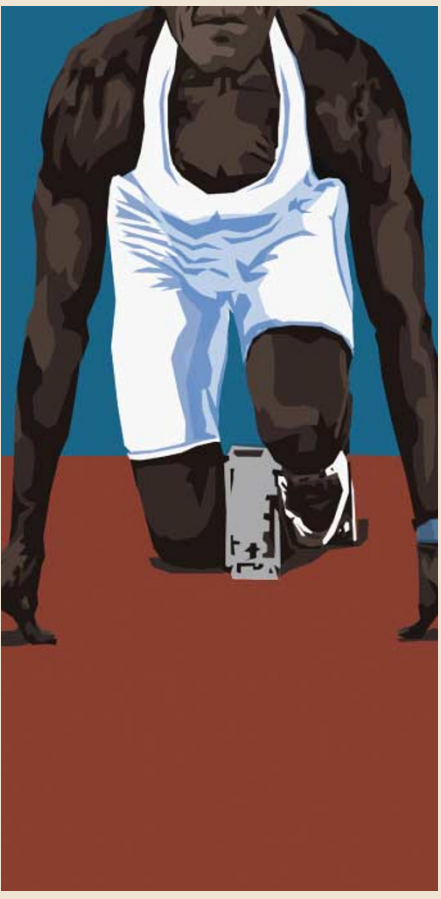
MreB was depleted and then re-expressed, although polar foci of PleC and DivJ were restored, these were located at the wrong pole in ~50% of cells. This indicates that when MreB is depleted, cells lose all memory of their initial polarity, so that when MreB is re-expressed polarity becomes randomized. MreB must therefore be required for the initial decision that determines which pole of the cell is which.

This study shows intriguing similarities between the establishment of polarity in *C. crescentus* and the corresponding processes in eukaryotic cells, in which actin has a central role. It will be interesting to see whether similar mechanisms operate in other bacteria.

Louisa Flintoft
Assistant Editor, Nature Reviews

References and links
ORIGINAL RESEARCH PAPER Gitai, Z., Dye, N. & Shapiro, L. An actin-like gene can determine cell polarity in bacteria. *Proc. Natl Acad. Sci. USA* **101**, 8643–8648 (2004)
WEB SITE
Lucy Shapiro's laboratory:
http://caulo.stanford.edu/shaplab/



CYTOSKELETON

A new pathway to explore

When placed in a new environment, a cell will extend long actin-rich structures called filopodia to help explore its surroundings, especially whilst it's spreading. The tyrosine kinase Abl promotes these finger-like filopodia, and Woodring *et al.* now suggest that this is achieved by Abl signalling to actin at the cell periphery through the phosphorylation of Dok1, which then recruits the adaptor protein Nck.

Filopodia formation is dependent on Abl being active, so the hunt for an Abl substrate began. Using the Src-homology-2 (SH2) domain of Abl as an affinity matrix in lysates from spreading fibroblasts, the authors detected a group of phosphotyrosine-containing proteins in the ~60-kDa range. The 62-kDa Dok1 protein is tyrosine phosphorylated in Abl-transformed cells, so the authors checked whether Dok1 might be one of the proteins in this group. A series of experiments confirmed that Dok1 was indeed a substrate for Abl during cell spreading.

How, then, does Abl affect Dok1? The authors studied this using a series of point mutants in which tyrosine residues were mutated to non-phosphorylatable phenylalanines. The mutants were expressed in spreading mouse embryonic fibroblasts that were null for both Abl and Arg (a related protein kinase), or in the same cells reconstituted with Abl. All the constructs apart from Y361F Dok1 had higher levels of tyrosine phosphorylation in the latter cell type, indicating that Abl-mediated phosphorylation of Dok1 Y361 occurs during cell spreading, a result that was confirmed by *in vitro* biochemical assays.

After finding that there was a positive correlation between Abl activity, the number of actin microspikes (filopodial precursors) and the amount of tyrosine-phosphorylated Dok1, Woodring *et al.* investigated whether Dok1 could enhance the number of filopodia that were induced by Abl in spreading fibroblasts. Overexpressing Dok1 could not increase filopodia formation in *Abl*- and *Arg*-null cells but could do when Abl was re-expressed in these cells. Expressing Y361F Dok1 did not enhance the number of filopodia in either case. The authors also noticed fewer filopodia in $Dok1^{-/-}$ fibroblasts, and this number could not be increased by Abl expression. Furthermore, treatment of wild-type fibroblasts, but not $Dok1^{-/-}$ fibroblasts, with an Abl inhibitor decreased the number of filopodia. So, Dok1 and Abl seem to be required for each other's effects in forming and maintaining filopodia during cell spreading. Consistent with this, Dok1 and Abl were both present along and at the tips of filopodia.

Finally, because Dok1 phosphorylated on Y361 can interact with p120RasGAP or Nck, Woodring *et al.* carried out immunoprecipitation assays using lysates from spreading cells and found an Abl-stimulated Dok1–Nck association. $Nck1^{-/-}$ $Nck2^{-/-}$-spreading cells had fewer filopodia and, whereas the Abl inhibitor reduced the number of filopodia in wild-type cells, it didn't in $Nck1^{-/-}$ $Nck2^{-/-}$ cells, implying that Nck is involved in the Abl-mediated formation of filopodia. There were hints that a ternary complex of Abl, Dok1 and Nck could form in cells, so the authors propose that Abl-mediated phosphorylation of Dok1 on Y361 results in the recruitment of Nck to the cell periphery where localized actin polymerization occurs. But how the cell decides where to extend a filopodium, and what lies downstream of the Dok1–Nck complex to drive actin assembly, are unknown entities.

Katrin Bussell

References and links
ORIGINAL RESEARCH PAPER Woodring, P. *et al.* c-Abl phosphorylates Dok1 to promote filopodia during cell spreading. *J. Cell Biol.* **165**, 493–503 (2004)
FURTHER READING Hernandez, S. *et al.* How do Abl family kinases regulates cell shape and movement? *Trends Cell Biol.* **14**, 36–44 (2004)