

Acting Like Actin: The Dynamics of the Nematode Major Sperm Protein (MSP) Cytoskeleton Indicate a Push-Pull Mechanism for Amoeboid Cell Motility

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The crawling movement of eukaryotic cells requires establishment of cell polarity, extension of the leading edge, attachment to the substratum, and retraction of the cell body. Each of these events depends on the dynamics of the actin cytoskeleton that are orchestrated by a host of signaling molecules and actin-binding proteins. Indeed, amoeboid cell motility involves so many biochemical components and requires such precise coordination that it can be difficult to formulate models to account completely for the integration of protrusion, adhesion, and retraction at the molecular level. However, the amoeboid sperm of nematodes are a simpler, more specialized system in which the role usually played by actin has been taken over by the 14-kD major sperm protein (MSP)¹. This model system has given insights into the general mechanism of how cells crawl, and has indicated that, at least in nematode sperm, locomotion appears to be produced primarily by a push-pull mechanism based on MSP assembly dynamics.

Nematode sperm not only provide a unique molecular perspective for studying amoeboid cell motility, but also offer advantages as an experimental system that, in many ways, complement those of actin-based cells. For example, many of the molecules that organize and regulate the actin cytoskeleton have been identified, and attention is shifting to understanding how those molecules interact to produce movement (for reviews see Machesky and Insall, 1999; Svitkina and Borisy, 1999; Borisy and Svitkina, 2000). This task is complicated by the versatility of actin, which, in addition to locomotion, is also engaged in determination of cell shape, establishment of polarity, endocytosis, movement of organelles, rearrangement of surface components, and cytokinesis. Nematode sperm, by contrast, are simple cells that use their MSP motility system exclusively for locomotion. Moreover, in *Ascaris* sperm, the cytoskeleton is organized so that it can be observed directly in crawling cells. This combination of features has made it possible to take apart and rebuild the MSP machinery and compare its operation to that of actin-based cells as a way of identifying the fundamental principles of amoeboid cell motility.

Although nematode sperm contain no F-actin, the cells display the classic features of amoeboid locomotion. For example, *Ascaris* sperm extend a persistent flattened lamellipodium that attaches to the substrate and pulls along a trailing, organelle-packed cell body. The lamellipodium is packed with filaments that assemble along the leading edge and flow rearward as the cell progresses in the same general pattern observed for the actin cytoskeleton in a number of other crawling cells (for reviews see Mitchison and Cramer, 1996; Theriot, 1996). Indeed, MSP- and actin-based cell crawling are so nearly identical that, although the two systems use different sets of molecular components to generate movement, they must employ very similar mechanical principles.

MSP and Actin

Although MSP and actin lie at the core of similar motile systems, it is surprising how little the two proteins have in common. Both are abundant cellular components (*Ascaris* sperm contain ~4 mM MSP) that are capable of self-assembly, but the proteins have no sequence homology, no structural similarity, and form filaments with different structural and polymerization properties. MSP contains only 126 amino acids, and its structure is based on an Ig fold that is completely different from the structure of actin (King et al., 1992; Bullock et al., 1996). Moreover, unlike actin, MSP does not bind nucleotides, and the polymerizing unit is a dimer rather than a monomer (Haaf et al., 1996; Italiano et al., 1996). Both proteins assemble into two-stranded polymers but, in actin filaments, the subunits in each strand are arranged like beads on a string, whereas MSP filaments are constructed from two loosely connected helical subfilaments (Stewart et al., 1994).

The most striking difference between MSP and actin, from the standpoint of the mechanism of motility, lies in the polarity of the filaments they form. Actin filaments have a characteristic structural polarity that not only influences the pattern and regulation of cytoskeletal assembly, but also allows the directional operation myosin family molecular motors on the filaments. MSP filaments lack this polarity. The two chains in the dimers from which MSP filaments are constructed are related by twofold rotational symmetry (Bullock et al., 1996). In filaments, the

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¹Abbreviation used in this paper: MSP, major sperm protein.

