Asymmetric Division in Fucoid Zygotes Is Positioned by Telophase Nuclei

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The relative contributions of cell polarity and nuclear position in specifying the plane of asymmetric division in fucoid zygotes were investigated. In zygotes developing normally, telophase nuclei were positioned parallel to the polar growth axis, and the division plane bisected both axes. To assess division plane specification, the colinearity of the nuclear and growth axes was uncoupled by treatment with pharmacological agents. Spatial correlations between the growth axis, telophase nuclei, and the division plane were analyzed in the treated zygotes. In all cases, cytokinesis was oriented transverse to the telophase mitotic array and was less well aligned with the growth axis. Telophase nuclei also played a predominant role in positioning the division plane in polyspermic zygotes. Microtubules from the telophase nuclei interdigitated throughout the plane of subsequent cytokinesis, and we speculate that they specify the division plane. Morphological markers of the division plane were not observed before telophase; the earliest division marker detected was a plate of actin that assembled in the zone of microtubule overlap late in telophase. These findings are consistent with division plane specification at cytokinetic boundaries.

INTRODUCTION

Asymmetric cell division is fundamental to development in all higher eukaryotes because it produces unequal daughter cells of different fates. Examples include early divisions in C. elegans embryos (White and Strome, 1996), neural development in Drosophila melanogaster (Ceron et al., 2001), reproduction in the green alga Volvox carteri (Kirk et al., 1993), and several formative divisions in higher plants (Gallagher and Smith, 1997; Scheres and Benfey, 1999; Smith, 1999; Sylvester, 2000). In most instances, these divisions are positioned precisely with respect to the polarity of the cell or tissue. Positioning cytokinesis is a matter of choosing a plane of division, and there are two general mechanisms for specifying the division plane. In the first mechanism, the orientation of the spindle (or the position of nuclei) determines the plane of division. This mechanism specifies division in metazoan, protist, and some plant cells (White and Strome, 1996; Field et al., 1999; Otegui and Staehelin, 2000; Brown and Lemmon, 2001). To correctly orient asymmetric division, the spindle (or nuclei) must be positioned in a precise location and orientation within the cell, presumably in accordance with polarity markers. In cells that divide centripetally by furrowing (e.g., metazoan cells), the spindle is thought to signal to the cortical division site, perhaps using astral microtubules (Hales et al., 1999; Glotzer, 2001). A variation on this theme occurs during cellularization in some plant tissues, including nuclear endosperm and female gametophytes. During cellularization, radial microtubules from nuclei define cellular spaces and centrifugally expanding phragmoplasts deposit cell plates at the boundaries (Pickett-Heaps et al., 1999; Otegui and Staehelin, 2000; Brown and Lemmon, 2001).

In the other general mechanism for positioning cytokinesis, cell polarity directly determines the division plane in accordance with localized cortical cues (Fowler and Quatrano, 1997). Once the division plane has been specified, the spindle need only align well enough to ensure that telophase nuclei are partitioned to different daughter cells. This mechanism often is used by cells that determine the division plane before mitosis, such as fission yeast, budding yeast, and somatic plant cells (Field et al., 1999; Smith, 1999; Sylvester, 2000). Asymmetric division in Azolla root cells (Gunning et al., 1978) and in onion guard mother cells (Mineyuki and Palevitz, 1990) provides clear examples: a preprophase band of microtubules forms in the cell cortex, marking the plane of future division, and the nucleus migrates to this site before spindle formation. These two general mechanisms are not mutually exclusive, and in many divisions in higher plants, both cellular asymmetry and nuclear position contribute to division plane specification (Gallagher and Smith, 1997; Smith, 1999; Sylvester, 2000; Baluska et al., 2001).

