Model of Magnetic Field-Induced Mitotic Apparatus Reorientation in Frog Eggs

James M. Valles, Jr.
Department of Physics, Brown University, Providence, Rhode Island 02912 USA

ABSTRACT Recent experiments have shown that intense static magnetic fields can alter the geometry of the early cell cleavages of *Xenopus laevis* eggs. The changes depend on field orientation, strength, and timing. We present a model that qualitatively accounts for these effects and which presumes that the structures involved in cell division are cylindrically symmetric and diamagnetically anisotropic and that the geometry of the centrosome replication and spreading processes dictates the nominal cleavage geometry. Within this model, the altered cleavage geometry results from the magnetic field-induced realignment of mitotic structures, which causes a realignment of the centrosome replication and spreading processes.

INTRODUCTION

Studies of the response of living systems to uniform physical fields (i.e., electric, gravitational, and magnetic) have yielded novel insight into a variety of biological processes (Zhao et al., 1999; Helmstetter, 1997; Henderson et al., 1998; Yokota et al., 1992; Gerhart et al., 1989; Denegre et al., 1998). These fields serve as a symmetry-breaking perturbation that can be applied with a well-defined and variable axis, variable strength, and flexible timing, and thus can be ideal for investigations of natural symmetry-breaking or structure-formation mechanisms. For example, Zhao et al. (1999) showed that the application of static electric fields to dividing human corneal epithelial cells causes the division planes to orient. Consequently, they suggested that physiological electric fields help position daughter cells during morphogenesis and other biological processes. Novel gravitational fields of variable magnitude were applied to manipulate the position of the first horizontal cell-division plane (Yokota et al., 1992) and of variable orientation to change the direction of cortical rotation in *Xenopus laevis* eggs (Gerhart et al., 1989). Those experiments suggested how some of the factors critical to development are localized and activated within the egg. Helmstetter (1997) and Henderson et al. (1998) varied the orientation of dividing cells relative to gravity and obtained insight into the factors determining cell-division geometry. Relatively recently, magnetic force fields have been applied and oriented to cancel or modify the uniform force of gravity on biological systems in efforts to probe gravitationally sensitive steps in growth and development (Valles et al., 1997; Brooks et al., 2000). Finally, and most germane to this work, static magnetic fields were used to reorient the early cell cleavages in *X. laevis* eggs (Denegre et al., 1998) to test factors determining their normal cleavage geometry (Valles et al., 2002). Those investigations implied that processes occurring before the start of the third cell cycle exert a strong influence on the orientation of the third cleavage plane. In this paper, we propose a model of how magnetic fields accomplish cleavage reorientation in *Xenopus* that implicates what process governs their early cleavage geometry. The field orientation, timing, and strength dependencies of the data served as essential guides for this model.

The well-described, nominal geometry of the early cell divisions or cleavages of amphibian eggs is beautifully symmetric and very regular (Nieuwkoop and Faber, 1967), but its origin is incompletely understood (see Fig. 1). The first and second cleavages are perpendicular, bisect one another, and align parallel to the animal-vegetal (AV) axis. The third cleavage plane forms perpendicular to the first and second and thus, perpendicular to the AV axis. Bjerknes accounted for this geometry with a model in which the shape of the egg and its internal yolk gradient play a central role (Bjerknes, 1986). These "shape" factors control the alignment of the mitotic apparatus (MA) in a dividing cell, which, in turn, determines the orientation of the cell-division plane (Bjerknes, 1986; Inoue and Salmon, 1995; Rappaport, 1996; Sharp et al., 2000; O'Connell and Wang, 2000). Alternatively, the geometry of the centrosome replication and spreading cycle that precedes the formation of the MA (described in more detail below) can also account for the egg's early cleavage geometry (Costello, 1961; Hyman and White, 1987). Neither of these models, however, had been explicitly tested.

Recently, a novel way to perturb the cleavage geometry was discovered (Denegre et al., 1998) and used to investigate factors influencing it (Valles et al., 2002). The experiments showed that the application of an intense static magnetic field, **B**, alters this simple geometry without changing cell shape. The effects depend on the strength and orientation of **B** relative to the egg. An AV axis parallel magnetic field, for example, can cause all of the third

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Address reprint requests to James M. Valles, Jr., Brown University, Dept. of Physics, Providence, RI 02912. Tel.: 401-863-7559; Fax: 401-863-2024; E-mail: valles@holley.physics.brown.edu.

