

Animal Cytokinesis: From Parts List to Mechanisms

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Abstract

The mechanism underlying cytokinesis, the final step in cell division, remains one of the major unsolved questions in basic cell biology. Thanks to advances in functional genomics and proteomics, we are now able to assemble a “parts list” of proteins involved in cytokinesis. In this review, we discuss how to relate this parts list to biological mechanism. For easier analysis, we split cytokinesis into discrete steps: cleavage plane specification, rearrangement of microtubule structures, contractile ring assembly, ring ingression, and completion. We report on the advances that have been made to understand these steps and how they can be integrated into a global understanding of cytokinesis. We also discuss the extent to which classic questions have been answered and identify major outstanding questions.

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INTRODUCTION AND RESEARCH APPROACHES

Cytokinesis, the physical separation of one cell into two, is the last step in the cell cycle. It requires coordinated actions of the cytoskeleton, membrane systems, and the cell cycle engine, which are precisely controlled in space and time. Cytokinesis was described more than 100 years ago, and the roles of actin and myosin in cleavage, as well as that of the mitotic spindle in specifying the cleavage plane, were discovered more than 20 years ago (reviewed in Reference 1). The last few years have seen intensive focus on the identification of proteins involved in cytokinesis, using classical genetics and biochemistry. This effort, coupled with recent systematic RNA interference (RNAi) screens, has culminated in a more or less complete “parts lists.” The next challenge, and the focus of this review, is to convert such lists into an interlinked system of molecular mechanisms.

In this review, we focus on cytokinesis in animal cells, especially the two model organ-

isms whose parts lists are now available, nematode (*Caenorhabditis elegans*) embryos and cultured insect (*Drosophila*) cells. Other important systems include cultured mammalian cells, echinoderm and amphibian (*Xenopus*) eggs, and *Drosophila* embryos and spermatocytes. For recent reviews of cytokinesis in fungi and plants, see References 2 and 3. Each model system has unique features, including distinct regulatory and mechanical challenges faced by particular cell types to effect cytokinesis, as well as the more obvious technical pros and cons for different research methods. In cytokinesis research, as in other areas of basic cell biology, our molecular understanding has progressed to the point where the differences between cell types are becoming as interesting as their conserved features (4). For example, large eggs require longer microtubules to position spindles and furrows, and they may sacrifice accuracy-enhancing checkpoints in exchange for faster division. Also, the amount of new plasma membrane required to create two cells from one varies with the size and surface area of a given cell type, leading to differing levels of importance for vesicular trafficking pathways. These biological differences require that conserved mechanisms be implemented in distinct ways in different cells and may, perhaps, also require additional or different mechanisms in particular cell types. When controversies arise in the field, it is important to determine which differences arise from genuine differences between systems and which arise from personal interpretation of particular experiments in a fast-developing field.

Some differences between systems hinge primarily on the technical ease of implementing the three most important approaches in modern cell biology: genetics/genomics, microscopy, and biochemistry. For genetics/genomics, *C. elegans* embryos and *Drosophila* tissue culture cells are currently the most advanced, thanks to genome-wide RNAi screens. RNAi is far from a perfect genetic technology, typically providing an incomplete knockdown of protein levels that may take

RNAi: RNA interference

several days in cultured cells. It is also difficult to score a role in cytokinesis using RNAi for a protein that is important for earlier steps in the cell cycle, such as mitosis. Thus, parts lists determined solely by RNAi screens are incomplete. However, the power of this technology and its applicability to systems that lack traditional genetics have led to an explosion of interest and information. Given the current availability of technology and RNAi libraries, we expect publication of a list of proteins involved in cytokinesis in human cancer cells soon. These will be accomplished first for the commonly used HeLa cell line, originally derived from a cervical carcinoma, which is extensively adapted for life in culture and thus not truly representative of any normal human cell type. Generating parts lists for cytokinesis in untransformed human cells, or cancer cells in situ, will be more difficult.

Protein lists are not by themselves mechanistically informative, but the phenotypes observed using RNAi and traditional genetics provide functional information, especially in conjunction with high resolution microscopy. Microscopy has always been an important tool in cytokinesis research, and the past few years have seen dramatic improvement in optical methods. Genetically encoded fluorescent proteins of different colors are now in routine use in many systems, and the dynamic information they provide is being enhanced by methods for localized photobleaching and photoactivation. Instrumentation has also advanced, with highly sensitive digital cameras and new confocal technologies widely available. Together, these imaging methods are allowing the field to observe molecular events at play inside living cells. The confluence of microscopy with perturbations using genetics/genomics methods, or in some cases physical perturbation using microneedles or UV microbeams, is currently driving the field forward.

The biochemistry of cytokinesis, in contrast, has seen slower progress in the past few years. An extract system for all or part of cy-

tokinesis has yet to be reported, and reconstitution with purified components has been achieved for only a few processes and proteins (5, 6). We believe this lack of biochemistry may become limiting in the quest for molecular mechanism, particularly as quantitative understanding is sought through modeling. For mathematical models to approach reality, it will be necessary to use real values for protein concentrations and association constants. Recently, a first attempt at protein quantitation has been made in fission yeast (6a).

Two less commonly used research approaches deserve comment. Modeling has a distinguished history in cytokinesis research (e.g., 7), and its importance is likely to grow in the future, driven in part by an influx into basic cell biology of young scientists with physical and mathematical training. Small molecule approaches have also been historically important, notably in the discovery of the role of actin in cytokinesis via the cytochalasins (reviewed in Reference 8). Because cytokinesis is a rapid and dynamic process that occurs during a small portion of the cell cycle, small molecules that can rapidly enter living cells and perturb specific processes are especially valuable tools. We also believe that interfering with specific aspects of cytokinesis may provide novel and useful anticancer therapeutics, with the recently introduced Aurora kinase inhibitors as examples (9–11). Despite their dual importance for research and potential therapeutics and the large number of potential protein targets, the number of well-characterized small molecules available for cytokinesis research is small (**Table 1**). We carried out a screen for small molecule inhibitors of cytokinesis and identified 50 small molecules that caused the formation of binucleate cells in *Drosophila* tissue culture cells (20). We are currently investigating the cellular targets and biochemical mechanisms of many of these compounds and hope to provide the field with more diverse and useful small molecule tools to study cytokinesis in the future.

Table 1 Small molecules that affect cytokinesis

| Small molecule | Mechanism of action | Reference |
|---|---|-----------|
| Cytochalasins | Actin depolymerization: | (12) |
| Latrunculin | Bind barbed ends of actin | (13) |
| Swinholide | Sequesters actin monomers | (14) |
| Jasplakinolide | Severs actin filaments | (15) |
| (-) Blebbistatin ^a | Stabilization of actin filaments | (16) |
| Hesperadin, ^b ZM447439, ^b VX-680 ^b | Inhibition of myosin II ATPase | (16) |
| Y27632 ^b | Inhibition of Aurora B kinase | (9–11) |
| W7, ^b ML7 ^b | Inhibition of Rho kinase | (17) |
| | Inhibition of myosin light chain kinase | (18, 19) |

^aBlebbistatin is sensitive to UV and blue light.

^bPlease note that these drugs target the active ATPase site of the kinase and can also inhibit other kinases, especially at higher concentrations.

A PARTS LIST FOR CYTOKINESIS

A prerequisite for a complete understanding of cytokinesis is a parts list, or inventory, of all the molecules involved. Effective RNAi technology and complete genome sequence information have allowed a preliminary listing of the full complement of genes involved in cytokinesis for two cell types and more fragmentary lists for several others (**Table 2**). Because of the limitations of RNAi mentioned above, we believe that the most significant omissions from current lists may be proteins involved both in mitosis and cytokinesis, for which the cytokinesis defect can be difficult to score. For example, Polo kinase has a central role in cytokinesis but does not score in the screens because it is required for mitosis.

Proteomics provides a complementary strategy for genome-wide understanding. It has the potential to supply more direct information on biochemical mechanisms by providing data on relative protein concentrations, posttranslational modifications, and the composition of protein complexes. The methodology for making such measurements on a global scale using mass spectrometry is only now emerging and has yet to be applied to cytokinesis. A major limitation in applying proteomic methods to cytokinesis has been the challenge of isolating relevant structures and complexes from defined cell cycle states.