Zygot es of fucoid algae, genera Fucus and Silvetia (formerly Pelvetia; Serrao et al., 1999), provide an excellent model system for investigating the processes that establish cell polarity and position asymmetric division (Fowler and Quatrano, 1995; Kropf, 1997; Kropf et al., 1999; Belanger and Quatrano, 2000; Brownlee et al., 2001). Symmetry is broken at fertilization, and the sperm entry site defines the rhizoid pole of a growth axis (Hable and Kropf, 2000). This initial axis can be overridden by vectorial cues in the environment until germination, when growth becomes localized to the rhizoid pole. Rhizoid elongation gives the zygote an asymmetric, pear-shaped morphology.
Before mitosis, the nucleus rotates, bringing the axis defined by the centrosomes into crude alignment with the growth axis (Bisgrove and Kropf, 1998). Because centrosomes become spindle poles in mitosis, the metaphase spindle is aligned approximately with the growth axis. During spindle elongation at anaphase and telophase, the mitotic apparatus comes into perfect registry with the growth axis, and cytokinesis bisects both the growth axis and the axis defined by telophase nuclei (Bisgrove and Kropf, 2001). Rhizoid and thallus cells resulting from this division differ in morphology, cellular composition, and developmental fate; the rhizoid cell is the progenitor of the holdfast, whereas the thallus cell gives rise to much of the stipe and frond tissues. The orientation of the first asymmetric division is important for subsequent embryogenesis; treatments that misorient this division result in developmental arrest (Bisgrove and Kropf, 1998).

Because the growth axis and telophase mitotic array are colinear at the onset of cytokinesis, it is difficult to assess the relative contributions of the two axes in determining the division plane. We addressed this issue experimentally by perturbing the orientation of the mitotic apparatus, effectively uncoupling the two axes, and measuring the spatial alignment of division. The results indicate that the orientation of telophase nuclei is the primary determinant of the division plane. We conclude that cell polarity is used to position telophase nuclei, which in turn specify the division plane, probably via interdigitating microtubules.

**RESULTS**

**Division Plane Markers**

Because the mitotic apparatus is not positioned transverse to the subsequent cytokinetic plane until telophase (Bisgrove and Kropf, 2001), identification of markers at the division site before telophase would indicate that cell polarity is primarily responsible for specifying the plane of the first asymmetric division. Mitosis and cytokinesis last for several hours in *Silvetia compressa* zygotes, making it possible to conduct detailed temporal and spatial analyses of putative markers. The plane of future division is marked by unique cytoskeletal arrays in many eukaryotic cells (Field et al., 1999; Wasteneys, 2002), so microtubule and actin arrays were examined. Microtubules were labeled with antibodies throughout G2 and mitosis, and thousands of images were examined. In no case was a conspicuous microtubule array found in the cortex at the division site. A preprophase band of microtubules, which marks the division plane in somatic plant cells (Otegui and Staehelin, 2000; Brown and Lemmon, 2001; Kost and Chua, 2002), was not present. Instead, microtubules radiated from two centrosomes into the cortex of premitotic cells (Figure 1A). These microtubules are thought to be captured at the cortex and to participate in nuclear rotation (Bisgrove and Kropf, 2001). In metaphase (Figure 1B) and anaphase (Figure 1C), the crudely aligned spindle nucleated short astral microtubules that did not extend to the cortex. A vast cytoplasmic array reformed at telophase, and microtubules from daughter nuclei interacted with the cell cortex and interdigitated in the midzone of the disassembling spindle (Figure 1D).

Actin filaments were present throughout the cortex in interphase (Kropf et al., 1992), metaphase, and anaphase but showed no preferential localization or orientation with respect to the division site (data not shown). The first actin structure that indicated the division plane was a plate of F-actin that assembled in the cytokinetic plane; the actin plate formed after the zygotes had entered telophase but several hours before partition membrane deposition (Figures 2A, 2C, and 2D). At approximately the same time, the plane of subsequent division became optically more translucent than adjacent cytoplasm (Figures 2B and 2D). The clear zone likely resulted from the exclusion of pigmented organelles and granules. Actin plates and clear zones persisted at fully mature cross-walls.

Cellular organization also was examined by transmission electron microscopy, but structural markers of the division plane were not apparent before telophase (data not shown). Because indicators of the division plane were not detected until zygotes had entered telophase, these data do not discriminate cell polarity from nuclear position as the primary determinant of the division plane.
Uncoupling Nuclear Position from Cell Polarity

In a second approach to assess the relative contributions of cell polarity and the division plane, the orientation of division was examined in zygotes in which telophase nuclei were not colinear with the growth axis. In any population of untreated zygotes, a few cells fail to complete nuclear alignment, causing the nuclear axis to be skewed from the growth axis (Figures 3A and 3B). We examined 4732 cells and found 63 zygotes (1.3%) with skewed relative positions. The division plane was measured with respect to the growth axis and the axis defined by the telophase nuclei in these zygotes. In every cell examined, the division plane was more transverse to the nuclear axis than to the growth axis. On average, the division plane was oriented 84.3° ± 4.3° to the nuclear axis and 66.1° ± 12.7° to the growth axis (Figure 3C).

