Cytokinesis in the *C. elegans* Embryo: Regulating Contractile Forces and a Late Role for the Central Spindle

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**Abstract.** Genetic and molecular studies in the nematode *Caenorhabditis elegans* have identified multiple essential pathways that regulate and execute cytokinesis in early embryonic cells. These pathways influence both the microfilament cytoskeleton and the microtubule cytoskeleton. Microfilaments are enriched throughout the cell cortex at all times during the cell cycle in embryonic cells. Cortical microfilaments are required for multiple processes in embryonic cells, including polar body extrusion during meiosis, anterior-posterior axis specification by the sperm-donated microtubule-organizing center, and cytokinesis during mitosis. In addition to contractile apparatus proteins that are required positively for cleavage furrow ingression, the Nedd8 ubiquitin-like protein modification pathway negatively regulates contractile forces outside the cleavage furrow during cytokinesis. Another pathway that acts positively during cytokinesis involves the mitotic spindle. The central spindle, where anti-parallel non-kinetochore microtubules overlap and are cross-linked, is required for a late step in cytokinesis, and other pathway(s) involved in membrane addition during cytokinesis may also require the central spindle. The amenability of *C. elegans* to classical genetics, the ease of reducing gene function with RNA interference, the completion of the genome sequence, and the availability of transgenic GFP fusion proteins that render the cytoskeleton fluorescent, all serve to make the early worm embryo an especially promising system for further advances in the identification of cytokinesis pathways, and in defining their interactions.

**Key words:** cortical cytoplasm/microfilaments/microtubules/ubiquitin

*Localized activation of the *C. elegans* contractile machinery during cytokinesis*

During cytokinesis in some cell types, including the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*, a microfilament-rich contractile ring assembles specifically within the cleavage furrow and mediates furrow ingression. During mitosis in such cell types, microfilaments are largely restricted to the contractile ring at the leading edge of the cleavage furrow (reviewed in Field *et al.*, 1999). However, in embryonic cells of the soil dwelling *Caenorhabditis elegans* nematode, microfilaments are enriched throughout the cytoplasmic cortex, with no detectable enrichment within cleavage furrows during mitosis (Strome and Wood, 1996; Aroian *et al.*, 1999). Along with microfilaments, a class II non-muscle myosin heavy chain required for cytokinesis in the early embryo, called NMY-2, is present throughout the cortex of embryonic cells, at all points in the cell cycle, with no enrichment in cleavage furrows (Guo and Kemphues, 1996; Shelton *et al.*, 1999). More recently, a profilin required for embryonic cytokinesis, called PFN-1, also has been shown to be present throughout the cortex, with no furrow enrichment (AFS and BB, unpublished data). Thus proteins required for cytokinesis are distributed throughout the microfilament-rich cell cortex, with no localized assembly of a contractile ring structure apparent during embryonic cell divisions. Presumably cytokinesis in these embryonic cells requires the localized activation, rather than assembly, of a contractile apparatus during cytokinesis. Activation of the contractile apparatus could involve the recruitment of additional proteins to the cleavage furrow, or the localized modification of contractility protein(s) that like microfilaments are present throughout the cortex at all times.

Only one protein known to be required for cytokinesis during *C. elegans* embryogenesis becomes enriched specifically within cleavage furrows during cell division. A formin homology protein called CYK-1 was detected only late in cytokinesis, at the leading of the cleavage furrow in divid-
ing embryonic cells (Swan et al., 1998). However, CYK-1 is required early in cytokinesis, before it accumulates to detectable levels at the leading edge of ingressed furrows (A. Severson and B. Bowerman, unpublished data). Thus early in cytokinesis the formin protein CYK-1 is probably present at the cortex below detected levels early in cytokinesis, whether it is enriched at presumptive furrows or, like microfilaments and myosin, present throughout the cortex. The enrichment of CYK-1 observed at the leading edge of cleavage furrows late in cytokinesis might reflect a second, distinct requirement for CYK-1 in terminating cytokinesis. A termination role might require higher protein levels than are needed for initiating cytokinesis. Consistent with a distinct late role, partial loss-of-function mutations in cyk-1 result in a late cytokinesis defect (Swan et al., 1998). More complete removal of CYK-1 results in an early defect, as observed when other contractile ring components are depleted (A. Severson and B. Bowerman, unpublished data). Alternatively, the accumulation of CYK-1 could simply be a functionally irrelevant by-product of its earlier role(s) in producing an active contractile apparatus within the cleavage furrow.

