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The mysterious nature of bacterial surface (gliding) motility: A focal adhesion-based mechanism in *Myxococcus xanthus*

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ABSTRACT

Motility of bacterial cells promotes a range of important physiological phenomena such as nutrient detection, harm avoidance, biofilm formation, and pathogenesis. While much research has been devoted to the mechanism of bacterial swimming in liquid via rotation of flagellar filaments, the mechanisms of bacterial translocation across solid surfaces are poorly understood, particularly when cells lack external appendages such as rotary flagella and/or retractile type IV pili. Under such limitations, diverse bacteria at the single-cell level are still able to "glide" across solid surfaces, exhibiting smooth translocation of the cell along its long axis. Though multiple gliding mechanisms have evolved in different bacterial classes, most remain poorly characterized. One exception is the gliding motility mechanism used by the Gram-negative social predatory bacterium *Myxococcus xanthus*. The available body of research suggests that *M. xanthus* gliding motility is mediated by trafficked multi-protein (Glt) cell envelope complexes, powered by proton-driven flagellar stator homologues (Agl). Through coupling to the substratum via polysaccharide slime, Agl–Glt assemblies can become fixed relative to the substratum, forming a focal adhesion site. Continued directional transport of slime-associated substratum-fixed Agl–Glt complexes would result in smooth forward movement of the cell. In this review, we have provided a comprehensive synthesis of the latest mechanistic and structural data for focal adhesion-mediated gliding motility in *M. xanthus*, with emphasis on the role of each Agl and Glt protein. Finally, we have also highlighted the possible connection between the motility complex and a new type of spore coat assembly system, suggesting that gliding and cell envelope synthetic complexes are evolutionarily linked.

Contents

1. Introduction 144
   1.1. Bacterial motility on surfaces 144
   1.2. *Myxococcus xanthus* 144
      1.2.1. *M. xanthus* social (S) motility 144
2. Myxobacterial gliding motility 145
   2.1. Focal adhesion complexes 145
   2.2. A-motility gliding and the bacterial cytoskeleton—MglA, MreB, and AglZ 145
   2.3. Cell polarity and reversals—MglB, RomR, and the Frz system 145
   2.4. The AglRQS molecular motor 146
3. The gliding transducer (Glt) complex 148
   3.1. Cytoplasmic components—GltI 148
   3.2. Inner-membrane components—GltG and GltJ 148

Abbreviations: IM, inner membrane; OM, outer membrane; OMV, outer-membrane vesicle; T4P, type IV pili; EPS, exopolysaccharide; S motility, social motility; A motility, adventurous motility; FA, focal adhesion; PMF, proton-motive force; Glt, gliding transducer; MASC, major spore coat polymer; TPR, tetratricopeptide repeat; ECM, extracellular matrix; Nfs, necessary for sporulation.

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1084-9521/

3.3. Periplasmic components—GltD, GltE, and GltF 148
3.4. Outer-membrane components—GltK, GltB, GltA, GltC, and GltH 150
3.5. Additional Glt components 150
3.6. The gliding motility track 150
4. The role of slime 151
5. Proposed gliding mechanism 151
6. Beyond gliding motility—Conclusions 151
6.1. Spore coat compaction—Role of the Nfs complex 151
6.2. The core complex—Evolution of gliding motility in the *Myxococcales* 152
6.3. Future perspectives 152
Acknowledgements 152
References 152

1. Introduction

The ability of numerous bacterial species to move and change location is a fundamental physiological characteristic that strongly promotes the survival of a given strain in biotic and abiotic environments. Bacterial motility can lead to the detection of food sources in liquid and solid settings, active avoidance of harmful stimuli, spore formation for strain preservation, and for pathogens, infection of host systems. Multiple methods of bacterial motility have arisen over evolutionary time, with each conferring different abilities to suit a particular environment and/or lifestyle. As such, motility systems and associated regulation systems are present in nearly all bacterial clades. Historically, bacterial motility has largely been investigated in the context of swimming in viscous environments through the rotation of flagellar appendages, while cell migration on surfaces has been much less studied. As such, this review will discuss bacterial motility on surfaces.

1.1. Bacterial motility on surfaces

Arguably, the most extensively-studied prokaryotic motility apparatus has been the bacterial flagellum, a rotary machine found in both Gram-negative and Gram-positive bacterial species. The rotation of flagella (which can be reversed) is powered by an inner (cellular) membrane (IM) motor energized via an ion gradient across the IM. Additional components reside in the peptidoglycan for both Gram-positive and Gram-negative cells, and also in the outer membrane (OM) for the latter. Flagella allow individual bacteria to swim and swarm collectively on moist surfaces [1–4], but as they have been extensively discussed in numerous review articles, they will not be discussed herein.

Often referred to as “twitching”, motility mediated by Type IV pili (T4P) has also been broadly studied. This type of surface motility involves the extension, adhesion, and retraction of an extruded polar filament in both Gram-negative and Gram-positive cells. As with the flagellum, multiple proteins in each subcellular compartment constitute the T4P machinery [5,6]. T4P have been adapted for roles in motility, virulence, acquisition of DNA, electrical conductance, secretion of protein substrates, biofilm formation, and attachment to a wide range of surfaces; however, as with flagella, T4P have also been extensively reviewed in the literature [6,7] and will not be discussed in detail herein.

Intriguingly, numerous bacteria have also been shown to move across surfaces in the absence of detectable appendages such as flagella and/or T4P [8,9]; this phenomenon has been broadly termed “gliding” motility and has been observed in a range of phylogenetically-distinct bacterial phyla and/or classes including the *Deltaproteobacteria*, *Cyanobacteria*, *Mollicutes*, and *Bacteroidetes* [10]. Incidentally, studies reveal that distinct gliding mechanisms appear to operate among these different types of bacteria [8,9]. Moreover, certain eukaryotic cell types such as the Apicomplexa move by yet an entirely different mechanism of gliding motility unrelated to any of the known bacterial mechanisms [11], further illustrating the diversity of gliding systems. In this review, we will focus on synthesizing current knowledge and recent advances towards understanding the mechanism of bacterial gliding motility in the Gram-negative bacterium *Myxococcus xanthus*.

1.2. Myxococcus xanthus

Much progress has recently been made regarding the elucidation of motility in the Gram-negative rod-shaped model gliding bacterium *M. xanthus* (order: *Myxococcales*; class: *Deltaproteobacteria*). *M. xanthus* as well as most other identified myxobacteria have been isolated from soils and are not known to be pathogenic. However, a novel tick-borne myxobacterium has recently been identified as the etiologic agent of epizootic bovine abortion (foothill abortion) in pregnant cattle [12,13], illustrating a largely unexplored potential for myxobacteria and disease.

M. xanthus is often referred to as a social bacterium as it displays coordinated behaviours of individual cells in a swarm group (Fig. 1A). When nutrients are scarce, the bacteria can engage in predatory behaviour, resulting in the killing of a range of Gram-positive and Gram-negative prey bacteria and saprophytic usage of the degradation products. In addition, under conditions of nutrient limitation, an alternative developmental cycle is initiated wherein cells aggregate to form fruiting body structures (Fig. 1B), which eventually mature to contain myxospores. Once nutrient conditions ameliorate, these myxospores have the capacity to germinate and form new vegetative cells [14].

1.2.1. M. xanthus *social (S) motility*

The collective motility of swarms, as well as the motility of single cells, are central to the aforementioned phenotypes, and are mediated by distinct motility systems. On soft surfaces (e.g. 0.5%

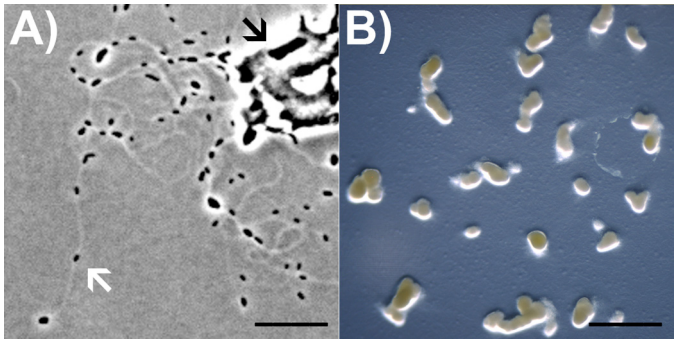

Fig. 1. (A) Micrograph of wild-type *M. xanthus* DZ2 population displaying both T4P-mediated S-motility of cell aggregates (black arrow) and Agl–Glt motor complex-mediated gliding A-motility of single cells on agar (1.5%), with some following a previously-deposited slime trail (white arrow). Scale bar: 50 μm. (B) Fruiting body formation after 72 h on starvation medium. Scale bar: 4 mm.

agar in the laboratory), *M. xanthus* cell aggregates (Fig. 1A) employ T4P-mediated movement to translocate in large cell groups, a process known as social (S) motility. This locomotion is similar to twitching motility studied in *Pseudomonas aeruginosa* and *Neisseria* species and involves the retraction of T4P [15,16]. However, in *M. xanthus* the T4P machinery cooperates with a secreted exopolysaccharide (EPS) that covers the cell and provides an attachment site for T4P from an adjacent cell; in this manner, cells are able to aggregate through collective binding of assembled T4P to neighbouring cells followed by pilus retraction [17–19]. A similar motility system has recently been elucidated for filamentous cyanobacteria, wherein the secretion of a polysaccharide plays a role in priming the substratum, but that the force for cell locomotion comes from the extrusion and retraction of T4P [20,21]; this is noteworthy as prior to this study, filamentous cyanobacteria were described as being "gliding" bacteria that used polysaccharide extrusion to propel the filaments forward. As such, filamentous cyanobacteria should probably be removed from discussion on "gliding" organisms.

M. xanthus EPS is likely produced via a Wzx/Wzy-dependent mechanism [22,23]; this involves translocation of undecaprenyl pyrophosphate-linked oligosaccharide repeat units from the cytoplasmic to the periplasmic leaflets of the IM by a Wzx flippase [24–27], followed by periplasmic polymerization of repeat units by Wzy [28–30], and ultimately export of the EPS polymer outside of the cell through a Wza translocon [31]. During S motility, EPS is believed to provide a matrix on adjacent cells to which extruded T4P can adhere [17]. Mutants deficient in EPS production are also deficient for S motility, supporting this link [32].

