

Making the Cut: The Chemical Biology of Cytokinesis

G. Ekin Atilla-Gokcumen^{†,§}, Adam B. Castoreno^{†,§}, Sofia Sasse^{†,*}, and Ulrike S. Eggert^{†,*}

[†]Dana-Farber Cancer Institute and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115 and [‡]Westfälische Wilhelms-Universität Münster, Germany. [§]These authors contributed equally to this work.

Cytokinesis is the physical division of cells and occurs after mitosis, the division of chromosomes. Successful completion of cytokinesis, with the cleavage furrow exactly bisecting the axis of chromosome segregation, involves the coordinated action of the cytoskeleton, cell cycle and membrane machineries of cells (1). Here, we review how small molecule membrane components, *i.e.*, lipids, participate in cytokinesis and briefly report what is known about other small molecule metabolites. We will also discuss how small molecules have been used as probes to study the mechanism of cytokinesis.

Mechanism of Cytokinesis. Cytokinesis occurs immediately after mitosis, the division of DNA. The mechanism of cytokinesis has been reviewed in detail elsewhere (1–3). Here, we will briefly introduce key steps to provide context for this review. During mitosis, chromosomes line up in a metaphase plate at the center of the mitotic spindle. As the chromosomes are dividing, a dramatic and poorly understood rearrangement of microtubules takes place and a contractile ring begins to assemble (Figure 1). Contact of the cellular cortex (and possibly the plasma membrane) with microtubule structures is essential for the correct positioning of the contractile ring. Several possible mechanisms have been proposed, although recent studies suggest that multiple mechanisms may occur simultaneously (2, 4, 5). There are several main signaling pathways, for example, those centered around Aurora B and Polo kinases and the small GTPase Rho, that operate and are essential throughout cytokinesis (1). As the location of the cleavage furrow is being specified, the contractile ring begins to assemble. The temporal sequence of protein recruitment to the ring is a significant but poorly understood mechanistic feature of early cytokinesis. The complete composition of the ring is not known, but the plasma

ABSTRACT Cytokinesis is the last step in the cell cycle, where daughter cells finally separate. It is precisely regulated in both time and space to ensure that each daughter cell receives an equal share of DNA and other cellular materials. Chemical biology approaches have been used very successfully to study the mechanism of cytokinesis. In this review, we discuss the use of small molecule probes to perturb cytokinesis, as well as the role naturally occurring small molecule metabolites such as lipids play during cytokinesis.

*Corresponding author,
ulrike_eggert@dfci.harvard.edu.

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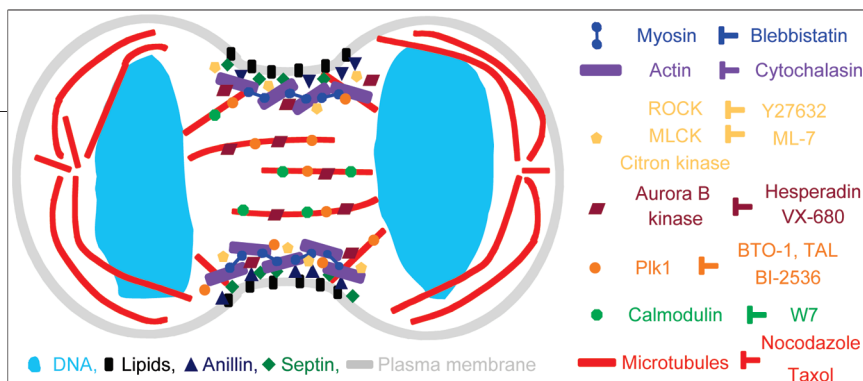


Figure 1. Illustration of a cell during an early stage of early cytokinesis. During cytokinesis, a contractile ring assembles. It is located just beneath the plasma membrane and includes a network of actin and myosin II filaments as well as septins and Anillin. Phosphorylation and activation of myosin II at the cleavage furrow is regulated by several kinases, including ROCK, MLCK, and Citron kinase. The mitotic kinases Plk1 and Aurora B both localize to the cleavage furrow as well as midzone microtubules where they function as key regulators of cytokinesis. An expanding collection of small molecules has been used to dissect cytokinesis regulation in both time and space. Key cytokinesis protein and their corresponding small molecule inhibitors are shown. For clarity, only localizations at the cleavage furrow and midzone microtubules are shown. Not drawn to scale.

membrane, actin, myosin II, and Anillin are clearly important (Figure 1) (6–8). It is not known how the contractile ring is linked to the plasma membrane during ring assembly or ingression. The contractile ring then ingresses, with actin and myosin providing much of the mechanical force (1). We have partial information for the stages of cytokinesis described so far, but the last step, called completion or abscission, remains the least understood. At the end of ingression, a small structure, the

midbody, forms. Severing between the daughter cells, which has to be very carefully controlled to avoid the generation of a hole in the cell, occurs in this structure (9). Targeted vesicle transport is thought to play a role both during contractile ring ingression and in generating a membrane barrier that seals the daughter cells during severing (10–15).

