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NOTES

The Motors Powering A-Motility in *Myxococcus xanthus* Are Distributed along the Cell Body[▽]

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Two models have been proposed to explain the adventurous gliding motility of *Myxococcus xanthus*: (i) polar secretion of slime and (ii) an unknown motor that uses cell surface adhesion complexes that form periodic attachments along the cell length. Gliding movements of the leading poles of cephalexin-treated filamentous cells were observed but not equivalent movements of the lagging poles. This demonstrates that the adventurous-motility motors are not confined to the rear of the cell.

The gram-negative bacterium *Myxococcus xanthus* glides on surfaces using two independent propulsive engines: (i) S (social)-motility, which is similar to twitching motility in *Pseudomonas aeruginosa* (4), is driven by extension, adhesion, and retraction of polar type IV pili (3); and (ii) A (adventurous)-motility, which is driven by an uncharacterized engine hypothesized to be associated with slime secretion (8). In 1924, Jahn proposed that the A-motility motor was powered by extrusion and hydration of slime (2). Recently, Wolgemuth et al. showed that a slime extrusion engine could theoretically produce enough force to drive a bacterium at the observed speed (7). This model was consistent with the observation that in a *Phormidium* sp., a gliding cyanobacterium, the rates of slime secretion and cell movements were similar (1). Furthermore, putative nozzles for slime secretion were observed clustered at the cell poles (8), suggesting that if slime extrusion powered A-motility, the A-motility engines should be located at the cell posterior, pushing cells forward.

Recently, an A-motility protein labeled with yellow fluorescent protein, AglZ-YFP, was used to track protein complexes in living cells as cells moved forward and in reverse (5). In moving cells, AglZ-YFP was found to be associated with transient adhesion complexes that remained at fixed positions relative to the substratum as cells moved forward. Interestingly, the periodic spacing of the AglZ clusters was similar to the helical period of MreB in *Escherichia coli* and *Bacillus subtilis*, which suggests that these clusters may be associated with the bacterial cytoskeleton. On the basis of these observations, Mignot et al. (5) proposed that an uncharacterized protein motor attaches to bacterial "focal adhesion complexes" to propel the cell. An important aspect of this model is that the propulsion forces are distributed periodically along the bacterial axis and are not focused primarily to the posterior of the cells as proposed in the slime extrusion hypothesis.

In this study, we sought to distinguish between distributed A-motility motors and posterior motors. The location of the A-motility motors should help us distinguish between the motility models. The slime extrusion mechanism is unlikely to utilize motors distributed along the cell length, because slime secretion is localized mostly at the cell poles (8). Moreover, if slime propulsion motors were distributed, they would require tilted nozzles along the cell body that could change their direction of tilt at the moments of cell reversal or to switch between two populations of such tilted nozzles. Either would require an extremely complicated mechanism. In contrast, propulsion using the observed substrate-fixed focal adhesions requires adhesion points approximately equally distributed along the cell body, that is, a distributed engine.

Sun et al. (6) addressed the issue of rear- versus distributed-force generation by measuring the velocity of cells as cells became elongated (filamentous) following treatment with nonlethal concentrations of the antibiotic cephalexin. $A^+ S^-$ mutant cells (i.e., cells with only the A-motility motor) moved at a constant speed regardless of cell length. In contrast, the $A^- S^+$ cells (i.e., cells with only the S-motility motor) slowed dramatically as they become longer. This finding is consistent with the A-motility motor being distributed and the S-motility motor being polar. However, it is still possible that when they become filamentous, $A^+ S^-$ mutant cells acquire stronger engines, for example, because they secrete more slime. Additionally, speed may depend nonlinearly on the motor force, as observed for other molecular motors. For example, if the A-motility engine is very strong, it may operate in the regime where the cell speed is nearly constant regardless of the cell length. In this case, the speed is not limited by the friction force but instead is limited by the processivity of the motor itself, for example, by the slime secretion rate.

Using filamentous cells treated as described by Sun et al. (6), we sought additional evidence regarding the distribution of the A-motility motor. For our studies, we used strains derived from wild-type strain DZ2: the DZ2 Δ*pilA* and DZ2 Δ*pilA* AglZ-YFP mutants (5). The cells were grown to mid-exponential phase in rich medium, plated on hard agar containing 1/2-
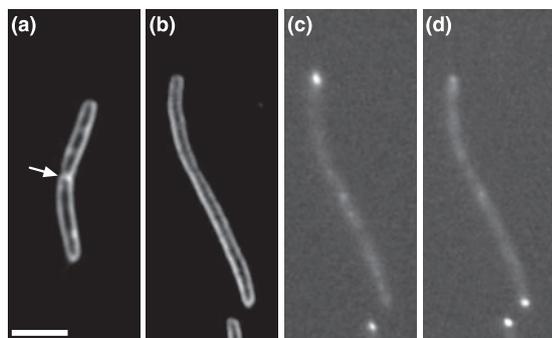

FIG. 1. Analysis of cephalexin-treated *M. xanthus* filaments for septation and cytoplasmic continuity. (a) A septum (arrow) between a dividing non-cephalexin-treated cell stained with FM4-64; (b) a typical cephalexin-treated 20-μm-long cell; (c and d) the same cell as that in panels a and b, showing localization of AglZ-YFP as the cell reversed (images were taken at 1-min intervals). These images indicate polarization along the whole cell and the continuity of the cytoplasm. Bar, 3 μm.

