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Regional Differences in the Lateral Mobility of Plasma Membrane Lipids in a Molluscan Embryo

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Regional and temporal differences in plasma membrane lipid mobility have been analyzed during the first three cleavage cycles of the embryo of the polar-lobe-forming mollusc *Nassarius reticulatus* by the fluorescence photobleaching recovery (FPR) method, using 1,1'-ditetradecyl 3,3,3',3'-tetramethylindocarbocyanine iodide (C_{14}diI) as a fluorescent lipid probe. During this period of development the lateral diffusion coefficient of membrane lipids is consistently greater in the vegetal polar lobe area as compared to the animal plasma membrane area (on average 30%), demonstrating the existence of an animal–vegetal polarity in plasma membrane properties. At third cleavage, the differences between animal and vegetal plasma membrane region become even more pronounced; in the four animal micromeres the diffusion coefficient (D) and mobile fraction (MF) are $2.9 \pm 0.2 \times 10^{-9}$ cm^2/sec and $51 \pm 2\%$, respectively, while in the four vegetal macromeres $D = 5.0 \pm 0.3 \times 10^{-9}$ cm^2/sec and MF = $78 \pm 2\%$. Superimposed upon the observed animal–vegetal polarity, the lateral diffusion in the polar lobe membrane area shows a cell-cycle-dependent modulation. The highest mean values for D are reached during the S phase (ranging from 7.0 to 7.8×10^{-9} cm^2/sec in the three cycles measured), while at the end of G_2 phase and during early mitosis mean values for D have decreased significantly (ranging from 5.0 to 5.9×10^{-9} cm^2/sec). Diffusion rates in the animal membranes of the embryo are constant during the three successive cell cycles ($D = 4.3$–5.0×10^{-9} cm^2/sec), except for a peak at the S phase of the first cell cycle ($D = 6.0 \times 10^{-9}$ cm^2/sec). These results are discussed in relation with previously observed ultrastructural heterogeneities in the *Nassarius* egg plasma membrane. It is speculated that the observed animal–vegetal polarity in the organization of the egg membrane might play an important role in the process of cell diversification during early development.

INTRODUCTION

In the eggs of many species, a polar distribution of cytoplasmic components has been observed, which often manifests itself as distinct cytoplasmic localizations in certain regions of the egg cell. Cytoplasmic localizations are thought to be essential for the establishment of different cell populations within the later embryo, since they become distributed differently over the blastomeres formed during cleavage and may be responsible for the generation of different patterns of gene activity within separate blastomeres (see Davidson, 1976). The main factor determining the spatial organization of the egg cytoplasm most likely resides in the plasma membrane/cortex complex (see Kühn, 1965).

In recent years a number of polar phenomena have been found to exist at the level of the plasma membrane or cortical cytoskeleton in egg cells. Topographical differences corresponding to polar organization have been found in the surface architecture of amphibian, mammalian, molluscan, and annelid eggs (Monroy and Baccetti, 1975; Eager *et al.*, 1976; Dohmen and van der Mey, 1977; Elinson, 1980; Dohmen, 1983; Speksnijder and Dohmen, 1983), in the affinity for lectins in ascidian and mammalian eggs (Johnson *et al.*, 1975; Ortolani *et al.*, 1977; Honegger, 1982), in the distribution of intramembrane particles in the plasma membrane of fucoid, amphibian, and molluscan eggs (Peng, 1976; Bluemink and Tertoolen, 1978; Speksnijder *et al.*, 1984b, 1985), in the lateral mobility characteristics of plasma membrane components in amphibian and mouse eggs (Wolf and Ziomek, 1983; Dictus *et al.*, 1984), in the distribution of ion channels in the plasma membrane of eggs of various species (Jaffe, 1982; Nuccitelli, 1983), and in the cortical cytoskeleton of ascidian eggs (Jeffery and Meier, 1983).

The eggs of a number of molluscan species are particularly interesting for a further study of the determination of spatial organization in an egg cell due to a close relationship between regional differences in the organization of the egg surface and the localization of surface-bound morphogenetic plasms at their vegetal pole (Dohmen, 1983). These eggs are further characterized by the formation of a so-called polar lobe at the vegetal pole during the first three cleavages. By the formation of this transient protrusion, the polar lobe contents are temporarily set apart and excluded from the cleavage process. At the end of the cleavage

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cycle, the polar lobe is resorbed into one of the two daughter cells, thus shunting its contents into one cell only. Deletion experiments have demonstrated the developmental importance of the polar lobe (reviewed by Dohmen, 1983; Verdonk and Cather, 1983). Centrifugation experiments (Verdonk, 1968; Clement, 1968) have demonstrated that the morphogenetic determinants present in the polar lobe are very likely to be closely associated with the egg surface. The occurrence of a locally different organization of the egg surface on the polar lobe of several species may play a role in this association (Dohmen and van der Mey, 1977; Dohmen, 1983; Speksnijder and Dohmen, 1983).