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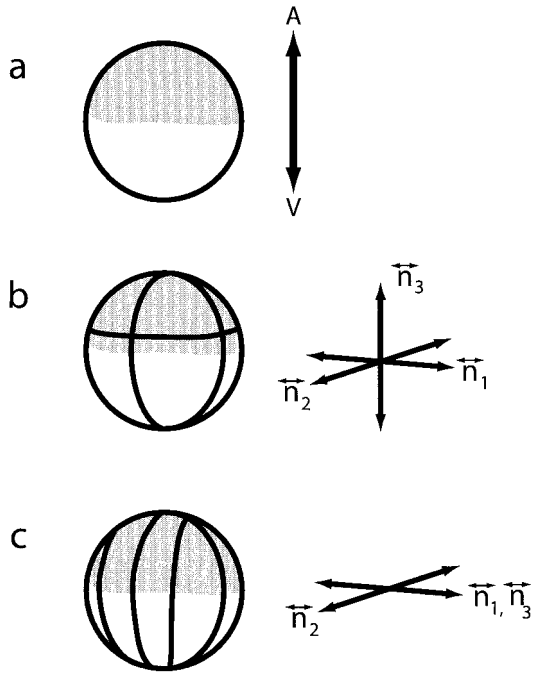

FIGURE 1 Sketch of *Xenopus* eggs and orientations of the first three cleavages. (*a*) Egg just after fertilization has an axis of approximate cylindrical symmetry, the AV axis. (*b*) Egg exhibiting the normal cleavage geometry at the eight-cell stage. The axes, $\vec{n}_1$, $\vec{n}_2$, and $\vec{n}_3$ are perpendicular to the first, second, and third cleavage planes, respectively. (*c*) Egg exhibiting vertical third cleavages similar to those exhibited by eggs exposed to an intense magnetic field during the first and second cell cycles. Note that $\vec{n}_3$ is perpendicular to $\vec{n}_2$ and parallel to $\vec{n}_1$.

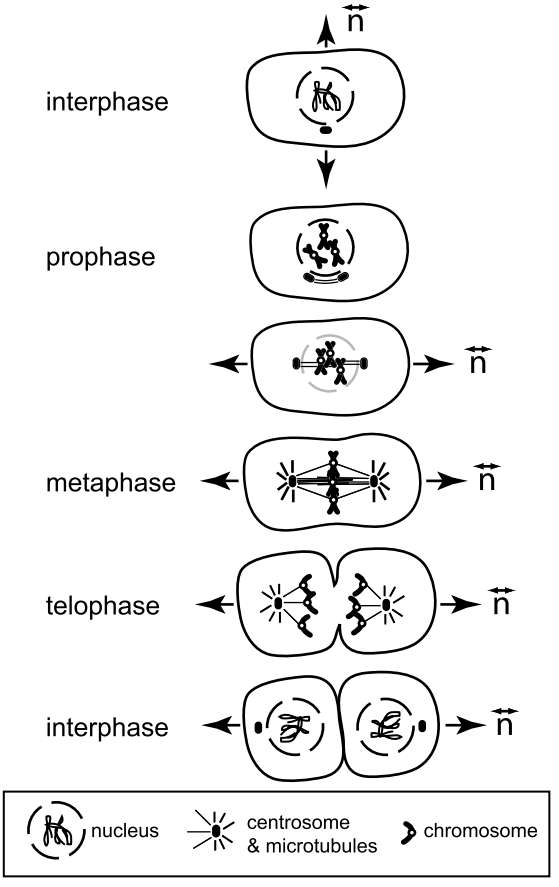

FIGURE 2 Mitosis schematic and definition of axes. In a simple picture of mitosis, a cell begins in interphase with a single nucleus containing a single set of chromosomes and a centrosome attached to the nuclear membrane. In the first step, both the centrosomes and chromosomes replicate and the centrosomes separate and spread to opposite poles of the nucleus (prophase). After the nuclear membrane disappears, the centrosomes serve as organizing centers for microtubules that form asters and a spindle that runs between the centrosomes. The structure consisting of the asters, spindle, and chromosomes is referred to as the mitotic apparatus (MA). The spindle microtubules and associated motor proteins organize the chromosomes into a plate-like structure at metaphase. During anaphase, the members of each pair move apart along spindle microtubules, toward one of the centrosomes. In telophase, the spindle ceases to exist, and the cell-division furrow forms perpendicular to the anaphase spindle axis orientation. Finally, the nuclear membrane reappears and two cells similar to the original obtain. The axis, $\vec{n}$, corresponds to the axis of approximate cylindrical symmetry of the mitotic structures.

