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Lateral Mobility of Plasma Membrane Lipids in *Xenopus* Eggs: Regional Differences Related to Animal/Vegetal Polarity Become Extreme upon Fertilization

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Regional differences in the lateral mobility properties of plasma membrane lipids have been studied in unfertilized and fertilized *Xenopus* eggs by fluorescence photobleaching recovery (FPR) measurements. Out of a variety of commonly used lipid probes only the aminofluorescein-labeled fatty acids HEDAF (5-(*N*-hexadecanoyl)-aminofluorescein) and TEDAF (5-(*N*-tetradecanoyl)-aminofluorescein) appear to partition into the plasma membrane. Under all experimental conditions used these molecules show partial recovery upon photobleaching indicating the existence of lipidic microdomains. In the unfertilized egg the mobile fraction of plasma membrane lipids (~50%) has a fivefold smaller lateral diffusion coefficient ($D = 1.5 \times 10^{-8}$ cm^2/sec) in the animal than in the vegetal plasma membrane ($D = 7.6 \times 10^{-8}$ cm^2/sec). This demonstrates the presence of an animal/vegetal polarity within the *Xenopus* egg plasma membrane. Upon fertilization this polarity is strongly (>100×) enhanced leading to the formation of two distinct macrodomains within the plasma membrane. At the animal side of the egg lipids are completely immobilized on the time scale of FPR measurements ($D \ll 10^{-10}$ cm^2/sec), whereas at the vegetal side D is only slightly reduced ($D = 4.4 \times 10^{-8}$ cm^2/sec). The immobilization of animal plasma membrane lipids, which could play a role in the polyspermy block, probably arises by the fusion of cortical granules which are more numerous here. The transition between the animal and the vegetal domain is sharp and coincides with the boundary between the presumptive ecto- and endoderm. The role of regional differences in the plasma membrane is discussed in relation to cell diversification in early development.

INTRODUCTION

The animal/vegetal polarity of the amphibian egg and the sperm entrance point determine the embryonic axes (Nieuwkoop, 1973, 1977; Gerhart *et al.*, 1981). In many studies eggs of *Xenopus laevis* have been used as a model system to investigate the role of polarity in amphibian development, not only because of the ease with which these large eggs can be manipulated (diameter ± 1.5 mm), but also because axis formation during embryonic development can be followed easily. Those studies have demonstrated that fertilized *Xenopus* eggs exhibit animal/vegetal polarity within the cytoplasm, as judged from the differential distribution of, e.g., RNA (Capco and Jeffery, 1981; Capco, 1982; Phillips, 1982), cortical granules (Grey *et al.*, 1974; Campanella and Andreucetti, 1977; Goldenberg and Elinson, 1980), yolk, pigment granules, and cytoskeletal elements (for Refs. see Nieuwkoop, 1973, 1977). In addition, it has been demonstrated by freeze-fracture electron microscopy of fertilized eggs that the distribution of intramembranous particles (IMPs) and of so-called IMP-free domains differs between the animal and vegetal half of the egg (Bluemink and Tertoolen, 1978). Animal/vegetal differences in surface structure have been demonstrated also by scanning electron microscopy (Elinson, 1980). These studies demonstrate that animal/vegetal polarity is associated with regional differences in the distribution of cytoplasmatic components as well as in cell surface properties.

A variety of studies have shown that the plasma membrane of fertilized *Xenopus* eggs is peculiar in various respects. Using freeze-fracture electron microscopy, IMPs were found to have a mean diameter of 12 nm, i.e., larger than generally observed in biological membranes. Moreover, again in contrast to most other membranes, these IMPs are mainly localized at the exoplasmic face (Bluemink *et al.*, 1976; Bluemink and Tertoolen, 1978). In addition the membrane has a very high specific resistance (74 kohm/cm^2; de Laat *et al.*, 1973; de Laat and Bluemink, 1974), lacks permselectivity for cations (de Laat *et al.*, 1975), and is very insensitive to cytochalasins (Bluemink, 1971a,b, 1978; Bluemink and de Laat, 1973; de Laat *et al.*, 1973; de Laat and Bluemink, 1974), all in contrast to most other biological membranes. During cytokinesis new intercellular membrane is formed in the cleavage furrow. Although the newly formed membrane is in direct continuity with the

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preexisting membrane, it shows a very different ultrastructural organization (Bluemink and de Laat, 1973) and permeability properties (de Laat and Bluemink, 1974; de Laat *et al.*, 1974). Since no evidence was found for any sort of structural barrier between the preexisting and newly- formed membrane, this indicates that the plasma membrane of fertilized *Xenopus* eggs cannot be considered as a continuous fluid mosaic membrane (Singer and Nicolson, 1972).

With respect to unfertilized eggs, studies on the distribution of RNA, cortical granules, yolk, pigment, and cytoskeletal elements have shown that at the cytoplasmic level animal/vegetal polarity already exists before fertilization (Capco and Jeffery, 1981; Capco, 1982; for other Refs. see Nieuwkoop, 1973, 1977). The observations that sperm always penetrates the egg via the animal half (Elinson, 1975) and that in this membrane region the sensitivity to progesterone is also higher (Gerhart *et al.*, 1981), suggest that polarity also exists at the membrane level. Relatively little is known with respect to the membrane organization of unfertilized eggs, but freeze-fracture electron microscopy reveals similar large particles having a similar (inverse) distribution among the inner and outer half of the plasma membrane as in fertilized eggs (Bluemink *et al.*, 1983).

