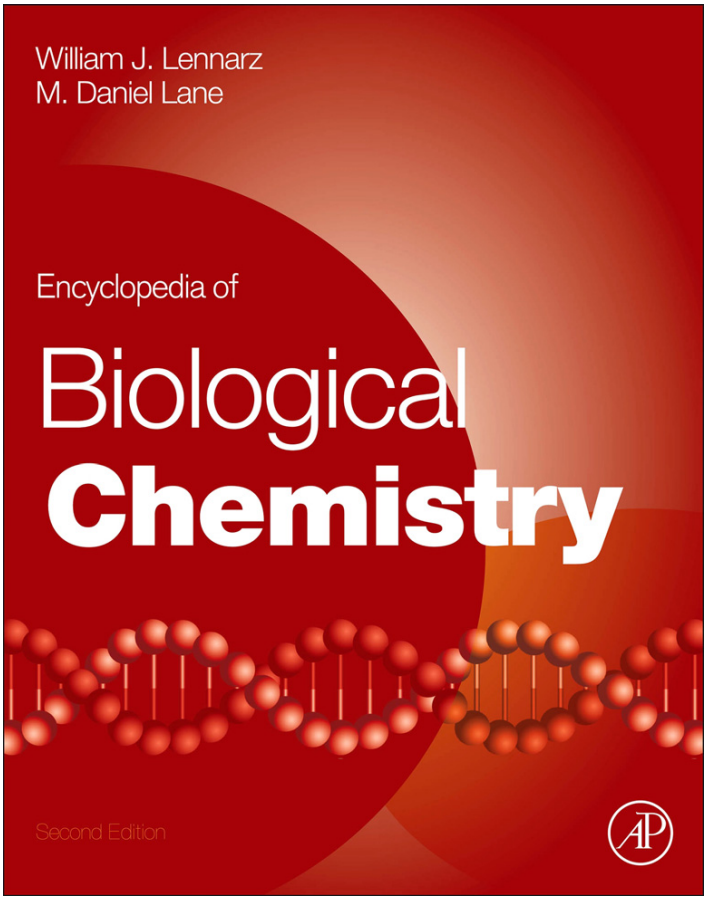

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Cytokinesis

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Glossary

Abscission The final severing of the intercellular bridge, which is formed through ingression of the cleavage furrow.
Asters The radial array of microtubules originating from each spindle pole that extends toward the cell cortex.
Cell cortex The network of actin filaments and other proteins in close proximity to the cell membrane.
Central spindle The microtubule network extending between the two microtubule organizing centers of a dividing cell.
Cleavage furrow The region of cortex of a dividing cell where the cell constricts to form the intercellular bridge.
Contractile ring A dense array of actin and myosin II arranged in a ring-like structure in the cleavage furrow of many dividing cells.
Cortical tension The energy cost per unit increase in the cell surface area.
Cytokinesis The process of physical separation of a dividing cell into daughter cells.
Intercellular bridge The narrow, cylindrical connection between two daughter cells.
Laplace pressure The pressure generated at a curved fluid surface due to the surface tension of the fluid.
Midbody A compact protein-rich structure that contains a dense anti-parallel microtubule array, which is found in the intercellular bridge during late cytokinesis.

Cytokinesis

Subsequent to chromosome segregation during mitosis, the cell cytoplasm and other organelles are partitioned into two daughter cells through the process of cytokinesis. Correct cytokinesis is relevant for both normal development and disease. The uniform partitioning of cellular material is critical for normal cell proliferation, while asymmetric cell division is important in processes such as stem cell maintenance. In addition, cytokinesis defects have been implicated in many diseases, including cancer. Thus, detailed studies aimed at identifying the mechanisms that control cytokinesis are necessary to understand cellular behavior and for therapeutic applications.

In order to understand the mechanisms that regulate cytokinesis, it is essential to answer the following questions:

1. When and where does the division occur?
2. What are the factors responsible for this division?
3. How do those factors interact with each other to help the cell divide?

