Cell division: **Plant-like properties of animal cell cytokinesis**

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Recent evidence that a syntaxin is required for cytokinesis in *Caenorhabditis elegans* embryos suggests that the mechanism of cell division in plant and animal cells may be more similar than previously imagined.

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Current Biology 1999, 9:R658–R660
http://biomednet.com/elecref/09609822009R0658

0960-9822/99/$ – see front matter

Past studies of cytokinesis in plants and animals have suggested that two key processes, contractile ring function and membrane addition at the cell surface, contribute to the partitioning of daughter cells at the end of mitosis [1]. Genetic and molecular studies of cytokinesis in vertebrate, insect, yeast and slime mold systems have provided ample *in vivo* evidence that the actin cytoskeleton and a contractile ring are critical for cell division [2]. Genetic studies in plants have demonstrated a requirement for membrane addition, though not cortical contraction [3]. Now, a study of cytokinesis in the nematode *Caenorhabditis elegans*, published recently in *Current Biology* [4], has shown that a syntaxin — a type of protein known in other contexts to play a part in specific membrane fusion — is necessary for cell division. This new work provides genetic evidence that membrane addition may be required during cytokinesis in an animal cell, and shows that, in spite of their apparently very different cytokinetic strategies, plants and animals may use conserved genetic programs to incorporate new membrane during cytokinesis.

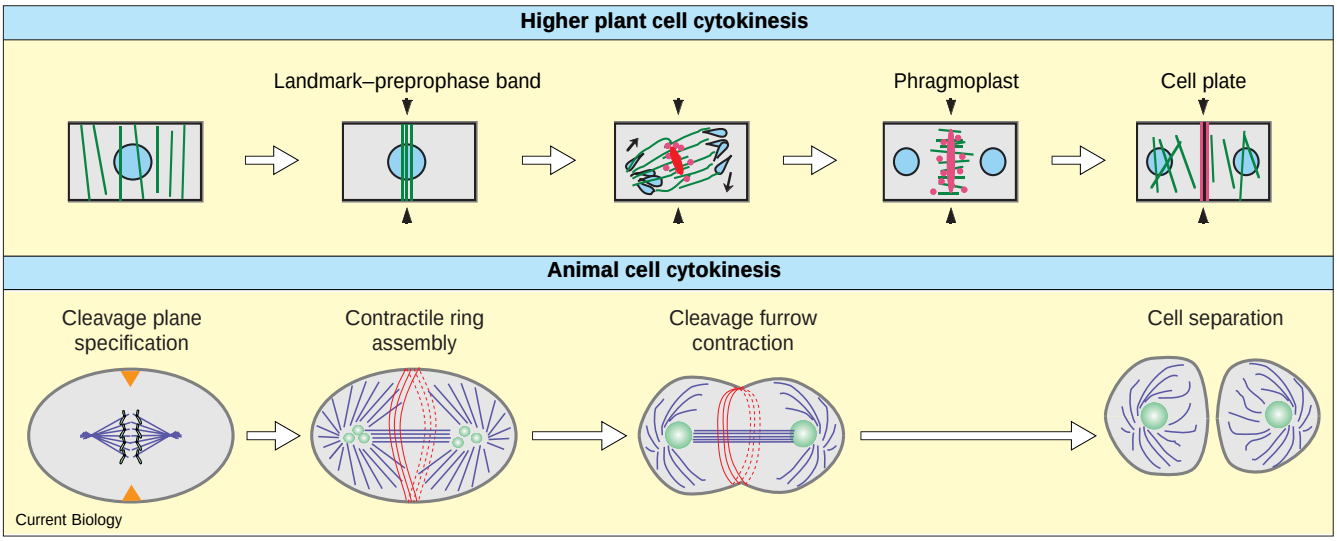
At first blush, cytokinesis in animal cells appears substantially different from plant cell cytokinesis (Figure 1) [3]. In the more malleable animal cells, a ring of actin and myosin anchored to the cell membrane generates force, constricting the cleavage furrow and ultimately bisecting the midzone of the mitotic spindle during late anaphase and telophase to generate two daughter cells. By contrast, almost the reverse occurs in plant cells. A dense network of microtubules and microfilaments grows out from the spindle midzone towards the cell surface, forming a large and complex structure called the phragmoplast. Phragmoplast construction, including the apparent transport and targeting of membrane vesicles by microtubules, appears to build the nascent cell wall from the cell center outward, eventually producing the rigid wall characteristic of higher plants. In short, animal cells appear to emphasize the use of a contractile ring during cytokinesis, while plant cells appear more partial to membrane addition.