cleavage planes to be vertical, rather than horizontal (see Fig. 1 *c*). Additional experiments showed that this effect could be attributed to **B** inducing the third cleavage MA, which always forms with its axis perpendicular to the cell-division plane, to form nearly perpendicular to its normally vertical orientation. Also, **B** induces these alterations by affecting processes that occur before second cleavage.

In this paper, we propose a model for this magnetic field-induced cleavage reorientation that provides at least a qualitative account of all of the observations. Central to the model is the presumption that the geometry of the centrosome replication and spreading cycle (CRSC) (Costello, 1961; Hyman and White, 1987), rather than shape effects, is the primary determinant of the normal cell-division pattern. In addition, we presume that the mitotic structure (MS), which consists of chromosomes, microtubules, and centrosomes, is approximately cylindrically symmetric and its magnetic properties are anisotropic with the same symmetry. The model's success provides strong support for the idea that the early cleavage geometry in *Xenopus* is governed by the geometry of the CRSC. It also suggests magnetic fields as a useful tool for perturbing and investigating cellular processes that involve large biomolecular assemblies.

THE MODEL

The conclusion from the magnetic field-induced cleavage-alteration experiments, that events prior to first and second cleavage influence the third cleavage-plane orientation, suggests the process responsible for the normal cleavage geometry in *Xenopus*. Other than cell shape effects, the only process in mitosis known to control the relative orientations of successive cell divisions is the CRSC (Rappaport, 1996; Costello, 1961; Hyman and White, 1987). Figures 2 and 3 illustrate the geometry of this cycle. Referring to Fig. 2,

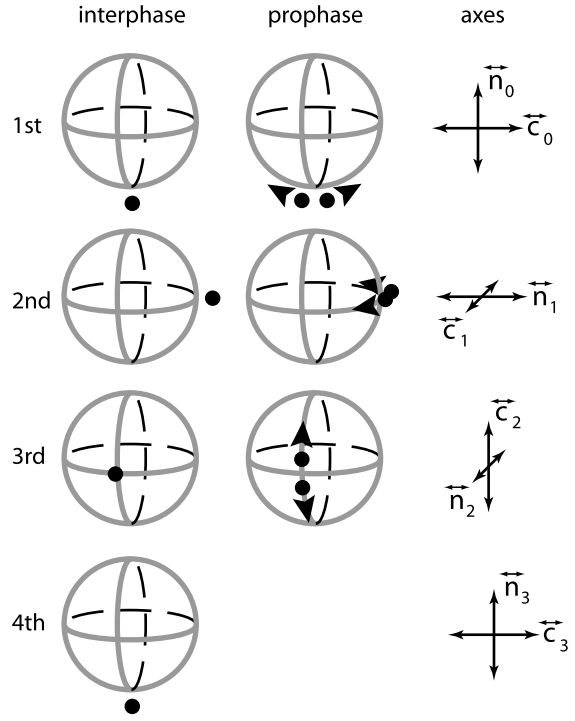

FIGURE 3 Sketch of the centrosome replication and spreading cycle. The first column depicts the orientation of the nucleus and centrosome complex at interphase of the first four cell cycles. The second column depicts the geometry of the spreading of the pair of centrosomes during prophase in each of those cell cycles. The third column defines the orientation of the axes, $\vec{c}_i$ and $\vec{n}_i$ relative to the centrosome–nucleus complexes.

