Crawling cell locomotion revisited

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Cell crawling is of primary importance for such fundamental biological processes as embryonic development, wound healing, inflammation, and cancer metastasis (1). Motion of cells along extracellular substrates or matrices requires an apparently very simple condition: a fairly small force has to be applied to the cell to move it against the friction between the cell surface and the surrounding liquid. However, under common biological circumstances, there are no external fields or factors that would generate such forces. As a result, this force must be produced by the cell itself. This represents a substantial complication as, if a force-generating device is a part of the cell body, it cannot produce the body movement on its own. Indeed, in contrast to the Baron Münchhausen experience, one would fail moving him/herself by pulling his/her own hairs. One needs legs to transmit the generated force to the ground. These legs—in the cell's case, the cell-substrate adhesions—have to fulfill two apparently contradictory conditions. First, they have to transmit the force (the momentum) to the substrate and, hence, fuel the cell movement. However, they should not remain persistently stuck to the substrate, as, in such a case, they would resist the cell motion. The adhesions must emerge and then disintegrate and be recycled at different stages of the cell movement. One of the major challenges of biology and biophysics of cell motility is to understand the molecular structures a cell uses to generate and transmit force and how it removes and recycles these structures after their functions are completed and they become an obstacle to cell movement. The study by Ofer et al. in PNAS (2) suggests and substantiates experimentally an elegant and simple mechanism for the latter process.

The cell-moving machinery is designed as two self-assembling molecular systems undergoing persistent intracellular turnover: the actin cytoskeleton (3), which consists of filaments made of protein actin and numerous accessory proteins and represents the force-producing engine, and the dynamic cell adhesions—the multimolecular complexes spanning the plasma membrane and mediating a mechanical link between the cytoskeleton and the extracellular substrate (4) (Fig. 1). According to the necessity to compose the force-producing machinery and recycle it

Fig. 1. A scheme depicting a model for crawling locomotion of keratocyte fragment. Crawling proceeds through protrusion of the cell leading edge, which is driven by polymerization of the actin network interacting with the substrate through cell adhesions. The actin network density decays toward the cell rear, where its remnants get crashed through force application by the cell membrane. This force is produced by the actin assembly at the cell leading edge and transmitted to the cell rear through the membrane. The cell movement is enabled by detachment and recycling of the adhesion at the rear edge.

at a later stage, the cell movement is commonly seen as consisting of two phases: protrusion of the cell leading edge driven by a concerted self-assembly of the actin filament network at the cell front and the adhesions underneath, and retraction of the cell rear requiring disassembly and removal of the two systems from the back part of the cell. The mechanisms by which the actin cytoskeleton self-assembles at the cell front and acts to generate the forces have been thoroughly explored (3, 5, 6). A less resolved question concerns the decomposition and recycling of these two systems at the cell rear during the retraction phase.

The work by Ofer et al. (2) presents a detailed experimental analysis of the simplest cell derived system able to crawl, fragments of fish keratocytes (7) lacking microtubules and cellular organelles (8, 9). Measurements of keratocyte fragment morphology speed and the F-actin distribution revealed two interesting phenomena: exponential decay of the actin network density from the front to the rear of the fragment, and a correlation between fragment velocity and the distance between the front and rear edges. Based on the observation that the actin network does not completely vanish at the rear edge, their model would suggest that the essence of the retraction phase consists of force-driven disintegration of the actin network remnants facing the rear edge of

the cell. The major idea is that a pushing force has to be applied to this residual actin network to decompose it completely and enable the retraction phase. Essentially, this force is suggested to be produced by the same machinery that drives the cell movement, namely, by the actin assembly at the cell front. Finally, the force transmission from the cell front to the rear and application to the remnants of the actin network is proposed to be mediated by the plasma membrane (Fig. 1).