2. Myxobacterial gliding motility

While *M. xanthus* uses S motility to promote group movement on surfaces, single gliding cells are typically found at the swarm vanguard (Fig. 1A). Similar to flagellated swimming bacteria in liquid, which use periods of runs and tumbles to change cell orientation and advance towards nutritionally-favourable locales, *M. xanthus* on solid surfaces can employ single-cell gliding to explore new locales and change direction through reversal events. These cells leave behind trails (see Section 4) that can be followed by other single cells as well as rafts containing multiple cells (Fig. 1A). The mechanism for single-cell gliding is independent of the S-motility system. Instead, the process is mediated by so-called adventurous (A) motility in *M. xanthus*. Through a variety of mutagenesis screens, more than 40 genes have been implicated in *M. xanthus* A motility [33–35], many of which are discussed below (see Section 3).

2.1. Focal adhesion complexes

The overall mechanism of *M. xanthus* A motility was originally developed through work on AglZ, a cytoplasmic protein that is essential for single-cell gliding [36]. (The specific function of AglZ is discussed in Section 2.2). When a fluorescently-labelled variant of AglZ was tracked in live single cells, it was found to accumulate at the leading pole. In A-motile cells, AglZ clusters were also found to localize with a constant periodicity at sites that remained fixed relative to the underlying substratum; these clusters were subsequently dissociated upon reaching the lagging cell pole (Fig. 2A) [37]. These clusters were proposed to contain trafficking A-motility protein complexes that had strongly adhered to the underlying substratum. The net effect of rearward transport of substratum-fixed complexes would be the forward propulsion of the remainder of the bacterial cell body, thus providing a testable explanation for *M. xanthus* A-motility gliding (Fig. 2B) [37,38].

During *Myxococcus* gliding, cell–substrate interactions appear to involve specific adhesions, a conclusion supported by cell–cell collision studies in which forces required to re-engage a lost focus of adhesion were analyzed [39]. This finding was also supported via direct application of opposing loads to surface-attached beads with an optical trap, a device which uses a highly-focused laser to hold and move objects on a microscopic scale. Together, these findings indicated a strong adhesive attachment and elastic cell–substratum coupling [39] (Fig. 2B). Therefore, by analogy to adhesive eukaryotic motility complexes, we will refer to the adhered *Myxococcus* clusters as bacterial Focal Adhesions (FAs).

2.2. A-motility gliding and the bacterial cytoskeleton—MglA, MreB, and AglZ

Though early transposon mutagenesis screens identified several classes of mutants with different motility defects, only the *mglA* (mutual function for gliding protein A) gene was shown to be important for both *M. xanthus* single-cell gliding A motility and group S motility [40]. MglA is a cytoplasmic Ras-like GTPase [41–43] (Figs. 3 and 4), a class of proteins that have been extensively investigated in eukaryotes and shown to mediate cell migration, transport, and signal transduction [44–46]. When *mglA* is deleted, both A- and S-motility proteins display defective localization [41]. The role of MglA in S motility is not understood, but consistent with its importance for A motility, MglA-GTP was found to interact both with AglZ and MreB (bacterial actin) [47], which serves as an organizing factor for macromolecular complexes in bacteria such as those involved in peptidoglycan biosynthesis [48].

Independent of peptidoglycan assembly, the MglA–MreB interaction thus stimulates assembly of the gliding machinery at the leading cell pole, possibly via several protein interactions including the recruitment of AglZ. AglZ contains an unusually long coiled-coil structure (Fig. 3) which contributes to its propensity to oligomerize [36]. This extended coiled-coil domain (>320 residues in length) could serve as a cytosolic assembly platform for the motility complex (see Section 3.1). MglA–MreB interactions are also maintained at bacterial FA sites that mediate A-motility gliding [47], further illustrating their functional significance.

2.3. Cell polarity and reversals—MglB, RomR, and the Frz system

The localization of MglA-GTP at the leading cell pole is promoted by the exclusionary localization of its cognate GTPase-activating protein (GAP) MglB at the lagging cell pole, preventing MglA from binding at the same site [42,49]. Interestingly, despite the importance of MglA-GTP, a cognate guanine nucleotide exchange factor (GEF) has yet to be identified. Based on MglA and MglB partitioning between poles, a polarity axis is created inside of cells that directly affects the direction of A- (and S-) motility for a given cell (Fig. 2C). Importantly, the presence of MglB at the lagging cell pole is responsible for the spatial disassembly of the A-motility complex. In this process, MglB activates MglA-GTP hydrolysis, dissociating MglA from MreB, AglZ, and the rest of the motility complex [47]. Synchronous pole-switching by MglA and MglB results in an inverted polarity, causing a reversal event in which A-motile cells slow down, stop, and finally accelerate in the opposite direction [42,49,50] (Fig. 2C). Regulation of MglA/B pole-switching is mediated by the Frz (frizzy protein) signal transduction cascade, a chemosensory-like pathway involving a partially-resolved cascade of receptor-dependent phosphorylation events that controls reversal frequency and ultimately cell–cell coordination in groups [42,49–53] (Fig. 2C).

Recruitment of MglA to the leading cell pole is mediated by RomR, a protein essential for A (and S) motility and which also contains a two-component-type REC receiver domain (Fig. 3); the RomR REC domain contains the critical phosphorylation residues and could thus be directly phosphorylated by the Frz kinase and link MglA localization to signal transduction. The exact nature and

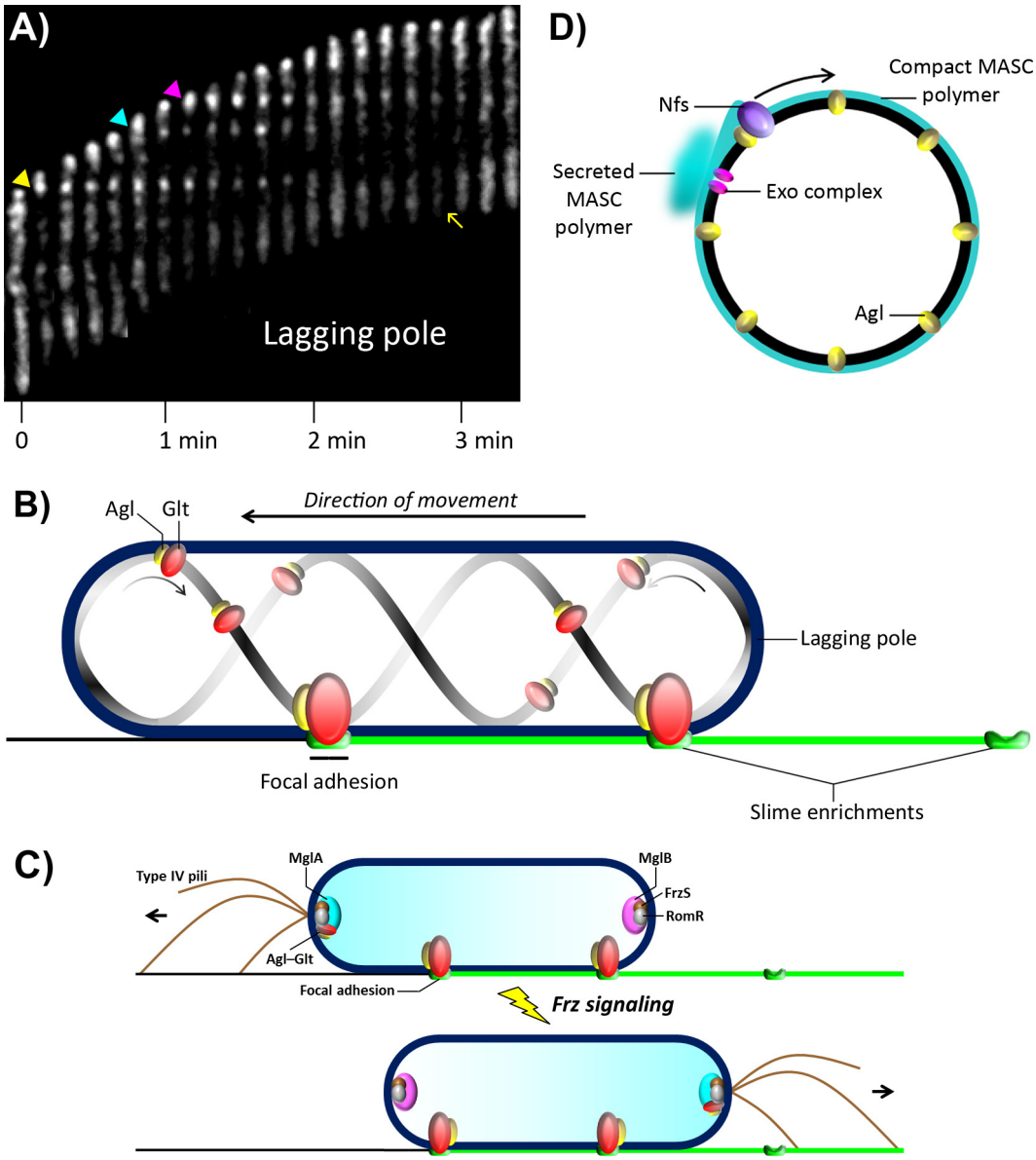

Fig. 2. (A) AglZ-YFP clusters in a gliding *M. xanthus* cell. Each coloured arrowhead denotes a new focal adhesion that has been formed, which remains immobile relative to the substratum. The cell is moving towards the top of the panel relative to the stationary focal adhesions. "Lagging pole" denotes the trailing end of the advancing cell in each timeframe. The initial focal adhesion (yellow arrow) is dispersed once it reaches the lagging cell pole. (B) Proposed focal adhesion model of gliding motility. The cytoplasmic, IM, and periplasmic components of the Agl–Glt motors move along a helical track within the cells. Once this trafficking complex engages the OM Glt components required for motility, the entire Glt apparatus becomes fixed to the substratum, forming a focal adhesion (FA). The engagement with the substratum is mediated by extracellular matrix "slime". Through continued trafficking of the now-fixed complex along the helical track, the cell body moves forward. Depending on the hardness of the substratum, Agl–Glt complexes can accumulate at FAs. (C) Mechanism of cell polarity and reversal. MglA localizes at the leading pole via recruitment by RomR, activating Agl–Glt-mediated A motility (aided by AglZ) and T4P-mediated S motility (aided by FrzS). Through co-localization with RomR and FrzS at the lagging pole, MglB presence impedes MglA accumulation at this site. Signaling through the Frz pathway results in pole-swapping by MglA and MglB, thus inverting the polarity of the cell body and activating both A and S motility in the opposite direction leading to a reversal. (D) Spore coat compaction model. MASC polymer is secreted outside of the spore via the Exo complex, remaining loosely associated with the spore surface. Following interaction of Nfs with MASC polymer, active spreading via mobile Agl–Nfs motor complexes results in MASC becoming tightly wound around the spore, ultimately producing a durable spore coat.

sequence of events that leads to switching is still not understood [54,55].