Skop et al. (25) approached this by isolating midbodies, representing a very late stage of cytokinesis, from synchronized Chinese hamster ovary cells. Five hundred seventy-seven proteins enriched in midbodies were identified, and 160 candidates were tested for relevance by RNAi of orthologs in *C. elegans* (25). Some of the cytokinesis proteins identified in this study are included in **Table 2**.

Detailed examination of recently published screens (20–25, 29, 31, 34, 41, 49, 74) reveals that certain proteins appear in every screen, whereas many proteins were only found in one or a few screens. **Table 2** lists those that scored in two or more screens, as well as well-described cytokinesis proteins from the literature. Proteins that scored in only one screen are presumably a mixture of conserved proteins that escaped detection in other screens for technical reasons, and proteins required in only a subset of systems. [See Supplemental **Tables 1–3** for a summary of hits (follow the Supplemental Material link from the Annual Reviews home page at <http://www.annualreviews.org>)]. Only two screens, one in worms (23) and one in flies (20), covered most of a genome so it is currently difficult to distinguish these possibilities. Given the unprecedented rate of new data generation, **Table 2** will soon be out of date. The European MitoCheck consortium

Table 2 Parts list of proteins involved in cytokinesis^a

| Mammalian gene | <i>Drosophila</i> gene | <i>C. elegans</i> gene | Predicted protein function | Screen reference | Reference |
|-------------------------------|------------------------|------------------------|--------------------------------------|------------------------------|-----------|
| Actin cytoskeleton | | | | | |
| <i>Actin</i> | <i>Act5C</i> | <i>act-1</i> | Actin | (20–23) | (12) |
| <i>Myosin heavy chain</i> | <i>zip</i> | <i>nmy-2</i> | Nonmuscle myosin II heavy chain | (20, 21, 24, 25) | (26–28) |
| <i>MRLC</i> | <i>sqb</i> | <i>mhc-4</i> | Myosin II regulatory light chain | (20–23, 25, 29) | (30) |
| <i>Anillin</i> | <i>ani</i> | <i>ani-1</i> | Anillin, actin binding | (20, 21, 24, 29, 31) | (32, 33) |
| <i>Arp3</i> | <i>arp66B</i> | <i>arx-1</i> | Actin nucleation | (20, 25) | |
| <i>CAPZ</i> | <i>cpb</i> | <i>cap-2</i> | Capping protein | (20, 25) | |
| <i>Profilin</i> | <i>cbic</i> | <i>pfu-1</i> | Binding of actin monomers | (21, 23–25, 34) | (35) |
| <i>Cofilin</i> | <i>tsr</i> | <i>unc-60</i> | Actin severing | (20, 21, 24, 25, 29, 31) | (36) |
| <i>mDia</i> | <i>dia</i> | <i>cyk-1</i> | Formin-Rho effector, actin nucleator | (20–25, 31) | (37, 38) |
| <i>RhoA</i> | <i>Rho1</i> | <i>rho-1</i> | Rho GTPase | (20, 21, 24, 29, 31) | (39) |
| <i>Ect2</i> | <i>pbl</i> | <i>let-21</i> | RhoGEF | (20, 21, 23, 25, 29, 31) | (40) |
| <i>MgcRacGAP</i> | <i>RacGAP50C</i> | <i>cyk-4</i> | RhoGAP | (20–24, 29, 41) | (42, 43) |
| <i>Citron kinase</i> | <i>CG10522</i> | <i>F59A6.5/W02B8.2</i> | Kinase-Rho effector | (20, 21, 24, 25, 31) | (44) |
| <i>ROCK</i> | <i>rok</i> | <i>let-502</i> | Rho kinase-Rho effector | (20, 21) | (45) |
| Microtubule associated | | | | | |
| <i>Tubulin</i> | <i>tub84D</i> | <i>tba-2</i> | Tubulin | (21, 25) | |
| <i>γTubulin</i> | <i>γTub</i> | <i>tbg-1</i> | Microtubule nucleation | (25, 41) | |
| <i>PRC1</i> | <i>feo</i> | <i>spd-1</i> | Microtubule bundling | (20, 21) | (46, 47) |
| <i>CLASPI/2</i> | <i>orbit</i> | <i>cls-2</i> | Microtubule-tip binding | (25) | (48) |
| <i>MKLP1</i> | <i>pav</i> | <i>zen-4</i> | Kinesin-6, microtubule motor | (20, 21, 23, 25, 29, 31, 49) | (50–52) |
| <i>MKLP2/rab kinesin6</i> | | | Kinesin-6, microtubule motor | (49) | (53) |
| <i>Kif4A/B</i> | <i>klp3A</i> | <i>klp-12, klp-19</i> | Kinesin-4, microtubule motor | (25, 49) | (54) |
| <i>KIFC1</i> | <i>ncd</i> | <i>klp-16</i> | Kinesin-14, microtubule motor | (20, 41) | |
| <i>KIF18</i> | <i>klp67A</i> | | Kinesin-8, microtubule motor | | (55) |
| Vesicle transport | | | | | |
| <i>Clatrin heavy chain</i> | <i>cbc</i> | <i>cbc-1</i> | Endocytosis | (20, 25) | (56) |
| <i>Dynamin</i> | <i>sbi</i> | <i>dyn-1</i> | Endocytosis | (20, 25, 31) | (57) |
| <i>Syntaxin 1A</i> | <i>syx1A</i> | <i>unc-64</i> | Vesicle fusion | (21, 29) | |
| <i>Syntaxin 5</i> | <i>syx5</i> | <i>syn-3</i> | Vesicle fusion | (20, 21) | (58) |
| <i>betaCOP</i> | <i>betaCOP</i> | <i>Y25C1A.5</i> | COP1 coatomer | (20, 25) | |
| <i>gammaCOP</i> | <i>gammaCOP</i> | | COP1 coatomer | (20, 21, 24, 31) | |
| <i>NSF attachment protein</i> | <i>SNAP</i> | <i>pbi-29</i> | SNARE-mediated membrane fusion | (20, 21) | |
| <i>Arfophilin/Fib3-Rab11</i> | | | Recycling endosome | | (59) |

(Continued)

Table 2 (Continued)

| Mammalian gene | <i>Drosophila</i> gene | <i>C. elegans</i> gene | Predicted protein function | Screen reference | Reference |
|--|--|--|-------------------------------|------------------|-----------|
| Regulation | | | | | |
| <i>Aurora B kinase</i> | <i>ial</i> | <i>air-2</i> | Aurora B kinase complex | (20, 21, 31) | (60) |
| <i>INCENP</i> | <i>INCENP</i> | <i>icp-1</i> | Aurora B kinase complex | | (61) |
| <i>Survivin</i> | | <i>bir-1</i> | Aurora B kinase complex | | (62) |
| <i>Borealin</i> | <i>Borr</i> | <i>csc-1</i> | Aurora B kinase complex | (20) | (63–65) |
| <i>Cyclin B3</i> | <i>cycB3</i> | <i>cyb-3</i> | Cyclin | (41) | (66) |
| <i>CyclinB</i> | <i>cycB</i> | <i>cyb-1</i> , <i>cyb2.1</i> | Cyclin | (41) | (66) |
| <i>Polo</i> | <i>polo</i> | <i>plk-1</i> | Polo kinase | | (67) |
| Other | | | | | |
| <i>Annexin11</i> | | | Annexin | | (68) |
| <i>BRC A2</i> | | | Oncogene | | (69) |
| <i>centriolin</i> | | | Centrosome binding | | (70) |
| <i>Nir2</i> | <i>rdgB</i> | <i>M01F1.7</i> | PI transferase | | (71) |
| <i>Orc6</i> | <i>orc6</i> | | Initiation of DNA replication | | (72) |
| <i>PI4 kinase</i> | <i>fwd</i> | | PI4 kinase | (20) | (73) |
| <i>SEPT2^b SEPT9^b</i> | <i>Sep2^b</i> , <i>pnut^b</i> | <i>unc-59^b</i> , <i>unc-61^b</i> | Septin | (21) | (73a–c) |
| <i>SNW1</i> | <i>Bx42</i> <i>CG7236</i> <i>Tra1</i> | | Splicing factor | (20, 74) | |
| | | | Kinase | (20, 21, 31) | |
| | | | Transcription factor | (20, 21) | |
| | | <i>lin-5</i> | Unknown | (41) | (75) |
| | | <i>spk-1</i> | Kinase | (22, 23) | |

^aGene products that scored in a screen and at least one other study as well as proteins identified in detailed studies are included. Putative human, *Drosophila*, and *C. elegans* orthologs are shown for each protein. If several copies of a gene are present in the genome (for example actin), only one is shown for clarity. For a full list of screening hits, see Supplemental Tables 1–3. Follow the Supplemental Material link from the Annual Reviews home page at <http://www.annualreviews.org>. Genes implicated in cytokinesis are shown in bold font; orthologs that have not yet been implicated are shown in regular font.

