Mini-Review

Fibroblasts on the Move

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There are widely divergent views about how animal cells, as exemplified by fibroblasts, move over a substrate. All models assume that fibroblasts are anchored to the substratum by plasma membrane receptors which bind transiently to specific components of the extracellular matrix. It is through these individual molecular feet (not to be confused with focal contacts) that force has to be generated: the cell is moved forward over the substratum as it pushes against those feet that are, at that moment, attached to the substratum. This, of course, will lead to the accumulation of feet towards the rear of the cell: some mechanism must exist to return the feet to the leading front of the cell to attach it to the substratum. But what provides the driving force to push the cell forward? This is where there is most disagreement.

Cytoskeletal models (see, for example, Darnell et al., 1986) presume that the cell's feet span the plasma membrane and are attached (perhaps via other proteins) to the actin network in the cell cytoplasm. Force is somehow applied to those feet, by perhaps actin–myosin interactions, which pulls the cell forward. This could be analogous to a ship maneuvering by pulling on its anchor.

An alternative membrane flow model (Bretscher, 1984) is completely different. In this there exists a continual flow of plasma membrane from the front of the cell towards the rear, both on the dorsal and ventral cell surfaces. It is this flow which pushes on those feet attached to the substrate and thereby produces the force to move the cell forwards. This flow arises from the cell's endocytic cycle: specific receptors and lipids are endocytosed by randomly distributed coated pits on the cell surface, and these molecules are later returned by exocytosis to the cell surface at the cell's leading edge. It is this separation between the sites of endocytosis and exocytosis which generates the flow. Furthermore, exocytosis of membrane at the leading front enables the cell to extend itself forward, with respect to the substratum. A crude mechanical analogy compares the cell with a tank, the lower tread is the plasma membrane with feet attached to the substratum, the upper tread being membrane cycled through the cell to the leading front.

A curious feature of this model is that the cyclic flow of membrane from the front of the cell to its back and through the cell again is restricted to just those molecules which are endocytosed by coated pits. Other plasma membrane proteins, which are excluded from coated pits and therefore do not recycle (Bretscher et al., 1980), may therefore find themselves being continually swept backwards to the rear of the cell. Whether such a noncirculating protein does get swept backwards or not depends on how fast it can diffuse by Brownian motion, and how fast the flow is. Calculation shows that if such a protein has a high diffusion coefficient (D $\sim 10^{-8}$ cm^2/s), the flow on a fibroblast would be too slow to sweep it effectively towards the cell's rear – Brownian motion would win out – and the distribution of the protein over the cell's surface would be roughly uniform. By contrast, a slowly diffusing protein would be swept towards the rear of the cell. In this way, the molecular feet of fibroblasts, the fibronectin receptors, whilst tethered to the substrate fibronectin and therefore unable to diffuse at all, would be pushed backwards with respect to the advancing front of the cell. On stationary cells, this model presumes that there is no net membrane flow because, although the endocytic cycle remains, both endocytosis and exocytosis occur randomly over the cell's surface.

A most interesting paper by Ishihara, Holifield, and Jacobson (1988) occurs elsewhere in this issue. In this the authors study a protein, GP80, on the surface of fibroblasts. The interest in this protein is that it has an unusually low diffusion coefficient (Jacobson et al., 1984) and is known not to participate in the endocytic cycle (Bretscher et al., 1980). They find that, when the fibroblasts move, the GP80 protein is concentrated towards the rear of the cell. When the cells stop moving, the protein randomizes itself by diffusion.

The observation that GP80, which is noncirculating, is swept backwards on motile cells, but not stationary ones, fits nicely into the membrane flow scheme outlined above. However, does the magnitude of the gradient observed match that estimated by calculation? Ishihara et al. (1988) find that on a typical cell (50-μm long) moving at 0.5 μm/min, GP80 is ~20-fold more concentrated at the rear of the cell than at its front. Calculation of the effective diffusion coefficient for GP80 which would give this gradient gives D $\sim 7 \times 10^{-10}$ cm^2/s which is in reasonably good agreement with that previously measured (3.2×10^{-10} cm^2/s, Jacobson et al., 1984).

Further observations of Ishihara et al. (1988) are concerned with what happens when a fibroblast held at 0°C, where GP80 is uniformly distributed, is warmed up to 37°C. They find that GP80 is absent from any newly formed lamellae, that the boundary of GP80 between these bare regions and the rest of the cell is initially rather sharp, and that, at short times after the warm up, the new boundary does not move appreciably backwards with respect to the substrate, although it obviously does with respect to the new and advancing leading edge. Ishihara et al. (1988) suggest that the lack of rearward movement of the boundary (with respect to the substratum) is incompatible with a rearward membrane flow scheme. This is not so. When the cells are warmed up, two extreme situations could exist. Exocytosis of membrane (lacking GP80) at the new front could lead to a new lamella which adheres to the substratum. Alternatively, actual extension may not occur because the reinserted membrane fails to attach to the substratum – that is, "slippage" occurs. In the former case, a boundary would exist between the new bare lamella and the rest of the GP80-covered cell: this boundary would be stationary with respect to the substrate. In the latter slipping case, the GP80 would be swept rearwards by the exocytosing membrane, but as no new attachments are formed and the front of the cell cannot advance, the boundary would move rearwards with respect to the substrate. In both cases – and this is the only certain point – the boundary must move rearwards with respect to the leading front. Of course, once the cell reaches a "steady state" and translocates, the boundary will move forward (with the cell) with respect to the substrate.

