

# Chapter 11

## Asymmetries in Cell Division, Cell Size, and Furrowing in the *Xenopus laevis* Embryo

Jean-Pierre Tassan, Martin Wühr, Guillaume Hatte, and Jacek Kubiak

**Abstract** Asymmetric cell divisions produce two daughter cells with distinct fate. During embryogenesis, this mechanism is fundamental to build tissues and organs because it generates cell diversity. In adults, it remains crucial to maintain stem cells. The enthusiasm for asymmetric cell division is not only motivated by the beauty of the mechanism and the fundamental questions it raises, but has also very pragmatic reasons. Indeed, misregulation of asymmetric cell divisions is believed to have dramatic consequences potentially leading to pathogenesis such as cancers. In diverse model organisms, asymmetric cell divisions result in two daughter cells, which differ not only by their fate but also in size. This is the case for the early *Xenopus laevis* embryo, in which the two first embryonic divisions are perpendicular to each other and generate two pairs of blastomeres, which usually differ in size: one pair of blastomeres is smaller than the other. Small blastomeres will produce embryonic dorsal structures, whereas the larger pair will evolve into ventral structures. Here, we present a speculative model on the origin of the asymmetry of this cell division in the *Xenopus* embryo. We also discuss the apparently coincident asymmetric distribution of cell fate determinants and cell-size asymmetry of the 4-cell stage embryo. Finally, we discuss the asymmetric furrowing during epithelial cell cytokinesis occurring later during *Xenopus laevis* embryo development.

### 11.1 Introduction

The cell-size asymmetry and the asymmetric distribution of cell fate determinants have been shown to be coupled in diverse model organisms. However, it is not clear to what extent the two asymmetries are really linked and/or co-dependent.

---

J.-P. Tassan (✉) • G. Hatte • J. Kubiak  
CNRS UMR 6290, Rennes, France  
Université de Rennes 1, Institut de Génétique et Développement de Rennes, Rennes, France  
e-mail: [jean-pierre.tassan@univ-rennes1.fr](mailto:jean-pierre.tassan@univ-rennes1.fr)

M. Wühr  
Department of Molecular Biology and the Lewis-Sigler Institute for Integrative Genomics,  
Princeton University, Princeton, NJ, USA

*Xenopus laevis*—a model used in developmental biology research for more than a century—allowed discovering basic principles of early development in vertebrates including asymmetric distribution of cell fate determinants, which leads to dorsalization of the embryo. Although the asymmetric cell-size division of the early *Xenopus* embryo was described long ago, it is still, in contrast to other systems such as *C. elegans* or *D. melanogaster*, poorly understood. This is mostly due to the large size of the embryo and its blastomeres—a fantastic advantage for micromanipulation studies but an obstacle for cell biology studies, especially those involving microscopy approaches. In the first part of this chapter, we present a speculative model on the origin of the asymmetry of the second embryonic cell division. We also discuss the apparently coincident asymmetric distribution of cell fate determinants and cell-size asymmetry of the 4-cell stage embryo. In the second part of this chapter, we describe the asymmetric furrowing in epithelial cell cytokinesis and its regulation during early embryo development.

## 11.2 Asymmetric Cell Division

### 11.2.1 Unfertilized Egg

Before discussing asymmetric cell division in the early *Xenopus* embryo, we will overview the organization of the fertilized egg because it profoundly influences subsequent zygotic divisions.

The unfertilized *Xenopus* egg is a very large (1.2 mm in diameter) spherical and highly polarized cell. In wild type (non-albino *Xenopus*), the egg polarity is readily visible thanks to the dark pigment, which is asymmetrically concentrated in the animal hemisphere. Thus, the animal hemisphere is black to light brown, whereas the vegetal hemisphere, which contains much less pigment, appears whitish. The egg displays symmetry around the animal–vegetal axis with all pole-to-pole meridians of the egg surface seemingly identical and indistinguishable before fertilization. This symmetry is often referred to as radial symmetry or cylindrical symmetry. The egg organization roughly overlaps with the embryo germ layers' organization: the region of the animal and vegetal hemispheres corresponds to the future ectoderm and endoderm, respectively, while the equator corresponds to the future mesoderm (reviewed in Gerhart and Keller 1986). The animal and vegetal hemispheres also differ in their organelles and molecular content. For example, the yolk platelets of the vegetal hemisphere are larger and have a much higher density (Danilchik and Gerhart 1987). Massive accumulation of yolk in the vegetal hemisphere displaces egg cytoplasm toward the animal hemisphere. This asymmetric distribution of yolk and cytoplasm has clear consequences on the positioning of the third zygotic division plane and potentially also on the two first cell divisions (see below). In fully grown oocytes the large cell nucleus (called germinal vesicle) is located at the vicinity of the animal pole. This position facilitates polar body extrusion during meiosis I and II. *Xenopus* oocyte meiotic divisions are among the most asymmetric cell divisions,

not only in terms of the 200-fold difference in linear size between the egg and polar bodies but also in a functional sense—the oocyte survives beyond meiosis while the polar bodies do not. At the molecular level, the animal and vegetal hemispheres differ greatly. In the *Xenopus* egg, the cortex of the vegetal hemisphere is a repository for developmental information, much of which is in the form of localized RNAs (Kloc and Etkin 1994; King et al. 1999; Mowry and Cote 1999).

### 11.2.2 *From Fertilization to the Third Division*

The asymmetry between animal and vegetal hemisphere of the egg is not restricted to its constituents' distributions. There is also a functional asymmetry; the sperm enters the egg only at the animal hemisphere (Elinson 1980 cited in Gerhart et al. 1989). The position at which the sperm enters the egg is called “sperm entry point” (SEP). Shortly after fertilization, the egg vitelline envelope hardens to become the fertilization envelope, which protects egg against polyspermy. Because of the asymmetric distribution of yolk, the more dense vegetal hemisphere rotates downward until the animal–vegetal axis aligns with the gravity vector and becomes vertical.

During oogenesis, *Xenopus* oocytes lose their centrioles but maintain centrosomal proteins allowing acentriolar meiotic divisions. Once the sperm has entered the egg, its centrosome organizes a huge microtubular aster (sperm aster), which functions to draw the male pronucleus toward the center of the animal hemisphere (Stewart-Savage and Grey 1982). After completion of meiosis and extrusion of the second polar body, the female pronucleus is captured by the sperm aster and migrates toward the male pronucleus. To reach the common position, the two pronuclei generally move in the three-dimensional space of the egg: in the X–Y plane (plane parallel to the equator) and also along the animal–vegetal axis. They always end up within the animal hemisphere probably because of the much lower density of yolk platelets in this hemisphere. They thus become situated between the egg's geometric center and the animal pole. The mechanisms regulating the geometric centering in such a huge cell as one-cell embryo are not fully understood. In small-sized cells, e.g., in vitro cultured somatic cells, microtubule asters are believed to center via pulling forces from the cellular cortex (Glotzer 1997; Grill and Hyman 2005). However, these mechanisms can't explain centering of pronuclei in the one-cell *Xenopus* embryo. It was proposed that microtubule asters center via a dynein-dependent mechanism. Dynein, anchored to cytoplasm, exerts length-proportional pulling forces onto microtubules and thus tends to pull the microtubule aster in the direction of the longest microtubules (Wühr et al. 2009; Mitchison et al. 2015).