Putting the exception of CYK-1 aside, cytokinesis in the early C. elegans embryo appears to involve the localized activation, rather than localized assembly, of contractility factors distributed uniformly throughout the cell cortex. Are the ubiquitous cortical distribution of microfilaments and associated proteins, and the localized activation of contractile forces during cytokinesis, typical of many animal cell types? Or do these properties instead reflect the specialized requirements of early embryonic cells in C. elegans? Even without answering these questions, the widely held view that cytokinesis involves the localized assembly of a contractile ring already appears limited in scope. It will be interesting to learn if assembly of the contractile apparatus has been uncoupled from activation in other cell types, and if any such uncouplings occur in the same or in different ways.

Multiple requirements for the cortical contractile machinery in early embryonic cells

It is not surprising that proteins required for microfilament-mediated contractile forces during cytokinesis are dispersed throughout the cortex of embryonic cells in C. elegans. Some of the initial investigations of embryogenesis in C. elegans took advantage of chemicals that interfere with cytoskeletal function to demonstrate that microfilament-based contractile forces are required, well before cytokinesis, both for polar body extrusion and for establishment of the anterior-posterior body axis (reviewed in Bowerman and Shelton, 1999). Specifically, disruption of the microfilament cytoskeleton, using brief pulses of exposure to cytochalasin, first implicated the actin cytoskeleton in axis formation at the one cell stage (Hill and Strome, 1990). Subsequent studies identified a microfilament-dependent cytoplasmic flux, in which cortical cytoplasm flows anteriorly while deeper cytoplasm moves posteriorly (Hird and White, 1993). This fountain or flux of cytoplasmic movement carries embryonic determinants to the posterior pole after fertilization, where they apparently are somehow captured or fixed (Hird et al., 1996). The posterior pole, towards which internal cytoplasm moves, is specified after fertilization by the position of the sperm pronucleus and its associated asters (Goldstein and Hird, 1996). Signals from the sperm-donated centrosome, possibly involving astral microtubule contact with the cell cortex, appear to initiate the microfilament-dependent flux and thereby specify the anterior-posterior body axis (O’Connell et al., 2000; Wallenfang and Seydoux, 2000). Thus cortical microfilaments fulfill multiple functions in the one cell embryo, with different spatial regulation occurring at different times to mediate distinct processes that include polar body extrusion, axis formation and cytokinesis during mitosis.

Recent studies have shown that in addition to requiring microfilaments, the generation of a cytoplasmic flux and the establishment of an anterior-posterior axis of cell polarity require the same motor proteins that mediate cytokinesis. These include the heavy chain NMY-2 and the regulatory myosin light chain MLC-4 (Guo and Kemphues, 1996; Shelton et al., 1999). Intriguingly, NMY-2 binds the cytoplasmic flux, in which cortical cytoplasm flows anteriorly while deeper cytoplasm moves posteriorly (Hird and White, 1993). This fountain or flux of cytoplasmic movement carries embryonic determinants to the posterior pole after fertilization, where they apparently are somehow captured or fixed (Hird et al., 1996). The posterior pole, towards which internal cytoplasm moves, is specified after fertilization by the position of the sperm pronucleus and its associated asters (Goldstein and Hird, 1996). Signals from the sperm-donated centrosome, possibly involving astral microtubule contact with the cell cortex, appear to initiate the microfilament-dependent flux and thereby specify the anterior-posterior body axis (O’Connell et al., 2000; Wallenfang and Seydoux, 2000). Thus cortical microfilaments fulfill multiple functions in the one cell embryo, with different spatial regulation occurring at different times to mediate distinct processes that include polar body extrusion, axis formation and cytokinesis during mitosis.