The colinearity of the growth axis and the telophase mitotic apparatus was perturbed experimentally by disrupting nuclear rotation. Nuclear rotation occurs before mitosis and is driven by microtubules that interact with the cell cortex (Bisgrove and Kropf, 2001). Agents that perturb the cell cortex prevent rotation, resulting in mispositioned telophase nuclei. The cell cortex was disrupted in two ways, by plasmolysis and by treatment with latrunculin B, which depolymerizes cortical actin arrays (Alessa and Kropf, 1999; Bisgrove and Kropf, 2001). Although cytokinesis was blocked in latrunculin B–treated zygotes, a clear zone formed between daughter nuclei, indicating that a division plane had been chosen. Two treatment regimes were used to analyze the spatial relationships between the clear zone, cell polarity, and the nuclear position at first telophase. First, zygotes were treated with latrunculin B before germination (6 to 9 h after fertilization [AF]), and angles between the clear zone and the growth axis, or the clear zone and the mitotic axis, were measured on randomly selected zygotes. The clear zone formed transverse to the axis of the daughter nuclei but was not well aligned with respect to the growth axis (Figure 4A). In the second protocol, zygotes were allowed to germinate before treatment (latrunculin B added at 13 h AF), and only zygotes with severely misaligned nuclei were analyzed. In controls, telophase nuclei were in register with the growth axis, and the clear zone formed transverse to both axes (Figure 4B). In treated cells with severely misaligned nuclei (Figure 4C), the
clear zone also formed transverse to the nuclear axis but was skewed with respect to the growth axis (Figures 4C and 4D). Plasmolysis also was used to disrupt the cortex and misposition telophase nuclei (Henry et al., 1996; Bisgrove and Kropf, 2001). Zygotes in artificial sea water (see Methods) were transferred to artificial sea water containing 0.6 M sucrose at 12 to 13 h AF, and relevant angles were measured at 28 to 30 h AF. Cell plates formed between telophase nuclei without regard to the orientation of the growth axis (Figure 5A).

Treatment with oryzalin depolymerizes microtubules and inhibits rotational alignment, presumably by preventing interaction between the nucleus and the cortex (Bisgrove and Kropf, 2001). Because chronic microtubule depolymerization blocks the cell cycle and prevents mitosis (Corellou et al., 2000), 1 μM oryzalin was applied in a pulse treatment. Zygotes were exposed to oryzalin during the period of rotational alignment, from 15 to 19 h AF, and then rinsed back into artificial sea water. Not all zygotes recovered from the treatment, and those that did developed slowly, so measurements were made at 39 h AF, when many of the surviving zygotes had completed the first division. The division plane was oriented nearly randomly with respect to the growth axis (alignment angle of 45.0 ± 19.7°) but was transverse to the nuclear axis (Figure 5B).

Polyspermy

Polyspermy severely alters the stereotypical spatial relation between nuclei and cell polarity. Centrioles are inherited paternaly in fucoid algae (Motomura, 1994), and polyspermy induced by fertilization in low-Na+ seawater introduces supernumerary centrioles (Brawley, 1987; Nagasato et al., 1999). Labeling of cytoplasm extruded from squashed, polyspermic zygotes has shown that the supernumerary centrosomes nucleate a multipolar spindle at first mitosis (Nagasato et al., 1999). To investigate the spatial aspects of division in polyspermic zygotes, we labeled microtubules, actin, and partition membrane in intact cells. At telophase, each spindle pole organized a radial microtubule array and actin plates formed approximately equidistant between poles (Figure 6). Subsequently, partition membranes and cell plates formed in the zones containing actin plates (data not shown).

Positioning of division planes between daughter nuclei in untreated, polyspermic, and treated zygotes strongly indicates that nuclear position is the primary factor in determining the division plane.

Microtubules and Division Plane Specification

How might telophase nuclei position the division plane? In green algae and cellularizing plant tissues, nuclei specify the division plane by defining a cytoplast (also termed a nuclear

Figure 4. Analysis of the Division Plane in Zygotes Treated with Latrunculin B.