^bSeptins have been implicated in cytokinesis in a number of systems, but the ortholog relationships are unclear at this point.

Cleavage furrow or cytokinetic furrow or contractile ring:

forms at cell equator during anaphase and ingresses during cytokinesis. Contains actin, myosin and other proteins

(<http://www.mitocheck.org>) is making a systematic effort to identify and annotate all proteins involved in mitosis and cytokinesis in human cells and has created a growing database. This resource will incorporate the results of genome-wide screening in human cells starting some time in 2006. **Table 2**, and related lists, are the first effort toward a complete parts list for cytokinesis.

Not all the molecules involved in cytokinesis will be proteins that can be identified by techniques such as RNAi; small molecule metabolites and nonprotein macromolecules will also have important roles. Because cytokinesis intimately involves the plasma mem-

brane and organelles, we expect specific roles for lipids or their metabolites. Recent studies show that phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P₂] accumulates at the cleavage furrow and is required for cytokinesis in HeLa cells and in *Drosophila* spermatocytes (76, 77), and **Table 2** includes two enzymes of lipid metabolism, the PtdIns transfer protein Nir2 and a PtdIns 4 kinase. Also, a glycosphingolipid, psychosine (1-β-D-galactosylsphingosine) is known to negatively regulate cytokinesis in certain cell types (78, 79). Further analysis of the role of specific lipids and other metabolites might provide interesting insights.

SUBPROCESSES IN CYTOKINESIS

In the rest of this review, we address new mechanistic information on various subprocesses in cytokinesis and relate these to the parts list in **Table 2**. **Figure 1** shows a breakdown of cytokinesis into a series of subprocesses based on timing and morphology. This is the traditional method of making cytokinesis more manageable from a reductionist perspective, but caution is required because biochemical subsystems may not fit neatly into categories defined by morphology and timing. For example, the biochemically defined Rho pathway is probably involved in several subprocesses, and some subprocesses that appear unitary, such as completion, may in fact be highly complex, requiring multiple biochemical pathways. The timing and physical requirements for cytokinesis were extensively explored in classic experiments, notably in echinoderm embryos by Rappaport (1). These constitute a platform for current research that has been extensively reviewed elsewhere (80). Determining the molecular mechanisms underlying the Rappaport phenomenology can be viewed as the central goal of modern cytokinesis research.

Timing Cytokinesis

An important Rappaport conclusion was that although the whole cell cortex can support assembly of a cleavage furrow, this ability is tightly restricted in time. In echinoderm embryos, the ability to form a cleavage furrow is only expressed in a short window starting after anaphase onset. Later, Margolis and coworkers (81) found that inhibition of cytokinesis in tissue culture cells with a drug that blocks actin depolymerization was reversible but only if the drug was washed out within a window of ~45 min following initiation of cytokinesis. Canman et al. (82) extended this work and coined the term “C phase” for the period during the cell cycle in which cytokinesis can occur. C phase is not part of the

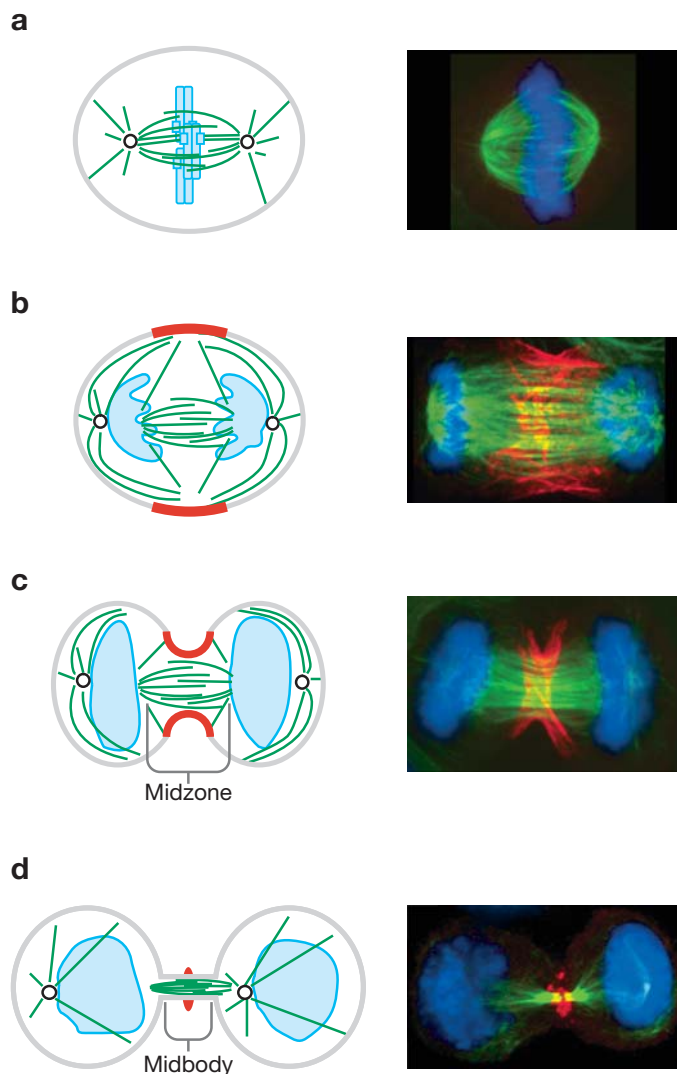


Figure 1

Schematic and immunofluorescence illustrations showing different stages of cytokinesis: DNA (*light blue*), microtubules (*green*), and the cleavage furrow protein Anillin (*red*) are shown. (a) Cell is in metaphase. Chromosomes are aligned at the metaphase plate by the microtubules of the mitotic spindle. (b) Cell is in late anaphase. Microtubules have elongated and contact the cortex. They have also rearranged to create a region of bundled microtubules between chromosomes termed the midzone. Cleavage furrow components (*red*) have assembled at the equator. (c) Cell is in early telophase. The cleavage furrow ingresses. (d) Cell is in late telophase. The cleavage furrow has fully ingressed compressing the midzone and creating an intercellular bridge containing a microtubule midbody. Completion occurs when the intercellular bridge is resolved creating two daughter cells.

Cell cortex: a meshwork attached to the plasma membrane that contains actin and other proteins

C phase: the time (~1 h) during which the cortex remains capable of contraction after anaphase onset

GEF: guanine nucleotide exchange factor

fundamental cell cycle oscillator like S or M phase but responds to that oscillator and appears to be a conserved aspect of animal cytokinesis. None of the proteins in **Table 2** has an obvious role in timing cytokinesis, suggesting C phase regulation may involve proteins required elsewhere in the cell cycle.

