REVIEW ARTICLE

From individual cell motility to collective behaviors: insights from a prokaryote, Myxococcus xanthus

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Abstract
In bird flocks, fish schools, and many other living organisms, regrouping among individuals of the same kin is frequently an advantageous strategy to survive, forage, and face predators. However, these behaviors are costly because the community must develop regulatory mechanisms to coordinate and adapt its response to rapid environmental changes. In principle, these regulatory mechanisms, involving communication between individuals, may also apply to cellular systems which must respond collectively during multicellular development. Dissecting the mechanisms at work requires amenable experimental systems, for example, developing bacteria. Myxococcus xanthus, a Gram-negative deltaproteobacterium, is able to coordinate its motility in space and time to swarm, predate, and grow millimeter-size spore-filled fruiting bodies. A thorough understanding of the regulatory mechanisms first requires studying how individual cells move across solid surfaces and control their direction of movement, which was recently boosted by new cell biology techniques. In this review, we describe current molecular knowledge of the motility mechanism and its regulation as a lead-in to discuss how multicellular cooperation may have emerged from several layers of regulation: chemotaxis, cell-cell signaling, and the extracellular matrix. We suggest that Myxococcus is a powerful system to investigate collective principles that may also be relevant to other cellular systems.

Introduction
How thousands of cells self-assemble and differentiate to generate a specialized organ is a fundamental problem in developmental biology. During embryogenesis, cell migration events are regulated, so that differentiation occurs correctly in time and space. Incorrect regulation has dramatic consequences, for example, an increased risk of cancer metastasis. Despite a great deal of effort, our understanding of these processes remains relatively primitive. In higher organisms, progress is often impeded by the complexity of the experimental models and sometimes, ethical issues. For these reasons, unicellular microorganisms are often powerful model systems to study the developmental progression and directed cell migration. Studies of the social amoeba Dictyostelium discoideum in particular have been crucial to unraveling the general principles of chemotaxis and its role in multicellular development (Chisholm & Firtel, 2004).

Bacteria have been very powerful model systems to address the molecular mechanisms that underlie biological functions since the dawn of molecular biology. However, bacteria have long been considered single-cell organisms, and microbiologists have thus regarded pure cultures as the normal state of these organisms. This appreciation is now changing with the discovery of bacterial cell-cell communication (i.e. quorum sensing, Bassler & Losick, 2006) and biofilm formation, a widespread sessile lifestyle where the bacterial cells grow adhered to surfaces and form architecturally complex communities (Lopez et al., 2009). Growing evidence suggests that a biofilm is a differentiated entity where specialized cell types contribute specific functions locally (Lopez et al., 2009). Biofilm development often relies on motility and
its regulation (Klausen et al., 2003). A model organism that displays this behavior is the bacterium *Myxococcus xanthus* using motility to form large spore-filled upright fruiting bodies. In this organism, starvation induces a stringent response (Singer & Kaiser, 1995) which then triggers a developmental program leading thousands of *Myxococcus* cells to initiate fruiting body formation. Collective motility behaviors constitute an essential facet of the *Myxococcus* lifestyle and drive many processes, including vegetative swarming and predation on other microorganisms (Kaiser, 2003; Berleman & Kirby, 2009; Luciano et al., 2011). *Myxococcus* is thus a powerful model system to study how thousands of cells achieve coordinated migration.

A true understanding of the mechanisms leading to *Myxococcus* multicellular behaviors requires a multiscale analysis, linking molecular mechanisms to collective phenomena. First and foremost, an in-depth understanding of the motility mechanisms employed by individuals and how they are regulated is needed. Second, this knowledge must then be used and expanded to understand how large cell groups coordinate and respond synchronously to environmental signals. In this review, we attempt to provide an overview of current knowledge about *Myxococcus* motility and group behaviors and present selected future challenges to understanding this fascinating social organism.

**Myxococcus motility is driven by two distinct macromolecular systems**

On standard agar substrates, *Myxococcus* cells can move as large coordinated groups or as single isolated cells. Early genetic work by Hodgkin & Kaiser (1979) showed that these two motility behaviors are genetically separable: two categories of mutants showed defects either in single cell or in group motility. Thus, two distinct gene systems drive motility, suggesting that separate motility machineries independently drive movement. Based on these distinctions, coordinated group movement was termed Social (S)-motility in contrast to single-cell motility, named Adventurous (A)-motility. Social motility has been shown to be driven by Type-IV pili (T4P). In other bacteria, such as *Pseudomonas aeruginosa* and *Neisseria* sp., this form of motion is termed twitching motility (Merz et al., 2000; Skerker & Berg, 2001). In *Myxococcus*, T4P are assembled at the leading cell pole and also mediate twitching motility, for example, when single A ’S’ cells are spotted on methylcellulose (Sun et al., 2000). The *Myxococcus* T4P cluster contains all known core genes typically required for T4P assembly (Pellic, 2008). Molecular knowledge of structure–function relationships is abundantly described elsewhere (Pellic, 2008); thus in this review, we will briefly summarize knowledge about the *Myxococcus* T4P.

In *Myxococcus* as in other bacterial systems, the pilus body is composed of the core PilA, B, C, D, T, M, N, O, P, and Q proteins (Fig. 1a) (Pellic, 2008). Typically, prepilin precursors (PilA) are secreted via the general secretion pathway and cleaved by the PilD peptidase. Mature pilin subunits are then polymerized to form the pilus fiber, a 5- to 8-nm-thin filament that can be as long as several cell lengths when it is extruded (Pellic, 2008). Pilus assembly is promoted by the extension ATPase PilB (Jakovljevic et al., 2008), and the growing fiber penetrates the outer membrane through the PilQ secretin, a dodecameric complex whose stable assembly depends on Tgl, a lipoprotein (Nudleman et al., 2006). The pilus retraction motor is distinct from the extension motor and involves PilT, an ATPase that presumably acts to remove pilin subunits at the fiber base (Jakovljevic et al., 2008). Localization and genetic studies suggest that both PilB and PilT...
function in the cytosol (Jakovljevic et al., 2008). Therefore, PilB and PilT may not interact with pilin subunits directly but through a membrane component, presumably one of the six basal body proteins PilC, D, M, N, O, and P (Carbonnelle et al., 2006).

