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Extra View

# Cytokinesis and the Spindle Midzone

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cytokinesis, spindle midzone, centralspindlin, cortical contractile element

**ABBREVIATIONS**

ASS asymmetric spindle severing

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## ABSTRACT

At the end of the cell cycle a cell physically divides into two daughter cells in a process called cytokinesis. Cytokinesis consists of at least four steps: (1) The position of the presumptive cytokinesis furrow is specified. (2) A contractile ring is formed. (3) The contractile ring contracts, resulting in furrow ingression. (4) Cytokinesis completes with sealing of the membranes. The mitotic spindle positions the cytokinesis furrow at the cell cortex midway along the longitudinal axis of the spindle, which is both the mid-point between the two asters and the location of the spindle midzone. The mitotic spindle emits two consecutive signals that position the furrow: Microtubule asters provide a first signal; the spindle midzone provides a second signal.

Our results support the view that the spindle midzone is dispensable for completion of cytokinesis. However, the spindle midzone can negatively affect aster-positioned cytokinesis, possibly because the aster- and midzone-positioned furrows compete for contractile elements.

## CYTOKINESIS FURROW POSITIONING BY THE MITOTIC SPINDLE

The idea that a mitotic spindle positions the cytokinesis furrow has been extensively tested and verified over the last 100 years, primarily by using micromanipulation experiments. The clearest demonstration is that if a spindle is mechanical displaced, the position of the furrow always coincides with the new position of the spindle.[1] Further studies have tried to identify the spindle substructure that positions the furrow, primarily by assessing the sufficiency of various spindle parts to support furrowing. At the time of cytokinesis, a spindle can be thought of as two microtubule asters connected together by a microtubule-based structure called the spindle midzone. The spindle midzone is a set of bundled non-kinetochore microtubules that forms between the chromosomes after chromosome segregation. Numerous experiments in various systems have shown that either the asters alone or the midzone alone can specify a furrow.[2,3] How is the activity of these two different structures coordinated so that they form one furrow? There are four extreme models as to how the midzone and the asters could contribute to furrow positioning. Two models assume a dominant mechanism and two assume a redundant mechanism: (1) The asters dominate the position of the cytokinesis furrow. (2) The midzone dominates the position of the cytokinesis furrow. (3) Both the asters and the midzone contribute to one furrow-positioning signal. (4) Both the asters and the midzone provide independent signals.

The problem with resolving these models is determining the relative contributions from the two different parts of the spindle. A cue from the asters would position the furrow midway between them; a cue from the midzone would position the furrow at the same place. Recent work in *C. elegans* has suggested that the midzone and centrosome separation play redundant roles in furrow formation.[4] We have developed a laser-based assay to distinguish the relative roles of asters and the midzone in forming the cytokinesis furrow in *C. elegans* embryos. In this assay, we sever the mitotic spindle between one aster and its associated chromatin at early anaphase, after the formation of the midzone. The two unequal spindle parts are pulled apart by intrinsic cortical pulling forces, which normally contribute to elongation of the spindle. Thus in this assay, the position midway between the asters and the position of the spindle midzone are spatially separated. We term this assay asymmetric spindle severing (ASS)[5] (Fig. 1A and B). After ASS, we observed that a furrow first formed midway between the two asters. This first furrow did not complete. A second furrow formed slightly later and ingressed toward the spindle midzone. The two furrows met and cytokinesis completed (Fig. 1B). We also showed that microtubule asters

position the first furrow, and that the spindle midzone positions the second furrow. From these experiments, we concluded that the position of the cytokinesis furrow is specified by two consecutive signals, the first from the asters and the second from the midzone.

## IS THE SPINDLE MIDZONE DISPENSABLE FOR CYTOKINESIS FURROW INGRESSION AND COMPLETION?

These experiments showed that both the midzone and the asters can specify the cytokinesis furrow position. However, they leave open the question of whether the midzone is necessary for furrow ingression, or whether it is also required specifically to complete cytokinesis so that two separate daughter cells are formed. Completion of cytokinesis requires that the cell membranes of the constricting furrow fuse together, and this process is poorly understood.

