2.4. Concluding remarks

The bacterial flagellum was historically viewed as an apparatus that enables motility. New research has expanded that view by identifying a role for the flagella in surface sensing and other related phenomena. As discussed, several challenges exist in determining the molecular mechanisms by which flagella trigger the transition from planktonic to surface-associated states. Advances in genetic engineering, microscopy, and mechanical stimulation techniques will be necessary to address some of those challenges.

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3. Gliding motility of the social bacterium *Myxococcus xanthus*

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3.1. Status

Bacterial gliding motility refers to the smooth movements of cells on solid surfaces unaided by flagella or pili. Gliding movements in divergent bacterial groups rely on distinct mechanisms. In the past two decades, gliding motility has become a gold mine for the discovery of novel molecular mechanisms. In fact, the protein complexes driving gliding in *M. xanthus*, *Flavobacterium johnsoniae* and mycoplasmas all represent new types of molecular machineries [11]. The gliding of *M. xanthus*, a rod-shaped biofilm-forming bacterium, is arguably the best studied, because most, if not all, of the components in the gliding complex have already been identified.

About 20 proteins form the core gliding complex of *M. xanthus*, among which a three-component proton channel in the inner membrane functions as the motor (figure 2). Other motor-associated components reside in four different compartments of the cell: the cytoplasm, inner membrane, periplasm and outer membrane [12]. The gliding motors of *M. xanthus* transport partially assembled gliding complexes (dynamic complexes) rapidly along helical tracks. Current evidence suggests that filaments of the bacterial actin homolog MreB provides the platforms for the assembly of motor complexes and might serve as the tracks for their helical motion [13–15]. At the sites where cells contact the substrate surfaces, dynamic complexes assemble with additional motor-associated proteins to form force-generating complexes that span the whole cell envelope [12, 16, 17]. Probably due to resistance from the cell wall, fully assembled gliding complexes reduce their velocity, aggregate and appear nearly static with respect to the substratum (static complexes) [12, 14, 16]. Through either deforming the cell surfaces or directly binding with the substratum, these static complexes exert force between the helical track and the substratum, and drive a corkscrew-like motion of the helical track. As a result, the cell also moves forward like a corkscrew (figure 2) [12]. After transient stalls, static complexes quickly disassemble and resume rapid motion [16].

The motor of *M. xanthus* gliding is remarkably versatile. The motor connects to other cellular machineries and facilitates multiple functions beyond motility. For example, the gliding motor carries a secretion system to deposit polysaccharides on the surfaces of developing spores during *M. xanthus* sporulation [18]. Another unexpected feature of *M. xanthus* gliding is its connection with the synthesis of the peptidoglycan (PG) cell wall through MreB. While moving along MreB filaments, gliding motors also transport MreB [13]. As MreB coordinates the synthesis of the PG cell wall by the rod complex, the major PG synthesis machinery for cell elongation, its transport by the gliding motors affects the distribution and activity of the cell wall synthesis machineries and plays an important role in *de novo* establishment of the cell's rod shape [13, 19]. Interestingly, rod complexes also drive MreB filaments to rotate circumferentially around the long axis of the cell with nm s^{-1} velocities, which is two orders of magnitude slower than the helical transportation of MreB by the gliding motors [13]. It is still unclear if gliding motility and cell wall synthesis are coupled to each other and how MreB accommodates these two functions with distinct velocities and trajectories.

3.2. Current and future challenges

Under regular fluorescence microscopy, most of the gliding-related proteins, including the subunits of the motor, localize diffusively and display rather chaotic movements [13, 14]. Such chaos reflects the sum of fluorescence signals from individual molecules that switch between different behaviors, such as stationary, diffusion and directed motion [13, 16]. Due to this fluid nature, it is technically difficult to dissect the assembly of the gliding complexes. While one could presume that the stationary molecules are assembled into the static complexes, functions of the molecules that undergo diffusion and directed motion remain to be investigated. Most importantly, it is still unclear how the static complexes transmit the proton motive force from the inner membrane to the cell surface. Another challenge for understanding the assembly