**Twitching mechanism**

Optical trap experiments showed that T4P are exceptionally strong motors: a single pilus exerts up to 150 piconewton forces (Clausen et al., 2009) and bundles of up to 10 filaments retracting cooperatively, can even reach nanonewton forces (Biais et al., 2008). When PilT levels are low, a force-dependent switch reverses pilus retraction to elongation when forces > 100 pN are applied (Maier et al., 2004). This suggests that the activities of PilB and PilT are controlled by a force-sensing mechanism to balance extension and retraction appropriately. The pilus fiber may act as sensor to recruit one or the other motor depending on the external forces. Other mechanisms may also coordinate PilB and PilT activities. In *Myxococcus*, PilT was shown rather counterintuitively to localize mostly to the nonpiliated pole (Bulyha et al., 2009). Upon closer examination, bursts of PilT accumulation were observed at the piliated pole, which may be sufficient for retraction (Bulyha et al., 2009). Spatial segregation of PilT and PilB at opposite poles may thus participate in coordination and possibly ensure the complete absence of active pili at the back of the cells. In conclusion, T4P convey twitching motility by retraction, but the molecular mechanism allowing coordinated extension and retraction cycles remains to be established.

**Gliding motility**

**Gliding motility is powered by distributed motors**

The identification of T4P as the molecular machinery that promotes S-motility revealed unambiguously that A-motility and S-motility are powered by distinct molecular systems. However, while genetic screens readily identified the twitching motility apparatus, similar genetic screens did not reveal a conspicuous gliding motility apparatus, thereby complicating the identification of the exact motility mechanism (Youderian et al., 2003; Yu & Kaiser, 2007). In the absence of more genetic evidence, microscopic investigations have attempted to image motility organelles directly. However, these studies were not very conclusive: even if ordered structures could be observed, they could not be identified and assigned to specific functions (Burchard et al., 1977; Freese et al., 1997; Lunsdorf & Schairer, 2001). In many bacterial species, gliding motility is associated with a mucus or slime, a putative polysaccharide that forms visible trails laid by the motile cells (Wolgemuth et al., 2002). Slime secretion could directly propel gliding motility. For example, if secretory organelles were located at the back of the cells, propulsion would occur when carbohydrate chains hydrate as they are transported through the outer membrane (Wolgemuth et al., 2002). However, recent advances prove that the motility machinery is distributed along the cell body in a definitive manner and makes this hypothesis unlikely. Slime may not contribute to the propulsion mechanism but rather provide a preferential substratum or a way to guide the cells.

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**Fig. 1.** Type-IV pili drive S-motility in *Myxococcus xanthus*. (a) Twitching motility by T4P extension–retraction cycles and EPS-driven cell–cell coordinations. Pili extrusion and binding to a neighboring cell surface (1) are proposed to trigger a regulatory loop increasing EPS expression via the Dif system in one cell and T4P retraction in the other cell (2). The structural pilus and Dif proteins are represented according to the current knowledge in *Myxococcus xanthus* and other bacterial systems (see text for details). (b) Polar TFP observed by AFM. Image reprinted with the permission of publisher from Pelling et al. (2005). (c) S-motility-driven colony expansion on soft agar.
A breakthrough in the understanding of *Myxococcus* gliding motility came from cytological experiments investigating the live dynamics of a known gliding motility protein, AglZ-YFP (Mignot et al., 2007). AglZ-YFP formed fluorescent clusters that were distributed regularly along the cell body (Fig. 2a). Strikingly, the clusters maintained fixed positions with respect to the agar surface; these clusters appeared at the front of the cell and dispersed when they reached the back of the cells. To appear fixed, the clusters must be moving opposite to the cell movement and at a similar velocity. It was thus proposed that AglZ accumulates within motility complexes acting like eukaryotic-like focal adhesions where substrate-bound adhesins are coupled to the actomyosin network (Mignot et al., 2007). Based on this principle, an as yet unidentified molecular motor (distinct from AglZ, which was shown to be a regulator rather than a structural component of the machinery) would interact with an adhesive envelope complex and produce thrust tracking on the internal cytoskeleton (Fig. 2b and c). The *Myxococcus* gliding motor would thus function like the Apicomplexan gliding motor, translocating adhesive proteins along the cell surface of the parasite to translocate on the host cell (Sibley, 2004).

To test whether AglZ-YFP localizes in a force-producing cell, cells were immobilized on a substrate where the motility complex cannot attach to the substratum. As expected, AglZ-YFP clusters were not fixed under these conditions, and they were transported unidirectionally by the unattached motility machinery at velocities that matched the gliding velocities (Sun et al., 2011). When applied directly to the cell surface of these cells, polystyrene beads were first immobile but occasionally collided with trafficking clusters that were always accompanied by processive transport of the beads along the exterior of the cell (Fig. 2b) (Sun et al., 2011). Thus, AglZ-YFP associates with an active transport system that transduces force to the cell exterior. Attachment of the complex to the substratum would then translocate the cell, consistent with the focal adhesion hypothesis.

**The gliding motility machinery**

**The cytoskeleton**

The AglZ-YFP clusters are regularly spaced, suggesting that they are anchored along an intracellular periodic scaffold (Mignot et al., 2007). In bacterial cells, the actin-like MreB cytoskeleton forms helical filaments running along the axis of a cell, which is important to guide the assembly of cell wall synthetic complexes (Carballido-Lopez, 2006). Several arguments suggest that MreB is directly involved in the positioning of the motility clusters: (i) Rapid MreB depolymerization reversibly blocked motility. Coincidently, assembly of the AglZ-YFP clusters also depended on MreB cables, (ii) bead transport required MreB polymerization (Sun et al., 2011), and (iii) MreB was shown to interact directly with AglZ in vitro (Mauriello et al., 2010). It remains to be shown whether the motility machinery actively tracks on rigid MreB polymers to generate force.