(A) Zygotes treated chronically with 30 nM latrunculin B beginning at 6 to 9 h AF. Black bar, angle between the growth axis and the clear zone in solvent-treated control zygotes; white bar, angle between the nuclear axis and the clear zone in latrunculin B–treated zygotes; hatched bar, angle between the growth axis and the clear zone in latrunculin B–treated zygotes. Error bars indicate s.d. (B) and (C) Treatments begun at 13 h AF. Solvent-treated control (B) and latrunculin B–treated (C) zygotes were labeled with fluorescein diacetate at 26 h AF to visualize clear zones (arrowheads) and nuclear positions. Images are overlays with bright-field images. Bar in (C) = 25 μm for (B) and (C).

(D) Angles between the clear zone and the nuclear axis (white bar) and the clear zone and the growth axis (hatched bar) in 94 latrunculin B–treated zygotes. Error bars indicate s.d.
cytoplasmic domain (Brown and Lemmon, 2001)) using radial microtubules, with cytokinesis occurring in the zone of microtubule overlap (Pickett-Heaps et al., 1999; Baluska et al., 2001; Brown and Lemmon, 2001). The organization of microtubule arrays in polyspermic S. compressa zygotes is consistent with this mechanism; radial microtubule arrays extending outward from nuclei interdigitate in the plane of subsequent division (Figure 6). Treatments with pharmacological agents that alter microtubule arrays were not informative in investigating the role of microtubules in specifying the division plane because the treatments invariably blocked cell cycle progression (Corellou et al., 2000; S.R. Bisgrove, unpublished observations). Instead, microtubule arrays in untreated zygotes were examined in detail throughout telophase. Early in telophase, microtubules nucleated at centrosomes associated with the reforming nuclei interdigitated in the midzone between the two nuclei (Figures 1D and 7A). As telophase progressed, the region of interdigitating microtubules expanded outward to the cortex (Figures 7B and 7C). Interdigitating microtubules persisted throughout cytokinesis. At later stages of telophase, microtubules parallel to the cytokinetic plane (transverse to the radial array) became prevalent in the region of microtubule overlap (Figures 7D and 7E). Microtubules residing in, and parallel to, the cleavage plane are present in various groups of green algae and collectively form a structure termed a phycoplast, which is thought to form a barrier that keeps daughter nuclei apart during cytokinesis (Pickett-Heaps, 1975). Finally, an actin plate formed in the cytokinetic plane, where both interdigitating microtubules and phycoplast microtubules were present (Figure 7F).

**DISCUSSION**

Although previous work has shown that the division plane can be skewed from the growth axis (Shaw and Quatrano, 1996; Bisgrove and Kropf, 2001), in this report we have investigated the spatial relation between the division plane and telophase nuclei. Analysis of cell polarity, nuclear position, and cytokinesis in individual cells demonstrates that nuclear position is the primary determinant of the division plane. This conclusion is based on the observation that whenever the nuclear axis is uncoupled from the growth axis, either naturally or experimentally, cytokinesis bisects the nuclear axis. Cellular polarity is fundamental in positioning the telophase nuclei; therefore, it plays an important, albeit indirect, role in division plane specification.
Cellular polarity provides cortical spatial cues that guide both phases of nuclear alignment. Early in development, the paternally inherited centrosomes migrate to opposite sides of the zygotic nuclear envelope; the axis defined by the centrosomes initially is oriented randomly with respect to the growth axis (Bisgrove and Kropf, 1998). Before entry into metaphase, microtubules from the perinuclear centrosomes extend into the cellular cortex preferentially at the rhizoid pole, although some microtubules clearly associate with the thallus cortex. Microtubules are thought to be captured at specific sites in the cell cortex and to exert force; because most capture sites are in the rhizoid cortex, the centrosome is effectively pulled toward the rhizoid (Kropf, 1997). One centrosome, presumably that with the most anchored microtubules, wins this tug-of-war and is translocated toward the rhizoid; the other centrosome moves in the direction of the thallus. Because the centrosomes are embedded in the nuclear envelope, the result is a rotation of the entire nucleus. Although the nature of the cortical capture sites is uncertain, they apparently involve transmembrane adhesions. Membrane wall adhesions from cortical actin to extracellular protein are concentrated in the rhizoid (Henry et al., 1996), and treatments that disrupt these cortical adhesions prevent nuclear rotation (Bisgrove and Kropf, 2001). Available evidence suggests that nuclear rotation in S. compressa is mechanistically similar to spindle alignment processes associated with asymmetric division in yeast and C. elegans embryos (White and Strome, 1996; Adames and Cooper, 2000; Tirnauer and Bierer, 2000).