It is known from studies on various organisms that the physical properties and composition of the egg plasma membrane may change upon fertilization (Peters and Richter, 1981; Grey *et al.*, 1974; Epel, 1972; Wolf *et al.*, 1981a,b). Most likely this results from the fusion of cortical granules with the plasma membrane during the fertilization process. Wolf *et al.*, (1981a,b) have shown that fluorescence photobleaching recovery (FPR) is an excellent technique for studying changes in membrane physicochemical properties during fertilization, since only a single cell is required for these measurements. Using this technique, by which the lateral mobility of proteins and lipids in membranes can be measured (Axelrod *et al.*, 1976), a strong decrease in lipid lateral mobility upon fertilization has been observed in sea urchin eggs (Wolf *et al.*, 1981a; Campisi and Scandella, 1978; Peters and Richter, 1981) and mouse eggs (Wolf *et al.*, 1981b; Klausner and Wolf, 1980).

The large size of the *Xenopus* egg allows for the study of regional differences of plasma membrane properties. In the present study we have measured the lateral mobility of the plasma membrane lipids along the animal/vegetal axis in fertilized and unfertilized eggs of *X. laevis*. The results demonstrate the existence of an animal/vegetal polarity in fertilized as well as unfertilized eggs, with a lower lipid lateral mobility in the animal half. Fertilization strongly reduces the rate of lipid lateral mobility and results in almost complete lipid immobilization in the animal half, demonstrating again the unusual features of the *Xenopus* egg plasma membrane. The potential role of this animal/vegetal polarity in the plasma membrane for early morphogenesis and cell diversification is discussed.

MATERIAL AND METHODS

Materials

Eggs of *X. laevis* were collected in modified amphibian Ringer solution (MMR; Kirschner *et al.*, 1980) from females stimulated by human chorionic gonadotrophin (Physec LEO Pharm.). Eggs were fertilized as described by de Laat *et al.* (1973). After chemical removal of the jelly layer with 2% L-cystein/HCl and 0.2% papain (modified after Spiegel, 1951) in 25% MMR at pH 7.8, the eggs were rinsed three times with 25% MMR. Fertilized eggs were selected under the dissecting microscope by the appearance of a sperm entrance point. The vitelline membrane was removed with forceps. The eggs were kept in 25% MMR in Falcon petri dishes containing a 4% agar bottom coloured with a few droplets of India ink. To prevent activation, all treatments with unfertilized eggs were done in Ca^{2+}/Mg^{2+}-free media. *Xenopus* albino mutants were obtained from the Hubrecht Laboratory collection. The eggs were collected and treated as described above for wild-type eggs.

Labeling

The 5-(*N*-hexadecanoyl)aminofluorescein or 5-(*N*-tetradecanoyl)aminofluorescein (abbreviated as HEDAF and TEDAF respectively; Molecular Probes, Plano, Tex.) 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 for fertilized eggs, and in 25% Ca^{2+}/Mg^{2+}-free MMR for unfertilized eggs, resulting in a saturated stock solution. The stock solution was centrifuged for 1 min at 700*g* to precipitate undissolved fluorescent lipid probe. Incubation media were made by 30-fold dilution of the saturated supernatant in 25% MMR or 25% Ca^{2+}/Mg^{2+}-free MMR, for fertilized and unfertilized eggs, respectively. The eggs were labeled for 10 min at room temperature and washed three times with 25% MMR (or Ca^{2+}/Mg^{2+}-free MMR) containing 0.1% fatty acid-free albumin, and again three times with medium without albumin. Autofluorescence of unlabeled eggs was always less than 5% of the total fluorescence intensity of labeled eggs.

FPR Measurements

Lateral mobility characteristics of membrane lipids were measured using the fluorescence photobleaching recovery method (FPR) 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, focussed 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 equipped with a photomultiplier (EMI 9863/100, EMI, Hayes Middlesex, U. K.) and an ORTEC 9302 amplifier–discriminator (ORTEC Inc., Oak Ridge, TN, USA) as described by Boonstra *et al.* (1982). In the illuminated region, 40–60% of the fluorescence intensity was bleached by a short (20–60 msec) approximately a 1000- to 2500-fold increase in laser intensity. The duration of the bleach pulse, the intensity of the laser beam, and the recording of the fluorescence recovery were controlled with an Apple II Plus microcomputer. This 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). Measurements were made along the animal/vegetal axis of unfertilized and fertilized eggs. To reduce movements of the eggs they were placed between two platinum wires and slightly compressed under the weight of a thin glass coverslip. All measurements were made at room temperature. It took about 15 min to fertilize and decapsulate the eggs *in vitro* and another 15 min to label and rinse the eggs. The earliest possible FPR measurement was made about 35 min after fertilization. The time period for the FPR measurements was between 35 min after fertilization and first cell division (ca. 80 min postfertilization at 20°C room temperature).