Like many biological processes, cytokinesis is a complex phenomenon involving a large number of players that interact with each other through multiple overlapping biochemical and mechanical pathways. Thus, cytokinesis is a great example of a biological control system, where the various feedback loops act in tandem to ensure the fidelity and robustness of cell division. Although the exact biochemical interactions are still unresolved to a large extent, a great deal is now known about the underlying mechanisms.

In spite of large cell-specific variations in the details of cytokinesis, there are certain universal characteristics of the process. Typically, cytokinesis proceeds through a series of stereotypical cell shape changes where the cell first rounds up, then elongates forming a cleavage furrow near the middle, whose constriction finally results in the separation of the daughter cells (Figure 1). These cell shape changes imply that cytokinesis is fundamentally a mechanical process that requires major reorganization of the cytoskeleton and associated proteins to promote cellular contractility at the cleavage site. Hence, it is essential to supplement biochemical and genetic information with biophysical and mechanical studies to understand force generation, sensing, and transduction in a dividing cell.

Proteins That Regulate Cytokinesis

Methods

A variety of genetic, biochemical, and biophysical techniques have been used to identify and characterize cytokinesis genes. A traditional genetic approach involves phenotypic screening of mutant cell lines for cytokinesis defects, such as an increase in the number of bi- or multi-nucleated cells. Partial knockdown of protein expression using RNA interference (RNAi) has allowed functional analysis of essential genes, whose deletion can be lethal. By contrast, overexpression of genes has helped identify proteins that possess dominant-negative activities. Finally, live cell imaging of fluorescently labeled proteins gives information about their localization and dynamics *in vivo*. These techniques provide a powerful toolset for the functional analysis of protein dynamics, and have been combined with high-throughput methods for genome-wide screening to identify genes that regulate cytokinesis. In addition, directed screens and genetic suppression have helped map out biochemical pathways. However, to uncover entire pathways, these studies need to be supplemented with detailed characterization of protein interactions and *in vitro* biochemical reconstitution. Additionally, the use of pharmacological agents that modify the activity of specific proteins has allowed real-time phenotypic manipulation. This approach is especially useful for studying elements that are essential for cell survival or those that are required at multiple stages during the cell cycle. For example, cytokinesis defects were observed in post-mitotic cells treated with the microtubule-destabilizing compound, nocodazole. This established the involvement of

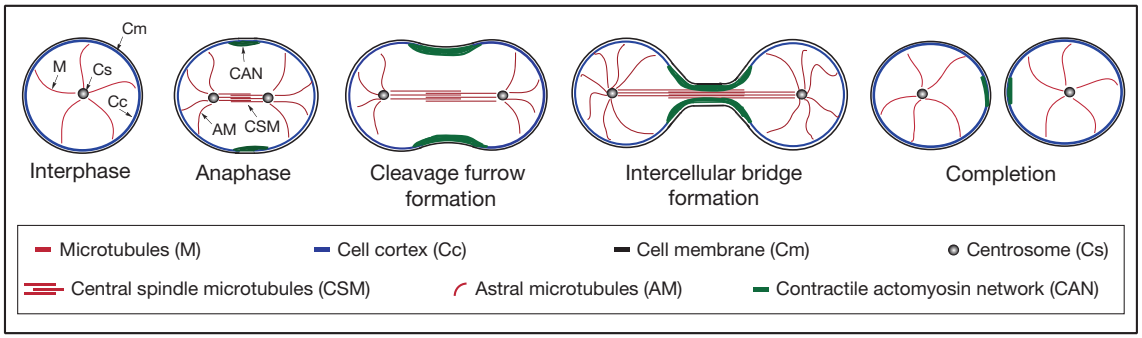

Figure 1 Stages of cytokinesis: a schematic diagram depicting the progression of cytokinesis in an amoeboid cell. Note that the contractile actomyosin network (CAN) is found throughout the cell cortex, but is enriched in the cleavage furrow.

microtubules in cytokinesis completion, apart from their role in chromosome segregation and cleavage site selection. Using a combination of various genetic and biochemical studies, a partial parts list of cytokinesis proteins has been compiled for many model organisms. Many of these are cytoskeleton-related proteins and kinases, while a large number of genes still remain uncharacterized.

