DEVELOPMENTAL BIOLOGY 104, 210-218 (1984)

Lateral Mobility of Plasma Membrane Lipids in Dividing *Xenopus* Eggs

P. A. T. TETTEROO, J. G. BLUEMINK,[1] W. J. A. G. DICTUS, E. J. J. VAN ZOELEN, AND S. W. DE LAAT

Hubrecht Laboratory, International Embryological Institute, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

Received November 8, 1983; accepted in revised form February 10, 1984

The lateral mobility of plasma membrane lipids was analyzed during first cleavage of *Xenopus laevis* eggs by fluorescence photobleaching recovery (FPR) measurements, using the lipid analogs 5-(*N*-hexadecanoyl)aminofluorescein ("HEDAF") and 5-(*N*-tetradecanoyl)aminofluorescein ("TEDAF") as probes. The preexisting plasma membrane of the animal side showed an inhomogeneous, dotted fluorescence pattern after labeling and the lateral mobility of both probes used was below the detection limits of the FPR method ($D \ll 10^{-10}$ cm^2/sec). In contrast, the preexisting plasma membrane of the vegetal side exhibited homogeneous fluorescence and the lateral diffusion coefficient of both probes used was relatively high (HEDAF, $D = 2.8 \times 10^{-8}$ cm^2/sec; TEDAF, $D = 2.4 \times 10^{-8}$ cm^2/sec). In the cleaving egg visible transfer of HEDAF or TEDAF from prelabeled plasma membrane to the new membrane in the furrow did not occur, even on the vegetal side. Upon labeling during cleavage, however, the new membrane was uniformly labeled and both probes were mobile, as in the vegetal preexisting plasma membrane. These data show that the membrane of the dividing *Xenopus* egg comprises three macrodomains: (i) the animal preexisting plasma membrane; (ii) the vegetal preexisting plasma membrane; (iii) the new furrow membrane.

INTRODUCTION

Early development of amphibians is characterized by a process of rapid successive cell divisions or cleavages, following fertilization of the egg. Eggs of *Xenopus laevis* (diameter ca. 1.5 mm) can readily be obtained, are easy to manipulate, and provide a suitable experimental system for analyzing the cell cycle. In *Xenopus* eggs the first division starts approximately 80 min after fertilization. Up to the midblastula stage each successive cleavage takes about 30 min. During this period development proceeds in the absence of DNA transcription, while DNA replication and synchrony of cell divisions are controlled by a cytoplasmic clock mechanism (Hara *et al.*, 1980). Evidence is now available that transcription of the embryonic genome is switched on when a critical DNA/cytoplasmic ratio is reached at the so-called midblastula transition, or MBT (Newport and Kirschner, 1982a,b).

Apart from DNA replication the early divisions of the *Xenopus* egg require the rapid assembly of large amounts of plasma membrane material to constitute the intercellular membranes. It has been estimated that the first three divisions involve the formation of approximately 1.3 mm^2 of new plasma membrane for each cycle (Bluemink and de Laat, 1973). Earlier studies from this laboratory have demonstrated that in the dividing *Xenopus* egg new membrane formation is restricted to the furrow region, where plasma membrane growth takes place by fusion and incorporation of membrane material from the underlying cytoplasm (Bluemink and de Laat, 1973; Bluemink and de Laat, 1977). Under experimental conditions which prevent the formation of junctions between the blastomeres, i.e., removal of the vitelline envelope or exposure of the dividing egg to cytochalasin B (Bluemink, 1971a; de Laat *et al.*, 1973), the preexisting surface membrane and the newly formed membrane constitute a continuous plasma membrane (Bluemink and de Laat, 1973). Nevertheless, striking structural, biochemical, and physiological differences are being maintained between these membrane domains by mechanisms as yet unknown. The egg surface occupied by the preexisting membrane is rough and is underlain by a cortical cytoplasmic layer of filamentous material and pigment granules (Bluemink, 1971b). In ultrathin sections the preexisting plasma membrane shows a well-defined trilaminar organization; however, the surface made up by the newly formed membrane is smooth, not underlain by filaments or pigment, and exhibits an ill-defined trilaminar organization (Bluemink and de Laat, 1973; de Laat and Bluemink, 1974). Upon freeze fracturing, the preexisting membrane region shows a high density of intramembranous particles (IMPs), particularly on the E face, whereas the IMP density in the newly formed membrane is strikingly lower (Bluemink *et al.*, 1976; Bluemink and Tertoolen, 1978; Sanders and DiCaprio, 1976).

In addition to ultrastructural differences, the two membrane regions exhibit completely different physiological properties. The preexisting, outer plasma mem-

[1] To whom correspondence should be addressed.

0012-1606/84 $3.00

brane is very impermeable to ions and molecules, in contrast to the new membrane (de Laat *et al.*, 1973; de Laat and Bluemink, 1974). Also in normally developing embryos, where junction formation is not prevented, a difference in ionic conductance of more than an order of magnitude is being maintained between the outer membrane and the intercellular membrane, at least up to the fourth cleavage (de Laat and Barts, 1976; de Laat *et al.*, 1976). Furthermore, the preexisting membrane shows no permselectivity for cations, whereas the newly formed membrane in the furrow is relatively permeable to K^+ ions, as expected for plasma membranes of animal cells (de Laat *et al.*, 1974). Relatively little is known about the biochemical characteristics of the two membrane regions, but differences have been reported in their binding properties for lanthanum (Bluemink and de Laat, 1973), ruthenium red (Singal and Sanders, 1974), and lectins (Geuskens and Tencer, 1979; Nosek, 1978; Roberson and Armstrong, 1979, 1980). Together, these data demonstrate that the regional assembly of the furrow membrane, although it occurs in continuity with the preexisting surface membrane, results in the appearance of two distinct membrane domains.