In previous papers, we reported on the existence of a distinct animal-vegetal polarity in the plasma membrane organization of the polar-lobe-forming egg of *Nassarius reticulatus* (Speksnijder *et al.*, 1984b, 1985). Using freeze-fracture electron microscopy, it was demonstrated that the numerical distribution of intramembrane particles (IMPs) in the egg membrane shows considerable regional differences. More particularly, the vegetal pole of the fertilized, uncleaved egg is characterized by a much higher IMP density.

In the present study we have measured the lateral mobility of plasma membrane lipids along the animal-vegetal axis during early development of the same species. The results clearly demonstrate the existence of an animal-vegetal polarity in the organization of the egg plasma membrane at least up to the eight-cell stage. The lipid lateral mobility in the polar lobe membrane area is significantly greater than in the animal membrane area of the embryo and shows a cell-cycle-dependent modulation. The difference between the animal and vegetal membrane area is enhanced at third cleavage, when four micromeres are formed at the animal pole. The potential role of these phenomena for polar-lobe-dependent morphogenesis and cell diversification during early development is discussed. An abstract of part of these results has been published (Speksnijder *et al.*, 1984a).

MATERIALS AND METHODS

Embryos

Adult specimens of the snail *N. reticulatus* were obtained from Roscoff (Britanny, France) and kept at 14–16°C in tanks with running seawater. The females deposit their eggs in capsules, each containing 100–150 fertilized eggs, on the bottoms and walls of the tank. At the desired stage of development the capsules were opened and the embryos were washed several times with filtered seawater (FSW) before further treatment.

Labeling Procedure

The fluorescent lipid analogues C_{18}diI (1,1'-dioctadecyl-3,3,3'3'-tetramethylindocarbocyanine iodide) and C_{14}diI (1,1'-ditetradecyl-3,3,3',3,-tetramethylindocarbocyanine iodide) were purchased from Molecular Probes Inc. (Plano, Tex.). Stock solutions were prepared by dissolving 1 mg diI in 1 ml ethanol. Just before labeling, 20 μl of the stock solution was added to 1 ml FSW under vigorous stirring. In this solution 50–75 embryos were incubated at room temperature for 15 min. After labeling, the embryos were washed three times in FSW and attached onto poly-L-lysine-coated glass coverslips in order to reduce movements of the egg. Control measurements on membrane areas not in contact with the poly-L-lysine-coated glass gave similar results, which is in accordance with observations made by Peters and Richter (1981). All measurements were made at 19°C.

Fluorescence Photobleaching Recovery Measurements

Lateral mobility characteristics of membrane lipids were measured using the fluorescence photobleaching recovery (FPR) method (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 514 nm, focussed with a Leitz 50×/1.0 water-immersion lens to a spot with a $1/e^2$ radius of 1.23 μm. 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, Tenn.) 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 3000-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, which 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).

In each experimental group, 5–18 embryos were measured and on each embryo 2–3 measurements were done on different places in one particular membrane area. A total of 583 measurements on 190 eggs were made. An analysis of variance (see Snedecor and Cochran, 1967) was used for the statistical comparison of the different groups.

Staining of Nuclei with Gallocyanin/Chromalum

The timing of mitosis during the cell cycle was performed on gallocyanin/chromalum-stained prepa-

rations of the embryos during the first three cleavage cycles (see Sandritter *et al.*, 1963). For that purpose, eggs were allowed to develop at 18°C and fixed at successive intervals in Zenker's fixative for 24 hr, washed in tap water for at least 90 min, and stained in a solution of 1% gallocyanin in 4% chromalum, pH 0.84, for 2.5 hr. After a 30-min rinse in distilled water, the eggs were dehydrated in a graded series of ethanol, transferred to xylol, and mounted in Canada balsam. The preparations were analyzed in a Zeiss microscope using Nomarski optics.

Feulgen Cytophotometry

Timing of DNA synthesis in the first cleavage cycle was assessed by cytophotometry of Feulgen-stained nuclei (Swift, 1950). At intervals of 5 min, starting at the beginning of first cleavage, eggs were fixed in ethanol 96%/acetic acid (3:1) for 30 min and subsequently in acetic acid/ethanol 96% (3:1) for another 30 min. With a small drop of solution the eggs were transferred to a clean slide; evaporation of the ethanol/acetic acid fixed the egg firmly to the slide. The slides were dipped in a solution of 2% agar at 60°C, dried thoroughly, and placed in ethanol 96% for at least 24 hr. After drying, hydrolysis was performed in 5 *N* HCl for 30 min. The samples were washed for 30 min in tap water and stained for 1 hr with Schiff's reagent, washed three times for 2 min in SO_2-water, and finally in tap water for 15 min. After dehydration the slides were mounted in DePeX.