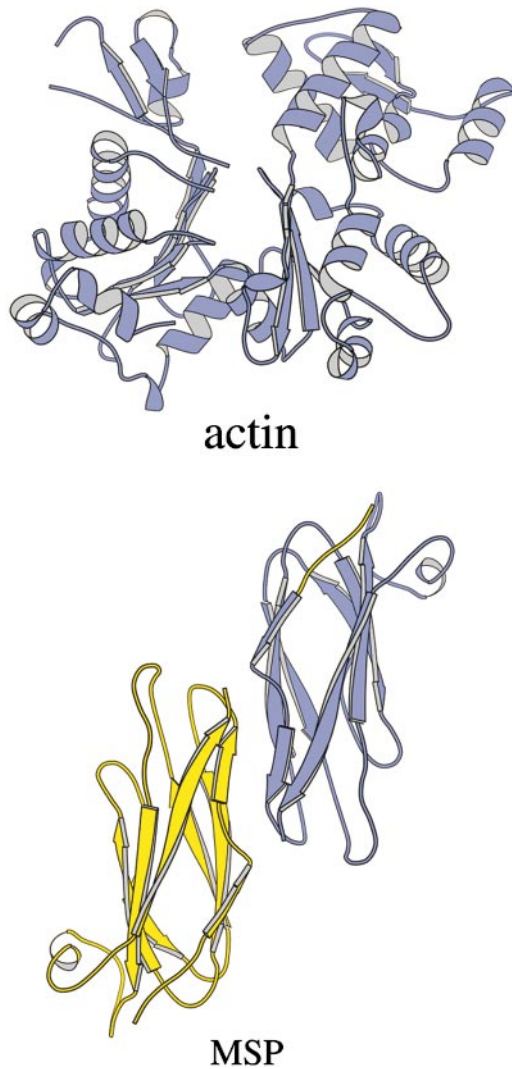


Figure 1. Ribbon diagrams of *Dictyostelium discoideum* G-actin (Matsuura et al., 2000) and the *Ascaris suum* α -MSP dimer (Bullock et al., 1996) at the same magnification. Actin consists of four subdomains that surround a nucleotide-binding cleft. The G-actin molecule is asymmetric, so that when it polymerizes, the filaments it forms have a characteristic polarity and its two ends differ structurally. By contrast, MSP contains no nucleotide binding site, and the polymerizing unit is a dimer in which the two MSP molecules are related by twofold rotational symmetry. Polymerization produces filaments comprised of two helical subfilaments in which the dimers' twofold axes are oriented perpendicular to the helix axis. Consequently, the MSP helices have no polarity and the subfilament ends are identical structurally (Bullock et al., 1998).

dimer twofold axes are parallel to the subfilament helix axis (Bullock et al., 1998). This results in the subfilaments being nonpolar, and the filaments formed from these subfilaments also have no overall polarity. Therefore, in contrast to F-actin, both ends of MSP filaments are the same and so polymerization must be controlled by external factors. Moreover, it is unlikely that an apolar filament can support the action of a molecular motor protein analogous to myosin. This observation has focused attention on the continuous cytoskeletal remodeling that accompanies

sperm locomotion as the source of the forces required for motility.

Locomotion Is Coupled to Cytoskeletal Assembly and Disassembly

Migrating cells display a characteristic pattern of cytoskeletal rearrangement. Filaments are assembled and cross-linked into meshworks along the advancing front, and flow rearward where they disassemble so that subunits can be recycled to the leading edge for reassembly. The cytoskeleton in *Ascaris* sperm can be imaged directly in live cells without resorting to the labeled probes that are often needed to detect actin filaments. The MSP filaments are arranged into long branched meshworks, called fiber complexes, which span the entire length of the lamellipodium. Along each fiber complex, filaments extend radially to interact with similar filaments from adjacent complexes, so that the entire cytoskeleton functions as an interconnected unit (Seppenwol et al., 1989). The action takes place at the ends of the fiber complexes, which are assembled in small protrusions along the leading edge and taken apart at their opposite end, at the base of the lamellipodium adjacent to the cell body. Thus, as the cell moves along, the fiber complexes treadmill from front to rear through the lamellipodium without a detectable change in shape or filament density (Fig. 2 a).