As these domains are maintained even in the absence of apparent structural membrane specializations at their borderline, the question arises what physico-chemical properties are involved in establishing and maintaining them. Earlier we have approached this question by studying possible regional differences in the lateral mobility of membrane lipids in both preexisting and newly assembled plasma membrane regions, using the fluorescence photobleaching recovery (FPR) method (Axelrod *et al.*, 1976) with fluorescein-conjugated fatty acids as lipid probes. These experiments have demonstrated once again the peculiar nature of the *Xenopus* egg plasma membrane (Dictus *et al.*, 1983). In the unfertilized egg an animal-vegetal gradient in the lateral diffusion of membrane lipids is detectable, the diffusion coefficient (D) being fivefold smaller in the animal ($D = 1.5 \times 10^{-8}$ cm^2/sec) than in the vegetal plasma membrane ($D = 7.6 \times 10^{-8}$ cm^2/sec). Upon fertilization this polarity is strongly (100×) enhanced, probably by the preferential incorporation of membrane from cortical granules into the animal plasma membrane. As a result two distinct macrodomains are established within the plasma membrane of the uncleaved egg, the transition being sharp and roughly coinciding with the boundary between the presumptive ectoderm and endoderm. Within the animal region lipids are completely immobilized ($D \ll 10^{-10}$ cm^2/sec), whereas their mobility is only slightly reduced on the vegetal side ($D = 2.8 \times 10^{-8}$ cm^2/sec) (Dictus *et al.*, 1983).

In the present study we demonstrate that the regional assembly of the furrow membrane, under conditions in which junction formation between blastomeres is prevented, results in the formation of a third macrodomain, in which membrane lipids can diffuse rapidly ($D = 2.2 \times 10^{-8}$ cm^2/sec) without, however, exchanging with lipids of the preexisting plasma membrane.

MATERIALS AND METHODS

Materials. Eggs of *Xenopus laevis* were collected in modified amphibian Ringer solution (MMR; Kirschner *et al.*, 1980) from females stimulated by human chorionic gonadotropin (Physec LEO Pharm.). Fertilized eggs were obtained as described before (de Laat *et al.*, 1973) and selected by the appearance of a dark sperm entrance point after the cortical reaction. Jelly coats were digested with 2% L-cysteine/HCl and 0.2% papaine in 25% MMR at pH 7.8 (modified after Spiegel, 1951). Vitelline envelopes were removed with forceps. Eggs were kept in 25% MMR in Falcon petri dishes with a 4% agar bottom stained with a few droplets of india ink. In eggs devoid of their vitelline envelope and kept at room temperature, first cleavage started approximately 80 min after fertilization. The initial assembly of the cleavage membrane becomes manifest 7 min later. The interval between successive cleavages is 30 min. In some experiments blastomere formation (cytokinesis) was prevented by exposing the egg to 10 μg cytochalasin B/ml (Bluemink and de Laat, 1973).

Fluorescent labeling. 5-(*N*-Hexadecanoyl)aminofluorescein or 5-(*N*-tetradecanoyl)aminofluorescein (HEDAF and TEDAF, respectively; Molecular Probes, Plano, Texas) were used as fluorescent lipid probes and were dissolved in ethanol up to approximately 10 mg/ml and stored in the dark at −20°C. Prior to labeling, this solution was diluted 100-fold in 25% MMR, resulting in a saturated stock solution. The stock solution was centrifuged for 1 min at 700g to precipitate undissolved probe. Incubation media were made by 30-fold dilution of the saturated supernatant in 25% MMR. After removing the vitelline envelope the eggs were labeled for 10 min at room temperature and washed three times with 25% MMR containing 0.1% fatty acid-free albumin, and again three times with medium without albumin. Eggs labeled during cleavage were kept at 10°C to inhibit further membrane growth or, alternatively, mildly prefixed in 4% formaldehyde about 20 min after the onset of cleavage (30 min at room temperature).

FPR measurements. Lateral mobility characteristics of membrane lipids were measured using the fluorescence photobleaching recovery method (FPR), essentially as described before (Axelrod *et al.*, 1976; Jacobson *et al.*, 1976; Koppel *et al.*, 1976), using the beam-splitting system as described by Koppel (1979). The fluorescent probe molecules in the egg membrane were excited with

a laser beam (Argon ion laser, Coherent CR-4) in the Gaussian mode at 488 nm, focused with a Zeiss 25×/0.8 Plan Neofluor water immersion lens to a spot with an $1/e^2$ radius of 3.7 μm, giving rise to a power of 2.9–4.6 W/cm^2 in the plane of the plasma membrane. Laser mode and radius were calibrated by scanning a fluorescent glass fiber through the laser beam with known velocity (L. G. J. Tertoolen, unpublished). The fluorescence intensity was measured by a single photon counting system, as described earlier (Boonstra *et al.*, 1982). In the illuminated region 40–60% of the fluorescence intensity was bleached by a brief (20–60 m/sec), approximately 1000- to 2500-fold increase in laser intensity. An Apple II Plus microcomputer controlled the duration of the bleach pulse and the intensity of the laser beam, and was also used for on-line analysis of the data. Final calculations were done following the method described by Axelrod *et al.* (1976), using the mathematical analysis of van Zoelen *et al.* (1983). To reduce movements of the eggs they were placed between two platinum wires and slightly compressed under a thin glass coverslip. Measurements were made at room temperature (20°C), except for measurements in the region of the cleavage membrane of unfixed eggs, which were made at 10°C to prevent further membrane growth.

