CELL BIOLOGY

Shrinking Gels Pull Cells

Alex Mogilner and George Oster

In 1675, van Leeuwenhoek described "pleasing and nimble" motions of extending and contracting "living atoms." He was, of course, observing motile cells migrating across his microscope slide. Cell migration, we now know, is essential during embryogenesis and wound healing (*1*). Our current understanding of how cells crawl on solid surfaces began in the 1970s when Abercrombie discovered that migration can be dissected into three distinct stages. First, the cell develops a protrusion at the leading edge; then it strengthens its adhesions at the leading edge and weakens them at the trailing edge; finally, it develops contractile forces that pull the rear of the cell forward (*2*). Of these three stages, the last, called retraction, is the least well understood, both mechanically and at the molecular level (*2*). On page 1405 of this issue, Miao and colleagues (*3*) advance our knowledge of retraction by reconstituting the phenomenon in vitro for the first time.

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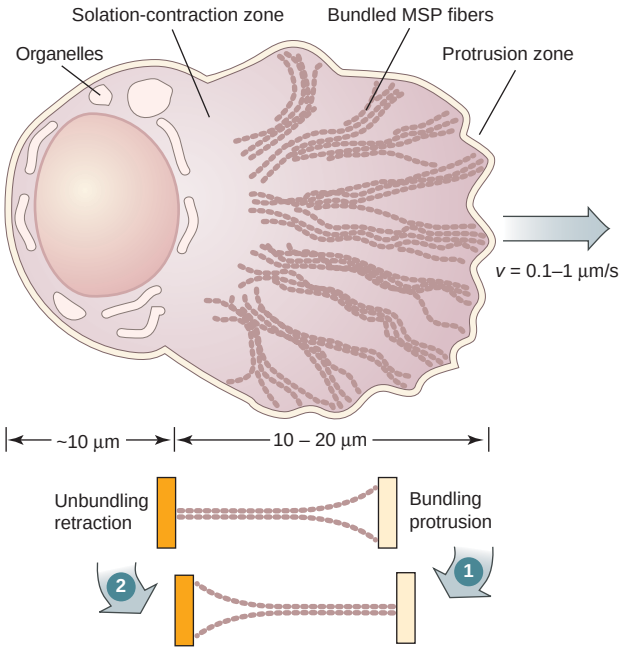
Most eukaryotic cells commence the crawling cycle by extending lamellipodia at their leading edge. These are broad, thin protrusions in front of the cell body that contain a dynamic, polarized network of actin filaments that grow at the leading edge and disassemble at the rear (*1*, *2*, *4*). The force driving protrusion is generated at points where the growing barbed ends of actin filaments abut the cell membrane, most likely by a ratchet mechanism generated as actin monomers are added to the growing filaments (*4*, *5*). According to this model, the bending of filaments due to thermal fluctuations makes room for actin monomers to polymerize onto their barbed ends. This allows the elongated filaments to recoil and exert an elastic pushing force on the membrane.

The nature of the contractile force at the rear pulling on the cell body is more mysterious. In the fast-gliding keratocyte cells of fish, the lamellipodial actin network weakens by disassembly at the rear, and is collapsed into muscle-like bundles by the contractile force of myosin motors (*6*). The sliding action between actin filaments and myosin motors is likely to be a major part of the contractile force in many types of motile cells. However, it is not the only mechanism for generating the force of retraction.

Sperm of the nematode *Ascaris suum* provide an important system with which to study cell crawling, because the locomotion machinery of these amoeboid-like cells is dramatically simplified (*7*). Instead of actin, movement is powered by a cytoskeleton built from filaments of major sperm protein (MSP). MSP monomers associate into symmetrical dimers that polymerize into helical filaments. Hydrophobic and electrostatic interfaces allow these filaments to bundle together, forming thick rope-like fibers that extend from the front to the rear of the lamellipod (see the figure). The sperm maintain a pH gradient that decreases from the front to the rear of the cell and has an important regulatory function. In the more alkaline environment at the leading edge, MSP assembles into filaments. These filaments then bundle together into fibers and drive finger-like protrusions from the cell surface. Each lamellipod adheres to the substratum, but the strength of adhesion decreases toward its middle. In the acidic cytoplasm at the rear, the fibers lose their adhesion to the substratum, unbundle, and disassemble (*8*). This produces the "treadmilling" of fibers that accompanies forward translocation of the cell.

In the new work, Miao, Roberts, and their colleagues used extracts from *Ascaris* sperm cells to investigate the forces exerted as the sperm crawl (*3*). They extracted cytoplasm (containing vesicles) derived from the leading-edge membrane of the sperm, which directs the local assembly of MSP fibers that propel the vesicles forward (*3*). The investigators identified a pH-sensitive soluble factor and a membrane protein that were required for MSP assembly (*9*). ATP was also essential for MSP assembly, although its involvement remains unclear because MSP does not bind to or hydrolyze ATP.

MSP filaments attached to the vesicles disassembled when a phosphatase was added to the sperm extract (*3*). During disassembly, the filaments shrank in length and diameter, and a plastic bead attached to the end of an MSP fiber was pulled toward the vesicle attached at the fiber's other end. This observation indicated that disassembly of MSP filaments generated a contractile force. ATP was not required for the contraction, so energy for retraction must be stored in



When speed counts. The "push-pull" model for locomotion of nematode sperm. The cell body is a passive cargo, but it generates a pH gradient that regulates gelation and solation of MSP fibers. At the leading edge, where the pH is high, the growing MSP filaments bundle into thick fibers. This bundling extends the filaments beyond their equilibrium length, pushing the cell front out (**bottom**), and at the same time storing elastic energy. At the rear, where the pH is low, the interfilament interactions weaken, and the filaments unbundle and contract to their equilibrium length. Because the cell front adheres to the substratum, this provides the contractile force to pull the cell body forward.