The sperm aster microtubules are also believed to bias the orientation of the subset of cortical microtubules on the opposite site of the sperm entry point (Gerhart et al. 1984). During the first cell cycle following fertilization, three concentric layers can be distinguished in the zygote. From the outside toward the inside, these layers are the cortex, the shear zone, and the deep core cytoplasm. Microtubules are involved in the cortical rotation, an event which corresponds to the loosening of the embryo cortex from the deep cytoplasm. The cortex rotates,

about 30° relative to the core of cytoplasm and away from the SEP (Vincent et al. 1986). The resulting shear modifies the pigment distribution at the equator, producing a gray crescent, which corresponds to the embryo presumptive dorsal side. The gray crescent is also observed in some other amphibian species such as *Rana pipiens*. Although the position of the SEP, which can be simply visualized due to the pigment concentration, is a relatively good indicator of the prospective dorsal–ventral axis, it is not absolutely reliable (Black and Gerhart 1985). In fact, the direction of the cortical rotation more accurately predicts the dorsal side of the embryo (Vincent et al. 1986). In the absence of sperm, the egg can be activated parthenogenetically, for example, by pricking, and the cortical rotation still occurs, but in this case it happens randomly. This indicates that the sperm aster is dispensable for cortical rotation, but it is necessary to polarize and direct the rotation.

Cortical rotation is intimately linked to the establishment of the embryo dorsal–ventral asymmetry. Indeed, it allows asymmetric redistribution of developmental factors associated with the cortex from the vegetal pole toward the equator opposite to SEP and will determine the new dorsal–ventral axis of the embryo, which is generated orthogonally to the preexisting animal–vegetal axis of the oocyte (Vincent and Gerhart 1987). It is along these primary axes that the three germ layers form. Therefore, fertilization leads to a symmetry breakage, which transforms the cylindrical asymmetry of the unfertilized egg into a bilateral symmetry of the embryo (Gerhart et al. 1989). Similarly to the cortical rotation, the translocation of the dorsal determinants depends on a parallel array of microtubule bundles situated in the vegetal hemisphere. These microtubules appear in the shear zone of the egg about midway through the first cell cycle (Elinson and Rowning 1988). They are of multiple origins: some originate from the sperm centriole, others from unknown sources deep in the cytoplasm, and some appear to be assembled in the vegetal shear zone (Houliston and Elinson 1991; Schroeder and Gard 1992). The rotation involves dynein and kinesin microtubule motors (Marrari et al. 2004). Cortical rotation is essential for embryo dorsalization. Indeed, when it is experimentally blocked, for example, with microtubule inhibitors, then dorsal development is prevented (Vincent and Gerhart 1987). However, such embryos in which cortical rotation has been prevented can be rescued by tipping, which mimics the natural cortical rotation (Scharf and Gerhart 1980).

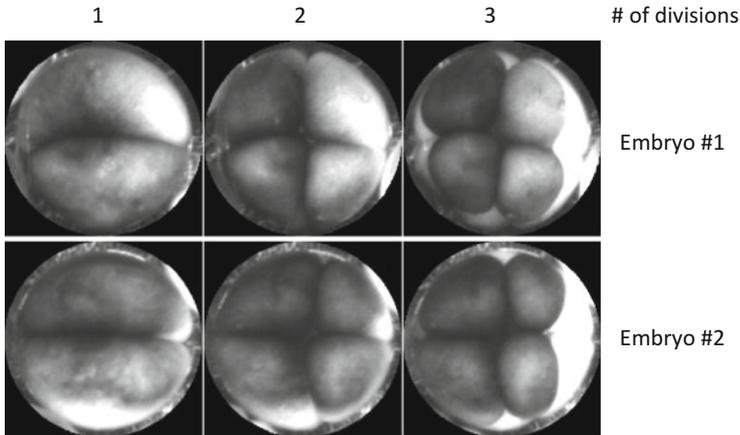
Cortical rotation coincides with the translocation of the maternal dorsalizing activity from the vegetal pole toward the prospective dorsal side of the embryo, in the same direction as cortical rotation (Kageura 1997; Kikkawa et al. 1996; Sakai 1996). It results in the asymmetrical activation of the Wnt/ $\beta$ -catenin signaling pathway and the enrichment of  $\beta$ -catenin in dorsal nuclei (Larabell et al. 1997; Schneider et al. 1996). In this chapter, we will not discuss the mechanisms of embryo dorsalization because pathways involved in the embryo dorsalization have been recently reviewed (Houston 2012; Carron and Shi 2016). Here, we will focus on a novel topic: the apparent relationship between the asymmetric distribution of cell fate determinants and the cell-size asymmetry in the 4-cell *Xenopus* embryo.

The asymmetric positioning of the mitotic apparatus is manifested externally by “Surface Contraction Waves” (SCWs) during embryo cell divisions. SCWs are

waves of pigmentation changes at the embryo surface. They propagate from the animal toward the vegetal pole just prior to cytokinesis (Rankin and Kirschner 1997). These SWCs are associated with MPF (M-phase Promoting Factor, the complex of CDK1 with cyclin B) activation–deactivation waves within the cytoplasm (Pérez-Mongiovi et al. 1998; Beckhelling et al. 2000). The activation of MPF is initiated at the centrosomes (originating from the sperm centrosomes and duplicated during the first cell cycle within the zygote) and propagates centripetally through the cytoplasm toward the cell periphery (Chang and Ferrell 2013). As the centrosomes (together with the mitotic spindle) are not situated in the geometric center of the one-cell embryo, but are clearly displaced toward the animal pole, the radially propagating MPF activation wave reaches the cell cortex with a slight delay along the cell surface. This creates local reactions of the cytoskeleton and in consequence the appearance of wave of pigment responding to the MPF activation. Therefore, in the one-cell embryo, the SWCs are the manifestations of the cell asymmetry and especially of the displaced centering of the mitotic spindle along the A–V axis. Thus, SWCs represent an easily observable readout of displaced cell center at this stage of embryo development. However, the SWCs are not restricted to the first cell cycle (despite that they are very well visible during the first cell division of the embryo), which indicates that nuclei of blastomeres are not in their geometric centers also in the following cell cycles. This point is of special importance for our further analysis and hypotheses presented below.

The first three cell divisions of the *Xenopus* embryo are stereotyped as the majority of embryos display the same division pattern. The first division plane will tend to pass close to the sperm entry point. Whether the first division plane defines the embryonic bilateral symmetry of the embryo has been a matter of debate (Klein 1987; Danilchik and Black 1988). However, for most embryos, the first cleavage furrow identifies a meridian, which approximately coincides with the embryo midline (Masho 1990). It has been shown that the sperm aster has an elongated shape with its long axis parallel to a tangent (a straight line or plane that touches a curve or curved surface at a point) at the embryo surface at the SEP (Wühr et al. 2010). This particular shape results from the inability of microtubules to grow at the SEP because they are blocked by the cell cortex, whereas they are free to grow in other directions into the oblate volume of the animal hemisphere containing the majority of egg cytoplasm (Fig. 11.2). Thus, because of this peculiar geometry, the sperm aster senses the cell shape and defines the position and orientation of the first division plane by following the so-called long-axis rule (Hertwig 1893). This rule predicts that the division plane bisects the cell through its longer axis. Interestingly, the orientation of centrosomes during the preceding cell cycle defines the orientation of the division plane of the subsequent division. Together with the orientation of the sperm aster along the long axis, the two of them nicely explain the observation that the first cleavage furrow tends to bisect the one-cell embryo close to SEP.