One of the interesting features of cytokinesis is that there can be considerable mechanistic variation between cell types (1). Often multiple pathways operate in parallel, and the importance of individual pathways varies across different systems. This mechanistic plasticity may have evolved to

allow cytokinesis in cells with very different characteristics, ranging from very large embryos with a large cell surface area to smaller cells. Some aspects of cytokinesis have been extensively studied in the popular model organisms budding (*S. cerevisiae*) and especially fission yeast (*S. pombe*) (16, 17). Insights into the timing of contractile ring assembly during cytokinesis have come from studies in *S. pombe* (18). Since fungi have many organism-specific features, however, we will focus

mostly on cytokinesis in higher eukaryotes.

Small Molecule Probes of Cytokinesis

Small molecules are versatile tools to probe dynamic cellular processes such as cytokinesis because they enable spatial and temporal control. It has been challenging to study cytokinesis by traditional methods because it is a rapid and dynamic process that occupies only a small portion of the cell cycle. Many key cytokinesis proteins also perform important functions earlier in the cell cycle, especially during mitosis, and their removal leads to an arrest prior to cytokinesis. Small molecules act rapidly and can be added at specific points in the cell cycle. In addition, the effects of small molecules are often reversible upon washout, allowing even greater control because treatments can be combined sequentially. Although the collection of small molecules that are available

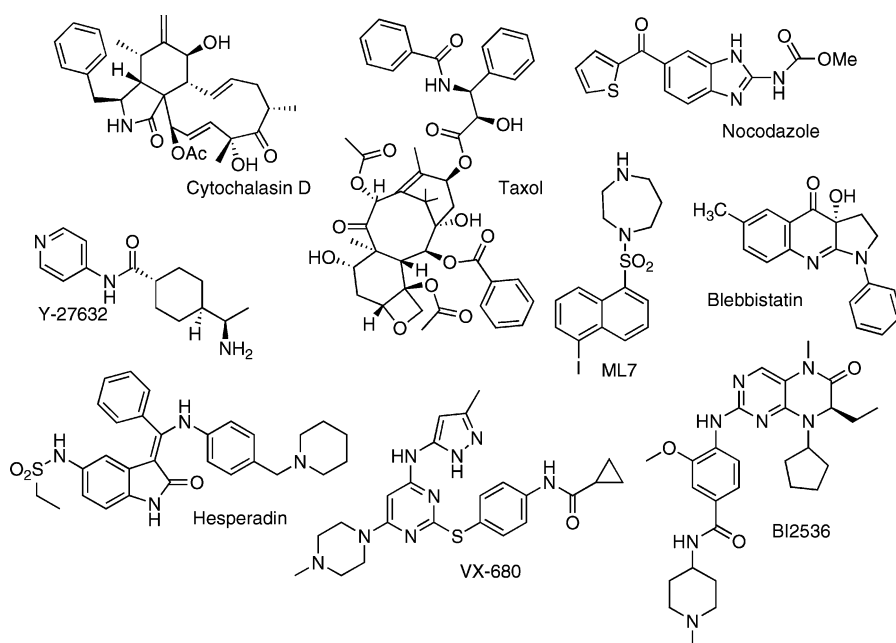


Figure 2. Chemical structures of small molecules that have been used to probe the mechanism of cytokinesis.

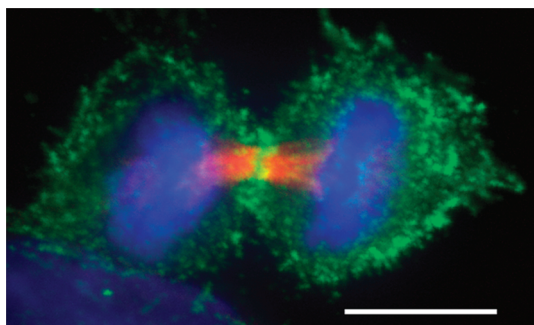


Figure 3. Dividing HeLa cell during early cytokinesis. Phosphorylated myosin II accumulates at the cleavage furrow. Microtubules (red) were stained with antitubulin (Sigma T6199), phospho-myosin (green) was stained with Phospho-Myosin Light Chain 2 (Ser19) antibody (Cell Signaling, 3671 L), DNA (blue) with DAPI. Scalebar represents 10 μ M.

to study cytokinesis is being expanded (19), it is still small at present (Figure 2) but has been used to great effect.

Probing the Role of the Cytoskeleton in Cytokinesis.

Precise positioning of the cleavage furrow is specified by the mitotic apparatus and allows ingression to occur along a plane that bisects segregating chromosomes (20). Thus, microtubules as well as the actin-based cytoskeleton are essential for cytokinesis, and small molecules have been used to probe their involvement. In particular, small molecules targeting actin have a distinguished history in cytokinesis research (21). For example, natural products in the cytochalasin family cause the depolymerization of actin filaments (22). Because the cytochalasins inhibited multiple processes such as cell migration, ruffling, and division (23), they were used to show that the same protein, actin, was involved in these processes. Cytochalasin inhibited cellular division but not nuclear division, providing evidence for the first time that the two processes could be decoupled from each other (23).