Proteins

In most eukaryotic cell types, actin and the motor protein myosin II are known to form a contractile structure in the equatorial region of a dividing cell, whose ingression drives cytokinesis. Actin controls the cell mechanics by forming a highly dynamic network of semi-flexible filaments (Figure 2). During the myosin II power stroke, myosin pulls on the actin filaments as it releases the adenosine triphosphate (ATP) hydrolysis products, thereby generating mechanical force. Two myosin heavy chains combine with two essential and two regulatory light chains (ELC and RLC, respectively) to form a myosin II hexameric monomer, which then assembles into functional bipolar thick filaments (BTFs). Rho-kinase (ROCK) directs BTF assembly by activating myosin II through the phosphorylation of RLC. Additionally, heavy chain phosphorylation also controls BTF assembly and disassembly, both of which are required for normal cytokinesis. Although myosin II is the major mechanoenzyme during cytokinesis, it is not necessary for cytokinesis. Adherent cells can divide fairly normally in the absence of myosin II using traction forces to help with the initial cell elongation followed by cortical tension-driven furrow thinning. The effects of cortical tension, which serves to minimize the surface area-to-volume ratio, are highly reminiscent of the surface tension of a liquid droplet, which also helps drive droplet breakup.

In addition, several other actin-binding proteins, such as anillin and α-actinin in animals and cortexillin in *Dictyostelium*, help form a cross-linked actin network in the cell cortex (Figure 2), thereby regulating mechanical properties of the cortex. These proteins differ in their structure, actin-binding kinetics, force sensitivity, and cellular location. Collectively, these promote load-dependent force generation by myosin II and thereby cellular contractility. Some cross-linkers also contain lipid-binding domains (such as the pleckstrin-homology (PH) domain) that facilitate membrane attachment of the actin network. In animal cells, anillin is a potential scaffolding

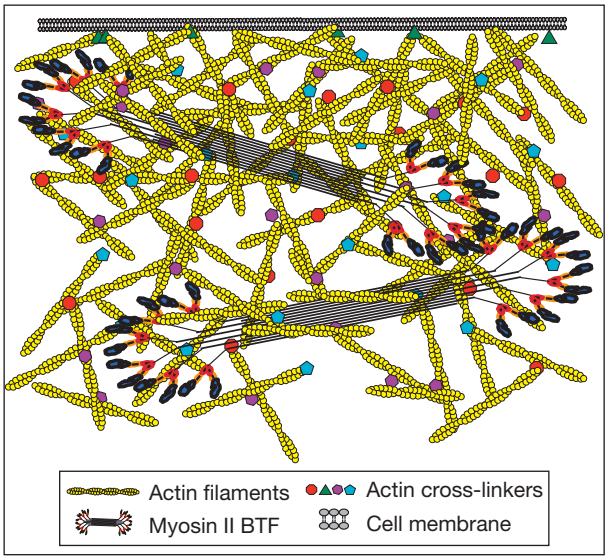

Figure 2 A stylized diagram showing major components of the actin cytoskeleton in the cell cortex. Actin filaments form a dynamic highly cross-linked network that is connected through various actin cross-linking proteins. These proteins can regulate the tension in the network, thereby controlling the contractility of the cortex. The actin network is also attached to the cell membrane through some actin-binding proteins. The mechanoenzyme myosin II forms bipolar thick filaments and generates mechanical stress within the network by pulling on actin filaments.

protein that may provide membrane anchoring and link Rho, actin, and myosin II in the furrow. Rho is a major regulator of animal cytokinesis, as it controls both actin polymerization through formins and myosin II activation through ROCK. The levels of guanosine triphosphate (GTP)-bound active Rho in the cleavage furrow are controlled by the guanidine-exchange factor (GEF) ECT2 and the GTPase-activating protein (GAP) MgcRacGAP.