The two first embryonic divisions, which are perpendicular to each other and parallel to the animal–vegetal axis, generate two pairs of blastomeres (4 blastomeres total), which usually differ in size and also frequently differ in pigmentation (Nieuwkoop and Faber 1967, Fig. 11.1). The pair of dorsal blastomeres is smaller



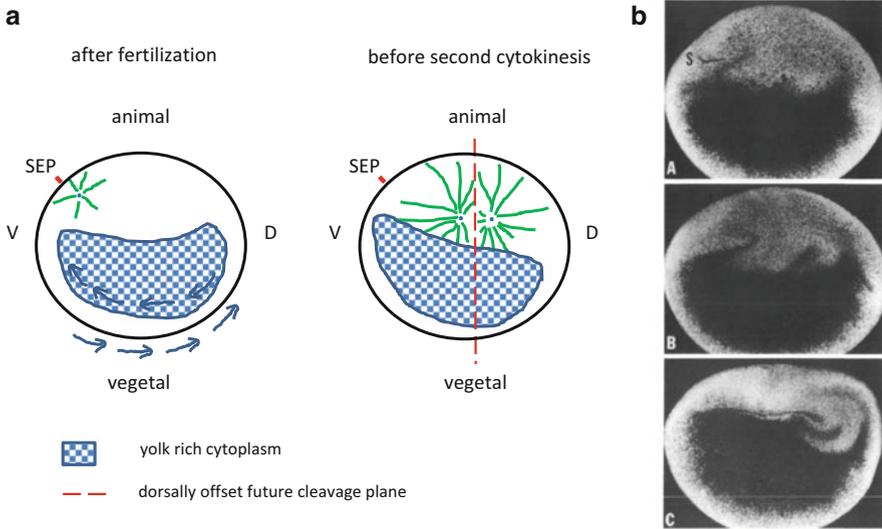
**Fig. 11.1** The variability of cell divisions in the early *Xenopus* embryo in regard to the pigment distribution. Images are taken from a movie showing two embryos at the 2-, 4-, and 8-cell stage with the pigmented animal hemisphere in front of the viewer. The embryo shown in the *upper row* represents an asymmetric distribution of pigments at the 2-cell stage. The first cleavage furrow equally bisects the *lighter* and *darker* zones. The second division produces two darkly pigmented ventral blastomeres (left) and two lightly pigmented dorsal blastomeres (right). This embryo exemplifies a limited cell-size asymmetry of the first four blastomeres. This kind of embryos is usually selected for the cell fate studies (Grant et al. 2013). In the same clutch, some embryos, like the one shown in the *bottom row*, do not show a marked pigmentation asymmetry. Although the first division appears symmetric, or only slightly asymmetric, the second one results in clearly asymmetric embryo with two larger and two smaller blastomeres. This difference in cell size is maintained after the third division

and less pigmented and the ventral pair is larger and darker. At the 8-cell stage, the cell-size asymmetry can be visually even more pronounced. Following fertilization, the pigment concentrates toward the SEP indicating the future ventral side of the embryo. However, it is important to note that there can be considerable variations between clutches of embryos (Grant et al. 2013). The pigmentation asymmetry at the 2-, 4-, and 8-cell stage is easily perceptible by simple observation. The orientation of the first cleavage furrow relative to pigment distribution has been used as a selection criterion to produce fate maps (Moody and Kline 1990; Grant et al. 2013) or to study molecular mechanisms involved in the embryo asymmetry (Domenico et al. 2015). The cell-size asymmetry is also variable from clutch to clutch but can be frequently observed at the 4-cell stage (Fig. 11.1). However, the mechanisms leading to cell-size asymmetry of the two first cell divisions have been poorly studied. This is mainly due to the large size and opacity of the embryo at this stage, which allows analysis of fixed samples, but does not allow live imaging necessary to analyze and understand the highly dynamic process of cell division. Other biological models allowing live imaging, such as zebrafish, have been preferred (Wühr et al. 2010). One could assume that the cell-size asymmetry at the 4-cell stage could simply be randomly biased by a relatively neutral factor such as yolk distribution. However, this would be difficult to reconcile with the fact that this is

observed with a very high frequency. Only a small proportion of embryos from the same clutch present a pronounced cell-size asymmetry at the 2-cell stage, which could be simply explained by an imperfect alignment of the sperm aster in relation to the SEP-center radius. The centering of the sperm aster in the X–Y axis is not perfect; it has been proposed that the sperm aster is placed closer to the site of sperm entry than to the opposite side (Wühr et al. 2009, 2010). Conversely, at the 4-cell stage, embryos often have blastomeres asymmetric in size (Fig. 11.1, Nieuwkoop and Faber 1967). The imperfect positioning of sperm aster and the resulting slightly eccentric position of the zygote nuclei after the first division could explain why the second cell division produces two smaller blastomeres closer to the SEP and two larger blastomeres on the opposite side. The two smaller blastomeres will transform into the dorsal and the larger into the ventral side of the embryo. Because the dorsal side is opposite to the SEP, the initial asymmetric positioning of the sperm aster farther from the SEP cannot explain the cell-size asymmetry of dorsal and ventral cells. Therefore, below, we put forward a speculative model that could reconcile these apparently conflicting observations. As discussed above, upon the cortical rotation the cortical cytoplasm rotates upward and away from the SEP, while the subcortical cytoplasm rotates in the opposite direction, upward and toward the SEP (Vincent et al. 1986; Hausen and Riebesell 1991) This cytoplasmic movement brings yolk-rich vegetal cytoplasm in the animal hemisphere close to the SEP. In contrast, on the future dorsal side the yolk moves away (Fig. 11.2a). This movement generates a ventral–dorsal yolk-density asymmetry. Staining experiments of yolk and cytoplasm at this stage (Fig. 11.2b, Danilchik and Denegre 1991) are roughly consistent with the proposed model shown in Fig. 11.2a. The microtubule aster’s centering is biased against yolk granules, the size of which is apparently greater in the vegetal than it is in the animal hemisphere (see below). It seems plausible that the sperm aster and/or anaphase asters of mitotic spindles from the first and second cell division sense this asymmetry and center with a dorsal bias. In addition, the yolk asymmetry effectively reduces the amount of cytoplasm in the vegetal hemisphere. In conjunction with the cortex-induced asymmetry of the sperm aster discussed above, this cytoplasmic asymmetry could explain how the sperm aster directs the first cleavage plane, typically to cut through the sperm entry point. It finds the long axis of the animal hemisphere taking into consideration the yolk asymmetry.

How does the outlined hypothesis reconcile with the previous proposal that the sperm aster and anaphase asters’ centering are biased toward the sperm entry point (Wühr et al. 2010)? These propositions were based on fixed images, and the location of the SEP relative to the asters was only inferred but not directly observed. Taking into consideration the arguments put forward here, it seems likely that this inference was mistaken.

The third division plane is orthogonal in relation to the two first division planes. In addition, because the nuclei resulting from the two first cell divisions are positioned eccentrically relatively to the geometric cell centers of blastomeres and displaced along the animal–vegetal axis (presumably due to differential yolk density), the third division plane becomes asymmetrically positioned and shifted toward the animal pole giving rise to four small animal blastomeres and four large vegetal blastomeres.



