CELL MIGRATION

Coordinating calcium signalling

Directed cell migration requires local Ca^{2+} pulses near the leading edge of the cell to activate myosin and to modulate focal adhesions. This study details the coordination between, and polarized spatial localization of, components of the calcium signalling machinery that is required to promote migration.

The details of calcium signalling are well known. Briefly, receptor tyrosine kinase activation by chemoattractants induces phospholipase C (PLC) to generate inositol-1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (DAG). The binding of $InsP_3$ to its receptor in the endoplasmic reticulum (ER) releases Ca^{2+} from ER stores, which activates myosin light chain kinase (MLCK; also known as MYLK). The Ca^{2+} pulse is terminated by the removal of Ca^{2+} from the cytoplasm through the plasma membrane by PMCA (plasma membrane Ca^{2+} ATPase) or its return to the ER by SERCA (sarcoendoplasmic reticulum Ca^{2+} ATPase). Stromal interaction molecule 1 (STIM1) also restores ER Ca^{2+} levels by activating Ca^{2+} influx channels at plasma membrane–ER junctions. However, it was not known how Ca^{2+} signalling is coordinated to achieve directional migration.

Using live cell imaging of the collective migration of human umbilical vein endothelial cells in response to basic fibroblast growth factor, the authors showed that phosphotyrosine signals were highest in the front of leader cells (those cells at the front of the group). This indicates that receptor tyrosine kinase signalling, and therefore PLC activation, might be polarized in leader cells. Indeed, levels of membrane-localized DAG were higher in the front than at the back of leader cells, and there was a higher frequency of local Ca^{2+} release in the front of these cells.

Surprisingly, however, the average cytosolic Ca^{2+} level was lower in the front than at the back of leader cells. Inhibition or overexpression of Ca^{2+} regulators to increase or decrease cytosolic Ca^{2+} levels showed that there is an inverse relationship between basal Ca^{2+} levels and migration speed. The authors suggest that lower basal Ca^{2+} levels at the leading edge prevent persistent MLCK activation and enable smaller pulses of local Ca^{2+} release to have a functional effect. They showed that an increase in Ca^{2+} level of less than twofold at the front of the cell can activate MLCK and hence myosin. Low basal Ca^{2+} levels at the front of cells were maintained by PMCA; inhibition of PMCA, but not of SERCA, in migrating cells decreased the Ca^{2+} gradient between front and back and also decreased migration. The pump activity of PMCA was calculated to be significantly higher at the front than at the back of migrating cells.

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The increased frequency of Ca^{2+} release from the ER at the front of leader cells resulted in a decrease in Ca^{2+} in the ER lumen. ER Ca^{2+} levels must be replenished if migration is to be maintained; in support of this, the enrichment of STIM1 in the front of migrating leader cells was required to support cell migration. The polarization of STIM1 localization was mediated by its binding to the microtubule plus-end protein EB1. Also, STIM1 accumulated to a greater extent at ER–plasma membrane junctions in the front of cells, which is indicative of increased Ca^{2+} influx.

Finally, the authors showed that the DAG gradient from the front to the back of leader cells also helps to increase the speed of migration. Increasing the intracellular concentration of DAG augmented the speed of leader cells in a concentration-dependent manner. Inhibiting the DAG target protein kinase Cβ (which normally promotes actin polymerization) decreased migration speed.

So, the results suggest a model in which localized receptor signalling and PLC activation lead to increased DAG production and a higher frequency of Ca^{2+} pulses at the front of leader cells, both of which support increased migration. The efficacy of intracellular Ca^{2+} signalling in promoting such migration requires the coordinated activities of PMCA and STIM1 to decrease cytosolic Ca^{2+} levels and to replenish ER Ca^{2+} stores, respectively.

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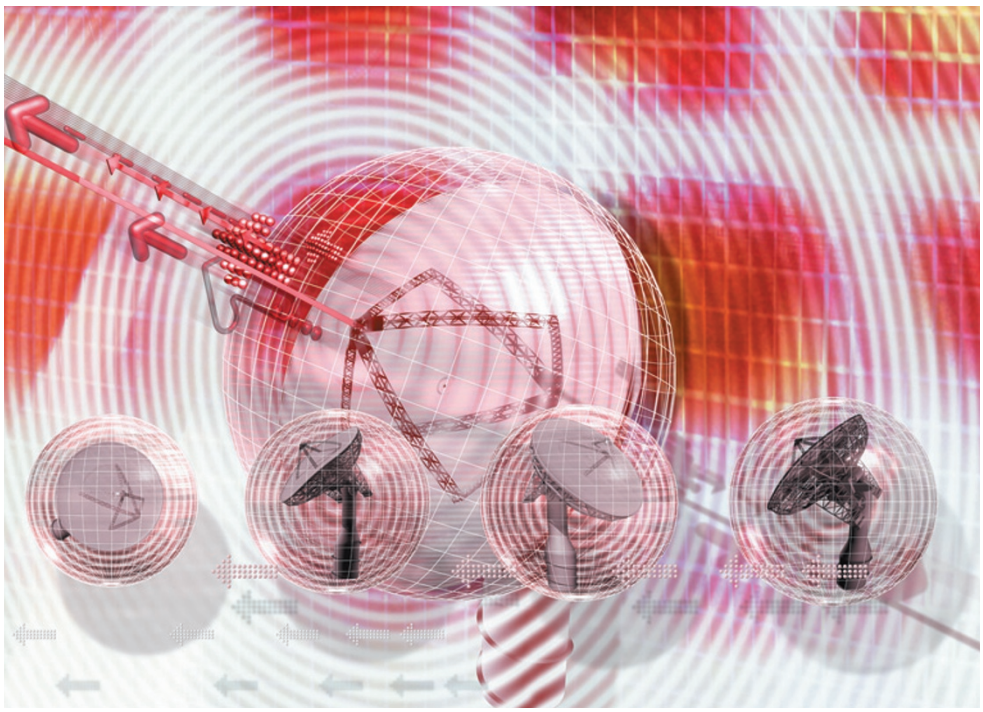

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ORIGINAL RESEARCH PAPER Tsai, F.-C. *et al.* A polarized Ca^{2+}, diacylglycerol and STIM1 signalling system regulates directed cell migration. *Nature Cell Biol.* **16**, 133–144 (2014)