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 70–105-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

Dejellied unfertilized and fertilized eggs without vitelline membrane were fixed for 1 hr in 25% MMR containing 2.5% glutaraldehyde. After washing three to four times in 25% MMR the eggs were postfixed for 1 hr in 25% MMR containing 2% osmium tetroxide at 4°C. After washing three to four times with distilled water the eggs were kept overnight in distilled water, washed three to four 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 with a Cambridge Stereoscan 600 M. Preparation tables with eggs were kept in a vacuum exsiccator above phosphorus pentoxide.

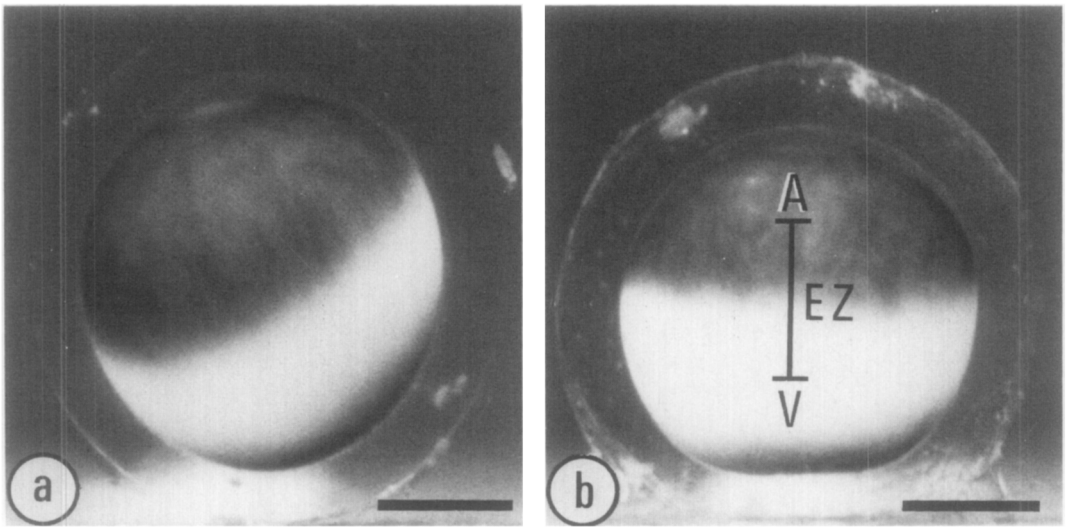

FIG. 1. Wild-type eggs of *Xenopus laevis* within the egg capsule. Photomicrographs of the side view of unfertilized (a) and fertilized egg (b), showing the pigmented half (A), the unpigmented half (V), and the equatorial zone (EZ). The latter is partly pigmented and partly nonpigmented. Bar, 200 μm.

RESULTS

Cell Surface Architecture

Figures 1a,b show an unfertilized and a fertilized egg of *X. laevis*. In both eggs a distinct pigmented and unpigmented half can be distinguished, separated by a sharp transition in the equatorial zone. These two halves will be referred to as the animal and vegetal half of the egg, respectively. Figures 2a–d show scanning electron micrographs of the cell surface at the animal and vegetal poles of unfertilized and fertilized eggs. At the animal pole of the unfertilized egg, long villi or finger-like protrusions (0.5–2 μm long, 0.2–0.4 μm diameter) are observed, which upon fertilization are converted to short protrusions (length and diameter about 1 μm). At the vegetal pole short, point-like protrusions (length and diameter about 0.3 μm) are seen, with a higher density before than after fertilization. The density of protrusions is always higher at the animal than at the vegetal pole (see also Monroy and Baccetti, 1975). The cell surface architecture changes gradually from the animal to the vegetal pole, without a sharp transition in the equatorial