**Fig. 2.** A distributed envelope machinery drives *Myxococcus xanthus* gliding A-motility. (a) Time-lapse showing accumulation of AglZ-YFP within distributed fixed clusters (arrows). Reprinted from Mignot et al. (2007) with the permission from publisher. (b) Surface-transported polystyrene beads are associated with AglZ-YFP clusters. Shown are time series of fluorescence (lower panel) and fluorescence overlaid on phase contrast images (upper panel). White dots indicate the position of the bead in the fluorescence images. (c) Current motility model. Motility motors are proposed to translocate along an endless closed loop. Directionality (yellow or green arrows) may be regulated by specific activation of the motors at the corresponding leading pole (yellow or green) and deactivation at the lagging pole. Activated/deactivated complexes thus run in the opposite directions. (d) Proposed gliding motility complex. The structural proteins are represented based on available interaction and localization data (see text for details). See (e) for question marked circles at the bottom of the cartoon. (e) The focal adhesion (model 1) and the cell wall constraints in the crawling snail (model 2) hypotheses.
Studying the in vivo dynamics of MreB during motility will likely be critical for a better understanding of the motility functions of the cytoskeleton.

The gliding motility motor

Two independent studies unambiguously demonstrated that gliding motility is not powered by ATP but directly by the pH gradient across the inner membrane, indicating that the gliding motor is akin to the flagellar motor, MotAB (Nan et al., 2011; Sun et al., 2011). In bacteria, the MotAB/TolQR/exbBD proteins constitute a large family of proton-conducting motors that energize various processes in the cell envelope (Lloubes et al., 2001). Interestingly, two gene regions encoding putative MotAB/TolQR/exbBD proteins are found in a collection of mutants that lack gliding motility (Youderian et al., 2003). One insertion (aglX) is within a region encoding a bona fide Tol-Pal-like system. AglX is unlikely to be a true component of the gliding motor because Tol-Pal is involved in the general envelope processes including outer membrane (OM) integrity, transmembrane transportation, and cell division (Gerding et al., 2007; Yeh et al., 2010). Furthermore, an aglX mutant is also defective in S-motility (Youderian et al., 2003). For these reasons, we favor that Tol-Pal defects create general cellular defects, which generally impacts motility. Two other transposon insertions (aglR and aglS) mapped in a locus encoding another predicted motor complex. Based on the knowledge from MotAB and TolQR, the locus contains three genes, aglRQS-encoding proteins with all necessary residues for lumen and H+ conductance. In TolQR, a functional H+ channel results from the trimeric assembly of two TolR molecules with one TolQ molecule (Goemaere et al., 2007). In AglRQS, pairwise combinations suggest that AglR (TolR-like) may form functional motors with AglQ (TolQ-like) and AglS (TolS-like) homodimers but also possibly with an AglQS heterodimer. The AglRQS complex colocalizes with AglZ-YFP and is essential for its processive transport, an activity that depends on a conserved H+-binding Asp residue in the predicted lumen of the channel (Sun et al., 2011). Thus, AglRQS may power motility directly by interacting with the motility machinery in the distributed clusters.

The motility complex

Recently, a search for genes that coevolved with the aglRQS genes identified the motility machinery for the first time (Luciano et al., 2011). It was thus found that the A-motility machinery evolved from a widespread conserved ancestral complex (CAC) of unknown function (Luciano et al., 2011). Phylogenomics revealed that the CAC was expanded by several acquisitions of new functional modules during evolution of the deltaproteobacteria (where Myxococcus belongs), terminally differentiating into a motility machinery. In the past, the A-motility machinery was not readily identified because its structural genes were scattered in three distinct regions of the Myxococcus genome; in fact, functional linkage of the machinery genes only becomes apparent when their co-occurrence and spatial proximity are studied in ancestor bacteria genomes (Luciano et al., 2011).

It is now apparent that motility is driven by a trans-envelope spanning complex of at least 11 motility proteins, GltA-K (Gliding transducer, Luciano et al., 2011). Bioinformatics predictions and genetic and interaction studies suggested that GltA-K forms a complex that spans the entire cell envelope and interacts with AglRQS in the inner membrane through GltG (Nan et al., 2010; Luciano et al., 2011). A low resolution architecture of the motility machinery can be proposed: an inner membrane AglRQS-GltGJ platform may be connected to GltH, GltZ, MglA, and MreB on the cytosolic side and extend to the cell exterior through GltD, E, B, C, H, A, K and CglB (Fig. 2d, Nan et al., 2010; Luciano et al., 2011). The dynamics of GltD and GltF (previously AgmU and MXAN4868) were studied in larger detail. As expected, both proteins localized to the focal adhesion complexes, proving that the motility machinery is assembled at those sites in a definitive manner. Upon closer examination, deconvolution and photobleaching studies suggested that, in fact, GltD traffics along a twisted endless loop that spans the entire length of the cell (Nan et al., 2011). In live cell assays, the helix seemed to rotate, which required both the proton motive force (PMF) and an intact MreB cytoskeleton (Nan et al., 2011).

Force transduction to the cell surface and current motility models

A motility mechanism may be proposed based on cytoskeletal trafficking of Glt-bound AglQRS motors (Fig. 2d). Conceivably, thrust is produced when complex-bound adhesins in the outer membrane latch onto the substratum and immobilize the motility machinery (Fig. 2e: model 1). However, in this scenario, the motility complex must accommodate the cell wall and ‘burn’ through the peptidoglycan layer as the cell moves. An alternative ‘crawling snail model’ overcomes this difficulty (Nan et al., 2011): the motor-attached Glt complex would not span the peptidoglycan but rather deform it locally, creating drag forces on the ventral side of the cell (Fig. 2e: model 2). Viewed externally, motor-generated transverse waves would thus propagate at the cell surface toward the trailing pole and push on the substrate. Active motility complexes (‘high drag cargoes’) would be
preferentially loaded at the leading cell pole and replaced by inactive motility complexes (‘low drag cargoes’) at the lagging pole, creating robust directional translocation of the cell (Nan et al., 2011). According to this view, the observed fixed motility clusters would not be ‘focal adhesions’ in the eukaryotic sense but ‘traffic jams’ that occur when the ‘high drag’ motility complexes collide on the ventral side of the cell. Total internal reflection fluorescence (TIRF) microscopy and atomic force microscopy (AFM) studies suggest that zonal contact points with the substrate may exist on the ventral side of the cell (Pelling et al., 2005; Nan et al., 2011). However, proofs that these structures are related to motility are still lacking.

