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Determination of Cleavage Planes

Minireview

Susan Strome
Department of Biology
Indiana University
Bloomington, Indiana 47405

Most biologists appreciate the precision with which the mitotic spindle separates the duplicated chromosomes into two identical sets. Not as many realize that precision in the subsequent cleavage of the cell into two daughter cells is also important. Selection of the proper orientation and placement of the cleavage plane determines the relative positions of the daughter cells and ensures that each will receive the proper complement of cytoplasmic and membrane material, along with a nucleus.

The importance of cleavage plane choice is especially apparent in developing embryos. In many animal species, the orientations of embryonic divisions determine the overall organization of the embryo and the positions of embryonic cells and tissues relative to each other. Furthermore, some divisions in early embryos are "determinative," producing daughter cells with different developmental fates. A specific orientation of the cleavage plane can differentially partition localized membrane or cytoplasmic components to the daughter cells, and an asymmetric position of the furrow relative to the ends of the cell can generate daughters of different sizes. Such qualitative and quantitative differences, along with differential cell–cell interactions, determine a cell's developmental potential. Although this article focuses on animal cells, the requirement for controlling the orientation of division planes is even more stringent in plants. Because the cells of multicellular plants are prevented from moving by their cell walls, correct morphogenesis requires that cells be generated in their proper positions.

The Position of the Mitotic Spindle Dictates the Position of the Cleavage Plane

In all animal cells, the cleavage furrow forms equidistant between the two poles of the spindle (Figure 1). To test the role of the spindle in cytokinesis, Conklin (1917) altered the position of the spindle by subjecting Crepidula eggs to centrifugal force. Displacement of the metaphase spindle resulted in a corresponding displacement of the cleavage furrow. Displacement of spindles during anaphase did not displace the furrow. Conklin's results provided three important insights: first, the position of the mitotic spindle dictates the position of the cleavage furrow. Second, this occurs before or during anaphase. Third, multiple regions of the cortex are capable of furrow formation. Hiramoto (1956) subsequently tested the ability of sea urchin eggs to divide after removal of the mitotic spindle. Aspiration of the spindle during anaphase or later did not prevent cytokinesis, confirming that the determination of cleavage furrow position by the spindle is complete by anaphase and demonstrating that the spindle need not remain present for cytokinesis to occur.

To determine which elements of the spindle are important in specifying the position of the cleavage furrow, Rappaport (1961) used a glass needle to generate donut-shaped sea urchin eggs (Figure 2). The first cleavage of these eggs generated a horseshoe-shaped, binucleate cell. During the next cell cycle, two separate mitotic spindles formed in the two arms of the horseshoe. This configuration led to the formation of three cleavage furrows, two in their normal positions bisecting the two spindles and a third furrow between the two spindles (Figure 2). This elegant experiment indicates that it is the asters of the spindle that signal where the cleavage furrow will form.

The two favored models for how such signaling occurs both invoke stimulation of the cortex by astral microtubules. The models differ in whether microtubules stimulate the cortex near the spindle poles to relax, or stimulate the cortex around the spindle equator to contract. Regardless of which model is correct (discussed in reviews by Rappaport, 1986; White and Borisy, 1983), it is well established that the asters of the spindle dictate where the cleavage furrow will form. Thus, the question of how the position of the cleavage plane is determined becomes a question of how the position of the spindle is specified.

Control of Spindle Orientation

The "default" orientation of a spindle is 90° to the previous spindle. This can be explained by the movements of the centrosomes: prior to mitosis, the centrosome in a cell duplicates, and the two daughter centrosomes migrate away from one another to opposite sides of the nucleus, where they serve as the microtubule-organizing centers of the mitotic spindle. Deviations from this 90° pattern require alternative or additional movements of the centrosomes.