Photographs. Fluorescence photographs were made with a Leitz Orthoplan fluorescence microscope with an image intensifier type XX 1340 (B. V. Delft, Roden, the Netherlands) connected to a Nikon 70- to 105-mm F 2.8 zoom objective. An HBO 100-W mercury arc lamp provided the illumination. Kodak Plus X Pan 35-mm negative film was used and processed in Rodinal.

Scanning electron microscopy. After removal of the jelly coat and vitelline membrane eggs were fixed for 1 hr in 25% MMR containing 2.5% glutaraldehyde, washed 3–4 times in 25% MMR, and postfixed for 1 hr in 25% MMR containing 2% osmium tetroxide at 4°C. After washing 3–4 times with distilled water the eggs were kept overnight in distilled water, washed 3–4 times in distilled water, and dehydrated with 2,2-dimethoxy propane/HCl (Maser and Trimble, 1977). After critical-point drying with liquid carbon dioxide, the eggs were fixed on preparation tables and sputtered with gold at 750 V during 6 min from a distance of about 2 cm, using argon in a vacuum of 0.05 Torr. The eggs were examined and photographed in a Cambridge Stereoscan 600 M. Preparation tables with eggs were kept in a vacuum exsiccator above phosphorous pentoxide.

RESULTS

Cell Surface Architecture

The *Xenopus* egg normally develops inside a vitelline envelope. This provides a mechanical support for the large egg (diameter ca. 1.5 mm) and thus prevents the daughter cells from separating upon cleavage (Fig. 1a). First cleavage is essentially a two-phase process. It is initiated by the contraction of a microfilamentous ring underlying the plasma membrane, which leads to the formation of the cleavage furrow. New membrane assembly, from membrane precursors present in the underlying cytoplasm, starts 7 min after the onset of cleavage (20°C) at the tip of the advancing cleavage furrow. The result is the formation of a double sheet of intercellular membrane which separates the two blastomeres (Bluemink and de Laat, 1973). Under normal conditions the newly formed membrane is not directly accessible from the outside (Fig. 1a). It can be exposed, however, by removing the vitelline envelope before first cleavage. The egg then flattens under gravity, the daughter cells consequently separate, and the newly formed (normally intercellular) membrane is exposed in the open furrow (Fig. 1b). No indications have been found that the structural or physiological properties of this membrane region are modified under these conditions (Bluemink and de Laat, 1973; de Laat and Bluemink 1974; de Laat *et al.*, 1978).

Scanning electron micrographs show that the surface architecture of the preexisting and the new membrane differ markedly (Fig. 1c) and that the transition between the two regions is sharp (Fig. 1d). The preexisting outer surface is rough, extending numerous protrusions, which at the animal pole are approximately 1 μm in length and in diameter. Toward the vegetal pole the protrusion become shorter and less numerous (not shown). In contrast, the newly formed membrane is very smooth. It should be noted that a thin band of rough surface is detectable within the new membrane area in the middle of the furrow (Fig. 1c; see also Fig. 2f). This probably represents a remnant of preexisting membrane material (see below).

Fluorescence Labeling

Incubation of eggs before first cleavage with either one of the fluorescein-conjugated fatty acids, HEDAF or TEDAF, results in the incorporation of these lipid probes into the egg membrane. On the animal side a dotted pattern is seen, with fluorescent dots of approximately 1 μm in diameter (Fig. 2a), while a homogeneous distribution is observed on the vegetal side of the egg (not shown). As shown elsewhere (Dictus *et al.*, 1983) the transition between dotted and homogeneous fluorescence is sharp and coincides with the transition in pigmentation in the equatorial zone. We have argued that the distribution of the lipid probes is not correlated with cell surface architecture, since the pattern of protrusions changes gradually along the animal–vegetal