Negative regulation of cortical contractility outside the cleavage furrow

Because the contractile properties of the cortical microfilament cytoskeleton are used for multiple processes, a dynamic regulation of contractile forces must influence the cortex during early development. Positive signals from the mitotic spindle are thought to regulate the assembly or activation of cleavage furrows, although the signal(s) remain unknown. Genetic studies of cell division in the early C. elegans embryo now indicate that negative regulation of contractility
outside the cleavage furrow also is important during cytokinesis (Kurz et al., 2002). A Nedd8 Ubiquitin-like protein conjugation pathway is required for negative regulation of contractile activity, and this negative regulation occurs throughout the cortex of early embryonic cells (Kurz et al., 2002). Mutational inactivation of the Nedd8 pathway in C. elegans resulted in ectopic and prolonged cleavage furrows, both during axis formation and during cytokinesis. All ectopic furrowing required known cleavage furrow genes, including mny-2 and a myosin regulatory light chain gene mhc-4. Depletion of these gene functions by RNA interference suppressed all furrow formation. Moreover, the formin protein CYK-1 was ectopically localized to multiple furrows during mitosis in mutant embryos, consistent with ectopic activation of contractile furrowing in the absence of Nedd8 pathway function. These results indicate that the widely conserved Nedd8 ubiquitin-like protein modification pathway regulates the cytoskeleton, in addition to its previously documented role in regulating cell cycle progression. Presumably, negative regulation of contractility targets cleavage furrow component(s) or activator(s) for ubiquitin-mediated degradation, as the only known role for neddylation is in the regulation of some E3 ubiquitin ligase complexes. It will be interesting to learn if the Nedd8 protein modification pathway or other pathways negatively regulate contractile forces outside the cleavage furrow during cytokinesis in other cell types.

To summarize our current understanding of contractile force regulation during cytokinesis in the early C. elegans embryo, a combination of positive and negative regulation mediates the different applications of contractile force within the microfilament-rich cell cortex. At least much of the same contractile machinery is used for multiple processes, including meiosis, anterior-posterior axis specification and cytokinesis. At least one pathway, the Nedd8 ubiquitin-like protein modification pathway, is used in spatially distinct patterns at two different times to mediate negative regulation, once during axis specification and again during cytokinesis. Presumably other pathways will be identified that mediate positive regulation, and additional pathways may be found to influence negative regulation. Whether this combination of negative and positive regulation is conserved during cytokinesis in other organisms requires further investigation, but the Nedd8 pathway is highly conserved from yeast to humans.

**A Late Contribution to Cytokinesis by a Central Spindle Pathway in C. elegans**

In addition to contractile apparatus proteins, another group of central spindle proteins mediate a poorly defined but distinctly late step in cytokinesis. The central spindle, or midzone, consists of bundles of anti-parallel, overlapping MTs that form during anaphase and persist after division as the midbody. Two central spindle cytokinesis genes, called zen-4 and cyk-4, encode a Mitotic Kinesin-like Protein (MKLP) and a Rho-GTPase Activating Protein (RhoGAP) that require each other for localization to the central spindle and are both required for integrity of the central spindle (Jantsch-Plunger et al., 2000; Powers et al., 1998; Raich et al., 1998). A vertebrate relative of ZEN-4, called MKLP-1, can cross-link anti-parallel MTs in vitro (Nislow et al., 1992), suggesting that this kinesin subfamily is directly involved in central spindle assembly. ZEN-4 and CYK-4 form a tetrameric complex that likely mediates central spindle assembly by cross-linking of anti-parallel, non-kinetochore microtubules (Mishima et al., 2002). While reducing the function of a contractile ring component results in an early cytokinesis defect, with no membrane ingression, disrupting CYK-4 or ZEN-4 results in a late cytokinesis defect, with cleavage furrows regressing only after extensive ingestion. Chromosome segregation appears normal in many central spindle mutants, suggesting that this spindle structure is required more for cytokinesis than for chromosome segregation. MKLP-1 and MgcRacGAP, human orthologues of ZEN-4 and CYK-4, form a similar complex, and depleting MgcRacGAP in a human cell line by RNA interference disrupted central spindle assembly and cytokinesis (Mishima et al., 2002). Thus this complex has been functionally conserved from worms to humans.