**Fig. 11.2** (a) A model attempting to explain the typically observed dorsal shift of the second cleavage plane during *Xenopus laevis* first cell division. *Left*: While the egg is rotationally symmetric to the animal–vegetal axis, this symmetry is broken by the sperm entry and formation of the sperm aster. *Right*: The position of the sperm aster determines the orientation of cortical microtubules (not shown here for simplicity), which results in the rotation of cytoplasm relative to the cortex. We hypothesize that this rotation brings yolk-rich vegetal cytoplasm up and near the sperm entry point (*SEP*) and might also remove a significant part of yolk from the dorsal side. Such an asymmetry of a dorsal–vegetal yolk density might be sensed by the microtubule asters, and as a consequence, the second cleavage plane might shift dorsally. *V*: ventral, *D*: dorsal. (b) Observed yolk platelet movement in embryos seems consistent with this model. Shown are micrographs of yolk (dark) and cytoplasmic (light) rearrangements at 30 min (top), 45 min (middle), and 90 min (bottom) after fertilization. A major cytoplasmic swirl develops on the side opposite to the *SEP*. Orientation of the embryo as in (a). *S*: sperm trail which is a trace of pigment formed during the male pronucleus migration in the cytoplasm. Figure reprinted from Danilchik and Denegre (1991)

### 11.2.3 Asymmetric Divisions in Blastulae and Gastrulae

One of the earliest cell diversifications during *Xenopus* development is the production of polarized epithelial cells at the embryonic surface and nonpolar cells internally in the embryo. The outer, apical, membrane domains of these early polarized cells derive from the original egg membrane, while their basolateral membrane domains are newly formed (Fesenko et al. 2000). Asymmetric division along the apical–basal axis (divisions perpendicular to the embryo surface) occurs by rotation of the division plane (Chalmers et al. 2003). Again, this transition is stereotyped since it invariably occurs at the 32- to 64-cell stage (sixth zygotic division). The perpendicular divisions are detected until late blastula (stage 10, Nieuwkoop and Faber 1967) and are absent in gastrula (stage 11). Cell divisions

plane can be perpendicular, tangential, or oblique to the embryo surface. Whereas divisions occurring perpendicular to the apical–basal axis symmetrically divide cells, those occurring tangentially give rise to an epithelial cell and an internal cell. In this case, only the daughter cell situated in the external cell layer (superficial cell) inherits the apical membrane from the mother cell. Because atypical protein kinase C (aPKC), an important phylogenetically conserved polarity protein (Martin-Belmonte and Perez-Moreno 2011), is localized at the egg membrane, it is preferentially inherited by the superficial daughter cells when the mother cells divide perpendicularly. Moreover, superficial cells express the bHLH gene *ESR6e*, whereas inner cells do not. Thus, perpendicular divisions, by allowing asymmetrical segregation of the polarity protein aPKC, and other factors such as *ESR6e*, generate diversification of the superficial and inner cell fates (Chalmers et al. 2003). The correlation between the orientation of cell divisions and the cell surface area (area of the apical membrane) suggests that the orientation of blastomere divisions (parallel or oblique versus orthogonal) is simply dictated by geometric rules. This indicates that the “long-axis rule” (Hertwig 1893), which controls division plane orientation in the early embryo, is also operant in blastulae. This also suggests that the “long-axis rule” could bring plasticity to the embryo and correct size asymmetry produced by the first two cell divisions, thus equilibrating cell size. This could also explain the robustness of the early developmental pattern in *Xenopus*, which would rely on geometric properties.

### ***11.2.4 Does Cell-Size Asymmetry Produced by the Two First Cell Divisions Have a Role?***

Cell-size asymmetry and the early dorsal–ventral polarization of the embryo appear intermingled: the small blastomeres correspond to the future dorsal structures, and, reversely, the large blastomeres correspond to the embryo ventral side. This raises the question whether the early embryonic cell-size asymmetry has a functional role or developmental implication. If dorsal–ventral polarization and cell-size asymmetry are indeed independent, then could it be possible to uncouple these two events? This question has not been directly raised by developmental biologists. However, studies on the establishment of the dorsal–ventral axis relative to the SEP might provide some clues to answer this question. Indeed, the dorsal–ventral axis appears to be topologically linked to the SEP since in normal conditions the dorsal side of the embryo will roughly correspond to the opposite of the SEP position. Although correlated, are the two asymmetries definitively linked? Based on results published by others, we again propose a completely speculative model, which, however, is consistent with results obtained in the insect model system.

Experiments of reorienting embryos along the gravity vector before the first cleavage may provide some important information. Indeed, as previously mentioned, shortly after fertilization the fertilized egg freely rotates in its fertilization envelope, thus aligning its animal–vegetal axis with gravity. Then, the 30° cortical

rotation leads to redistribution of inner constituents relative to the zygote cortex. As already discussed, unfertilized eggs have a stereotyped distribution of yolk platelets, which is modified in the embryo (Danilchik and Denegre 1991; Ubbels et al. 1983; Gerhart et al. 1981). By reorienting the one-cell embryo, Gerhart and collaborators (Gerhart et al. 1981) prevented the naturally occurring redistribution of yolk and cytoplasm. Interestingly, this manipulation of the one-cell zygote apparently also displaced the dorsal structure of the embryo from the expected position, which is usually opposite to the SEP. When the SEP was oriented upward, the embryo dorsal side now coincided with the SEP side. Thus, by briefly reorienting the fertilized egg relatively to the gravity field it was possible to uncouple the natural topology of SEP and the embryo dorsal side. The effect of reorientation relative to gravity on embryos depended on when it was applied: the one-cell embryo became refractory to reorientation while progressing through the first cell cycle. This led the authors (Gerhart et al. 1981) to propose that during the early period of the first cell cycle, gravity might be able to interfere with the embryo dorsal–ventral polarization, which is naturally generated later during the first cell cycle. The low impact of gravity on *Xenopus* development was subsequently demonstrated by Souza et al. (1995). The embryos obtained in microgravity during a Space Shuttle mission showed minor developmental perturbations such as displacement of the blastocoel toward the vegetal hemisphere and thickening of the blastocoel roof. Both could be explained by the displacement of the third division plane toward the vegetal hemisphere. None of these perturbations led to apparent defect of embryo development. As previously suggested, in normal conditions, the embryo would be protected from adverse effects of gravity which could occur early during the first cell cycle by the free rotation of the embryo in the perivitelline space (Gerhart et al. 1981).