during prophase, the axis along which the centrosomes separate to opposite nuclear poles lies perpendicular to the eventual cell-division plane. If no other factors come into play, then this separation axis specifies the division-plane orientation. Given the initial position of the centrosomes in the daughter cells, the separation axis of their centrosome pairs is perpendicular to their predecessor's. Thus, two successive cell-division planes tend to be orthogonal. Hyman and White (1987) made the further observation that the axes describing three successive separations of centrosome pairs in the AB line of cells in *Caenorhabditis elegans* are mutually orthogonal as shown in Fig. 3. That is, not only do the centrosomes spread to opposite poles, but also they spread to specific opposite poles dictated by the previous two divisions. Guided by the experiments, we presume that this CRSC accounts for the mutually orthogonal geometry of the egg's early cleavages (Rappaport, 1996; Costello, 1961; Hyman and White, 1987).

To mathematically describe the CRSC, we define two axes. The first, $\vec{n} = \hat{n}\hat{n}$, captures the approximate cylindrical symmetry of the mitotic structure through the phases of mitosis (see Fig. 2). It passes through one or both centrosomes and the center of mass of the chromosomes, lying along the spindle during metaphase and anaphase. It is perpendicular to the cleavage plane that forms during telophase. The second axis, $\vec{c} = \hat{c}\hat{c}$, specifies the directions of the impending separation of the centrosomes. It is shown in Fig. 3. Successive orthogonal orientations of these axes can be generated using the following relations:

$$\vec{n}_{i+1} = \vec{c}_i$$

$$\vec{c}_{i+1} = (\hat{n}_i \times \hat{c}_i)(\hat{n}_i \times \hat{c}_i) \qquad (1)$$

where $\vec{c}_i$ and $\vec{n}_i$ are the axes for the ith cell cycle. We represent the above operations as G_{i+1}. An equivalent representation of this operator is

$$G = \begin{pmatrix} 0 & 1 & 0 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \end{pmatrix} \quad \text{so that} \quad \hat{n}_{i+1} = G\hat{n}_i.$$

Each new generation of axes appears at the end of interphase (see Fig. 2), when the two centrosomes start to spread to opposite poles. Setting the AV axis along $\vec{z}$, $\vec{n}_0 = \vec{z}$ and $\vec{c}_0 = \hat{y}\hat{y}$, successive operations yield, $\vec{n}_1 = G_1\vec{z} = \vec{y}$, $\vec{n}_2 = G_2\vec{y} = \vec{x}$, and $\vec{n}_3 = G_3 x = \vec{z}$, three mutually orthogonal cleavages (see Fig. 1 *b*).

The second presumption of the model is that the MS is diamagnetically anisotropic, and thus, experiences a torque in a sufficiently intense magnetic field (Maret and Dransfield, 1985; Maret, 1990). Individual components of the MS, such as microtubules (Chabre, 1986; Bras, 1995; Bras et al., 1998) and chromosomes (Maret, 1990), align with magnetic fields comparable to those applied based on estimations of their diamagnetic anisotropies (Maret, 1990). For the case of microtubules, the size of their diamagnetic anisotropy is such that 5 μm long microtubules become completely aligned in a field of ~10 T (Bras, 1995; Bras et al., 1998). Because this field is comparable to those used in the cleavage plane-alteration experiments and the MS consists of many microtubules and chromosomes, it seems reasonable to presume that fields in excess of 10 T can align the MS.

How the MS aligns depends on how the diamagnetic anisotropies of its individual components align within it. In general, the axes of symmetry of a structure coincide with the principal axes of its magnetic susceptibility tensor (Maret and Dransfield, 1985). Because of its nearly cylindrical symmetry, only the susceptibilities along $\vec{n}$, χ_{nn} and along an axis perpendicular to $\vec{n}$, χ_{cc}, are required. The magnetic anisotropy of the MS is given by the susceptibility difference between these axes, $\Delta\chi = \chi_{nn} - \chi_{cc}$, and the torque it experiences in a magnetic field is given by

$$\mathbf{N}_B = \frac{\Delta\chi B^2}{2} \sin 2\theta(\hat{n} \times \hat{B});$$

$\theta < \pi/2$ is the angle between $\vec{n}$ and **B**.