The absorption of light by individual Feulgen-stained nuclei is a measure for the total amount of DNA present, and was determined by the method described by van den Biggelaar (1971). Measurements were carried out using a Zeiss microphotometer 01 with a scanning object stage coupled to a Hewlett-Packard 9825T desktop computer. For the photometric measurements the Zeiss computer program APAMOS was used. Analysis and evaluation of the data were performed using programs developed in the Department of Lightoptical Registration of the Zoological Laboratory (M. Terlou, unpublished).

In each group, 10–36 nuclei were measured.

RESULTS

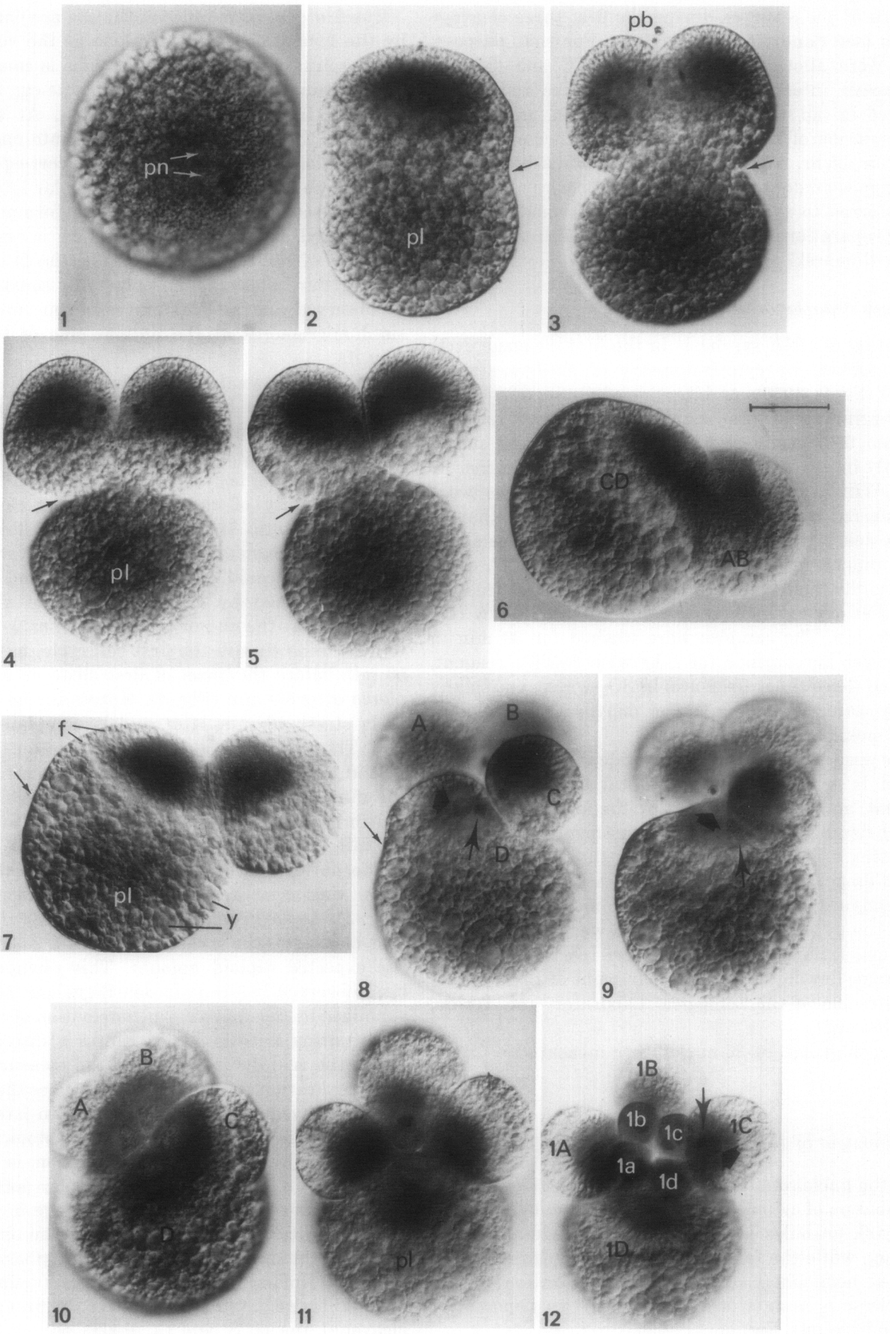
Development of Nassarius Eggs

In the uncleaved, fertilized egg of *Nassarius* a polar distribution of cytoplasmic components can be observed. The yolk granules are present in the vegetal part of the egg, while the fat droplets are concentrated in the animal hemisphere. This polar organization of the cytoplasm is maintained during early development of the embryo (see Fig. 1).