2.4. The AglRQS molecular motor

In order to drive rearward transport of substratum-fixed FAs, a motor component was needed [37]. A molecular motor is a device, powered by a source of energy, which generates a cyclic mechanical stroke; here, to move the motility complex directionally towards the lagging pole. While most molecular motors are powered via ATP hydrolysis, this is not the case for A-motility gliding; in A-motile cells, disruption of the proton motive force (PMF) resulted in a rapid and reversible cessation of gliding motility [56,57]. On substrata where the motility complex cannot attach, motor activity could be detected when beads attached to the *M. xanthus* surface were transported along the length of the cell body towards the rear pole; this process was also arrested upon disruption of the PMF [57]. Together, these results suggested that a proton channel-like motor was responsible for energizing the A-motility gliding process.

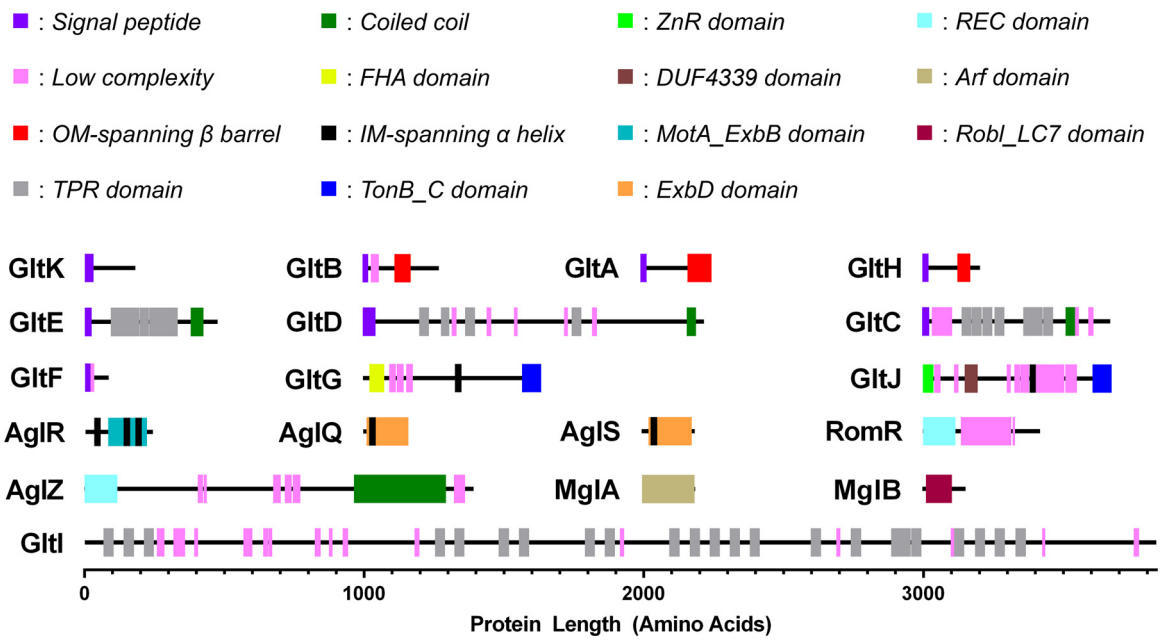

Fig. 3. Domain organization of Glt proteins. Protein domains were detected using profile hidden Markov model (HMMER) searching [98] via the Simple Modular Architecture Research Tool (SMART) [99], complemented with Phyre2 fold analysis [100]. All protein and domain lengths are depicted to scale. Consensus α-helical TMS were verified via TOPCONS analysis [101]. Detailed domain analysis of AglRQS was recently presented elsewhere [62]. TPR, tetratricopeptide repeat; FHA, forkhead-associated; TonB_C, C-terminus of TonB; ZnR, zinc ribbon; DUF, domain of unknown function; REC, CheY-homologous receiver domain; Arf, Arf-like small GTPase; RobI_LC7, roadblock/LC7.

PMF-driven motor systems are numerous in bacteria, such as MotAB which powers flagellar rotation [58], F_1F_O which energizes ATP synthesis [59], TolQR which triggers trans-envelope macromolecule transport [60], and ExbBD which drives import of iron siderophore molecules [61]. Through use of these sequences, a genome search of *M. xanthus* uncovered the *aglRQS* locus; AglR was identified as a homologue of TolQ/ExbB/MotA, with three α-helical transmembrane segments (TMS), whereas AglQ and AglS were found to be homologous to TolR/ExbB/MotB, each with only one TMS [57]. Disruption of these genes was found to compromise A motility, but not S motility; moreover, AglRQS was found to be enriched at FAs [34,57]. Furthermore, amino acid substitutions in AglRQS that prevent proton transit through the putative channel paralyze the motility complex [57,62]. Together, these results are all consistent with AglRQS motor activity [34,57,62].

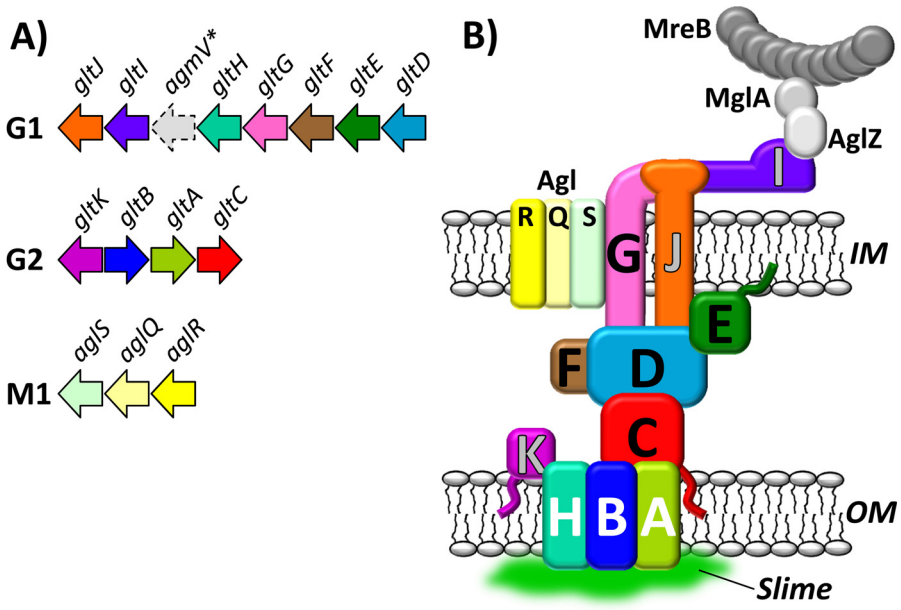

Fig. 4. The genes and proteins of the Glt apparatus. (A) Chromosomal organization of the G1, G2, and M1 clusters that encode the known components of the Agl–Glt gliding motility apparatus. The *agmV* locus (*), originally corresponding to *mxan_4864* and *mxan_4865* together, likely represents reading frames denoting pseudogenes that have been improperly annotated, and as such are not believed to encode components of the Glt complex [64]. (B) Schematic of the Agl–Glt complex architecture. Directly-interacting components have been represented as faithfully as possible based on reported findings (Table 1). Protein colours match their respective genes in panel (A). Proteins with black lettering denote "core complex" constituents, paralogues of which are found in both the Glt and Nfs systems. White lettering denotes proteins in addition to the core complex that are paralogous between the Glt and Nfs systems. Grey lettering denotes proteins that are unique to the Glt apparatus and as such likely represent gliding motility-specific factors.

3. The gliding transducer (Glt) complex

Of the >40 known proteins important for *M. xanthus* A motility [33,34,63], 11 were found to be structural components of a gliding machine and have co-evolved together as well as with the AglRQS motor. These 11 proteins are encoded across two gene clusters termed G1 and G2 (Fig. 4A) [62,64]. Most of these genes had already been randomly knocked out during various transposon mutagenesis screens; however, each investigation attributed a different nomenclature for the genes identified in their respective screen [34,35]. For example, despite these 11 co-evolved genes being part of the same apparatus (and for some the same operon), individual components had been given gene prefixes as varied as *cgl* (contact or conditional gliding defect), *agl* (adventurous gliding defect, class A), *agm* (adventurous gliding motility), *agn* (adventurous gliding), and *pgl* (partial gliding) (Table 1). To eliminate the confusion, and to unify the nomenclature based on a physiological function (rather than a qualitative anthropomorphism), the 12 genes coding for the A-motility apparatus were renamed *glt* (gliding transducer). As most identified *glt* genes were found to be paralogous to the *nfs* (necessary for sporulation) machinery required for compaction of the major spore coat (MASC) polymer in *M. xanthus* [65] (see Section 6.1), the *glt* suffixes were matched against their respective *nfs* paralogues (Table 1) [64].

3.1. Cytoplasmic components—GltI

At >400 kDa, GltI is at least three times larger than any other Glt protein in the motility complex and possesses upwards of 23 tetratricopeptide repeat (TPR) motifs; in general, these domains are overrepresented in the motility complex (Fig. 3). TPR motifs typically display 34-residue-long tracts that are minimally conserved around the consensus sequence W_4-L_7-G_8-Y_{11}-A_{20}-F_{24}-A_{27}-P_{32} [66]. Structurally, TPR domains adopt a solenoidal conformation via a pair of α-helical tracts of equal length. The occurrence of 3–16 TPR motifs in a tandem array confers a superhelical tertiary structure possessing right-handed helicity. The amphipathic channel through the TPR superhelix can then act as an interaction platform for the structurally-compatible motif of a target protein [66].

In addition to its high TPR domain content, the size and cytoplasmic localization of GltI would support a role as a nucleating scaffold/platform on which multiple copies of cytoplasmic and/or IM-spanning factors could be recruited (Fig. 4B). It has already been shown that as the hardness of the substratum increases, the relative size of fluorescent FA clusters increases, consistent with the concept of modulatable Agl–Glt content at FA sites [67]. Lack of GltI results in altered localization of AglZ and MglA away from FA sites, suggesting possible GltI–AglZ and GltI–MglA interactions [47,68]. Taken together, these data are consistent with the acquisition of *gltI* contributing exclusively to A-motility gliding (see Section 6.2).

