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Membrane Protein Redistribution during *Xenopus* First Cleavage

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Abstract. A large increase in surface area must accompany formation of the amphibian embryo first cleavage furrow. The additional membrane for this areal expansion has been thought to be provided entirely from cytoplasmic stores during furrowing. We have radioiodinated surface proteins of fertilized, pre-cleavage *Xenopus laevis* embryos and followed their redistribution during first cleavage by autoradiography. Near the end of first cleavage, membrane of the outer, pigmented surface of the embryo and a short band of membrane at the leading edge of the furrow displayed a high silver grain density, but the remainder of the furrow membrane was lightly labeled. The membrane of the cleavage furrow is thus mosaic in character; the membrane at the leading edge originates in part from the surface of the zygote, but most of the membrane lining the furrow walls is derived from a

source inaccessible to surface radioiodination. The furrow membrane adjacent to the outer, pigmented surface consistently showed a very low silver grain density and was underlain by large membranous vesicles, suggesting that new membrane derived from cytoplasmic precursors is inserted primarily in this location, at least during the later phase of cleavage. Radioiodinated membrane proteins and surface-attached carbon particles, which lie in the path of the future furrow, contract toward the animal pole in the initial stages of cleavage while markers in other regions do not. We suggest that the domain of heavily labeled membrane at the leading edge of the definitive furrow contains the labeled elements that are gathered at the animal pole during the initial surface contraction and that they include membrane anchors for the underlying contractile ring of microfilaments.

T HE UPPER surface of the *Xenopus laevis* zygote appears dark due to pigment granules in the cortex of the animal hemisphere. Cortical pigment is sparse beneath the membrane of the first cleavage furrow so that the apical border of the furrow is externally visible as a white slash bisecting the dark animal hemisphere. Numerous differences between the membrane of this "unpigmented" furrow surface and the pigmented surface membrane have been described (1, 6, 7). Measurements of local ion currents during cleavage demonstrate that current leaves the unpigmented furrow region and enters the pigmented surface, suggesting a heterogeneous ion channel distribution between the two plasma membrane domains (13). Freeze fracture electron microscopy has revealed a higher intramembranous particle density in the pigmented surface membrane as compared with the furrow membrane (4, 20). The morphological distinctions between the unpigmented furrow and pigmented outer surfaces remain throughout the cleavage divisions. At the 32-64 cell stage, concanavalin A receptors are preferentially localized in the unpigmented (basolateral) surfaces of amphibian blastomeres (18), and this surface is more adhesive than the pigmented surface (11, 19).

Complete division of the amphibian embryo into two cells requires a substantial increase in surface area. Particle marking studies have provided evidence that the pigmented surface

of the zygote does not change sufficiently in area to account for the increase in furrow surface area (3, 23). This observation, and the striking structural and functional differences between the basolateral membranes of the blastomeres and the membrane of the external surface, have suggested that the furrow membrane is constructed entirely from internal sources, and that the "pre-existing" surface does not contribute plasma membrane to the furrow (see reference 5 for review). As described, this conclusion is based largely on inferential evidence; integral membrane probes have not been used to directly follow the membranes in question. We have used iodinated membrane proteins as integral markers of the pigmented zygote surface to re-examine the contribution of this surface to the expanding membrane of the furrow.

Proteins in the plasma membrane of fertilized *Xenopus* embryos were labeled with ^{125}I before cleavage. The embryos were allowed to develop to specific stages of first cleavage and were then fixed and processed for light and transmission electron microscope (TEM)[1] autoradiography. The zygote plasma membrane labels heavily, but a dilution of label in the furrow region supports the hypothesis that cytoplasmic stores are the principle source of new furrow membrane. Contrary to this hypothesis, however, we find that some

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1. *Abbreviation used in this paper:* TEM, transmission electron microscopy.

proteins labeled at the zygote surface before first cleavage relocate to the leading edge of the furrow at an advanced stage of cytokinesis. A cortical contraction moves surface markers toward the animal pole in line with the nascent furrow in the first few minutes of cleavage. This subset of markers may be the same as those found at the leading edge of the furrow near the end of first cleavage and may represent in part membrane "anchors" for contractile ring microfilaments.

Materials and Methods

Preparation of Zygotes

Xenopus laevis females were injected with 1,000 IU of human chorionic gonadotropin (Sigma Chemical Co., St. Louis, MO) to induce ovulation. About 12 h after injection, eggs were stripped into a few drops of F-1 medium (10) containing 41 mM NaCl, 1.8 mM KCl, 0.5 mM Na$_2$HPO$_4$, 2 mM NaOH, 2.5 mM Hepes, 0.25 mM CaCl$_2$ and 0.06 mM MgCl$_2$, pH 7.8. A sperm suspension from freshly minced testes was added to fertilize the eggs. Fertilized eggs were dejellied by swirling in 2% cysteine-HCl in F-1, pH 7.8 for 3–5 min at 15°C. Fertilization envelopes were removed manually with watchmaker's forceps. Embryos without their envelopes can survive to form normal tadpoles. Denuded embryos were placed in shallow wells formed in a bed of 0.5% agarose (Sigma Type VI) in 12-mm diam × 5-mm high plastic carriers in preparation for radioiodination.

Radioiodination

Iodo Gen (Pierce Chemical Co., Rockford, IL)-coated beakers were prepared as follows: 0.5 ml of chloroform containing 0.5 mg Iodo Gen was placed in a 15-ml Pyrex beaker. The chloroform was evaporated in an N$_2$ atmosphere while rotating the beaker at an angle to coat the walls. Coated beakers were rinsed, then filled with 4 ml F-1 at 15°C. A plastic carrier containing three denuded, pre-cleavage zygotes was transferred to each coated beaker and the radioiodination reaction was initiated by the addition of 0.3 mCi of carrier-free ^{125}I (Amersham Corp., Arlington Heights, IL). The beakers were agitated gently on a rotating table shaker at room temperature. The reaction was stopped after 15 min by transferring the carriers to 250 ml F-1 + 5 mM NaI. After two 10-min rinses, embryos were transferred from the carriers to fresh agarose beds and fixed at defined stages (below).

Radioiodination with Iodo Gen has been shown to label only those proteins exposed at the outer surface of intact cells (8, 15, 16). This is supported by the present study in which the autoradiographic signal greater than background was always located over the plasma membrane at the light and electron microscope levels. In comparison, the grain density over the cytoplasm was seldom appreciably above background. Lopo and Vacquier (15) have demonstrated that some radioactivity is incorporated into lipids with this method. Lipid-associated ^{125}I is not likely to contribute to the autoradiographic response described here because most membrane lipids are extracted by the organic solvents used in histological processing.