Small molecules' ability to rapidly disrupt cytoskeletal organization was also used to establish the importance of microtubules in cleavage furrow positioning and ingression. Studies using the microtubule depolymerizer nocodazole to disrupt microtubule organization have demonstrated the importance of midzone microtubules in defining the cleavage plane (24). Disruption of microtubule dynamics during anaphase with the microtubule stabilizing agent taxol led to a reduction in the

rate of furrowing (25). Work by Shannon *et al.* showed that taxol-stabilized microtubules were sufficient to initiate furrow formation; however, the taxol-mediated disruption of microtubule dynamics delayed ingression and prevented cytokinesis completion (26). Using a combination of small molecules including taxol and the historically important hexylene glycol and urethane (20), Strickland *et al.* differentially perturbed the dynamic state of microtubules. They determined that successful furrow initiation was positively regulated by the physical contact between the astral microtubule plus end and the cell cortex, rather than the dynamic state of the microtubules (27).

Using Small Molecules to Study the Regulation of Cytokinesis. In addition to helping elucidate the critical role of actin and microtubules, small molecules have provided insight into the function of key regulators of cytokinesis, especially by taking advantage of their high temporal control and often reversibility. For example, washout studies following blebbistatin (see below), nocodazole, or cytochalasin treatment have been used to identify the approximately 1 h timing window in which cells remain competent to carry out cytokinesis, termed C-phase (28, 29).

The phosphorylation and activation of myosin II regulatory light chain (MRLC) by several kinases including Rho kinase (ROCK), myosin light chain kinase (MLCK; see the section on calcium for a discussion on the activation of MLCK) and Citron kinase is important for proper ingression of the cleavage furrow (Figure 3). Small molecule inhibitors of ROCK and MLCK have been used to dissect their respective functions and possible functional redundancies during cytokinesis (30–32). HeLa cells treated with the ROCK inhibitor Y-27632 exhibited a reduced MRLC phosphorylation, enhanced accumulation of ROCK at the cleavage furrow, and a significantly prolonged ingression time (31). In contrast, acute inhibition of MLCK with ML7 redistributed myosin and actin away from the equatorial cortex and caused subsequent furrow regression (32). Lucero *et al.* used ML7 and the ROCK inhibitor H-1152 to tease apart the temporal requirements for ROCK and MLCK activities during cytokinesis in sea urchin eggs (33). While both MLCK and ROCK were required for furrow ingression, only MLCK was necessary for the global activation of myosin II at the metaphase-anaphase transition. Thus, treatment with ROCK and MLCK inhibitors leads to different cytokinesis defects, suggesting they have different functions

and cannot compensate for each other. Adding even more complexity is Citron kinase. RNAi and overexpression studies suggest that Citron kinase induces the diphosphorylation of MRLC and is important during the later stages of cytokinesis (34, 35). Currently there are no small molecule inhibitors of Citron kinase. Future studies using small molecules will continue to provide information about how these kinases differentially regulate myosin phosphorylation at the cleavage furrow.

Polo-like kinase (Plk1) regulates several aspects of cell division including mitotic entry, bipolar spindle formation, chromosome segregation, and cytokinesis (36). Perturbing Plk1 function in animal cells leads to a failure in bipolar spindle assembly and arrest in early mitosis. The lack of mitotic progression upon Plk1 disruption greatly complicated studies on the role of Plk1 during late mitosis and cytokinesis. Only recently was the role of Plk1 in cytokinesis dissected in more detail through the use of chemical genetics and small molecule inhibitors, where Plk1 could be rapidly inactivated in synchronized cells (37–40). Inhibition of Plk1 activity during anaphase disrupted Plk1-mediated phosphorylation of HsCdk-4 RhoGAP and subsequent recruitment of Ect2 RhoGEF to the central spindle, events necessary to initiate contractile ring assembly and to activate Rho GTPase, one of the master regulators of cytokinesis (41, 42). Future experiments to characterize additional Plk1 targets will be important to further elucidate the role of Plk1 during cytokinesis.

Like Plk1, Aurora B kinase regulates a variety of critical events during mitosis, including chromosome alignment, bipolar and central spindle assembly, and cytokinesis (43). Hesperadin, a small molecule inhibitor of Aurora B, has been

used to elucidate the role of Aurora B during cytokinesis (44, 45). Miyauchi *et al.* treated HeLa cells with Hesperadin and observed mis-localization of a number of key cytokinesis proteins, as well as disorganized astral and midzone microtubules. Hesperadin treatment during anaphase/telophase was used to establish a role for Aurora B in the phosphorylation and activation of MKLP1, a kinesin motor protein needed during cytokinesis (46). Plk1 can also phosphorylate MKLP1, so it will be interesting to determine if and how coordinated phosphorylation of MKLP1 by both kinases is necessary for central spindle function and proper cytokinesis.

The Rho GTPase pathway is another key regulator of cytokinesis, but no specific inhibitors of the Rho pathway exist apart from the ROCK inhibitors mentioned above. We have developed small molecules that target the Rho pathway in cytokinesis, using a chemical variant of a classical modifier screen (Castoreno and Eggert, unpublished data). Our compounds inhibit cellular functions of the Rho pathway and likely target different proteins within the Rho pathway.

As we discuss below, membranes and also membrane trafficking are important in cytokinesis but poorly understood (10–15). Unfortunately, there are fewer small molecules available that target membrane trafficking than there are inhibitors of the cytoskeleton or signaling cascades. Brefeldin A, an inhibitor of post-Golgi trafficking, inhibits cytokinesis in some (but not all) cell types and has been used to show that membrane accumulation at the abscission point is important for completion (13, 15, 47).