Another major player in cytokinesis is the mitotic spindle, which helps define the axis of cell division and is involved in many signaling pathways. The spindle can deliver signals to the cell cortex that modulate cortical mechanics and direct cleavage-site selection and actomyosin contractile structure formation. This signal can either be in the form of a biochemical factor or be in the form of a purely mechanical cue like a change in

cortical tension or membrane potential. Many microtubule-based proteins such as the kinesin-6 family of proteins (mitotic kinesin-like protein-1 (MKLP-1)) are known to be important in cytokinesis and may localize differentially in a dividing cell. These proteins are believed to promote communication between the central spindle and the cell cortex.

Even though the membrane is thought to have a relatively limited contribution to the cellular mechanical properties, proteins involved in membrane dynamics, membrane fission and fusion, and vesicle transport are important in cytokinesis. The surface area of a dividing cell increases significantly as the furrow constricts. This requires the deposition of new membrane in the furrow region. In addition, constant membrane remodeling is required to relieve mechanical stress.

As the search for new genes that regulate cytokinesis continues, many nonprotein factors, including lipids and small metabolites, are also being examined for their role in cytokinesis. For example, the phosphoinositol-4,5-bisphosphate (PIP_2) is enriched in the cleavage furrow and can control the accumulation and retention of PIP_2-binding proteins during cytokinesis. In addition, many mechanical parameters, such as those described in the section Mechanics of cytokinesis, can affect the kinetics of cell division. To ensure robustness of cytokinesis, current models support the existence of multiple interacting, as well as parallel, mechanisms, thereby making the compilation of a comprehensive cytokinesis parts list challenging.

Spatiotemporal Events during Cytokinesis

Cytokinesis is tightly coupled to the cell cycle to ensure the proper segregation of genetic material into daughter cells. The onset of anaphase triggers major restructuring of the cytoskeleton, which initiates cytokinesis. No obvious factors for controlling the timing of cytokinesis initiation have been identified yet, but it is thought that some of the factors involved in the mitotic phase also regulate cytokinesis initiation. For example, Cdc2/Cdk1 inactivation during early anaphase or anaphase-promoting complex (APC)-dependent proteolysis could help initiate cytokinesis. The mitotic-exit network (MEN) and septation-initiation network (SIN) are other pathways that are known to regulate cytokinesis in some systems, and are relatively conserved across yeast, fungi, and plants.

Along with the temporal control of cytokinesis, the process is also regulated spatially. In most cells, cytokinesis occurs in the plane perpendicular to the cellular long axis generally close to the center of the cell. Thus, even from a purely geometrical perspective, the mitotic spindle is likely to be important in symmetry breaking and cleavage-site selection. Both the central spindle and astral microtubules are required for the correct positioning of the furrow, and likely deliver chemical signals to the cell cortex. The microtubule-dependent symmetry breaking can be achieved by the following mechanisms: motor-based transport of molecules, tracking of differential microtubule density in the cortex, or signals from the plus ends of microtubules themselves. Two classical models have been proposed to explain the role of spindle and the nature of these signals. The polar relaxation model proposes that signals inhibiting contractility are delivered to the polar cortex by the asters, while the equatorial simulation model argues for positive cues being delivered to the equatorial cortex by either the central spindle or astral microtubules. Recent evidence suggests that both mechanisms most likely act synergistically to ensure fidelity of furrow initiation, though the exact signaling molecules involved remain unknown.

