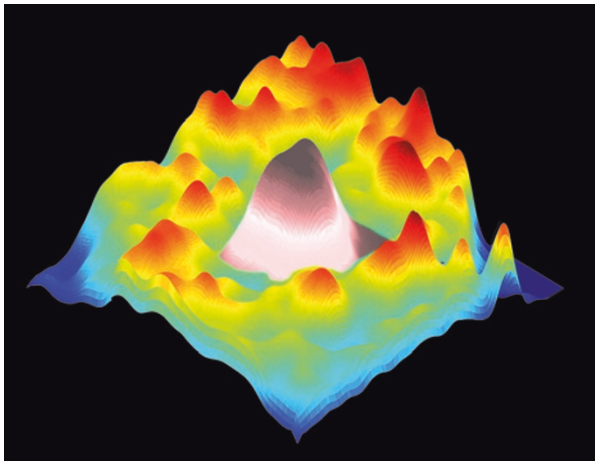
CYTOSKELETON

RhoC invades cofilin's space

> p190RhoGAP and p190RhoGEF spatially regulate RhoC activity at invadopodia.

Invadopodia are membrane protrusions containing filamentous actin that produce matrix metalloproteinases to degrade extracellular matrix (ECM) during tumour cell invasion. The actin-severing activity of cofilin, which creates free barbed ends for actin polymerization and filament turnover, is essential for the maturation of invadopodia. The phosphorylation of cofilin inhibits its severing activity, and Bravo-Cordero *et al*. now reveal that RhoC activity promotes cofilin phosphorylation around, but not inside, invadopodia, to restrict where actin polymerization takes place.

As RhoC expression positively correlates with tumour invasion, the authors investigated its role in invadopodial protrusion. Small interfering RNA (siRNA)-mediated depletion of RhoC from MTLn3 carcinoma cells decreased invasion and the length of invadopodia. Surprisingly, the invadopodia of RhoC-depleted cells were more efficient at degrading ECM. In an effort to explain this discrepancy, the authors analyzed the ultrastructure of these invadopodia. They did not protrude deeply into the matrix and, as opposed to the focal and unbranched invadopodia of control cells, their structures were branched and unfocused. Thus, RhoC-depleted cells are unable to protrude into the matrix to degrade it.



RhoC activity increases around the invadopodial core structure, as shown by this plot of the maximum projection over time of RhoC activity. Pseudocolour shows low RhoC activity levels (blue) to high RhoC activity levels (red) in relation to low (white) and high (brown) cortactin intensity; cortactin is an invadopodium marker. Image courtesy of J. J. Bravo-Cordero, Albert Einstein College of Medicine of Yeshiva University, USA.

As RhoGTPases are spatiotemporally regulated, the authors next looked at the localization of RhoC activity using a novel fluorescence resonance energy transfer biosensor. RhoC activity surrounded invadopodia but was largely excluded from the protrusions themselves. Depletion of p190 Rho GTPase-activating protein (p190RhoGAP), a negative regulator of RhoC, resulted in active RhoC in invadopodia. p190 Rho guanine nucleotide exchange factor (p190RhoGEF) was enriched around invadopodia, where it positively regulated RhoC activity. Thus, p190RhoGAP and p190RhoGEF spatially regulate RhoC activity at invadopodia.

But, what is spatially regulated by RhoC at invadopodia, and how does this influence invadopodial structure? RhoGTPases activate Rho-associated coiled-coil kinase (ROCK), which activates LIM domain kinase (LIMK) to phosphorylate cofilin on Ser3 (pcofilinS3). The authors found that, in control cells, cofilin and pcofilinS3 are enriched inside and outside invadopodia, respectively. Depletion of RhoC decreased pcofilinS3 outside these structures, suggesting that RhoC spatially regulates pcofilinS3. As knockdown of LIMK1 and LIMK2 also reduced pcofilinS3 levels, a RhoC–ROCK–LIMK pathway probably leads to the phosphorylation and inactivation of cofilin. To confirm that RhoC influences cofilin activity, the authors assessed the actin barbed ends in the invadopodia of cells in which RhoC was depleted. These had an increased number of actin barbed ends, probably owing to the inability of these cells to inhibit the severing activity of cofilin outside the invadopodia. The authors propose that: "Cells that cannot focus their cofilin activity would show defects in actin polymerization within the invadopodia core, leading to abnormal branched invadopodia …"

Thus, RhoC activity is spatially regulated to ensure that cofilin remains unphosphorylated in the invadopodial core to generate cofilin-dependent barbed ends in a focused manner. It will be interesting to see whether this mechanism contributes to the role of RhoC in metastasis.

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ORIGINAL RESEARCH PAPER Bravo-Cordero, J. J. *et al.* A novel spatiotemporal RhoC activation pathway locally regulates cofilin activity at invadopodia. *Curr. Biol.* **21**, 635–644 (2011)