One might expect that onset of C phase is triggered by the reduction of Cdc2/Cdk1 kinase activity that accompanies anaphase onset because lowered Cdc2 activity is thought to trigger other cytoplasmic rearrangements at anaphase, such as nuclear envelope reformation (83). Consistent with this expectation, inhibiting Cdc2 in mammalian cells with drugs rapidly triggers cytokinesis-associated changes in cortical activity (83a). Studies in echinoderm embryos, however, suggest a different role for Cdc2. When anaphase spindles were pushed close to the cortex in echinoderm embryos in which Cdc2 kinase activity was kept artificially high, furrowing was induced (84). Thus, lowering Cdc2 kinase may not regulate the cortex directly in that system, rather it may function to increase the length of astral microtubules required for signaling (discussed below). The same manipulation did not induce furrowing when the anaphase-promoting complex (APC) was inhibited (85). APC is the E3 ligase that regulates exit from mitosis by ubiquitination of cyclin B and securin (86). Thus C phase entry may be induced by APC-dependent proteolysis. C phase exit may also involve ubiquitin-mediated proteolysis. A pharmacological study in cultured human cells found that, out of many drugs tested, the only one that altered the duration of C phase was a proteasome inhibitor, which approximately doubled its length when added after C phase was initiated (16).

Rape & Kirschner (87) recently proposed that the duration of G1 is regulated by ordered proteolysis of different APC substrates, with the ordering generated by different ubiquitination kinetics for varied substrates. Perhaps ordered proteolysis, mediated by APC and proteasomes, regulates both onset and

exit from C phase in a similar manner. Consistent with this idea, Lindon & Pines (88) found that ordered proteolysis of mitotic regulators such as Polo-like kinase can contribute to the timing of mitotic exit and can influence the duration of C phase, and Echard & O'Farrell (66) proposed that sequential degradation of cyclin B and cyclin B3 controls the timing of C phase in *Drosophila*, possibly by regulating the Rho guanine nucleotide exchange factor (GEF) Ect2/pebble. In a screen for APC substrates in mammalian cells, two important cytokinesis proteins, the actin-myosin II-binding protein Anillin (89) and Aurora B kinase (89a), were found to be ubiquitinated and degraded late in the M to G1 transition. It seems likely that regulation of C phase involves both kinase-phosphatase and regulated proteolysis systems. Dissecting their relative contributions might resolve apparent discrepancies in the literature.

Cleavage Plane Specification: Evaluation of Classic Models

Cleavage always occurs perpendicular to the axis of chromosome segregation, thus ensuring equal partition of the genome. Typically, it occurs in the middle of the cell, partitioning the cytoplasm equally. Unequal cleavage, following off-center positioning of the spindle, is common during embryonic development and in stem cell divisions during adult homeostasis. The special mechanisms involved in unequal division have recently been reviewed (90, 91), so we do not discuss them. It has long been known that cleavage plane specification involves communication between microtubules and the actin cortex (1), and the molecular mechanisms involved are now the subject of intense research efforts in many laboratories (92–95).

In both classical models and recent research, a large effort has been made to distinguish possible contributions of different types of signals from microtubules to the cortex (see **Figure 2**). One distinction focuses on the nature of the signal delivered. *Polar*

relaxation refers to a negative signal from microtubules to the cortex at the poles (red arrows in **Figure 2**), preventing furrow assembly there, and specifying assembly at the equator by default. *Equatorial stimulation* refers to a positive signal from microtubules to the cortex at the equator (blue arrows in **Figure 2**), directly stimulating furrow assembly there. A second distinction focuses on the identity of the microtubules delivering the signal, and the primacy of asters vs midzones (**Figure 2**). *Asters* (pale green lines in **Figure 2**) are approximately radial microtubule arrays nucleated by centrosomes on the outsides of the spindle. *Midzones* (dark green lines in **Figure 2**) are antiparallel arrays of microtubules that assemble in between the separated chromosome masses during cytokinesis. Midzone organization mechanisms are discussed below. The two debates (relaxation vs stimulation, asters vs midzone) are related in the sense that any relaxing signal at the poles would presumably be delivered only by astral microtubules because only these come near the polar cortex. A stimulating signal at the equator, in contrast, could come from either type of microtubule, or both, because plus ends of astral and midzone microtubules intermix near the equatorial cortex in many cell types (see **Figure 2** [this review] and figure 5 in Reference 96). Although these hypotheses were classically seen as alternatives, it now seems likely that all of them may in fact operate in a single cell.

The asters vs midzone debate was recently resolved by an experiment proving that both arrays send signals to the cortex. These signals were distinguished by physically separating the two arrays, causing the induction of two distinct furrows (see **Figure 3**) (97). A UV microbeam was used to sever the connection between one aster and the midzone early in cytokinesis in *C. elegans* embryos (**Figure 3a**). The strong pulling forces that act on cortical microtubules in this system then caused the single aster and the midzone with the attached aster to move further apart than during

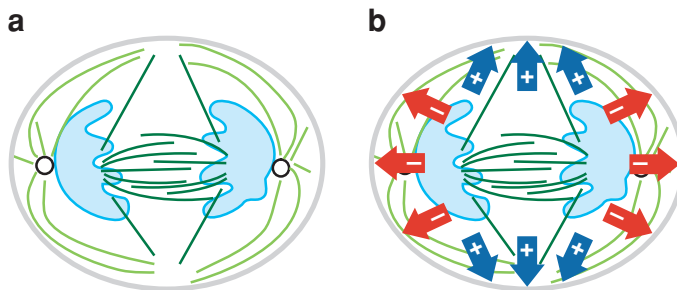


Figure 2

Organization of anaphase microtubule arrays and models for their roles in signaling to the cortex. Adapted from immunofluorescence images of PtK2 cells (99). (a) In anaphase, astral microtubules (light green) emanating from the centrosomes (circles) have elongated and many of their plus ends extend to the equatorial cortex. The midzone microtubule array (dark green) is made up of bundled, overlapping microtubules that extend between the chromatin masses (light blue). Note that some of the midzone microtubules extend to the equatorial cortex. The midzone microtubule array is typically more dense but has been reduced for clarity. (b) Models of how anaphase microtubules signal to the cortex. Polar relaxation signals (red arrows) and equatorial stimulation signals (blue arrows) are shown.

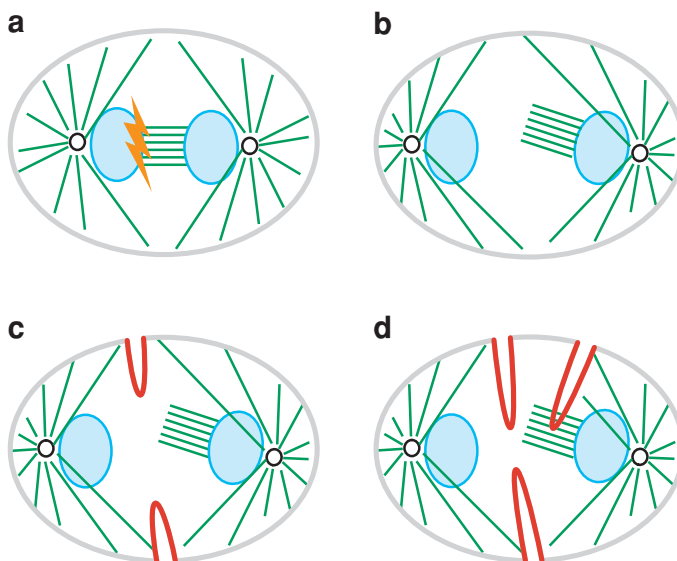


Figure 3

Cartoon of the experiments performed by Bringmann & Hyman (97). (a) Asymmetric spindle severing. (b) Severed aster is pulled away from the midzone and other aster. (c) First furrow ingresses at a point equidistant to both asters. (d) A second furrow ingresses at the middle of the midzone.

Midzone: bundled microtubule array between separating chromosomes, first formed during anaphase, sometimes called central spindle

Actomyosin: structures containing actin and nonmuscle myosin II

GAP: GTPase-activating protein

conventional cytokinesis (**Figure 3b**). The cell responded by first assembling a furrow equidistant between the two asters, ignoring the midzone (**Figure 3c**). This aster-promoted furrow ingressed deeply, but later it tended to regress and appeared incapable of completing cytokinesis alone. A few minutes after the first furrow formed, a second furrow assembled at the center of the midzone (**Figure 3d**). This midzone-promoted furrow ingressed rapidly, and remarkably, it often appeared to change direction to link up with the aster-promoted furrow, allowing cytokinesis to progress toward completion. Using RNAi, the authors began to assign some of the proteins from **Table 2** into roles in triggering each type of furrow. As expected, the proteins required for midzone assembly blocked only midzone-promoted furrows. Interestingly, some proteins, e.g., the kinesin Zen4, were involved in triggering both types of furrow, implying some overlap in signaling and/or delivery mechanisms.