Once the cleavage furrow position is established, the actin cytoskeleton must reorganize to form the contractile actomyosin network. In animals, the activation of Rho GTP-binding proteins in the furrow region stimulates actin polymerization and myosin activation. For the enrichment of active Rho in the furrow, its diffusion must be restricted. One hypothesis argues for the role of anillin as a scaffold protein, as it can specifically bind to PIP_2 as well as activated Rho. Another theory supports microtubule-dependent accumulation of equatorial proteins.

The organization of actin and myosin II filaments varies with cell type. Actin and myosin II form a well-defined contractile ring-like structure (**Figure 3(a)**) in a number of cell types including the fission yeast *Schizosaccharomyces pombe*, while in many cells such as fibroblasts and *Dictyostelium*, actin forms a contractile meshwork (**Figure 3(b)**). The length and orientation of individual filaments are also cell-type dependent, ranging from ~1 μm long, roughly concentric filaments in *S. pombe*, to a more disorganized network of short ~100 nm actin filaments in *Dictyostelium*. Myosin II is a force-sensitive mechanoenzyme whose actin-binding lifetime and force generation depend on the tension in the actin filament. Thus, by forming junctions in the actin network and tethering it to the plasma membrane, actin cross-linkers help generate tension in the filaments, thereby increasing the overall myosin II-dependent contractility of the network, which drives constriction of the furrow cortex. The dynamic properties of these cross-linkers regulate the kinetics and mechanics of furrow ingression. Actin cross-linkers localizing in the cleavage furrow generally promote ingression, while other global cross-linkers inhibit contractility. After the initial accumulation, the furrow concentration of actin and myosin II remains largely unchanged as the furrow volume decreases, highlighting the importance of cytoskeletal disassembly during contraction. In summary, while actin and myosin II together form the active contractile force generation machinery responsible for furrow ingression, other actin-binding proteins act as regulators of cellular contractility by assisting or inhibiting cleavage furrow constriction.

As furrow ingression proceeds, a thin intercellular bridge is formed connecting the daughter cells. During later stages, the midzone microtubules, which are the microtubules between segregated chromosomes, condense to form a dense protein-rich structure called the 'midbody' within the bridge. The midbody is comprised of condensed anti-parallel microtubules and several other spindle-associated proteins such as the components of the chromosome passenger complex (CPC; including proteins such as Aurora B kinase and inner centromere protein (INCENP)) and the centralspindlin (MKLP1 and MgcRacGAP). A dividing cell can continue to exist at the bridge stage for long periods of time (from minutes to hours, depending on cell type) before the final separation, which may enable the cell to ensure proper segregation of cellular content.

Multiple models have been proposed to explain abscission, which is the final severing of the intercellular bridge. The final push of cytoplasm from the bridge may be a largely physical process driven by Laplace pressure that favors minimization of surface area to volume ratio. A second model