Particle Marking

Blood charcoal (E. Merck, Darmstadt, FRG) was suspended in 0.1% poly-L-lysine, rinsed in distilled water, and resuspended in 0.01% glutaraldehyde. This treatment was necessary for the particles to stick firmly to the surface of the embryo. A fine suspension of rinsed particles was applied to the upward-facing surface of newly fertilized eggs. After 5 min the zygotes were rinsed with medium expelled from a drawn out pipet and transferred to a clean dish to remove non-adhering particles. This procedure resulted in the tight adherence of 500–1,000 particles of 5–10-μm diam. Some particle-marked embryos were photographed on a dissecting microscope at 30-s intervals from the initiation of cleavage to the appearance of unpigmented surface in the furrow. Micrographs were printed at a final magnification of 85× and reliably oriented for tracing particle movement by markers embedded in the agarose substrate. Other embryos were fixed (as below) at the late first cleavage stage, dehydrated in a graded ethanol series, embedded in paraffin, and sectioned at 10 μm to determine the distribution of particles attached to the unpigmented furrow surface.

Histology

Embryos were fixed with 2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M Na$_2$HPO$_4$ (phosphate buffer), pH 7.8 at four stages: pre-cleavage, early first cleavage stage (at the first appearance of unpigmented surface in the furrow), late first cleavage stage (marked by the beginning of second cleavage), and

blastula stage. After overnight fixation at room temperature, embryos were rinsed in phosphate buffer, postfixed for 2 h in 2% OsO$_4$ in phosphate buffer (where indicated), rinsed again, and dehydrated in a graded acetone series and propylene oxide. They were then infiltrated overnight and embedded in Spurr's epoxy resin (Polysciences, Inc., Warrington, PA). Serial 5-μm sections were cut on a Porter-Blum MT-2 ultramicrotome and placed alternately on two sets of glass slides. One set of alternating serial sections was processed for light microscope autoradiography (14) using Kodak NTB-2 photographic emulsion. Thick section autoradiograms were photographed with a Zeiss WL microscope, using plane-polarized epiillumination which passed through a second polarizing filter after the objective. This technique allowed us to distinguish silver grains, which reflected the light, from cortical pigment granules, which remained black; they were otherwise indistinguishable in bright or dark field illumination.

Sections displaying a silver grain pattern of interest were used to select adjacent serial sections from the second set of slides for re-embedding and thin sectioning on a Reichert OMV3 ultramicrotome with a diamond knife. Pale gold thin sections were prepared for TEM autoradiography by the loop method described by Stevens (26) using Ilford L-4 photographic emulsion (Polysciences, Inc.) with the addition of 0.1% dioctyl sodium sulfosuccinate (Sigma Chemical Co.) to increase the strength of the emulsion film as suggested by Nagata et al. (17). The emulsion was removed by treatment with 20% acetic acid for 12 min at 32°C, and sections were stained with uranyl acetate and lead citrate. Undeveloped emulsion films were checked periodically in the TEM to ensure that the silver bromide crystals were distributed on the grid as a uniform layer.

Results

Surface Events during First Cleavage

A brief review of *Xenopus* first cleavage is necessary to define events that pertain to our observations. The early cleavage divisions are assymmetrical; that is, the furrow begins in the animal hemisphere and progressively encircles the embryo. First cleavage is preceded by surface contraction waves that radiate from the future initiation site of the furrow toward the vegetal hemisphere (9). Pigment granules then aggregate into a narrow stripe (the initiation site) located near the animal pole, and the radially symmetrical pigment cap begins to change shape. Cortical pigment granules, which lie near the plane of cleavage (defined as the plane of the future furrow), move toward the initiation site (23) leading to a constriction of the pigment cap in the plane of cleavage (Fig. 1). Transverse wrinkles radiate from the constricted region, indicating that the surface is under tension. These changes occur during the first 6–8 min of cleavage at 20°C and are followed by the appearance of white, unpigmented surface in the cleavage furrow. This event makes the "early first cleavage stage."

We used the beginning of second cleavage (referred to as the "late first cleavage stage") as a standard timepoint at which to fix embryos to compare results between different batches of eggs. At this stage, unpigmented surface is externally visible (Fig. 2) to a variable extent. The lateral walls of the furrow are closely apposed and the daughter blastomeres remain connected by a cytoplasmic bridge near the center of the embryo. We fixed embryos at the late first cleavage stage and broke apart their blastomeres to determine the circumference of this cytoplasmic bridge. The average circumference was 432 μm ± 133 SD as measured from 17 embryos. The cortical pigment of the outer surface usually ends abruptly at the furrow, creating a sharply defined border between this surface and the mostly unpigmented surface of the furrow. We will refer to this boundary as the "border" region.

Redistribution of Markers at the Early First Cleavage Stage

Surface-attached carbon particles redistributed in a pattern that paralleled the movement of cortical pigment granules.

Most dramatic was the directed movement of particles from equatorial regions in the plane of the future cleavage furrow toward the animal pole (Fig. 3). Particles in other regions of the embryo surface often moved perpendicularly away from the plane of cleavage. These movements were largely due to a flattening of the embryo and the progressive separation of the blastomeres during cleavage rather than to lateral movement in the membrane and will not be considered further.

Radiolabeled membrane proteins redistributed in a fashion that was predicted by the movement of surface-attached particles. Pre-cleavage *Xenopus* zygotes radioiodinated by the Iodo Gen method displayed a silver grain distribution restricted to the membrane of the hemisphere that faced the medium; the lower surface, which rested on the agarose support, did not label, resulting in a partial labeling of the embryo circumference (~50%) in autoradiograms. Zygotes incubated with ^{125}I but without Iodo Gen showed a low silver
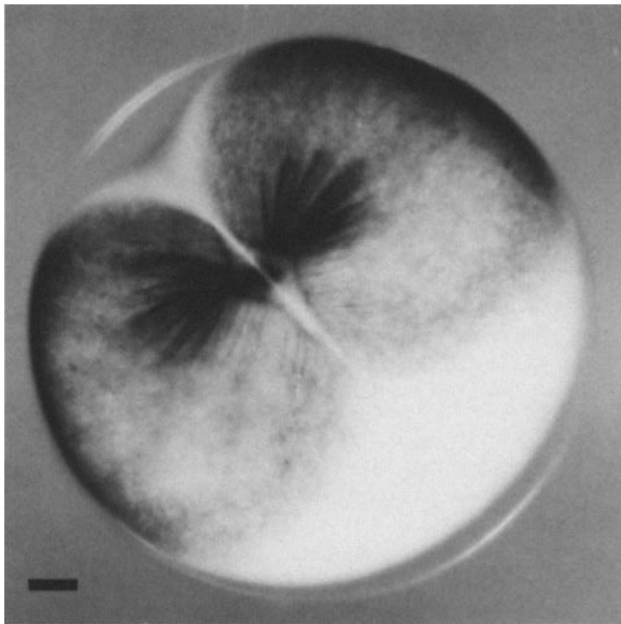

