On the shape of migrating cells — a 'front-to-back' model

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Summary

The wide range of shapes that are seen in stationary animal cells is believed to be the result of an interplay between giant filamentous complexes – largely the microfilaments and microtubules – although how this is achieved is unknown. In a migrating cell these large elements are also important, but here I suggest an additional factor: the cell surface distribution of those molecules that attach the cell to the substratum. As an animal cell advances, the attachments it makes with the substratum necessarily move backwards with respect to the cell. A fresh supply of these attachments – usually integrin molecules – is required at the cell front so that new attachments can be made. This supply is believed to be provided by the endocytic cycle, which enables the collection of integrins and other molecules from elsewhere on the surface of the cell to be recirculated to the front end of the cell. The rate at which a particular integrin cycles will determine its distribution on the ventral surface of the cell and this, in turn, might help to determine the shape of the cell. I also propose that adhesion molecules that have a slow rate of cycling will produce a flattish phenotype, as seen in fibroblasts, whereas a more rapid cycling will lead to a more snail-like shape. In addition, this model suggests why membrane ruffling occurs and that large non-circulating surface molecules move towards the back of the cell where they might assist in detaching the back end of the cell.

Key words: Cell shape, Integrin, Cytoskeleton, Membrane recycling, Leading lamella, Endocytosis, Exocytosis, Fibroblast, Front-to-back model

Introduction

Observation of the shape of individual mammalian cells is largely limited to using cells that are growing in culture, and shows that a single cell can rapidly change its shape and appearance. How does this happen? Is there some molecular hierarchy determining cell shape? The key molecules that are responsible for effecting the shape of a cell are well known: they are primarily the cytoskeletal structures – microfilaments, microtubules, intermediate filaments and their accessory proteins. These giant filamentous complexes can extend over distances of many micrometres, which is comparable to the size of most cells. To the outside observer, they clearly have a crucial influence in what a cell looks like. But how is their disposition achieved? A set of factors inside the cell are likely to act in a spatially determining way to set these shape-determining components on a particular path. These intracellular factors or signals will depend, in part, on extracellular information in the medium, from neighbouring cells or from basement membranes. At present, we know little about these signalling networks and the framework within which they operate, although some advances have been made in stationary cells (Bakal et al., 2007). In addition, a major screen using RNA-interference techniques has identified about 160 genes in *Drosophila melanogaster* cells that have a role in determining cell shape (Kiger et al., 2003), which indicates the complexity of the problem.

Despite this, there is one aspect of cell shape I should like to consider, and it concerns migrating cells. Again, although migrating cells come in many different forms, they mostly have a common feature – the leading lamella. This structure extends the cell forwards and appears to be the main motor for cell movement. Opinions differ over quite how this extension is caused, whether it is simply by actin polymerisation (Le Clainche and Carlier, 2008) or whether the addition of recycled membrane is also important (Bretscher, 1996a). Most are agreed that some recycled membrane is added at the lamella (Bretscher, 1984) or very close by (Hopkins et al., 1994). Not only is there experimental evidence for this, but an endocytic or exocytic cycle in which membrane is added to the leading tip during exocytosis would help to explain how those adhesion molecules that act as the 'feet' of the cell (usually integrins) can be replenished at the front of the cell (Fabbri et al., 1999; Lawson and Maxfield, 1995; Pierini et al., 2000). Without such a replenishment, the feet that are attached to the substrate would be moved towards the back of the cell as the cell advances, leaving the leading edge at the front of the cell devoid of adhesion molecules for making fresh attachments.

The actual mechanism by which integrins are internalised by the cell and returned to the front for making new attachments is mostly unknown, although it is widely assumed that some are taken up by clathrin-coated pits (and possibly other structures) and returned to the cell surface via the endocytic cycle. However, it is clear that different integrins on the surfaces of cells in suspension do circulate into and out of cells at quite different rates (Bretscher, 1989; Bretscher, 1992). Here, I pose the question of whether the rate of circulation of the feet of the cell – by whatever mechanism – contributes to the shape of a moving cell.