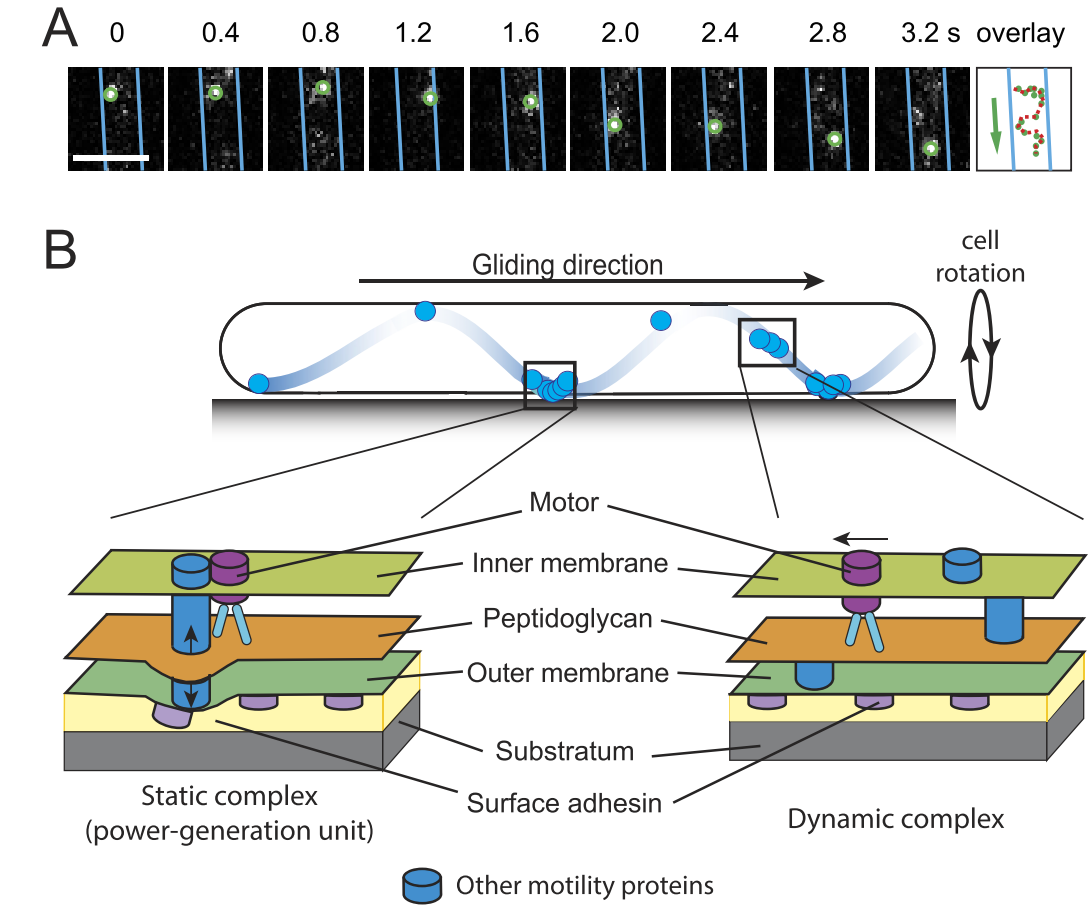

Figure 2. The gliding motility of *M. xanthus*. (A) 2D trajectory of a single gliding motor was recorded at 200 ms intervals. The cell boundaries were marked with blue lines. The overlay shows the positions of the motor in consecutive frames. Scale bar, 1 μm. Adapted from reference [16]. (B) A schematic model for *M. xanthus* gliding. Motors carrying incomplete gliding complexes move rapidly along helical paths but do not generate propulsion (dynamic complexes). These motors stall and become nearly static relative to the substratum when they assemble into complete gliding machineries with other motor-associated proteins at the ventral side of the cell (static complexes). The static complexes exert force against putative outer membrane adhesins. As the adhesins slide, the cell moves forward and the cell body rotates. Adapted with permission from reference [17].

of the gliding complexes stems from the complexity of the machinery itself. Whereas mutagenesis of the gliding-related genes and pairwise colocalization of the components in the gliding complex have provided important information on the assembly process [20], it is challenging to dissect the dynamic interactions among 20 different proteins.

M. xanthus gliding used to be considered as the motility for cells that move as individuals. However, mutants lacking gliding motility are usually not able to form mature biofilms (i.e. fruiting bodies), which is a multicellular process. In addition, the localization and dynamic behaviors of gliding-related proteins are regulated by external mechanical cues, such as substrate stiffness (and potentially the physical contacts with neighboring cells) [16]. Thus, gliding might be part of the mechanism by which cells sense their environment and colony mates. The critical roles of gliding in biofilms remain to be understood.