Using Sequential Small Molecule Treatments To Study Cytokinesis. A major advantage of small molecules is that they can be added sequentially or combined to dissect different steps within a process. Using small molecules to synchronize cells at different stages of the cell cycle is an important application in many different areas of research. Several of the studies described in the previous section used a variety of small molecules to synchronize cells in mitosis, both to increase the pool of cells about to enter cytokinesis and to focus specifically on the role of proteins such as Aurora B and Plk-1 in cytokinesis rather than mitosis. In addition to dissecting the role of these kinases, combined small molecule treatments have also proven useful in studying contractile ring assembly.

Blebbistatin, a reversible inhibitor of myosin II ATPase activity, has enabled a detailed study of contractile

KEYWORDS

Actin: A key constituent of the cortical cytoskeleton and one of the main players of cytokinesis. It exists in globular (G-actin) or filamentous form (F-actin).

Cleavage furrow/contractile ring: The contractile ring, the actin-based structure responsible for ingression and cleavage during cytokinesis, assembles at the cleavage furrow. It contains actin, myosin II, Anillin, septins, and many other proteins.

Cytokinesis: The last step in the cell cycle, where daughter cells separate.

Metabolites: Small molecules that naturally occur in cells and are processed (or metabolized) by cellular machineries for a variety of purposes, including signaling.

Midzone: The main microtubule-based structure during cytokinesis. It is formed by rearrangement of the mitotic spindle and is located between the separated chromosomes.

Myosin: A family of motor proteins responsible for actin-based motility. Myosin II is one of the key players in cytokinesis.

Phosphatidylinositol 4,5-bisphosphate (PIP2): A signaling lipid in the phosphatidyl inositol family that is an important regulator of the actin-based cytoskeleton.

Small molecule probes: Small molecules that are used to study biological pathways or mechanisms.

Tubulin: Tubulin is a key constituent of the mitotic spindle during mitosis and the midzone during cytokinesis. Microtubules are tubulin polymers.

ring assembly and dynamics. In the presence of blebbistatin, components of the cleavage furrow including microtubules, myosin II, and Anillin (a contractile ring scaffolding protein) organize properly, but contractile ring ingression is blocked (29). Straight *et al.* combined blebbistatin treatment with additional small molecules to probe the signaling pathways necessary to maintain components at the cleavage furrow following assembly. The addition of cytochalasin or nocodazole to blebbistatin-treated cells caused the delocalization of myosin and Anillin, while small molecule inhibition of ROCK (using Y-27632) or Aurora B kinase functions perturbed myosin but not Anillin localization, indicating alternate pathways for the maintenance of myosin and Anillin at the cleavage furrow (29).

Combined small molecule treatments have also been used to study how cells with monopolar spindles are able to form furrows, which can be used as a lower-complexity model to dissect contractile ring assembly and ingression. Work by Canman *et al.* showed that cytokinesis could occur in cells without a bipolar spindle (48). To extend these studies, Hu *et al.* developed a drug synchronization method with two small molecules to trigger monopolar cytokinesis in HeLa cells. After arresting cells in mitosis using the kinesin-5 inhibitor *S*-trityl-L-cysteine and then forcing cells into cytokinesis using the Cdk1 inhibitor purvalanol A, they observed a strong polarization of microtubules and a large cellular protrusion. Subsequently, a furrow formed between the protrusion and the cell body (49). To probe the requirements for polarization in monopolar cells, a third inhibitor was introduced. The addition of nocodazole, latrunculin B, blebbistatin, or the Aurora B inhibitor VX-680 disrupted polarization and furrowing in monopolar cells, demonstrating a requirement for microtubules, actin, myosin II, and Aurora B during monopolar cytokinesis.

Using Small Molecules To Study Spatial Regulation in Cytokinesis. The ability to locally perfuse small molecules to spatially distinct areas of a dividing cell has provided a deeper understanding into how equatorial and polar regions regulate furrow ingression.

Local application of cytochalasin D to the polar region, not the equatorial region, of dividing epithelial cells blocked cytokinesis, indicating a global coordination of contractive and cortical activities for proper ingression (50). Following up on these results, Guha *et al.* applied blebbistatin to the equator close to the cell cortex and observed inhibited ingression in most cells,

along with a strong accumulation of equatorial actin filaments. In contrast, application of blebbistatin to the polar region caused minimal cytokinesis failure. Many cells, however, displayed problems with furrow ingression and positioning, suggesting a coordinating role for myosin activity in both regions.

In complementary studies, Murthy *et al.* performed FRAP experiments in GFP-actin-expressing cells and observed decreased actin turnover in the assembled contractile ring the presence of blebbistatin. These results provide evidence for a second role for myosin in regulating actin dynamics at the cleavage furrow (32, 51).

Small Molecule Metabolites and Cytokinesis. Given the complexity of cytokinesis, it is not surprising that naturally occurring small molecule metabolites are important participants in this process. Much less is known about the functions of small molecule metabolites than about key proteins, in part because it can be difficult to detect many metabolites in their cellular context. Here, we will focus mostly on the roles of lipids and of calcium in cytokinesis (Figure 4), mainly because the role of other metabolites has not yet been explored.