Figure 1. Early first cleavage stage. Dejellied embryo within its fertilization envelope. A narrow band of unpigmented surface is visible in the furrow. The animal hemisphere pigment cap is deformed toward the animal pole at each end of the furrow. Transverse surface wrinkles radiate from the furrow. Bar, 100 μm.
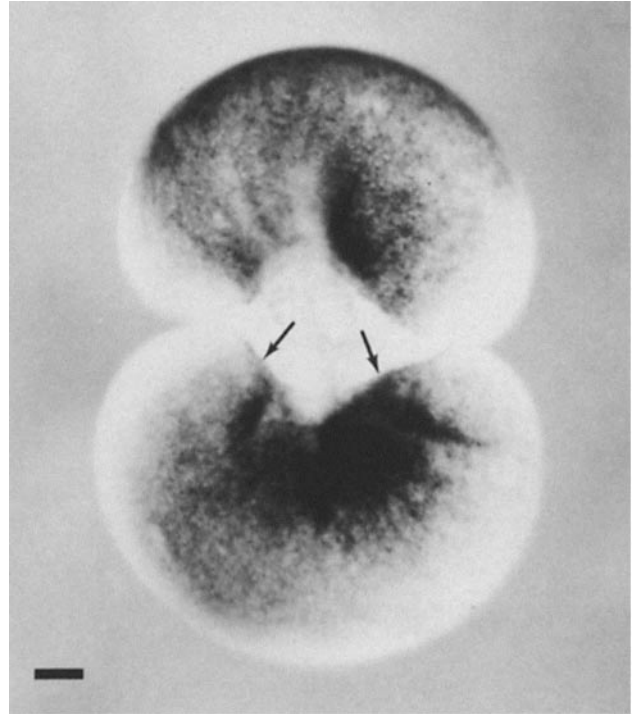

Figure 2. Late first cleavage stage. Dejellied and denuded embryo. Unpigmented surface is visible in the furrow. The "border" region is clearly defined (*arrows*). Bar, 100 μm.
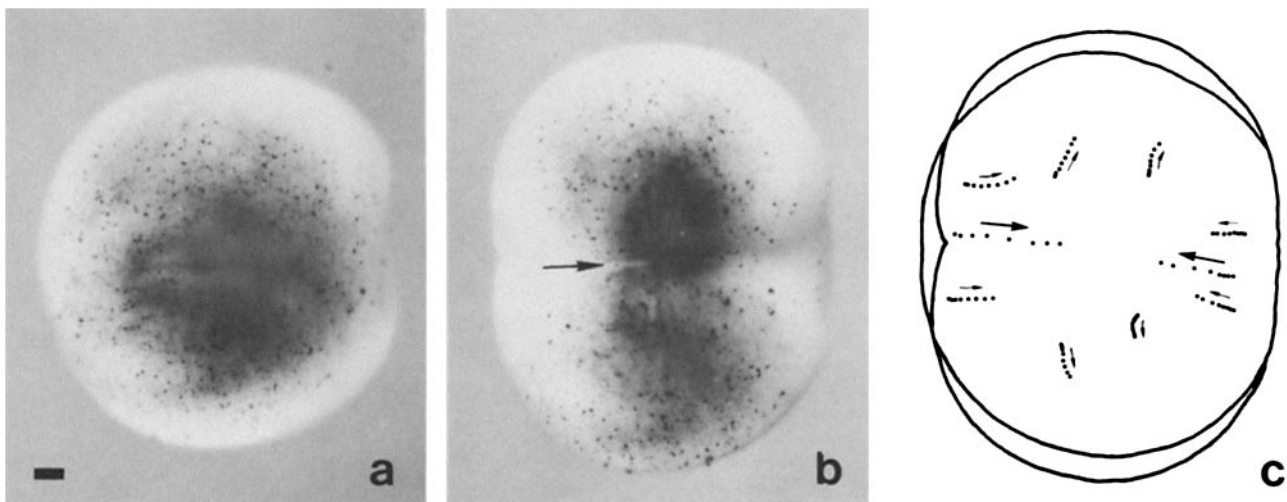