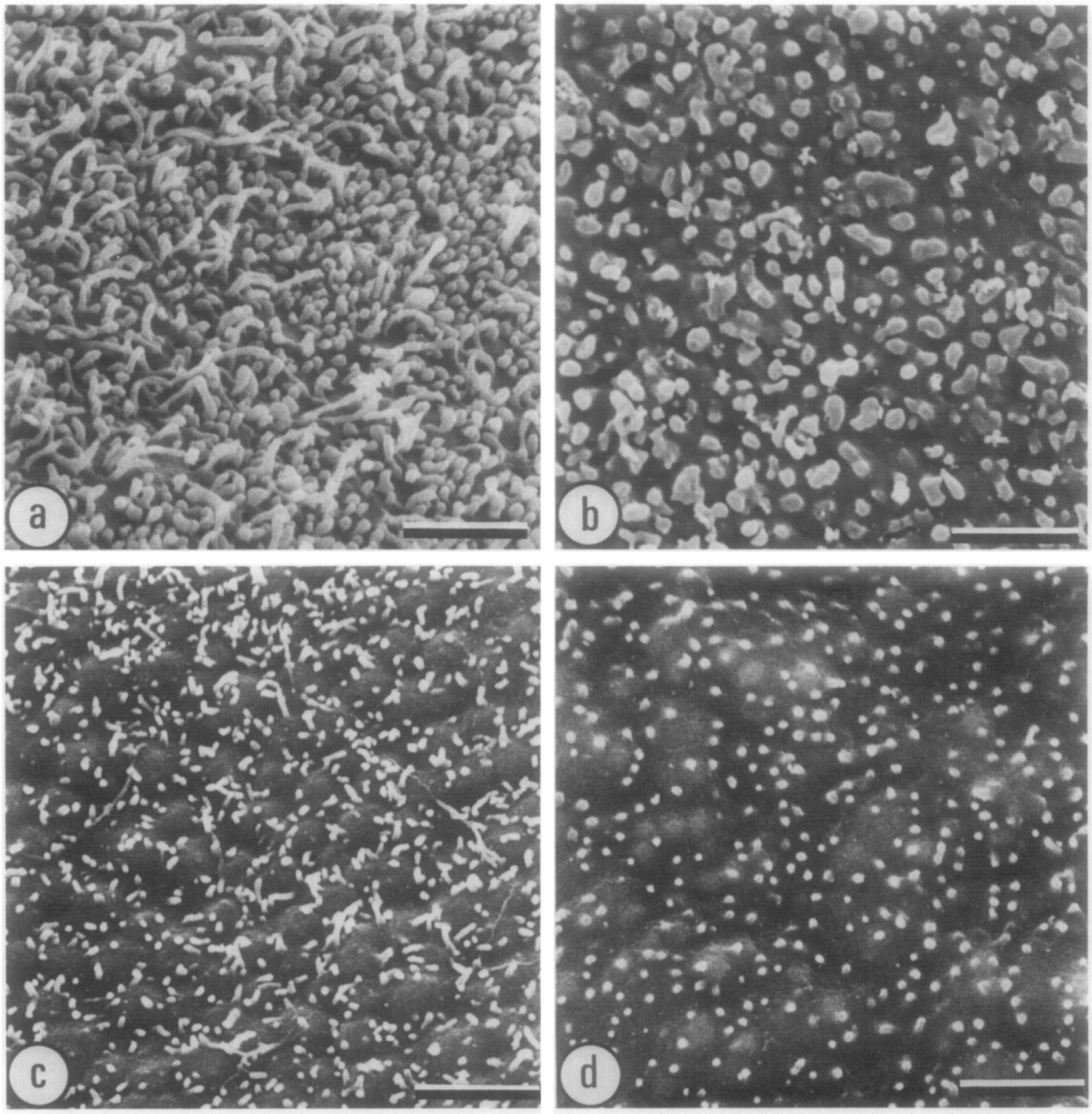

FIG. 2. Scanning electron microscopy of the animal and vegetal poles of unfertilized and fertilized eggs. (a) Animal pole of unfertilized egg. (b) Animal pole of fertilized egg. (c) Vegetal pole of unfertilized egg. (d) Vegetal pole of fertilized egg. Bar, 4 μm.

zone (data not shown; see also Elinson, 1980). These observations are in agreement with the results of Elinson (1980) on eggs of *Rana*, and provide additional evidence for the existence of animal/vegetal polarity at the cell surface, both in unfertilized and fertilized eggs.

Fluorescent labeling

Figure 3 shows the fluorescence pattern of fertilized and unfertilized *Xenopus* eggs of the animal and vegetal half after labeling with the fluorescent lipid probe HEDAF. A speckled fluorescence pattern consisting of dots of approximately 1 μm in diameter is seen on the animal half, both before and after fertilization, while a homogeneous fluorescence pattern is observed on the vegetal half in both cases. The transition between the speckled fluorescence on the animal half and the homogeneous fluorescence on the vegetal half is sharp, and coincides with the transition in pigmentation in the equatorial zone (not shown). This shows that the speckled fluorescence is not correlated with cell surface architecture, and therefore that the pattern at the animal pole is not related with the presence of microvilli or protrusions. Neither is it related with the presence of pigment granules, as could be demonstrated on albino eggs (see below).

The fluorescence pattern after labeling of the egg with TEDAF is similar to that observed with HEDAF (not