Although these authors did not examine cell-size asymmetry in the reoriented embryos, they described, in addition to yolk redistribution, the positions of aster and pronuclei in the fertilized egg. Interestingly, in the summarizing schemes they presented (Fig. 4 in Gerhart et al. 1981; Fig. 12 in Ubbels et al. 1983), they clearly showed that pronuclei were still displaced away from the SEP side (the side which should have formed the prospective dorsal side if the embryo would not have been reoriented). Although these embryos were fixed for microscopy analysis slightly before the first embryonic cleavage ( $t = 0.7$  of normalized time in Gerhart et al. 1981) when the sperm aster depolymerizes and the first mitotic spindle forms (Wühr et al. 2009), it is reasonable to anticipate that the two pronuclei and centrosome deposited at this position away from SEP will determine the position of the subsequent cleavage plane. At the second cleavage, these embryos would be expected to cleave asymmetrically as normal embryos would do, thus producing two large and two small blastomeres. However, in this case cell-size asymmetry and fate would be uncoupled. Although clearly speculative, this interpretation raises the possibility that in the 4-cell *Xenopus* embryo, cell-size asymmetry would not be involved in the embryonic polarization but would be coincident with the asymmetric distribution of cell fate determinants. It would be possible that this early cell-size asymmetry is subsequently attenuated by divisions following the “long-axis rule” as discussed previously, thus reducing cell-size differences and homogenizing cell

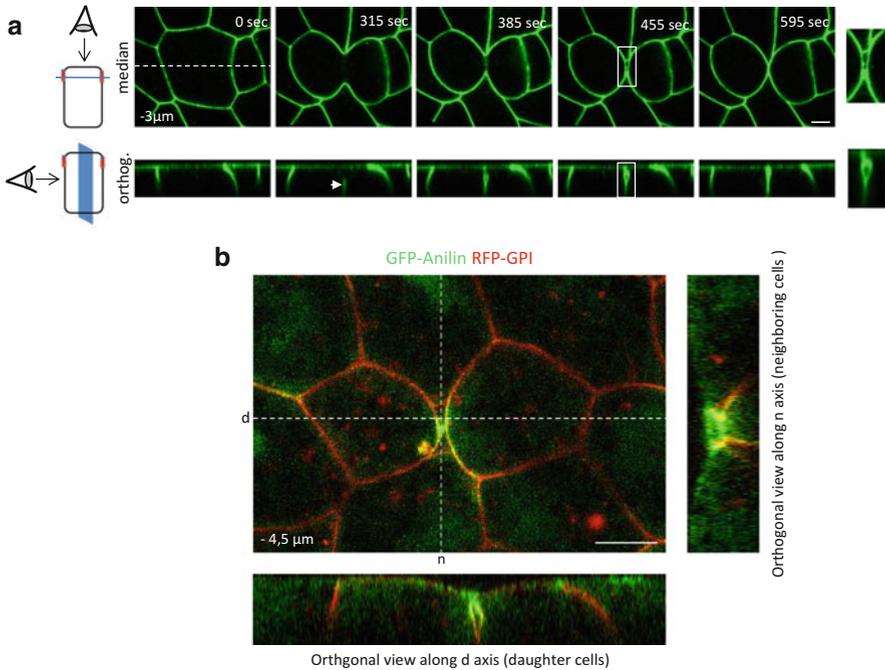
size in each hemisphere. Obviously, to be proven, the veracity of this speculative proposition needs experimental proof. Interestingly, such independence between asymmetric segregation of cell fate determinants and cell-size asymmetry, which we speculate for the *Xenopus* early embryo, was previously reported in *Drosophila* (Albertson and Doe 2003).

## 11.3 Asymmetric Furrowing During Epithelial Cytokinesis

In the second part of this chapter, we will discuss the asymmetric furrowing by which polarized epithelial cells divide in the *Xenopus* embryo.

### 11.3.1 Asymmetric Furrowing During Cytokinesis

In dividing one-cell embryos, cytokinesis starts with the formation of the division furrow at the zygote's animal pole. The oolemma ingresses progressively and the furrow expands circumferentially toward the vegetal pole. When the furrow has completed ingression at the cell surface, more deeply in the zygote the membrane is not yet fully closed because the furrow advances more rapidly through the animal than the vegetal hemisphere. Closure completes during the second cell division (Fesenko et al. 2000). By the end of the first embryonic division, the tight junction between blastomeres is implemented. This allows formation of a cavity located between blastomeres, which ultimately becomes the blastocoele. Consequently, all blastomeres until stage 16–32 cells, when internal cells are produced within the embryo (see below), are polarized. These cells inherit the “old” oolemma, which corresponds to the apical membrane. Concomitantly, their basal–lateral membranes become newly assembled (Bluemink and de Laat 1973; de Laat and Bluemink 1974), via the localized fusion of exocytotic vesicles near the furrow base (Danilchik et al. 1998, 2003). Thus, the external blastomeres form an epithelium at the surface of the embryo. From this time point forward, until the late blastula stage, during epithelial cells divisions, the cytokinetic furrow ingression proceeds from the apical to basal pole of these cells (Le Page et al. 2011). This pattern begins to change during pregastrular stages, and furrows begin to initiate on the basolateral membrane. In 16-cell stage embryos cytoplasmic bridges (midbodies) have been observed by electron microscopy at the proximity to the basal plasma membrane (see Fig. 2d in Danilchik et al. 2013). However, in 128-cell stage embryos, circumferential cytokinetic furrow could be observed in blastomeres of the animal hemisphere (Danilchik, personal communication). Cytoplasmic bridges with an approximately central position in the cell were also observed in 16-cell stage embryos (see Fig. 2d in Danilchik et al. 2013) and positioned more apically in 32-cell stage embryos, suggesting that in some cells the transition in the polarity of furrow ingression might occur early in development. In Le Page et al. (2011), only animal cells of blastula were studied by confocal microscopy. Thus, it is possible that



**Fig. 11.3** The example of an asymmetric furrowing during epithelial cell cytokinesis. **(a)** Cell division of the epithelial cell expressing the fluorescent membrane marker GFP–GPI was followed by confocal microscopy. *Upper row (median)* shows a time-lapse *en face* view of the epithelium. The *dotted line* on the image at the time 0 s indicates the X–Y position of the plane used for the orthogonal projection shown in the *bottom row (orthog.)* **(a)**. It shows that the cytokinetic furrow, indicated by a *white arrow* at the 315 s time point, ingresses asymmetrically from the basal side toward the apical side. White rectangles at the 455 s time point images indicate the regions, which are enlarged and shown on the *right*. This series of images shows that during cell division the gap forms between the two daughter cells. **(b)** GFP–anillin (*green*) localizes at the leading edge of the gap during epithelial cell cytokinesis in *Xenopus laevis* gastrula. The plasma membrane is visualized by the fluorescent membrane marker RFP–GPI (*red*). The orthogonal view along the neighboring cells’ (*n*) axis shows the cytokinetic ring, while the orthogonal view along the daughter cells’ (*d*) axis shows the gap between the two daughter cells. Scale bar: 10  $\mu\text{m}$

the change in the direction of furrowing takes place at different times in different region of blastula. Further analyses including both electron microscopy and live imaging will be necessary to determine the exact timing and location of the cytokinetic furrow polarity transition during the embryo development. In epithelial cells of the gastrula, the ingression of the cytokinesis furrow is still asymmetric. However, at this stage it progresses from the basal side toward the apical membrane (Fig. 11.3a). In blastulae, the tight junctions are not localized close to the apical membrane as typically found in epithelial tissue but localize laterally up to 200  $\mu\text{m}$  away from the apical surface (Merzdorf et al. 1998). In contrast, in gastrulae, tight junctions occupy the typical position near the apical membrane. This correlation between the position of the tight junction along the apical–basal axis and the polarity of the furrowing

allows speculating that the apical junctional complex is indeed involved in regulation of the cytokinetic furrow ingression polarity in epithelial cells.