The first cleavage cycles of the egg are characterized by the formation of a polar lobe at the vegetal pole. During first cleavage, this polar lobe is constricted off almost completely from the rest of the egg and is thus excluded from the cleavage process. At the end of cleavage, the polar lobe is resorbed into one cell only, the CD blastomere. As a result, the corresponding AB blastomere is completely devoid of polar lobe material. During second cleavage, polar lobe formation is less pronounced, but again the polar lobe is resorbed into only one of the two daughter cells, the D blastomere. During third cleavage, no polar lobe constriction can be observed and all blastomeres form small, equally sized micromeres at the animal pole of the embryo (Fig. 1).

Using gallocyanin/chromalum-stained cytological preparations, we have studied changes in the morphology of the nuclei during the first three cleavage cycles of the egg (Fig. 1). From the four-cell stage onward, Balbiani bodies (Guraya, 1979) can be observed in the vicinity of the nuclei. During cleavage, the different mitotic stages are clearly visible (Fig. 1). Mitosis was considered to start as soon as chromosome condensation could be distinguished. End of mitosis was determined by the appearance of the nuclear membrane at the end of telophase. Thus we were able to determine that in the first cell cycle, mitosis starts 50 min before the onset of first cleavage and is completed after 60 min (Fig. 3). Mitosis in the second cell cycle starts 60 min later and is completed after 60 min. Thus the interval between the onset of first and second mitosis is 120 min. During the third cell cycle the duration of mitosis is 60 min as well, and again the interval between the second and third mitosis is 120 min (Fig. 3).

The pattern of DNA synthesis during the two-cell stage was measured using Feulgen cytophotometry (Fig. 2). At early telophase (5 min after the onset of first cleavage), the measured values are smaller than the expected diploid amount. This phenomenon has been observed before (van den Biggelaar, 1971) and is explained by the nonuniform distribution of the Feulgen stain during mitosis, which causes a discrepancy between the real dye content and the measured absorption. Replication starts about 10 min after the beginning of first cleavage, when the nuclei are in late telophase (stage 3, Fig. 1). Apparently, the blastomeres do not pass through a G_1 phase. Replication is completed after 35 min. At this time the polar lobe is being resorbed into the CD blastomere (stage 5, Fig. 1). As the next mitosis starts 70 min after the onset of first cleavage, the nuclei pass through a G_2 phase of 25 min (Fig. 3). During the two-cell stage (defined as the interval between the beginnings of the first and second cleavage), the M, S, and G_2 phases occupy 50, 29, and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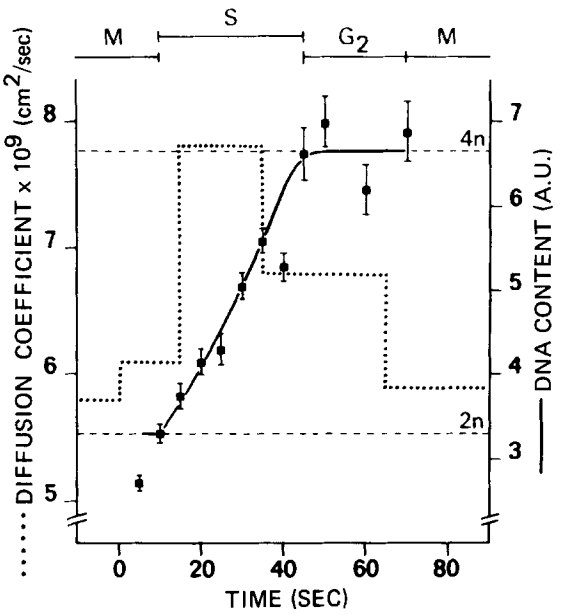

FIG. 2. DNA duplication during the first cell cycle of the *Nassarius* egg as determined by Feulgen cytophotometry, and the diffusion coefficient of plasma membrane lipids in the polar lobe membrane during the first cell cycle. Indicated are means ± SEM (vertical bars) for the DNA content (in arbitrary units), and the calculated values for the DNA content before (2n) and after (4n) duplication. At $t = 0$ first cleavage starts. Indicated at the top are the phases of the cell cycle.

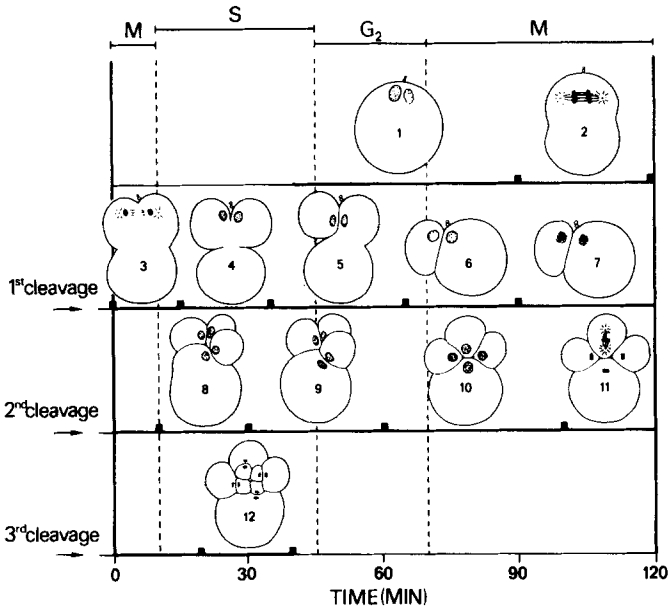

FIG. 3. Schematic diagram demonstrating the 12 developmental stages measured with FPR, and the time course of the different phases of the cell cycle during the first three cleavage cycles.