Role of Lipids in Cytokinesis. Surprisingly, given the important link between the membrane and the actin cortex, little is known about the role of the plasma membrane and lipids in cytokinesis. It is known that some lipids localize to the cleavage site and that membrane transport is required to relieve the cortical tension that occurs during separation. However, the precise roles of these lipids are still unclear, leaving a number of important outstanding questions: Are any lipids actively involved in cytokinesis, and is their role structural or do they have a signaling function? Some early clues suggest that both of these scenarios are true (52, 53). How do membranes influence cell shape changes that occur during cytokinesis? How are the actin cortex and more specifically the contractile ring linked to the plasma membrane? How are different lipids transported to the cleavage furrow? How are they maintained there? What is the role of membranes in the midbody, and how do they seal the hole in the cell wall that inevitably occurs during cleavage? We will discuss first steps that have been made to address some of these questions.

It has been reported that cholesterol (54–56), phosphatidyl ethanolamine (PE) (57, 58), phosphatidylinositol 4,5-bisphosphate (PIP₂) (59, 60), and ganglioside GM₁ (54) localize to the cleavage furrow, suggesting an involvement in cytokinesis. In addition, the sphingolipid

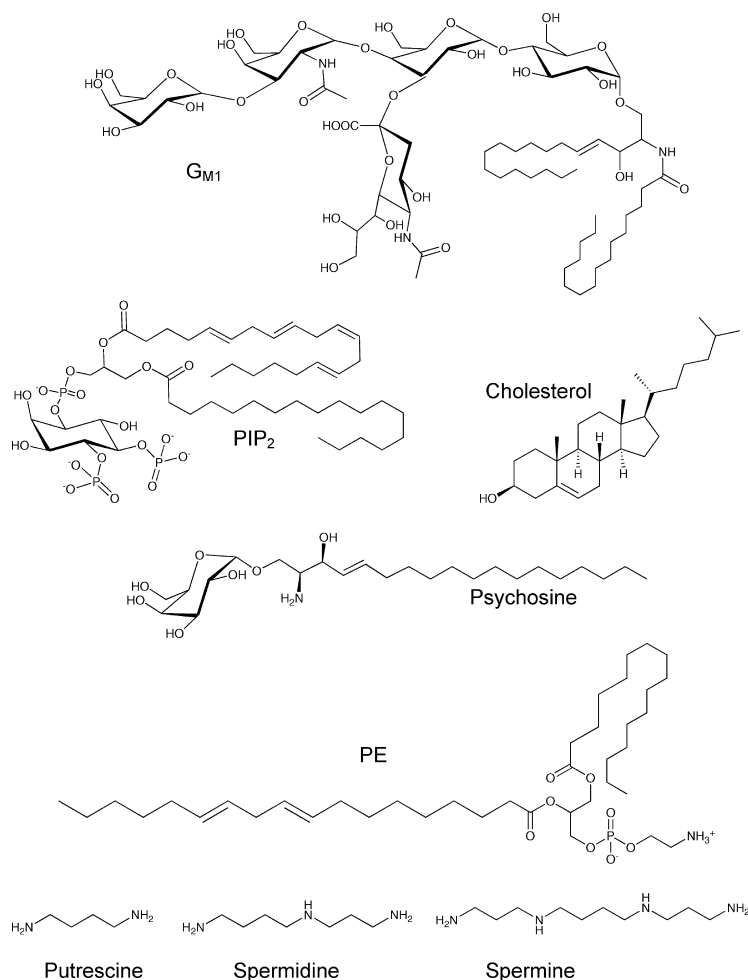


Figure 4. Chemical structures of lipid and polyamine metabolites that are thought to participate in cytokinesis.

psychosine has also been found to participate in cytokinesis (61). It has been possible to link these lipids to cytokinesis because they can be visualized with fluorescent probes. The development of new tools to detect lipids both in live and fixed cells would be extremely useful and would contribute significantly to unraveling which lipids are involved in cytokinesis. It is very likely that there are other undiscovered lipids that play significant roles in the process since recent RNAi screens targeting cytokinesis identified lipid modifying enzymes (19, 62, 63).

Phosphatidylinositols. Phosphatidylinositols (PtdIns) are important signaling molecules that regulate the actin cytoskeleton, for example, during cell polarization

and division (53, 64). PIP₂ is the best-studied lipid in cytokinesis and was first shown to be involved in cytokinesis when injection of *X. laevis* embryos with PIP₂ antibodies inhibited furrow ingression (65). Similarly, the treatment of crane fly spermatocytes with inhibitors of PtdIns recycling, particularly PI3 kinase and Phospholipase C (PLC), slowed down or inhibited furrow ingression, demonstrating that PtdIns metabolism is required for cleavage (66).