Figure 3. Movement of surface-attached particles. Carbon particles were attached to the upward-facing surface before cleavage. This embryo was photographed at 30-s intervals from the initiation of cleavage until the early first cleavage stage. (*a*) Zero timepoint. (*b*) Final timepoint, 7 min, 30 s after *a*. Unpigmented surface is apparent in the furrow (*arrow*). The movement of selected particles on the same embryo is presented in *c*. Each point marks the position of a particle at 1-min intervals; arrows indicate the direction of travel. The initial and final cell outlines are shown. Particle movement was more rapid and extensive in the plane of cleavage than in other regions of the embryo and was directed toward the animal pole. Particles that moved away from the cleavage plane followed the gradual flattening and separation of the blastomeres but were displaced little with respect to one another. Bar, 100 μm.
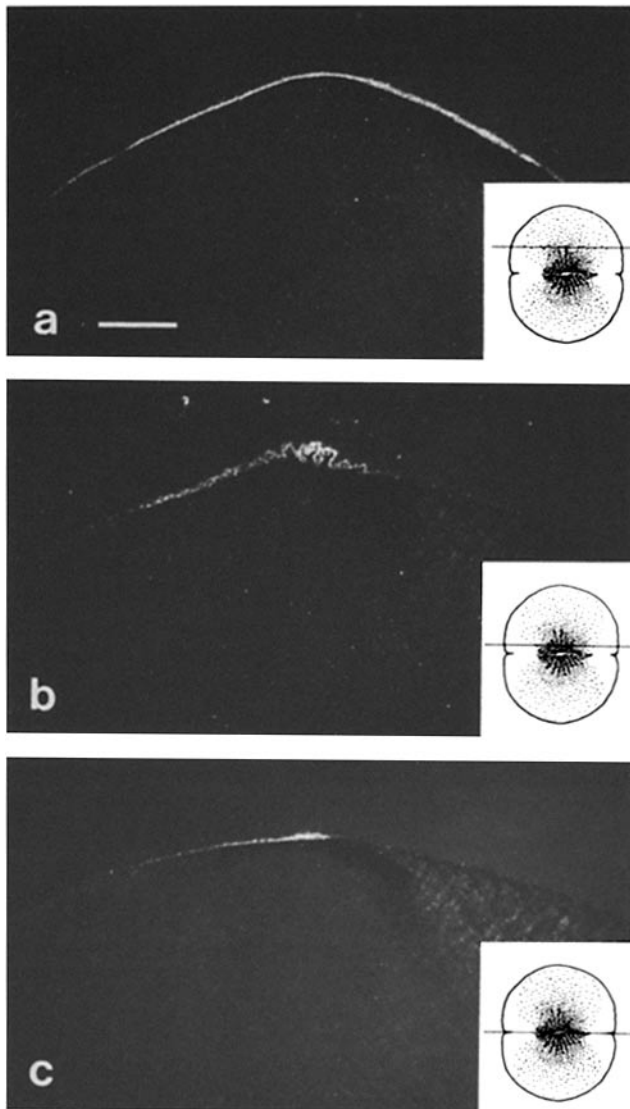
Figure 4. Autoradiography at the early first cleavage stage. This embryo was radioiodinated before the initiation of cleavage, processed as indicated in Materials and Methods, and sectioned parallel to the plane of cleavage. The autoradiogram was photographed with reflected light so that silver grains appear white. The insets show the plane of sectioning on a schematic top view of the early first cleavage embryo (refer to Fig. 1). (*a*) Section through the middle of one blastomere. (*b*) Section through the region of stress folds near the plane of cleavage. (*c*) Section very close to the plane of cleavage. The extent of labeling about the equator is reduced in *b* and *c*, corresponding to the reduction in the extent to which pigmented cortex surrounds the embryo in the plane of cleavage at this stage (Fig. 1). Bar, 50 μm.

grain density over the entire cell. At the early first cleavage stage, it can be seen in sections taken parallel to the plane of cleavage that the equator of the embryo is labeled to a lesser extent in sections close to the cleavage plane (Fig. 4, *b* and *c*) than in sections through the blastomeres (Fig. 4*a*). This reduction in the amount of equatorial label in the plane of cleavage is presumably due to the gathering of labeled membrane proteins toward the initiation site in a manner that parallels the movement of surface-attached particles. Taken together, the concerted movements of pigment granules, sur-

face-attached particles, and radioiodinated membrane proteins reveal an assymetric surface contraction in the first few minutes of cleavage, which is restricted to the plane of cleavage and directed toward the animal pole.

Redistribution Patterns at the Late First Cleavage Stage

Our marking studies have revealed the cleavage furrow membrane to be mosaic in character, with the furrow walls containing low densities of labeled elements and a distinct pattern of higher density label at the leading edge. We frequently observed a higher density of pigment granules at the leading edge of the furrow compared to the furrow walls. Carbon particles that were attached to the surface of zygotes before first cleavage were found in the furrow at the late first cleavage stage after fixation and serial sectioning. Of 59 embryos, 37 had particles associated with the unpigmented surface of the furrow. 33 of a total of 128 such particles appeared at the leading edge of the furrow. Membrane proteins that were iodinated before cleavage were also found in the furrow at the late first cleavage stage. Three membrane regions could be distinguished on the basis of label intensity; the pigmented surface, the lateral walls of the furrow, and the leading edge of the furrow. In autoradiograms of sections taken perpendicular to the furrow, the pigmented surface membrane was heavily labeled (Fig. 5). This heavy label intensity ended abruptly at the border region (Fig. 6), and the membrane of the furrow walls was lightly labeled (Figs. 5 and 7). The furrow membrane immediately adjacent to the pigmented/unpigmented border was smooth and underlain by large vesicular membrane profiles (Fig. 7*a*). These vesicular profiles were rare under the pigmented surface and in furrow regions distant from the border.