The asymmetric furrowing occurring in *Xenopus* embryo is also encountered in other biological model animals including *Drosophila* (Founounou et al. 2013; Guillot and Lecuit 2013; Herszterg et al. 2013; Morais-de Sà and Sunkel 2013), ascidian embryo (Prodon et al. 2010), mouse intestine (Jinguji and Ishikawa 1992), and cultured MDCK cells (Reinsch and Karsenti 1994). During epithelial cells division, the asymmetric furrowing correlates with the asymmetric localization of apical junctional complexes. This suggests that the two processes are closely related. Indeed, in *Drosophila melanogaster* the knockdown of  $\alpha$ -catenin, an important player in the adherent junction, interferes with the asymmetric furrow constriction (Guillot and Lecuit 2013). However, asymmetric furrowing also occurs independently of preexisting cell–cell junctions. It happens in one-cell *Xenopus* and *Caenorhabditis elegans* embryos (Audhya et al. 2005; Maddox et al. 2007). This indicates that the initiation of the asymmetric furrowing in the one-cell embryo does not involve cell–cell junctions. In the *Xenopus* embryo, apical junction appears soon after cytokinesis initiation but while the furrow still ingresses (Kalt 1971a, b). In the mouse oocyte, the polar body extrusion occurs through a unique combination of an asymmetric division and asymmetric furrowing (Ibanez et al. 2005; Wang et al. 2011). The question arises of whether this is correlated to the cell polarity and the proximity of the mitotic spindle creating a favorable cytoplasmic environment? The fact that asymmetric furrowing is observed in so many different systems suggests that this mode of cytokinesis may play an important role in embryo development and beyond (e.g., epithelial cells of adults).

### **11.3.2 Detailed Observations of Asymmetric Furrowing in *Xenopus* Embryo**

Recently, we further characterized asymmetric furrowing during epithelial cell division in the ectoderm of *Xenopus laevis* gastrula (Hatte et al. 2014). We showed that the plasma membranes of the two daughter cells during cytokinesis are usually separated by a gap (Fig. 11.3a). In most cases, daughter cells form new contacts a few microns basally in relation to the furrow tip. This allows the formation of a volume tightly associated with the furrow, which we named “inverted teardrop” after the teardrop-shaped furrow canal during cellularization in *Drosophila melanogaster* (Lecuit and Wieschaus 2000). The complex organization of the cleavage furrow during the first *Xenopus* embryonic division and cleavages was described in detail by Kalt (1971a, b). These electron microscopy studies beautifully showed that formation of the presumptive blastocoele is not formed afterward but is intimately linked to the cytokinesis process as early as the first division. It also showed that the furrow continues to ingress below the presumptive blastocoele toward the embryonic vegetal pole. On the animal side, the plasma membranes are

closely apposed, but between these contacts and the furrow tip, membranes diverge thus forming a dilatation. The strong similarity between cleaving embryos (Kalt 1971a, b) and gastrulae (Hatte et al. 2014) suggests that the “inverted teardrop” is not specific of gastrulae. It will be interesting to know if this organization is also found in epithelial cells at later developmental stages. Kalt (1971b) reported the presence of finely granular material in the dilated portion of the furrow tip. Whether a fluid fills the inverted teardrop of gastrulae and the composition of this putative fluid are currently unknown although during cytokinesis we could detect fibronectin below the furrow tip (JP Tassan, unpublished result). The cleavage furrow progresses with two different phases characterized by different speeds along the apical–basal axis: the fast one at the beginning of cytokinesis and the slow one when it reaches the neighborhood of the apical junctional complex. This difference in the dynamics of furrowing along the apical–basal axis could be linked to the fact that at some point the cytokinetic ring must cross the apical actin filaments. Also the size of the “inverted teardrop” evolves during its progression along the apical–basal axis. It is small when situated basally and enlarges progressively when it approaches the apical part of the dividing cell. It may be correlated with the presence of pulling forces, which spread plasma membranes and could be higher when the cytokinetic furrow reaches the apical adhesion belt. The inverted teardrop is resorbed at the end of the cell division. Therefore, it is a transient structure.

It is still unknown how the gap forms. However, several proteins actively involved in cytokinesis, including anillin (Fig. 11.3b), actin, and myosin II heavy chain, are localized both at the tip and lateral to the inverted teardrop, which suggests that membrane spreading is an active process. The presence of the teardrop gap during asymmetrically furrowing cell division raises the question of why the daughter cell membranes are transiently separated. The leading edge of the cytokinetic furrow could induce the plasma membrane-specific bending, thus creating a gap. It was shown that in *Xenopus* one-cell embryo, the leading edge of the cytokinetic furrow is mainly inherited from the surface plasma membrane, whereas the lateral side of the furrow is mainly formed by new membranes (Bluemink and de Laat 1973; de Laat and Bluemink 1974; Byers and Armstrong 1986; Danilchik et al. 1998). The membrane spreading at the leading edge of the cytokinetic furrow could create two independent membrane domains, where newly formed membranes are added. Thus, cytokinetic proteins localized at the leading edge of the inverted teardrop could be involved in the regulation of forces necessary to limit membrane spreading.

In gastrulae, during the phase of cytokinesis when epithelial cells are still dividing apically, the two daughter cells enter in contact basally, creating the “inverted teardrop.” This mechanism coupled with asymmetric furrowing may contribute to the maintenance of epithelial integrity by ensuring cell–cell cohesion before completion of cytokinesis. Because the gap is transient, eventually the two daughter cells will be in close contact. However, this general scheme has an exception, which is the intercalation of neighboring cells. Although it may not be the unique mechanism, the intercalation of neighboring cells during cytokinesis participates in cell mixing during *Xenopus* gastrulation.

We also showed that intercalation of neighboring cells between daughter cells occasionally occurs during cytokinesis. The plasma membrane separation below the cytokinetic furrow forming the gap could be an active process and involves cytokinetic proteins. This suggests that these proteins could be involved in cell intercalation. It has been shown that remodeling of cell–cell contacts allows cell intercalation during convergent extension during elongation of the *Xenopus* embryo during later development (Keller 2002). Cytokinesis provides favorable conditions for cell intercalation and thus participates in cell mixing in the ectodermal epithelium of *Xenopus* gastrula. This raises the question of how cell–cell junctions are remodeled during intercalation. In *Xenopus*, cell mixing is restricted to clone borders (a group of cells stemming from the division of the same blastomere) (Bauer et al. 1994). This limited cell intermingling explains why the *Xenopus laevis* fate map is more consistent than in other model organisms including zebrafish and mouse, in which massive cell mixing happens during embryogenesis. Interestingly, it was shown in mouse small intestine that the neighboring cells can infiltrate the space in the cytokinetic furrow (Jinguji and Ishikawa 1992). This suggests that neighboring cell intercalation between dividing cells may be a general feature and occur also in mammalian tissues.

## 11.4 Conclusions

The *Xenopus* embryo displays multiple facets of asymmetric mechanisms during cell divisions. Some of these events, such as asymmetric localization of mRNAs and proteins, clearly participate in the development of the embryo. The understanding of mechanisms including asymmetric furrowing and its role during development and possibly in pathological processes still needs further investigation. The *Xenopus* early embryo offers a unique opportunity to study the role of these asymmetries in situ in a developing vertebrate embryo.

