DEVELOPMENT

A joint movement

During the formation of the vertebrate heart, a simple tube of contracting muscle cells — the heart tube — arises from two populations of cells in the lateral-plate mesoderm. Although the tube then undergoes further developmental processes before the mature heart is formed, relatively little is actually known about the cellular mechanisms that regulate the movement of the myocardial precursors that form the initial tube. Attempting to get to the heart of the matter, Le Trinh and Didier Stainier have found that the extracellular matrix protein fibronectin is required for myocardial precursors to stick together in an epithelial sheet on their journey towards the midline in zebrafish.

Using confocal microscopy, the authors first examined the cellular architecture of the migrating myocardial cells and found them to actively migrate as a coherent population of polarized epithelia, rather than as individuals. Because a certain mutation — *nat* — shows defective myocardial migration, the authors took a closer look at this locus. Although myocardial differentiation proceeded normally, *nat*-mutant cells remained some distance away from the midline compared with wild-type cells. They also had problems sticking together through their cell–cell adherens junctions.

Using several molecular approaches and some intuition (fibronectin is implicated in the morphogenesis of other tissues), the authors found that *nat* encoded the *fibronectin* gene. Antisense oligonucleotides targeting *fibronectin* reproduced the myocardial cell migratory defect seen in *nat* mutants. Fibronectin is normally deposited around the basal surface of the anterior-lateral-plate mesoderm tubules, which contain the myocardial precursors, and at the midline between the endoderm and the endocardial precursors. In *nat* mutants, no fibronectin was deposited in these regions of the embryo. Surprisingly, fibronectin at the midline was required for the temporal regulation of myocardial migration, but was dispensable for migration itself, as myocardial precursors eventually reached their target.

CYTOKINESIS

At a right angle

In eukaryotic cells, the mitotic spindle is positioned perpendicular to the axis of cell division during mitosis. Reporting in *EMBO Journal*, Gachet *et al.* now propose a mechanism for coordinating spindle orientation and cell division in fission yeast.

The authors used a strain in which the α2-tubulin gene *atb2* was tagged with green fluorescent protein (GFP) to study spindle dynamics *in vivo*. They had previously shown *in vitro* that by disrupting the actin cytoskeleton (using the actin inhibitor latrunculin), spindle orientation was inefficient and that anaphase onset — when the sister chromatids separate — was delayed. These findings were now confirmed in live cells.

When analysing the behaviour of the astral microtubules that spring from the two spindle pole bodies, Gachet *et al.* noted that spindle orientation normally requires the simultaneous binding of astral microtubules from both poles to a defined region on opposite sides of the medial cell cortex — which they named the astral microtubule interaction zone (AMIZ). However, in latrunculin-treated cells, the astral microtubules did not seem to contact the cell cortex and spindle positioning was inefficient.

Is astral-microtubule binding to the AMIZ essential for spindle orientation? All the data seem to point that way. The analysis of a mutant strain that is defective in astral-microtubule nucleation from spindle pole bodies showed that spindle orientation was inefficient and anaphase onset was delayed. A high proportion of misorientated spindles with a corresponding delay in anaphase onset were also seen in an actin-mutant strain, as well as in latrunculin-treated cells.

These data also confirmed that an actin-containing complex at the medial cell cortex, which is necessary for astral-microtubule interactions, is important for spindle rotation and anaphase onset. Moreover, the authors showed that astral microtubules interact with actin at the medial cell cortex via two type-V myosins, Myo51 and Myo52, both of which are necessary for spindle rotation.

To examine more precisely where astral microtubules interact with the cell cortex, the authors used a GFP fusion of Cdc15, a protein that locates exclusively to the cytokinetic actomyosin ring (CAR) in mitosis. This ring structure forms in the central plane of the dividing cell and coincides with the centre of the AMIZ. When being constricted, CAR directs the assembly of the division septum. Gachet *et al.* showed that astral microtubules first contacted the AMIZ and then seemed to move towards the CAR.

The anillin homologue Mid1 is required for the placement of the division septum in the centre of the cell and, therefore, for the central positioning of the CAR. In the absence of Mid1, spindles rotate but fail to stabilize, which delays anaphase onset.

So, Gachet *et al.* suggest a two-part mechanism for spindle positioning in fission yeast — spindle orientation requires a rotational force that is provided by the simultaneous binding of astral microtubules with the AMIZ at opposite sides of the cell cortex and, once correctly aligned, the interaction of astral microtubules from at least one spindle pole body with CAR stabilizes this position.

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPER Gachet, Y. *et al.* Mechanism of controlling perpendicular alignment of the spindle to the axis of cell division in fission yeast. *EMBO J.* **23**, 1289–1300 (2004)

Because *nat*-mutant cells had problems adhering to each other, Trinh and Stainier again inspected their architecture. Some cell-junctional components such as atypical protein kinase C and zonula occludens-1 were missing, and the cells were disorganized. So cell adhesion to fibronectin somehow influences the formation and/or integrity of junctions in myocardial precursors. Cell attachment to fibronectin is also required for the myocardial epithelia to mature, as none of the cell-shape changes that are associated with this process occurred in *nat* mutants.

Fibronectin, therefore, seems to be required for getting myocardial precursor cells together as polarized epithelia and choreographing their movement to the midline. The authors propose that this goes beyond the more traditional role of fibronectin as a substrate for cell migration.