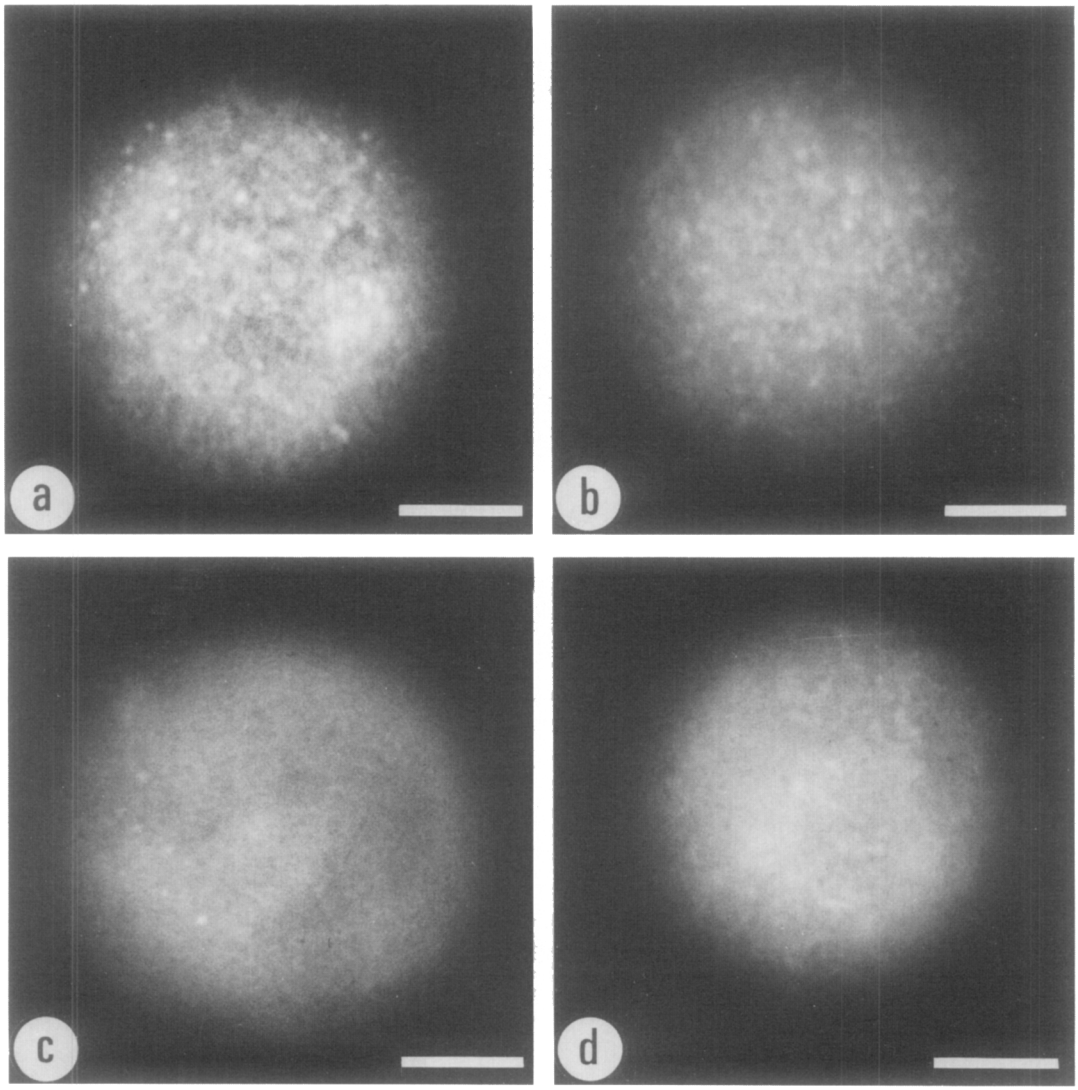

FIG. 3. Fluorescence pattern of wild-type *Xenopus* eggs labeled with HEDAF, showing the spotty fluorescence of a part of the animal half of the unfertilized (a) and the fertilized egg (b), and the homogeneous fluorescence on the vegetal half of the unfertilized (c) and the fertilized egg (d). Only a small region of the eggs is shown. Bar, 40 μm. (Note that the total diameter of the egg is about 1500 μm.)

shown). Attempts to label the egg plasma membrane with other lipid probes such as the 3,3'-diacylindocarboxyamino iodides (C14-dil, C16-dil, and C18-dil) and the fluorescently labeled gangliosides GM1 and GM2 (a generous gift from Dr. S. Spiegel, Rehovot, Israel) were not successful. This again illustrates the peculiar nature of the *Xenopus* plasma membrane.

Fluorescence Photobleaching Measurements

Table 1 shows the lateral mobility characteristics of the plasma membrane of unfertilized and fertilized *Xenopus* eggs labeled with HEDAF. A laser spot of 7.4 μm in diameter was used, which is large compared to the size of the surface protrusions. In the case of unfertilized eggs the vegetal half shows a four to fivefold higher lateral diffusion coefficient than the animal half, demonstrating that in the unfertilized egg animal/vegetal polarity exists with respect to the physicochemical properties of the membrane lipids. In both halves only partial recovery of the lipid probe was detected (40–50%). Upon fertilization a strong decrease in lipid lateral mobility is observed in the animal half of the egg, resulting in nearly complete immobilization of the lipid probe. After bleaching of a small area in the animal half a dark spot is seen in the fluorescence pattern (see also Fig. 5). The bleached area remains visible for at least 15 min, from which a maximal value for the lateral diffusion coefficient was estimated. Fertilization also results in a reduction of the lipid diffusion coefficient in the vegetal half of the egg, though less pronounced than in the animal half. Moreover, in the vegetal half an increase in the mobile fraction of the lipid probe was observed as a result of fertilization.

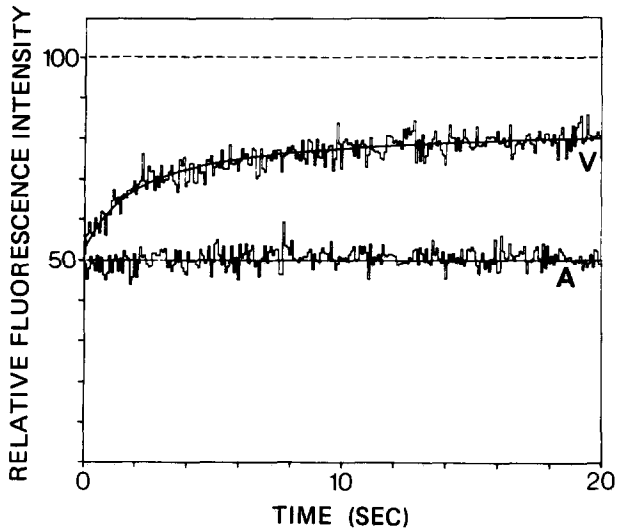

FIG. 4. Example of typical fluorescent photobleaching recovery curves of a HEDAF-labeled fertilized *Xenopus* egg. (A) Animal half. (V) Vegetal half.

