MEK signalling tunes actin treadmilling for interstitial lymphocyte migration

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The EMBO Journal (2010) 29, 2861–2863. doi:10.1038/emboj.2010.183

When lymphocytes follow chemotactic cues, they can adopt different migratory modes depending on the geometry and molecular composition of their extracellular environment. In this issue of *The EMBO Journal*, Klemke *et al* (2010) describe a novel Ras-dependent chemokine receptor signalling pathway that leads to activation of cofilin, which in turn amplifies actin turnover. This signalling module is exclusively required for lymphocyte migration in three-dimensional (3D) environments, but not for locomotion on two-dimensional (2D) surfaces.

Since Abercrombie established the classical textbook paradigm of cell motility in 1970, not only have many variants of the proposed 'protrusion–adhesion–contraction' cycle been described, but also entirely different principles of force generation and transduction have been discovered—such as collective cell migration and blebbing motility (Friedl and Wolf, 2010). Intuitively it makes sense that different types of cells that reside and migrate in different tissue environments use different locomotory strategies: a keratocyte is optimized for migration on 2D substrates, whereas a fibroblast usually migrates in a scaffold of 3D extracellular matrix fibres; epithelia often migrate as cell sheets and leukocytes always as single cells. It is, however, less intuitive that one specific cell type can adopt different modes of motility depending on the type of environment it is exposed to. From a teleological perspective it seems somewhat curious that a fibroblast can migrate efficiently on a 2D glass cover slip, although it never sees a flat and stiff surface within the organism. For leukocytes, such an inbuilt plasticity program appears to make more sense: these cells are not organized into stable organs but rather behave like single-celled organisms that constantly swarm through the whole body. To do so they have to interact with various extracellular environments, sometimes planar, sometimes 3D. For example, the extracellular compositions of the central nervous system versus mesenchymal tissues have only few molecular components in common, yet leukocytes migrate with similar efficiency once embedded in either substrate.

One scenario how the cells could adapt to different tissues is biochemical accommodation: here, the cells 'feel' their environment and modulate their proteome (specifically their cytoskeletal machinery) accordingly. As such adaptation is slow and costly, it would be much more effective to employ a machinery that can instantaneously shift between different

locomotion modes. Indeed, this is what leukocytes do: they move from 2D to 3D environments and back without making breaks for accommodation (Renkawitz *et al*, 2009).

In this issue of *The EMBO Journal*, Klemke *et al* (2010) describe a novel signalling module that dissects some features of the migratory plasticity of lymphocytes: inhibition of this pathway leaves T-cell migration on 2D surfaces undisturbed, while migration in 3D interstitial environments is almost completely blocked. It is shown that the chemokine stromal cell-derived factor-1, via the G α i subunit of CXCR4, triggers the small GTPase Ras, the activity of which remains constrained to the leading edge of the cell. Ras then activates the mitogen-activated protein kinase kinase (MEK), which finally leads to dephosphorylation and hence activation of cofilin (Figure 1). Active cofilin has actin severing activity and is known to amplify actin treadmilling by two possible mechanisms: generating free barbed ends at the leading edge of the cell and releasing monomeric actin, which is then available for filament elongation or novel nucleation (DesMarais *et al*, 2005; Le Clainche and Carlier, 2008).

The MEK-dependent signalling module is only one signalling branch that is triggered by active Ras. Entirely inactivating Ras, which is likely to be a very proximate output of chemokine receptor signalling (Thelen and Stein, 2008; Charest *et al*, 2010), blocks any form of motility. However, blocking MEK activity and thereby cofilin activation (or knocking down cofilin itself) only affects lymphocyte migration in 3D environments but not on 2D surfaces, even though the molecular composition of the substrate remained identical.

What causes such selective requirement for cofilin activity in 3D? One obvious possibility is that lymphocytes turn over less actin in 2D than in 3D. Indeed, one recent study showed that on low adhesive 2D surfaces lymphocytes adopt a migratory mode termed 'walking'. Here the cells move in a caterpillar-like manner by alternating cycles of leading edge extension and trailing edge contraction (Jacobelli *et al*, 2009). Although it remains to be shown exactly how the protrusions are formed in walking cells, such motility obviously lacks the retrograde streaming of actin filaments seen in cells that move with a classical lamellipodium. When contractility was blocked in walking cells they started 'sliding', meaning that they switched to the lamellipodial principle. Klemke *et al* (2010) show that the sliding mode is cofilin- (and MEK-) sensitive, while the walking mode is not. They further

Figure 1 A Ras-MEK-cofilin module exclusively regulates 3D T-cell migration. Schematic views of 3D versus 2D migration modes of T lymphocytes. Shown are side views of a T lymphocyte migrating in 3D interstitial space (top) or on a flat 2D surface such as an endothelial lining (bottom). The direction of movement is indicated by an arrow. During 3D migration, T cells make multiple protrusions at the leading edge and 'slide' through the narrow interstitial pores. Binding of the chemokine SDF-1 to CXCR4 triggers Ras activation via G α i and this in turn leads to MEK-dependent cofilin dephosphorylation. Migration in 3D is mainly driven by actin polymerization and therefore the localized severing and depolymerizing activity of dephosphorylated cofilin is critical to initiate cellular protrusions. Chemokine-induced T-cell migration on 2D surfaces on the other hand is guided by an interplay of adhesion and contraction, resulting in a 'walking' mode that requires Ras, but not necessarily MEK activation and cofilin dephosphorylation.

demonstrate that when G-actin levels are pharmacologically lowered, the cofilin dependence increases. All this strongly suggests that high actin turnover rates are required for migration in 3D environments and that under conditions of high actin turnover MEK-dependent cofilin activation gains importance.

Apart from the mechanistic implications for lymphocyte motility, this paper teaches an important lesson about studying cell migration: you have to know your cells very well in order to correctly interpret the outcomes of genetic or pharmacological interference. Cells can adapt not only to changes of the external environment but also to perturbation of their own machinery by adopting an alternative migration style. Such responses can certainly not be captured simply by counting cells in the lower compartment of a transwell assay. Even video observation of cell morphology has its limits and the ultimate goal should always be to observe cytoskeletal dynamics directly. In this respect there is still

much to learn about lymphocytes: it will be important to see how exactly force is generated when T cells migrate in different environments. However, it is technically extremely demanding to study actin flow in 3D, and techniques like speckle microscopy are still restricted to studies in 2D.

Going forward, it would be interesting to know how the cofilin activation cycle is tuned when the cells switch between migratory modes. Jacobelli *et al* demonstrated that adhesiveness is also involved in the walking/sliding switch. Does adhesion eventually feed into the MEK pathway to tune actin treadmilling? Another potential regulator that dampens cofilin activity is ROCK signalling, which is closely coupled to mechanoresponses and would therefore be well suited to physiologically regulate actin treadmilling. Finally, the position of Ras in chemokine receptor signalling is still poorly investigated in lymphocytes and the finding that inhibition of Ras itself blocks any kind of motility begs the question which other downstream modules are triggered.

Acknowledgements

MW is supported by the Humboldt Foundation and MS by the Peter Hans Hofschneider Foundation for Experimental Biomedicine.

Conflict of interest

The authors declare that they have no conflict of interest.

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