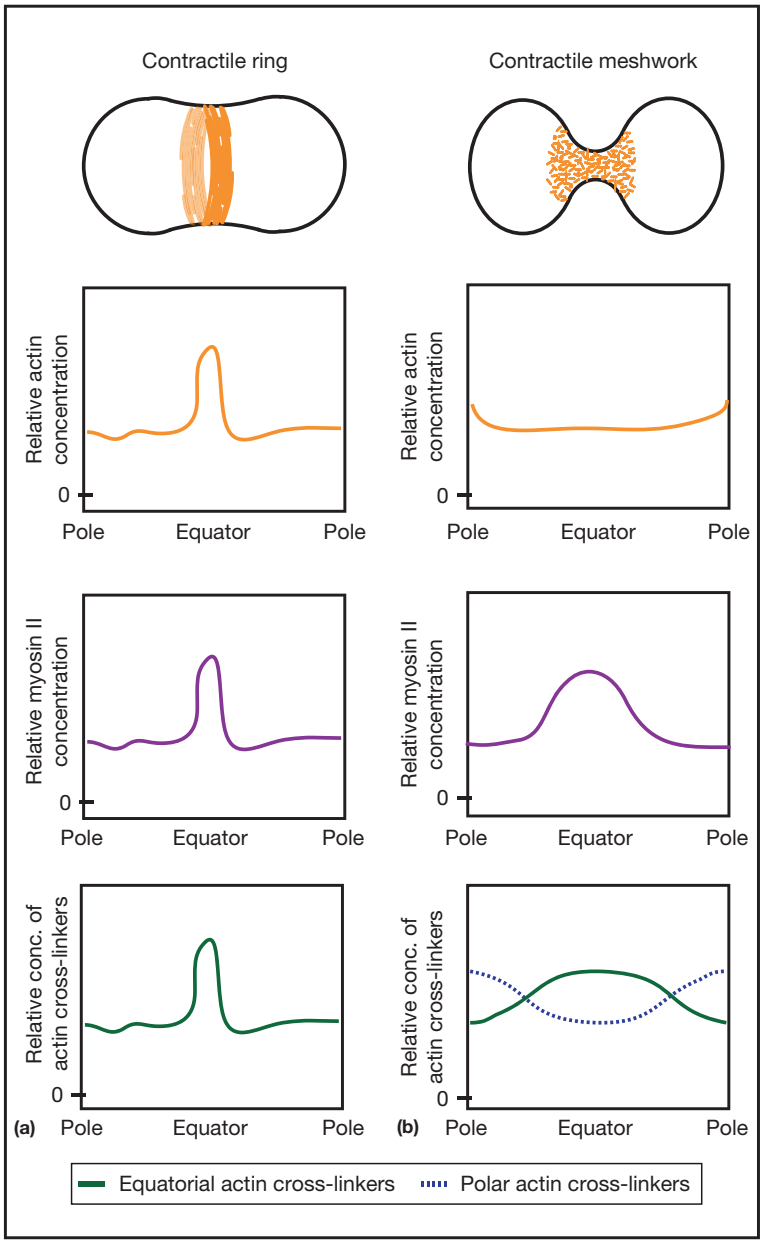

Figure 3 A comparison of actin cytoskeleton structure in cells forming a contractile ring (a) or a contractile meshwork (b). Note that the actin structures are shown to emphasize the cleavage furrow contractile organization, and the global actin network is not shown. The relative concentrations of actin, myosin II, and actin cross-linkers in the polar or equatorial cortex are also shown for both structures.

suggests that cell separation is promoted by the accumulation of secretory and endocytic vesicles adjacent to the midbody. Vesicle transport is also necessary to prevent membrane tearing as the furrow ingresses, increasing the total cellular surface area. A third model proposes that the invagination of the plasma membrane results first in hemifusion and finally cell cleavage. There is some evidence of differential membrane compositions in the equatorial versus polar cortices and in the inner versus outer membrane leaflet, which can activate signaling cascades and/or help in membrane fusion. Recently, the direct involvement of the endosomal sorting complex required for transport (ESCRT) in abscission has been shown using high-resolution imaging. However, similar to other steps in cytokinesis, multiple mechanisms are likely to be working in parallel during abscission to ensure robustness.

Mechanics of Cytokinesis

Cytokinesis is a mechanical process during which a cell undergoes major mechanical deformation. Thus, an in-depth understanding of the effects of mechanical signals on cell mechanics and biochemistry is required. A diverse tool set is available to study cell mechanics during cytokinesis, allowing characterization of various mechanical parameters. While micropipette aspiration (MPA) studies are used to determine the elastic modulus and effective cortical tension, atomic force microscopy (AFM)

measures the bending modulus. The elastic modulus quantifies the deformability of the cell surface, and the cortical tension is a complex parameter that measures the energy cost per unit increase in cell surface area. The bending modulus reflects the stress required for bending a material. During cytokinesis, the initial deformation of a roughly spherical cell requires deviation from its quasi-steady state. This is resisted by the cortical surface tension, which favors a spherical cell. However, as the furrow continues to ingress, the curvature in the cleavage furrow changes so that the Laplace pressure eventually favors bridge thinning and abscission. Laser tracking microrheology (LTM) can be used to measure cortical viscoelasticity noninvasively. Viscoelasticity represents the time-dependent cellular response to stresses and affects the kinetics of furrow ingression by dampening the mechanical deformation, thereby allowing sufficient time for activation and stabilization of biochemical factors.