21% of the cleavage cycle. Similar values have been found for the first three cell cycles in the egg of the mollusc *Lymnaea*, viz., 48, 27, and 25% (van den Biggelaar, 1971). The lack of a G_1 phase is characteristic for embryonic cells and has been reported for the eggs of various species (see van den Biggelaar, 1971).

Since the duration of the second and third cell cycle and their mitotic phases are similar to the values determined for the first cycle, we extrapolated our measurements on the S phase to the second and third cell cycle. This extrapolation is also justified by observations of van den Biggelaar (1971) on the first cell cycles during development of the egg of the mollusc *Lymnaea*.

Figure 3 gives a schematic representation of 12 successive developmental stages of the *Nassarius* embryo with the time course of the different phases of the cell cycle.

Fluorescent Labeling

Incubation of *Nassarius* eggs with the lipid analogue C_{18}diI results in a very weak fluorescent labeling, which is unsuitable for performing FPR measurements. After labeling with C_{14}diI, the eggs show a uniform distribution of the fluorescent probe after focussing on the contour of the egg (not shown). No difference in fluorescence intensity could be observed between the animal and the vegetal membrane region of the embryo after focussing on the egg surface, but generally a slightly more intense bleach pulse was required for the animal region in order to obtain bleach levels of ±50%. Possibly this is due to a reflection of the laser light from the yolk granules, which are present only in the vegetal part of the embryo, resulting in additional bleaching of the fluorescent label. Development of the embryos was not impaired by labeling with the lipid analogue.

Kinetics of Diffusion

At all developmental stages, the fluorescence recovery after photobleaching followed two-dimensional diffusion kinetics (Axelrod *et al.*, 1976), as determined after curve-fitting of the data (van Zoelen *et al.*, 1983). As an example, Fig. 4 shows typical fluorescence photobleaching recovery curves of the plasma membranes of the animal micromeres, the vegetal macromeres, and the polar lobe area of the eight-cell stage embryo. The diffusion coefficients calculated from these curves range from 2.9 to 7.8×10^{-9} cm^2/sec, which is in accor-

FIG. 1. Gallocyanin/chromalum-stained cytological preparations of early developmental stages of the *Nassarius* embryo from uncleaved egg up to the 8-cell stage (Nomarski optics; bar = 50 μm). Stage 1, view on the animal pole of the egg; stages 2-12, lateral view of the embryo. y, yolk granules; f, fat droplets; pn, pronuclei; pb, polar body; pl, polar lobe; small arrow, constriction of the polar lobe; pointed arrow, nucleus; arrowhead, Balbiani body; AB-CD, blastomeres of the 2-cell stage; A-D, blastomeres of the 4-cell stage; 1A-1D, macromeres and 1a-1d, micromeres of the 8-cell stage. See text for further explanation.

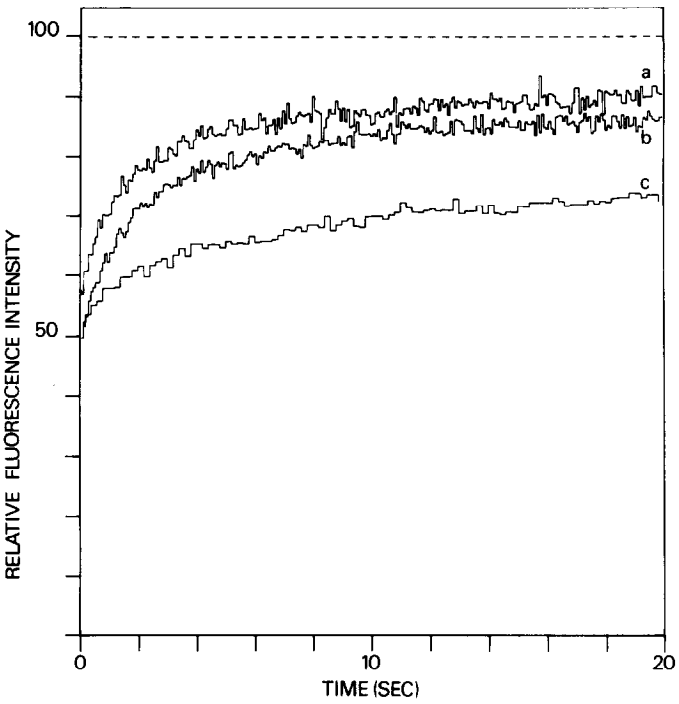

FIG. 4. Typical recovery curves obtained after bleaching of the fluorescent lipid probe in the plasma membrane of (a) the polar lobe, (b) the vegetal macromeres, and (c) the animal micromeres of the 8-cell stage.