**Acknowledgments** We thank Michael V. Danilchik (Oregon Health and Sciences University, Portland, OR, USA) and Malgorzata Kloc (the Houston Methodist Hospital, Houston TX, USA) for helpful discussion and comments on the manuscript. We also thank the Microscopy Rennes Imaging Center (MRic, BIOSIT, IBiSA). Work in our lab was supported by le Centre National de la Recherche Scientifique (CNRS) and l'Agence Nationale de la Recherche (ANR, KinBioFRET). G.H. was supported by the MENESR and partly by a grant from the Ligue Nationale contre le Cancer. MW was supported by Princeton University start-up funding.

## References

- Albertson R, Doe CQ (2003) Dlg, Scrib and Lgl regulate neuroblast cell size and mitotic spindle asymmetry. *Nat Cell Biol* 5:166–170
- Audhya A, Hyndman F, McLeod IX et al (2005) A complex containing the Sm protein CAR-1 and the RNA helicase CGH-1 is required for embryonic cytokinesis in *Caenorhabditis elegans*. *J Cell Biol* 171:267–279

- Bauer DV, Huang S, Moody SA (1994) The cleavage stage origin of Spemann's organizer: analysis of the movements of blastomere clones before and during gastrulation in *Xenopus*. *Development* 120:1179–1189
- Beckhelling C, Pérez-Mongioli D, Houlston E (2000) Localised MPF regulation in eggs. *Biol Cell* 92:245–253
- Black SD, Gerhart JC (1985) Experimental control of the site of embryonic axis formation in *Xenopus laevis* eggs centrifuged before first cleavage. *Dev Biol* 108:310–324
- Bluemink JG, de Laat SW (1973) New membrane formation during cytokinesis in normal and cytochalasin B-treated eggs of *Xenopus laevis*. I. Electron microscope observations. *J Cell Biol* 59:89–108
- Byers TJ, Armstrong PB (1986) Membrane protein redistribution during *Xenopus* first cleavage. *J Cell Biol* 102:2176–2184
- Carron C, Shi DL (2016) Specification of anteroposterior axis by combinatorial signaling during *Xenopus* development. *Wiley Interdiscip Rev Dev Biol* 5:150–168
- Chalmers AD, Strauss B, Papalopulu N (2003) Oriented cell divisions asymmetrically segregate aPKC and generate cell fate diversity in the early *Xenopus* embryo. *Development* 130:2657–2668
- Chang JB, Ferrell JE Jr (2013) Mitotic trigger waves and the spatial coordination of the *Xenopus* cell cycle. *Nature* 500:603–607
- Danilchik MV, Black SD (1988) The first cleavage plane and the embryonic axis are determined by separate mechanisms in *Xenopus laevis*. I. Independence in undisturbed embryos. *Dev Biol* 128:58–64
- Danilchik MV, Denegre JM (1991) Deep cytoplasmic rearrangements during early development in *Xenopus laevis*. *Development* 111:845–856
- Danilchik MV, Gerhart JC (1987) Differentiation of the animal-vegetal axis in *Xenopus laevis* oocytes. I. Polarized intracellular translocation of platelets establishes the yolk gradient. *Dev Biol* 122(1):101–112
- Danilchik MV, Funk WC, Brown EE, Larkin K (1998) Requirement for microtubules in new membrane formation during cytokinesis of *Xenopus* embryos. *Dev Biol* 194:47–60. doi:10.1006/dbio.1997.8815
- Danilchik MV, Bedrick SD, Brown EE, Ray K (2003) Furrow microtubules and localized exocytosis in cleaving *Xenopus laevis* embryos. *J Cell Sci* 116:273–283
- Danilchik M, Williams M, Brown E (2013) Blastocoel-spanning filopodia in cleavage-stage *Xenopus laevis*: potential roles in morphogen distribution and detection. *Dev Biol* 382:70–81
- De Domenico E, Owens ND, Grant IM, Gomes-Faria R, Gilchrist MJ (2015) Molecular asymmetry in the 8-cell stage *Xenopus tropicalis* embryo described by single blastomere transcript sequencing. *Dev Biol* 408:252–268
- de Laat WS, Bluemink JG (1974) New membrane formation during cytokinesis in normal and cytochalasin B-treated eggs of *Xenopus laevis*. II. Electrophysiological observations. *J Cell Biol* 60:529–540
- Elinson RP (1980) The amphibian egg cortex in fertilization and early development. In: *The cell surface: mediator of developmental processes*, pp 217–234
- Elinson RP, Rowning B (1988) A transient array of parallel microtubules in frog eggs: potential tracks for a cytoplasmic rotation that specifies the dorso-ventral axis. *Dev Biol* 128:185–197
- Fesenko I, Kurth T, Sheth B, Fleming TP, Citi S, Hausen P (2000) Tight junction biogenesis in the early *Xenopus* embryo. *Mech Dev* 96:51–65
- Founounou N, Loyer N, Le Borgne R (2013) Septins regulate the contractility of the actomyosin ring to enable adherens junction remodeling during cytokinesis of epithelial cells. *Dev Cell* 24:242–255
- Gerhart J, Keller R (1986) Region-specific cell activities in amphibian gastrulation. *Annu Rev Cell Biol* 2:201–229
- Gerhart J, Ubels G, Black S, Hara K, Kirschner M (1981) A reinvestigation of the role of the gray crescent in axis formation in *Xenopus laevis*. *Nature* 292:511–517