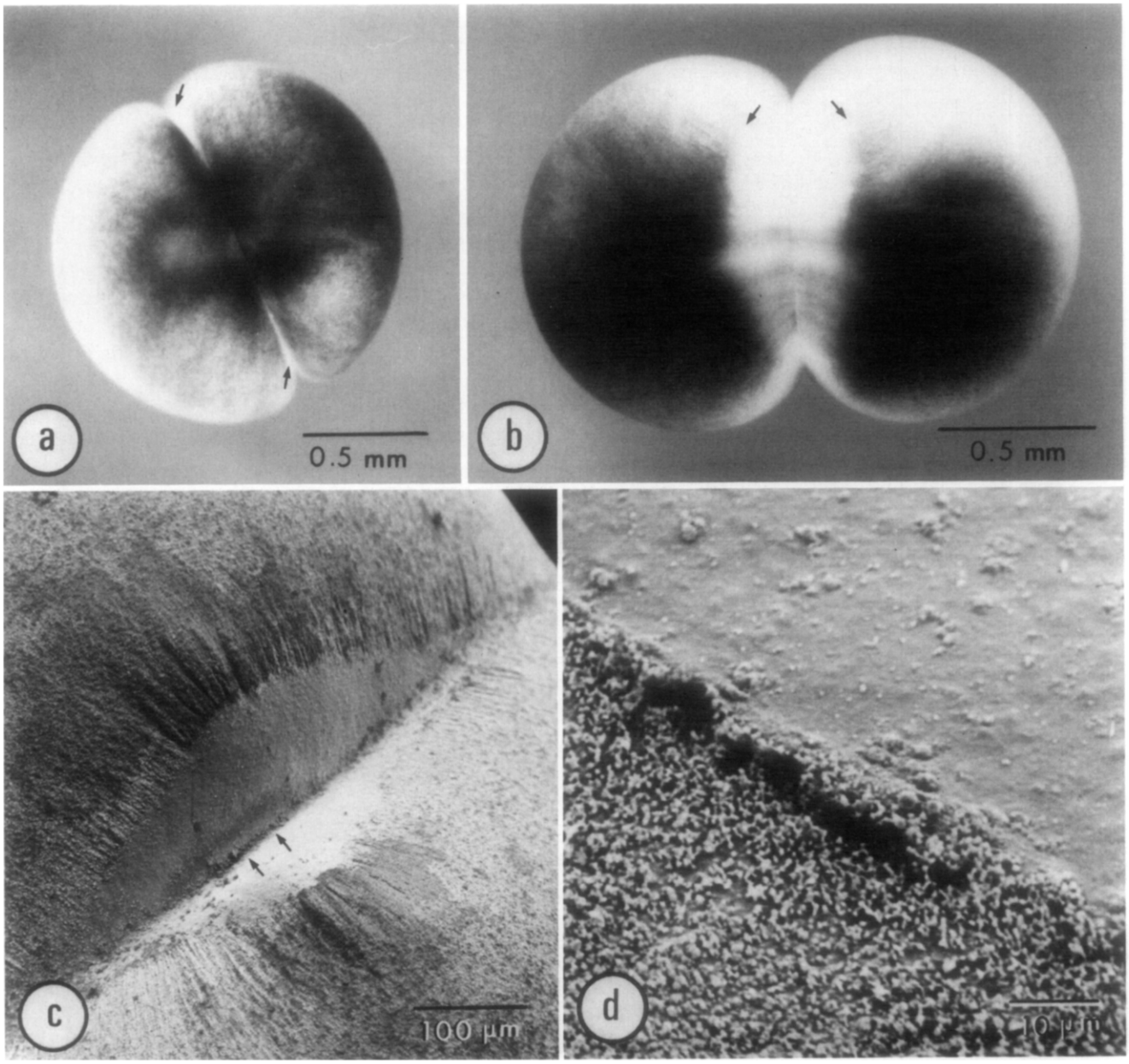

FIG. 1. *Xenopus* eggs during first cleavage. (a) Egg with vitelline envelope intact. The two blastomeres are closely apposed and hardly any new membrane (unpigmented surface, arrows) is visible. ×40, bar 0.5 mm. (b) Egg without vitelline envelope. The two blastomeres have separated, new membrane in the furrow (unpigmented zone) is exposed to the medium. Arrows point at the borderline. ×50, bar 0.5 mm. (c) Scanning electron micrograph of an open furrow during first cleavage. The borderline between the rough surface of preexisting membrane and the smooth surface of new membrane is evident. Arrows point at a narrow zone of preexisting membrane (rough surface) at the bottom of the furrow. ×175, bar 100 µm. (d) Scanning electron micrograph of the sharp borderline between new membrane (smooth surface, upper right) and preexisting membrane (rough surface, lower left). ×1375, bar 10 µm.

axis. It is also not due to the presence of pigment granules, since albino mutant eggs show a similar distribution (Dictus *et al.*, 1983). It may thus be concluded that the properties of the animal and vegetal halves of the *Xenopus* egg membrane differ in their uptake properties for lipid probes, and that within the animal egg membrane subdomains are present in which they are incorporated preferentially.

When labeled during first cleavage the newly formed membrane shows a homogeneous distribution of HEDAF (Fig. 2b) or TEDAF, whereas the labeling of the preexisting membrane is still as described. Measurements of the local fluorescence intensity, immediately after labeling, indicated that probe uptake in the preexisting and the newly formed membrane regions is approximately equal (Table 1a). However, with time the

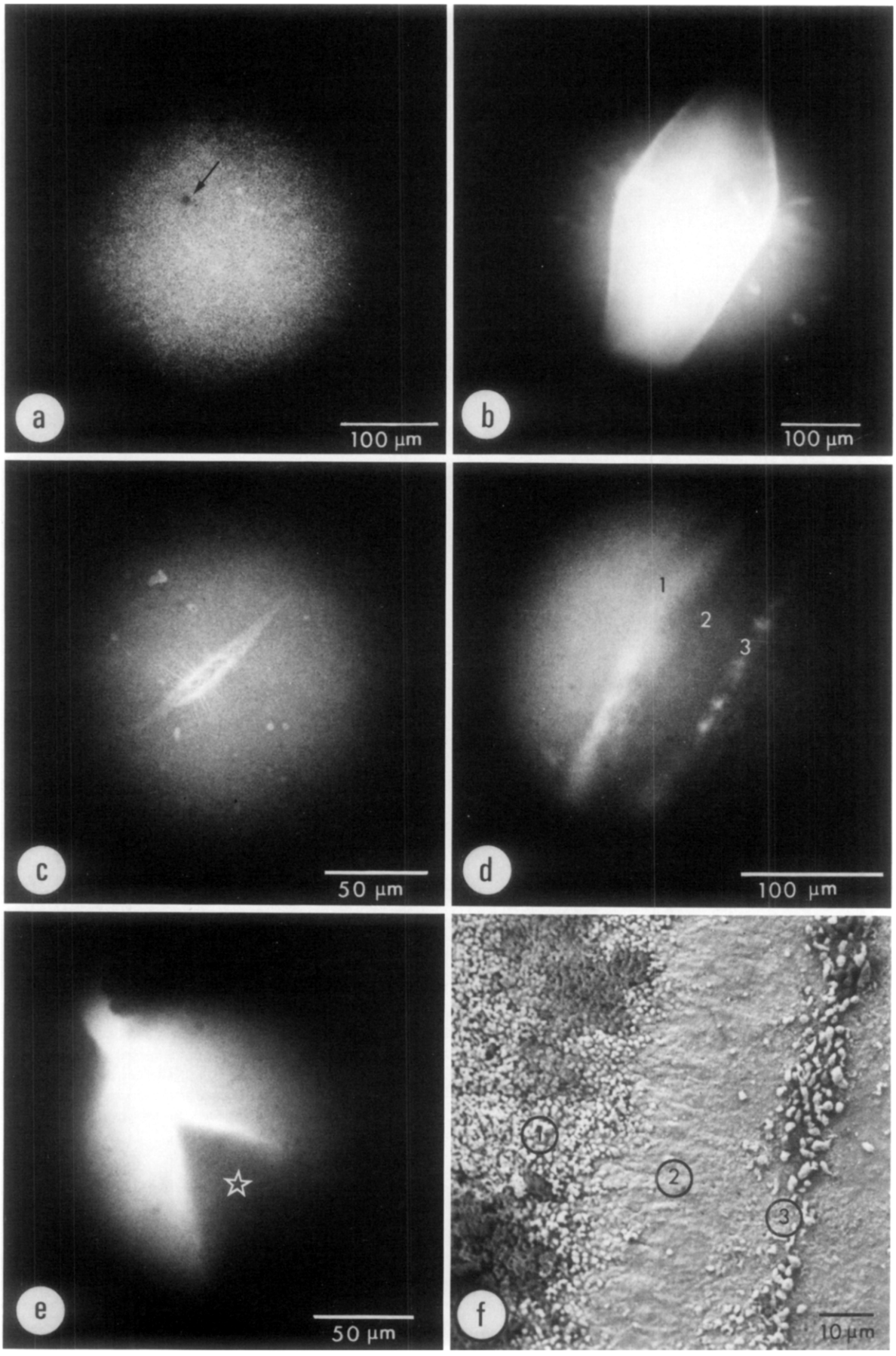
a
100 µm
b
100 µm
c
50 µm
d
1
2
3
100 µm
e
50 µm
f
1
2
3
10 µm