Not all crawling cells exhibit such a close correlation between cytoskeletal dynamics and locomotion. Often, as in fibroblasts (Wang, 1985) and *Aplysia* neuronal growth cones (Forscher and Smith, 1988), the rate of cytoskeletal treading outpaces the speed of translocation. In fish epithelial keratocytes, the rate of localized actin cytoskeletal assembly matches that of leading edge protrusion, but cytoskeletal disassembly occurs throughout the lamellipodium (Theriot and Mitchison, 1991). In the *Ascaris* sperm, cytoskeletal assembly and disassembly occur at opposite ends of the lamellipodium, 15–20 μm apart, but at the same rate. Thus, elongation of the fiber complexes appears to push the plasma membrane forward, allowing the leading edge to advance while simultaneously the cell body is pulled forward as the cytoskeleton disassembles at the base of the lamellipodium. Methods have now been developed to uncouple MSP cytoskeletal assembly and disassembly and explore their independent contributions to sperm locomotion.

Reconstitution of Lamellipodial Protrusion In Vitro

In both nematode sperm and actin-based cells, the localized cytoskeletal assembly that occurs at the leading edge suggests that this process itself may drive protrusion. This hypothesis has been confirmed directly by reconstituting lamellipodial protrusion in cell-free extracts of *Ascaris* sperm (Italiano et al., 1996). Addition of ATP to this material induces the formation of discrete meshworks of MSP filaments, called fibers, each of which has a membrane vesicle at one end (Fig. 2 b). Growth of these fibers is due to assembly of filaments at the vesicle-bearing end, which produces vectorial movement of the vesicle. Immunolabeling indicates that the vesicles that build fibers derive from

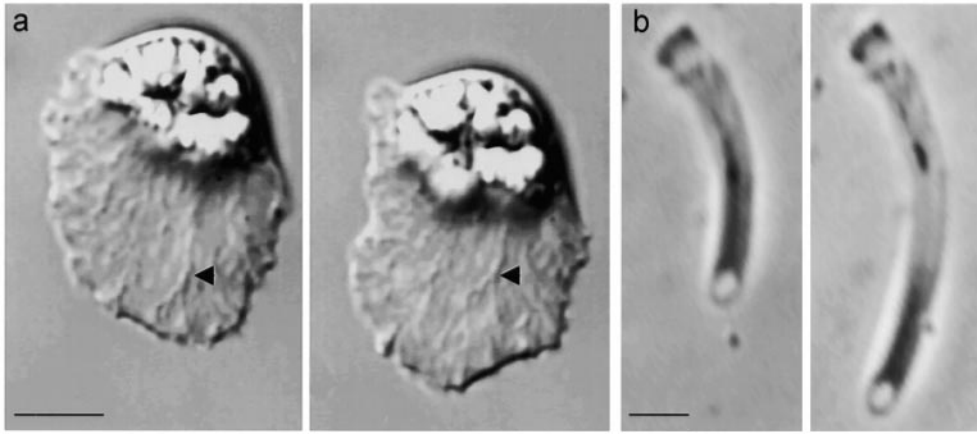


Figure 2. Cytoskeletal dynamics can be observed directly by light microscopy in crawling *Ascaris* sperm and reconstituted in vitro in cell-free extracts. (a) Two images, taken 10 s apart, of a sperm crawling on a glass coverslip. The MSP fiber complexes are readily visible in the lamellipodium, so their dynamics can be followed in real time. The rate of cytoskeletal assembly along the leading edge and disassembly at the base of the lamellipodium is tightly coupled to locomotion. Thus, fiduciary mark-

ers, such as branches in the fiber complexes (arrowhead), flow centripetally through the lamellipodium but remain nearly stationary with respect to the substratum. (b) Leading edge dynamics can be reconstituted in vitro such that vesicles from the plasma membrane induce the assembly of MSP filament meshworks, called fibers, that push the vesicle forward as they elongate. The two images were taken 10 s apart. Bars: (a) 5 μm ; (b) 2.5 μm . Frame a was reproduced from *J. Cell Biol.* 1999. 146, pp. 1087–1095 by copyright permission of the Rockefeller University Press.

the plasma membrane at the leading edge of the lamellipodium. Thus, simply adding ATP to a crude cell extract can reconstitute lamellipodial protrusion: a fragment of the leading edge membrane triggers polymerization and bundling of a meshwork of filaments that moves a vesicle in the same way as elongation of the fiber complexes seems to push the lamellipodial membrane forward in crawling cells. MSP does not bind ATP and is not phosphorylated. Thus, ATP appears to be used indirectly, but its exact role in protrusion still needs to be defined.