These data demonstrate that the animal/vegetal difference in lipid lateral mobility present in the unfer-

TABLE 1
LATERAL DIFFUSION COEFFICIENT AND MOBILE FRACTIONS OF HEDAF AND TEDAF IN *Xenopus laevis* EGGS

	Diffusion coefficients[a]	Mobile fraction	Number of experiments[b]
HEDAF			
Wild-type eggs			
Unfertilized egg vegetal half	7.6 ± 1.3	0.44 ± 0.03	11 (4)
Unfertilized egg animal half	1.5 ± 0.3	0.49 ± 0.04	10 (3)
Fertilized egg vegetal half	2.8 ± 0.4	0.66 ± 0.05	19 (5)
Fertilized egg animal half	≪0.01	≪0.05	30 (10)
Fertilized egg equatorial zone: unpigmented region	4.4 ± 0.5	0.63 ± 0.03	19 (8)
Fertilized egg equatorial zone: pigmented region	≪0.01	≪0.01	12 (4)
Albino mutant eggs			
Fertilized egg vegetal half	4.3 ± 0.7	0.66 ± 0.05	16 (5)
Fertilized egg animal half	≪0.01	≪0.05	20 (7)
TEDAF			
Wild-type eggs			
Fertilized egg vegetal half	2.4 ± 0.2	0.64 ± 0.02	14 (4)
Fertilized egg animal half	≪0.01	≪0.05	11 (4)

[a] Diffusion coefficients in 10^{-8} cm^2/sec.
[b] Number of eggs in parentheses.

tilized egg is strongly enhanced after fertilization. This is illustrated in Fig. 4, which shows typical fluorescence photobleaching recovery curves of HEDAF in the animal and vegetal half of fertilized eggs. Measurements of lipid lateral mobility in the equatorial zone of fertilized eggs show a correlation with the presence of pigment granules. In the pigmented part a low lipid diffusion coefficient was measured, similar to that observed in the animal half. In the nonpigmented part, however, a high lipid mobility was found, generally even higher than in the vegetal half (see Table 1).

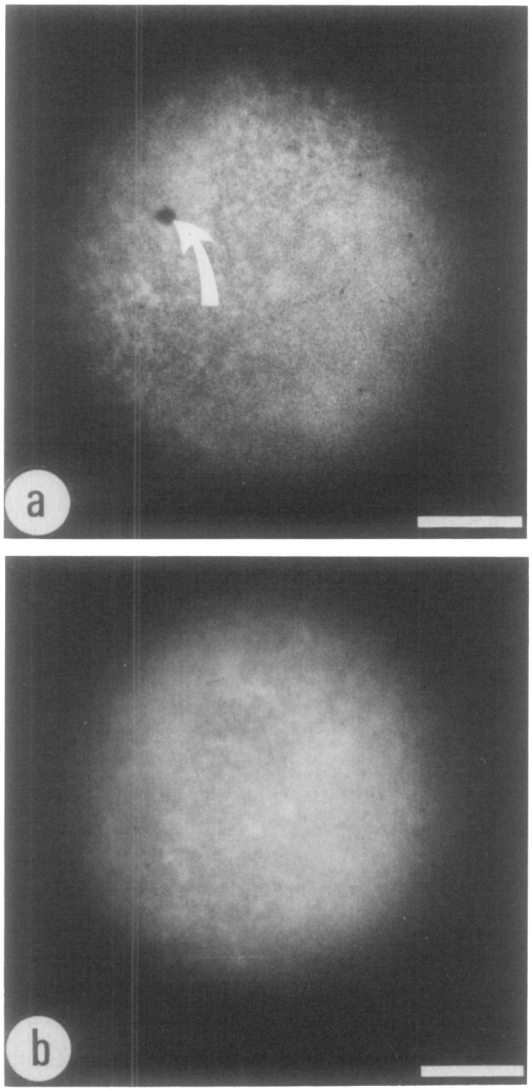

FIG. 5. Fluorescence pattern of albino mutant *Xenopus* eggs labeled with HEDAF. After removal of the vitelline membrane the animal pole of the egg was identified by the opaque maturation spot and stained with "crystals" of Nile blue (modified from Kirschner and Hara, 1980). (a) Spotty fluorescence of animal half. (b) Homogeneous fluorescence of vegetal half. (Arrow: bleached spot.) Bar, 40 μm.

To check the extreme differences in the diffusion coefficient and mobile fraction of HEDAF in the animal and vegetal halves of fertilized eggs a second lipid probe, TEDAF, was used. Table 1 shows that the mobility characteristics of TEDAF in the plasma membrane of the animal and vegetal half of the fertilized egg are comparable to those of HEDAF. In addition, the bleached spot in the animal half of TEDAF-labeled fertilized eggs also remained visible for at least 15 min.