TABLE 1
RELATIVE DISTRIBUTION OF HEDAF OVER THE PREEXISTING AND NEW MEMBRANE IN DIVIDING *Xenopus laevis* EGGS

	Relative fluorescence intensity	
	Measured	Corrected[a]
a. Eggs labeled during first cleavage	1.2 ± 0.4	0.8 ± 0.3
b. Eggs labeled before first cleavage	12 ± 4.0	7.7 ± 2.9

Note. Relative probe distribution is expressed as the ratio of the fluorescence intensity in an area of 5-μm radius in the preexisting membrane over that in the new membrane, 12 min after the onset of first cleavage. Data are expressed as mean values ± SEM (n = 3).

[a] Corrected for differences in membrane area per unit of cell surface area, see de Laat and Bluemink (1974).

fluorescence intensity per unit area of new membrane decreases, probably as a result of continued membrane growth and consequent dilution of the probe molecules.

A first indication for the existence of a diffusion barrier between the preexisting and the newly formed membrane was obtained by monitoring the lipid probe distribution during cleavage in eggs labeled before first cleavage. At the onset of cleavage such eggs show a bright fluorescent stripe at the animal pole (Fig. 2c) due to the local contraction of the preexisting egg surface and the consequent condensation of fluorescent membrane material. New membrane appears as a nonfluorescent region distinct from the fluorescent, preexisting membrane (Fig. 2d), and remains so for at least about 25 min, when the formation of the cleavage membrane has proceeded to the vegetal pole (Fig. 2e). Interestingly, a narrow fluorescent band remains visible in the plane of cleavage (Fig. 2d), bisecting the cleavage membrane. This band probably represents preexisting membrane material (cf. Fig. 2f) originally located at the site of surface contraction at the onset of cleavage. Its position suggests that the cleavage membrane is formed by bilateral incorporation of new membrane material on either side of the plane of cleavage. The restriction of probe redistribution during cleavage was quantified by measurements of the local fluorescence intensity (Table 1b). After correction for the differences in membrane area (de Laat and Bluemink, 1974) the fluorescence intensity per unit membrane was about eightfold greater in the preexisting membrane than in the newly formed cleavage membrane.

Taken together, these fluorescence labeling characteristics indicate that the preexisting and the new membrane region are distinct domains: although the lipid probes (HEDAF and TEDAF) are incorporated equally well into both regions, their distribution within the domains differs and their diffusion from the preexisting to the new membrane is strongly restricted.

Lateral Mobility of Plasma Membrane Lipids

As described in detail elsewhere (Dictus *et al.*, 1983) plasma membrane lipid mobility shows a pronounced animal/vegetal polarity in the *Xenopus* egg. In the unfertilized egg the diffusion coefficient (D) of HEDAF was found to be fivefold smaller in the animal part of the plasma membrane than in the vegetal part (1.5 and 7.6×10^{-8} cm^2/sec, respectively). Upon fertilization this polarity is strongly enhanced (see Table 2, preex. membrane). Probably as a result of the preferential incorporation of cortical granule membrane into the animal plasma membrane, membrane lipids in this region become completely immobilized on the time scale of FPR measurements ($D < 10^{-10}$ cm^2/sec), while only a slight reduction is seen in the vegetal plasma membrane ($D = 2.8 \times 10^{-8}$ cm^2/sec). Thus, two macrodomains are established in the plasma membrane, which possibly foreshadow their different fates in the ecto- and endoderm, respectively, of the later embryo (Dictus *et al.*, 1983).