FIG. 2. Two time-lapse series of cephalexin-treated cells moving on hard agar stained with FM4-64. (a) First cell, with images taken at 100-s time intervals; (b) second cell, with images taken at 40-s time intervals. Empty arrows indicate the rear ends of the cells; solid white arrows indicate the front ends. Bar on first frame of panel b, 3 μm. The movies are published at our website (http://mcb.berkeley.edu/faculty/BMB/zusmand.html).

diluted CTT medium (1.5% agar, 0.5% Casitone, 10 mM Tris, 8 mM $MgSO_4$, 1 mM KPO_4), and covered with a coverslip. The cells were then treated with cephalexin at a 100 μM concentration starting approximately 6 hours before the imaging was done and continuing during the imaging, as described previously (6). The cells were imaged by fluorescence microscopy as described previously (5).

To confirm that our cephalexin-treated cells did not have septa, we stained cells with a membrane dye, FM4-64 (Invitrogen), which can clearly stain septa in nontreated cells; one such cell is shown in Fig. 1a. The vast majority of the cephalexin-treated cells did not have septa, although there were occasional exceptions, but no more than one septum per 100 cells. To further confirm the continuity of the cytoplasm in the filamentous cells, we monitored the localization of AglZ-YFP. Previous studies showed that AglZ-YFP is localized initially to the front of a cell; as the cell reverses, AglZ-YFP relocalizes to the opposite pole (5). Similar results were found with motile AglZ-YFP-containing filamentous cells (Fig. 1c and d). This result demonstrates the continuity of the cytoplasm and that the filaments do not contain barriers to the movement of AglZ complexes or nodes that may function like cell poles.

Figure 2 shows some typical results of our cell motility observations. These images are frames from time-lapse movies available at our website (http://mcb.berkeley.edu/faculty/BMB/zusmand.html). They show filamentous cells stained with the membrane dye FM4-64 visualized by fluorescence microscopy. We observed that in these cells, the anterior portions of cells moved forward using their A-motility motors and that the posterior portions lagged behind or did not move. Since elastic energy stored in sharp folds can potentially affect motility, we selected cells in which the curvature and the number of folds of the cell body remained constant; for these cells, elastic forces could not affect the motion. The cells also did not change their lengths: the first cell measured 20.6 ± 0.1 μm (Fig. 2a) and the second one measured 13.15 ± 0.1 μm (Fig. 2b) throughout the duration of the experiment. These movies show that since the A-motility engine provides the only driving force, it must
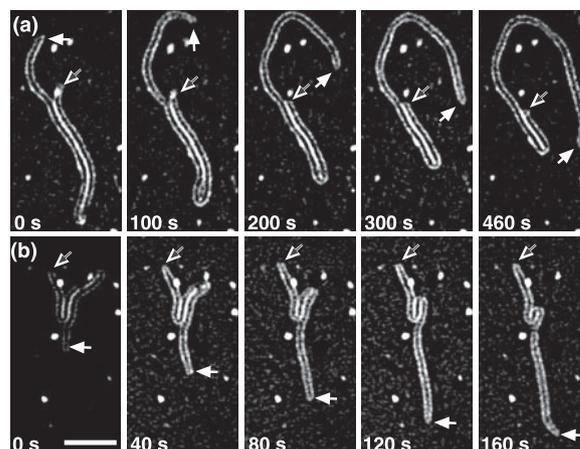
be localized not at the cell posterior but distributed along the cell body. This behavior was common for filamentous cells; we recorded movies of 27 unfolding cells, at least 14 of which unambiguously showed distributed force production.

Our observations clearly show that the A-motility engine is distributed (but not necessarily uniformly) along the cell body of filamentous cells with no signs of intermediate septa, rather than being localized at the rear pole. The data do not eliminate the possibility that slime extrusion may contribute to the propulsive force. However, we consider it unlikely because the putative slime extrusion pores appear clustered at the cell poles. The data are consistent with the "focal adhesions" model but do not provide proof, for they do not address the nature of the propulsive engine. Thus, the A-motility motor awaits better characterization of its exact mechanism.

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