dance with values obtained in other biological membranes (Peters, 1981). Therefore we are confident that our FPR measurements provide information on the lateral mobility of plasma membrane lipids. A fractional recovery or mobile fraction (MF) of approximately 76% was observed at all sites measured, except in the plasma membrane of the micromeres at the eight-cell stage, where the MF was 51% (see Fig. 4).

Animal-Vegetal Polarity

In the uncleaved, fertilized egg, the lateral diffusion coefficients of plasma membrane lipids in the animal, equatorial, and vegetal zone of the egg are 4.3 ± 0.4, 4.7 ± 0.2, and 5.0 ± 0.4 × 10^{-9} cm^2/sec, respectively. A slight increase in the mean values for D can be observed along the animal-vegetal axis of the egg. However, an analysis of variance did not demonstrate a significant difference between the values for the animal and vegetal areas of the egg ($P = 0.15$).

Figure 5 shows the lateral diffusion coefficient in the plasma membrane of the uncleaved egg up to the eight-cell stage. We have compared the lipid lateral mobility in the polar lobe membrane—either separated from the egg by the polar lobe constriction, or resorbed into a blastomere in between the cleavages—with the animal part of the embryo at 12 successive developmental stages (Figs. 1, 3). These stages were distinguished on basis of the morphology of the egg, particularly on the extent of constriction or resorption of the polar lobe during the three cleavages. In almost all stages measured—except in the fertilized, uncleaved egg (stage 1, Fig. 1) and in the four-cell stage (stage 10, Fig. 1), the diffusion coefficient in the plasma membrane of the polar lobe is significantly higher than in the animal plasma membrane area of the embryo ($P < 0.001$)(Fig. 5). The difference between the two parts of the embryo can amount up to a 45% higher value for D in the polar lobe membrane. The mobile fraction measured in the polar lobe membrane is identical to or slightly higher than that in the animal membrane area (see Fig. 4, curves a and b).

No significant regional differences in mobility characteristics are observed within the animal region of the embryo (with the exception of the eight-cell stage, which will be discussed later). For example, during the resorption of the first polar lobe (stage 5, Fig. 3), the diffusion coefficients in the plasma membrane of the AB blastomere and in the animal part of the CD blastomere are identical (data not shown), but they both differ significantly ($P < 0.001$) from the value measured in the vegetal part of the CD blastomere (= polar lobe area).

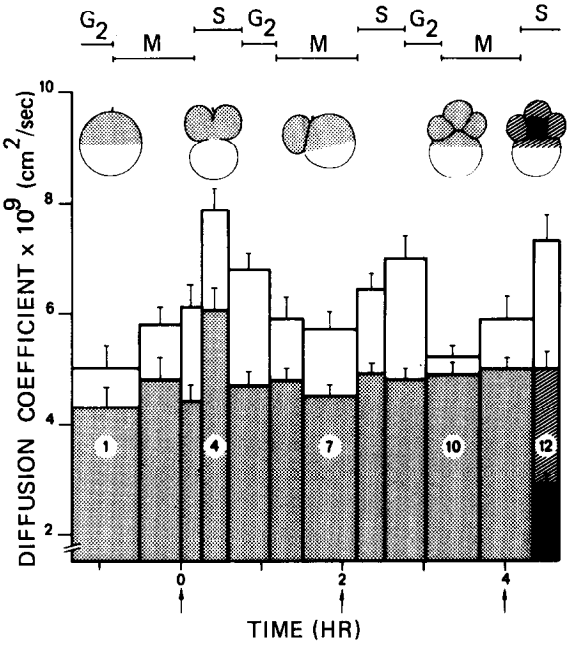

FIG. 5. Lateral diffusion coefficient of plasma membrane lipids during early development of the *Nassarius* egg. Indicated are means ± SEM (vertical bars). □, polar lobe; ▨, animal area; ■, micromeres of the 8-cell stage; ▩, macromeres of the 8-cell stage. The arrows indicate the time of first, second, and third cleavage, and the numbers refer to the 12 developmental stages measured. Indicated at the top are the phases of the cell cycle.

These data all clearly demonstrate the presence of an animal-vegetal polarity in the dynamic organization of the *Nassarius* egg plasma membrane.