Nuclear rotation crudely aligns the centrosomal axis with the growth axis, and at metaphase, a spindle with short astral microtubules is assembled. The spindle is brought into perfect registry with the growth axis as the spindle elongates during anaphase and telophase. Interestingly, dynamic microtubules interact with the cortex during this phase of alignment, but cortical adhesions are not required (Bisgrove and Kropf, 2001). Although the mechanism of postmetaphase alignment is unclear, nuclear centering mechanisms may participate. Centering involves dynamic centrosomal microtubules that either push or pull on the cell cortex (Hill and Kirschner, 1982; Bjerknes, 1986; Holy et al., 1997; Reinsch and Gonczy, 1998). In the asymmetrically shaped S. compressa zygote, centrosomal centering forces that act on the separating spindle poles could align the spindle with the long axis of the cell (Bjerknes, 1986). There is ample evidence in plant cells that cell morphology can affect spindle position (Oud and Nanninga, 1992; Palevitz, 1993; de Ruijter et al., 1997).

The current findings indicate that once positioned along the growth axis, the telophase nuclei specify the division plane. Specification of the cytokinetic plane must occur late in telophase, because only then do the daughter nuclei attain a position that is transverse to the subsequent cell plate (Bisgrove...
and Kropf, 2001). This conclusion is supported by the absence of division plane markers at earlier times. The late specification of the division plane is in contrast with that in many algae, yeast, and somatic plant cells, which establish a division plane before mitosis (Field et al., 1999; Pickett-Heaps et al., 1999). Instead, the timing of division plane specification in S. compressa is more similar to that in metazoan cells, in which the anaphase spindle orients subsequent division by a mechanism involving unknown signals from the spindle midzone to the cell cortex (White and Strome, 1996; Hales et al., 1999; Glotzer, 2001). In S. compressa, the mechanism of cytokinesis is not well understood; if it occurs centrifugally, as recent evidence suggests (Belanger and Quatrano, 2000a), the telophase mitotic apparatus need not signal the cell cortex. Instead, the expanding cell plate may fuse with the parental wall wherever contact is made.

The telophase array may specify the division plane via interdigitating microtubules that define cytok plast boundaries. A cytok plast is a tese getal unit in which compression-resistant struts integrate with tension elements in the cell periphery (Ingber, 1993). In the most evolutionarily conserved arrangement, the struts are microtubules emanating from the nucleus region and a network of cortical actin constitutes the tension elements (Ingber, 1993; Pickett-Heaps et al., 1999; Baluska et al., 2000). At cytokinesis, microtubules from daughter nuclei overlap in the region of the remnant spindle midzone, thereby defining a new cytok plast border, and the cytokinetic machinery assembles there.

The first asymmetric division in S. compressa zygotes is consistent with the cytok plast concept. Throughout interphase, radial microtubules extend into the cellular cortex, which contains a meshwork of actin filaments. In telophase, remnant spindle microtubules interdigitate in the midzone between nuclei, and with time this structure expands centrifugally such that microtubules eventually interdigitate across the entire cell. We speculate that the interdigitating microtubules specify the cytokinetic plane. An actin plate assembles in the plane of interdigitating microtubules, ensuring that both thallus and rhizoid cells inherit a complete tesegetal unit when cytokinesis is complete. It should be noted that an actin plate is not required for division plane specification, because a clear zone forms between telophase nuclei in latrunculin B-treated zygotes. Unfortunately, the requirement for microtubules in division plane specification is more difficult to assess with physiological agents, because microtubule depolymerization or stabilization during mitosis blocks cell cycle progression (Corellou et al., 2000; S.R. Bisgrove, unpublished observations).

Among photosynthetic organisms, division plane specification at cytok plast boundaries defined by interdigitating radial microtubules occurs in the most ancient taxa, most notably green and red algae (Pickett-Heaps et al., 1999), and recent findings indicate that it also may be common in brown algae (Karyophyllis et al., 2000; Nagasato and Motomura, 2002). In the plant lineage, this mechanism has been retained in multinucleate tissues, such as meiocytes, nuclear endosperm, and female gametophytes (Otegui and Staehelin, 2000; Baluska et al., 2001; Brown and Lemmon, 2001), which cellularize after repeated rounds of karyokinesis. During cellularization, each nucleus forms an extensive radial microtubule array, and cell plate deposition occurs in the zone of microtubule overlap by means of a centrifugally expanding phragmoplast (or several miniature phragmoplasts). As in S. compressa zygotes, preprophase bands generally are not formed, and there is no evidence that cortical division sites are specified before cytokinesis.