**Outstanding questions for the future**

While the general features of gliding motility appear clearer, much work remains to determine the exact motility mechanism. Both the crawling snail and the focal adhesion hypotheses invoke a continuous helical scaffold (Fig. 2c). MreB is an obvious rotor candidate because it is required for motility complex positioning and assembles helical polymers in *Myxococcus* and other bacteria (Carballido-Lopez, 2006; Mauriello et al., 2010). Yet, there is currently no evidence that these assemblies form continuous closed loops, spanning the entire cell length. In fact, converging studies in other bacteria indicate that MreB rather forms short dynamic filaments rather than continuous cables (Kim et al., 2006; Dominguez-Escobar et al., 2011; Garner et al., 2011; Swulius et al., 2011).

The motility machinery may not be inserted in the peptidoglycan but only deform it. However, there is evidence that the machinery spans the entire cell envelope. Co-tracking of polystyrene spheres with AglZ-YFP clusters shows that the beads are somehow connected to the cytosolic parts of the complex despite the presence of the cell wall (Sun et al., 2011). Also, many outer membrane proteins are important for A-motility, some of which are part of the Glt complex (GltA, K and GltH). In *E. coli*, outer membrane lipoproteins regulate cell wall synthetic complexes directly (Paradis-Bleau et al., 2010; Typas et al., 2010). Several OM lipoproteins of unknown function are tied to motility (Youderian et al., 2003; Nudleman et al., 2005); some of these proteins may thus regulate machinery insertion into the cell wall. Alternatively, the motility complex could interact directly with a cell wall hydrolase to promote digestion of the peptidoglycan layer locally. Finally, attachment to the substrate may be specifically mediated by motility complex outer membrane proteins, possibly GltH, A, K, and CglB (Luciano et al., 2011).

Finally, how the motor generates torque is unresolved. By analogy with known channels like MotAB or TolQR, AglIR is expected to transduce motor work to the cell envelope motility parts, which is supported by the direct interaction between AglR and GltG (Luciano et al., 2011). However, whether this work generates direct traction on MreB is a major open question. This question also relates to the question of directionality. We discuss this particular aspect in detail below because gliding *Myxococcus* cells can switch their directionality very rapidly.

**Spatial regulation of motility**

During development, thousands of *Myxococcus* cells must direct their motility to aggregate and form multicellular fruiting bodies. Understanding this phenomenon requires first to decipher how single cells regulate their direction of movement. *Myxococcus* cells reverse their direction of movement through a process whereby the poles exchange roles (Blackhart & Zusman, 1985): following a short pause, the leading pole is rapidly converted into the lagging pole allowing the cell to move back in the exact opposite direction. In the following sections, we discuss the signaling networks that allow *Myxococcus* cells to switch their polarity axis in a matter of seconds, allowing cells to change the directionality of A-motility and S-motility machineries synchronously.

**Biased random walk by regulated cellular reversals?**

Swimming bacteria orient in three-dimensional space in response to spatiotemporal chemical gradients, a phenomenon known as chemotaxis. In enteric bacteria, ligand-induced activation of a methyl-accepting chemoreceptor protein (MCP) activates autophosphorylation of the CheA histidine kinase, which then transfers a phosphoryl group to the CheY response regulator. Phosphorylated CheY then binds directly to the flagellar switch complex, inducing clockwise rotation of the flagellum (for extensive reviews, see Wadhams & Armitage, 2004; Kirby, 2009).

How do bacteria orient their motility when crawling across solid surfaces? In theory, tactic behaviors would emerge if cells biased their reversal period in response to external cues. *Myxococcus* gliding is slow (\(\mu\text{m min}^{-1}\) as opposed to \(\mu\text{m s}^{-1}\) for swimming bacteria) suggesting that cells may only sense very stable chemical gradients. However, several lines of evidence suggest that *Myxococcus* motility can be chemotactic. First, the *Myxococcus* genome is equipped with an arsenal of chemosensory pathways: eight complete chemosensory-like systems and up to 21 predicted methyl-accepting proteins (Goldman et al., 2006). Mutations in three of the four systems under experimental studies impair motility and multicellular development to various degrees (Kirby, 2009). In particular, one of the chemotaxis-like pathways (Frz) regulates...
the reversal frequency of the cells: loss of function mutations results in severely lowered reversal frequencies, while a subclass of gain-of-function receptor mutations leads to increased reversal frequencies (Blackhart & Zusman, 1985; Bustamante et al., 2004). Second, treatment with attractant lipids or toxic compounds, such as isoamyl alcohol, lead to changes in the reversal periods, which depends on signaling (through Dif and Frz) and specifically, when Frz is involved, adaptation by receptor methylation (McBride et al., 1992; Kearns & Shimkets, 1998). Third, while clear taxis behaviors remain to be observed in individual cells, multicellular swarms show directed responses toward a nutrient or prey bacteria (Shi et al., 1993; Berleman et al., 2008; Taylor & Welch, 2008).

The Frz signal transduction pathway

The *Myxococcus* reversal cycle is controlled by the Frz signal transduction pathway. Similar to the Che pathway in enteric bacteria, activation of the MCP-like FrzCD receptor leads to the phosphorylation of a CheY-like response regulator involving a CheW-like protein (FrzA) and a CheA-like histidine kinase (FrzE). The pathway is also adapted by methylation/demethylation through the activity of FrzF (methyltransferase) and FrzG (methylesterase) (Fig. 3c). However, unlike chemotaxis pathways in swimming bacteria, the output of the signaling cascade is not flagellum rotation but polarity switching of the motility engines. This regulation is linked to several unique features of the Frz pathway: (i) The FrzCD receptor is cytosolic and lacks standard MCP ligand–binding and transmembrane domains (Bustamante et al., 2004), (ii) the FrzE kinase is a hybrid kinase linked to a response regulator domain (Inclan et al., 2008), and (iii) FrzE phosphorylates at least three distinct receiver domains (the FrzE receiver domain, the FrzZ tandem receiver domains) (Fig. 2c) (Inclan et al., 2007, 2008).