The leading edge of the furrow showed an unexpected silver grain pattern at the late first cleavage stage. At this stage, the leading edge forms a ring that defines the perimeter of the cytoplasmic bridge. Because only the animal hemisphere is labeled under our radioiodination conditions, we will consider only that portion of the furrow that originates in the animal hemisphere. Serial sections perpendicular to the cleavage furrow showed a variable, light silver grain density in the peripheral regions of the leading edge, on either side of the central region (Fig. 5, *a* and *b*). Sections, which included the central region, revealed a domain of very intensely labeled membrane at the leading edge (Fig. 5, *c* and *d*). This heavy labeling extended along the length of the leading edge for a distance of ~40–110 μm as calculated by counting serial sections of known thickness. The mean length of this labeled band was 78 ± 28 μm for nine serially sectioned embryos. Fig. 8 schematically illustrates the distribution of silver grains at the late first cleavage stage. In TEM autoradiograms, microvilli at the leading edge of the furrow in the central region of the embryo consistently showed a high label density (Fig. 7*c*). These microvilli increased the membrane area in the leading edge region, thus amplifying the label density there artifactually in light microscope autoradiography, but in TEM autoradiograms the label density per unit area of membrane was still much higher at the leading edge of the furrow than over the walls of the furrow (Fig. 7*b*) in the same section. The membrane of the pigmented apical surface remained heavily labeled at least until the blastula stage at 6 h, 30 min after
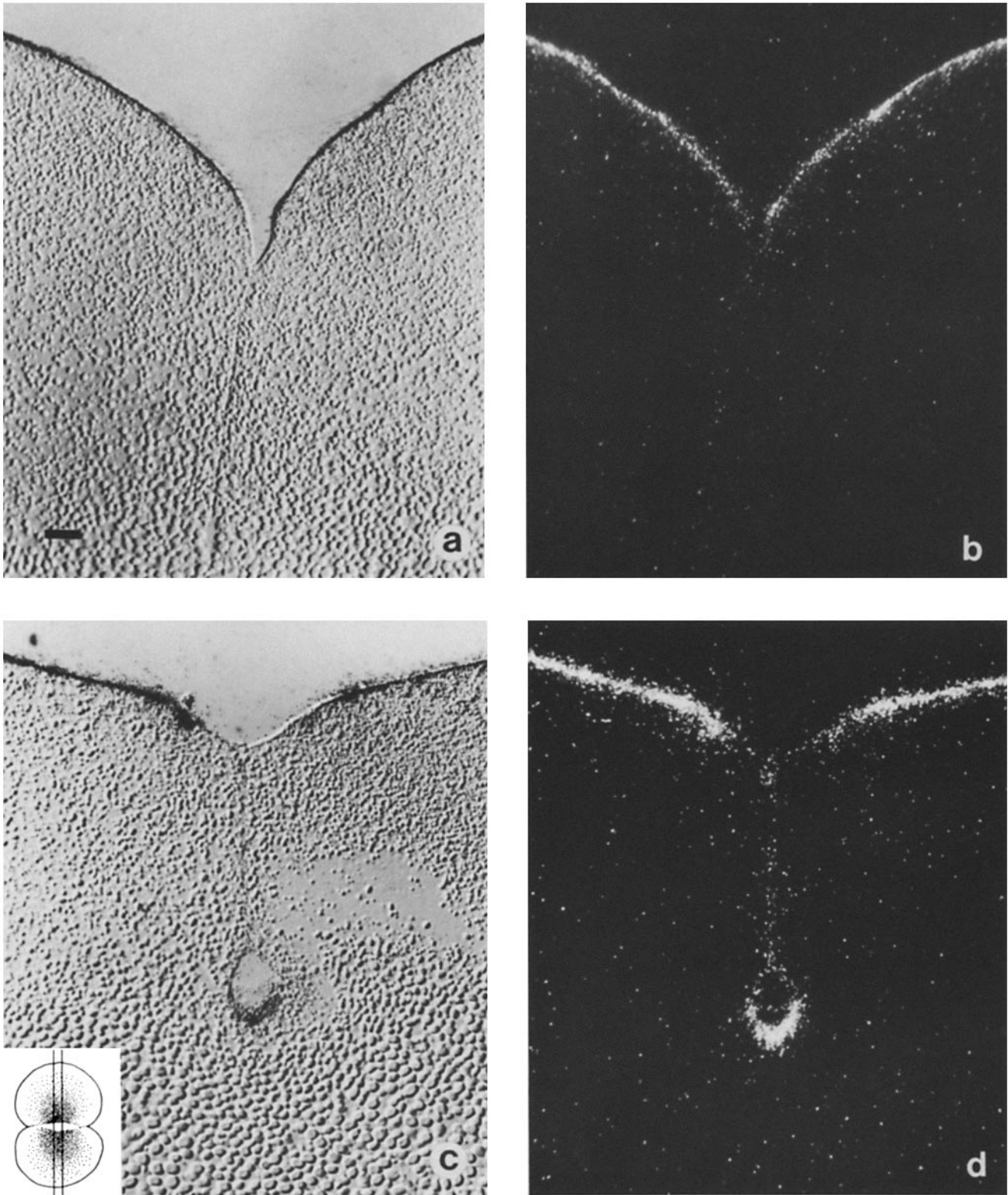
Figure 5. Light microscope autoradiography at the late first cleavage stage. This embryo was radioiodinated before the initiation of cleavage and was sectioned perpendicular to the cleavage furrow. The inset is a schematic top view of the late first cleavage embryo; the parallel lines illustrate the plane of sectioning and delineate the central region of the embryo. (*a* and *b*) Section taken outside the central region of the embryo. The furrow of the animal hemisphere is shown. The outer, pigmented surface is more heavily labeled than the lateral walls of the furrow. (*c* and *d*) Section taken from the central region of the embryo. The furrow displays a high density of silver grains at its leading edge. The darkness at the outer surface of the embryo in *a* and *c* is due to a combination of pigment granules and silver grains. (*a* and *c*) Differential interference contrast; (*b* and *d*) reflected light. Bar, 10 μm.
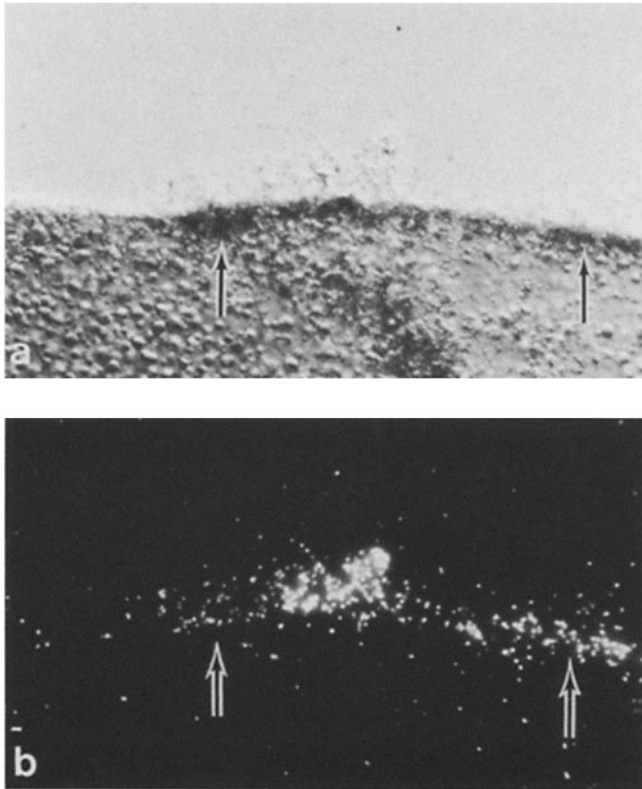
Figure 6. Co-distribution of silver grains and pigment granules in the border region. A portion of the new, unpigmented surface is visible on the left hand side of this section from a late first cleavage stage embryo; conditions as in Fig. 5. Silver grains may be distinguished in both micrographs, but cortical pigment granules (*arrows*) are visible only in *a*. In general, the regions of heavy cortical pigment and high silver grain density are closely co-distributed at the border between the furrow and outer surfaces.

fertilization (Fig. 9). The much lighter silver grain density over the basolateral membranes was more evenly distributed than at the late first cleavage stage.