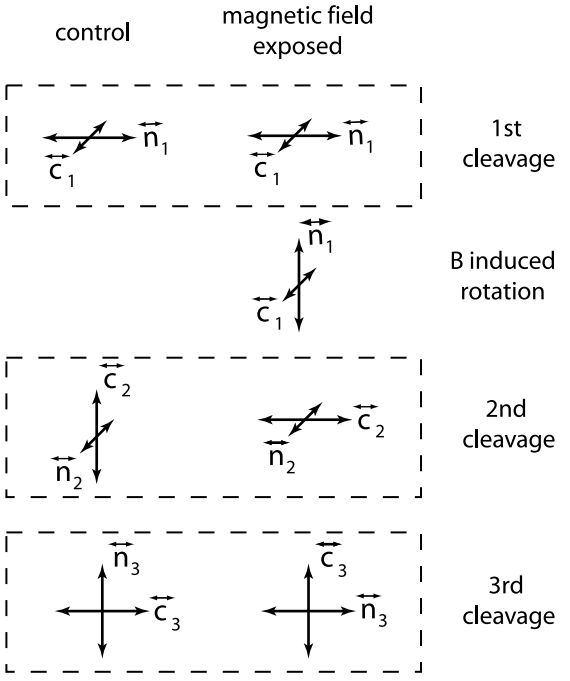

FIGURE 4 Magnetic field-induced realignment of the mitotic structures. The axes in the control column exhibit the normal progression of the first three cell cycles. A rotation of the axes by magnetic field after first cleavage, as shown in the magnetic field exposed column, leads to a reorientation of the axes at second and third cleavage. Note, however, that the orientation of the second cleavage plane does not differ from the control, whereas the orientation of the third cleavage plane is perpendicular to the control.

RESULTS AND DISCUSSION

With the above two elements, it is possible to describe how a magnetic field can reorient third cleavage to vertical. To illustrate how, we first begin with a simple scenario. Consider a magnetic field momentarily applied to an egg from the beginning of telophase of the first cell cycle until the end of interphase in the second cell cycle and assume $\Delta\chi > 0$. As shown in Fig. 4, $\vec{n}_1$, which is initially parallel to $\vec{y}$, tends to rotate around $\hat{n}_1 \times B\hat{z}$ to align with **B**. If the telophase half spindle or the interphase centrosome–nucleus complex are free to rotate and **B** is sufficiently intense then $\vec{n}_1$ reaches perfect alignment, becoming parallel with $\vec{z}$. This rotation does not alter $\vec{c}_1$ because it is parallel with the rotation axis. Applying G_2 to $\vec{n}_1$ produces an unaltered second cleavage plane, $\vec{n}_2 = \vec{y}$. Applying G_3 to $\vec{n}_2$, however, produces an altered third cleavage plane that is vertical and perpendicular to the second cleavage plane, $\vec{n}_3 = \vec{x}$.

For the above example to work, two choices were made. First, **B** was only applied from first cell-cycle telophase until the end of second cell-cycle interphase. This procedure is equivalent to applying **B** from first cell-cycle telophase until the end of second cell-cycle anaphase and presuming that **B** can only induce rotation of the MS when the bipolar spindle is not formed. Suppose this presumption were not true, and, for example, **B** reoriented the second cell-cycle metaphase MA. That reorientation would cause the second cleavage plane to reorient toward horizontal: an outcome never observed in experiments. Second, it was necessary to choose $\Delta\chi > 0$ so that $\vec{n}$ aligned with **B**. If the opposite held, then application of **B** during this period would exert no effect on cleavage geometry, contrary to observations. Thus, the experimental observations indicate that the MS can only be induced to rotate during telophase and interphase and it rotates to bring $\vec{n}$ into alignment with **B**.

To compare directly to the measurements of MA orientation at third cleavage, we need to extend the model. First, in the experiments, **B** was applied from the beginning of first mitosis until third cell-cycle metaphase and consequently, rotations of the MS during more than one cell cycle must be included. Second, the rotations of these structures do not necessarily bring them into full alignment. Cytoplasmic drag and cytoskeletal anchoring oppose the rotation of any cellular structure. Consequently, the effects of rotations that are a fraction of 90° must be considered.