Input into the Frz pathway

In the cytosol, FrzCD may sense signals with two distinct domains: a unique N-terminal extension and a C-terminal region that shows homologies with the methylation and signaling domain of the enteric MCPs (Bustamante et al., 2004). Deletion of the FrzCD N-terminal region does not result in significant motility defects, suggesting that most sensing involves the C-terminal domain (Bustamante et al., 2004). Accordingly, several mutations in the methyl-accepting region of FrzCD lead to a characteristic deregulated hyperactive phenotype (Bustamante et al., 2004). Thus, methylation may be a major input into the Frz pathway. *In vitro*, FrzF is regulated by an unusual extension carrying multiple tetratricopeptide repeat motifs (TPR) suggesting that protein–protein interactions with FrzF modulate Frz signaling (Scott et al., 2008).

A function of the N-terminal domain of FrzCD was unmasked when it was discovered that its deletion somewhat rescued the A-motility defect of an aglZ mutant.
(Mauriello et al., 2009a). Thus, A-motility proteins such as AglZ act to alleviate a Frz-dependent motility block mediated by the N-terminal region of the FrzCD receptor. The biological function of this effect remains to be explored, but it reveals intriguing connections between the motility and the regulation machineries. Finally, Frz recruitment of other MCPs or even Che-like systems may yield other signaling routes into the Frz system. For example, genetic work shows that FrzCD may cooperate with DifA when cells respond to phosphoethanolamine (Xu et al., 2008).

Output from the Frz pathway

The Frz pathway contains up to three receiver domains (FrzE-CheY, FrzZ1, and FrzZ2), and three other critical motility proteins, RomR, FrzS and AglZ, also contain receiver-like domains, so in total, up to six receiver proteins may contact the FrzE kinase. Deletion of the full-length frzZ recapitulates a frzE-null mutation; thus, FrzZ likely acts as the output response regulator (Fig. 3c, Inclan et al., 2007). In vitro, FrzE transfers a phosphoryl group to both FrzZ receiver domains, which seem to act redundantly because individual domain deletions only display subtle phenotypes (Inclan et al., 2008). Intriguingly however, phosphotransfer to FrzZ is only observed if the phosphoacceptor site of FrzE-CheY is mutated, which in vivo only generates moderate motility defects (Inclan et al., 2008). In vivo, FrzE-CheY probably acts to inhibit the phosphate flow to FrzZ. How this regulation is alleviated and what protein(s) is the target of phosphorylated FrzZ remain to be discovered.

As mentioned earlier, FrzS, AglZ, and RomR may also be targets of FrzE. However, genetics do argue that these proteins do not act immediately downstream from FrzE but rather act downstream from MglA to regulate S-motility (FrzS) and A-motility (AglZ, RomR). Also, the FrzS and AglZ receiver domains are unusual and lack conserved phosphoacceptor residues making them unlikely phosphorylation substrates (Yang et al., 2004; Fraser et al., 2007). The primary sequence of RomR indicates that it could be phosphorylated (Leonardy et al., 2007). Interestingly, a constitutive RomR mutation (a phosphorylated mimic) bypassed the FrzE requirement for reversals (Leonardy et al., 2007). RomR is specifically involved in A-motility and thus how it fits in the pathway is unclear. As for AglZ, FrzS and RomR may also act in feedback signaling rather than deliver Frz signaling directly (Fig. 2c).

Generating cellular reversals

How does Frz signaling create cellular reversals? In order to reverse, cells must switch the direction of the A- and S-machineries simultaneously. Recent cytological experiments suggest that directional switching is implemented by pole-to-pole oscillations of critical motility regulators and structural motility proteins (i.e. PilB and PilT). Every reversal is preceded by a c. 30-s pause preceding movement in the opposite direction. During this pause, regulator proteins belonging to each motility system (FrzS and AglZ) relocate simultaneously to the opposite pole (Mignot et al., 2005, 2007). Conversely, proteins localizing at the back pole, for example RomR, oscillate in the opposite direction (Leonardy et al., 2007). Protein spatial oscillations involve physical protein exchange between the cell poles and do not require de novo protein synthesis (Mignot et al., 2005; Bulyha et al., 2009). Thus, Frz signaling provokes a rapid inversion of the polarity axis, changing the direction of movement.

Twitching motility reversals

A simple way to switch the polarity of T4P would be to activate preassembled T4P in a pole-specific manner. For example, Tgl only localizes to one cell pole suggesting that the PilQ secretin that localizes at both poles is only active at one pole (Nudleman et al., 2006). Detailed dynamics of several other structural pilus components show several localization patterns: PilM, PilC, and PilQ are distributed symmetrically (Bulyha et al., 2009), while other proteins accumulate asymmetrically at the leading (PilB and FrzS) or at the lagging pole (PilT) (Mignot et al., 2005; Bulyha et al., 2009). All these proteins are relocated asymmetrically when cells reverse. Thus, symmetry breaking is probably achieved by regulated localization of multiple T4P components. This apparent redundancy may explain the tight regulation of pilus localization, for example, despite a large number of studied twitching motility genes, no mutant has thus far been shown to display pili at both cellular poles.

Gliding motility reversals

Directional switching of the gliding motility apparatus is not intuitively trivial because how the motility complexes are transported unidirectionally and rarely backtrack is not understood (Sun et al., 2011). AglZ clusters are assembled at the leading pole and dispersed at the lagging pole, suggesting that directionality involves activation of the A-motility machinery at the leading pole and deactivation/dispersal at the lagging pole. Supporting this idea, a mutant in which the motility complex cannot be dispersed (mglAQ82L) becomes trapped in an endless pendulum motion, systematically reversing when the gliding motor (visualized by AglZ-YFP) reaches the cell poles (Zhang et al., 2010). Thus, artificial accumulation of
A-motility proteins at the back of cells triggers a directional switch, suggesting that indeed directionality is dictated at the cell poles. If the gliding motor tracks along an endless loop structure (Nan et al., 2011), failure to inactivate the machinery at the back of the cells would indeed lead to reversal.