The idea that interaction between the cytokinesis furrow membranes and the midzone is required for completion is an attractive idea. However, classic data collected over the last 100 years suggest that, at least in some systems, the spindle midzone is dispensable for cytokinesis furrow ingression. In 1912, Yatsu cut ctenophore eggs so that they contained the leading edge of the furrow but not the spindle. Although the cells did not contain spindles, the cleavage furrow still completed cytokinesis.[6] Hiramoto aspirated the spindle away after specification, but before ingression, of the cytokinesis furrow in sea urchin eggs. In this experiment as well, the cytokinesis furrow completed.[7] These experiments suggested that the mitotic apparatus is dispensable for furrow ingression and completion of cytokinesis. Once initiated, the furrow is able to ingress without a spindle.

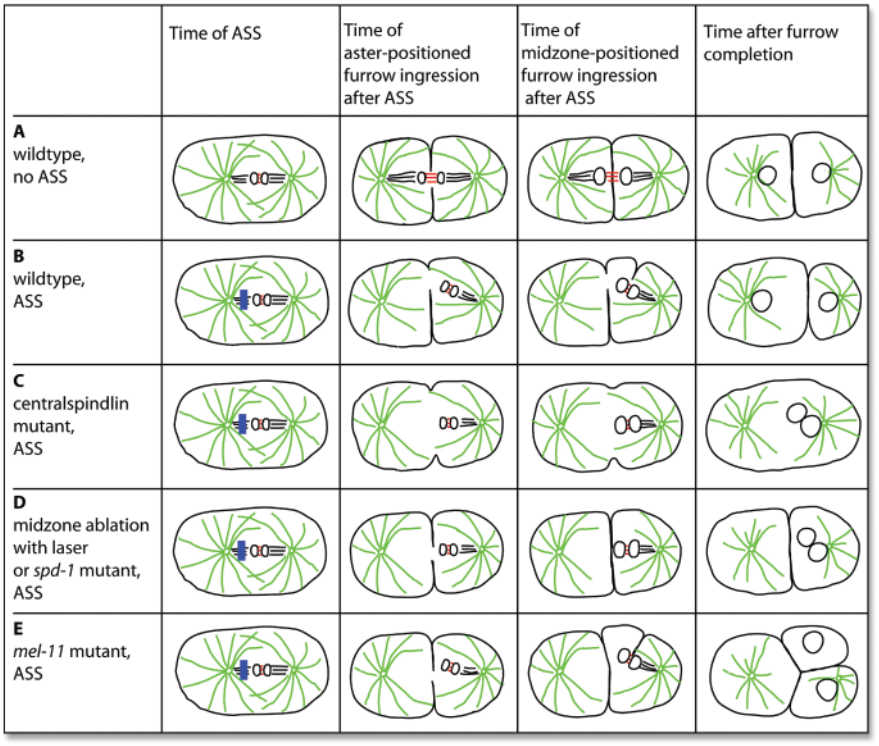


Figure 1. The figure shows cartoons of (A) cytokinesis in a wild type embryo, and cytokinesis after ASS in (B) wild type, (C) centralspindlin mutants, (D) midzone-null mutants (*spd-1* mutant and laser ablation of the midzone), (E) and nonlimiting contractility conditions (*mel-11* mutant). Timepoints shown are at the time of ASS, aster-positioned furrow ingression, midzone-positioned furrow ingression, and after the completion of cytokinesis. The laser-irradiated region is shown as a blue bar, asters are shown in green, and the midzone is shown in red.

In contrast to the micromanipulation data, genetic data collected over the last ten years has suggested that a part of the mitotic spindle, the spindle midzone, is required for the ingression of the furrow (reviewed in ref. 8). In *C. elegans* embryos, most of the evidence for this idea comes from analysis of the centralspindlin complex, which localizes to the central region of the midzone.[9] Mutations in centralspindlin genes disrupt both the formation of the spindle midzone and furrow ingression and completion of cytokinesis.[10,11] This genetic data suggested an indispensable role of the spindle midzone in completion of furrow ingression.

