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SPOTLIGHT

Do migrating cells need a nucleus?

Rhoda J. Hawkins 

How the nucleus affects cell polarity and migration is unclear. In this issue, Graham et al. (2018. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201706097>) show that enucleated cells polarize and migrate in two but not three dimensions and propose that the nucleus is a necessary component of the molecular clutch regulating normal mechanical responses.

The physical properties of the nucleus play increasingly recognized roles in cellular processes such as polarization and migration in addition to the nucleus containing DNA and gene regulatory machinery. The presence, position, and material properties of the nucleus and its connections with the cytoskeleton make it an important mechanical component of cells. However, experiments on cell fragments established the ability of cells to migrate on 2D substrates without a nucleus (Verkhovskiy et al., 1999). So what is the role of the nucleus in migration and is it essential? This question arises within the rapidly growing field exploring the interplay between mechanical and biochemical responses of cells. In particular, what are the mechanisms regulating mechanotransduction and the mechanosensitivity of cells? In this issue, Graham et al. show that the nucleus is not essential for 2D migration but is important for a cell to respond to its mechanical environment and migrate in 3D. Graham et al. (2018) revisit an older technique of removing the nucleus (Wigler and Weinstein, 1975) to produce cytoplasts, i.e., cells without nuclei (Fig. 1). They then use a variety of different modern assays to investigate the behavior of these cytoplasts.

First they establish that the nucleus is not required for cell polarization by measuring the position of the centrosome and Golgi on different micropatterns (using the technique pioneered by Théry et al. [2006]). This implies that the position of the nucleus is an effect rather than a cause of cell polarity. Graham et al. (2018) then verify that the nucleus is not essential for random nor directed 2D migration. The cytoplasts polarize and migrate on a 2D substrate by forming lamellipodia and undergo rear retraction just like intact cells. The cytoplasts are also able to migrate along gradients (e.g., of platelet-derived growth factor in a microfluidic setup). This shows that their chemotactic abilities are not reliant on the presence of the nucleus. In a scratch wound assay, cytoplasts are able to close the wound, albeit more slowly than intact cells. Interestingly, if the fibronectin density on the substrate is increased the cytoplasts are able to move as fast as intact cells. This difference

in fibronectin density-dependent velocity can be explained by a reduction in the number of integrins proportional to the smaller cell size of the cytoplasts compared with intact cells. Migration of cytoplasts on 2D micropatterned lines is similar to intact cells. However, consistent with the hypothesis of Petrie et al. (2017) that 3D migration requires the physical presence of the nucleus, Graham et al. (2018) find that cytoplasts are mostly immotile in 3D collagen gel compared with intact cells. Why are cytoplasts unable to migrate in 3D? The difficulty in answering this question arises from the fact that several factors are changed when comparing most 2D versus 3D experimental setups. Not only is the dimensionality changed but the amount of confinement, geometrical symmetries, and often matrix density and rigidity are too. In the study by Graham et al. (2018), they chose to focus on the fascinating question of how rigidity affects migration by investigating the 2D migration of their cytoplasts on a variety of substrate stiffnesses.

Migration velocity depends nonmonotonically on adhesion density and rigidity (Peyton and Putnam, 2005) with a velocity peak at intermediate values. Graham et al. (2018) show that removing the nucleus moves the velocity peak to higher density and rigidity. The peak velocity for intact cells is on a substrate rigidity of 8 kPa but this peak is surprisingly shifted to 25 kPa for the cytoplasts. The effect of removing the nucleus on migration velocity is mimicked by blebbistatin, implying that the contractility of the actomyosin cytoskeleton is also important. Graham et al. (2018) therefore suggest the increased rigidity necessary to achieve optimal migration speeds of cytoplasts is a result of their reduced contractility. In biaxial cyclic strain experiments, both intact cells and cytoplasts show increased phosphorylation of FAK after strain but this is at a lower level in cytoplasts, implying that the mechanosensitivity of focal adhesions decreases in the cytoplasts. Therefore, the nucleus may be necessary for regulation of contractility and mechanosensitivity such that a higher rigidity is necessary to activate mechanosensory pathways in its absence.

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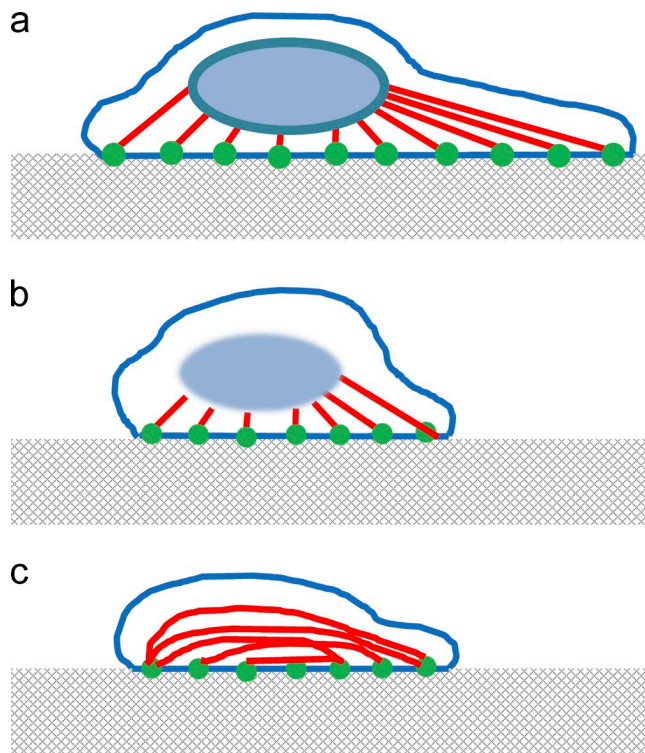