3.2. Inner-membrane components—GltG and GltJ

Despite the availability of numerous methods for experimentally determining the topology of an integral IM protein [69], GltG and GltJ have yet to be characterized in this manner. However, topology prediction analyses for GltG and GltJ indicate a strong propensity for the presence of a single TMS in each, with the N-termini in the cytoplasm and the C-termini in the periplasm [64]. While each has a different N-terminal composition for its cytoplasmic domain, each possesses a TonB_C domain in the periplasm (Fig. 3); these domains are known to bind a conserved region known as a Ton-box on target proteins typically on the periplasmic side of the OM [70]. However, the periplasmic binding partners of GltG and GltJ remain unknown as no canonical Ton-boxes have been identified to date in any of the known Glt proteins. This indicates that there is either a novel Ton-box containing Glt protein remaining to be discovered, or that a non-standard TonB-box-like domain is present in one of the existing Glt components. Intriguingly, interaction of the TonB_C domain with its target Ton-box (in the context of nutrient transport across the OM into cells) is proposed to generate force via mechanical pulling to drive active transport [70]; this may provide a tantalizing analogy and framework for how force generated at the IM (via AglRQS) is transduced to the rest of the Glt apparatus. Consistent with this, AglR (the TolQ homologue) has been shown to interact directly with GltG, likely via TMS–TMS interactions [64]. Thus, it is through the interaction of GltG with AglR that mechanical force (via AglRQS proton translocation) could be transmitted to effect work on the remainder of the Glt complex.

Despite their similar C-terminal (periplasmic) domain arrangements, GltJ and GltG display distinct cytoplasmic domains. As part of its cytoplasmic architecture, GltJ possesses a predicted zinc ribbon (ZnR) domain (Fig. 3), one of the main families of folds classified as a zinc finger. ZnR domains are typically the least spatially complex type of zinc finger structure, and interact with a wide range of ligands, including DNA, RNA, and proteins [71]. Consequently, the binding target of the cytoplasmic domain of GltJ remains unknown, but could include cytoplasmic factors important for A motility such as GltI (Fig. 4B), especially since GltJ and GltI co-occur across various genomes [62].

As part of its cytoplasmic domain, GltG possesses a forkhead-associated (FHA) domain (Fig. 3); FHA motifs typically mediate interactions with phospho-peptides that have been modified with a phosphate by serine/threonine kinases [72]. This may indicate that, in the cytoplasm, GltG interacts with a phosphorylated cytoplasmic domain on GltI, GltJ, or some unknown additional protein that has a role in A motility. However, the kinase (if it exists) has yet to be discovered; this may be a difficult task since the *M. xanthus* genome encodes more than 100 Ser/Thr-type kinases, many of which display redundant functions [73].

3.3. Periplasmic components—GltD, GltE, and GltF

In some instances, the assignment of periplasmic localization may be due to ambiguity in the detected localization patterns of various proteins. For instance, GltD possesses a Type I signal peptide, which would suggest that it is translocated from the cytosol and across the IM via the Sec translocon, with the N-terminal signal peptide being cleaved by signal peptidase I (i.e. no post-cleavage modification with a lipid moiety to form a lipoprotein). This would result in soluble GltD in the periplasm. However, the determined subcellular localization for GltD has not been consistent. In the native *M. xanthus* host, Western immunoblotting of crude membranes compared with purified OM vesicles (OMVs) detected an abundance of GltD in crude membranes, and no signal from the OMV sample, suggesting localization at the periplasmic face of the IM [64]. Moreover, heterologous expression of NfsD (GltD paralogue) in an *Escherichia coli* background, followed by sucrose gradient density centrifugation revealed that the paralogue of GltD was enriched in IM-derived fractions [74]. However, two separate proteomics investigations carried out in the native *M. xanthus* background have revealed that GltD can be readily detected via mass spectrometry of OMVs and/or biotinylated OM samples from cells grown across multiple conditions (Table 1) [75,76]; in these investigations, a protein's presence in OMVs may denote localization in (or at) the OM, and/or in the bulk volume of the periplasm. Given the convincing data for GltD affinity for both IM- and OM-associated Glt factors, this would support a role for GltD in directly linking the proximal and distal membrane components of the Glt machinery (Fig. 4B). In support of this notion, GltD has been shown to localize at FAs in A-motile cells [56,64,68,77]. Moreover, when used as bait in a pull-down assay, whether through direct or indirect

Table 1
Glt components and their Nfs paralogues.

Gliding transducer (Glt) proteins									Necessary for sporulation (Nfs) proteins						
Gene name	Former name(s)	Locus tag	Genbank accession	Protein length (aa)	Detected in OM/OMVs[a] [Ref.]	Position in cell via fluorescence microscopy [Ref.]	Subcellular localization via fractionation[b] [Ref.]	Direct protein interaction partner [Ref.]	Glt paralogue	Paralogue locus tag	Genbank accession	Protein length (aa)	Position in cell/spore via fluorescence microscopy [Ref.]	Subcellular localization via fractionation[b] [Ref.]	Direct protein interaction partner [Ref.]
gltK	*cglC, agmO*	MXAN_2538	ABF91201	184	√ [76]	–	–	–	(No identified paralogue)	–	–	–	–	–	–
gltB	–	MXAN_2539	ABF91576	275	√ [75]	[79]	–	GltA, GltC [79]	*nfsB*	MXAN_3372	ABF88895	442	–	OM [74]	–
gltA	–	MXAN_2540	ABF92837	256	√ [75]	[79]	–	GltB, GltC [79]	*nfsA*	MXAN_3371	ABF92004	294	–	OM [74]	–
gltC	*agnA*	MXAN_2541	ABF87056	673	√ [75]	[79]	–	GltA, GltB [79]	*nfsC*	MXAN_3373	ABF88255	512	–	OM [74]	–
gltJ	*agmX*	MXAN_4862	ABF87224	674	–	–	–	–	(No identified paralogue)	–	–	–	–	–	–
gltI	*agmK*	MXAN_4863	ABF86482	3822	–	[47]	–	–	(No identified paralogue)	–	–	–	–	–	–
gltH	*cglE, agmV*	MXAN_4866	ABF86354	209	√ [75]	–	OM [64]	–	*nfsH*	MXAN_3378	ABF90837	200	–	–	–
gltG	*pglI*	MXAN_4867	ABF90228	640	–	–	IM [64]	AglR [64]	*nfsG*	MXAN_3377	ABF92113	682	–	IM [74]	AglR [77]
gltF	*cglF*	MXAN_4868	ABF88669	89	–	[64]	–	–	*nfsF*	MXAN_3376	ABF89690	96	–	–	–
gltE	*aglT*	MXAN_4869	ABF87666	478	–	–	IM [64]	–	*nfsE*	MXAN_3375	ABF89042	499	–	IM [74]	–
gltD	*agmU*	MXAN_4870	ABF89124	1218	√ [75]	[56,64,68,77]	C,P [68]	–	*nfsD*	MXAN_3374	ABF89098	1219	[77]	IM [74]	–
aglR	–	MXAN_6862	ABF90777	245	–	[56,67]	–	GltG [64,77] NfsG [77]	(Same as used by Glt system)						
aglQ	–	MXAN_6861	ABF88330	162	√ [75]	[57,77]	–	–	(Same as used by Glt system)						
aglS	–	MXAN_6860	ABF86057	194	–	–	–	–	(Same as used by Glt system)						

[a] Identified from a minimum of three unique peptides in at least two replicate mass spectrometric analyses.
[b] C, cytoplasm; IM, inner membrane; P, periplasm; OM, outer membrane.

interactions, numerous Glt proteins have been isolated [68]. This propensity for interaction with other proteins is undoubtedly due to its multiple TPR and coiled-coil domains (Fig. 3).

GltE possesses a Type II signal peptide; as such, once the signal peptide has been removed following passage across the IM, it is believed to be modified with a N-terminal lipid moiety to form a lipoprotein, exposed on the periplasmic leaflet of the IM. Via Western immunoblot, GltE can be detected in total-membrane samples, but not OMVs, from *M. xanthus* [64]. Through sucrose gradient membrane fractionation in *E. coli*, heterologously-expressed NfsE (GltE paralogue) is also enriched in IM fractions [74]. However, unlike GltD, GltE is not readily detected via mass spectrometry in biotinylated OM samples or OMVs. As such, these data reflect the presence of GltE, anchored via a lipid tail, in the periplasmic leaflet of the IM (Fig. 4B). Through its multiple TPR domains and C-terminal coiled-coil (Fig. 3), GltE also has a strong predisposition for interaction with other proteins; however, direct interacting partners have yet to be identified.

Of the identified Glt components to date, GltF is perhaps the most mysterious. It contains a Type I signal peptide (Fig. 3), and as such should be translocated across the IM to the periplasm. Consistent with these results, it has been detected in the soluble fraction of *M. xanthus* cell lysates [64]. However, a different group has reported that the A-motility defect of a Δ*gltF* mutant can be rescued by treating the cells with exogenously-added synthetic GltF peptide, and that this result supports GltF being tightly bound on the cell surface [78]. However, the latter report did not preclude the notion of the exogenous GltF getting past the OM. Moreover, despite 18 different mass spectrometry experiments (from different laboratories), testing a range of conditions and samples, including fractionated OMs and OMVs [75,76], only a single GltF peptide from a single experiment was detected; incidentally, this was derived from a sample representing the combined IM and OM fractions [76]. As such, it is more likely that GltF resides in the periplasm (Fig. 4B), as opposed to on the cell surface. Its exact role in A-motility gliding remains to be determined.

3.4. Outer-membrane components—GltK, GltB, GltA, GltC, and GltH

Of the various Glt proteins identified to date, GltA, GltB, GltC, GltH, and GltK have been assigned as OM components based on results of signal peptide prediction, cell envelope fractionation experiments and/or their unambiguous detection in outer membrane vesicles [64,75,76,79]. GltA, GltB, and GltH are all predicted to contain OmpA-like folds, and as such are believed to span the OM via beta-barrel architecture (Fig. 4B). Interestingly, GltA and GltB interact with and stabilize each other, so if one is lacking from the OM, so is the other [79]. In other bacteria, OmpA (and related proteins) have been shown to function in numerous roles, including in cell adhesion, as a receptor for bacteriophage, and as a contributor to biofilm formation [80]. Though the exact roles of GltA, GltB, and GltH remain to be characterized, it is tempting to speculate that one or more may mediate adhesion due to their OmpA-like folds.