It is not known if the CYK-4/ZEN-4 complex, called centralspindlin, is directly involved in the completion of cytokinesis. Centralspindlin might instead influence cytokinesis only indirectly, by mediating central spindle assembly and thereby making it possible for other central spindle components to participate directly in the termination of cytokinesis. Possible scenarios include the following. The RhoGAP in centralspindlin could antagonize Rho function, which is required for furrow ingression (Jantsch-Plunger et al., 2000), and thereby promote the disassembly or deactivation of the contractile ring as it contacts the spindle midzone late in cytokinesis. Alternatively, the central spindle could provide a scaffold for anchoring the contractile ring to the spindle during the completion of cytokinesis. If so, MKLPs might stabilize cleavage furrows directly, by tethering the contractile ring to the midbody. Consistent with this possibility, ZEN-4 may be required to stabilize furrows well after the apparent completion of cytokinesis (Severson et al., 2000). Finally, it is also possible that the central spindle mediates the delivery of new membrane vesicles to the cleavage furrow to promote the final membrane fusion events that ultimately partition daughter cells (reviewed in Bowman and Severson, 1999).

Centralspindlin is conserved in humans, but the general significance of its late role in cytokinesis is not clear, though it is well documented in C. elegans. Unlike ZEN-4, the Drosophila MKLP Favarotti is required not only for central spindle assembly but also for assembly of a contractile ring early in cytokinesis (Adams et al., 1998). The central spindle in Drosophila can be a large disc that spans the entire...
diameter of a dividing cell, while in the early *C. elegans* embryo, the central spindle is narrow relative to cell diameter. Perhaps centralspindlin acts late in cytokinesis in all animal cells, but there may also be early requirements when large central spindles are involved in specifying the assembly or activation of a contractile ring. Whether the early role in *Drosophila* requires centralspindlin directly, or other central spindle proteins, is not known. Nor is it known if depleting centralspindlin in human cell lines causes an early cytokinesis defect, as in *Drosophila*, or a late defect, as in *C. elegans*.

**Polar body extrusion during meiosis also requires the contractile apparatus proteins that mediate cytokinesis during mitosis**

Some cytokinesis genes in *C. elegans* are also required for the highly asymmetric cell divisions that occur during meiosis. Prior to its first mitosis, the nematode zygote undergoes two meiotic divisions that extrude small polar bodies, usually at the anterior end of the embryo (Albertson, 1993). Contractility genes identified thus far are also required for meiosis: reducing the function of *cyk-1, mlc-4*, or *nmy-2* resulted in failures to extrude polar bodies during meiosis (Swan et al., 1998; Shelton et al., 1999). Based on analyses of non-conditional mutants, it thus is not possible to determine if the gene products are required directly for cytokinesis, or if such mitotic defects are caused indirectly by the earlier failures during meiosis. However, the identification of temperature-sensitive alleles for some cytokinesis genes has made it possible to demonstrate requirements during mitosis after bypassing any earlier requirements at the permissive temperature (Severson et al., 2000). The continued application of different genetic approaches to the study of cytokinesis in *C. elegans* should make it possible to further define the different pathways that control cytokinesis in a developing animal embryo.

The availability of multiple genetic approaches and the transparency of the early nematode embryo greatly facilitate the study of cell division in *C. elegans*. Much insight can be gained from easily obtained time-lapse movies using Nomarski DIC optics to view cell division in the cytologically appealing early embryonic cells. Moreover, sensitive time-lapse imaging methods, and GFP fusion proteins that render the cytoskeleton fluorescent, now provide dynamic views of specific molecular structures during mitosis in live embryonic cells. Continued screens for functionally important loci, and sophisticated analyses of their mutant phenotypes, will continue to define the pathways that regulate and execute cytokinesis in *C. elegans*. It should be possible to identify additional factors that participate in known pathways to regulate contractile forces in the microfilament cortex, to control the assembly and function of the central spindle, and to mediate membrane addition at the cleavage furrow. Genetic analyses may reveal how these different pathways interact, and are also likely to identify conserved genes not currently known to have important roles in cytokinesis.

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