Cell-Cycle-Dependent Modulation

Apart from an animal-vegetal polarity in the lateral mobility characteristics of plasma membrane lipids, we also observed a modulation of the diffusion coefficient in the polar lobe membrane through the 12 stages measured (D varies between 5.9 and 7.8 $\times$ 10^{-9} cm^2/sec; see Fig. 5). Comparing the lateral diffusion coefficient in the polar lobe membrane during early development with the time course of the different phases of the cell cycle (Figs. 2, 5), it is evident that the two parameters are interrelated. In each cell cycle the lowest value for the diffusion coefficient is reached in late G_2/early mitosis. At the end of mitosis the value for D starts to increase and becomes highest during the S phase. An analysis of variance demonstrates significant differences between the lowest and highest values for D during each cell cycle ($P < 0.001$).

The diffusion coefficient in the animal membrane area of the embryo does not show such a cell cycle-dependent modulation. However, during the S phase of the first cell cycle (stage 4, Fig. 3), the diffusion coefficient of membrane lipids is significantly higher than in the animal membrane area of all other stages measured ($P < 0.001$) (Fig. 5).

Formation of the Micromeres

The difference between the mobility characteristics of the animal membrane area and the polar lobe membrane is strongly enhanced at third cleavage, when four micromeres are formed at the animal pole of the embryo (stage 12, Fig. 3). The diffusion coefficient in the micromeres is considerably smaller (D = 2.9 $\pm$ 0.3 $\times$ 10^{-9} cm^2/sec) than in the macromeres and the polar lobe, in which values similar to those obtained in earlier stages are measured (D = 5.0 $\pm$ 0.3 $\times$ 10^{-9} cm^2/sec in the macromeres and 7.3 $\pm$ 0.5 $\times$ 10^{-9} cm^2/sec in the polar lobe; Fig. 5). In addition to this profound decrease of the diffusion coefficient in the plasma membrane of the micromeres at the eight-cell stage, also the fraction of lipid molecules that are mobile is reduced considerably in these cells. In the macromeres and in the polar lobe of the eight-cell stage, values for the mobile fraction are similar to those measured in earlier developmental stages (78 $\pm$ 2%, and 79 $\pm$ 2%, respectively), but in the micromeres the MF is reduced to 51 $\pm$ 2% (see Fig. 4).

DISCUSSION

In the present study we have shown that (i) the lateral diffusion coefficient of plasma membrane lipids is up to 45% greater in the vegetal polar lobe membrane than in the animal membrane area of the *Nassarius* embryo up to the eight-cell stage; (ii) the lipid lateral mobility in the polar lobe membrane shows a cell-cycle-dependent modulation, with highest values for the diffusion coefficient during the S phase; (iii) the mobility of lipids is restricted in the plasma membrane of the micromeres formed during third cleavage, as inferred from a considerable decrease in both the diffusion coefficient and the mobile fraction.

These results demonstrate the existence of an animal-vegetal polarity in the mobility characteristics of plasma membrane lipids in the egg membrane. So far, only one other report on regional differences in membrane lipid lateral mobility related to animal-vegetal polarity in egg cells has been published. In the unfertilized *Xenopus* egg the lateral diffusion coefficient of membrane lipids is smaller in the animal half than in the vegetal half of the egg. This difference is strongly enhanced upon fertilization, as a result of complete lipid immobilization in the animal plasma membrane, which persists after first cleavage up to the 32-cell stage (Dictus *et al.*, 1984). In contrast, no regional differences in lipid lateral mobility have been observed in unfertilized sea urchin eggs (Peters *et al.*, 1984). No data are available on lateral mobility characteristics after fertilization in these eggs.

In a previous study, we demonstrated by freeze-fracture electron microscopy that in the uncleaved fertilized *Nassarius* egg regional differences exist in the numerical distribution of intramembrane particles (IMPs) in the egg membrane (Speksnijder *et al.*, 1984b, 1985). More particularly, the vegetal zone of the egg is characterized by a higher IMP density on both E and P face of the plasma membrane. Since the IMPs in the *Nasarius* egg membrane most likely represent intrinsic membrane proteins, this result has been interpreted either as a polar accumulation of membrane proteins in the vegetal zone of the egg, and/or as a regional difference in lipid composition, causing vertical displacement of membrane proteins in relation to the plane of fracturing (Speksnijder *et al.*, 1985).

With respect to the lateral diffusion of membrane lipids in the uncleaved egg, only slight differences can be observed when comparing the animal, equatorial, and vegetal zone of the egg. Application of an analysis of variance fails to demonstrate a significant difference between the animal and the vegetal zone of the egg. Apparently, the phenomena responsible for the existence of regional differences in the numerical IMP distribution in the membrane of the uncleaved egg do not cause any detectable differences in the lipid lateral mobility as measured by the FPR method.

However, during further development of the egg,

prominent changes can be observed in the lateral diffusion of membrane lipids in the egg membrane. At the time when the polar lobe preceeding first cleavage is being formed, the diffusion coefficient in the polar lobe area starts to increase, resulting in a significant difference between the animal and vegetal zone of the egg. This difference persists at least up to the eight-cell stage.