In addition to each other, GltA and GltB also interact with and stabilize GltC, a soluble protein that is robustly detected in OM fractions and OMVs, likely through association with GltA/B (Fig. 4B) [75,76,79]. The propensity for GltC to interact with other proteins is high, in particular due to its numerous TPR motifs (Fig. 2). As such, GltC is an ideal candidate protein for coupling the cytoplasmic-IM-periplasmic module to an OM module to complete the connection of the AglRQS-powered trans-envelope Glt assembly/complex to the substratum.

The role of GltK remains unclear. However, it is evident that it is important for A motility. It is worth noting that the paralogous Nfs system for sporulation does not encode a protein similar to GltK (see Section 6.1). Protease protection assays reveal that GltK is located on the periplasmic face of the OM [79] (Fig. 4B). Unfortunately, no identifiable domains can be detected based on its amino acid sequence (Fig. 2). *De novo* tertiary structure modelling (S.T. Islam and T. Mignot, unpublished) displays loose similarities with existing pilotin structures [81]; this may suggest a role for GltK in facilitating the insertion of particular proteins into the OM. In support of this idea, GltK has been shown to stimulate the integration of GltA and GltB into the OM [79].

In A-motile cells, GltA, GltB, (and GltC) were shown to localize at FA sites; however, when this recruitment to FAs was inhibited, instead of in punctate spots, GltA and GltB (and presumably GltC) displayed diffuse localization in the OM [79]. This would suggest that, unlike the cytoplasmic-IM-periplasmic Glt assembly, the OM module does not traffic by itself around the cell periphery in the absence of surface coupling; instead, numerous copies of the OM module could be waiting in the OM to become engaged by the remainder of the Glt machinery, thus forming a FA. It is worth noting that GltA, GltB, GltC, and GltK are all encoded in the G2 cluster, whereas all other Glt proteins are encoded by the G1 cluster (Fig. 4A).

3.5. Additional Glt components

In addition to the 11 *glt* genes and their translated products already discussed, up to 30 other genes have been implicated in A-motility gliding through mutagenesis screens [33–35]. Many of these hits may be in genes that are either involved in the regulation of A motility or that impact key physiological functions. However, others could encode thus far unconfirmed structural factors of the Glt complex. Clean genetic deletions for these genes as well as single-cell motility analysis would aid greatly in confirming their importance for gliding. Detailed phylogenomics data to support co-occurrence would also be highly informative if genes are confirmed as important for A motility.

The proteins CglB and CglD are two such proteins that are important for A-motility gliding [33,78,82]. Both are associated with the OM and are predicted lipoproteins, and both could be transferred between cells via surface–surface contact and Tra system-dependent OM fusion and exchange [83]. CglB is essential for A-motility gliding; however, it is not encoded in either the G1 or G2 gene cluster (described above), nor is its role in A-motility known. CglD is also not encoded in the G1 or G2 clusters. Though insertion-mutant cells lacking CglD were initially reported to be completely defective for A-motility [33], a subsequent investigation working with a clean genetic deletion mutant has reported A-motility in a subset of single cells [78]. As such, the role of CglD in gliding is unclear.

3.6. The gliding motility track

To explain the periodicity of AglZ-YFP fluorescence first observed (Fig. 2A), a rigid helical filament running the length of the cell body was proposed [37]. MreB in the cytoplasm was initially considered a track candidate as immunofluorescence experiments suggested that it forms helical structures in the cytoplasm with similar periodicity to AglZ-YFP [41]. However, the concept of a continuous cytoplasmic MreB helical filament is a matter of debate because different high-resolution microscopy studies suggest that MreB can form patches, short filaments, and long filaments [84]. Thus, MreB may form a cytosolic scaffold for protein assembly rather than an elongated track for motor movements. In addition, the predicted IM topology of AglRQS [62] is consistent with the generation of a power stroke in the periplasm (and not in the cytoplasm); this would be consistent with the similarities of AglRQS

with TolQR (discussed in Section 2.4), as the latter is responsible for powering envelope processes in the periplasm [60].

Therefore the motility track remains to be identified. It may indeed be helical in shape because recent single-particle tracking of AglR over short distances supports helical motion of the motor [67] (Fig. 2B). Moreover, deconvolution imaging of *M. xanthus* cells expressing a fluorescently-tagged variant of GltD suggested motor organization along a closed-loop helical structure in the periplasm [56]. However, deconvolution processing of fluorescence imaging data is highly susceptible to artifacts, with a relevant example being the interpretation in earlier studies of MreB fluorescence patterns and the proposition of large helical structures in the cell.

4. The role of slime

Following A motility on hard agar surfaces, gliding *Myxococcus* species have long been known to leave behind a trail of extracellular matrix (ECM) "slime" that can be followed by subsequent cells (Fig. 1A) [85]. As such, slime has been proposed to serve an important role for swarm populations, potentially coordinating collective bacterial behaviours on a single-cell level via stigmergic regulation [86]. Previously, slime secretion from the cell poles had been proposed as a theoretical source of propulsion for A motility [35,87]; however, this model has been definitively ruled out based on extensive experimentation [37,41,47,55–57,64,67,68,79,88,89].

Slime trails are composed of an as-yet-uncharacterized secreted polysaccharide, along with material from previous cells including OM vesicles and tubes, thus representing a very heterogeneous microenvironment [90]. When slime trails (deposited by gliding cells) were imaged on functionalized glass surfaces using specialized high-contrast optical microscopy (Wet–SEEC), different slime thicknesses were detected at different positions in the trail [88]. Single-cell gliding speed measurements of the trail-depositing bacteria indicated that the cells moved at slower speeds over trail tracts corresponding to thicker slime; this is consistent with slime serving an adhesive, rather than a propulsive, role in gliding motility [88].

These patches of slime enrichment were found to occur at regular intervals matching the spatial periodicity of the above-described FAs. In addition, the positions of fluorescent FAs in gliding cells were matched with patches of enriched slime. Finally, fluorescently-stained polysaccharide enrichments were found to traffic towards the lagging cell pole at speeds equivalent to FAs. Although the structure of slime and the presence of possible receptors have yet to be elucidated, the results described in this section suggest that slime is deposited underneath cells by the Agl–Glt motility complexes as they become fixed to the substratum [88] (Fig. 2B). Thus, FAs may transport and deposit their own "glue", allowing adherence for gliding motility on various types of surfaces.

5. Proposed gliding mechanism

Based on findings from the various investigations described (see Sections 2 and 3), the following model for *M. xanthus* single-cell gliding motility can be proposed. Through Frz-dependent signalling, polarity of the cell is set, with MglA at the leading pole (Fig. 2C). There, MglA becomes connected to the cell cytoskeleton (MreB) as well as the Agl–Glt apparatus (through AglZ and GltI). GltI serves as a nucleation scaffold to recruit additional AglRQS as well as IM-spanning GltG and GltJ. On the periplasmic face of the IM, GltD, GltE, and GltF interact with GltGJ, to form a subcomplex (Fig. 4B). The AglRQS motor then moves the complex directionally towards the lagging pole. Displacement of this Agl–Glt subcomplex is constrained through association with a potentially helical track in the cell (Fig. 2B).

Once this trafficked subcomplex (containing cytoplasmic, IM, and periplasmic components) engages the OM Glt module (composed of GltA, GltB, GltC, GltK, and GltH), the entire Glt apparatus becomes fixed to the substratum, forming a FA site (Fig. 4B); this FA remains stationary relative to the substratum (Fig. 2A). Coupling of Agl–Glt to the substratum is mediated by ECM slime (Figs. 2B and 4B). The net rearward transport along the cell length of adhesive slime creates propulsion (Fig. 2B). Once the lagging pole reaches the FA site, the FA is dissociated, allowing the cell to continue advancing through the generation of a new FA at the leading pole to repeat the cycle of directional slime transport (Fig. 2A). Ultimately, this gliding motility mechanism may represent an example of a polysaccharide (slime) transport system that evolved to become a motility apparatus (see Section 6.2).

While the model is attractive in principle, it must be tested by addressing several major questions:

(i) If FAs are indeed formed by an integral, trans-envelope complex, how does this complex dynamically span the peptidoglycan? Indeed, peptidoglycan forms a continuous sacculus in the periplasm and any contact between the IM and OM must therefore occur through this meshwork [91]. It is possible that the motility complex digests peptidoglycan locally if, for example, it is associated with a cell wall hydrolase. Alternatively, transient contact could also occur between the IM and OM parts of the complex if the motility motor provokes conformational changes in the periplasmic proteins.
(ii) What is the motility track and how does the motility motor traffic directionally?
(iii) How does the motility complex adhere to the substratum and how is this process dynamically regulated to uncouple FA–substratum interactions as the cell moves away from the adhesion point?

Answering these questions will likely not only explain the motility mechanism but also improve our understanding of general dynamic processes of the bacterial cell envelope.

6. Beyond gliding motility—Conclusions

6.1. Spore coat compaction—Role of the Nfs complex

Among characterized Gram-negative bacterial species to date, the ability to form desiccation- and heat-resistant spores is a rarity. While the mechanism of *M. xanthus* sporulation is not well understood, it differs markedly from the well-characterized Gram-positive processes giving rise to aerial filament spores in *Streptomyces* species [92] and endospores in *Bacillus* species [93]. Importantly, in *M. xanthus* the reorganization process from vegetative rod cell to spherical spore does not require septation (unlike in *Streptomyces* and *Bacillus*). In addition, assembly of the protective spore coat takes place outside of the OM [94].

The MASC polymer is produced and secreted by the Exo machinery [94], another Wzx/Wzy-dependent assembly pathway [22] requiring a Wza translocon [31] for transport of the respective polysaccharide across the spore envelope to the outer surface. Once outside the spore, the Nfs machinery is required to correctly distribute and compact the secreted MASC polymer (Fig. 2D), a critical step for spore maturation [77,94].

Remarkably, each identified component of the *M. xanthus* Nfs machinery is paralogous to a known Glt machinery component [62,64,65,77] (Table 1). The Nfs system also shares the same phylogenomic distribution as the Glt system, suggesting recent evolutionary divergence [62]. Similar to the dynamic nature of Glt complexes in vegetative cells, Nfs complexes were found to

colocalize with MASC polymer and to move around the periphery of spores in an AglRQS motor-dependent manner [62,77] (Fig. 2D).