Discussion

Our results demonstrate a dynamic, directed contractile event at the surface of the *Xenopus* embryo that occurs just before the furrow begins to penetrate the cytoplasm. Surface-attached particles and labeled membrane proteins in the path of the future furrow move toward the furrow initiation site at the animal pole. Particles lying outside the future furrow plane do not redistribute significantly with respect to one another. The furrow deepens after this contraction and carries carbon particles and ^{125}I-labeled proteins at its leading edge into the center of the embryo, markers that were originally attached to zygote surface elements before cleavage. At the same time, new membrane appears to be inserted into the lateral walls of the furrow to allow for the expansion of surface area concomitant with cleavage.

Origin of New Furrow Membrane

There are two possible sources of membrane for the new furrow surface. The "pre-existing" zygote surface may supply membrane to the furrow through a flattening of surface projections, or the furrow membrane may arise by the insertion of cytoplasmic precursors. The sharp reduction in label density in passing from the pigmented outer surface to the unpigmented furrow wall eliminates the possibility that unaltered "pre-existing" membrane is used to form the membrane of the furrow walls. Our observations support the hypothesis that cytoplasmic precursors (inaccessible to surface iodination) are the principle source of membrane for furrow expansion, and that insertion of new membrane occurs during cleavage.

Membrane proteins that were labeled at the zygote surface before cleavage were, however, present in the furrow at the late first cleavage stage. A low concentration of such proteins were present in the lateral walls of the furrow, and the leading edge showed a discrete band of label in the central region of the embryo with an intensity similar to that of the outer surface membrane. The presence of iodinated membrane proteins in the furrow calls for a modification of the current hypothesis, which states that membrane from the outer "pre-existing" surface does not contribute to the membrane of the furrow in amphibian first cleavage (5). Also, this result, coupled with the finding that a much lower density of intramembranous particles is found in the furrow walls in freeze fracture electron microscopy (4, 20), argues that the newly inserted membrane may be composed primarily of membrane lipids.

Location of Precursor Insertion

The location of cleavage-related membrane insertion has not been previously established. Singal and Sanders (24) have postulated two sites, the lateral walls of the furrow and the leading edge. They suggested that folds and microvilli at the leading edge may be the result of an excess of recently inserted membrane. The high silver grain density associated with membrane folds at the leading edge, however, effectively rules out insertion at that location. Our results implicate the furrow membrane adjacent to the border between the furrow and outer surfaces as a probable site of membrane insertion at least during the later stages of cleavage. This is suggested both by the consistently light label intensity and the presence of vesicular membrane profiles beneath the plasma membrane in that location. Structures similar to these have been interpreted as cytoplasmic membrane elements en route to insertion in the plasma membrane (3, 25).

Amphibian cleavage stage blastomeres have distinct apical and basolateral domains typical of polarized epithelial cells (see reference 1 for review). Local insertion of new membrane in the furrow during cytokinesis provides a mechanism for the establishment of this polarized membrane organization. The polarized distribution of membrane proteins iodinated before cleavage is maintained on superficial blastomeres at least until the blastula stage where the outer (apical) membrane retains much more ^{125}I label than the basolateral membrane (Fig. 9).

Redistribution of Markers during Cleavage

A directed surface contraction appears to precede penetration of the furrow in the amphibian embryo. Selman and Waddington (23) measured the movement of clusters of cortical pigment granules from time-lapse films of cleaving newt embryos and documented a movement of cortical pigment in
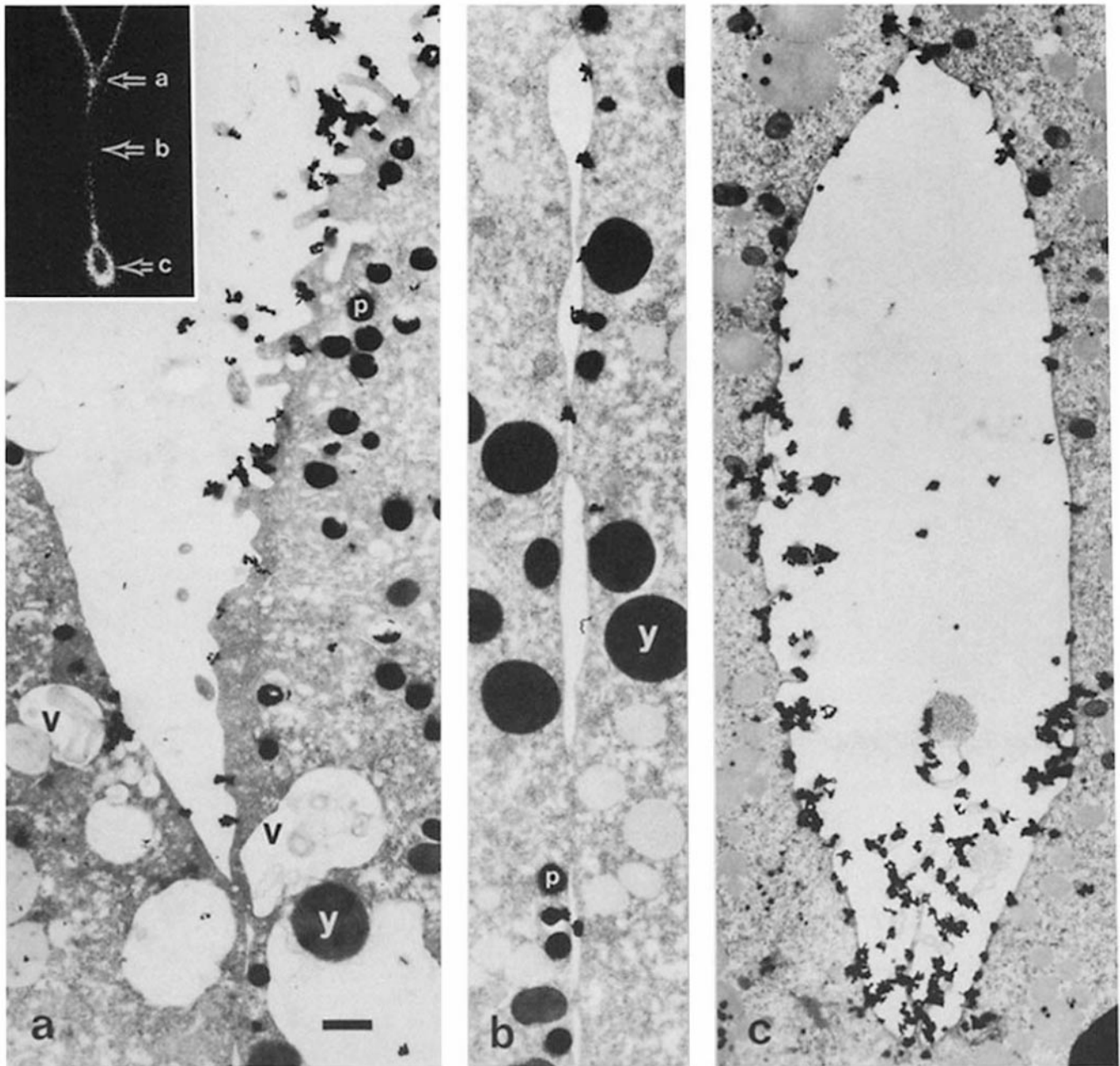
Figure 7. TEM autoradiography at the late first cleavage stage. The arrows on the inset demonstrate the regions of the furrow from which *a–c* were derived. These embryos were radioiodinated before first cleavage and processed as indicated in Materials and Methods, including OsO₄ post-staining. (*a*) Furrow groove showing the border region. The pigmented surface (pigment, *p*) is folded and shows a high density of silver grains. The "unpigmented" surface is smooth, has a very low silver grain density, and is underlain by vesicular membrane profiles (*v*); (yolk granules, *y*). The lateral walls of the furrow (*b*) are very lightly labeled, but the leading edge (*c*) is heavily labeled in the central region of the embryo, particularly on folds located there. The silver grain density per unit area of membrane was always much higher in this region than over the lateral walls of the furrow. Bar, 1 μm.