Surface distribution of circulating receptors

Different cell-surface receptors spend quite different lengths of time on the cell surface between rounds of endocytosis, hereafter referred to as the 'residence time'. This time is not easily measured, but estimates exist that clearly show that residence times vary from ~1

minute for the most rapidly circulating receptors, such as that for low-density lipoprotein (LDL) on fibroblasts (Anderson et al., 1976), to ~5 minutes for the transferrin receptor on HeLa (and many other) cells (Bleil and Bretscher, 1982), to ~10-20 minutes for the integrin αvβ3 in fibroblasts (Roberts et al., 2004) and ~20-70 minutes for integrin α5β1 in fibroblasts (Bretscher, 1989; Caswell and Norman, 2006; Roberts et al., 2004). If one examines the distribution of the most rapidly circulating receptors on large motile cells, the receptor is greatly concentrated towards the leading edge (Bretscher, 1983; Bretscher and Thomson, 1983). This is because the residence time is short compared with the time it takes for a particular receptor to diffuse well away from the leading edge. Thus, after exocytosis at the front of the cell, the receptor diffuses towards the back of the cell but, before it can get very far, it is endocytosed and returned to the leading edge; the receptor has become kinetically trapped near the front of the cell. This gives rise to a receptor gradient that is high at the leading edge and very low towards the back of the cell (see Fig. 1A for a cell in which the receptor is highly concentrated in the endocytic step, and Fig. 1B for a cell in which the receptor is less highly concentrated there). The steepness of this gradient might be expected to depend on several factors: most important are the residence time of the receptor (which is reflected in its concentration in the endocytic structure), the length of the cell and the diffusion coefficient of the receptor. In this context, 'length' means the contour length of the plasma membrane from front to back. If this distance is greater than that in which a free protein can diffuse during a residence time (for example, about 20 μm in 4 minutes for a membrane protein that has a diffusion coefficient of 3×10^{-9} cm^2/second), a gradient of that protein will exist. This is true not only for the LDL or transferrin receptors on motile mammalian cells (Bretscher, 1983), but also for the circulating vesicle-membrane-soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (v-SNARE) Snc1p in stationary (but polarised) cells of the budding yeast (Valdez-Taubas and Pelham, 2003).

