Previews

Cytokinetic Pyrotechnics

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Cytokinesis, the final step in cell division, is dependent on formation and closure of a ring of actin filaments (F-actin) and myosin-2 which is, in turn, dependent on activation of the small GTPase, RhoA, at the cell equator. Four new papers, including two in this issue of *Developmental Cell* (Petronczki et al., 2007; Birkenfeld et al., 2007), provide new insights into how RhoA activation at the equator is initiated and maintained.

Cytokinesis is the final step in cell division, wherein the nascent daughter cells are physically separated from each other. In animal cells and a variety of fungi, cytokinesis is driven by an array of actin filaments and myosin-2 that assembles at the cell equator and subsequently closes inward, bringing the plasma membrane with it (Eggert et al., 2006). Assembly of the actomyosin array is controlled by spindle microtubules, which somehow direct the activation of the small GTPase, RhoA, in a precisely defined zone at the equator (Bement et al., 2005). RhoA promotes actin filament assembly and myosin-2 activation, so the cytokinetic RhoA activity zone can be thought of as a local fire that promotes rapid remodeling of the cortical actomyosin cytoskeleton.

How do the microtubules start the fire? The favored model posits that ignition is triggered by the RhoGEF (guanine nucleotide exchange factor) Ect2. Ect2 is thought to activate RhoA at the equator by virtue of its interaction with the centralspindlin complex (Somers and Saint, 2003). Centralspindlin is a heterotetramer comprised of MKLP, a kinesin, and MgcRacGAP (Mishima et al., 2002). MgcRacGAP can bind to Ect2 (Somers and Saint, 2003), so the idea is that MKLP transports MgcRac-GAP along microtubules to the equatorial region, where it interacts with Ect2 and thereby promotes local Rho activation (Somers and Saint, 2003; Piekny et al., 2005).

Is building the cytokinetic fire as simple as that? As it turns out, no. Three recent papers (Burkard et al., 2007; Petronczki et al., 2007; Brennan et al., 2007) show that Plk1, a mitotic

kinase previously implicated in spindle assembly, is essential for ignition, while a fourth (Birkenfeld et al., 2007) reveals that GEFH1 (also known as Lfc), a microtubule-binding RhoGEF, is required to maintain the fire. Collectively, these studies both expand our understanding of cytokinesis and provide a particularly dramatic demonstration of the strength of the so-called chemical genetic approach.

Plk1 acts as a regulator of spindle assembly (Barr et al., 2004), a role that previously precluded direct analysis of its role in cytokinesis, since loss-of-function approaches to disrupt Plk1 produce severe spindle phenotypes and resultant cell-cycle arrest prior to anaphase, due to invocation of the spindle assembly checkpoint. To overcome this problem, three different groups turned to chemical genetics. Burkard et al. (2007) engineered a Plk1 that was selectively sensitive to inhibition by an ATP analog and introduced it into cells in which wild-type Plk1 was depleted by RNAi. In the absence of the analog, mitotic progression and cytokinesis were normal, but when the analog was applied in anaphase, the normal patterns of Ect2 and RhoA distribution were perturbed, and cytokinesis failed. Importantly, the ATP analog had no effect on cytokinesis in cells lacking the engineered Plk1, confirming the specificity of this manipulation. In complementary work, Petronczki et al. (2007) showed that BI 2536, a highly potent and specific Plk1 inhibitor inhibits RhoA accumulation at the equator in anaphase. They further showed that BI 2536 sharply suppressed the interaction of Ect2

and MgcRacGAP, providing a likely mechanistic explanation for this phenotype. Finally, Brennan et al. (2007) used both BI 2536 and another, structurally unrelated Plk1 inhibitor, BTO-1 and showed not only do these agents prevent RhoA localization and cytokinesis in anaphase, but also suppress spindle pole separation.