- Gerhart JC et al (1984) Localization and induction in early development of *Xenopus*. *Philos Trans R Soc Lond B Biol Sci* 307:319–330
- Gerhart J, Danilchik M, Doniach T, Roberts S, Rowning B, Stewart R (1989) Cortical rotation of the *Xenopus* egg: consequences for the anteroposterior pattern of embryonic dorsal development. *Development* 107(Suppl):37–51
- Grant PA, Herold MB, Moody SA (2013) Blastomere explants to test for cell fate commitment during embryonic development. *J Vis Exp*. doi:10.3791/4458
- Glotzer M (1997) The mechanism and control of cytokinesis. *Curr Opin Cell Biol* 9:815–823
- Grill SW, Hyman AA (2005) Spindle positioning by cortical pulling forces. *Dev Cell* 8:461–465
- Guillot C, Lecuit T (2013) Adhesion disengagement uncouples intrinsic and extrinsic forces to drive cytokinesis in epithelial tissues. *Dev Cell* 24:227–241
- Hatte G, Tramier M, Prigent C, Tassan JP (2014) Epithelial cell division in the *Xenopus laevis* embryo during gastrulation. *Int J Dev Biol* 58:775–781
- Hausen P, Riebesell M (1991) The early development of *Xenopus laevis*: an atlas of the histology. Springer, New York
- Herszterg S, Leibfried A, Bosveld F, Martin C, Bellaiche Y (2013) Interplay between the dividing cell and its neighbors regulates adherens junction formation during cytokinesis in epithelial tissue. *Dev Cell* 24:256–270
- Hertwig O (1893) Ueber den Werth der ersten Furchungszellen fuer die Organbildung des Embryo. Experimentelle Studien am Frosch- und Tritonei. *Arch Mikr Anat* xlii:662–807
- Houliston E, Elinson RP (1991) Evidence for the involvement of microtubules, ER, and kinesin in the cortical rotation of fertilized frog eggs. *J Cell Biol* 114:1017–1028
- Houston DW (2012) Cortical rotation and messenger RNA localization in *Xenopus* axis formation. *Wiley Interdiscip Rev Dev Biol* 1:371–388
- Ibanez E, Albertini DF, Overstrom EW (2005) Effect of genetic background and activating stimulus on the timing of meiotic cell cycle progression in parthenogenetically activated mouse oocytes. *Reproduction* 129:27–38
- Jinguji Y, Ishikawa H (1992) Electron microscopic observations on the maintenance of the tight junction during cell division in the epithelium of the mouse small intestine. *Cell Struct Funct* 17:27–37
- Kageura H (1997) Activation of dorsal development by contact between the cortical dorsal determinant and the equatorial core cytoplasm in eggs of *Xenopus laevis*. *Development* 124:1543–1551
- Kalt MR (1971a) The relationship between cleavage and blastocoel formation in *Xenopus laevis*. I. Light microscopic observations. *J Embryol Exp Morphol* 26:37–49
- Kalt MR (1971b) The relationship between cleavage and blastocoel formation in *Xenopus laevis*. II. Electron microscopic observations. *J Embryol Exp Morphol* 26:51–66
- Keller R (2002) Shaping the vertebrate body plan by polarized embryonic cell movements. *Science* 298:1950–1954
- Kikkawa M, Takano K, Shinagawa A (1996) Location and behavior of dorsal determinants during first cell cycle in *Xenopus* eggs. *Development* 122:3687–3696
- King ML, Zhou Y, Bubunenko M (1999) Polarizing genetic information in the egg: RNA localization in the frog oocyte. *Bioessays* 21:546–557
- Klein SL (1987) The first cleavage furrow demarcates the dorsal-ventral axis in *Xenopus* embryos. *Dev Biol* 120:299–304
- Kloc M, Etkin LD (1994) Delocalization of Vg1 mRNA from the vegetal cortex in *Xenopus* oocytes after destruction of Xlirt RNA. *Science* 265:1101–1103
- Larabell CA, Torres M, Rowning BA, Yost C, Miller JR, Wu M, Kimelman D, Moon RT (1997) Establishment of the dorso-ventral axis in *Xenopus* embryos is presaged by early asymmetries in beta-catenin that are modulated by the Wnt signaling pathway. *J Cell Biol* 136:1123–1136
- Le Page Y, Chartrain I, Badouel C, Tassan JP (2011) A functional analysis of MELK in cell division reveals a transition in the mode of cytokinesis during *Xenopus* development. *J Cell Sci* 124:958–968
- Lecuit T, Wieschaus E (2000) Polarized insertion of new membrane from a cytoplasmic reservoir during cleavage of the *Drosophila* embryo. *J Cell Biol* 150:849–860

- Maddox AS, Lewellyn L, Desai A, Oegema K (2007) Anillin and the septins promote asymmetric ingression of the cytokinetic furrow. *Dev Cell* 12:827–835
- Marrari Y, Rouvière C, Houliston E (2004) Complementary roles for dynein and kinesins in the *Xenopus* egg cortical rotation. *Dev Biol* 271:38–48
- Martin-Belmonte F, Perez-Moreno M (2011) Epithelial cell polarity, stem cells and cancer. *Nat Rev Cancer* 12:23–38
- Masho R (1990) Close correlation between the first cleavage plane and the body axis in early *Xenopus* embryos. *Dev Growth Differ* 32:57–64
- Merzdorf CS, Chen YH, Goodenough DA (1998) Formation of functional tight junctions in *Xenopus* embryos. *Dev Biol* 195:187–203
- Mitchison TJ, Ishihara K, Nguyen P, Wühr M (2015) Size scaling of microtubule assemblies in early *Xenopus* embryos. *Cold Spring Harb Perspect Biol* 7:a019182
- Moody SA, Kline MJ (1990) Segregation of fate during cleavage of frog (*Xenopus laevis*) blastomeres. *Anat Embryol (Berl)* 182:347–362
- Morais-de-Sá E, Sunkel C (2013) Adherens junctions determine the apical position of the midbody during follicular epithelial cell division. *EMBO Rep* 14:696–703
- Mowry KL, Cote CA (1999) RNA sorting in *Xenopus* oocytes and embryos. *Faseb J* 13:435–445
- Nieuwkoop PD, Faber J (1967) Normal table of *Xenopus laevis* (Daudin). North-Holland, Amsterdam
- Pérez-Mongiovi D, Chang P, Houliston E (1998) A propagated wave of MPF activation accompanies surface contraction waves at first mitosis in *Xenopus*. *J Cell Sci* 111(Pt 3):385–393
- Prodon F, Chenevert J, Hebras C et al (2010) Dual mechanism controls asymmetric spindle position in ascidian germ cell precursors. *Development* 137:2011–2021
- Rankin S, Kirschner MW (1997) The surface contraction waves of *Xenopus* eggs reflect the metachronous cell-cycle state of the cytoplasm. *Curr Biol* 7:451–454
- Reinsch S, Karsenti E (1994) Orientation of spindle axis and distribution of plasma membrane proteins during cell division in polarized MDCKII cells. *J Cell Biol* 126:1509–1526
- Sakai M (1996) The vegetal determinants required for the Spemann organizer move equatorially during the first cell cycle. *Development* 122:2207–2214
- Scharf SR, Gerhart JC (1980) Determination of the dorsal-ventral axis in eggs of *Xenopus laevis*: complete rescue of uv-impaired eggs by oblique orientation before first cleavage. *Dev Biol* 79:181–198
- Schneider S, Steinbeisser H, Warga RM, Hausen P (1996) Beta-catenin translocation into nuclei demarcates the dorsalizing centers in frog and fish embryos. *Mech Dev* 57:191–198
- Schroeder MM, Gard DL (1992) Organization and regulation of cortical microtubules during the first cell cycle of *Xenopus* eggs. *Development* 114:699–709
- Souza KA, Black SD, Wassersug RJ (1995) Amphibian development in the virtual absence of gravity. *Proc Natl Acad Sci U S A* 92:1975–1978
- Stewart-savage J, Grey RD (1982) The temporal and spatial relationships between cortical contraction, sperm trail formation and pronuclear migration in fertilized *Xenopus* eggs. *Wilhelm Roux Arch Dev Biol* 191:241–245
- Ubbels GA, Hara K, Koster CH, Kirschner MW (1983) Evidence for a functional role of the cytoskeleton in determination of the dorsoventral axis in *Xenopus laevis* eggs. *J Embryol Exp Morphol* 77:15–37
- Vincent JP, Gerhart JC (1987) Subcortical rotation in *Xenopus* eggs: an early step in embryonic axis specification. *Dev Biol* 123:526–539
- Vincent JP, Oster GF, Gerhart JC (1986) Kinematics of gray crescent formation in *Xenopus* eggs: the displacement of subcortical cytoplasm relative to the egg surface. *Dev Biol* 113:484–500
- Wang Q, Racowsky C, Deng M (2011) Mechanism of the chromosome-induced polar body extrusion in mouse eggs. *Cell Div* 6:17
- Wühr M, Dumont S, Groen AC, Needleman DJ, Mitchison TJ (2009) How does a millimeter-sized cell find its center? *Cell Cycle* 8:1115–1121
- Wühr M, Tan ES, Parker SK, Detrich HW 3rd, Mitchison TJ (2010) A model for cleavage plane determination in early amphibian and fish embryos. *Curr Biol* 20:2040–2045