3.3. Advances in science and technology to meet challenges

Single-particle tracking is a technology that allows the collection of rich data on protein dynamics in live cells with unprecedented spatial and temporal resolutions (figure 2). These data reveal features not available from regular fluorescence imaging. For example, single-particle tracking is able to record complex dynamic behaviors, such as different subpopulations of the same protein moving in different modes [13, 16]. The current limit of this technique lies in relatively short trajectories of particles due to the short life time of individual fluorescence tags. Most analyses performed to date have been limited to mean-squared displacement, which is not an ideal parameter for analysing short trajectories. Furthermore, these methods emphasize generic modes of motion, such as Brownian diffusion, anomalous diffusion and directed motion. New methodology development in both experimentation and data analysis is needed to dissect more intricate processes expected in gliding, for instance, the transition of a molecule from one state to another.

Despite the latest advances in microscopy, it remains impossible to simultaneously track a large number of proteins that are typically involved in *M. xanthus* gliding [12]. Thus, experimental data

only represent fragmented snapshots of the system, which do not readily lead to coherent mechanistic understanding. Mathematical modeling is a powerful tool for studying the gliding complexes from a systems perspective. Mathematical models can weave fragmented data with basic laws of physics and chemistry, which could suggest mechanistic frameworks and inspire new experiments. A previous mechanochemical model, for example, has successfully brought many critical features of *M. xanthus* gliding under a coherent framework, such as the helical motion of motors, the formation of static force-generating complexes, the rotation of the cell body, the gliding velocity and even the sensitivity of motor clustering to substrate stiffness [14]. Building on new experimental observations, future modeling efforts will play a key role in understanding gliding motility by bridging the gap between complex biological observations and their underlying mechanisms.

3.4. Concluding remarks

The machineries of bacterial gliding motility are brand-new additions to the collection of force-generating protein complexes. The behaviors of gliding-related proteins in *M. xanthus* suggest a novel surface-sensing mechanism. Studying such a gliding system offers a rare opportunity to understand a fluid machinery that switches between a chaotic, non-functional form and an organized, force-generating form. Understanding the *M. xanthus* gliding complexes, especially the mechanisms of their assembly and force generation, will advance our knowledge far beyond motility itself. Studying gliding will also provide new insights in biofilm formation from the aspect of individual cells. Building upon new experimental techniques and modeling approaches, we expect major breakthroughs in the near future in the research of gliding in *M. xanthus* and many other organisms.

Acknowledgments

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4. Dynamics and mechanics of type IV pili

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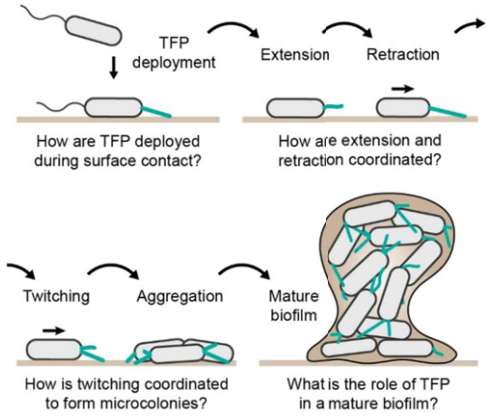

Figure 3. Known and hypothesized contribution of TFP and twitching during different stages of biofilm formation. TFP must rapidly deploy upon surface contact. Rounds of extension and retraction drive twitching motility, which ultimately lead to the formation of cellular aggregates. These microcolonies eventually mature into large, 3D biofilm in which the presence and potential function of TFP remain unclear.

4.1. Status

Adhesion and motility are crucial ingredients in the initial steps of biofilm formation. Adhesion allows cells to stay on the surface to grow into biofilms, while motility promotes aggregation and surface encounters. Adhesins play a central role in establishing stable attachment when transitioning from swimming to sessile states. In particular, protein polymers that extend from the cell surface called pili promote rapid adhesion upon surface contact. One class of such filaments called type IV pili (TFP) are essential in initiating biofilm formation in *P. aeruginosa* (*Pa*). In addition to adhesion, single *Pa* cells extend and retract TFP to generate traction and displacements on a surface, thus driving a motility mode known as twitching. *Pa* twitches to explore surfaces and to aggregate into microcolonies that eventually mature into biofilms (figure 3).

TFP are dynamic: they extend over several micrometers and actively retract all within seconds, generating forces up to 100 pN. The motor proteins PilB and PilT function respectively as polymerase and depolymerase at the base of the pilus by shuttling single PilA monomer subunits between the inner membrane and the filament. Successive rounds of extension, attachment and retraction power twitching. This mode of motility is slow compared to flagella-mediated swimming (a few micrometers per minute compared to several micrometers per second for swimming) but allows single cells to move while remaining on a surface.

Pa optimizes TFP movement by synchronizing retraction with contact of the pilus tip with the surface (figure 4 top), efficiently converting chemical energy into movement [21]. This suggests that