MSP-driven vesicle motility resembles a number of specialized actin-based motile systems typified by the movement of *Listeria monocytogenes* (for review see Machesky, 1999). This intracellular bacterial pathogen commandeers proteins from its host cell to build a columnar meshwork of actin filaments. Elongation of this column pushes the bacterium forward in the same way as growth of an MSP fiber moves its associated vesicle (Fig. 3). Like the MSP in vitro system, movement of *Listeria* is thought to be a simplified version of leading edge dynamics in crawling cells, and identification of properties shared by these two systems reveals important clues about the mechanism of lamellipodial protrusion. For example, both use the same general mechanism to build their motile apparatus. In *Listeria*, a membrane protein, ActA, recruits soluble proteins to the bacterial surface to initiate localized filament assembly (for review see Beckerle, 1998), whereas in the MSP system, an integral membrane phosphoprotein interacts with at least one cytosolic protein other than MSP to trigger filament assembly at the vesicle surface (Roberts et al., 1998). In both systems, filaments appear to be assembled de novo by a nucleation-elongation reaction rather than by addition of subunits to the ends of existing filaments. The Arp2/3 protein complex, which is a nucleator of actin polymerization (Mullins et al., 1998), is a key component for *Listeria* movement (Welch et al., 1997), which is required for reconstitution of motility from purified components (Loisel et al., 1999). The analogous pro-

teins for MSP polymerization remain to be identified. However, assays of the effects of hydrostatic pressure on fiber growth have shown that increased pressure reduces both the number of filaments assembled at the vesicle surface and their rate of polymerization (Roberts et al., 1998). Thus, MSP filament assembly also involves a site-directed nucleation-elongation reaction. Moreover, in both systems, the newly formed filaments are rapidly cross-linked and remain stationary within the meshwork as assembly proceeds and the vesicle or bacterium moves away.

Even in these simple, reconstituted systems the precise mechanism of propulsion remains a vexing problem. *Listeria* motility can be reconstituted in vitro without myosins (Loisel et al., 1999) so neither system seems to require motor proteins and, instead, movement appears to be associated with polymerization and bundling of filaments. Mogilner and Oster (1996) have proposed an elastic Brownian ratchet mechanism to account for this movement whereby the thermal writhing of a filament allows it to move away from an object sufficiently to add a subunit, then the elastic restoring force of the lengthened filament pushes the object forward. The ability to reconstitute both MSP- and actin-based systems in vitro provides a powerful comparative basis to test this model and to evaluate the relative contributions of filament nucleation, elongation, and cross-linkage to force production.

Retraction Is also Required for Crawling

Recent studies of both nematode sperm and actin-driven crawling cells have emphasized that protrusive force at the leading edge is necessary but not sufficient for cell crawling. A second force, independent of that involved in protrusion, is required to pull the cell body forward as the cell advances. For example, analyses of cells crawling on flexible substrates have shown that traction forces are produced well behind the leading edge (Harris et al., 1980; Lee et al., 1994; Pelham and Wang, 1997). Moreover,