Mutations of *knolle*, a syntaxin-encoding gene of the plant *Arabidopsis thaliana*, were found to result in cytokinesis defects during embryogenesis [5]. The encoded protein localizes to the phragmoplast during cell division, but is undetectable during interphase [6]. As syntaxins are thought to provide target specificity during the transport and docking of intracellular vesicles, the observations on *knolle* mutants indicate that membrane addition may be required specifically for cytokinesis. Further support for this possibility has come from ultrastructural studies, which have shown that membrane vesicles accumulate at presumptive sites of cell wall synthesis in *knolle* mutant embryos [6]. Other processes requiring targeted vesicle fusion, such as the growth of root hairs and pollen tubes, appear normal [5]. Therefore the cytokinesis defect is probably not caused by depletion of an important transmembrane protein due to a general block in vesicle transport. Presumably other syntaxins in the *Arabidopsis* genome participate in vesicle transport unrelated to phragmoplast formation and cytokinesis.

Studies of cytokinesis in embryos of the amphibian *Xenopus* have provided compelling evidence that membrane addition also is important during cytokinesis in animal cells. In *Xenopus* embryos, the addition of new membrane during cytokinesis is easily monitored, as the newly added membrane in the cleavage furrow is unpigmented and hence easily distinguished from the more highly pigmented pre-existing plasma membrane [7]. Consistent with membrane addition occurring during furrow progression, transmission electron microscopy studies revealed accumulations of membrane vesicles near the leading edge of cleavage furrows [7]. Disrupting microfilament function in early *Xenopus* embryos by treatment with the inhibitor cytochalasin D [7], or by microinjection of modified forms of the small GTPases Rho or Cdc42 [8], blocked furrow ingression but did not prevent unpigmented membrane from accumulating at the presumptive site of furrow formation, suggesting that contractile ring assembly and membrane addition are separable.

These elegant studies indicate that the new membrane required for the increase in surface area as a cell divides is added during division and targeted specifically to cleavage furrows, apparently independently of contractile ring function. The similarity of cytokinesis in animal and plant cells is further reinforced by the observation that the microtubule depolymerizing drug nocodazole blocks addition of new membrane during cytokinesis in *Xenopus*

Figure 1



Cytokinesis in higher plants (top) requires vesicle fusion to generate the cell plate, while animal cell cytokinesis (bottom) requires contraction of an acto-myosin ring.

embryos [9]. Thus, as for the plant phragmoplast, the targeting of membrane vesicles by microtubule-mediated motor proteins may mediate the addition of new membrane during cytokinesis in animal cells.

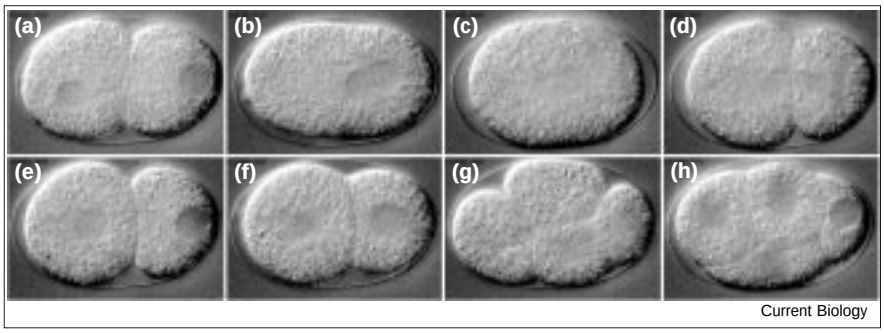
Studies of cytokinesis in the early *C. elegans* embryo now provide loss-of-function genetic evidence that membrane addition is required for cytokinesis in an animal cell. This advance comes from Jantsch-Plunger and Glotzer [4], who have taken advantage of the nearly complete genome sequence of *C. elegans* that is now available, and the powerful technique of RNA-mediated interference (RNAi), to survey all known *C. elegans* syntaxin genes for requirements during embryonic cytokinesis. They inactivated eight different syntaxin genes by RNAi and found one, called *syn-4*, that appears to be required both for cytokinesis and for the reassembly of nuclei after the completion of cell division.

C. elegans embryos lacking *syn-4* function were found to exhibit partially penetrant defects in cytokinesis: approximately two-thirds of the embryos analyzed failed to complete the first two rounds of embryonic cytokinesis. Some embryos failed to form ingressing furrows, whereas in others the furrows ingressed substantially but ultimately regressed. Many of the *syn-4*-deficient embryos also exhibited defects in the extrusion of polar bodies during meiosis, apparently as a result of defects in meiotic cytokinesis. Finally, mitotic spindles appeared to segregate chromosomes normally in the absence of *syn-4* function, but supernumerary nuclei sometimes formed as chromosomes decondensed and attempted re-assembly into daughter nuclei.

