a membrane-anchored form, RNAi of Dlp didn't block the signal response, which indicates that Dlp might usually concentrate the Hh signal.

Indications from the library screens were that CK1α might control basal pathway activity, as dsRNA increased basal reporter activity. Ci was required for this increase, whereas Smo and Fu were not, implying that CK1α is upstream or at the level of Ci, but downstream of Smo and Fu.

As a regulator of the basal activity of Hh and Wg signalling pathways, CK1α could function as a tumour suppressor in several cancers that are associated with overactivation of one or the other pathway. Similarly, deletion of a human chromosomal region that contains GPC6, a mammalian glycipan member that is closely related to Dlp, is associated with many human malformations. So, too, are mutations in one of the two remaining genes identified in the screen that had not previously been implicated in Hh signalling. Hopefully, discovering the functions of these genes in the Hh pathway won't be too prickly a problem!

*Katrin Bussell*


**References and links**

**ORIGINAL RESEARCH PAPER** Lum, L. *et al.* Identification of Hedgehog pathway components by RNAi in *Drosophila* cultured cells. *Science* **299**, 2039–2045 (2003)


CYTOKINESIS

# Time to separate?

At the end of cell division, daughter cells separate from each other by a process known as cytokinesis. Now, in a report in *Science*, Timothy Mitchison and colleagues establish the usefulness of a fast-acting and reversible inhibitor in beginning to uncover the mechanisms involved in the spatial and temporal control of cytokinesis in mammalian cells.

Using a high-throughput screening assay to find small-molecule inhibitors of nonmuscle myosin II — a major component of the cytokinesis furrow that provides the force needed for furrow ingression — the authors identified blebbistatin. They found that blebbistatin disrupted cytokinesis in vertebrate cells by the rapid and reversible inhibition of ingression. But assembly of the furrow and the microtubules that position it was similar to controls. The timing of mitotic exit was also unaffected, with chromosomal decondensation and nuclear envelope re-formation taking place as normal.

Mitchison and colleagues then investigated the timing of C phase — the period during which cytokinesis can occur. At around one hour, the duration of C phase in blebbistatin-arrested cells was similar to that in cells treated with an actin-depolymerizing drug, and the authors concluded that "…an unidentified cell-cycle signal terminates C phase…". C phase is triggered by the start of anaphase and requires the anaphase-promoting complex and ubiquitin-mediated proteolysis. So, Mitchison and colleagues tested whether a proteasome inhibitor, MG132, had any effect. Indeed, in the presence of blebbistatin, MG132 significantly increased the percentage of binucleate cells retaining a myosin II ring, and the authors estimated that MG132 more than doubles the length of C phase. In addition, these cells showed abnormal organization and localization of myosin II and another major furrow component, anillin. So, ubiquitin-dependent proteolysis is important for C-phase exit in mammalian cells.

Next, the authors examined the spatial control of cytokinesis. They arrested cells in mitosis using monastrol (an inhibitor of the mitotic kinesin Eg5), released them into blebbistatin to initiate anaphase and furrow assembly, and then tested the effects of several drugs. By depolymerizing microtubules with nocodazole, Mitchison and colleagues showed that continuous microtubule-to-cortex communication is necessary for maintaining the localization of furrow components. In these cells, separated nuclei collapsed back together, indicating that the midzone is necessary to keep the nuclei apart until cytokinesis is completed.

The addition of staurosporine, a broad-spectrum kinase inhibitor, caused extensive disorganization of midzone microtubules. So, the authors looked at the effects of inhibiting the kinase aurora B, which has been implicated in midzone organization. ATP competitive inhibitors of aurora B produced similar results to staurosporine. In addition, staurosporine and a Rho-kinase inhibitor both showed that localization of myosin II to the cytokinesis furrow is controlled independently of anillin localization. Neither cyclin-dependent kinase (CDK) inhibitors nor monastrol affected the cytology of blebbistatin-arrested cells. This suggests that Eg5 might not be necessary for organizing spindle bipolarity once anaphase has been initiated, and that, although CDKs might be involved in C-phase timing, they do not have a central role in maintaining the contractile ring after anaphase.

So, the use of blebbistatin has allowed the further dissection of the proteins and processes involved in cytokinesis, and "New drugs that target guanosine triphospates, membrane dynamics, and mitotic motors will be useful in further dissecting the logic of cytokinesis".

*Natalie Wilson*


**References and links**

**ORIGINAL RESEARCH PAPER** Straight, A. F. *et al.* Dissecting temporal and spatial control of cytokinesis with a myosin II inhibitor. *Science* **299**, 1743–1747 (2003)