Two recent studies focused on the role of PIP₂ in mammalian cells (59, 60). Both studies showed that PIP₂ localizes to the cleavage furrow during ingression (Figure 5) and, more importantly, that interference with PIP₂ results in cytokinetic defects. Field *et al.* investigated the effects of PLC-PH overexpression in mammalian cells. The PH domain of PLC, an enzyme that hydrolyses PIP₂, binds to PIP₂. GFP-PLC-PH can be used to study the localization of PIP₂ and to sequester its function when overexpressed. Ten-fold overexpression of GFP-PLC-PH resulted in separation of actin from the plasma membrane, indicating that PIP₂ connects actin to the plasma membrane at the cleavage furrow. Furthermore, a decrease in PIP₂ levels by overexpression of PLC-PH or a kinase-inactive form of PI(4)P-5-kinase, the enzyme that synthesizes PIP₂, induces the formation of binucleated cells. Field *et al.* suggest a model in which PIP₂ provides the connection between the contractile ring and the plasma membrane by binding to plasma membranes and/or different components of the contractile ring.

Giotto, a class I PITP (PtdIns transfer protein), is enriched at the cleavage furrow and the endoplasmic reticulum. It mediates the transfer of PtdIns monomers to the equatorial membrane in *Drosophila*, leading to a local increase in the levels of PtdIns. Giotto mutants fail cytokinesis and show abnormal localization of vesicle-like structures. The contractile ring forms but cannot constrict. The cytokinetic arrest phenotypes in this mutant are similar to that of the fwd (PI4 kinase) and fws (four way stop, a Cog5 homologue in *Drosophila*) mutants. On the basis of these results, the authors suggested that changes in membrane composition might contribute to vesicle formation, which results in targeted delivery of proteins and/or membrane addition (67).

PIP₂ binds to septins and ERM (ezrin/radixin/moesin) family proteins *in vitro* (68). These proteins are thought to be mainly scaffolding proteins that may be involved in linking the actin cortex to the plasma membrane. It

was shown recently that PIP₂ levels are directly correlated to the amount of membrane-bound ERM proteins in cells, possibly through regulation of PLC (69). An intriguing study in *S. cerevisiae* has shown that PIP₂ binding is a mechanism by which Rho GTPase can localize to the bud neck, further supporting the notion that PIP₂ is an important signaling molecule in cytokinesis (70).

Cholesterol and Phosphatidyl Ethanolamine. It has been possible to hypothesize that cholesterol is involved in cytokinesis because it can be visualized in cells with the fluorescent macrolide filipin (71). Filipin binds to sterols and sequesters their activity. Using filipin, it was shown that cholesterol is involved in cytokinesis in *S. pombe* (56). In a similar study in sea urchin eggs, the Burgess group has shown that the formation of an equatorial band enriched with G_{M1} and cholesterol is coupled to contractile ring formation and assembly. The same study also demonstrated that filipin addition during cleavage furrow formation results in the retraction of the furrow (54). Methyl- β -cyclodextrin has also been used to investigate the effects of cholesterol depletion in various cellular events. With a hydrophilic external and a hydrophobic internal face, cyclodextrins are able to extract cholesterol from the plasma membrane. Cholesterol depletion in zebrafish blastomeres with methyl- β -cyclodextrin inhibits endocytosis and prevents completion of cytokinesis, eventually resulting in multinucleated cells at high concentrations (72). Another study in human leukemia cells, focusing on the role of cholesterol in mitosis, revealed that cholesterol starvation and inhibition of cholesterol synthesis induced cytokinesis failure. Reintroduction of cholesterol to the growth medium abolished this effect, reinforcing the idea that the abnormalities were due to cholesterol deficiency (55). These data suggest an involvement of cholesterol in cytokinesis; however, its precise role, be it structural or signaling, is not known.

PE is an abundant lipid in most cells. It has also been reported to participate in cytokinesis, although its function is not clear. The cell surface localization of PE, which is normally localized at the inner leaflet of the plasma membrane, appears to be important for the completion of cytokinesis. Reduced PE can lead to cytokinetic arrest, which can be reversed by the addition of PE or ethanolamine, a precursor of PE (57). A study investigating the localization of PE using a peptide that recognizes PE showed that its surface exposure at the cleavage furrow is specific to the late telophase of cyto-

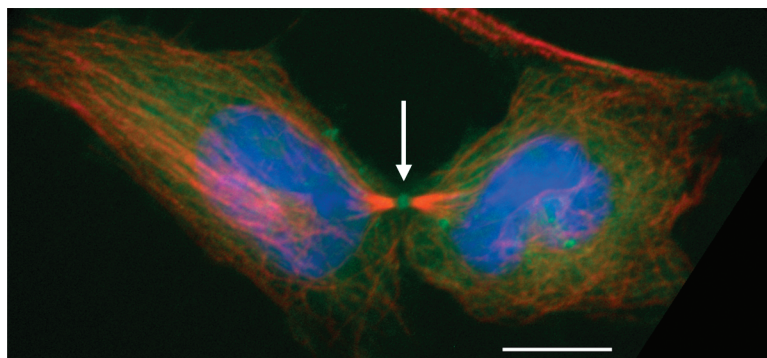


Figure 5. Dividing HeLa cell during the final stage of cytokinesis. PIP₂ localizes to the midbody (white arrow). HeLa cells were transfected with GFP-PLC-PH (green, vector kindly provided by Tamas Balla, NIH). Microtubules (red) were stained with antitubulin (Sigma T6199), DNA (blue) with DAPI. Scalebar represents 10 μ M.

kinesis. Cell surface immobilization of PE with a streptavidin analogue of the peptide blocked cytokinesis (58).