The genetic evidence that the spindle midzone is required for the late stages of cytokinesis, however, seems contradictory to historic experiments in which the spindle was removed and cytokinesis completed (as discussed above). In *C. elegans* it is also inconsistent with a recent study of the midzone component SPD-1. *spd-1* mutants do not form a cytologically detectable midzone, but they do ingress and complete a cytokinesis furrow.[12] Two hypotheses could explain the contradictory data: either the spindle midzone is required for completion of cytokinesis and the *spd-1* mutant is not a "midzone null", or the spindle midzone is not required for completion of cytokinesis and components of the centralspindlin complex have midzone-independent roles in furrow ingression.

Using ASS, we were able to directly test both hypotheses. We measured the ingression of aster- and midzone-positioned cytokinesis furrows in wild type and centralspindlin mutants following ASS (Fig. 1C). As expected, a midzone-positioned furrow did not form in centralspindlin mutants. An aster-positioned furrow did form. However, the aster-positioned furrow in centralspindlin mutants ingressed less than the furrow in wildtype embryos. We thus concluded that the centralspindlin components must have at least two roles: the known role in the formation of the midzone, and a second role in ingression of an aster-positioned furrow. Thus it seems unlikely that the centralspindlin mutant phenotype is a specific "midzone null" phenotype. This result is consistent with the finding that the centralspindlin components do not only localize to the spindle midzone but also localize to the ingressing furrow. The furrow localization of the centralspindlin components has been shown to be independent of a spindle midzone.[12]

Why does the furrow still ingress weakly in centralspindlin mutant embryos? One possible explanation is that the centralspindlin phenotype in *C. elegans* embryos is hypomorphic, and complete-loss-of function of centralspindlin would cause a complete absence of furrowing. The other possibility is that there is an independent pathway responsible for the weak ingression of the furrow. In centralspindlin mutants of *Drosophila* and human cells, no furrow ingression is visible,[13,14] supporting the idea of hypomorphic phenotypes in *C. elegans* centralspindlin mutants. However, in *spd-1* mutants, centralspindlin components localize to the aster-dependent furrow late in the course of ingression but can not be detected on the early furrow,[12] consistent with a centralspindlin-independent pathway that can support weak furrow ingression.

## THE SPINDLE MIDZONE IS DISPENSABLE FOR CYTOKINESIS

To directly test whether the midzone is required for the ingression of the cytokinesis furrow, we performed ASS in *spd-1* mutants, which do not form a spindle midzone but complete cytokinesis (Fig. 1D). We found that the first aster-positioned furrow is specified and completes, but a midzone-positioned furrow does not form. This finding shows that the defective midzone in *spd-1* is not able to specify a furrow, supporting the idea that the *spd-1* mutant is a "midzone null" in terms of furrow positioning. The assay also suggests that the midzone is not required for completion of cytokinesis. To further address this idea, we ablated the midzone using the UV laser after ASS in wildtype embryos. The midzone was irradiated before the midzone-positioned furrow was initiated. Following midzone ablation, the aster-positioned furrow was specified and completed, but a midzone-furrow did not form (our unpublished results). Thus the *spd-1* phenotype resembles a midzone-ablation phenotype and in both cases, cytokinesis completes. We conclude that the midzone is not required for the completion of cytokinesis.

## THE SPINDLE MIDZONE INHIBITS ASTER-POSITIONED FURROWING

If both the midzone and the asters can specify a cytokinesis furrow, why does a cell divide into two cells rather than three after ASS? Completion of two furrows is prevented because the first furrow stops and incorporates into the midzone-positioned furrow. Why does the first furrow stop? Our data suggests that the first furrow does not complete because the midzone inhibits the activity of the first furrow. The strongest piece of evidence supporting this idea is that in midzone-null embryos, the aster-positioned furrow ingresses completely at twice the speed of wild type embryos. This result suggests the midzone exerts a negative effect on the aster-positioned furrowing.