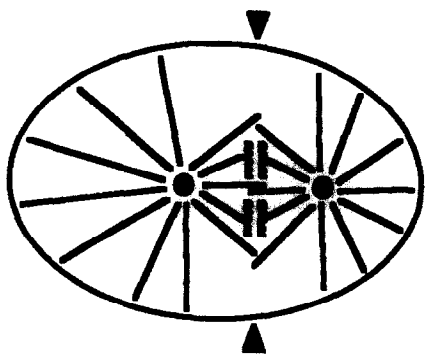

Figure 1. Spindle Organization

In animal cells, the components of a typical spindle are the centrosomes at the poles of the spindle, the astral microtubules that emanate from the centrosomes toward the cell surface, the microtubules that extend from the centrosomes to the chromosomes, and the microtubules that extend from the centrosomes and interdigitate with each other to mediate the separation of the spindle poles during anaphase. The cleavage furrow forms equidistant between the spindle poles. This cell would undergo an unequal division (at the arrowheads) as a result of the asymmetric position of the spindle.

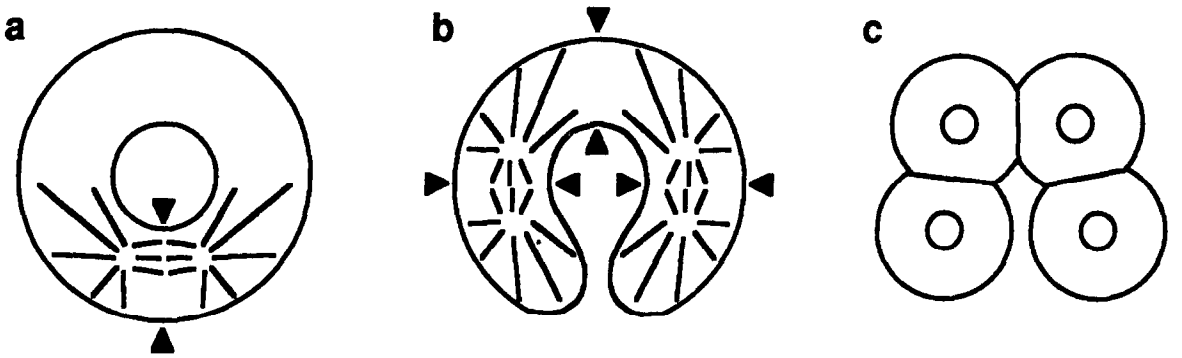

Figure 2. Demonstration That the Asters of the Spindle Determine the Position of the Cleavage Furrow

A donut-shaped sea urchin egg generated by inserting a needle through the center of the egg (a) divides into a horseshoe-shaped cell with two nuclei. The two spindles that form in such a horseshoe-shaped cell (b) direct the formation of three cleavage furrows (arrowheads), two that bisect the two spindles and one that forms between the two spindles (c). Adapted from Rappaport (1961).

One particularly well-studied example of deviation from the 90° rule is seen in two-cell Caenorhabditis elegans embryos (Figure 3). The AB cell shows the default orthogonal pattern of division, while the P1 cell divides along the same axis as the previous division. The P1 pattern of division is due to a 90° rotation of the centrosome–nucleus complex following centrosome duplication and migration (Figure 3; Hyman and White, 1987). This rotation appears to be mediated by microtubules extending from the centrosome to a site at the anterior cortex, because the rotation is sensitive to microtubule inhibitors and to laser irradiation of the zone between the centrosome and the anterior cortex (Hyman and White, 1987; Hyman, 1989). In fact, additional results suggest that microtubules emanating from both centrosomes initially interact with the anterior cortex, one set of microtubules wins the interaction contest, and the shortening of those microtubules swivels the centrosome–nucleus complex by 90° (Hyman, 1989).

Similar interactions between a specialized cortical site and microtubules emanating from a centrosome may dictate the unusual division pattern of the vegetal four cells in eight-cell sea urchin embryos. As a result of migration of the centrosome–nucleus complex to a specified cortical position in these cells, they divide unequally and approximately along the same axis as the previous division (Dan, 1979; Schroeder, 1987).