the plane of the future cleavage furrow, directed toward the initiation site. Similarly, Sawai (21) observed that pieces of cortex grafted onto cleaving newt embryos underwent changes consistent with a contraction of the cortex in the plane of cleavage, also toward the initiation site. An analogous movement during early stages of *Xenopus* cleavage gives rise to the constricted appearance of the pigment cap and surface wrinkles perpendicular to the cleavage plane (Fig. 1). We report a redistribution of surface-attached particles during the first few minutes of cleavage complementary to the movement of

underlying pigment granules and find that iodinated membrane proteins appear to redistribute in the same pattern. In sum, these observations reveal a concerted movement during the initiation of furrowing of only those cortical and surface elements that lie in the plane of the future cleavage furrow. This movement may be explained by a meridional contraction of aligned cortical filaments toward the animal pole. One consequence of this asymmetrical surface contraction is that the equator of the embryo shows a reduced extent of labeling in the future plane of cleavage (Fig. 4). This condition appears
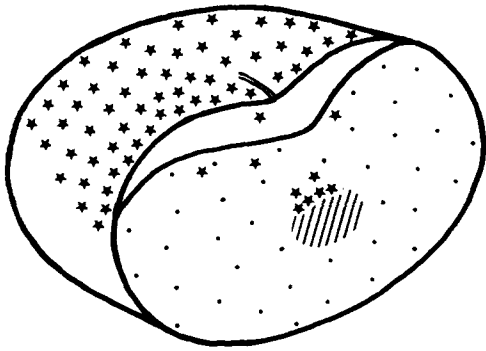
Figure 8. Late first cleavage stage silver grain distribution, explanatory diagram. One blastomere has been removed to reveal the surface between the blastomeres. Stars approximate the distribution of silver grains, cross-hatch represents the area of cytoplasmic continuity between the blastomeres, and stippling indicates the region where the membranes between the blastomeres are closely apposed. About 18% of the perimeter of the cytoplasmic bridge is labeled at this stage.
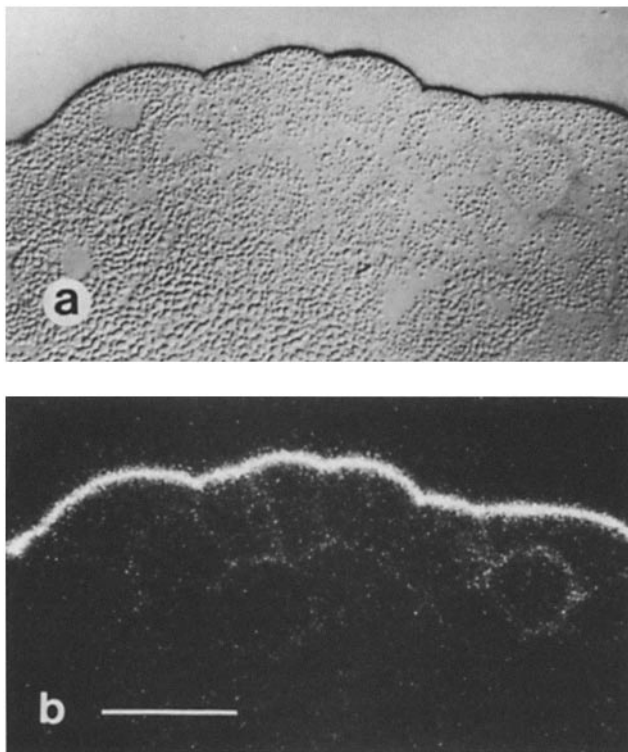

Figure 9. Blastula stage. This embryo was radioiodinated before first cleavage and fixed 6 h 30 min after fertilization (at 18–20°C). The outer (apical) surfaces remain heavily labeled. The basolateral membranes of the blastomeres display a low silver grain density, but the cells were found to be distinctly outlined by silver grains after longer autoradiographic exposures. (*a*) Differential interference contrast. (*b*) Reflected light. Bar, 50 μm.

to be maintained at least until the late first cleavage stage for the following reason. Before the initiation of cleavage at least 50% of the equator of the embryo is labeled in cross-section (the lower part of the embryo, which contacts the agarose, does not label). At the late first cleavage stage the labeled band at the leading edge of the furrow encompasses ~18% of the perimeter of the cytoplasmic bridge. There is, therefore, a

reduction in the extent of equatorial label in the plane of cleavage from 50% to 18%.