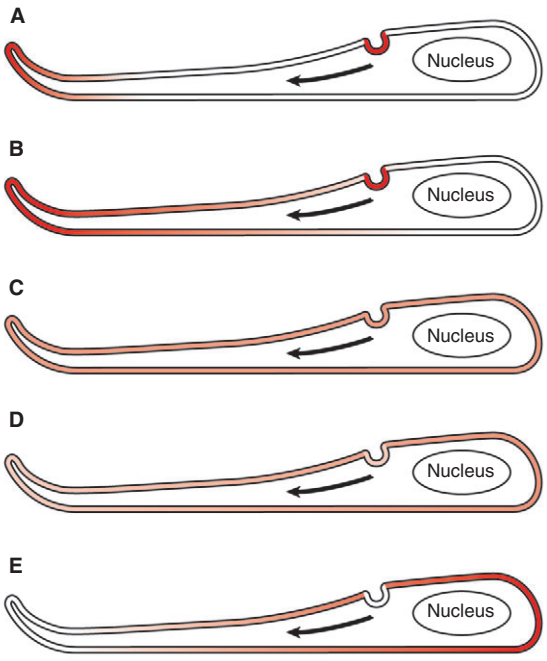

Fig. 1 Distribution of a circulating receptor on a locomoting cell. Shown in each part are an endocytic structure that contains a varying concentration of a recycling receptor (in brown) and the consequent concentration of this receptor on the cell surface. The expected distributions of: (A) a rapidly circulating receptor; (B) a more slowly circulating receptor; (C) a receptor that is neither concentrated nor depleted during endocytosis; (D) a receptor that is somewhat depleted from the endocytic cycle; (E) a receptor that is excluded from the endocytic cycle. The shapes of these gradients depend on the sites of endocytosis being randomly spread over the entire surface of the cell. In practice, the sites of endocytosis by clathrin-coated pits in tissue-culture cells are roughly randomly distributed, hence circulating receptors on moving cells are collected from all regions of the cell surface and transported to the cell front. The steepness of the gradient of a surface protein is expected to depend on its diffusion coefficient, the length of the cell and how long it resides on the cell surface compared with the time it takes for the cell to endocytose its entire surface. If the residence time is short, the surface protein will have a positive gradient (as in A); if it is very long, it will have a negative gradient (as in E). In either case, the steepness should be more accentuated in longer cells and by receptors that have lower diffusion coefficients.

A corollary of this kinetic trapping of circulating receptors is that a poorly circulating protein (Fig. 1D) or a non-circulating protein (Fig. 1E) should actually be swept backwards by the polarised endocytic cycle so that it has a reverse gradient (Bretscher, 1996b); the observation that a non-circulating protein is swept backwards was first demonstrated in yeast (Valdez-Taubas and Pelham, 2003). Again, the extent to which such a surface protein is swept backwards would be anticipated to depend upon the rate at which the endocytic cycle causes membrane lipids to flow backwards, the length of the cell and, most importantly, the diffusion coefficient of the receptor. Interpolating between these extremes, a very slowly circulating plasma membrane molecule – one that is neither concentrated nor depleted with respect to the endocytosed material – would be expected to have a flat gradient (Fig. 1C).

Adhesion-receptor circulation and cell shape

Now, turning to the circulation of integrins, how they circulate will affect where on the surface of a moving cell they are, and this may have an interesting bearing on cell shape. Thus, it follows that if a cell is attached to the substrate by a very slowly circulating integrin (Fig. 2C) – one that is neither concentrated nor depleted in endocytic structures (as in Fig. 1C) – the distribution of this integrin would be anticipated to be uniform and, provided it is active in binding to the substratum wherever it is on the cell, would lead the cell to be flattened out on the substrate. By contrast, if the integrin were more concentrated in the endocytosed and exocytosed membrane (and therefore more rapidly circulating when not attached to the substratum) the integrin would have a graded distribution: higher at the front of the cell and lower as one moves away from the front. Again, if all these surface integrins were active in binding the substratum the front of the cell would be firmly attached, but this attachment would weaken away from the front because of the paucity of receptors. In other words, the cell might have a more 'snail-like' shape: this is shown schematically in Fig. 2A,B, in which a steeper (Fig. 2A) and an intermediate (Fig. 2B) integrin distribution is shown.

We see cells that display the two extreme differences of shape in migrating fibroblasts and growth cones – which are usually remarkably flat – on the one hand, and neutrophils (and many other cells) – which have a much more lumpen character with a less extensive leading front – on the other hand (see Campello et al., 2006 and figure 2 within for an example).

At the other end of the range, in which the integrin barely circulates at all (as in Fig. 1D,E), the surface density of the integrin at the front may be so low that attachments there are tenuous or

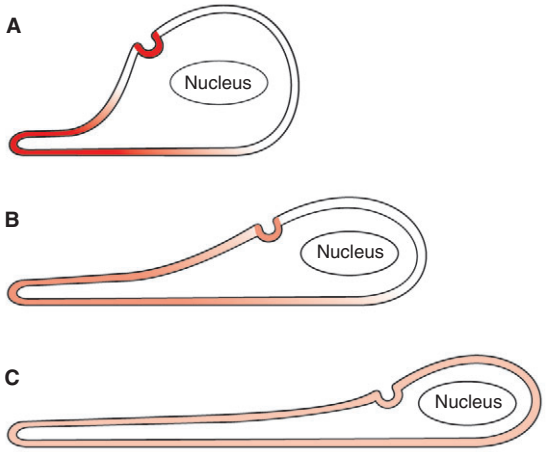

Fig. 2. Proposed dependence of cell shape on the endocytic properties of the attaching integrin. The circulating integrin is shown in brown and the cell is attached to the substratum only where the integrin has a sufficient concentration on the ventral aspect. (A) A quickly circulating integrin. (B) A more slowly circulating integrin. (C) An integrin that is neither concentrated nor depleted during endocytosis. The cell becomes less snail-shaped as the rate of circulation of the integrin decreases until the cell is quite spread out and flat.