Schematically, we take these two factors into account and predict the final orientation of the third cell cycle mitotic apparatus using the series of operations represented by the expression

$$\hat{n}_3 = G_3R_3G_2R_2G_1R_1\hat{n}_0.$$

R_{i+1} represents the field-induced rotation of $\vec{n}_i$ about the axis $(\hat{n}_i \times \vec{B})(\hat{n}_i \times \vec{B})$ through an angle α_i. G_i is the generator operation given by Eq. 1. In this notation, the first cleavage furrow forms perpendicular to $\hat{n}_1 = G_1R_1\hat{n}_0$, the second to $\hat{n}_2 = G_2R_2\hat{n}_1$, and the third to $\hat{n}_3 = G_3R_3\hat{n}_2$.

Table 1 gives the evolution of the axes $\vec{n}_i$ and $\vec{c}_i$ in an AV parallel magnetic field through the first three cleavages. Starting at first interphase, $\alpha_1 = 0$ because $\vec{n}_1$ is parallel to **B**. α_2 and α_3, in contrast, are each nonzero, which leads to a change in the third cleavage geometry. It is turned from its normal $\vec{z}$ orientation to the axis defined by $\hat{n}_3 = \cos\alpha_2 \sin\alpha_3\hat{x} + \sin\alpha_2\hat{y} + \cos\alpha_2 \cos\alpha_3\hat{z}$. We can estimate α_2 and α_3 from the experimental data (Valles et al., 2002). In those experiments, the angle between the third cleavage metaphase MA, which coincides with $\vec{n}_3$, and the AV axis was measured. That angle corresponds to

$$\theta = \tan^{-1}\left(\frac{\tan\alpha_2}{\cos\alpha_3}\right) = \tan^{-1}\left(\frac{n_y}{n_z}\right).$$

For eggs exposed to 21.8 T through first mitosis until metaphase in the third cell cycle, θ assumed a distribution of values with an average of 72° and a standard deviation of 15°. If the amount of rotation induced is independent of cell cycle, (i.e., $\alpha_2 = \alpha_3$) then this average θ corresponds to an average magnetic field-induced rotation through 58° in each of the two cell cycles. The corresponding third cleavage plane theoretically would have oriented 62° from the second cleavage plane.

TABLE 1 Evolution of the axes, $\vec{n}$ and $\vec{c}$ in an AV parallel magnetic field

Operation	Mitotic Apparatus Symmetry Axis*	Centrosome Spreading Axis†
Initial	$\hat{z}$	$\hat{y}$
$R_1(\alpha_1 = 0)$	$\hat{z}$	$\hat{y}$
G_1	$\hat{y}$	$\hat{x}$
$R_2(\alpha_2)$	$\hat{y}\cos\alpha_2 + \hat{z}\sin\alpha_2$	$\hat{x}$
G_2	$\hat{x}$	$\hat{y}\sin\alpha_2 + \hat{z}\cos\alpha_2$
$R_3(\alpha_3)$	$\hat{x}\cos\alpha_3 + \hat{z}\sin\alpha_3$	$\hat{x}\cos\alpha_2\sin\alpha_3 + \hat{y}\sin\alpha_2 + \hat{z}\cos\alpha_2\cos\alpha_3$
G_3	$\hat{x}\cos\alpha_2\sin\alpha_3 + \hat{y}\sin\alpha_2 + \hat{z}\cos\alpha_2\cos\alpha_3$	

*The mitotic structure symmetry axis, $\vec{n}$, is given by the outer product of each unit vector in this column with itself. The operator in a given row operates on the vectors in the row above it to produce the vectors in its row. The orientation of the ith cleavage plane is perpendicular to the vector generated by G_i.

†This axis, $\vec{c}$, is given by the outer product of each unit vector in this column with itself. The operator in a given row operates on the vectors in the row above it to produce the vectors in its row.

It was also shown that a magnetic field perpendicular to the AV axis could change the alignment of the first or second cleavage planes. Twenty-four percent of the planes became horizontal or oblique to one another. This model can produce each of these effects. Table 2 shows the prediction for the orientation of the first and second cleavage planes for the specific case in which $\mathbf{B} = B\hat{x}$, $\hat{n}_0 = \hat{z}$, and $\hat{c}_0 = \hat{y}$. The first cleavage plane is vertical, and the second cleavage plane orientation as given by $\hat{n}_2$ can be either horizontal or oblique to the first.