Given the requirement of Nfs proteins for spore coat compaction, and through analogy with the Agl–Glt complex-dependent transport of slime polysaccharide in A-motile vegetative cells (described above), it has been proposed that Nfs complex rotation around the spore periphery serves to stretch out and wind the spore coat polysaccharide around the spore surface, similar to winding loose yarn into a tight ball [62] (Fig. 2D).

6.2. The core complex—Evolution of gliding motility in the Myxococcales

Despite the restricted phylogenomic detection of the 12 Glt protein sequences in only certain members of the order *Myxococcales*, a "core complex" subset (analogous to GltCDEFG–AglRQS) was found to be encoded by a divergent range of bacteria, with a large representation in the class *Gammaproteobacteria* [62,64]. Though the original function of the "core complex" remains unknown, it could also be linked to polysaccharides and therefore, both the Glt and Nfs systems may have originated from an ancestral system for polysaccharide capsule assembly [62]. As such, the paralogous nature of the Nfs and Glt systems may provide a starting point for evolutionary studies of similar macromolecular protein complexes [62]. For the Glt and Nfs machineries, this would have involved accessorization of the "core complex" with additional interacting components or modules to yield the current configuration required to effect gliding motility and MASC polymer compaction, respectively (Fig. 2B and D).

Within the core complex, the requirement to maintain the GltG-like component may stem from its ability to interact directly with the motor component AglR (Fig. 4B), as seen for both GltG and NfsG [64,77]. The capacity to directly interact with a molecular motor would provide a strong selective pressure to maintain a GltG-like protein as part of the conserved apparatus. The occurrence of GltC paralogues in the core complex is also intriguing, for as previously discussed with the Glt system, GltC is a candidate for coupling the OM Glt proteins to the remainder of the Glt apparatus. However, GltA and GltB were necessary for stabilizing GltC [79], so this may indicate for the "core complex" that there are other OM-spanning components/modules that may serve to stabilize the C component in their respective backgrounds. Alternatively, it may suggest that GltC specifically evolved to have a high affinity for GltA and GltB in the context of the gliding apparatus.

Between the closely-related Glt and Nfs machineries, in addition to the Agl–CDEFG "core complex" constituents, paralogues are also encoded for the OM-spanning HBA components. As discussed for the Glt system, this may be the site of interaction with ECM slime on the cell surface; for the Nfs system, this could represent the interface for coupling of the MASC-spreading machinery of the spore to the MASC polymer itself.

As GltK (present on the periplasmic side of the OM) has been shown to stimulate insertion of β-barrel proteins GltA and GltB into the OM [79], it is puzzling that the Nfs system does not similarly encode a paralogous "NfsK" protein to similarly stimulate OM-insertion of NfsA and NfsB. One explanation is that there are potentially key differences between the vegetative cell and spore envelopes, such as the presence/absence of peptidoglycan, which for spores may preclude the need for a pilotin in NfsA and NfsB insertion. Another possibility is that GltK represents a gliding motility-specific differentiation between the Glt and Nfs machineries. Similarly, GltI and GltJ are also exclusive to the gliding motility system [62] (Fig. 4B, Table 1). Since GltI and GltJ have extended cytoplasmic domains, they may connect the gliding machinery with cytoplasmic structures. It is worth noting that the Nfs system does not require the cytoplasmic factors MglA, AglZ, or MreB, and therefore this cytoplasmic module may be linked to gliding specificity (Fig. 4B).

6.3. Future perspectives

The gene content and phylogenetic distribution of the "core complex" suggest that a class of ancestral transporters may have been accessorized over evolutionary time. This in turn gave rise to the current-day systems specialized for spore coat compaction (Nfs) and single-cell gliding motility (Glt) in *M. xanthus* [62]. Future studies on the functional specialization of the "core complex" will yield important insights into the evolution of these macromolecular structures. For example, one additional *agl*-like and two additional *glt*-like gene clusters are present in the *M. xanthus* genome; however, when deleted, none have an effect on motility or sporulation [64]. Thus, determining the function of these *agl*/*glt* homologues could shed light on the general function of these systems.

It has long been known that members of the phylum *Bacteroidetes* (such as *Flavobacterium* species) also use gliding motility to translocate across surfaces [8]. Intriguingly though, a different machinery for single-cell gliding is used by *Bacteroidetes*, compared to the Agl–Glt system of *M. xanthus*. Recently, in addition to potential surface adhesins [95], gliding by *Bacteroidetes* has been linked to a novel rotary motor [96], as well as a type IX secretion system implicated in the buildup of the microcrystalline S-layer on the surface of these cells [97]. Intriguingly, this may indicate yet another instance of a macromolecular complex involved in cell envelope biogenesis that has also become accessorized to play another role. If so, it would represent the convergent evolution of distinct macromolecular assemblies to effect the common function of bacterial gliding motility.

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References

[1] Minamino T, Imada K. The bacterial flagellar motor and its structural diversity. Trends Microbiol 2015;23:267–74, http://dx.doi.org/10.1016/j.tim.2014.12.011.
[2] Belas R. Biofilms, flagella, and mechanosensing of surfaces by bacteria. Trends Microbiol 2014;22:517–27, http://dx.doi.org/10.1016/j.tim.2014.05.002.
[3] Chaban BL, Hughes V, Beeby M. The flagellum in bacterial pathogens: for motility and a whole lot more. Semin Cell Dev Biol 2015, http://dx.doi.org/10.1016/j.semcdb.2015.10.032 (Manuscript number YSCDB-D-15-00111).
[4] Wolgemuth C. Flagellar motility of the pathogenic spirochetes. Semin Cell Dev Biol 2015, http://dx.doi.org/10.1016/j.semcdb.2015.10.015 (Manuscript Number: YSCDB-D-15-00113).
[5] Trokter M, Felisberto-Rodrigues C, Christie PJ, Waksman G. Recent advances in the structural and molecular biology of type IV secretion systems. Curr Opin Struct Biol 2014;27:16–23, http://dx.doi.org/10.1016/j.sbi.2014.02.006.
[6] Melville S, Craig L, Type IV. Pili in Gram-positive bacteria. Microbiol Mol Biol Rev 2013;77:323–41, http://dx.doi.org/10.1128/mmbr.00063-12.
[7] Giltner CL, Nguyen Y, Burrows LL. Type IV pilin proteins: versatile molecular modules. Microbiol Mol Biol Rev 2012;76:740–72, http://dx.doi.org/10.1128/mmbr.00035-12.

[8] McBride MJ. Bacterial gliding motility: multiple mechanisms for cell movement over surfaces. Annu Rev Microbiol 2001;55:49–75, http://dx.doi.org/10.1146/annurev.micro.55.1.49.

[9] Jarrell KF, McBride MJ. The surprisingly diverse ways that prokaryotes move. Nat Rev Microbiol 2008;6:466–76, http://dx.doi.org/10.1038/nrmicro1900.

[10] Burchard RP. Gliding motility of prokaryotes: ultrastructure, physiology, and genetics. Annu Rev Microbiol 1981;35:497–529, http://dx.doi.org/10.1146/annurev.mi.35.100181.002433.

[11] Heintzelman MB. Gliding motility in apicomplexan parasites. Semin Cell Dev Biol 2015, http://dx.doi.org/10.1016/j.semcdb.2015.09.020.

[12] King DP, Chen C-I, Blanchard MT, Aldridge BM, Anderson M, Walker R, et al. Molecular identification of a novel deltaproteobacterium as the etiologic agent of epizootic bovine abortion (foothill abortion). J Clin Microbiol 2005;43:604–9, http://dx.doi.org/10.1128/jcm.43.2.604-609.2005.

[13] Chen C-I, King DP, Blanchard MT, Hall MR, Aldridge BM, Bowen L, et al. Identification of the etiologic agent of epizootic bovine abortion in field-collected *Ornithodoros coriaceus* Koch ticks. Vet Microbiol 2007;120:320–7, http://dx.doi.org/10.1016/j.vetmic.2006.10.036.

[14] Dworkin M, Voelz H. The formation and germination of microcysts in *Myxococcus xanthus*. J Gen Microbiol 1962;28:81–5, http://dx.doi.org/10.1099/00221287-28-1-81.

[15] Burrows LL. *Pseudomonas aeruginosa* twitching motility: type IV pili in action. Annu Rev Microbiol 2012;66:493–520, http://dx.doi.org/10.1146/annurev-micro-092611-150055.

[16] Miller F, Lécuyer H, Join-Lambert O, Bourdoulous S, Marullo S, Nassif X, et al. *Neisseria meningitidis* colonization of the brain endothelium and cerebrospinal fluid invasion. Cell Microbiol 2013;15:512–9, http://dx.doi.org/10.1111/cmi.12082.

[17] Li Y, Sun H, Ma X, Lu A, Lux R, Zusman D, et al. Extracellular polysaccharides mediate pilus retraction during social motility of *Myxococcus xanthus*. Proc Natl Acad Sci USA 2003;100:5443–8, http://dx.doi.org/10.1073/pnas.0836639100.

[18] Yang Z, Lux R, Hu W, Hu C, Shi W. PilA localization affects extracellular polysaccharide production and fruiting body formation in *Myxococcus xanthus*. Mol Microbiol 2010;76:1500–13, http://dx.doi.org/10.1111/j.1365-2958.2010.07180.x.

[19] Black WP, Xu Q, Yang Z, Type IV. pili function upstream of the Dif chemotaxis pathway in *Myxococcus xanthus* EPS regulation. Mol Microbiol 2006;61:447–56, http://dx.doi.org/10.1111/j.1365-2958.2006.05230.x.

[20] Khayatan B, Meeks JC, Risser DD. Evidence that a modified type IV pilus-like system powers gliding motility and polysaccharide secretion in filamentous cyanobacteria. Mol Microbiol 2015 (advance online), http://dx.doi.org/10.1111/mmi.13205.

[21] Wilde A, Mullineaux CW. Motility in cyanobacteria: polysaccharide tracks and Type IV pilus motors. Mol Microbiol 2015 (advance online), http://dx.doi.org/10.1111/mmi.13242.

[22] Islam ST, Lam JS. Synthesis of bacterial polysaccharides via the Wzx/Wzy-dependent pathway. Can J Microbiol 2014;60:697–716, http://dx.doi.org/10.1139/cjm-2014-0595.

[23] Lu A, Cho K, Black WP, Duan X-y, Lux R, Yang Z, et al. Exopolysaccharide biosynthesis genes required for social motility in *Myxococcus xanthus*. Mol Microbiol 2005;55:206–20, http://dx.doi.org/10.1111/j.1365-2958.2004.04369.x.