The other debate, polar relaxation vs equatorial stimulation, has yet to be resolved decisively, but we favor the possibility that both hypotheses are again correct. There is abundant evidence that proteins enriched at the equator deliver positive signals (discussed below). There is currently no direct molecular evidence for a negative or relaxing signal delivered to the poles in cytokinesis. However, negative signals and/or the absence of proteins are harder to study. Nevertheless, strong phenomenological evidence has been produced for polar relaxation in cytokinesis. For example, a partially depolymerized microtubule array triggered flow of material away from the nearby cortex in *C. elegans* embryos (98), and abnormally strong contractions were noted when mammalian cells were forced to enter C phase in the absence of microtubules by overriding the mitotic checkpoint (99). Furthermore, microtubules have been shown to deliver a negative signal to cortical actomyosin in other systems (100), and one mechanism is thought to be sequestration of a GEF for the small GTPase RhoA (101).

If it is true that all the classically discussed mechanisms are in fact correct, and that they operate in parallel, why would cytokinesis be so complicated? An obvious idea is that overlapping mechanisms make site specification in cytokinesis robust to variation in the exact amounts of different proteins in the cell, the size and shape, or other variables that are difficult for a cell to control. Robustness—producing a well-defined outcome in the face of variation of input parameters—is a property of a molecular system that can be quantified if an appropriate model can be built, and it will be an interesting measure of future models of cytokinesis.

Cleavage Plane Specification: Molecular Mechanisms

The positive signal at the equator has been easiest to study; proteins that accumulate at the equator in a microtubule-dependent manner and are required for furrowing are candidates for participation in this signal. By these criteria, the Rho pathway, which regulates both myosin II activation and actin polymerization, is heavily implicated (4, 104). Rho (particularly RhoA in mammalian systems) and several Rho regulators, including the GEF Ect2/pebble and the GTPase-activating protein (GAP), MgcRacGAP, all accumulate at furrows and are required for cytokinesis (39, 40, 43, 102). The primacy of Rho in positive signaling is still controversial; enrichment of Rho at the furrow is clear in fixed cells by immunofluorescence (103), but reporters for active Rho in living cells have given somewhat contradictory results (104, 105). Furthermore, cytokinesis in the absence of RhoA function has been reported in strongly adherent mammalian cells, where it correlates with an absence of Rho activation using a fluorescence resonance energy transfer (FRET) reporter (106). Lack of a Rho pathway requirement in mammalian cells may correlate with the ability to cleave in the absence of myosin II function (discussed below).

Two kinases, Aurora B and Polo, also meet the criteria for involvement in the positive signal (reviewed in Reference 83). Their role in cytokinesis has been difficult to dissect because both are also involved in mitosis. The recent availability of small molecule Aurora B inhibitors (**Table 1**) may help elucidate its specific role in cytokinesis, and similar reagents for Polo are needed. To make progress in understanding the function of Aurora B and Polo in cytokinesis, we also need to identify substrates whose phosphorylation plays a specific role in regulating cytokinesis. There will probably be many such substrates. Two likely Aurora B substrates involved in cytokinesis are MgcRacGAP (107) and the kinesin Zen4/MKLP (108).

The mechanism by which microtubules spatially regulate the activity of the Rho pathway and the kinases is currently quite mysterious. Models under consideration include transport of signaling complexes along microtubules by motors, signaling by plus-end tracking complexes, and control of signaling simply by the local concentration of tubulin polymer. In support of the motor hypothesis, the plus-end-directed motor, kinesin-6 (Pavarotti/MKLP1/Zen4) has been implicated in cytokinesis in all systems examined (**Table 2**). This motor physically interacts with the Rho GAP, MgcRacGAP, (109) and participates in a defined molecular complex with the Rho GEF, Ect2/pebble, possibly through a Ect2/pebble-MgcRacGAP interaction (42, 110). Thus the motor protein could transport key Rho regulators to the cortex. In support of signaling by plus ends, a plus-end-tracking protein, CLASP/orbit, has been shown to be important for cytokinesis (48). However, taxol-stabilized asters can successfully signal (111, 112), implying dynamic plus ends are not always required. Local microtubule polymer concentration alone may also be important. An actin nucleator and essential cytokinesis protein, Diaphanous, binds to microtubules independent of Rho signaling and microtubule dynamics (113). Perhaps the presence of microtubules locally sequesters

this protein (or other signaling factors) away from the plasma membrane. We still have much to learn about how microtubules direct the signaling pathways required for cytokinesis.

Organization of Cytokinesis-Associated Microtubules

Given their role in delivering spatially restricted signals to the cortex, it is important that microtubules are properly organized in space and time during cytokinesis. Cytokinesis-associated microtubules are dominated by asters and midzones (**Figure 2**). Astral microtubules elongate greatly at anaphase in many systems, so they often touch the cortex and elongate toward the equator. This elongation plays a central role in morphogenesis of the microtubule cytoskeleton during cytokinesis, and it is thought to be important for microtubule signaling to the cortex (84, 112). Elongation may be driven by a decrease in catastrophe rate after anaphase (114). This is correlated with decreased Cdc2 levels, although exactly how Cdc2 regulates microtubule dynamics is not understood. Rappaport (1) measured that the microtubule-derived signal propagates to the cortex in echinoderm eggs at a rate of 6 to 7 $\mu\text{m}/\text{min}$, and perhaps anaphase microtubule elongation sets this rate.

The midzone is initially formed between the separating chromosomes by bundling of elongating overlapping microtubules associated with the spindle (see the movies in Reference 115). Later, the midzone becomes self-organizing, and it can persist for many minutes, if furrow contraction is blocked (16). Although initially formed by the reorganization of existing microtubules, it is likely that new microtubules are nucleated within the midzone (115a). Some authors refer to midzones as “central spindles,” which is confusing, as the same name has been applied to overlapping microtubules of the mitotic spindle during metaphase. Although midzones

Intercellular bridge: connects two daughter cells prior to abscission and is microtubule rich with the midbody at its center

Midbody: the center of the intercellular bridge. It contains microtubules and a high protein density area, the stembody

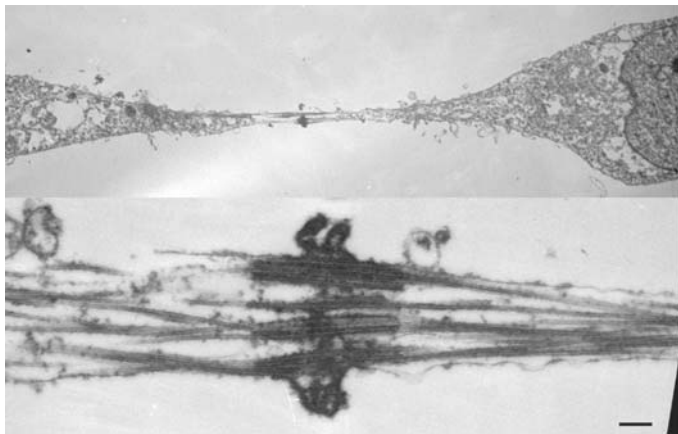


Figure 4

Thin section electron micrographs of a dividing HeLa cell. The upper panel shows an intercellular bridge between two daughter cells. The nucleus of the right-hand cell is visible. The lower panel is a higher resolution image of the same bridge. Note the bundled microtubules in the midbody and the electron-dense material (stembody) concentrated in a discrete zone at the center of the bridge (*lower panel*). Stembodies typically bulge outward at their center. The bar shown in the lower panel corresponds to 2 μm (*upper panel*) and 250 nm (*lower panel*). Images courtesy of Margaret Coughlin.

originate from spindle microtubules, distinct microtubule-associated proteins (MAPs) and motors organize the two arrays.