These findings are fascinating for several reasons. First, they argue that the connection between Plk, RhoA, and cytokinesis previously revealed in budding yeast (Yoshida et al., 2006) is conserved in animals. This is surprising because, unlike cytokinesis in animal cells, cytokinesis in budding yeast is independent of microtubules. Second, the results suggest that understanding the link between microtubules and RhoA activation will require a detailed understanding of the spatiotemporal patterns of Plk1 activation—is Plk1 preferentially activated at the cell equator and, if so, how is this controlled by microtubules? Third, the results provide a striking demonstration of the utility of chemical genetics. In this "why-don't-you-just-RNAi-it?" era, the advantages of chemical agents for basic biology research are often overlooked.

The findings of Birkenfeld et al. (2007) are just as fascinating. They show that GEFH1 localizes to the spindle region and is required for normal cytokinesis. They also demonstrate that the GEF activity of GEFH1 is suppressed in early mitosis via Aurora A kinase- and Cdk1-mediated phosphorylation and that this inhibition is relieved after the onset of anaphase, coincident with activation of RhoA.

Most remarkably, they found that depletion of GEFH1 prevents accumulation of active RhoA, while depletion of Ect2 does not. These results, obtained via biochemical analysis, were confirmed and extended by direct imaging of RhoA activity during cytokinesis: following GEFH1 depletion, a relatively minimal amount of active RhoA is observed at the equatorial plasma membrane, and it often fails to persist. Following Ect2 depletion, in contrast, robust RhoA activation occurs but is not confined to the equatorial plasma membrane and instead the fire spreads, resulting in abnormal contraction and abnormal or failed cytokinesis.

Clearly, these findings are not consistent with Ect2 serving as the major RhoA activator during cytokinesis, and instead argue that its primary role is to keep the fire restricted to the equator. How could this work?

One possibility is that a small pool of RhoA activated by Ect2 somehow primes more extensive RhoA activation by GEFH1. Birkenfeld et al. (2007) make an even more provocative suggestion, and argue that Ect2 doesn't serve as GEF in the usual sense at all, but instead acts as part of a scaffold that ensures localization of other players critical for local RhoA activation. Whatever the mechanistic explanation turns out to be, this study and the three that use chemical genetics to disrupt Plk1 function, suggest that it is time to revise our model for how RhoA is activated during cytokinesis.

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Cell Polarity from Cell Division

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Apical-basal polarity of epithelial cells is critical for their symmetric versus asymmetric division and commonly thought to be established in interphase. In a novel type of cell division termed "mirror-symmetric", apical cell constituents accumulate during M-phase at the cleavage furrow, resulting in epithelial daughter cells with opposite apical-basal polarity.

Neurulation in vertebrates is an ideal system to study the cell biological and genetic basis regulating the coordination of cell shape changes, cell divisions, and cell polarity during morphogenesis of an epithelium, which ultimately results in the formation of a tube. Primary neurulation in mammals occurs via invagination of the neural plate, during which the neuroepithelial cells maintain apical-basal polarity, independent of the phase of the cell cycle (Figures 1A and 1B). Neuroepithelial cell number is increased by symmetric divisions, with the cleavage plane oriented parallel to their apical-basal axis, resulting in the equal distribution of polarized apical and basal constituents to the daughter cells (Figure 1B). Cleavage planes deviating from the apical-basal axis result in an unequal inheritance of polarized constituents and, consequently, different fates of the daughter cells; in the most extreme (albeit rare) case of these asymmetric divisions, the cleavage plane is oriented perpendicular to the apical-basal axis (Figure 1B) (Götz and Huttner, 2005).

In contrast to mammals, most teleosts, including the zebrafish *Danio rerio*, undergo what is called secondary neurulation (Kimmel et al., 1995). The neural plate folds inward at the midline to build the neural keel, a solid structure, which shortly after rounds up and detaches from the overlying epidermis to form the lumenless neural rod. Cavitation finally results in the formation of the lumen of the neural tube (Figure 1C). Cleavage plane orientation differs during these stages: it is perpendicular to the neuroectodermal surface in the neural plate and to the