[24] Islam ST, Taylor VL, Qi M, Lam JS. Membrane topology mapping of the O-antigen flippase (Wzx), polymerase (Wzy), and ligase (WaaL) from *Pseudomonas aeruginosa* PAO1 reveals novel domain architectures. mBio 2010;1, e00189–10, http://dx.doi.org/10.1128/mBio.00189-10.

[25] Islam ST, Fieldhouse RJ, Anderson EM, Taylor VL, Keates RAB, Ford RC, et al. A cationic lumen in the Wzx flippase mediates anionic O-antigen subunit translocation in *Pseudomonas aeruginosa* PAO1. Mol Microbiol 2012;84:1165–76, http://dx.doi.org/10.1111/j.1365-2958.2012.08084.x.

[26] Islam ST, Eckford PDW, Jones ML, Nugent T, Bear CE, Vogel C, et al. Proton-dependent gating and proton uptake by Wzx support O-antigen-subunit antiport across the bacterial inner membrane. mBio 2013;4, e00678–13, http://dx.doi.org/10.1128/mBio.00678-13.

[27] Islam ST, Lam JS. Wzx flippase-mediated membrane translocation of sugar polymer precursors in bacteria. Environ Microbiol 2013;15:1001–15, http://dx.doi.org/10.1111/j.1462-2920.2012.02890.x.

[28] Islam ST, Gold AC, Taylor VL, Anderson EM, Ford RC, Lam JS. Dual conserved periplasmic loops possess essential charge characteristics that support a catch-and-release mechanism of O-antigen polymerization by Wzy in *Pseudomonas aeruginosa* PAO1. J Biol Chem 2011;286:20600–5, http://dx.doi.org/10.1074/jbc.C110.204651.

[29] Islam ST, Huszczynski SM, Nugent T, Gold AC, Lam JS. Conserved-residue mutations in Wzy affect O-antigen polymerization and Wzz-mediated chain-length regulation in *Pseudomonas aeruginosa* PAO1. Sci Rep 2013;3:3441, http://dx.doi.org/10.1038/srep03441.

[30] Taylor VL, Udaskin ML, Islam ST, Lam JS. The D3 bacteriophage α-polymerase-inhibitor (Iap) peptide disrupts O-antigen biosynthesis through mimicry of the chain length regulator Wzz in *Pseudomonas aeruginosa*. J Bacteriol 2013;195:4735–41, http://dx.doi.org/10.1128/jb.00903-13.

[31] Nickerson NN, Mainprize IL, Hampton L, Jones ML, Naismith JH, Whitfield C. Trapped translocation intermediates establish the route for export of capsular polysaccharides across *Escherichia coli* outer membranes. Proc Natl Acad Sci USA 2014;111:8203–8, http://dx.doi.org/10.1073/pnas.1400341111.

[32] Shimkets LJ. Correlation of energy-dependent cell cohesion with social motility in *Myxococcus xanthus*. J Bacteriol 1986;166:837–41.

[33] Hodgkin J, Kaiser D. Cell-to-cell stimulation of movement in nonmotile mutants of *Myxococcus*. Proc Natl Acad Sci USA 1977;74:2938–42.

[34] Youderian P, Burke N, White DJ, Hartzell PL. Identification of genes required for adventurous gliding motility in *Myxococcus xanthus* with the transposable element mariner. Mol Microbiol 2003;49:555–70, http://dx.doi.org/10.1046/j.1365-2958.2003.03582.x.

[35] Yu R, Kaiser D. Gliding motility polarized slime secretion. Mol Microbiol 2007;63:454–67, http://dx.doi.org/10.1111/j.1365-2958.2006.05536.x.

[36] Yang R, Bartle S, Otto R, Stassinopoulos A, Rogers M, Plamann L, et al. AglZ is a filament-forming coiled-coil protein required for adventurous gliding motility of *Myxococcus xanthus*. J Bacteriol 2004;186:6168–78, http://dx.doi.org/10.1128/jb.186.18.6168-6178.2004.

[37] Mignot T, Shaevitz JW, Hartzell PL, Zusman DR. Evidence that focal adhesion complexes power bacterial gliding motility. Science 2007;315:853–6, http://dx.doi.org/10.1126/science.1137223.

[38] Mignot T. The elusive engine in *Myxococcus xanthus* gliding motility. Cell Mol Life Sci 2007;64:2733–45, http://dx.doi.org/10.1007/s00018-007-7176-x.

[39] Balagam R, Litwin DB, Czerwinski F, Sun M, Kaplan HB, Shaevitz JW, et al. *Myxococcus xanthus* gliding motors are elastically coupled to the substrate as predicted by the focal adhesion model of gliding motility. PLoS Comput Biol 2014;10:e1003619, http://dx.doi.org/10.1371/journal.pcbi.1003619.

[40] Hodgkin J, Kaiser D. Genetics of gliding motility in *Myxococcus xanthus* (myxobacterales): two gene systems control movement. Mol Gen Genet 1979;171:177–91, http://dx.doi.org/10.1007/bf00270004.

[41] Mauriello EMF, Mouhamar F, Nan B, Ducret A, Dai D, Zusman DR, et al. Bacterial motility complexes require the actin-like protein, MreB and the Ras homologue, MglA. EMBO J 2010;29:315–26, http://dx.doi.org/10.1038/emboj.2009356.

[42] Zhang Y, Franco M, Ducret A, Mignot T. A bacterial Ras-like small GTP-binding protein and its cognate GAP establish a dynamic spatial polarity axis to control directed motility. PLOS Biol 2010;8:e1000430, http://dx.doi.org/10.1371/journal.pbio.1000430.

[43] Miertzschke M, Koerner C, Vetter IR, Keilberg D, Hot E, Leonardy S, et al. Structural analysis of the Ras-like G protein MglA and its cognate GAP MglB and implications for bacterial polarity. EMBO J 2011;30:4185–97, http://dx.doi.org/10.1038/emboj.2011.291.

[44] Hartzell P, Kaiser D. Function of MglA, a 22-kilodalton protein essential for gliding in *Myxococcus xanthus*. J Bacteriol 1991;173:7615–24.

[45] Hartzell PL. Complementation of sporulation and motility defects in a prokaryote by a eukaryotic GTPase. Proc Natl Acad Sci USA 1997;94:9881–6.

[46] Charest Pascale G, Firtel Richard A. Big roles for small GTPases in the control of directed cell movement. Biochem J 2007;401:377–90, http://dx.doi.org/10.1042/bj20061432.

[47] Treuner-Lange A, Macia E, Guzzo M, Hot E, Faure L, Jakobczak B, et al. The small G-protein MglA connects to the MreB actin cytoskeleton at bacterial focal adhesions. J Cell Biol 2015;210:243–56, http://dx.doi.org/10.1083/jcb.201412047.

[48] Randich AM, Brun YV. Molecular mechanisms for the evolution of bacterial morphologies and growth modes. Front Microbiol 2015:6, http://dx.doi.org/10.3389/fmicb.2015.00580.

[49] Leonardy S, Miertzschke M, Bulyha I, Sperling E, Wittinghofer A, Søgaard-Andersen L. Regulation of dynamic polarity switching in bacteria by a Ras-like G-protein and its cognate GAP. EMBO J 2010;29:2276–89, http://dx.doi.org/10.1038/emboj.2010.114.

[50] Bulyha I, Hot E, Huntley S, Søgaard-Andersen L. GTPases in bacterial cell polarity and signalling. Curr Opin Microbiol 2011;14:726–33, http://dx.doi.org/10.1016/j.mib.2011.09.001.

[51] Guzzo M, Agrebi R, Espinosa L, Baronian G, Molle V, Mauriello EMF, et al. Evolution and design governing signal precision and amplification in a bacterial chemosensory pathway. PLOS Genet 2015;11:e1005460, http://dx.doi.org/10.1371/journal.pgen.1005460.

[52] Mignot T, Merlie JP, Zusman DR. Regulated pole-to-pole oscillations of a bacterial gliding motility protein. Science 2005;310:855–7, http://dx.doi.org/10.1126/science.1119052.

[53] Moine A, Agrebi R, Espinosa L, Kirby JR, Zusman DR, Mignot T, et al. Functional organization of a multimodular bacterial chemosensory apparatus. PLOS Genet 2014;10:e1004164, http://dx.doi.org/10.1371/journal.pgen.1004164.

[54] Keilberg D, Wuichet K, Drescher F, Søgaard-Andersen L. A response regulator interfaces between the Frz chemosensory system and the MglA/MglB GTPase/GAP module to regulate polarity in *Myxococcus xanthus*. PLOS Genet 2012;8:e1002951, http://dx.doi.org/10.1371/journal.pgen.1002951.

[55] Zhang Y, Guzzo M, Ducret A, Li Y-Z, Mignot T. A dynamic response regulator protein modulates G-protein-dependent polarity in the bacterium

Myxococcus xanthus. PLOS Genet 2012;8:e1002872, http://dx.doi.org/10.1371/journal.pgen.1002872.

[56] Nan B, Chen J, Neu JC, Berry RM, Oster G, Zusman DR. Myxobacteria gliding motility requires cytoskeleton rotation powered by proton motive force. Proc Natl Acad Sci USA 2011;108:2498–503, http://dx.doi.org/10.1073/pnas.1018556108.

[57] Sun M, Wartel M, Cascales E, Shaevitz JW, Mignot T. Motor-driven intracellular transport powers bacterial gliding motility. Proc Natl Acad Sci USA 2011;108:7559–64, http://dx.doi.org/10.1073/pnas.1101101108.

[58] Guttenplan SB, Kearns DB. Regulation of flagellar motility during biofilm formation. FEMS Microbiol Rev 2013;37:849–71, http://dx.doi.org/10.1111/1574-6976.12018.

[59] Fillingame RH, Steed PR. Half channels mediating H^+ transport and the mechanism of gating in the Fo sector of *Escherichia coli* F_1F_o ATP synthase. Biochim Biophys Acta 2014;1837:1063–8, http://dx.doi.org/10.1016/j.bbabio.2014.03.005.

[60] Cascales E, Lloubès R, Sturgis JN. The TolQ–TolR proteins energize TolA and share homologies with the flagellar motor proteins MotA–MotB. Mol Microbiol 2001;42:795–807, http://dx.doi.org/10.1046/j.1365-2958.2001.02673.x.

[61] Lloubès R, Goemaere E, Zhang X, Cascales E, Duché D. Energetics of colicin import revealed by genetic cross-complementation between the Tol and Ton systems. Biochem Soc Trans 2012;40:1480–5, http://dx.doi.org/10.1042/bst20120181.