**Spatial coupling of the motility systems by MglA, a Ras-like small G-protein**

How are the spatial oscillations of A-motility and S-motility proteins synchronized? Recent works suggest that the MglA protein acts downstream from Frz to activate the motility systems at the leading cell pole. MglA defines a bacterial subclass of predicted Ras-like small G-proteins (Dong et al., 2007). In eukaryotes, Ras-like small G-proteins and their regulators are involved in central cellular processes including transcription, signal transduction, secretion, and motility regulation. In leukocytes, or in the amoeba *D. discoidum*, dynamic polarization during chemotaxis is achieved by a complex interplay of multiple small G-proteins at the front and the rear involving Ras, Rac, Cdc42, and Rho (see (Charest & Firtel, 2007) for a detailed review of these regulations). Remarkably, MglA-like proteins are widespread in bacteria, but knowledge of their function is completely lacking (Dong et al., 2007). Ras-like small G-proteins act as nucleotide-dependent molecular switches, cycling between a GDP-bound inactive state and a GTP-bound active state. In general, small G-proteins do not hydrolyze GTP effectively but are regulated at many levels, for example, by specific GTPase-activating proteins (GAPs) activating G-protein nucleotide hydrolysis and guanine nucleotide exchange factors facilitating GDP/GTP substitution in the G-protein catalytic site (Bos et al., 2007).

*In vitro*, MglA acts as a *bona fide* small G-protein: it binds GTP with a much higher affinity than GDP (Leonardy et al., 2010) and has a very slow intrinsic GTPase activity, similar to canonical Ras small G-proteins (Leonardy et al., 2010; Patryn et al., 2010; Zhang et al., 2010). An mglA mutant is completely nonmotile (Leonardy et al., 2010; Mauriello et al., 2010; Zhang et al., 2010). An MglA-YFP fusion localizes at the leading cell pole but also within the gliding motility clusters, suggesting that MglA is required for both synchronization and activity of the motility machineries (Mauriello et al., 2010; Patryn et al., 2010). In addition, MglA is required for proper localization of both A-motility and S-motility proteins (i.e. AglZ, FrzS, and RomR) (Leonardy et al., 2007; Mauriello et al., 2010). Thus, MglA may be an upstream regulator recruiting critical motility proteins to the motility complexes.

Obviously, leading pole localization of MglA must be important for directional motility. How is it maintained and dynamically switched to the opposite pole when cells reverse? The regulatory mechanism was recently unraveled in two recent studies (Leonardy et al., 2010; Zhang et al., 2010). The polarity of MglA is maintained by MglB, an MglA GAP. In this process, MglB localizes at the lagging cell pole and excludes MglA from that pole by activating transition to the MglA-GDP inactive form (Fig. 3a and b, Leonardy et al., 2010; Zhang et al., 2010). This way, the *Myxococcus* polarity axis is established by spatial segregation of a small G-protein inhibitor, a common theme in eukaryotic cell biology, for example, during yeast budding, *Dictyostelium* chemotaxis or *C. elegans* embryogenesis (Charest & Firtel, 2007; Tong et al., 2007; Anderson et al., 2008).

**Spatial regulation of motility: unsolved questions and perspectives**

In theory, *Myxococcus* reversals may simply result from switching the localization of MglB to the opposite pole, a process that may be activated directly by FrzZ-P. However, several lines of evidence suggest more complexity. First, MglA and MglB are reciprocally dependent for localization (Leonardy et al., 2010; Zhang et al., 2010). Second, the polarity switch is a stepwise process in which MglA and not MglB is first relocalized (Leonardy et al., 2010), suggesting that MglB does not trigger the switch but acts to maintain the polarity axis after reversal (Fig. 3b). Thus, reversals may be first initiated by MglA relocalization, a still mysterious mechanism that may be solved once a direct link between the Frz pathway and MglA/B is established. Conceivably, bursts of phosphorylated FrzZ (Igoshin et al., 2004) may act to detach MglA from the leading cell pole, initiating the polarity switch. How MglA then triggers MglB relocalization and whether that involves other proteins also need further clarification.

MglA-GTP is required for correct polarity of the motility proteins but not strictly for their binding at a cell pole. For example, in an mglA mutant, motility proteins such as FrzS and RomR do not switch but bind erroneously to the same pole (Leonardy et al., 2007; Mauriello et al., 2010). As is the case of polar flagella (Huitema et al., 2006; Lam et al., 2006), parts of the motility complexes may be assembled during the cell cycle at division septa, spatial activation may then result from MglA-GTP binding at one cell pole only. FrzS and AglZ are candidate direct MglA effectors because both interact with MglA in a pull-down assay (Mauriello et al., 2010). However, MglA is strictly required for motility while, as discussed earlier, both AglZ and FrzS are dispensable for A-motility and S-motility under specific conditions (Mignon et al., 2005; Mauriello et al., 2009a). Thus, MglA likely also acts through other unknown motility effectors.
Spatial regulations at the lagging pole are equally important: T4P must be kept inactive, and the gliding machinery must be disassembled. There are good indications that MglB plays a central role in this process. Whether MglB regulation restricts active pili at one cell pole has not been tested but could be the case because an mglB mutant has a strong S-motility defect and MglA is bipolar in this mutant (Zhang et al., 2010). MglB is clearly important to inactivate the A-motility machinery at the back of the cells: mglB mutants or a mutant expressing an MglA GTP-locked variant shows the pendulum motion. Thus, MglB presumably inactivates AglRQS-Glt-bound MglA-GTP when the motility complex reaches the back of the cells, leading to its disassembly (Fig. 3b) (Leonardy et al., 2010; Zhang et al., 2010).

From single cells to complex multicellular behaviors

As discussed earlier, the main features of the motility mechanisms and their regulation in single cells are starting to emerge. The next step will be to understand how thousands of reversing cells generate a variety of collective behaviors including predation and multicellular development (Fig. 4). Modeling studies indicate that changing the reversal period may affect the outflow of cells at the swarm edge, questioning the regulation mechanisms at work (Wu et al., 2009). In the next part of this review, we discuss how cooperative mechanisms such as extracellular matrix (ECM) recognition, chemotaxis, and cell–cell contacts may contribute to coordinated multicellular behaviors. To conclude this review, we propose experimental directions to achieve a systems-level understanding of the Myxococcus motility process in the future.