In both C. elegans and sea urchins, deviation from the 90° pattern of division is observed in cells that undergo determinative divisions to generate daughters with different developmental fates. In C. elegans, the determinative division of the P1 cell described above is preceded by the segregation of cytoplasmic structures, termed P granules, to the posterior end of P1 (Strome and Wood, 1983). The rotation of the spindle in P1 (Figures 3c–3e) ensures that only one daughter cell inherits the P granules. This illustrates the crucial role that the orientation of division can play in generating different daughter cells.

Control of Spindle Asymmetry

In most proliferating cells, the spindle is centrally located and equal-sized daughter cells are generated. However, as with spindle orientation, there are exceptions to this rule, and the exceptions are generally associated with determinative divisions. Three examples illustrate different ways in which centrosomes and microtubules interacting with the cortex can lead to asymmetric placement of the spindle. The unequal division of the vegetal cells of sea urchin embryos (see above) is due to movement of the nucleus, led by a centrosome, to a specified cortical site late in interphase (Dan, 1979; Schroeder, 1987). In C. elegans zygotes, the spindle becomes asymmetric during mitosis; one aster of the spindle remains fixed in position, while the other aster oscillates and becomes smaller as it moves closer to the cortex (Kemphues et al., 1988). Similarly, in Spisula zygotes, one aster of the spindle becomes centered, while the other aster oscillates and appears to search for a specific cortical anchorage site (Dan and Inoué, 1987).

Some of the best evidence for the existence of specialized cortical sites that interact with spindle microtubules comes from micromanipulation studies of meiotic spindles in Chaetopterus oocytes. Meiotic spindles are generally small and located close to the cell surface. If the meiotic spindle is displaced using a microneedle, it returns to its original cortical attachment site (Lutz et al., 1988). The observation that either pole of the spindle could lead the migration and reattach to the cortex provides evidence that the original attachment was severed by the microneedle and that either pole of the spindle is competent to interact with the cortical site. Pushing the spindle next to other regions of the cortex did not promote attachment, indicating the specialized nature of the cortex at the original attachment site.

Current and Future Directions

The nucleus and spindle movements that ultimately dictate the orientation and position of cleavage vary considerably between cells and species. Despite the variation in sequence of events, many of the movements may be mediated by a common mechanism: force-generating interactions between specialized cortical sites and microtubules emanating from the centrosomes. The questions that need to be tackled are obvious: What is the nature of the cortical site? How is the placement of the site determined? How do microtubules interact with the site? How does this interaction effect movement of the nucleus or spindle?