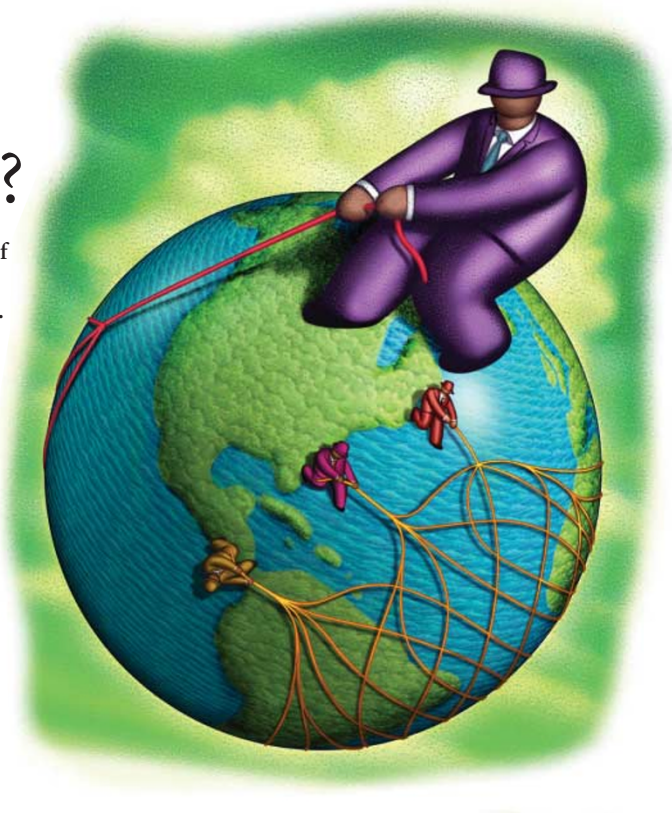
Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Trinh, L. A. & Stainier, D. Y. R. Fibronectin regulates epithelial organization during myocardial migration in zebrafish. *Dev. Cell* **6**, 371–382 (2004)

CELL CYCLE

The APC: a control freak?



Waves of ubiquitin-dependent degradation of key cellular regulators are required to help drive the cell cycle through its various phases. In *Nature*, two complementary papers by Bashir *et al.* and Wei *et al.* now show that the anaphase-promoting complex/cyclosome (APC/C), a well-known regulator of mitosis, in complex with the activator subunit CDH1 (APC/C^{CDH1}), is also required in late G1 phase to control the activity of its fellow ubiquitin-ligase complex SCF^{SKP2} (the SKP1/CUL1/F-box protein complex that contains the specific substrate-targeting F-box protein SKP2).

As components of an SCF ligase ($SCF^{SKP2–CKS1}$), SKP2 and its cofactor CKS1 regulate the degradation of the cyclin-dependent-kinase inhibitors p27 and p21. These, in turn, regulate the onset of S phase. SKP2 is an oncoprotein that is often overexpressed in human cancers and the two groups set out to clarify how SKP2 is regulated.

Both studies showed that the amount of SKP2 fluctuates during the cell cycle, being at its lowest during G1 phase. Bashir *et al.* also showed that CKS1 follows a similar pattern of abundance, and downregulation of both SKP2 and CKS1 is prevented by treatment with a proteasome inhibitor.

Overexpression of the APC/C subunit CDH1 by Bashir *et al.*, in conjunction with SKP2 and CKS1 overexpression, caused a considerable destabilization of SKP2 and CKS1, but this was not the case when the alternative APC/C activator subunit CDC20 was overexpressed. Similarly, Wei *et al.* showed that overexpression of CDH1 reduces the amount of SKP2, but that this SKP2 downregulation can be attenuated by proteasome inhibitors.

Short interfering RNAs (siRNAs) were then used in both studies to deplete the levels of CDH1 and SKP2 in synchronized cells. Wei *et al.* showed that *CDH1*-depleted cells contained an increased amount of SKP2, which led to attenuated p27 accumulation and accelerated entry into S phase. This accelerated cell-cycle progression in *CDH1*-siRNA-treated cells was abrogated by the simultaneous addition of *SKP2* siRNA. Similar results were obtained by Bashir *et al.* — so SKP2 must be an essential APC/C^{CDH1} target. In addition, when CDH1 is silenced, CKS1 is also stabilized in both cycling G1 cells and in cells withdrawing from the cell cycle.

Both groups also showed, using SKP2 mutants, that SKP2 interacts with CDH1 through its amino terminus, which contains a destruction-box (D-box) motif, and that APC/C^{CDH1} polyubiquitylates SKP2 in a D-box-dependent manner. Bashir *et al.* also noted that expression of a stable SKP2 mutant (at levels identical to the endogenous SKP2 protein) caused accelerated S-phase entry in these cells. But, wild-type SKP2 expressed at physiological levels was regulated by proteolysis and unable to speed up entry into S phase. Therefore, SKP2 destruction must be an essential event in cell-cycle control.

So, these studies show that, apart from its role in the control of mitosis, the APC/C complex is also important for preventing the unscheduled degradation of $SCF^{SKP2–CKS1}$ substrates during G1 phase and the consequent premature entry into S phase that could cause genetic instability. Indeed, Wei *et al.* suggest that APC/C^{CDH1} might have tumour-suppressor activity through its inhibition of SKP2, high concentrations of which correlate with the destabilization of p27 in human cancers.

Lesley Cunliffe

References and links

ORIGINAL RESEARCH PAPERS Bashir, T. *et al*. Control of the $SCF^{Skp2–Cks1}$ ubiquitin ligase by the APC/C^{Cdh1} ubiquitin ligase. *Nature* **428**, 190–193 (2004) | Wei, W. *et al*. Degradation of the SCF component Skp2 in cell-cycle phase G1 by the anaphase-promoting complex. *Nature* **428**, 194–198 (2004)

WEB SITES

Michele Pagano's laboratory:
http://www.med.nyu.edu/Path/Pagano

William Kaelin Jr's laboratory:
http://www.hhmi.org/research/investigators/kaelin.html