The model described here makes a number of other testable predictions. One of the more interesting and perhaps unique is that the magnetic field-induced vertical third cleavage planes can be perpendicular or parallel to the second cleavage plane depending on when the field is applied. Referring to Table 1, the former follows if $\alpha_3 = 0°$ and the latter if $\alpha_2 = 0°$. These dramatic differences are the direct result of the geometry of the CRSC. Of course, the most direct tests of this model require visualization of the centrosomes and their relation to the nucleus for different magnetic-field conditions.

Finally, we consider the plausible alternative model that a magnetic field induces third cleavage reorientation by breaking the symmetry of the "background" in which the MA resides rather than by acting directly upon the MS. The background elements we have in mind that can influence cleavage orientation are the external shape of the embryo and the microscopic structure of its cytoplasm. With regard to the former, it is well known that mechanically induced changes in cell shape can alter division geometry (Bjerknes, 1986; Inoue and Salmon, 1995; Rappaport, 1996; Sharp et al., 2000; O'Connell and Wang, 2000). Because magnetic fields can couple to cell membranes through their diamagnetic anisotropy (Maret and Dransfield, 1985), it is plausible that those magnetic fields induce cell-shape changes to affect cleavage orientation. In situ imaging of embryos, however, revealed that magnetic field-induced shape distortions were substantially smaller (less than a few percent) than the amount required (~10%) to reorient cleavages (Valles et al., 2002). With regard to the latter, it is also plausible that a magnetic field induces anisotropy in the mechanical properties of the cytoplasm by aligning molecular structures within it to bias the MA to orient perpendicular to the AV axis. However, for this scenario to account for the data, one must presume that the magnetic field induces anisotropy during either or both of the first two cell cycles that persists into the third cell cycle, and does not induce anisotropy during the third cell cycle. Although each of these presumptions seems reasonable on its own, it seems unreasonable to expect the cytoplasm to be more susceptible to a magnetic field during one cell cycle than during another (Valles et al., 2002). Thus, the experimental results do not support this alternative model.

Motivated by recent experiments, we have presented a model of magnetic field-induced cleavage and MA reorientation in *Xenopus* eggs. The model is built on the presumptions that the centrosome replication and spreading cycle determines the nominal cleavage geometry, and the magnetic field realigns this cycle by coupling to and turning the diamagnetically anisotropic mitotic structures. The model's predictions agree with the data if the mitotic structures are free to turn only during telophase or interphase and if the

TABLE 2 Evolution of the axes, $\vec{n}$ and $\vec{c}$ in an AV perpendicular magnetic field

Operation	Mitotic Apparatus Symmetry Axis*	Centrosome Spreading Axis†
Initial	$\hat{z}$	$\hat{y}$
$R_1(\alpha_1)$	$\hat{x}\sin\alpha_1 + \hat{z}\cos\alpha_1$	$\hat{y}$
G_1	$\hat{y}$	$\hat{x}\cos\alpha_1 + \hat{z}\sin\alpha_1$
$R_2(\alpha_2)$	$\hat{x}\sin\alpha_2 + \hat{y}\cos\alpha_2$	$\hat{x}\cos\alpha_1\cos\alpha_2 + \hat{y}\cos\alpha_1\sin\alpha_2 + \hat{z}\sin\alpha_1$
G_2	$\hat{x}\cos\alpha_1\cos\alpha_2 + \hat{y}\cos\alpha_1\sin\alpha_2 + \hat{z}\sin\alpha_1$	

*The mitotic apparatus symmetry axis, $\vec{n}$, is given by the outer product of each unit vector in this column with itself. The operator in a given row operates on the vectors in the row above it to produce the vectors in its row. The orientation of the ith cleavage plane is perpendicular to the vector generated by G_i.

†This axis, $\vec{c}$, is given by the outer product of each unit vector in this column with itself. The operator in a given row operates on the vectors in the row above it to produce the vectors in its row.

sign of the diamagnetic anisotropy is such that the axis of symmetry of the mitotic structures tends to align with the magnetic field. One of the notable properties of this model is that it does not depend on or specify the part of the MS to which the magnetic field couples to achieve reorientation. It only requires that a magnetic field can realign the axis of symmetry of those structures.

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