regulates the frequency of cell reversals. **D)** Mutations that affect the regulation of reversal frequency abolish the ability to form fruiting bodies under starvation conditions. Please refer to text for details.

Fig. 2. Cell polarity

A) The GTPase/GAP proteins MglA and MglB localize to opposite cell poles and switch their position on cell reversals (arrows). Time lapse microscopy (15 sec intervals) of fluorescent MglA (green) and MglB (red). Reprinted from [**24]. **B)** The subcellular localization of MglA depends on its GTP bound state and on RomR. MglB promotes GTP hydrolysis and prevents MglA accumulation at the lagging pole.

Fig. 3. Mechanism for S-motility

A) EPS is presumably the primary anchor that mediates polar type IV pili binding and retraction. **B)** The subcellular localization of pili proteins at the leading cell pole consists of membrane-bound PilA monomers that are assembled as they pass through the PilC and PilQ channels. Extension/retraction cycles are driven by the PilB ATPase and intermittent activity of PilT ATPase at the leading cell pole. EPS is synthesized by a large protein complex of which the inner membrane EpsZ and outer membrane EpsO channels are shown. EPS secretion requires FrzS and coordination of both EPS and pilin production requires the Che-like Dif pathway. **C)** S-motility mutants such as *frzS* can be rescued by the exogenous addition of purified EPS. Reprinted from [*39]. It remains to be determined how the EPS required for S-motility relates to the polysaccharide slime that mediates A-motility. They may or may not be the same substance. **D)** FrzS protein localizes to the leading cell pole changes its position at cell reversal. Time lapse microscopy (1.5 min intervals) of fluorescent FrzS, reprinted with permission from [8].

Fig. 4. A-motility mechanism

A) Motor complexes are distributed along the cell and appear static while the cell moves forward. Time lapse microscopy (30 s intervals) of fluorescent AgmU protein, reprinted from [47]. **B)** Current model for the gliding motility mechanism. A large protein complex spans the cell envelope and includes regulatory components and motor proteins. Presumably, the protein complex travels along a cellular track provided by cytoskeletal proteins (green arrows), resulting in cell rotation. Black arrows indicate the direction of cell movement. Two hypotheses have been proposed for force generation. Either the envelope-spanning complex could penetrate the peptidoglycan (PG), aided by a lytic enzyme (magenta). A surface protein is presumed to adhere to the substrate (“focal adhesion”). The protein complex translocates along the cellular track, pushing the cell forward. Alternatively, the protein complex could function inside the cell envelope. Translocation of the complex is stalled when in contact with a surface, which leads to local distortion of the cell envelope and the generation of drag force (“helical rotor”). IM, OM: inner, outer membrane respectively. Refer to references [47,50] for a complete account of the proteins of the envelope spanning complex.