How can a membrane transmit the force? The key notion here is the membrane tension. A lipid bilayer constituting the base of any biological membrane has properties of a 2D fluid. One of the fundamental features of any fluid is that, according to Pascal law, “pressure exerted anywhere in a confined incompressible fluid is transmitted equally in all directions throughout the fluid.” The tension in a lipid membrane is nothing but a 2D pressure in a 2D fluid. The only difference is in the convention concerning the sign. The pressure in an ordinary 3D fluid is defined as positive if the fluid is compressed by external forces and negative if the fluid is stretched. The membrane tension, on the contrary, is commonly defined to be positive if the membrane is stretched, as in the most biologically relevant situations, including the case of plasma membrane of a moving cell. Otherwise, the membrane tension behaves according to the common physical laws holding for pressure in regular fluids, including propagation throughout the whole membrane area according to the Pascal law. This means that the actin filaments assembly against the membrane at the leading edge generates a tension, which propagates isotropically and evenly throughout the whole membrane, including the membrane at the rear edge (10). The latter faces the remnants of the actin network and, because of the tension, pushes on them. According to the hypothesis by Ofer et al. (2), the pushing membrane crashes the residual actin filaments.

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The convincing mathematical modeling and experimentation by Ofer et al. (2) leave no doubts that the suggested mechanism for the actin network decomposition at the cell rear plays an essential role in the retraction phase of the cell motion. However, taking into account the variability of mechanisms in cells of different kinds and the relative simplicity of keratocyte fragments as a model for a whole cell, the important remaining questions are whether the process of crashing the remnants of the actin network is always the only or the major one enabling the advancing of the cell rear. Feasible candidates for producing a resistance to the cell rear retraction are, apart from the residual actin network, the remaining cell adhesions (Fig. 1). To allow for the cell motion, these adhesions have to be detached from the external substrate and recycled or moved along the substrate. Specifically, for the fish keratocytes, the major adhesions have been visualized in the lobes of the cell, which, apparently, have to be dealt with to enable the keratocyte movement (11, 12). It is plausible that the detachment or disintegration-recycling of the cell adhesions requires application of a force, similarly to the mechanism of the actin network decomposition assumed by Ofer et al. (2). Moreover, this force can come from the membrane tension directly or through actin filaments. According to the common physical rules, the force required for the adhesion decomposition or detachment would increase with growing velocity of the cell movement, which would mean a larger membrane tension in faster-moving

cells. The same tension/velocity relationship resulting in the observed increase in the cell front-to-rear distance is one of the implications of the Ofer et al. (2) hypothesis. Hence, the mechanism of the cell rear retraction based on the adhesion decomposition-detachment seems to result in a phenomenological behavior of the moving cell similar to that observed

The work by Ofer et al. presents a detailed experimental analysis of the simplest cell derived system able to crawl.

by Ofer et al. (2) and appears, therefore, to be a viable alternative to the suggested mechanism.

A corollary of any model proposing to explain a particular physiological process must, in principle, be a recipe for creation of an artificial "biomimetic" system that reproduces the essential features of the process. Does the work by Ofer et al. (2) provide us with an understanding sufficient for the design of an artificial system that could mimic cell crawling? Obviously, actin polymerization and depolymerization are needed, whereas, according to this work, the myosin contractile activity is redundant (2) and can be skipped. Further, Ofer et al. (2) propose that the coupling between protrusion at the leading edge and retraction at the rear is mediated

by the plasma membrane. As a result, what appears to be needed is a giant unilamellar liposome filled with actin and appropriate polymerization- and depolymerization-controlling proteins (13, 14) (we do not deal with a problem of ATP supply here). However, as discussed earlier, the force generated by actin within the liposome has to be transmitted to the external substrate through the membrane, which means one should worry about adhesion receptors spanning the membrane and undergoing some kind of friction-like or sticking interaction with actin. In addition, these receptors have to be dynamic and recyclable to mediate the force transduction but not prevent the liposome movement. The outstanding issue is how to couple the actin system with the adhesion receptors and how to recycle the receptors at the right time and place. In cells, such coupling is mediated by the focal adhesions, complex protein assemblies performing the environmental sensing (4, 15), which might not be necessary for just crawling locomotion. Thus, a question remains about a minimal actin-adhesion link sufficient for effective cell movement. Altogether, we are not yet ready to create an artificial crawling cell tomorrow, but it makes sense to begin planning.

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