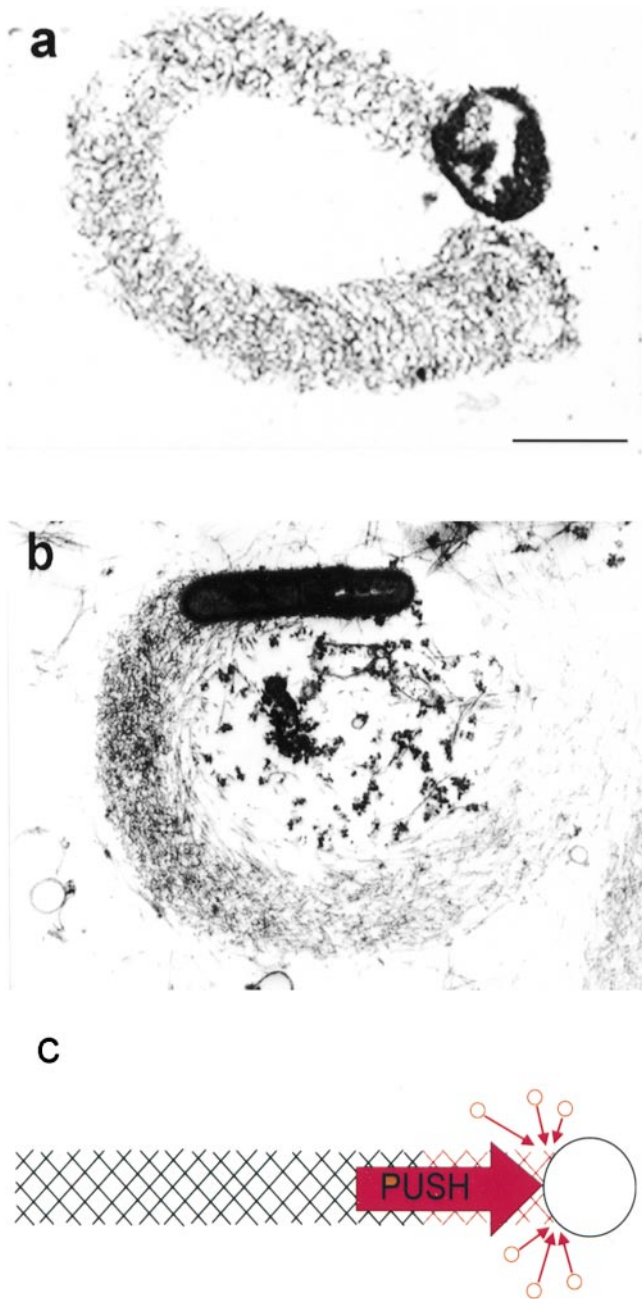


Figure 3. Thin section electron micrographs of an MSP fiber assembled *in vitro* (a) and an actin comet tail formed by *Listeria* (b) showing that, in both of these specialized systems, the motile apparatus is comprised of a columnar meshwork of filaments. In both cases (c), the object at the head of the column directs filament nucleation-elongation from a soluble pool of subunits (red circles) and this localized polymerization pushes the object forward. Frame a was reproduced from Italiano et al., 1996. *Cell* 84: 105–114 by copyright permission of Cell Press. (b) Reproduced with permission from the *Annu. Rev. Cell Dev. Biol.* 1995. Vol. 11, by Annual Reviews <http://www.AnnualReviews.org>

when actin polymerization in the lamellipodium of fish epithelial keratocytes is blocked by treatment with cytochalasin, the trailing cell body continues to retract (Anderson et al., 1996). In a series of elegant studies, Borisy and colleagues have shown that bipolar arrays of myosin II form

at the base of the lamellipodium of these cells (Svitkina et al., 1997). Similar arrays form at the trailing margin of polarized, motile fragments of the keratocyte lamellipodium (Verkhovskiy et al., 1998). Thus, in these cells, myosin is properly organized and situated to play a role in retraction, although there is not yet direct evidence that it performs this function.

Evidence for a specific retraction force in *Ascaris* sperm was obtained by exploiting the sensitivity of the MSP cytoskeleton to changes in intracellular pH (Italiano et al., 1999). Lowering intracellular pH in sperm below 6 causes a complete, but fully reversible, disassembly of the MSP cytoskeleton. By fine tuning this pH effect, cytoskeletal assembly can be uncoupled from disassembly, and so the role of each process in sperm motility can be studied independently. For example, at pH 6.35, filament assembly along the leading edge stops and the tips of the fiber complexes detach from the lamellipodial membrane. Localized disassembly at the base of the lamellipodium continues and the fiber complexes are pulled toward the cell body as they shorten. At a slightly higher pH of 6.75, assembly at the leading edge again stops, but the fiber complexes remain attached to the lamellipodial membrane. In this case, disassembly at the base of the lamellipodium continues but, instead of pulling the fiber complexes rearward, the cell body is pulled forward. These observations indicate that at the base of the lamellipodium, a force is generated that is associated with cytoskeletal disassembly, but which is independent of the protrusive force at the leading edge. This second force places the MSP cytoskeleton under tension, as illustrated by the recession of the fiber complexes toward the site of disassembly at pH 6.35. When the fiber complexes maintain their attachment at the leading edge, as in cells at pH 6.75 and in crawling sperm, this tension powers the retraction of the cell body.