Albino Mutant Eggs

The above data show that the animal/vegetal polarity in lateral mobility of plasma membrane lipids coincides with the distribution of pigment granules in the egg, both being characterized by a sharp transition in the equatorial zone. In order to investigate to what extent the dotted fluorescence pattern may be due to the presence of pigment granules in the underlying cortex, eggs from albino mutants of *X. laevis* were used, which lack premelanosomes and are thus devoid of pigment granules (Bluemink and Hoperskaya, 1975). Figure 5 shows that both the animal and the vegetal half of the fertilized albino egg yields a similar fluorescence pattern as those of the wild-type egg. Moreover, Table 1 shows that the values for the mobility characteristics of HEDAF in the plasma membrane of fertilized albino eggs are similar for the two types of wild-type eggs, including the pronounced animal/vegetal polarity (see also Fig. 5a). This shows that no causal relationship exists between the presence of pigment granules and the mobility characteristics of the membrane lipids. It also shows that pigment granules do not give rise to artifacts in fluorescence photobleaching recovery measurements.

DISCUSSION

In the present study lateral mobility of the plasma membrane lipids of unfertilized and fertilized *Xenopus* eggs was examined by measuring the fluorescence photobleaching recovery of the fluorescent lipid probes HEDAF and TEDAF. The results have demonstrated the existence of animal/vegetal polarity in the lateral mobility of the plasma membrane lipids in unfertilized eggs. This polarity is strongly enhanced upon fertilization, as a result of complete lipid immobilization in the animal plasma membrane.

For interpretation of these data it is important to establish whether the HEDAF molecules are indeed incorporated into the lipid phase of the egg plasma membrane. Although this is difficult to prove directly, the following argues in favour of an intramembrane localization:

(1) The order of magnitude of the lateral diffusion coefficients in the unfertilized egg and in the vegetal half of the fertilized egg is as expected for biological membranes.

(2) Extramembrane localization of the probe molecules at the animal side of the fertilized egg could cause an apparent immobilization in this region. This is, however, unlikely as under such conditions probe molecules would diffuse from the vegetal plasma membrane into the lipid phase of the animal plasma membrane, unless some physicochemical barrier would prevent them from doing so. However, no evidence for such a barrier is available. Thus, the absence of lipid mobility in the animal plasma membrane of the fertilized egg cannot be explained by assuming extramembrane localization of immobile probe molecules.

(3) The observation that similar fluorescence intensities are observed in the animal and vegetal half of the egg, as measured with an attenuated laser beam (data not shown) also argues against regional extramembrane probe localization.

(4) The possibility that apart from the plasma membrane also intracellular structures would have been labeled with HEDAF cannot be excluded, but selective focusing of the laser spot on the plasma membrane is easy to achieve. Based on these arguments we feel confident that the FPR measurements provide information on the lateral mobility of the plasma membrane lipids.

Our data demonstrate that the plasma membrane of the *Xenopus* egg differs in its organization from other biological membranes studied so far, and make clear that this membrane is not of a fluid mosaic nature (Singer and Nicolson, 1972). Out of a variety of commonly used lipid probes only the aminofluorescein-conjugated fatty acids HEDAF and TEDAF partition into the membrane. Furthermore, our data provide evidence for the existence of at least three types of domains within the lipid phase of the *Xenopus* egg plasma membrane.

Incorporation of HEDAF gave rise to a speckled fluorescence pattern in the animal half of the egg and a homogeneous fluorescence pattern in the vegetal half, with a sharp transition in the equatorial zone. This fluorescence distribution suggests the occurrence of intermediate domains (1–10 μm^2) in the animal plasma membrane. The origin of the speckled fluorescence in the animal half is unclear: (1) it follows the distribution of pigment granules in the egg but is not due to the presence of the granules; (2) no correlation exists between the occurrence of a speckled fluorescence and the presence of protrusions or microvilli on the cell surface, since the former shows a sharp transition in the equatorial zone and the latter not; (3) the fact that the animal pole of unfertilized eggs show a speckled fluorescence together with lateral mobility of the probe molecules, indicates that no relation exists between speckled fluorescence and immobility of the probe. Whether a relation exists between the speckled fluorescence pattern and the animal/vegetal distribution of IMP-free domains in freeze-fractured *Xenopus* egg plasma membrane (Bluemink and Tertoolen, 1978; Bluemink *et al.*, 1976) is an interesting possibility, which needs to be investigated further.

The observation that under all conditions tested only partial recovery of the lipid probe was observed indicates that lipid microdomains must be present (small compared to the laser beam size). The presence of lipid domains in the bilayer of the plasma membrane as detected by partial recovery of a lipid probe in an FPR measurement seems a characteristic of early embryonic cells (see also Wolf *et al.*, 1981b; Klausner and Wolf, 1980; and Peter and Richter, 1981, for mouse eggs; and Campisi and Scandella, 1980; and Wolf *et al.*, 1981a, for sea urchin eggs). Whether this involves the presence of gel-state lipids in the membrane is at present unknown. Klausner *et al.* (1980) and Klausner and Wolf (1980) have shown that the affinity of various fluorescent lipid probes for lipid domains depends on the fatty acid chain length of the probe molecules. Consequently the lateral mobility characteristics of plasma membrane lipids as measured by the FPR technique can strongly depend on the choice of the fluorescent lipid probe. We have therefore used a second probe, TEDAF, which has a shorter chain length than HEDAF. The results with TEDAF were similar to those obtained by HEDAF, indicating that our results, particularly those with respect to probe immobility in the animal half of the fertilized egg, are not strongly probe dependent.