FPR measurements in the region of new membrane formation are relatively difficult to accomplish. Not only is the dividing egg, when devoid of its vitelline membrane, extremely fragile and thus susceptible to damage during experimental manipulation, but problems are also posed by the process of membrane growth itself. As mentioned above, the formation of new membrane in the furrow occurs at room temperature at a rate of

FIG. 2. (a–e) Fluorescence micrographs of eggs without vitelline envelope, labeled with HEDAF. Only a small area of the total egg surface is in focus. (a) Dotted fluorescence seen in the preexisting plasma membrane on the animal side. The 7.5-μm dark spot (arrow) is due to photobleaching and remains visible for about 15 min. ×180, bar 100 μm. (b) Egg labeled during first cleavage; animal view. The preexisting plasma membrane and the new membrane in the furrow are both labeled. ×140, bar 10 μm. (c) Egg labeled before first cleavage, photographed at the beginning of cleavage. The bright fluorescent stripe at the animal pole is due to local surface contraction. ×340, bar 50 μm. (d) Egg labeled before first cleavage, photographed from the animal side in an advanced stage of cleavage. Distinct border between the fluorescent preexisting plasma membrane (1) and the nonfluorescent new membrane (2), with a fluorescent band of preexisting plasma membrane (3) in the furrow. See also Fig. 2f. ×240, bar 100 μm. (e) Egg labeled before first cleavage, photographed from the side at the end of first cleavage, showing fluorescent preexisting membrane and nonfluorescent new membrane in the vegetal furrow (asterisk). ×340, bar 50 μm. (f) Scanning electron micrograph of an open furrow on the animal pole. Preexisting membrane (rough surface, 1) is well delimited from the new membrane (smooth surface, 2), which contains a narrow zone of preexisting membrane (3) in the middle of the furrow. Compare with Fig. 2d. ×970, bar 10 μm.

TABLE 2
LATERAL MOBILITY PROPERTIES OF PLASMA MEMBRANE LIPIDS IN DIVIDING *Xenopus laevis* EGGS

	HEDAF			TEDAF		
	D	MF	*N*	*D*	MF	*N*
Preexisting membrane[a]						
Animal half (20°C)	<0.01	<0.05	30 (10)	<0.01	<0.05	11 (4)
Vegetal half (20°C)	2.8 ± 0.4	0.66 ± 0.05	19 (5)	2.4 ± 0.2	0.64 ± 0.02	14 (4)
Vegetal half (10°C)	1.6 ± 0.2	0.74 ± 0.04	11 (4)			
Cleavage membrane[b]						
Unfixed (10°C)	2.2 ± 0.2	0.74 ± 0.06	9 (2)	—	—	—
Fixed (20°C)	3.0 ± 0.7	0.60 ± 0.03	9 (5)	2.3 ± 0.2	0.59 ± 0.03	12 (3)

Note. Diffusion coefficients (D, 10^{-8} cm^2/sec) and mobile fractions (MF) of the lipid probes HEDAF and TEDAF are given as mean ± SEM. Number of experiments (N) are presented as number of measurements (number of eggs).

[a] Values from the related paper by Dictus *et al.* (1983).

[b] Plasma membrane lipid mobility in the newly formed cleavage membrane was determined under two conditions of experimentally restricted membrane growth (see text).

approximately 0.1 mm^2/min, so that even on the scale of FPR measurements (beam radius 3.7 μm, duration 10 to 30 sec) no steady state is approached. For that reason membrane growth was inhibited to allow for meaningful FPR measurements. Two inhibitory conditions were used: (i) eggs were kept consistently at 10°C during labeling and measurement; and (ii) eggs were incubated in 10 μg/ml cytochalasin B (CB) during initial cleavage for maximal exposure of new membrane in an open furrow (de Laat and Bluemink, 1974), and mildly fixed 20 min after the onset of cleavage, prior to FPR measurements at 20°C. Under both conditions FPR measurements made in the region of the new membrane yielded rapid recovery after photobleaching, with the characteristics of a true diffusion process although recovery of the fluorescence was only partial (Table 2). Essentially identical results were obtained with both lipid probes. Control experiments at 10°C showed that the decrease in temperature only slightly affects the lateral mobility as tested in the vegetal half (see Table 2). Under the same conditions the lateral mobility in the animal half remained undetectable (not shown).

DISCUSSION

The formation of the first cleavage membrane in eggs of *Xenopus laevis* takes place by local fusion and incorporation of membrane precursors from the underlying cytoplasm into the plasma membrane of the furrow region (Bluemink and de Laat, 1973, 1977). Earlier ultrastructural and electrophysiological studies have shown that under conditions where junction formation between the blastomeres is prevented, this newly formed membrane has properties very distinct from the preexisting plasma membrane, despite the fact that the two regions form a continuous membrane (Bluemink and de Laat, 1973; de Laat and Bluemink, 1974). The mechanism involved in maintaining these regional differences is unknown.

In the present study we have examined the lateral mobility properties of membrane lipids in the preexisting plasma membrane and in the furrow region by the FPR method, using the fluorescent fatty acid analogs HEDAF and TEDAF as probe molecules. Taking into account that no probe diffusion is detectable from the preexisting plasma membrane to the region of new membrane, even at the vegetal pole, our results show that the plasma membrane of the dividing *Xenopus* egg comprises three macrodomains: (i) the animal preexisting region (inhomogeneous probe distribution, no lipid mobility); (ii) the vegetal preexisting region (homogeneous probe distribution, high lipid mobility); and (iii) the newly formed membrane in the furrow region (homogeneous probe distribution, high lipid mobility).

The peculiar nature of the preexisting membrane and the possible developmental significance of the distinct features of the animal and vegetal plasma membrane regions have been discussed in detail elsewhere (Dictus *et al.*, 1983). In the same study arguments have been given that HEDAF and TEDAF are indeed incorporated into the lipid phase of the plasma membrane. Whereas the integrity of the macrodomains in the plasma membrane of the uncleaved fertilized egg can be explained by the physical state of the animal plasma membrane lipids, which prevents exchange of membrane components between the animal and vegetal plasma membrane regions (Dictus *et al.*, 1983), a more complex situation is encountered during first cleavage. Under the conditions used in the present study the new membrane in the furrow is formed in apparent continuity with the

preexisting plasma membrane. Within the new membrane in the furrow, as in the preexisting vegetal plasma membrane region, the majority of lipid molecules can diffuse rapidly. Although the D and MF values of the vegetal region are very similar to those of the new membrane region, probe molecules are unable to cross the boundary between these domains, as judged by the probe distribution in dividing eggs labeled before cleavage (Fig. 2e). No evidence exists for the presence of a physical barrier in the plasma membrane to separate these domains. Hence this finding does not readily fit in with the concept of the fluid mosaic membrane as formulated by Singer and Nicolson (1972).