Very-Long-Chain Fatty Acids. Very-long-chain fatty acids (VLCFA), with aliphatic tails consisting of 22 carbons or longer, are thought to participate in the stabilization of highly curved membrane domains (73). They are mostly found in sphingolipids (see next section) and are essential for the formation and the function of these lipids. Elovl enzymes are involved in the biosynthesis of VLCFAs. A loss of function mutation in *bond*, an Elovl protein in *Drosophila*, causes cytokinetic defects in spermatocytes (74). Szafer-Glusman and colleagues investigated this phenotype and showed that VLCFAs are crucial in early cytokinesis. Although contractile ring assembly was normal, ring constriction was impaired, leading to halted furrow ingression, regression, and finally failed cytokinesis. In addition, the authors observed collapse and detachment of the contractile ring from the membrane during late cytokinesis. These mutants were unable to form an organized midzone, which is directly related to furrow regression (75). These results suggest that defects in the elongation of the VLCFAs affect the contraction of the ring as well as the stabilization of the midzone microtubules by the ring during cytokinesis.

Sphingolipids. Sphingolipids, ubiquitous components of the cell membrane, play a variety of roles in cellular environments. A few studies, mostly using small molecule probes, have found tentative links between sphingolipids and cytokinesis. Serine palmitoyl transferase (SPT) catalyzes the first reaction during sphingolipid biosynthesis. Myriocin, an inhibitor of SPT, causes

cytokinetic defects in some cell types, which can be rescued by the addition of sphingosine (76, 77).

Psychosine, a metabolite of the sphingolipid pathway, induces multinucleate cell formation in many cell types (61, 78). This was an exciting finding because it showed, for the first time, that changes in membrane composition could somehow cause cell division failure. Other sphingolipid analogues, glucopsychosine, sphingosylphosphorylcholine, and lysosulfatide, also induced the formation of multinuclear globoid-like cells (61). Psychosine accumulates and multinuclear globular giant cells are observed in the brains of patients with globoid cell leukodystrophy, a neurological disorder (79).

Psychosine's mechanism of cytokinesis inhibition is not clear. TDAG8 (T cell death associated gene 8), a G protein-coupled receptor (GPCR), is a target of psychosine. Expression of TDAG8 in some cell types in the presence of psychosine induced the formation of multinuclear cells. Overexpression of TDAG8 or psychosine treatment of cells that did not express TDAG8 did not induce the formation of multinuclear cells (78). Subsequent papers, however, suggested that it is likely that psychosine acts through additional targets (80). The inhibitory binding constant of psychosine for TDAG8 at physiological conditions is 3 μM , which is significantly higher than the binding constants of other lysolipids to their GPCR receptors. Also, TDAG8 is now thought to be proton-sensing GPCR. Psychosine shifts TDAG8's (and other proton-sensing GPCRs from the same family) proton response toward a more basic pH, suggesting that protons and psychosine may bind the GPCRs simultaneously (81). Once psychosine's cell membrane receptor(s) have been confirmed, the next challenge is to understand how it signals to the cell division machinery in the cell's interior.

G_{M1} is another sphingolipid that has been connected to cytokinesis (54). Together with their findings on the localization of sterols, Ng *et al.* studied the localization of G_{M1} by using a fluorophore conjugate of cholera toxin subunit B. In sea urchin eggs, they found a 7-fold increase in G_{M1} levels in furrowing cells compared to metaphase cells as well as a localization to the equatorial band during contractile ring formation (54). These data suggest a possible involvement, but no functional analyses are available at this point.

Are Polyamines Involved in Regulating Cytokinesis?

Polyamines are another class of naturally occurring small molecules that appear to be involved in cytokinesis, but their functional connections are not entirely clear. Small molecule inhibition of polyamine biosynthesis, particularly spermine, spermidine, and putrescine (Figure 4) synthesis, causes cytokinesis defects that can be rescued when the polyamine is added back (82). Biophysical experiments showed that polyamines can affect actin polymerization *in vitro*, and actin staining patterns in cells are disrupted when polyamine biosynthesis is inhibited (82–84). Thus, it is possible that the role of polyamines on cell division is through their specific interaction with actin filaments.

Role of Calcium Signaling in Cytokinesis. It has long been thought that calcium signaling plays important roles in cytokinesis, although the precise molecular functions are still only partially understood. Several studies conducted in embryos of different species have detected transient calcium signals at different stages of cytokinesis. Embryos have been used as a model system because they undergo several division cycles and are larger than cultured cells, allowing better differentiation of events that are tightly regulated in time and space.

For example, in zebrafish embryos, Ca^{2+} elevation along the equator of the cleavage furrow precedes the initiation of furrow contraction, followed by additional Ca^{2+} elevations at different time points during subsequent cleavages (85). Transient Ca^{2+} waves have also been observed in *Xenopus* embryos, although their timing and positioning differs from that of zebrafish embryos (86, 87). While the details are unclear, it is likely that there are functional relationships between calcium waves and cytokinesis because calcium sequestration by Ca^{2+} chelators such as BAPTA (1,2-bis(amino-phenoxy)ethane-*N,N,N',N'*-tetraacetic acid) (85, 87, 88) caused cytokinesis failure and because extra furrows were formed when calcium release was stimulated (89). Variations in the characteristics of the Ca^{2+} signals and in their presence during diverse events of cytokinesis might not only be due to differences in the studied systems. Using different imaging techniques and most importantly different Ca^{2+} -sensitive indicators might have resulted in opposing or partially complementary results (90, 91).