Push-Pull Model for Nematode Sperm Amoeboid Motility

Ascaris sperm motility suggests a simple push-pull mechanism for locomotion (Fig. 4). We propose that two separate and distinct forces are required for movement: a protrusive force along the leading edge that pushes against the membrane and a traction force at the base of the lamellipodium that pulls the cell body forward. This model suggests that substrate attachments, which provide the traction needed to convert forces generated within the cytoskeleton into movement, also have another role, one of mechanical separation of the forces for protrusion and retraction. The organization of the motility apparatus in *Ascaris* sperm, where the forces are generated at the opposite ends of the fiber complexes, illustrates the need for such separation. The protrusive force at the leading edge would place a fiber complex under compression while the force generated at the rear places that same fiber complex under tension. Ordinarily these two forces would tend to cancel each other. However, between the regions of polymerization and depolymerization, there is a region where the membrane (and the cytoskeleton) is attached to the substrate. Without this attachment, directional movement, would not be possible.

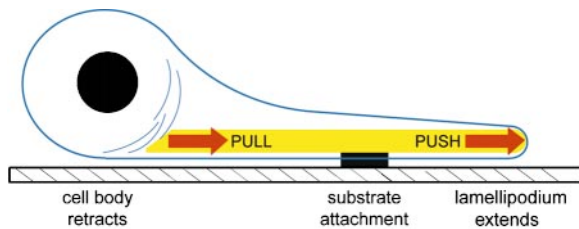


Figure 4. Proposed push-pull model for nematode sperm locomotion. Assembly and bundling of MSP filaments into fiber complexes (yellow band spanning the lamellipodium) pushes the membrane at the leading edge forward. At the same time a second force, which is associated with disassembly of the fiber complexes at the base of the lamellipodium, pulls the cell body forward. In this model, attachments where the cytoskeleton is linked to the membrane and the membrane anchored to the substratum establish traction and separate mechanically the forces produced at opposite ends of the fiber complexes. Thus, rather than canceling each other, these forces can be exerted independently against the substratum.

The principles of the push-pull model probably apply generally to amoeboid cell motility. Indeed, a consensus is developing that, in both sperm and actin-based crawling cells, the force for protrusion is derived from localized cytoskeletal assembly. However, as applied to nematode sperm locomotion, the model envisions that lamellipodial extension and cell body retraction are linked reciprocally to the polymerization state of the cytoskeleton, and that molecular motor proteins are not required for movement. The lack of structural polarity of MSP filaments, the precise localization of cytoskeletal polymerization and depolymerization at opposite ends of the fiber complexes, and insights gained from reconstitution of cytoskeletal dynamics, and motility *in vitro* and *in vivo* all support the conclusion that nematode sperm move without using motor proteins. We cannot rule out that myosins play a role in actin-based cell crawling and, indeed, may be required for cell body retraction in some cell types. However, our results suggest that it is plausible that a push-pull polymerization/depolymerization-based mechanism may contribute to the locomotion of at least some actin-based crawling cells.

Future Directions

Comparison of the MSP- and actin-based locomotory machinery has already yielded a number of insights into the basic mechanism of cell crawling and, for example, has emphasized the importance of vectorial assembly and filament bundling in protrusion. For the MSP system, a key goal is identifying the molecules that orchestrate the assembly and disassembly of the motility machinery. This should be possible to achieve by capitalizing on the simplicity of nematode sperm and the ability to reconstitute their motility *in vitro*, and perhaps also by exploiting the genomics and molecular genetics of *Caenorhabditis elegans*. It should be possible to address several key questions about this fascinating motility system: how the leading edge membrane directs MSP polymerization, how ATP is used to produce movement, how disassembly generates

tension, and how cytoskeletal assembly and disassembly are coupled. The answers to these questions should help pave the way to understand how reciprocal cytoskeletal events at opposite ends of the lamellipodium are coordinated to drive the migration of the cell, and illuminate the shared physical properties of MSP and actin cytoskeletons that are responsible for crawling movement.

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