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the MSP fiber. Contraction was not triggered directly by pH, but the pH may have affected phosphatase activity.

An important conclusion of this study is that the disassembly/unbundling mechanism driving retraction is completely separate from the assembly/bundling mechanism driving protrusion, and involves a different set of molecules and a different pH trigger. Although MSP filaments are not polar, the pH gradient in the motile sperm could, in principle, provide a directional cue for some undiscovered myosin-like motor. However, there was no pH gradient in the reconstituted sperm extracts, so motor-powered retraction is unlikely. What, then, is the physical nature of the contractile force?

The theoretical model illustrated in the figure suggests an answer (*10*, *11*). According to the theory, bundling of the flexible MSP filaments forces them to assume an end-to-end distance that is longer than it would be in solution. Thus, the bundles are stiffer than, and contain the stored elastic energy of, their constituent filaments. Weakening of the cohesive forces in the bundles allows individual MSP filaments to dissociate from the fiber complexes. As they do so, they tend to contract into their equilibrium end-to-end length with an increase in their entropy (molecular disorder). This solation process releases the elastic energy in the gel filaments that was stored by bundling at the cell front, generating a contractile stress. This solation-contraction mechanism can account for forces in the piconewton range per filament, or hundreds of piconewtons per fiber complex (*10*), more than enough to pull the cell body forward. Thus, rather than using complex mechanochemical cycles involving molecular motors, this cell employs a "one-shot" engine that does its increment of work at the cell rear, and then disassembles, only to reassemble at the front of the cell.

Many questions remain about the mechanism of retraction. In vitro, subpiconewton forces are sufficient to pull the bead against viscous drag. But in the moving cell, this is probably a tiny fraction of the force that must be developed against the greater load of cell adhesion. Future biophysical experiments (for example, using deformable substrata) can ascertain the actual forces developed during cell migration. Miao *et al*. (*3*) show that disassembly alone cannot account for retraction. Therefore, there must be some rearrangement of the remaining filaments to generate shortening. This can be due to unbundling or uncross-linking of the filaments. What is the role of the phosphatase in generating force? One possibility is that removal of a phosphate group from a hypothetical cross-linking protein alters its interactions with MSP filaments. How is the contractile force applied to the cell body? Attachment of the plastic bead to the shortening MSP fiber indicates that a very simple mechanism, such as entanglement of the cell body in the MSP cytoskeletal meshwork, could be responsible. What is the role of ATP in extending MSP fibers? What is the minimum essential set of molecules for MSP cytoskeleton treadmilling? This has been established for actin-based systems, but only for protrusion (*12*). This may be easier to answer for MSP-based cells because they are streamlined for locomotion and appear to rely on fewer accessory proteins than actin systems do.

Finally, what does the nematode sperm tell us about actin-based motile cells? Perhaps the most important lesson is that myosin-powered contraction may not be the whole story. Unlike MSP gel filaments, actin gel filaments are "semi-stiff" (*5*); nevertheless, actin gels can entropically contract and contribute to retraction forces. Entropic gel contraction may also be important in generating tissue-remodeling forces by highly adhesive motile cells, such as fibroblasts. There are other examples in nature where disassembling biopolymers and gels generate pulling forces, for example, microtubules that segregate chromosomes during mitosis (*5*) and the contracting gel of "forisome" proteins that plugs leaks in legume plants (*13*). To go beyond a mere inventory of essential molecular components, we need new experiments to generate quantitative data that can then be modeled by computer. Only this approach can bring us to a complete understanding of how whole cells crawl.

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NEUROSCIENCE

SPARring with Spines

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Nerve cells communicate with each other through contact points called synapses. The efficiency of information flow through synapses—synaptic transmission—determines the activity patterns of neuronal networks. Synapses are highly dynamic "plastic" structures, and corresponding dynamic changes in the efficiency of synaptic transmission are thought to represent the physiological basis of learning and memory. On page 1368 of this issue, Pak and Sheng (*1*) report their discovery of a new molecular mechanism through which synapses achieve plasticity.

Many aspects of synaptic plasticity can be explained by transient changes in the properties of the pre- and postsynaptic machinery mediating two key processes in synaptic transmission: neurotransmitter release from the presynaptic neuron, and signal reception and transduction by the postsynaptic neuron. It is plausible, however, that long-term storage of information is, at least in part, achieved by structural changes in neuronal connectivity—for example, in the structure and number of synapses. Consequently, recent demonstrations that structural rearrangements accompany synaptic plasticity have received much attention. This attention has centered on the rearrangement of a specialized structure found at many synapses in the mammalian central nervous system: the dendritic spine.

Dendritic spines are small postsynaptic protrusions that make contact with presynaptic nerve terminals that release the excitatory neurotransmitter glutamate (see the figure). The spine head harbors the postsynaptic membrane within which is the postsynaptic density rich in scaffolding and signaling proteins. The spine head is connected to the main neuronal process (dendrite) by a narrow neck. Although their exact role is unknown, dendritic spines are likely to act as spe-

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