The surface movements that we describe may be integrated into the contractile ring model of cytokinesis, with particular reference to theoretical predictions of the behavior of contractile ring filaments (27). The pigment stripe at the beginning of first cleavage is underlain by a band of cortical microfilaments oriented in the plane of cleavage (2, 12). As the band of microfilaments lengthens to encircle the embryo, it becomes the contractile ring. The contractile ring model assumes that the constituent filaments are mechanically coupled to the plasma membrane (22). The similar patterns of movement displayed by cortical pigment granules, surface-attached particles, and radioiodinated membrane proteins in the plane of cleavage suggests that surface and cortical elements may indeed be linked. Bearing in mind that our methods identify only the movements of surface membrane proteins and particle attachment sites, we propose that cytokinesis in the *Xenopus* embryo includes the following events: (*a*) Contraction of the band of cortical microfilaments under the pigment stripe provides the force for the lateral movement of cortex and tethered membrane proteins in the plane of cleavage and begins the indentation of the surface. Studies with cytochalasin B implicate actin microfilaments in the force generation required for furrowing (3). Because amphibian cleavage is unilateral and begins at the animal pole, the contraction is directed toward the cleavage initiation site. (*b*) Insertion of new membrane at the border of the pigmented and unpigmented surfaces allows the furrow to deepen. (*c*) Continued constriction by the contractile ring draws the leading edge ever deeper into the embryo. In this model the band of "pre-existing" membrane proteins, which we find at the leading edge of the furrow, represents a unique domain of surface membrane drawn into the furrow as a result of the mechanical attachment of trans-membrane proteins to microfilaments of the contractile ring.

References

1. Armstrong, P. B., M. M. Roberson, R. Nuccitelli, and D. Kline. 1982. Adhesion and polarity of amphibian embryo blastomeres. *Prog. Clin. Biol. Res.* 85B:235–287.
2. Bluemink, J. G. 1970. The first cleavage of the amphibian egg. An electron microscope study of the onset of cytokinesis in the egg of *Ambystoma mexicanum*. *J. Ultrastruct. Res.* 32:142–166.
3. Bluemink, J. G., and S. W. Delaat. 1973. New membrane formation during cytokinesis in normal and cytochalasin-B-treated eggs of *Xenopus laevis*. I. Electron-microscopical observations. *J. Cell Biol.* 59:89–108.
4. Bluemink, J. G., L. G. J. Tertoolen, P. H. J. TH. Ververgaert, and A. J. Verkleij. 1976. Freeze fracture electron microscopy of preexisting and nascent cell membrane in cleaving eggs of *Xenopus laevis*. *Biochem. Biophys. Acta.* 443:143–155.
5. Bluemink, J. G., and S. W. Delaat. 1977. Plasma membrane assembly as related to cell division. *In* The Synthesis, Assembly and Turnover of Cell Surface Components. G. Poste and G. L. Nicolson, editors. Elsevier/North Holland Biomedical Press, Amsterdam. 403–461.
6. Darribere, T., J. C. Boucaut, and V. Keil-Dlouha. 1982. Cell surface proteins in the early embryogenesis of *Pleurodeles waltii*. *Biochem. Biophys.*

Acta. 686:145–152.

7. Delaat, S. W., and J. G. Bluemink. 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.

8. Fraker, P. J., and J. C. Speck, Jr. 1978. Protein and cell membrane iodinations with a sparingly soluble chloramide, 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril. *Biochem. Biophys. Res. Commun.* 80:849–857.

9. Hara, K. 1971. Cinematographic observation of "surface contraction waves" (SCW) during the early cleavage of *Axolotl* eggs. *Wilhelm Roux's Arch. Dev. Biol.* 167:183–186.

10. Hollinger, T. G., and G. L. Corton. 1980. Artificial fertilization of gametes from the South African clawed frog, *Xenopus laevis. Gam. Res.* 3:45–57.

11. Holtfreter, J. 1943. Properties and functions of the surface coat in amphibian embryos. *J. Exp. Zool.* 93:251–323.

12. Kalt, M. R. 1971. The relationship between cleavage and blastocoel formation in *Xenopus laevis.* II. Electron microscopic observations. *J. Embryol. Exp. Morphol.* 26:51–66.

13. Kline, D., K. R. Robinson, and R. Nuccitelli. 1983. Ion currents and membrane domains in the cleaving *Xenopus* egg. *J. Cell Biol.* 97:1753–1761.

14. Kopriwa, B. M., and C. P. Leblond. 1962. Improvements in the coating technique of autoradiography. *J. Histochem. Cytochem.* 10:269–284.

15. Lopo, A. C., and V. D. Vacquier. 1980. Antibody to a sperm surface glycoprotein inhibits the egg jelly-induced acrosome reaction of sea urchin sperm. *Dev. Biol.* 79:325–333.

16. Markwell, M. A. K., and C. F. Fox. 1978. Surface-specific iodination of membrane proteins of viruses and eucaryotic cells using 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril. *Biochemistry.* 17:4807–4817.

17. Nagata, T., T. Nawa, and S. Yokota. 1969. A new technique for electron microscopic dry mounting radioautography of soluble compounds. *Histochemie.* 18:241–249.

18. Roberson, M. M., and P. B. Armstrong. 1979. Regional segregation of con-A receptors on dissociated amphibian embryo cells. *Exp. Cell Res.* 122:23–29.

19. Roberson, M. M., and P. B. Armstrong. 1980. Carbohydrate-binding component of amphibian embryo cell surfaces: restriction to surface regions capable of cell adhesion. *Proc. Natl. Acad. Sci. USA.* 77:3460–3463.

20. Sanders, E. J., and R. A. Dicaprio. 1976. A freeze-fracture and concanavalin-A binding study of the membrane of cleaving *Xenopus* embryos. *Differentiation.* 7:13–21.

21. Sawai, T. 1976. Movement of the cell surface and changes in surface area during cleavage in the newt's egg. *J. Cell Sci.* 21:537–551.

22. Schroeder, T. E. 1972. The contractile ring. II. Determining its brief existence, volumetric changes and vital role in cleaving Arbacia eggs. *J. Cell Biol.* 53:419–434.

23. Selman, G. G., and C. H. Waddington. 1955. The mechanism of cell division in the cleavage of the newt's egg. *J. Exp. Biol.* 32:700–733.

24. Singal, P. K., and E. I. Sanders. 1974. Cytomembranes in first cleavage *Xenopus* embryos. *Cell Tissue Res.* 154:189–209.

25. Spornitz, U. M. 1973. Lamellar bodies in oocytes of *Xenopus laevis* and their relation to the mode of fixation. *Experentia.* 29:589–591.

26. Stevens, A. R. 1966. High resolution autoradiography. *In* Methods in Cell Physiology. Vol. 2. D. Prescott, editor. Academic Press, Inc. New York. 255–310.

27. White, J. G., and G. G. Borisy. 1983. On the mechanisms of cytokinesis in animal cells. *J. Theor. Biol.* 101:289–316.