## HOW DOES THE MIDZONE-INDUCED FURROW INHIBIT THE ASTER-INDUCED FURROW?

Rappaport generated cylindrical sea urchin eggs and moved the spindle to a new location after a furrow had ingressed. He observed the regression of the old furrow and formation of a new furrow. Although a furrow can complete in the absence of a spindle, a furrow regresses if there is a competing furrow that continues to be stimulated by the spindle.[1] These experiments suggest that the control of furrow position is very dynamic and can respond to the changing position of the signal. White and Borisy suggested that cortical contractile elements with a high degree of cortical mobility increase the precision of cytokinesis furrow positioning.[15] Control of the flow of contractile elements would determine the position of the furrow.

One likely explanation for why the aster-positioned furrow does not complete is that a limitation of mobile contractile elements in the cortex could cause a competition between the aster-positioned furrow and the midzone-positioned furrow. In this model, an aster-positioned signal first starts recruiting contractile elements, and the furrow forms and begins to ingress. The midzone-positioned furrow is then specified and also starts recruiting contractile factors, thus competing with the aster-positioned furrow for cortical contractile elements. The midzone-positioned signal eventually gets stronger and outcompetes the aster-positioned signal it. The aster-positioned furrow stops ingressing and regresses if it is not incorporated into the midzone-positioned furrow.

Supporting evidence for this idea comes from the observation that NMY-2::GFP patches flow into the furrows after ASS. We first observed a flow of patches into the aster-positioned furrow. This flow then ceased when a massive flow into the midzone-positioned furrow started. We have observed NMY-2::GFP patches that moved from the aster-positioned furrow to the midzone-positioned furrow.

One way to test this model would be to increase the amount of contractility in the cortex and then examine the competition between the two furrows. *mel-11* encodes a myosin phosphatase that antagonizes contraction caused by myosin light chain phosphorylation.[16] Loss of MEL-11 activity, in theory, would lead to more contractility, and consistent with this, *mel-11* mutant embryos undergo cytokinesis twice as fast as wild type embryos.[4] After ASS in *mel-11* mutants, the aster-positioned furrow ingressed faster than in wild type and completed; a midzone-positioned furrow formed and also completed. The *mel-11* embryo thus divided into three cells following ASS: one cell contained one aster, but no nucleus; one cell contained one aster and one nucleus; and one cell contained only a nucleus. Other mechanisms are of course possible. For instance the midzone could actively inhibit the aster-positioned signal. In this model, the *mel-11* phenotype could be explained by the fact that the aster-positioned furrow ingresses fast enough to complete before the spindle midzone can actively inhibit the aster-positioned furrow.

## SUMMARY

The spindle midzone is defined as a structure composed of non-kinetochore microtubules that forms between the separating chromatin during anaphase. The midzone can position a cytokinesis furrow, but it seems dispensable for cytokinesis furrow ingression and completion. However, centralspindlin, defined largely by its essential role in midzone formation, is essential for cytokinesis furrow ingression. This apparent contradiction was resolved by the finding that centralspindlin has a nonmidzone role in aster-dependent furrow ingression.

The spindle midzone has the ability to correct furrows that are not directed at the midzone. The midzone can inhibit aster-positioned cytokinesis furrowing. Such a negative effect may result from a limited supply of contractile elements. The available contractility in the cell may be regulated by *mel-11*. The ability of one furrow to inhibit the other might help the cell to cleave under non-ideal conditions, primarily in situations where the aster- and midzone-positioned furrows are not superimposed. In such cases, it is essential that the final furrow separate the segregated DNA into separate cells. The position between the nuclei is marked by the midzone, and thus dominance by the midzone-positioned furrow is more likely to lead to efficient distribution of the genetic material.

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