Figure 1. Cartoon to show cells migrating on a 2D substrate. Wild-type cells (a), cells lacking lamin A (b), and cytoplasts (cells with their nucleus removed; c). The substrate is depicted as a gray mesh, the cell membrane as a blue line, the nucleus in light blue with its lamina in dark blue, actomyosin cytoskeleton/stress fibers as red lines, and focal adhesions as green circles.

The fact that cells have a maximum migration velocity on intermediate adhesion densities can be explained by a balance between the strengths of adhesion and contractility. There is an optimum cell substrate attachment–detachment rate. Too low an adhesion density is insufficient for the cell to generate traction. Conversely, too high an adhesion density slows, or even prevents, the detachment of rear adhesions necessary for the cell to migrate. Increasing or decreasing actomyosin contractility shifts the optimal density higher or lower, respectively (Gupton and Waterman-Storer, 2006). An increased contractile strength means the cell will pull itself off the substrate more easily and therefore the optimal speed will be reached at higher adhesion strength. The cytoplasts generated by Graham et al. (2018) are smaller than the intact cells (Fig. 1), which means a higher density of extracellular matrix is needed to achieve the optimum adhesion strength.

There is also an optimum substrate rigidity for cell migration, which increases monotonically for lower adhesion. If the substrate is too soft, a cell cannot generate enough traction for fast migration. This is partly because cells spread more with increasing rigidity so on soft substrates the cell has lower total adhesion. As the cell tries to move, it will deform a soft substrate instead of moving forward. Deformation of the substrate also prevents the mechanosensitive reinforcement of focal adhesions and stress fibers (Peyton and Putnam, 2005). These factors together mean the cell is unable to move fast on soft substrates. If the substrate is too stiff, the increased spreading and mechanosensitive

reinforcement of focal adhesions will mean the adhesion becomes too strong for the cell to detach quickly enough for fast migration. The optimum rigidity is achieved when the contractile tension generated between the nucleus and the substrate and the mechanosensitive reinforcement of focal adhesions are optimal for attachment and detachment. Without the nucleus, the optimum rigidity is stiffer and the contractile tension balance is now between the substrate and the cytoplasm. There is nothing but cytoplasm to pull off and the cytoplasm is very deformable, so the substrate will be deformed far less in comparison. This means generating traction will be harder. Therefore, a stiffer substrate is needed to activate the mechanosensitive strengthening of focal adhesions and stress fibers and generate traction. Additionally, the decreased size of the cytoplasts means the adhesion surface is smaller and therefore weaker, necessitating further enhancement on stiffer substrates.

These explanations for the increased adhesion and rigidity required for the cytoplasts to reach maximum speed are consistent with traction force microscopy measurements made by Graham et al. (2018). Cytoplasts are less able to contract collagen gel than intact cells and exert smaller traction stress, resulting in lower total strain energy in the substrate, which fits with the argument that cytoplasts deform themselves more so than the substrate. Therefore, this work suggests that the nucleus increases the traction forces a cell can exert on a substrate.

LINC complex (Sun1 and Sun2) depleted cells mimic the cytoplasts with decreased traction stress and strain energy, which is to be expected because these cells have lost the connection between their nucleus and cytoskeleton. Unexpectedly, removing lamin A to soften the nucleus, while decreasing the strain energy, did not decrease the traction stress. The reason for this is unclear but Graham et al. (2018) suggest the possibility that it is a result of the difference between a soft nucleus that is still attached to the cytoskeleton as opposed to one that is disconnected or absent. However, they acknowledge other possible explanations such as the removal of lamin A affecting other signaling pathways. Interestingly, Emerson et al. (2009) report that lamin A mutants spread less. This means Lamin A mutant cells have a lower adhesion strength, so they will require stiffer substrates to activate sufficient mechanosensitive strengthening of focal adhesions to achieve the optimum detachment rate. A smaller spread area will result in lower total strain energy even if the traction stress is not reduced. The presence of even a soft nucleus enables sufficient contractile tension to be generated between the nucleus and the substrate such that the resulting traction is the same for cells without lamin A as for wild-type cells. This suggests the cell contractility is regulated such that the contractile strength increases in the lamin A mutants to achieve the same traction force as wild-type cells.

In summary, the peak migration velocity on varying substrate stiffness increased (from 8 kPa to ≥ 25 kPa) upon loss of the nucleus, nuclear–cytoskeleton connections, lamin A, or contractility (by blebbistatin). Together, these findings imply a common force transduction mechanism determining the migration response to rigidity. Graham et al. (2018) postulate that the nucleus, LINC complexes, and lamina are part of an integrated “molecular clutch” (Case and Waterman, 2015) system with focal

adhesions and stress fibers. However, this clutch constitutes not just a “large-scale mechanosensing mechanism” (Trichet et al., 2012) but one on the scale of the whole cell.

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