In this context it is worth noting that the MF value in the new membrane region is also lower than might be expected for a lipid probe. Partial mobility of the lipid probe might be due to the existence of lipid microdomains but at present no evidence for this is available in the *Xenopus* egg. The occurrence of lipid microdomains as detected by partial recovery of a lipid probe, seems to be a characteristic of the plasma membrane in early embryonic cells and has been reported earlier for sea urchin eggs (Campisi and Scandella, 1980; Wolf *et al.*, 1981a) and mouse eggs (Wolf *et al.*, 1981b; Klausner and Wolf, 1980; Peter and Richter, 1981). From another study (Gadenne *et al.*, 1984) we know that in dissociated early blastula cells the MF value of the surface membrane is still 72.5 ± 4.6% but the D value is lower: 2.39 $\times 10^{-9}$ cm^2/sec. It seems that throughout the period of rapid cleavages the MF value remains constant whereas the D value decreases. More study will be required to find out whether the decrease in the diffusion coefficient is causally related to differentiation of the surface membrane and the expression of new cell behavior (see also Gadenne *et al.*, 1984).

It is not only the lateral mobility of membrane lipid that is restricted in the plasma membrane of the dividing *Xenopus* egg. Earlier studies by us have provided evidence that also a large difference in IMP density is maintained between the preexisting and new membrane regions (Bluemink *et al.*, 1976; de Laat *et al.*, 1978; Sanders and DiCaprio, 1976), suggesting that the exchange of membrane proteins between these domains is also restricted. As yet we have no explanation for this unique phenomenon. It seems likely, however, that specific cytoplasm/membrane interactions are involved. It is at the borderline between the preexisting and the cleavage membrane regions that the formation of tight junctions occurs during uninterrupted cleavage, and moreover this borderline coincides with a sharp boundary of the dense, cortical cytoplasmic layer underlying the preexisting plasma membrane, which is absent under the cleavage membrane. Further studies are required to elucidate this possible relationship.

We thank the instrumentation and electronics departments of this institute for their essential contribution to the realization of the fluorescence photobleaching setup, in particular Mr. J. Beeker, Mr. H. Krielen, Mr. W. Leurink, and Mr. H. Reitsma. We also thank Dr. J. Faber for editorial assistance and Ms. C. Kroon-Lobo for the preparation of the illustrations.

REFERENCES

AXELROD, D., KOPPEL, D. E., SCHLESSINGER, J., ELSON, E., and WEBB, W. W. (1976). Mobility measurements by analysis of fluorescence photobleaching recovery kinetics. *Biophys. J.* **16**, 1055–1069.

BLUEMINK, J. G. (1971a). Effects of cytochalasin B on surface contractility and cell junction formation during egg cleavage in *Xenopus laevis*. *Cytobiologie* **3**, 176–187.

BLUEMINK, J. G. (1971b). Cytokinesis and cytochalasin-induced furrow regression in the first cleavage zygote of *Xenopus laevis*. *Z. Zellforsch.* **121**, 102–126.

BLUEMINK, J. G., and DE LAAT, S. W. (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.

BLUEMINK, J. G., and DE LAAT, S. W. (1977). Plasma membrane assembly as related to cell division. *In* "The Synthesis Assembly and Turnover of Cell Surface Components. Cell Surface Reviews," (G. Poste and G. L. Nicolson, eds.), Vol. 4, pp. 403–461. North-Holland, Amsterdam.

BLUEMINK, J. G., and TERTOOLEN, L. G. J. (1978). The plasma membrane IMP pattern as related to animal/vegetal polarity in the amphibian egg. *Dev. Biol.* **62**, 334–343.

BLUEMINK J. G., TERTOOLEN, L. G. J., VERVERGAERT, P. H. J. TH., and VERKLEIJ, A. J. (1976). Freeze-fracture electron microscopy of preexisting and nascent cell membrane in cleaving eggs of *Xenopus laevis*. *Biochim. Biophys. Acta* **443**, 143–155.

BOONSTRA, J., NELEMANS, S. A., FEIJEN, A., BIERMAN, A., VAN ZOELEN, E. J. J., VAN DER SAAG, P. T., and DE LAAT, S. W. (1982). Effect of fatty acids on plasma membrane lipid dynamics and cation permeability in neuroblastoma cells. *Biochim. Biophys. Acta* **692**, 321–329.

CAMPISI, J., and SCANDELLA, C. J. (1980). Calcium-induced decrease in membrane fluidity of sea urchin egg cortex after fertilization. *Nature (London)* **286**, 185–186.

DE LAAT, S. W., and BARTS, P. W. J. A. (1976). New membrane formation and intercellular communication in the early *Xenopus* embryo. II. Theoretical analysis. *J. Membr. Biol.* **27**, 131–151.

DE LAAT, S. W., BARTS, P. W. J. A., and BAKKER, M. I. (1976). New membrane formation and intercellular communication in the early *Xenopus* embryo. I. Electrophysiological analysis. *J. Membr. Biol.* **27**, 109–129.

DE LAAT, S. W., and BLUEMINK, J. G. (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.

DE LAAT, S. W., BLUEMINK, J. G., and VAN DER SAAG, P. T. (1978). Membrane assembly during the cell cycle. *Biol. Cell.* **32**, 115–120.

