Animal cytokinesis: **Breaking up is hard to do**

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Recent studies have shed new light on how the physical association between sister cells is severed at the end of cytokinesis while the membrane is resealed. Comparisons with yeast suggest that daughter cell shape may feed back to regulate cytokinesis through the Bub2 checkpoint system.

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Breakups are complicated. In order to separate, numerous decisions and details accompany the severing of functional ties. For a dividing animal cell, the final decision to separate is the last step of cytokinesis, which cleaves a duplicated mother cell into two new daughter cells. This final moment requires resolving a slender tube of cytoplasm, a structure known as the midbody that forms an intercellular bridge between two incipient sisters. Once thought merely a remnant of the mitotic spindle caught by closing the contractile ring, work from several systems now points to an active, regulated role for the midbody in coordinating the transition from mitosis to G1 phase of the cell cycle. A pair of recent papers [1,2] — one published recently in *Current Biology* [2] — provides new insight into what is being regulated, and what criteria might be used to decide when two cells part ways. Together, these studies identify the moment of closure as a critical process that could play an important role in development and maintenance of spatial architecture in differentiated tissues.

Stuck in the middle: the midbody complex

The involvement of an actin–myosin contractile ring in the cleavage furrow has long been known. It is also clear that the position of the cleavage furrow — the belt of cell cortex that intersects the plane of the metaphase plate — depends on the position of the mitotic spindle. Nevertheless, experiments in several systems seem to rule out essential roles for spindle microtubules, centrosomes and even the nucleus in the completion of cytokinesis (reviewed in [3]). In contrast, other work shows that although the mitotic spindle is not required for cytokinesis — as the cortex will still contract in its absence — microtubules assembled during anaphase are required to coordinate cleavage furrow contraction [1,4,5]. These microtubules appear early after the metaphase–anaphase transition, assemble between the moving chromatin masses, and eventually form an array implicated both in force production for anaphase B and in assembly of the midzone complex for cortex contraction [5–7].

The midbody is created as the cleavage furrow closes, eventually bundling together the central spindle micro-tubules. In addition to the microtubule bundle, the mid-body contains a number of spindle and chromosome–derived proteins packed into the phase-dense ‘Flemming body’ at the center of the intercellular bridge (see Figure 1). Numerous proteins have been identified in the midbody, falling loosely into five groups: nuclear proteins, centromeric, chromosomal or nucleolar; motor proteins; signaling proteins, such as kinases, phosphatases, GTPases or proteases; cytoskeletal and associated components, such as actin, tubulin or intermediate filament proteins; and membrane proteins, such as septins, tight junction components and vesicle fusion proteins. These components are not simply innocent bystanders corralled by the contractile ring into the intercellular bridge, but may actively participate in the final step of cell separation.

The final cut

In a way, the midbody complex resembles the proverbial lad’s finger in the dike, and the predicament appears much the same: the physical obstacle of the midbody must be removed, while a hole is simultaneously repaired. In an animal cell, the hole is in the plasma membrane. Ingression of the cleavage furrow involves creating new membrane surface area and is facilitated by proteins used in vesicle transport and fusion. A recent study by Skop and coworkers in John White’s laboratory [2] has identified a distinctive membrane accumulation and transport process associated with the final step of midbody closure.

Skop *et al.* [2] asked how GTPase-regulated membrane transport influences cytokinesis in the well-characterized mitoses of early *Caenorhabditis elegans* embryos. Brefeldin-A (BFA), an inhibitor of ADP ribosylation factor (ARF)-dependent guanine nucleotide exchange activity, was used to disrupt intracellular membrane transport and secretion. BFA did inhibit cytokinesis, but surprisingly not by preventing ingression of the cleavage furrow as one might expect. Rather, BFA interfered specifically with closure of the intercellular bridge. Inhibition of bridge closure was also observed with RNAi-induced suppression of Rab11, a small GTPase involved in membrane targeting.

Time-lapse microscopy of the dividing *C. elegans* cells revealed vesicle movement through the central spindle and along the intercellular canal as cytokinesis progressed. In a dramatic four-dimensional movie sequence, the

cleavage furrow membrane is seen to surround an excluded zone — a black hole — that corresponds to the midbody, and then membrane material concentrates precisely around this annulus. This distinctive structure and the ARF and Rab11 dependence indicate that membrane closure at the intracellular bridge is a GTPase-regulated membrane remodeling process in animal cells, perhaps analogous to secretion as suggested by Skop *et al.* [2].