too weak to be useful. In this case, the cell may try to move forwards, but cannot adhere sufficiently to do so. The forming lamella fails to adhere properly and may detach from the substratum. It is well known that the leading edges of some cells, particularly migrating fibroblasts, have difficulty obtaining a sufficient grip on the substratum. When this occurs the detached lamella is lifted up to form a 'ruffle' as a new leading edge extends beneath it.

There might be a further potential, quite hypothetical, consequence of a polarised endocytic cycle for migrating cells. As a cell advances, the interactions it makes at the back of the cell with the substratum need to be undone. How this is achieved we do not know, although there are several different views. My impression is that most of the interactions that integrins make with their biological ligands – epitomised by the fibronectin–fibronectin-receptor pair with a K_m of ~10^{-6M} (Akiyama and Yamada, 1985) – are moderately weak and might be pulled apart as the segment of membrane in which the integrin resides is endocytosed. However, it is also possible that a more complex 'undoing' machinery exists at the rear of moving cells to facilitate this detachment. A molecule on the cell surface that does not circulate and diffuses slowly will be swept towards the back of a moving cell (see Fig. 1E). Amongst such molecules might be proteins that bear heparan sulphate chains; these glycosaminoglycans have large extracellular domains that would reduce the ability of an individual protein to diffuse, which makes them candidates for such a detachment role. It is noteworthy that several components of basement membranes – such as fibronection, vitronectin and laminin – have binding sites for heparan sulphate.

If the suggestions I made here have any real meaning, one would anticipate that, if a migrating cell can be made to express variants of a particular integrin that have quite different circulating properties, the distribution of these on the cell surface should be quite different. Provided that these variants are active in binding the cell with similar affinities to the substratum, the shape of the migrating cell when expressing them should be quite different. There are several problems, however, in obtaining reliable data that could shed light on these conjectures. Perhaps the most restrictive is that most cell lines (such as NIH 3T3 fibroblasts) barely move. As stationary cells are believed to return cycling proteins back to their surfaces randomly, they are poor material for studies of polarised surface proteins. Quantitative measurements of the rate of circulation of most surface proteins has not been undertaken, reflecting the somewhat cumbersome methods that are currently available for measuring them (Bretscher, 1989; Schmid and Smythe, 1991). This is especially true of integrins. Furthermore, cells in culture often express more than one adhesion protein so that there is usually uncertainty about which molecules actually bind the cell facing the substratum. And finally, we know that the avidity of adhesion molecules for their substrates can be regulated (Hynes, 2002). All this makes testing the ideas suggested here less than straightforward.

Conclusions

The shape of a cell is ultimately controlled by internal structures and how this cell interacts with its exterior. The proposals made here are solely concerned with migrating cells – they imply that the speed with which the cell circulates its adhesion sites will partly determine how flat and spread out it is. They may also provide a basis for the processes of ruffling at the front of the cell and de-adhesion at its rear. These ideas do not suggest an easy test for their validity: the shape effects need to be studied in a fast-moving cell by varying the circulation rate of an adhesive protein by mutagenesis. At present too little is known about the actual mechanism by which these molecules are endocytosed and the signals on their cytoplasmic domains that determine the rate at which they do so. A greater understanding of the role of cell-surface proteoglycans might tell us whether they have a role in de-adhesion. The value of the ideas offered here is, in my view, in the way of thinking about moving cells.

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