The midzone has several functions during cytokinesis. One is to help deliver the equatorial stimulation signal (discussed above), but this probably involves microtubules that elongate from the midzone to the cortex, rather than the midzone itself (48, 116). A second is to keep the separated genomes apart prior to completion; when microtubules were depolymerized before completion in mammalian cells, the nuclei collapsed back together (16). A third is to participate in completion and cell cycle regulation. These are functions of the midbody, a microtubule array within the intercellular bridge that connects two daughter cells. The midbody is a derivative of the midzone that forms by compression of the furrow during ingression, and its role is discussed below in the section on completion.

At the electron microscopy (EM) level, the midzone is dominated by electron-dense material that accumulates at the equator on

overlapping microtubule bundles starting at anaphase (117). The electron-dense material coalesces as the midzone microtubule bundles are compressed by the furrow, eventually forming a small disc that was called a stembody, from the German *Stemmkörper* “pushing body” (see **Figure 4**). Despite its dominance in EM views, the molecular nature of the electron-dense material has not been determined, and its precise function is unknown.

The principal microtubule-interacting proteins implicated in morphogenesis of the midzone are bundling factors and kinases. A conserved bundling factor, PRC1/Fascetto/spd-1 accumulates at the center of the midzone and ablating it blocks midzone assembly in all systems (**Table 2**). PRC1 binds and bundles microtubules *in vitro* and in cells (46). It is regulated by the cell cycle, possibly through phosphorylation by Cdc2-cyclin B. As mitosis progresses and Cdc2 activity decreases, PRC1 is dephosphorylated and becomes active (118). RNAi-mediated knock-down of PRC1 in mammalian cells prevented localization of other midzone markers and completely blocked midzone assembly (116). Signaling proteins such as Aurora B kinase still accumulated at the furrow, presumably because astral microtubules were unaffected. Ingression was normal, but completion failed. Given its biochemistry, localization, and genetics, PRC1 family members are probably the main bundling factor in midzones; an interesting unanswered question is whether they enforce antiparallel organization.

Two classes of plus-end-directed kinesins have been implicated in midzone assembly. In all systems, a kinesin-6 family member (MKLP1/Pavavotti/Zen4) accumulates at the center of the midzone and ablating it blocks midzone assembly (**Table 2**). MKLP1 is a plus-end-directed motor that can cross-link microtubules and slide one microtubule over another (52), making it an ideal candidate for organizing overlap interactions within the midzone. CHO1, a splice variant of MKLP1, may be the most relevant isoform for midzone morphogenesis in mammalian cells (119).

EM: electron microscopy

Stembody: the small electron-dense disk at the center of the midbody

A second plus-end-directed kinesin from the kinesin-4 family plays a less defined role in midzone assembly. Mammalian Kif4 is a chromokinesin during mitosis, with a poorly defined role in spindle assembly/function (120). At anaphase, Kif4 relocalizes to microtubule bundles and accumulates at the center of the midzone (120). Kif4 binds PRC1, with a preference for the dephosphorylated form, and may help to localize this microtubule-bundling protein correctly in the midzone (118).

Other proteins might also contribute to midzone formation. The plus-end-tracking protein CLASP/orbit may be involved (48). Annexin 11 is a new player in midzone organization in mammalian cells. Its biochemical function is not known, but it localizes strongly to midzones and removing it blocks their assembly (68).

Microtubule organization during cell division has typically been considered independent of the actin cytoskeleton, and signaling from microtubules to the cortex was classically considered unidirectional, but recent evidence has questioned this view. During mitosis, spindle organization depends on cortical actomyosin in some systems (121), and during cytokinesis, there may be feedback from the cortex to midzone organization. In *Drosophila* cells, damage to the actin cytoskeleton prevents assembly of a normal midzone (35); and, in mammalian cells, evidence from speckle imaging suggested that microtubule plus ends contacting the equatorial cortex are specifically stabilized against catastrophes (99). A feedback loop from the cortex to microtubule organization is appealing as a way to ensure robust self-organization of both cytoskeletal systems during cytokinesis.

Contractile Ring Assembly

The question of how the furrow assembles is closely connected to that of how microtubules signal to the cortex. The furrow consists of a contractile ring together with the plasma membrane to which it is connected.

We currently understand the biochemistry of the ring, which is dominated by actin and myosin II, much better than that of the membrane. Contractile rings are stable biochemical entities in the sense that they can be isolated and can be induced to contract in vitro (122), but these rings are highly dynamic in cells, with fast turnover of both actin and myosin (123, 124).

The organization of actin and myosin II in contractile rings is an unsolved problem. In **Figure 5a-c**, we depict three possible orthogonal alignments of actomyosin filaments to clarify their implications for force generation, noting that real cells presumably contain some mixture of these alignments. Schroeder proposed the “purse-string” model (**Figure 5a**) (125) on the basis of EM observations in echinoderm embryos. In this model, filament sliding shortens the ring, and ingression force comes from the component of the sliding force that is directed inward, which is given by contractile force in the ring multiplied by the reciprocal of the radius. In other words, this mechanism becomes more efficient as the ring gets smaller. Schroeder’s purse string dominates textbooks, yet there is surprisingly little structural evidence for alignment of contractile fibers in this orientation. Fishkind & Wang (126) observed actin bundles connecting the dorsal part of the furrow to the ventral surface in adherent mammalian cells, leading them to propose the model in **Figure 5b**. In this model, all contractile force is directed inward. Paradoxically, the majority of fluorescently-labeled actin filaments observed in mammalian cells tend to show the organization in **Figure 5c** (33, 126, 127). Contraction of filaments with this orientation has no inwardly directed force component, if anything it would tend to oppose ingression. Perhaps these filaments do not participate in force generation and align passively in response to ingression. Finally, we note that anisotropic organization could potentially generate inward force. Cytoplasmic extracts containing actin and myosin II undergo “gelation-contraction” in which an anisotropic F-actin gel contracts

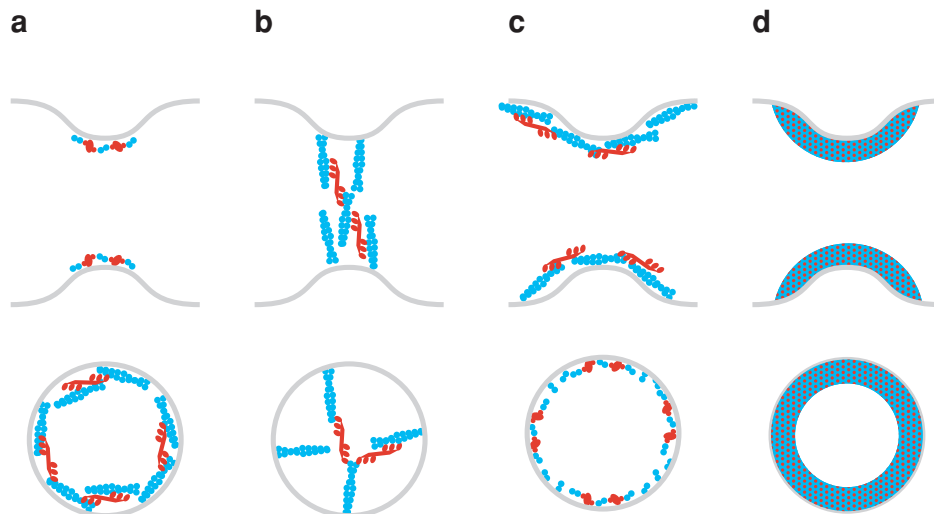


Figure 5

Models for different actomyosin alignments in the furrow. We cartoon three possible filament alignments to illustrate their implications for force production. Real cells presumably contain a mixture. The top row shows imaginary sections parallel to the axis of chromosome segregation, and the bottom row bisects that axis. Actin filaments are light blue and myosin motors red. (a) The purse-string model. Contraction of these filaments would promote ingress. (b) Filaments orthogonal to the ingressing membrane. Contraction of these filaments would also promote ingress. (c) Filaments parallel to the axis of chromosome segregation. Contraction of these filaments would, in theory, impede ingress, yet this is the major orientation observed in dividing cells. (d) Anisotropic orientation of filaments. Constriction could occur by gelation-contraction.

by myosin II activity (128). The structural basis by which an anisotropic gel contracts is unknown, and mixtures of pure F-actin and myosin II do not contract in the presence of ATP, rather they become more fluid (129). Nevertheless, observation of gelation-contraction implies that the contractile ring could contract even if its constituents are disorganized (**Figure 5d**), i.e., a mixture of the orientations in **Figure 5a-c**.