Two defining features of plant evolution are changes in the organization of the cytok plast and in the mechanism of division plane specification in vegetative, somatic cells (for an evolutionary perspective on these issues, see Pickett-Heaps et al., 1999). Microtubules, rather than forming radial struts from the nucleus, reside predominantly in the cell cortex. The division plane is specified in G2, before mitosis, and is marked in the cortex by a preprophase band of microtubules, from which actin becomes excluded (Wasteneys, 2002). The preprophase band disassembles during mitosis, leaving an actin-depleted zone to mark the cortical division site. Cell plate deposition progresses centrifugally by means of a phragmoplast that contains microtubules, actin, and many other proteins, including motor and signaling molecules (Otegui and Staehelin, 2000; Liu and Lee, 2001; Wasteneys, 2002). Phragmoplast actin is thought to guide the expanding cell plate to the predetermined cortical division site.

Although division plane specification in somatic cells is not well understood, analyses of several mutations in maize (e.g., tangled, discordia, warty, and brick) that affect the positioning of the cell plate have begun to identify the processes and molecules involved (Smith, 1999; Gallagher and Smith, 2000; Sylvester, 2000). As might be expected, some of the mutations reside in genes that encode proteins that interact with the cytoskeleton; TANGLED encodes a microtubule binding protein (Smith et al., 2001), and the DISCORDIA and BRICK proteins are thought to regulate actin arrays (Gallagher and Smith, 1999; Frank and Smith, 2002).

Although superficially similar, the phragmoplast of plant cells is distinct from the interdigitating microtubule array in S. compressa in several important respects. Microtubules in the S. compressa array are attached to perinuclear centrosomes, whereas phragmoplast microtubules are much shorter and have minus ends that are free in the cortical cytoplasm. In addition, phragmoplast microtubules in the center of the array disassemble as it expands outward, whereas the interdigitating array in Silvetia persists throughout the cytokinetic plane. Importantly, the phragmoplast of somatic cells does not determine the division plane but instead fuses with cortical sites occupied previously by the preprophase band (Wasteneys, 2002). Actin also is likely to have different functions in the two structures. Phragmoplast actin is thought to guide the expanding phragmoplast to the cortical division sites, whereas in Silvetia, the actin plate forms after the interdigitating microtubule array has expanded to the cell cortex; therefore, it is unlikely to play a role in guidance.

In summary, we suggest the following scenario for specifying the position of the first asymmetric division in S. compressa zygotes. Cell polarity participates indirectly by providing spatial cues for nuclear and spindle alignment during G2 and M phases, respectively. In telophase, a centrifugally expanding array of interdigitating microtubules defines a plane in which the cytokinetic machinery, including actin, assemblies. Ongoing studies are focused on identifying the molecules that link the radial microtub...
bulles to the cortical actin during nuclear alignment and on understanding cytoskeletal function during cell plate deposition.

**METHODS**

Sexually mature receptacles of the fucoid alga *Silvetia compressa* (Serrao et al., 1999) were collected near Santa Cruz, California, shipped cold, and stored at 4°C until use. To induce the release of zygotes, receptacles were placed in the light (100 µmol·m⁻²·s⁻¹) at 16°C in artificial sea water (ASW; 10 mM KCl, 0.45 M NaCl, 9 mM CaCl₂, 16 mM MgSO₄, and 0.040 mg/mL chloramphenicol, buffered to pH 8.2 with 10 mM Tris base) overnight and then transferred to the dark for 30 to 45 min. The time of fertilization was considered to be the midpoint of the dark period. All zygotes were grown at 16°C in unidirectional light.

The cytoskeleton was imaged by confocal microscopy using a monoclonal anti-α-tubulin antibody (DM1A; Sigma, St. Louis, MO) to label microtubules and rhodamine phalloidin to label actin filaments, as described previously (Alexa and Kropf, 1999; Bisgrove and Kropf, 2001). Actin also was labeled with a monoclonal anti-actin antibody (C4; ICN Biomedicals, Aurora, OH) after fixation in actin-stabilizing buffer at pH 6.9 (50 mM Na₂-Pipes, 0.75 M sucrose, 1 mM EGTA, 5 mM MgSO₄, and 0.05% Triton X-100) with 3% paraformaldehyde, 6.3 µM m-maleimidobenzoyl-

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