As yet, the underlying mechanisms responsible for the establishment of these regional differences in lipid lateral mobility are unknown. Both regional differences in protein and/or lipid composition, as well as differences in the interaction between membrane lipids and proteins might be involved. However, experimental evidence obtained from FPR measurements indicates that the lateral diffusion of membrane lipids is largely unaffected by the presence of proteins (Wolf *et al.*, 1977; Schindler *et al.*, 1980; Wu *et al.*, 1981). In contrast, lipid composition—cholesterol content more than fatty acid composition—appears to be a potent modulator of lipid lateral diffusion in biomembranes (Vaz *et al.*, 1982). Thus regional differences in lipid composition might be responsible for the observed local differences in lipid lateral mobility.

Superimposed upon spatial differences in lipid lateral mobility we also observed time-dependent modulations in lipid diffusion during early development of the egg. It appeared that the diffusion coefficient of the probe in the polar lobe membrane shows a cell-cycle-dependent modulation, with maximal values for D during the S phase of each cell cycle. A similar cell-cycle-dependent modulation of plasma membrane dynamics has been demonstrated in neuroblastoma cells (de Laat *et al.*, 1980; de Laat and van der Saag, 1982), and this reorganization of the plasma membrane has been interpreted as being pertinent for cell cycle progression (de Laat and van der Saag, 1982; de Laat *et al.*, 1984).

Similar mechanisms as in the neuroblastoma cells might be responsible for the observed modulations in lipid lateral mobility in the *Nassarius* egg membrane, and the correlation between these temporal modulations and the cell cycle may point to a role in cell cycle progression. However, another function is suggested by the exclusive occurrence of these modulations in the polar lobe area. This part of the egg plasma membrane differs from the rest of the egg in numerical IMP distribution patterns (Speksnijder *et al.*, 1984b, 1985) and in surface architecture (Dohmen and van der Mey, 1977; Dohmen, 1983). These regional specificities of the plasma membrane could reflect an intimate relationship between the plasma membrane and polar-lobe-specific morphogenetic determinants. This view is supported by centrifugation experiments, which show that there is a strong association between the egg surface at the vegetal pole and the lobe-specific determinants (see Verdonk and Cather, 1983). A number of polar lobe deletion experiments in various species suggest that during the early cleavage period resegregation of determinants out of the polar lobe occurs (e.g., Render, 1983), which is indicative of a dynamic process in the polar lobe.

Modulations in lipid lateral mobility—which are likely to reflect modulations in lipid composition—might be involved in such dynamic processes by influencing the organization of the cytoskeleton (see e.g., Hoover, 1981; Meier *et al.*, 1982), or by modulating the activity of intrinsic proteins (Edidin, 1981; Benga and Holmes, 1984) such as ion pumps and channels. Both the cytoskeleton and transcellular currents established by a nonrandom distribution of ion pumps and channels, have been named as being involved in localization phenomena (see, e.g., Jaffe, 1982; Nuccitelli, 1983; van den Biggelaar and Guerrier, 1983; Speksnijder and Dohmen, 1983; Jeffery, 1983). It remains to be solved, however, whether such mechanisms are also operative in the *Nassarius* egg.

Local changes in plasma membrane dynamics are not restricted to the polar lobe area. At the animal pole a considerable change is observed at third cleavage, when the four blastomeres of the embryo are divided into four macromeres at the vegetal pole and four micromeres at the animal pole. In these micromeres, both the diffusion coefficient and the mobile fraction have decreased significantly, indicating an important restriction on the lateral mobility of membrane lipids. The prominent difference in membrane lipid properties observed in the micromeres could thus determine major differences in plasma membrane functioning, which might play a role in cell diversification during early development.

This possibility is illustrated by deletion and other experiments on eggs of several species of gastropods, which clearly demonstrate that the first quartet of micromeres is committed to the formation of special structures (Verdonk, 1965; Clement, 1967; van Dam and Verdonk, 1982; Janssen-Dommerholt *et al.*, 1983; Verdonk and Cather, 1983). It is thought generally, that this commitment is brought about by the segregation of specific morphogenetic factors into the micromeres at third cleavage (see Verdonk and Cather, 1983). In addition, the acquirement of a plasma membrane with distinctly different properties could also be essential for the establishment of this specific cell line.

In conclusion, the present study demonstrates the existence of an animal–vegetal polarity in the dynamic properties of the *Nassarius* egg plasma membrane, in addition to previously observed regional differences in the structural organization of the plasma membrane

(Dohmen and van der Mey, 1977; Dohmen, 1983; Speksnijder *et al.*, 1984b, 1985). As suggested before (Wolf, 1983; Dictus *et al.*, 1984; Speksnijder *et al.*, 1985), such regional differences in plasma membrane properties could play an important role in early development.

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