Role of the ECM

Analogous to the ECM guiding cell migrations in higher eukaryotes, the Myxococcus ECM may affect cell–cell coordination, mediating specific adhesion, cell–cell cohesion, and even providing chemical signals to guide motility.

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Fig. 4. Multicellular behaviors and potential regulatory mechanisms. Fruiting body morphogenesis [reprinted and modified from Velicer & Vos (2009) with the permission of publisher], predataxis [reprinted and modified from Berleman & Kirby (2009) with the permission of publisher], and vegetative swarming (A. Ducret, shown is an artificially colored image with computed cell trajectories within the cell group. The inset shows a slime trail as detected by standard phase microscopy) involve multiple regulations: cell–cell contacts, chemotaxis, ECM, and slime trail guiding. Shown are selected examples from the literature: C-signal (p17, upper left panel), FrzCD alignments [middle left panel, reprinted and modified from Mauriello et al. (2009a, b) with the permission of publisher], outer membrane vesicles [upper right panel, reprinted and modified from Palsdottir et al. (2009) with the permission of publisher], and chemotaxis [middle and lower right panel, reprinted and modified from Taylor & Welch (2008) with the permission of publisher].
Moving *M. xanthus* cells deposits a mucus (slime) that appears as phase bright lines on agar surfaces under standard microscopy, and as large ribbons by EM (Wolgemuth *et al.*, 2002). Slime was initially proposed to propel A-motility by selective secretion at the back of the cells (Wolgemuth *et al.*, 2002), but this hypothesis appears unlikely now that the motility motors have been located along the cell body. Interestingly, slime trails deposited by scout cells moving at colony edges become favored motility paths for following cells (Burchard, 1982). In this process, *Myxococcus* cells may recognize adhesive molecules deposited by scout cells like ants deposit chemical pheromones, signaling environmental changes in the vicinity of the community. Solving the composition of slime, a suspected polysaccharide will be instrumental to test this hypothesis.

The *Myxococcus* ECM contains EPS, proteins, and lipid vesicles of various sizes. One particular EPS polymer has been studied in detail: it is distinct from slime and seems dedicated to S-motility (Lu *et al.*, 2005). This S-motility EPS contains glucosamine, galactose, rhamnose, and xylose to a lesser extent. Synthesis requires the eps and eas regions, carrying genes required for the assembly and export of the EPS polymer (Lu *et al.*, 2005). Accumulating evidence suggests that the S-motility EPS mediates T4P-dependent cell–cell contacts and retraction, explaining its importance for S-motility (Li *et al.*, 2003). A fraction of the EPS may be shed directly on the substratum and thus help guide twitching motility (Hu *et al.*, 2011). Supporting this hypothesis, EPS− cells do not perform S-motility, yet they twitch as single cells on artificial glucose or N-acetyl glucosamine polymers (Sun *et al.*, 2000; Hu *et al.*, 2011). Thus, EPS may act in combination with slime to guide the two motility systems. Another polymer, LPS, and more specifically the O-antigen portion contribute a mysterious role to S-motility (Bowden & Kaplan, 1998). Exposed at the cell surface, LPS O-antigen could also mediate important interactions with the ECM.

The ECM protein content was recently determined by mass spectrometry (Curtis *et al.*, 2007a). The functions of most of the ECM proteins remain unclear; one of them, FibA, a zinc metalloprotease of the elastase family, is extremely abundant. Genetic analysis shows that FibA is important for developmental progression (Bonner *et al.*, 2006) and very interestingly, tactic behaviors toward lipids (Kearns *et al.*, 2002), suggesting that indeed the ECM may also contain chemical signals.

**The role of chemotaxis during group behaviors**

Taylor & Welch (2008) suggested that *Myxococcus* chemotaxis may not be a property of single cells but the emergent property of a cell group, a swarm whose ‘intelligence’ is driven by signaling and cell–cell interactions. As discussed earlier, several reports provide unambiguous evidence that cell groups can show tactic behaviors toward nutrients, lipids, and prey cells. Under certain conditions, moving *Myxococcus* cells organize into rippling waves that do not interpenetrate each other but reflect off one another, oscillating back and forth with no net translocation (Fig. 4). When rippling, the reversal frequency of the cells sets the distance between two wave crests (Slušareno *et al.*, 2006). Rippling is a predatory behavior induced by the presence of prey cells (Berleman *et al.*, 2006). Berleman *et al.* (2008) have shown that the rippling period is adaptable and increases as the local concentration of prey decreases. At the molecular level, adaptation occurred through FrzCD methylation/demethylation (Berleman *et al.*, 2008). This ‘predataxis’ is thus a clear example of the chemosensory response of a *Myxococcus* cell group to prey availability.

In the absence of a characterized chemottractant, for example an equivalent of cyclic adenosine 3′,5′-monophosphate (cAMP) driving Dictyostelium aggregation (Gregor *et al.*, 2010), cell–cell contact mechanisms have been favored to guide cell aggregation and commitment to form fruiting bodies (Kaiser, 2003). However, chemotaxis and contact-dependent signaling are not mutually exclusive, and each could be involved at particular stages of the developmental cycle. As discussed earlier, FrzC may be activated internally by methylation or externally by coupling with other chemoreceptors; thus, the architecture of the Frz pathway conceivably accommodates both chemotaxis and contact-dependent regulations. Contact-dependent activation of the Frz pathway is further suggested by unusual properties of the FrzCD receptor.