Syn-4 protein, as detected by immunofluorescent labeling, localizes prominently to cleavage furrows and to small structures dispersed throughout the cytoplasm that concentrate around re-forming nuclei. Syn-4 may therefore be required for membrane addition at the cleavage furrow during cytokinesis, and for subsequent membrane fusion events that mediate nuclear re-assembly. Further supporting a role for membrane addition, the inactivation by RNAi of *rab-11* — which encodes a *C. elegans* member of the Rab family of small GTPases that have also been implicated in specific membrane fusion events — results in a cytokinesis defect similar to that caused by inactivation of *syn-4* (A. Skop and J. White, personal communication).

These results with *C. elegans* support the view that membrane addition is required during cytokinesis in animal cells, as in plant cells, but important issues remain to be addressed. One is the question of whether new membrane material is targeted specifically to the cleavage furrow during mitosis in *C. elegans* embryos. Further insight might come from membrane-labeling and ultrastructural studies of vesicle fusion or accumulation at the cleavage furrow during cytokinesis in wild-type and *syn-4*-deficient embryos. Indeed, it remains possible that Syn-4 and Rab-11 are required to transport transmembrane protein(s) essential for cytokinesis to the cell surface, in which case the phenotypes caused by loss of function of these proteins might not be due to defective membrane addition. Such an indirect explanation is supported by the observation that *syn-4* mutants fail to secrete a normal chitinous eggshell following fertilization [4], raising the possibility that Syn-4 might be required

Figure 2



Nomarski micrographs of the first two cell cycles in *C. elegans* embryos. (a) Pronuclear migration stage embryo. The oocyte pronucleus (left) migrates to meet the sperm pronucleus (right). A transient furrow, the pseudocleavage furrow, constricts the cell at this stage. (b) The pronuclei meet in the posterior of the embryo. The pseudocleavage furrow has relaxed. (c) The first mitotic spindle lies along the longitudinal axis of the embryo, and is posteriorly displaced. (d) The first cleavage furrow bisects the mitotic spindle, separating the reforming nuclei. Note the asymmetric shapes of the spindle poles. (e) The two cell stage. (f) The nuclear envelope has begun to break down in the anterior blastomere (left), which divides before the posterior daughter. (g,h) The second embryonic cytokinesis produces the four-cell stage embryo.

more generally for the fusion of all secretory vesicles at the cell surface.

The identification and analysis of temperature-sensitive alleles of *syn-4* may be necessary to dissect these different requirements. Meanwhile, examining the localization of transmembrane proteins expressed in the early embryo, such as the Delta and Notch homologs Glp-1 and Apx-1, might indicate whether *syn-4* is required more generally for vesicle transport. It will also be interesting to learn whether microtubules are involved in targeting new membrane addition in *C. elegans* embryos, and whether the midzone microtubules that become constricted by the contractile ring at the end of cytokinesis are in some ways similar to the phragmoplast of dividing plant cells.

Intriguingly, a protein that from its sequence is likely to be a member of the kinesin family of motor proteins is localized to the spindle midzone in early *C. elegans* embryos [10,11]. This protein is called Zen-4 or CeMKLP1, and inactivation of the *zen-4* gene was found to result in a late defect in cytokinesis, with cleavage furrows regressing only after substantial ingression. While the cytokinesis defect in *zen-4* mutant embryos might be caused indirectly by spindle defects, it also is possible that Zen-4/MKLP-1 is required to target membrane vesicles to the cleavage furrow late in cytokinesis, promoting the final separation of daughter cells at the spindle midzone or stem body. Perhaps membrane addition also occurs earlier, as *syn-4* and *rab-11* mutant embryos exhibit a range of both early and late cytokinesis defects, with variable degrees of furrow ingression.

While important questions remain, the genetic requirement for a syntaxin during cytokinesis in both *C. elegans* and in *Arabidopsis* supports the view that plant and animal cell cytokinesis may involve similar processes. The emergence of the early *C. elegans* embryo as a powerful genetic system for studying cell biology promises further insights. Using RNAi, one can go from gene to phenotype in days, while reverse genetic screens for deletions allow unambiguous determination of null-mutant phenotypes. Moreover, classical genetic screens for non-conditional and for temperature-sensitive mutations have begun to identify loci required for cell division processes, including cytokinesis. Equally appealing, the relatively large size of the *C. elegans* embryo makes it amenable to high-resolution cytological observation and experimental manipulation (Figure 2). While RNAi is largely responsible for the increasing use of the early *C. elegans* embryo in cell biology, a combination of powerful technologies should further inform our understanding of the genetic programs that regulate and execute cytokinesis in a nematode embryo.

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