Treatment with actin depolymerizing drugs such as Latrunculin-A has established that the actin cytoskeleton is the main contributor of cell mechanics, though the cell membrane and microtubules also make some contribution. The actin cytoskeleton also undergoes remodeling with the application of internal or external mechanical stresses. The impact of mechanical stresses has been uncovered using micropipette aspiration, which allows the application of an external stress on the cell, similar in magnitude to stresses generated internally during cytokinesis. Many mechanosensitive proteins such as myosin II, which localize to the cleavage furrow cortex, also accumulate at sites where mechanical stress has been applied.

In contrast to the mechanical activation of biochemical reactions, the mechanical properties of the cell can be controlled biochemically. Knockdown of some actin cross-linkers softens the cell cortex significantly, leading to altered furrow ingression kinetics and a reduced ability to perform cytokinesis in suspension culture (where cell–substrate adhesion is absent). Interestingly, the overall deformability of the furrow is lower than the polar cortex, even though furrow undergoes major deformation during cytokinesis, which is attributed to a differential cortical distribution of mechanosensitive proteins during cytokinesis. This further illustrates the intricate interplay between biochemical and mechanical pathways during cytokinesis.

Species-Specific Cell Division Mechanisms

Though the essential sequence of events during cytokinesis is more or less conserved across organisms, there are significant differences in the mechanisms in various cell types. These become especially important in furrow positioning, where various organisms use diverse mechanisms to induce the initial symmetry breaking. Although actin and myosin II form the core contractile machinery in yeasts, protozoans, and animals, a similar system has not been found in plants. Cytokinesis in plants proceeds through a microtubule-dependent mechanism involving two additional microtubule structures, apart from the mitotic spindle, known as the 'preprophase band (PPB)' and 'phragmoplast'. PPB is a ring-like microtubule and actin structure formed around the premitotic nucleus. During mitosis, actin dissociates from the PPB and the accumulation of a yet unknown factor marks the division site. The spindle then gives rise to the phragmoplast that expands toward the cell cortex. Subsequent membrane trafficking and fusion at its edge leads to cell plate formation.

Similar to plants, the budding yeast cleavage site is determined early during mitosis by the accumulation of the small Rho GTP-binding protein Cdc42. Thus, the mitotic spindle is not crucial for budding site determination; rather the spindle aligns itself according to the polarization by Cdc42. By contrast, the cleavage furrow in fission yeast is determined by the position of the nucleus and the cylindrical geometry of the cell, as repositioning of the nucleus before early mitosis causes a shift in furrow location. Precursors of the contractile ring (or nodes) first appear near the center of the cell, which are attached to the membrane. These nodes then condense to form the contractile ring.

Cytokinesis in bacteria differs greatly from most eukaryotes as they do not contain organelles and the eukaryotic cytokinetic components. Rather, bacteria use an ancestral tubulin-related FtsZ cytoskeleton and have developed diverse intricate mechanisms for cytokinesis.

Conclusion

Cytokinesis is an essential biological process. Understanding the molecular mechanisms that regulate cytokinesis has great potential in the development of molecular targets against both cancer and infectious pathogens. It is an elegant biological control system comprised of multiple overlapping and parallel biochemical and mechanical pathways, promoting fidelity and robustness of cell division. Thus, along with the obvious therapeutic applications, cytokinesis studies are important for appreciating the complexity of biological shape morphogenesis.

See also: **Cell Architecture and Function:** Actin-Related Proteins; Microtubule-Associated Proteins; Mitosis; Myosin Motors; Rho GTPases and Actin Cytoskeleton Dynamics.

Further Reading

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