Ishihara et al. (1988) propose an alternative mechanism for generating the observed gradients. They suggest that GP80 forms transient interactions with the cytoskeleton or extracellular matrix components, which would account for its low diffusion coefficient. To explain the observed gradient on moving fibroblasts, they propose that as the cell moves forward, the retracting tail provides surface membrane which flows forward, on the dorsal surface of the cell, leaving the tethered GP80 towards the rear. The authors test their observations against a model of this "retraction-induced-spreading" scheme, and find it can provide as good a fit as does the retrograde membrane flow model. They further feel that their model provides a more satisfactory explanation for the behavior of the boundary when cells are warmed up. As the authors note, the two models require diametrically opposed directions of surface flow. Abercrombie et al. (1970), who believed that they were not only observing but even measuring the rate of rearward flow in their carbon-particle experiments, would, I am sure, be surprised.

From what we know about GP80, there is no indication that this molecule acts as the cell's feet: however, the results of Ishihara et al. (1988) do demonstrate a problem for cytoskeletal models. If the feet are tethered inside the cell to the cytoskeleton, they, like GP80, would have a low diffusion coefficient and therefore might be more concentrated at the rear of the cell, and be nowhere near the leading front, which is where they are actually needed. In a flow model this problem is easily overcome by including the feet in the small group of recirculating proteins; once detached from the substratum, they would be transported through the cell to the leading lamella to be reused.

There are three additional points to note. (*a*) Two other rapidly diffusing proteins (Thy-1 and H-2) do not form a detectable gradient on moving fibroblasts. This can easily be interpreted in either scheme. (*b*) Moving cells may have a problem in undoing the interaction between their feet and the substratum. As mentioned above, cells need to attach to the substrate at their leading edges and detach at their rears. Cells moving on plastic frequently have long tails which seem to be stuck to the plastic at their rear-most tips. As the cell advances, the strain put on this tail increases, eventually causing the tail to snap. The cell then migrates away from its separated appendage. This behavior is likely to be an artifact of movement on plastic, in which nonbiological interactions between cell and plastic occur. When cells move in vivo or on a biological substrate (such as a thick layer of fibronectin), it is doubtful that they leave bits of themselves behind. If the interactions of the feet with a relevant substrate are strong at the leading front of the cell, there may have to exist a mechanism to undo those interactions at the rear of the cell. The fact that some proteins, such as GP80, can become localized to the rear of a moving cell could form the basis for such a mechanism. An "undoing" protein would be concentrated towards the rear of a moving cell, and in some unspecified manner assist in weakening the interaction between feet and substrate matrix. This would enable the detached feet to be moved once again to the front of the cell to be reused. (*c*) For those interested in endocytosis, there may be a hidden message. There are differing opinions on whether all endocytosis in fibroblasts is initiated by coated pits, or whether there is also substantial surface uptake by noncoated structures. This latter membrane internalization is widely assumed to involve random chunks of the plasma membrane; it is therefore nonselective and would include GP80 when it occurred in the rear region of a moving cell. If the membrane internalized by nonselective endocytosis were to be returned to the cell surface at the cell's leading edge, as is known to be the case for newly synthesized membrane (Marcus, 1962) and membrane internalized by coated pits (Bretscher, 1983), we should expect to find the endocytosed GP80 being returned to the cell surface at the leading edge. The paucity of GP80 at the front of a motile cell may therefore mean that noncoated pit endocytosis could, at most, account for only a small fraction of the total surface uptake by these cells.

Trying to figure out how an animal cell moves is necessarily an indirect science. You have to deduce from often rather remote observation how it may do so, and then test predictions for a particular model. Behind this is the belief that most animal cell locomotion – whether it be by fibroblasts, macrophages, or some amoebae – is based on the same set of molecular processes. Those who believed that the cytoskeleton, in the form of actin–myosin contractions, effects locomotion will have noted two papers (Knecht and Loomis, 1987; De Lozanne and Spudich, 1987) with surprise. In both, the major myosin gene of *Dictyostelium* has been inactivated, either by antisense RNA or by partial deletion. Although these cells have difficulty undergoing cytokinesis, locomotion appears to continue more or less normally. This latter observation reminds one of the earlier discovery that the highly motile amoeboid sperm of *Caenorhabditis elegans* have neither actin nor myosin (Nelson et al., 1982). This all suggests that these components of the cytoskeleton are not essential for driving the process of locomotion in some cells. Actin itself, I believe, plays a crucial role in providing a moving fibroblast with an internal structure. This structure, which determines the overall shape of the cell, including its leading lamella, could only be provided by a long polymeric molecule which has the abilities both to bundle and to assemble and disassemble rapidly. Actin seems to be ideal for this role.

There are relatively few observations that actually tell us something about how cells move. The paper by Ishihara et al. (1988) on page 329 is one of these.

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