**Coordination by cell–cell contacts**

During fruiting body formation, C-signal, a developmental specific signal is synthesized ∼6 h after starvation and acts upstream of a signal transduction cascade regulating both developmental gene expression and motility behaviors (Søgaard-Andersen *et al.*, 1996). In this later process, C-signal is proposed to suppress cellular reversals by a contact-dependent mechanism, leading cells to stream within initial aggregates and form fruiting bodies (Kim & Kaiser, 1990; Jelsbak & Søgaard-Andersen, 2002). During development, C-factor (the csgA gene product) is exported at the cell surface and processed by proteolysis (Lobedanz & Søgaard-Andersen, 2003; Rolbetzki *et al.*, 2008) to activate a receptor at the surface of neighboring cells, triggering downstream FrzCD methylation and thus suppressing reversals (Fig. 4) (Søgaard-Andersen & Kaiser, 1996). It now seems clear that C-factor is active at the cell surface (Rolbetzki *et al.*, 2008); however, the
predicted surface receptor remains elusive. Also, recent experimental tracking of aggregating cells did not show increased reversal period but rather suggested that cells slow down in the vicinity of the aggregates (Sluisarenko et al., 2007). This inconsistency with Jelsbak & Søgaard-Andersen (2002) may be due to differences in the experimental conditions, which would argue that fruiting body formation can be achieved by several mechanisms.

Cell–cell contacts may influence motility behaviors because cells tend to align when they collide, thus co-orienting cells in a purely physical way (Janulevicius et al., 2010). However, the dynamic behavior of a FrzCD-GFP chimera also suggests that collisions actively induce signaling events. Unlike most bacterial MCPs, which localize at the cell pole, FrzCD localizes in dynamic cytosolic clusters that tend to align when cells come in contact (Fig. 4) (Mauriello et al., 2009b). This alignment is not random because it does not occur in a frzE mutant, suggesting that it has a signaling function. Hence, Mauriello et al. (2009b) proposed that receptor alignment reflects a mechanism that synchronizes cell reversals upon cells collisions. The molecular basis of this phenomenon remains to be solved, but this example further supports the existence of contact-activated signaling to synchronize motile cells.

Social motility is a clear example of motility coordination by cell–cell contacts. As discussed earlier, EPS may trigger T4P retraction, and T4P-EPS interactions between cells may physically link large cell groups during motility. Interestingly, T4P themselves are required for EPS biosynthesis (Black et al., 2006), which attractively suggests that T4P interactions between cells are reinforced by a positive regulatory loop where the ligand (T4P) induces the expression of its own receptor (EPS). In this process, T4P stimulate the Dif-chemosensory pathway, which in turn activates EPS biosynthesis (Fig. 1a). Both the absence of pilin subunits and their abnormal accumulation as periplasmic monomers inhibit EPS synthesis (Black et al., 2006; Yang et al., 2010), while retraction defects lead to EPS overproduction (Black et al., 2006). Thus, assembled but not retractile pili may be sensed by the DifA receptor to activate EPS production.

Last, Myxococcus cells can transiently exchange outer membrane proteins in a mysterious phenomenon discovered by Hodgkin and Kaiser: it was observed that a subclass of S-motility or A-motility mutants are transiently stimulated back to motility when they are mixed with other motility mutants (Hodgkin & Kaiser, 1977). This stimulation does not result from mobile genetic elements but from the physical transfer of outer membrane proteins by cell–cell contact (Nudleman et al., 2005; Wei et al., 2011). Transfer is extremely efficient; it has been speculated to result from transient fusion events between outer membranes (Nudleman et al., 2005; Wei et al., 2011). So far, OM fusions have not been reported in CRYO-EM studies of Myxococcus (Palsdottir et al., 2009), but they were observed in Borrelia sp. where closely apposed cells could sometimes show a single outer membrane sheath (Kudryashev et al., 2011). Other mechanisms may also mediate protein transfer. Recently, extracellular lipid tubular extensions (nanotubes) have been proposed to transfer protein materials between Bacillus subtilis cells and potentially other bacteria (Dubey & Ben-Yehuda, 2011). Outer membrane vesicles can also deliver proteins (Kulp & Kuehn, 2010) and are very abundant in Myxococcus biofilms (Palsdottir et al., 2009). Until now, transfer has only been evident from experiments involving motility mutants; thus, it is presently unclear whether it is relevant to WT Myxococcus populations. Transfer may additionally support coordination, synchronizing the directionality of T4P and the gliding machinery within multicellular swarms.

**Concluding remarks—toward an integrated model of multicellular morphogenesis**

Studying Myxococcus fruiting body formation is a powerful means to address how thousands of cells self-organize to generate a multicellular organelle. As discussed in this review, Myxococcus cells have evolved a complex genetic network to direct their motility and integrate individual behaviors to collective environmental responses. In this context, we are interested in defining the minimum sequence of events required for morphogenesis. In the past, modeling studies have repeatedly attempted to reproduce the Myxococcus multicellular patterns. However, the models have been frequently inconsistent with each other. For example, Sluisarenko et al. (2007) computed that the slowing of the cell velocity accounts for the early stages of aggregation, while another study by Sozinova et al. (2005) suggested that aggregation is initiated by the suppression of cellular reversals. These discrepancies are likely due to our partial understanding of the mechanisms and thus reliance on specific assumptions that may be relevant under specific conditions only. For example, fruiting bodies can form under a multitude of conditions, on agar but also when submerged in liquid or at the boundaries of rippling waves (Welch & Kaiser, 2001), arguing that several pathways may in fact trigger fruiting body formation.

In this review, we discuss many potential coordination mechanisms, most of which inferred from genetic work and thus lacking spatiotemporal resolution. In this context, the inclusion of these mechanisms in the models can only be partial and largely hypothetical. We thus suggest that new experimental knowledge is needed before new
models are constructed. Recent time-lapse studies suggest that aggregates form when cells start moving on top of initial ‘traffic jams’ (Kaiser & Welch, 2004; Curtis et al., 2007b). In this process, large cell ‘rafts’ form tiers on top of the initial aggregates, and fruiting bodies are formed as tiers pile up on top of each other (Curtis et al., 2007b). Why are cells coalescing in traffic jams? How are rafts organized? What stabilizes a growing fruiting body? In the future, these studies may be completed by high-throughput digital tracking approaches (Xie et al., 2011) expanding the tools that were developed for single-cell analysis to track single cells and even proteins and polymers during the formation of the aggregates. This way, it should be possible to understand the individual contribution of the motility systems, the role of signaling (cell–cell contact and chemotaxis), and the local contribution of the ECM. The outcomes of these studies may reveal conserved principles driving primitive cellular assemblies, the foundation of all developmental processes in biology.

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Y.Z. and A.D. contributed equally to the work.

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