Recruitment of F-actin to the furrow might occur by nucleation in the furrow or by transport of filaments from elsewhere. Diaphanous, a formin, is a conserved, essential cytokinesis protein (**Table 2**), whose only known biochemical function is to nucleate actin filaments (130). The nucleation activity of Diaphanous is thought to be positively regulated by the Rho pathway (38). Thus it is a logical candidate to nucleate furrow actin. Whether it acts in furrows or nucleates else-

where, followed by transport of filaments to the furrow, is unclear. White & Borisy (131) proposed that cleavage furrow actomyosin self-organizes by contraction-driven flow in the cortex, and subsequent microscopy studies found evidence for such flow in mammalian cells (123, 132–134) as well as in nematode embryos (98). In *Xenopus* eggs, Noguchi & Mabuchi (135) observed F-actin polymerization directly in the furrow as patch-like accumulations. In summary, there may be multiple mechanisms for accumulating furrow actin, and the precise function of Diaphanous is an important topic for further research in this area.

Recruitment of myosin II to furrows does not require its ATPase activity (16, 136, 137), and recent studies used live imaging and RNAi to show that recruitment of myosin to the cleavage furrow and its continued localization depend on different pathways

(96, 126a). Phosphorylated myosin regulatory light chain is observed at the furrow during early anaphase (138) and is needed to assemble myosin into the ring. DeBiasio et al. (127) carried out a comprehensive analysis of myosin II during cytokinesis in live 3T3 cells. They found that myosin fibers flow toward the equator during anaphase and form a meshwork, which contains fibers that are both parallel and perpendicular to the plane of cleavage. Cortical flow of myosin has also been observed in *Xenopus* eggs (135). Myosin is very dynamic at the furrow; phosphorylation of the heavy chain is required to maintain its dynamic behavior (124). Myosin transport along microtubules (139) is an appealing idea but has not been substantiated by biochemistry. In summary, cortical flow appears to contribute to myosin localization but is unlikely to be the only mechanism given the observations of recruitment in the absence of its own motor activity.

Furrow Ingression

Furrow ingression proceeds by some combination of force generation from the cytoskeleton (**Figure 5**), coupled to an increase of plasma membrane surface area. The textbook view of cytoskeletal force generation focuses on the purse-string contraction model proposed by Schroeder. Questions concerning this model were discussed above, highlighting the need for more information on the structural organization of contractile rings. Inhibition of myosin II immediately blocks furrow ingression in some mammalian cells (16), and myosin II subunits score as conserved, essential cytokinesis proteins (**Table 2**). It is thus very puzzling how *Dictyostelium* cells and highly adherent mammalian cells, under some conditions, are capable of executing a form of cytokinesis with myosin II absent or greatly reduced in activity (28, 124, 140–142). Wang (143) has proposed an alternative model for furrow ingression, named “equatorial relaxation,” which posits that the whole cortex is under tension, and furrow in-

gression occurs at the equator because the cortex is softer there. Much of the evidence that supports this model comes from local drug perfusion experiments. Cytochalasin applied to the furrow tended to promote furrowing, whereas application to the poles inhibited it (144). Local application of the F-actin-stabilizing drug jasplakinolide, or the myosin II inhibitor blebbistatin, had essentially the opposite effects (136). These data support a model in which selective actin depolymerization at the equator is important for cytokinesis. However, direct measurements of cortical stiffness by atomic force measurements found increased stiffness at the equator (145). These observations might be reconciled if the equator was stiffer, yet depolymerized more rapidly, than the bulk cortex. In our view, the mechanics of cytoskeletal force production in cytokinesis is still an open question, and it is possible that more than one mechanism operates.

Independent of its effect on cortical stiffness, it is clear that actin depolymerization is an important aspect of furrow ingression. Actin and myosin both turn over rapidly in furrows (123, 124), implying that their local concentration depends on a dynamic balance between polymerization/recruitment and depolymerization/dissociation. Local concentration remains approximately constant during ingression, implying continuous decrease in contractile ring volume (125). The precise mechanism of actin depolymerization in cells is controversial, but proteins from the actin depolymerization factor (ADF)-cofilin family are key factors. Cofilin (twinstar in flies) is needed for cytokinesis (**Table 2**). In spermatocytes of *twinstar Drosophila* mutants, actin accumulates into abnormally large furrows, resulting in inhibition of cleavage (36). One model for keeping F-actin concentration constant while volume decreases would make the rate of recruiting new actin dependent on the surface area of the furrow, and the depolymerization rate dependent on the total amount of actin present. This could occur if recruitment depended on formin-driven nucleation within

the furrow or, perhaps, if it also depended on cortical flow from neighboring regions.

It has been known for years that furrow ingression can be coupled to deposition of new plasma membrane (146). The source of this membrane is not well characterized. Embryonic systems contain stored membranes in the form of vesicles, but in general this problem is unsolved, and recent investigations have focused on vesicle trafficking pathways. Actin-independent addition of new membrane has been observed in *Xenopus* eggs, where the membrane is delivered by exocytic vesicles that seem to travel along microtubules (147). New membrane addition was also observed in sea urchin eggs, using the extracellular matrix protein hyalin as a membrane marker (148). Shuster & Burgess (148) show that new membrane is added specifically to the ingressing furrow after mitotic exit and that membrane addition is dependent on astral microtubules and calcium. Both of these studies were carried out in eggs, which are larger than most cells and have a greater surface area, thus requiring more membrane to be inserted during ingression. *Drosophila* embryo cellularization is a special form of cytokinesis that exhibits a particularly large requirement for new surface area, and the process by which new membrane is inserted just behind the ingressing furrows in this system has been analyzed by genetics and microscopy (149). It is possible that specialized mechanisms have evolved in embryonic systems, but a general requirement for membrane addition during ingression seems likely. RNAi screens have revealed a conserved requirement for the endocytosis proteins clathrin and dynamin (Table 2), suggesting that plasma membrane recycling may be important during ingression, in addition to the addition of membrane from vesicular stores.

Completion

Completion, also termed abscission or scission, is the final step of cytokinesis. It is in some sense an optional step in cytokinesis and

the cell cycle. In embryos, blastomeres often remain connected by intercellular bridges for many cell cycles, perhaps explaining why some proteins involved in completion fail to score in cytokinesis screens performed in embryos (Table 2). Beyond its importance in cytokinesis, an emerging concept is that completion is a regulated process with a role in preventing accumulation of aneuploid cells (150). This exciting development may account for some of the biochemical complexity of completion. As the very last step in the old cell cycle or the first in the new one, it is logical to use completion as a sensor and regulator of cell cycle progression.

Completion occurs after the actomyosin ring has contracted and the cleavage furrow has ingressed to its fullest extent, creating an intercellular bridge. The bridge is packed with tightly bundled, antiparallel microtubules embedded in phase- and electron-dense material called the stembody (Figure 4). The morphology of completion in mammalian cells was described by live analysis and EM almost 30 years ago (151). When furrow ingression is complete, the intercellular bridge is approximately 1–1.5 μm in diameter. Separation of daughter cells is preceded by a reduction in the diameter of the bridge to approximately 0.2 μm . This occurs over the entire length of the bridge, except at the stembody, which retains its original diameter and projects out as a bulge in the center of the bridge (Figure 4). Microtubule bundles become further compacted and also begin to disappear across the entire length of the bridge, an observation duplicated recently by live imaging (21, 152).