The lateral mobility characteristics of the vegetal *Xenopus* plasma membrane are very remarkable. On the one hand the incorporated HEDAF molecules show only partial recovery after photobleaching, while on the other hand the rate of lateral diffusion of the "mobile fraction" is very high compared to that of other biological membranes and rather resembles that of fluid artificial lipid bilayers (for review see Peters, 1981). In order to check the validity of our experimental conditions and to verify the size of the laser spot in an independent way, we have measured the lateral diffusion coefficient at room temperature of egg phosphatidylcholine bilayers labeled with C18-diI. A lateral diffusion coefficient of 7.1×10^{-8} cm^2/sec was obtained, similar to values in the literature (Derzko and Jacobson, 1980).

The most prominent feature of the *Xenopus* egg plasma membrane is certainly the enhancement of animal/vegetal polarity upon fertilization, which results

in the formation of two distinct regions or macrodomains corresponding to the animal and vegetal half of the egg. Within the apparent continuous egg membrane an extreme regional difference in physicochemical properties is established and maintained with a sharp transition at the egg equator, although no indications have been observed for a structural barrier in this borderline. The integrity of these macrodomains is apparently maintained by the physical state of the lipid phase of the animal plasma membrane, thereby preventing exchange of membrane components between these domains. Lipid macrodomains have also been described in polarized mammalian cells, but in these cells they are maintained by the presence of tight junctions (Dragsten *et al.*, 1981).

In *Xenopus* sperm entry occurs preferentially on the animal side of the egg (Elinson, 1975). So far it is unclear what determines this regional specificity. Our results provide a possible clue hereto, as the regional differences in the mobility of plasma membrane components could provide a mechanism for controlling the localization of sperm recognition sites. As a result of sperm entry a rapid fusion of cortical granules takes place, specifically with the animal plasma membrane (Grey *et al.*, 1974). Consequently, a large amount of new membrane material is incorporated into this region of the plasma membrane. This leads to a structural membrane reorganization, as evidenced by freeze-fracture electron microscopy (Bluemink *et al.*, 1983) and is probably also the onset of the lipid immobilization in the animal plasma membrane of the fertilized egg. It is possible that this drastic change in the properties of the animal plasma membrane is functional in the block to polyspermy. An overall, but less spectacular, reduction in lipid mobility at fertilization has also been described for sea urchin eggs (Wolf *et al.*, 1981a; Peters and Richter, 1981), and was related to the fusion of cortical granules with the plasma membrane (Wolf *et al.*, 1981b).

From this study it is evident that animal/vegetal polarity in the *Xenopus* egg is expressed not only in the distribution of cytoplasmic components, but also in regional differences in the physicochemical properties of the plasma membrane. Therefore, cell diversification during the period of rapid cell division following fertilization can result from the differential distribution of cytoplasmic as well as plasma membrane constituents over the generated cells, depending on their particular position. Preliminary experiments have shown that the lipid immobility of the animal half persists after first cleavage up to the 32-cell stage. The significance of maternal cytoplasmic factors for early cell diversification is a long standing topic in the embryological literature (see Davidson, 1968), but the significance of plasma membrane-bound properties has received far less attention and should be considered in view of the present results.

The sharp transition between the animal and the vegetal macrodomain coincides with the demarcation line between the presumptive ectoderm and endoderm (see Nieuwkoop, 1973, 1977). This topographical relationship suggests that the observed regional differences in physicochemical membrane properties could play a role in this early step in cell diversification. There is abundant evidence that the lipid environment affects the properties and functioning of membrane proteins, such as enzymes, receptors and transport proteins (Warren *et al.*, 1975; Edidin and Petit, 1977; Sanderman, 1978; Rothfield and Romeo, 1971; Magee and Schlessinger, 1982; Boonstra *et al.*, 1983; van Zoelen *et al.*, 1983b; de Laat *et al.*, 1983). It seems thus plausible that the prominent animal/vegetal differences in membrane lipid properties could determine major regional differences in plasma membrane functioning. This could be achieved either by specification of the localization of particular membrane proteins, or by affecting membrane properties regionally. This possibility is illustrated by the reported polarized water transport in amphibian blastulae, which was ascribed to an animal/vegetal difference in plasma membrane properties leading to the formation of the blastocoelic cavity (Tuft, 1957, 1961a,b, 1962; Hamilton and Tuft, 1972). The blastocoelic cavity is a major determinant of the topographical relationships among the cells in the early embryo, and for that reason important for early morphogenesis (Nieuwkoop, 1973).

Previous experiments have shown that egg rotation through 180° leads to a complete cytoplasmic reorganization, which under certain conditions is followed by normal embryogenesis (Chung and Malacinski, 1982). Future experiments along similar lines offer the possibility to test the developmental significance of the presence of animal/vegetal polarity in the mobility properties of plasma membrane lipids as described here.

In conclusion, the present study not only gives further evidence for the peculiar nature of the *Xenopus* egg plasma but also provides the first evidence that regional differences in plasma membrane properties could play an important role in early amphibian morphogenesis.

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