DE LAAT, S. W., BUWALDA, R. J. A., and HABETS, A. M. M. C. (1974). Intracellular ionic distribution, cell membrane permeability and membrane potential of the *Xenopus* egg during first cleavage. *Exp. Cell Res.* **89**, 1–14.

DE LAAT, S. W., LUCHTEL, D., and BLUEMINK, J. G. (1973). The action of cytochalasin B during egg cleavage in *Xenopus laevis:* Dependence on cell membrane permeability. *Dev. Biol.* **31**, 163–177.

DICTUS, W. J. A. G., VAN ZOELEN, E. J. J., TETTEROO, P. A. T., TERTOOLEN, L. G. J., DE LAAT, S. W., and BLUEMINK, J. G. (1984). Lateral mobility

of plasma membrane lipids in *Xenopus* eggs regional differences related to animal/vegetal polarity become extreme upon fertilization. *Dev. Biol.*, **101**, 201-211.

GADENNE, M., VAN ZOELEN, E. J. J., TENCER, R., and DE LAAT, S. W. (1984). Increased rate of capping of Con-A receptors during early development is related to changes in protein and lipid mobility. *Dev. Biol.*, in press.

GEUSKENS, M., and TENCER, R. (1979). An ultrastructural study of the effects of wheat germ agglutinin (WGA) on cell cortex organization during the first cleavage of *Xenopus laevis* eggs; I. Inhibition of furrow formation. *J. Cell. Sci.* **37**, 47-58.

HARA, K., TYDEMAN, P., and KIRSCHNER, M. W. (1980). A cytoplasmic clock with the same period as the division cycle in *Xenopus* eggs. *Proc. Natl. Acad. Sci. USA* **77**, 462-466.

JACOBSON, K., DERZKO, Z., WU, E. S., HOU, Y., and POSTE, G. (1976). Measurements of the lateral mobility of cell surface components in single living cells by fluorescence recovery after photobleaching. *J. Supramol. Struct.* **5**, 565-576.

KIRSCHNER, M. W., GERHART, J. C., HARA, K., and UBBELS, G. A. (1980). Initiation of the cell cycle and establishment of bilateral symmetry in *Xenopus* eggs. *In* "The Cell Surface: Mediator of Developmental Processes" (S. Subtelny and N. K. Wessells, eds.), pp. 187-215. Academic Press, New York.

KLAUSNER, R. D., and WOLF, D. E. (1980). Selectivity of fluorescent lipid analogs for lipid domains. *Biochemistry* **19**, 6119-6203.

KOPPEL, R. D., AXELROD, D., SCHLESSINGER, J., ELSON, E. L., and WEBB, W. W. (1976). Dynamics of fluorescence marker concentration as a probe of mobility. *Biophys. J.* **16**, 1315-1329.

MASER, M. D., and TRIMBLE, J. J. (1977). Rapid hemical dehydration of biological samples for scanning electron microscopy using 2,2 dimethoxypropane. *J. Histochem. Cytochem.* **25**, 247-251.

NEWPORT, J., and KIRSCHNER, M. (1982a). A major developmental transition in early *Xenopus* embryos. I. Characterization and timing of cellular changes at the midblastula stage. *Cell* **30**, 675-686.

NEWPORT, J., and KIRSCHNER, M. (1982b). A major developmental transition in early *Xenopus* embryos. II. Control of the onset of transcripiton. *Cell* **30**, 687-696.

NOSEK, J. (1978). Changes in the cell surface coat during the development of *Xenopus laevis* embryos, detected by lectins. *Wilhelm Roux's Arch.* **184**, 181-193.

PETERS, R., and RICHTER, H. P. (1981). Translational diffusion in the plasma membrane of sea urchin eggs. *Dev. Biol.* **86**, 285-293.

ROBERSON, M. M., and ARMSTRONG, P. B. (1979). Regional segregation of Con A receptors on dissociated amphibian embryo cells. *Exp. Cell Res.* **112**, 23-29.

ROBERSON, M. M., and ARMSTRONG, P. B. (1980). Carbohydrate-binding component of amphibian cell surfaces: Restriction to surface regions capable of cell adhesion. *Proc. Natl. Acad. Sci. USA* **77**, 3460-3463.

SANDERS, E. J., and DICAPRIO, R. A. (1976). A freeze-fracture and concanavalin A-binding study of the membrane of cleaving *Xenopus* embryos. *Differentiation* **7**, 13-21.

SINGAL, P. K., and SANDERS, E. J. (1974). An ultrastructural study of the first cleavage of *Xenopus* embryos. *J. Ultrastruct. Res.* **47**, 433-451.

SINGER, S. J., and NICOLSON, G. L. (1972). The fluid mosaic model of the structure of cell membranes. *Science* **175**, 720-731.

SPIEGEL, M. (1951). A method for the removal of the jelly and vitellin membrane of the egg of *Rana pipiens*. *Anat. Res.* **111**, 544.

VAN ZOELEN, E. J. J., TERTOOLEN, L. G. J., and DE LAAT, S. W. (1983). A simple computer method for evaluation of lateral diffusion coefficients from fluorescence photobleaching recovery kinetics. *Biophys. J.* **42**, 103-108.

WOLF, D. E., KINSEY, W., LENNARZ, W., and EDIDIN, M. (1981a). Changes in the organization of the sea urchin egg plasma membrane upon fertilization: Indications from the lateral diffusion rates of lipid soluble fluorescent dyes. *Dev. Biol.* **81**, 133-138.

WOLF, D. E., EDIDIN, M., and HANDYSIDE, A. M. (1981b). Changes in the organization of the mouse egg plasma membrane upon fertilization and first cleavage: Indication from the lateral diffusion rates of fluorescent lipid analogs. *Dev. Biol.* **85**, 195-198.