The microtubule bundles and stembody in the intercellular bridge are required for completion. They form by compaction of the midzone during ingression of the furrow (16). Blocking midzone assembly prevents assembly of normal microtubule/stembody structures and causes completion to fail (68, 109, 116, 153). There have been recent suggestions that midbody microtubules are dynamic (153a), but the function of microtubules and

the stembody in completion is unknown. Current ideas focus on a possible role of microtubules in directing vesicles to the stembody and a possible role of septins, polymerizing GTPases that accumulate in the bridge (Table 2), in directing vesicle fusion (153b).

To study completion, it is important to distinguish it from late stages of ingression. One way to do this is to test for dependence on F-actin. During ingression and before bridge maturation, low doses of the actin depolymerizing compound Latrunculin B cause the furrow to reopen. Once the bridge matures and completion begins, it becomes Latrunculin insensitive (21), implying that the plasma membrane is linked to the midbody by a connection that does not involve dynamic F-actin.

A conserved cleavage furrow component that may be involved in bridge stability is Anillin. This multidomain protein binds to F-actin, myosin II, septin complexes, and perhaps also membranes via its pleckstrin homology (PH) domain (5, 33, 96, 154, 155). Anillin plays a nonessential role during ingression (96, 154) but is required for completion in several systems (21, 29, 96). Anillin and its interaction partners, the septins, remain in the maturing intercellular bridge after myosin II and most F-actin have dissociated (21, 32, 33). They also remain permanently in the narrow intercellular bridges that persist after incomplete cytokinesis in *Drosophila* spermatocytes (156). Microtubules are absent in these long-lived spermatocyte bridges, implying the existence of a stable, membrane-bound collar that does not depend on a microtubule scaffold. Biochemically, septins polymerize into relatively stable filaments that associate with, and may regulate, membrane trafficking proteins (reviewed in References 157–159). We hypothesize that Anillin and septins together assemble into a stable, filamentous array, which shapes the plasma membrane of the stable intercellular bridge, and that may also regulate the vesicle fusion required for completion.

Completion requires remodeling of plasma membranes to create sister cells, and

several recent reports focused on the role of vesicle trafficking components. Inhibition of the midbody-localized t-SNARE/v-SNARE pair syntaxin 2 and endobrevin/VAMP-8 by overexpression of dominant-negative constructs blocked completion but not ingression (160). Both the cellular localization and completion defects were specific for this SNARE pair. α -SNAP, part of a complex required for SNARE-mediated fusion, was found in a screen for completion defects in *Drosophila* cells (21). In SNAP-depleted cells, intercellular bridges formed normally but later disassembled without completion. Together, these studies imply that completion involves membrane fusion mediated by specific SNAREs, using mechanisms in common with other types of intracellular vesicle fusion. Whether these SNAREs promote direct fusion of plasma membranes, or work less directly by fusing transport vesicles to plasma membranes, remains to be determined, as does the possible role of midbody microtubules and septins in targeting fusion.

Centrosomes have long been implicated in cytokinesis biology as the nucleating sites of asters (Figure 2), but recently a new, and somewhat mysterious, role of centrosomes in completion has been discovered. Live imaging of GFP-tagged centrosomes revealed movement of one centrosome into the intercellular bridge late in cytokinesis. Completion correlated with this movement, suggesting a causal connection (152). The connection between centrosomes and completion was reinforced by the observation that RNAi depletion of the centrosome protein centriolin blocks completion (70). Centriolin localizes to centrosomes throughout the cell cycle, but during cytokinesis, it localizes in one or two spots adjacent to the stembody. Depletion of centriolin caused a defect in completion with persistent, elongated bridges and formation of multinucleated syncytia. Defects were also observed in cell cycle timing (70). Recently, centriolin has been shown to be required for the localization of exocyst

components and SNARE proteins to a ring on the stembody, indicating a role in both vesicle targeting and fusion (160a). A role for intercellular bridge components in cell cycle timing was also found in a microsurgery study, where cells that failed to inherit a bridge experienced cell cycle delays (161). The biological logic behind the centrosome/completion/cell cycle connection is not yet clear, but clues may come from what is known about mitotic exit in yeast cells (162, 163). The mammalian centriolin implicated in completion shares homology with the MEN/SIN proteins Nud1p (budding yeast) and Cdc11p (fission yeast) (70). The relevance of the yeast MEN/SIN mitotic exit pathways for mammalian biology has been unclear; these data suggest that a related pathway may regulate completion.

A new direction in completion research was opened by a paper describing a connection between mistakes in chromosome segregation and completion failure in cultured human cells (150). Spontaneously arising binucleate cells, which result from failed completion, were found to exhibit a high frequency of chromosome missegregation, and drug treatments that promoted missegregation increased the frequency of cytokinesis failure. This study implies that failure in chromosome segregation that escapes detection by the mitotic checkpoint can nevertheless be detected by the cell, which responds by blocking completion. Shi & King (150) propose that this mechanism evolved to reduce carcinogenesis because, in their view, single chromosome aneuploidy is more dangerous than tetraploidy. The molecular basis of the connection between missegregation and failed completion is unknown. BRCA2, a protein involved in genome stability and protection from cancer, was recently implicated in cytokinesis, providing a possible molecular clue (69).

CYTOKINESIS AND CANCER

Failure in cytokinesis very likely contributes to cancer progression. Many cancers are an-

euploid, facilitating genomic plasticity that allows rapid evolution of aggressive genotypes. Common solid tumors tend to exhibit polyploidy as well as single chromosome abnormalities, presumably resulting from failures in both mitosis and cytokinesis. The two may be closely connected both by chromosome missegregation triggering failure of completion (150) and by centrosome abnormalities, which cause defects in both spindle assembly and cytokinesis (reviewed in Reference 164). Pellman and coworkers (165) recently found that blocking cytokinesis causes primary cells lacking p53 to become much more carcinogenic in mice, directly demonstrating a causal connection between failed cytokinesis and carcinogenesis for the first time. Analysis of cytokinesis defects in human cancers is an important direction for future research.

Cytokinesis is also a point of possible therapeutic intervention in cancer. The first test of this idea will come from Aurora kinase inhibitors, currently in clinical trials (11). Cells treated with these drugs become polyploid before eventually dying, a mechanism of cell killing distinct from mitotic spindle poisons such as taxol. Given that blocking cytokinesis in p53⁻ cells can cause cancer in mice (165), there is a risk that this treatment will cause cancer as well as treat it, a concept familiar for DNA-damaging agents. Cancer drugs that target generic cell division mechanisms kill normal stem cells and thus cause bone marrow and gut toxicity, which limits their therapeutic dose. It would be better to find drugs that selectively blocked cytokinesis (or mitosis) in cancer cells while sparing normal stem cells. Cytokinesis is highly conserved, and we cannot expect major mechanistic differences. However, different cell types probably vary in the extent to which overlapping cytokinesis pathways are used, so selective inhibition is not out of the question. In that light, it will be useful to extend research on cytokinesis mechanisms to comparative studies of stem and cancer cells, using both genome-wide and detailed mechanism approaches.

SUMMARY POINTS

1. A parts list of genes involved in cytokinesis has been assembled through a combination of RNAi and previous efforts.
2. We dissect cytokinesis into six subprocesses and discuss mechanistic progress in each subprocess:
 - Timing: Proteolysis is involved in regulating C phase, the time during which the cortex can contract.
 - Cleavage plane specification: Multiple mechanisms operate in parallel; the Rho pathway and kinases are involved.
 - Rearrangement of microtubule structures: Microtubules rearrange into different arrays that have varied functions.
 - Ring assembly: Cortical flow and local nucleation contribute to assembly of actomyosin filaments.
 - Ring ingression: Force generation mechanisms for different orientations relative to the furrow are discussed. The traditional purse-string model is most likely an oversimplification.
 - Completion: Midzone microtubules, vesicle transport, and centrosomes are important for completion.
3. The relevance of cytokinesis to cancer is discussed.

FUTURE ISSUES TO BE RESOLVED

1. We are beginning to generate a parts list of all proteins involved in cytokinesis but still know little about how they interact with each other to accomplish this process.
2. We have partial information for most of the fundamental mechanisms underlying cytokinesis, but our understanding of biochemical mechanisms is only just emerging.

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ERRATA

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