CELL POLARITY

Polar exploration

Many bacteria show polar characteristics, such as the positioning of a flagellum at one end of the cell. However, the mechanisms involved in establishing this polarity are poorly understood. A recent study from Lucy Shapiro and colleagues has made important progress in understanding these processes by identifying a master regulator of polarity in *Caulobacter crescentus*.

The *C. crescentus* life cycle involves two different cell types, both with specialized structures located at one pole of the cell. Several proteins that are involved in the development of these structures and that have corresponding polar distributions have been identified, providing useful markers of polarity. Shapiro and colleagues made use of these features to investigate whether the actin-like protein MreB is required for polarization in *C. crescentus*.

MreB has a distinctive localization pattern, forming a spiral structure that extends along the length of *C. crescentus* cells. By analogy to eukaryotic actin, MreB molecules might have an intrinsic polarity, so the spirals they form could be used for the asymmetric localization of molecules required for the development of polar structures. To test this, the authors analysed the effect of MreB depletion on the distribution of four signalling proteins — PleC, DivJ, CckA and DivK — that are required for polar development in *C. crescentus*. Depletion of MreB abolished the polar foci that are usually formed by all four proteins at certain points in the cell cycle, consistent with a role for MreB as a global regulator of polarity.

Importantly, MreB seems to be actively required for specifying polarity, rather than having a passive role in protein localization. Unlike CckA and DivK, which form foci at both poles of *C. crescentus* cells, PleC and DivJ are asymmetrically distributed at certain points in the cell cycle, localizing to only one pole. When

CYTOKINESIS

A good place to start

A new report in *Science* describes the rapid identification and characterization of proteins that function in cytokinesis, using an approach that combines functional-proteomic and comparative-genomic analysis.

From synchronized Chinese hamster ovary (CHO) cells, Ahna Skop and colleagues isolated midbodies — microtubule-rich, transient structures that are derived from the spindle midzone and that exist after cell division, just before the daughter cells detach. The proteins in the midbody preparation were identified by a protein identification technology — MudPIT — that is used for the analysis of proteins in complex protein mixtures.

After eliminating proteins that have general housekeeping functions, 160 candidate midbody proteins remained, of which 57 were known cytokinesis proteins. Most candidate midbody proteins seemed to be conserved in evolution as 147 out of 160 proteins had clear *Caenorhabditis elegans* homologues. The functional relevance of the candidate midbody proteins became apparent when the authors analysed gene function by RNA interference using double-stranded (ds)RNA that corresponded to each of the *C. elegans* genes. Most of these dsRNAs produced a disrupted cytokinesis phenotype.

By analysing the various mutant phenotypes, the authors found that a significant percentage of midbody proteins that function in cytokinesis also produce defects in germline development when they are disrupted. They went on to show that gonad development and sterility mutant phenotypes are, in fact, frequently caused by defects in cytokinesis in the germline or in early embryos. In addition, 16 proteins that are essential for embryo and germline cytokinesis were also required for polar-body extrusion (the polarized, asymmetric cell division that occurs during meiosis). So, cytokinesis, gonad organization and polar-body extrusion seem to use a common set of proteins.

Although membrane and cytoskeletal proteins are known to be involved in cleavage-furrow formation, among other cytokinesis processes, how these proteins are recruited to the cleavage plane has been unclear. Skop and co-workers identified 40 membrane and cytoskeletal proteins, which include proteins that are involved in lipid-raft formation and vesicle trafficking. They suggest that raft-associated factors could target and activate specific membrane events in cytokinesis.

Among the midbody proteins, 24% were Golgi-associated proteins, which led the authors to speculate that there might be some parallels between cytokinesis in animals and plants, as Golgi-derived vesicles are involved in cell-wall formation after cell division in plants.

Finally, the functional analysis of midbody proteins revealed some unexpected phenotypes — 20% of the identified proteins caused defects in coordination, which implicates these proteins in muscle or neuronal development. In addition, 14% of the mammalian proteins that were identified are known to have a role in human diseases, mostly those that are associated with membrane and cytoskeletal pathologies. So this study provides plenty of starting points for further investigations — including into cytokinesis, development or disease mechanisms.

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References and links

ORIGINAL RESEARCH PAPER Skop, A. R. *et al.* Dissection of the mammalian midbody proteome reveals conserved cytokinesis mechanisms. *Science* 27 May 2004 (doi:10.1126/science.1097931)

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.

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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
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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.

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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)