If calcium signaling is important in cytokinesis, the origin of the calcium needs to be examined. There is evi-

dence that Ca^{2+} is released from internal stores through binding of IP_3 (inositol 1,4,5-triphosphate) to the IP_3 receptor. Manipulations of the IP_3 receptor or of IP_3 levels have an effect on cytokinesis (85, 86, 88, 92, 93) and endoplasmic reticulum proteins together with the IP_3 receptor co-localize to both sides of the ingressing cleavage furrow in zebrafish embryos (94). IP_3 is the hydrolysis product of the signaling lipid PIP_2 , which is also important for cytokinesis. The IP_3 receptor can be phosphorylated by the key cytokinesis regulator Plk1, leading to an increased sensitivity of the receptor toward IP_3 and therefore enhanced Ca^{2+} release (95). Ca^{2+} release from IP_3 mediated stores might also be regulated through the actin binding protein cofilin, which plays a role in cytokinesis (96, 97). During maturation and fertilization of starfish oocytes cofilin modulates Ca^{2+} release through IP_3 and other Ca^{2+} -releasing messengers (97).

It is not clear which proteins are involved in calcium regulation during cytokinesis. Some of the functions of calcium seem to be mediated by calmodulin. Calmodulin is a receptor for intracellular calcium and activates a diverse array of target proteins in a calcium-dependent manner (98). Calmodulin localizes to interzonal microtubules and the intercellular bridge during cytokinesis in HeLa cells (99). Loss of calmodulin or application of calmodulin inhibitors disrupted or prevented cytokinesis (100, 101). Calmodulin activates MLCK, one of the proteins that activate myosin regulatory light chain (see discussion above for a dissection of MLCK signaling using small molecules) (102). To study upstream modulation of MLCK activity during cytokinesis, Wong *et al.* inhibited PIP_2 hydrolysis to the second messengers DAG (diacylglycerol) and IP_3 , resulting in a redistribution of actin away from the cleavage furrow and disrupted ingression (88, 102). When the authors used ML7 and ML9 to inhibit MLCK activity, they observed a similar cytokinesis defect compared to inhibition of PIP_2 hydrolysis and proposed a model whereby PIP_2 hydrolysis regulates myosin activation and furrow ingression via calcium signaling and MLCK activity. Proteins such as dyneins or other with microtubule-associated proteins (101, 103) have also been suggested as possible calmodulin effectors. However, more work is needed to understand their precise nature or functions.

Several other possible downstream functions of Ca^{2+} signaling in cytokinesis have been described. Calcium-dependent vesicle transport and membrane remodeling

are important in some systems (15, 92). Interfering with the calcium concentration inhibited accumulation of new membranes during cytokinesis, using the extracellular matrix protein hyalin as a membrane marker in sea urchin eggs (15). VAMP-2 vesicle fusion at the ingressing furrow in zebrafish embryos depends on Ca^{2+} released from IP_3 receptor mediated stores (92). The Ca^{2+} -dependent calpain family might play a role in cytokinesis (92, 104, 105) as well as annexin XI, a Ca^{2+} -dependent phospholipid-binding protein. Annexin XI is recruited to the midbody in late telophase in some cell types and seems to be necessary for the formation of the midbody and the completion of cytokinesis, possibly by facilitating the interaction between the plasma membrane and the microtubules of the midbody (106).

A role for calcium signaling during cytokinesis in different systems seems to be apparent, but the molecular details, the spatiotemporal correlation, and functions are less clear. New imaging methods and more precise and sensitive Ca^{2+} reporters will help to shed light on the role of calcium in cytokinesis.

Can Cytokinesis Be a Target for Cancer

Chemotherapy? This review discusses how chemical biology approaches have been very useful in understanding different aspects of the mechanism of cytokinesis. In the first part, we focused on small molecules as biological probes. In addition to their use as probe compounds, however, small molecules also have therapeutic potential. One would expect small molecules that target cytokinesis to be important in the development of cancer therapeutics because improperly regulated cell division can be a cause or a consequence of cancer. Mitosis failure as a cause of cancer progression has been well documented, and a recent report also linked cytokinesis failure to carcinogenesis (107).

Since cancer cells proliferate (and hence divide) rapidly, drugs that target cell division have been very successful for therapeutic intervention in cancer treatment. The overwhelming majority of these drugs inhibit mitosis, for example, by binding tubulin directly like taxol or by targeting mitotic kinesins, which are promising targets for clinical development (108). Given the success of antimitotic drugs in cancer therapy, it is surprising that cytokinesis has not been targeted on a wide scale. Aurora kinase inhibitors, which target both mitosis and cytokinesis, are currently in clinical trials (109), although the mechanism by which they kill cancer cells is poorly understood. As we improve our understanding of the

mechanism of cytokinesis and develop more small molecule probes targeting different proteins within cytokinesis, we anticipate that it will soon be possible to explore the potential “druggability” of cytokinesis.

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