[62] Agrebi R, Wartel M, Brochier-Armanet C, Mignot T. An evolutionary link between capsular biogenesis and surface motility in bacteria. Nat Rev Microbiol 2015;13:318–26, http://dx.doi.org/10.1038/nrmicro3431.

[63] Stephens K, Hartzell P, Kaiser D. Gliding motility in *Myxococcus xanthus*: mgl locus, RNA, and predicted protein products. J Bacteriol 1989;171:819–30.

[64] Luciano J, Agrebi R, Le Gall AV, Wartel M, Fiegna F, Ducret A, et al. Emergence and modular evolution of a novel motility machinery in bacteria. PLOS Genet 2011;7:e1002268, http://dx.doi.org/10.1371/journal.pgen.1002268.

[65] Müller F-D, Treuner-Lange A, Heider J, Huntley SM, Higgs PI. Global transcriptome analysis of spore formation in *Myxococcus xanthus* reveals a locus necessary for cell differentiation. BMC Genomics 2010;11:264, http://dx.doi.org/10.1186/1471-2164-11-264.

[66] Cerveny L, Straskova A, Dankova V, Hartlova A, Ceckova M, Staud F, et al. Tetratricopeptide repeat motifs in the world of bacterial pathogens: role in virulence mechanisms. Infect Immun 2013;81:629–35, http://dx.doi.org/10.1128/iai.01035-12.

[67] Nan B, Bandaria JN, Moghtaderi A, Sun I-H, Yildiz A, Zusman DR. Flagella stator homologs function as motors for myxobacterial gliding motility by moving in helical trajectories. Proc Natl Acad Sci USA 2013;110:E1508–13, http://dx.doi.org/10.1073/pnas.1219982110.

[68] Nan B, Mauriello EMF, Sun I-H, Wong A, Zusman DR. A multi-protein complex from *Myxococcus xanthus* required for bacterial gliding motility. Mol Microbiol 2010;76:1539–54, http://dx.doi.org/10.1111/j.1365-2958.2010.07184.x.

[69] Islam ST, Lam JS. Topological mapping methods for α-helical bacterial membrane proteins—an update and a guide. Microbiol Open 2013;2:350–64, http://dx.doi.org/10.1002/mbo3.72.

[70] Shultis DD, Purdy MD, Banchs CN, Wiener MC. Outer membrane active transport: structure of the BtuB:TonB complex. Science 2006;312:1396–9, http://dx.doi.org/10.1126/science.1127694.

[71] Krishna SS, Majumdar I, Grishin NV. Structural classification of zinc fingers: survey and summary. Nucleic Acids Res 2003;31:532–50, http://dx.doi.org/10.1093/nar/gkg161.

[72] Pennell S, Westcott S, Ortiz-Lombardía M, Patel D, Li J, Nott TJ, et al. Structural and functional analysis of phosphothreonine-dependent FHA domain interactions. Structure 2010;18:1587–95, http://dx.doi.org/10.1016/j.str.2010.09.014.

[73] Pérez J, Castañeda-García A, Jenke-Kodama H, Müller R, Muñoz-Dorado J. Eukaryotic-like protein kinases in the prokaryotes and the myxobacterial kinome. Proc Natl Acad Sci USA 2008;105:15950–5, http://dx.doi.org/10.1073/pnas.0806851105.

[74] Holkenbrink C, Hoiczyk E, Kahnt J, Higgs PI. Synthesis and assembly of a novel glycan layer in *Myxococcus xanthus* spores. J Biol Chem 2014, http://dx.doi.org/10.1074/jbc.M114.595504.

[75] Kahnt Jr, Aguiluz K, Koch Jr, Treuner-Lange A, Konovalova A, Huntley S, et al. Profiling the outer membrane proteome during growth and development of the social bacterium *Myxococcus xanthus* by selective biotinylation and analyses of outer membrane vesicles. J Proteome Res 2010;9:5197–208, http://dx.doi.org/10.1021/pr1004983.

[76] Berleman JE, Allen S, Danielewicz M, Remis JP, Gorur A, Cunha J, et al. The lethal cargo of *Myxococcus xanthus* outer membrane vesicles. Front Microbiol 2014;5, http://dx.doi.org/10.3389/fmicb.2014.00474.

[77] Wartel M, Ducret A, Thutupalli S, Czerwinski F, Le Gall A-V, Mauriello EMF, et al. A versatile class of cell surface directional motors gives rise to gliding motility and sporulation in *Myxococcus xanthus*. PLOS Biol 2013;11:e1001728, http://dx.doi.org/10.1371/journal.pbio.1001728.

[78] Pathak DT, Wall D. Identification of the cglC, cglD, cglE, and cglF genes and their role in cell contact-dependent gliding motility in *Myxococcus xanthus*. J Bacteriol 2012;194:1940–9, http://dx.doi.org/10.1128/jb.00055-12.

[79] Jakobczak B, Keilberg D, Wuichet K, Søgaard-Andersen L. Contact- and protein transfer-dependent stimulation of assembly of the gliding motility machinery in *Myxococcus xanthus*. PLOS Genet 2015;11:e1005341, http://dx.doi.org/10.1371/journal.pgen.1005341.

[80] Smith SGJ, Mahon V, Lambert MA, Fagan RP. A molecular Swiss army knife: OmpA structure, function and expression. FEMS Microbiol Lett 2007;273:1–11, http://dx.doi.org/10.1111/j.1574-6968.2007.00778.x.

[81] Koo J, Burrows LL, Lynne Howell P. Decoding the roles of pilotins and accessory proteins in secretin escort services. FEMS Microbiol Lett 2012;328:1–12, http://dx.doi.org/10.1111/j.1574-6968.2011.02464.x.

[82] Rodriguez AM, Spormann AM. Genetic and molecular analysis of cglB, a gene essential for single-cell gliding in *Myxococcus xanthus*. J Bacteriol 1999;181:4381–90.

[83] Cao P, Dey A, Vassallo CN, Wall D. How myxobacteria cooperate. J Mol Biol 2015 (advance online), http://dx.doi.org/10.1016/j.jmb.2015.07.022 (in press).

[84] Errington J. Bacterial morphogenesis and the enigmatic MreB helix. Nat Rev Microbiol 2015;13:241–8, http://dx.doi.org/10.1038/nrmicro3398.

[85] Burchard RP. Trail following by gliding bacteria. J Bacteriol 1982;152:495–501.

[86] Gloag ES, Turnbull L, Whitchurch CB. Bacterial stigmergy: an organising principle of multicellular collective behaviours of bacteria. Scientifica (Cairo) 2015;2015:8, http://dx.doi.org/10.1155/2015/387342.

[87] Wolgemuth C, Hoiczyk E, Kaiser D, Oster G. How myxobacteria glide. Curr Biol 2002;12:369–77, http://dx.doi.org/10.1016/S0960-9822(02)00716-9.

[88] Ducret A, Valignat M-P, Mouhamar F, Mignot T, Theodoly O. Wet-surface-enhanced ellipsometric contrast microscopy identifies slime as a major adhesion factor during bacterial surface motility. Proc Natl Acad Sci USA 2012;109:10036–41, http://dx.doi.org/10.1073/pnas.1120979109.

[89] Nan B, Bandaria JN, Guo KY, Fan X, Moghtaderi A, Yildiz A, et al. The polarity of myxobacterial gliding is regulated by direct interactions between the gliding motors and the Ras homolog MglA. Proc Natl Acad Sci USA 2015;112:E186–93, http://dx.doi.org/10.1073/pnas.1421073112.

[90] Ducret A, Fleuchot B, Bergam P, Mignot T. Direct live imaging of cell–cell protein transfer by transient outer membrane fusion in *Myxococcus xanthus*. eLife 2013;2:e00868, http://dx.doi.org/10.7554/eLife.00868.

[91] Bui NK, Gray J, Schwarz H, Schumann P, Blanot D, Vollmer W. The peptidoglycan sacculus of *Myxococcus xanthus* has unusual structural features and is degraded during glycerol-induced myxospore development. J Bacteriol 2009;191:494–505, http://dx.doi.org/10.1128/jb.00608-08.

[92] McCormick JR, Flärdh K. Signals and regulators that govern *Streptomyces* development. FEMS Microbiol Rev 2012;36:206–31, http://dx.doi.org/10.1111/j.1574-6976.2011.00317.x.

[93] Tan IS, Ramamurthi KS. Spore formation in *Bacillus subtilis*. Environ Microbiol Rep 2014;6:212–25, http://dx.doi.org/10.1111/1758-2229.12130.

[94] Müller FD, Schink CW, Hoiczyk E, Cserti E, Higgs PI. Spore formation in *Myxococcus xanthus* is tied to cytoskeleton functions and polysaccharide spore coat deposition. Mol Microbiol 2012;83:486–505, http://dx.doi.org/10.1111/j.1365-2958.2011.07944.x.

[95] Nan B, McBride Mark J, Chen J, Zusman David R, Oster G. Bacteria that glide with helical tracks. Curr Biol 2014;24:R169–73, http://dx.doi.org/10.1016/j.cub.2013.12.034.

[96] Shrivastava A, Lele Pushkar P, Berg Howard C. A rotary motor drives Flavobacterium gliding. Curr Biol 2015;25:338–41, http://dx.doi.org/10.1016/j.cub.2014.11.045.

[97] Narita Y, Sato K, Yukitake H, Shoji M, Nakane D, Nagano K, et al. Lack of a surface layer in *Tannerella forsythia* mutants deficient in the type IX secretion system. Microbiology 2014;160:2295–303, http://dx.doi.org/10.1099/mic.0.080192-0.

[98] Finn RD, Clements J, Eddy SR. HMMER web server: interactive sequence similarity searching. Nucleic Acids Res 2011;39:W29–37, http://dx.doi.org/10.1093/nar/gkr367.

[99] Letunic I, Doerks T, Bork P. SMART: recent updates, new developments and status in 2015. Nucleic Acids Res 2015;43:D257–60, http://dx.doi.org/10.1093/nar/gku949.

[100] Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJE. The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc 2015;10:845–58, http://dx.doi.org/10.1038/nprot.2015.053.

[101] Tsirigos KD, Peters C, Shu N, Käll L, Elofsson A. The TOPCONS web server for consensus prediction of membrane protein topology and signal peptides. Nucleic Acids Res 2015, http://dx.doi.org/10.1093/nar/gkv485.