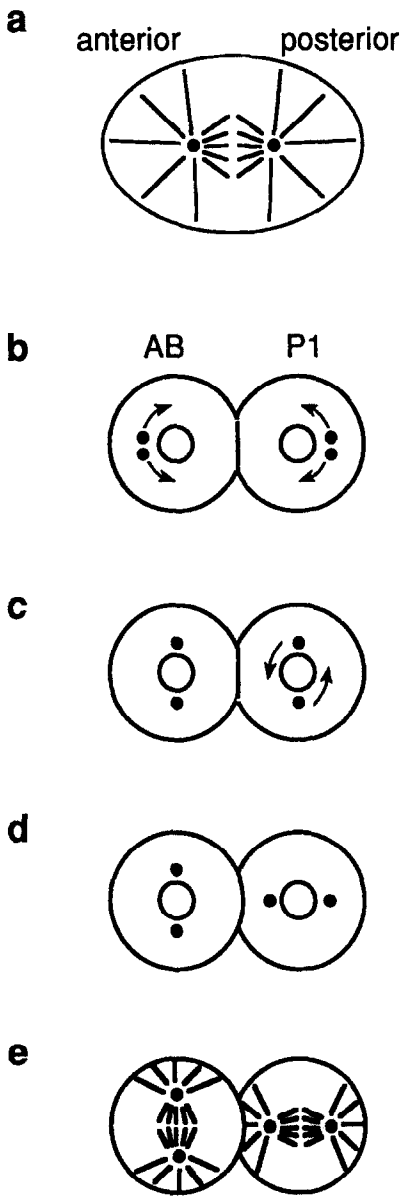

Figure 3. Centrosome Movements in C. elegans Embryos Control Spindle Orientation

After division of the zygote (a), the centrosome in each daughter cell divides, and the resulting centrosomes migrate to opposite sides of the nucleus (b). In the AB cell, this causes the spindle to be oriented 90° to the previous spindle ([c] and [e]). In the P1 cell, the centrosome–nucleus complex undergoes an additional rotation (c), causing the spindle to be aligned along the same axis as the spindle in the zygote ([d] and [e]).

Embryo manipulation and cytoskeleton perturbation experiments have provided some clues to answering these questions. In C. elegans, both rotation of the centrosome–nucleus complex and movement of the spindle to an asymmetric position are sensitive to microfilament inhibitors (Hyman and White, 1987; Hill and Strome, 1988). Thus, the actin cytoskeleton is probably involved in controlling both spindle orientation and placement. Actin filaments are known to be concentrated in the cortex (Strome, 1986), where they may participate in controlling spindle position either by interacting directly with microtubules or by localizing other cortical components with which microtubules interact. The cortical localization of microtubule motor proteins, such as kinesin or dynein, would offer a mechanism to bind and translocate microtubules, and thereby swivel or move the nucleus or spindle. This possibility can be investigated not only in C. elegans embryos, but also in zygotes of the alga Pelvetia. In these zygotes, as in the C. elegans P1 cell, there is a 90° rotation of the centrosome–nucleus complex, which is sensitive to microtubule and microfilament inhibitors and appears to be mediated by microtubules emanating from the rotating nucleus to a specific cortical site (Allen and Kropf, 1992).

One of the most promising approaches to answering the questions posed above and identifying some of the molecules involved is by screening for mutants with altered patterns of division. Such mutants have already been identified in C. elegans. Embryos produced by *par* mutant mothers show defects in spindle orientation and positioning and in partitioning of cytoplasmic components during the early divisions (Kemphues et al., 1988). Although the nature and functions of the *par* gene products are not yet known, the similar defects displayed by *par* mutant embryos and embryos treated with microfilament inhibitors (Hill and Strome, 1990) suggest that one or more of the five *par* genes may encode elements of the actomyosin cytoskeleton. Another possibility is that one or more of the *par* genes encode a microtubule motor, as suggested above.

Another system in which mutants in division pattern have been found is the budding yeast Saccharomyces cerevisiae. Although bud formation differs considerably from typical cell division, the mechanisms used to select, mark, and utilize specific cortical sites may be conserved. The selection and assembly of the cortical bud site are controlled by the products of at least 12 yeast genes, several of which are known to encode ras- and rho-type GTPases and proteins that regulate GTPase activity (reviewed by Drubin, 1991). After bud site selection and assembly, spindle position and orientation appear to be controlled by interactions between astral microtubules and the actin cytoskeleton (Palmer et al., 1992). The sophisticated genetics and molecular biology that are possible with yeast are enabling rapid progress in analyzing the functions and interactions of the familiar cytoskeletal components as well as the more recently identified bud pattern gene products. Furthermore, the bud pattern genes identified in yeast are providing access to homologous genes involved in cell division and proliferation in more complex eukaryotes.

Clearly, dissecting the mechanisms of cleavage plane determination is a formidable task. This review has focused on the first step in this process, specification of spindle orientation and position. Consideration of the next step, signaling of the cortex by the spindle, raises another set of intriguing questions, most notably where and how do the spindle asters signal the cortex, and how does this signaling modify the cortex such that the spindle is dispensable for the actual act of cytokinesis? Hopefully, the

answers to these questions will be the subject of future reviews.

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