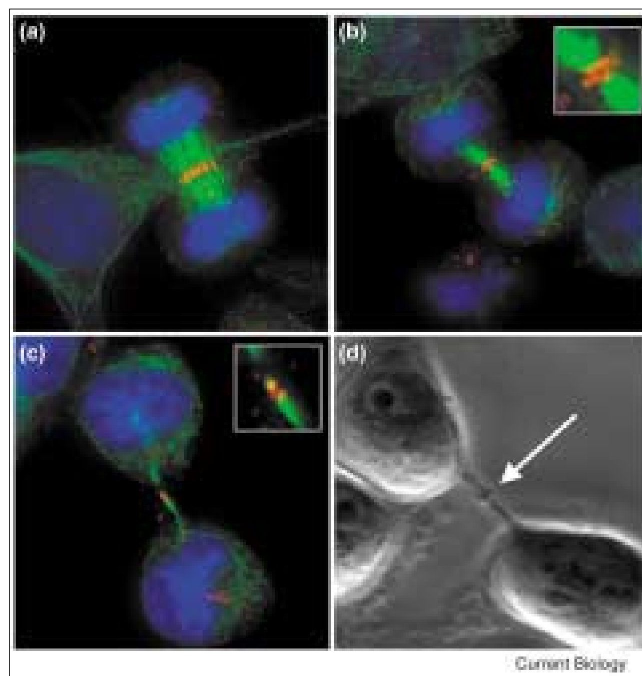
Pulling the plug

Removing the midbody itself is also necessary for closure. A remarkable set of observations by Piel and co-workers [1] in Michel Bornens' laboratory suggests that centrosomes, the microtubule-nucleating centers of cells, play an active role in this step. After labeling centrioles in HeLa cells with a fusion protein between centrin and the green fluorescent protein (GFP) [8], Piel *et al.* [1] filmed centrosome behavior during cytokinesis. As the post-mitotic cells spread, the paired centrioles at the heart of the centrosome are seen to split apart. The more brightly labeled mother centriole, the template in the last cell cycle, remains stationary near the cell center, while the daughter centriole tours the cell. Just before separation, or abscission, the mother centriole leaves the central region of the cell and travels straight to the midbody, where it dwells for 15–60 minutes. Soon after the mother centriole leaves the midbody region, midbody microtubules are released and cell separation occurs.

Does the centriole play an active role in resolving the midbody? Recent microsurgical experiments implicate the centrosome in completion of cytokinesis [9]. In agreement with this, Piel *et al.* [1] found that, in an acentrosomal *Drosophila* cell line, mitosis proceeded more or less normally, but cytokinesis failed in a high proportion of cells. Further, by careful administration of nocodazole after repositioning of the mother centriole to the midbody, but before cell separation, Piel *et al.* [1] have provided evidence that the microtubule network actively facilitates midbody microtubule release and returns the mother centriole to the cell center. It remains for future experiments to discern whether the centriole mediates microtubule-dependent forces to break the midbody or simply delivers a signal required for midbody resolution.

Why do animal cells execute such complex choreography to complete a process that is all but finished once the cleavage furrow has ingressed? One answer may lie in the requirement for differentiated cellular architecture during tissue development. Piel *et al.* [1] propose that the centrosome can 'examine' the spatial organization of the nascent cell, and integrate this information to monitor whether cell separation will succeed. The centrosome has been likened to a geometric computer and, indeed, is able to steer itself to the center of an enclosing volume by balancing within a microtubule network [10]. Can spatial information be fed back to the centrosome, like data into a geometric computer, and modify its functional properties? The centriole dance may now make sense, if the mother centriole carries a signal to the midbody. If an appropriate cell geometry were detected, an all-clear message would trigger execution of membrane closure and midbody microtubule resolution, completing cell division. If an inappropriate geometry were detected, a 'not ready' signal could arrest midbody resolution. In an animal tissue, apoptosis could be invoked to 'erase' the failed generation and try again.

Figure 1



Visualizing the central spindle and midbody in human cells. Microtubules are shown in green, DNA in blue and CENP-E in red. **(a)** CENP-E on the contractile ring; the central spindle is easily visible. **(b)** CENP-E in the Flemming body at the center of the intercellular bridge, with concomitant bundling of microtubules. **(c)** Tightly bundled microtubles at the point of breaking, with only residual CENP-E staining left in the midbody. **(d)** The Flemming body is clearly visible by phase microscopy (arrow).

(Un)binding arbitration

Checkpoints function to arbitrate cooperation between independent processes that must be coordinated. In the case of Bub2, this is accomplished by making spindle pole placement part of the checkpoint pathway. In yeast, this spindle positioning checkpoint is regulated by a distributed Ras family GTPase network. The GTPase itself, Tem1, and its cognate GAPs, Bub2/Bfa1, are restricted to the spindle pole body [11,12]. The guanine nucleotide exchange factor, Lte1, is present only in the bud. When

the spindle pole migrates into the bud, the circuit is closed, activating Tem1 and enforcing proper spindle geometry as a precondition for activating downstream Cdh1-dependent protein degradation [12,13]. Piel *et al.* [1] suggest that the centriole could serve an equivalent role in animal cells. This would mean that the mother centriole may be delivering proteins to the midbody, for example signaling proteins such as kinases, GTPases or proteases.

Could Bub2 arbitrate communication between the centrosome and the midbody in mammalian cells? The Bub2-dependent checkpoint seems to integrate input from upstream checkpoint systems during cytokinesis. Bub2 is required for maintaining cell-cycle arrest in response to DNA damage, kinetochore damage, spindle defects and spindle misorientation [11–15]. Bub2 binds to the septin Cdc3 in the budding yeast *Saccharomyces cerevisiae*, and is homologous to fission yeast *Schizosaccharomyces pombe* Cdc16, an essential protein required for cytokinesis in this yeast (reviewed in [16]). Though mammalian Bub2 has not yet been characterized, a straightforward hypothesis is that it will be present at the centrosome and play a similar role in regulating mitotic exit.

Most intriguingly, protein sequence comparisons show that budding yeast Bub2 shares a domain with the GTPase activating proteins (GAPs) Gyp6 and Gyp7, which activate Ypt/Rab family membrane transport GTPases [17]. Could Bub2 be a Rab GAP? This possibility suggests that a centrosomal Bub2-dependent checkpoint signal might directly regulate the membrane events described by Skop *et al.* [2]. Perhaps Bub2, like a good arbiter, integrates both sides of the story before issuing a decision to allow closure.

In summary, many signaling proteins are found in the midbody during cytokinesis, including GTPases, kinases and proteases — proteins also found at the centrosome or components of the DNA-damage or spindle-assembly checkpoints. Taken together, these observations suggest that earlier checkpoints may feed into the regulation of separation. After all, this is the last step before two individual daughter cells part ways. Everything must be divided appropriately by now, because it is the last chance at the end of the cell cycle: this is the checkpoint to end all checkpoints.

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