Journal of Fluorescence, Vol. 4, No. 4, 1994

Lateral Mobility of Plasma Membrane Proteins in Dividing Eggs of the Loach (*Misgurnus fossilis*): Regional Differences and Changes During the Cell Cycle

Valentina P. Bozhkova,[1] Monika Budayova,[2] Peter Kvasnicka,[2] Natalia Cigankova,[1] and Dusan Chorvat[2]

Received October 18, 1993

Regional differences in lateral diffusion rates of fluorescence-labeled proteins have been studied in the plasma membrane of dividing eggs of the loach (*Misgurnus fossilis*) by fluorescence recovery after photobleaching (FRAP). Apparent animal–vegetal differences in fluorescence intensity, lateral diffusion coefficients, and fractions of mobile proteins have been found, with all these quantities being higher in the animal pole region than in the yolk region. Cyclic changes in protein diffusion coefficients and mobile fractions during the first few cell cycles have also been recorded. Soon after the end of a cleavage, the diffusion coefficient reaches its minimal value and increases rapidly before the next cleavage.

KEY WORDS: Polarity; cell cycle; plasma membrane; translational mobility; photobleaching.

INTRODUCTION

The apparent correlation between growth behavior and changes of plasma membrane properties has been demonstrated by many studies (see, e.g., Refs. 1–4 and references therein) and has led to intense research of membrane role in growth control. Considerable efforts have been invested in the study of mobility of plasma membrane components in developing embryos [5–14]. It has become apparent that no universal conclusions can be made about the lateral mobility of plasma membrane components during early embryogenesis: In amphibian and molluscan embryos, membrane lipids have been found to be more mobile on the vegetative than on the animal pole, in terms of both diffusion coefficients and mobile fractions [10,11,14]. On the other hand, no such differences have been found in sea urchin and mouse embryos [8,13]. Mobility was found to decreased significantly immediately after fertilization, with the drop being generally more pronounced in the animal region. This may reflect differences in mechanisms of maintaining the animal–vegetal polarity in embryos of different species [3,6,7,10]. The lateral diffusion behavior of proteins is often different from that of lipids and proteins [9], indicating that more specific mechanisms than mere membrane fluidity are involved in the control of lateral mobility of proteins, so that changes in membrane fluidity do not tell significantly on the mobility of proteins.

Speeksnijder *et al.* [14] have found cyclic changes in lateral mobility of a lipid probe in the membrane of a vegetal blastomere of the mollusk *N. reticulatus,* with a significant increase in the diffusion coefficient in the S-phase. de Laat *et al.* [5] report cyclic changes in lateral diffusion of both lipids and proteins during the cell cycle of neuroblastoma cells. For lipids, the diffusion coefficient was found to be minimal in mitosis, it increased

[1] Institute for Information Transmission Problems, Russian Academy of Sciences, Moscow, Russia.

[2] Department of Biophysics and Chemical Physics, Faculty of Mathematics and Physics, Comenius University, Bratislava, Slovakia.

1053-0509/94/1200-0369$07.00/0

Table I. The Ratio of Fluorescence Intensities from Various Parts of an Embryo, 1 and 2 h After Labeling[a]

	Time of incubation	
	1 h	2 h
FB/FC	1.51	3.13
FB/FV	1.84	3.19

[a]FB, FC, and FV are fluorescence intensities on the blastomeres, in the yolk center, and at the vegetal pole, respectively.

in the G_1 phase, and after being constant during the S phase, it dropped back again at the beginning of mitosis. For proteins, the diffusion coefficient behaved differently in that it slowly decreased in the S and G_2 phases to a minimum in mitosis.

In this study we used eggs of loach (*Misgurnus fossilis*) as a model system to investigate animal–vegetal differences in distribution and translational mobility of proteins in embryonal plasma membranes and their variations during cell cycles at early stages of embryonal development.

MATERIALS AND METHODS

Materials. Mature eggs were collected from females of loach (*Misgurnus fossilis*), stimulated by human chorionic gonadotropin. To prevent activation, manipulations with nonfertilized eggs were done in a calcium-free medium. The eggs were fertilized by sperm, and prior to labeling, the vitelline membranes were carefully removed by a needle. The manipulation with the eggs was not difficult because of their large dimensions (> 1 mm in diameter).

Fluorescence Labeling. Proteins in the egg cytoplasmic membranes were labeled nonspecifically by with FITC, dissolved in calcium-free Hotfreter solution at pH 10 to a concentration of 3 mg/ml. The eggs were incubated with the dye solution for 10 min at 20°C, and then they were rinsed thoroughly in Holtfreter solutions of pH 10 and 7.2 and in tap water. Labeling at a high pH prevented internalization of the dye [6,13].

Preparation of Samples. The eggs were placed in small holes, formed in a thin (2-mm) 1% Bacto agar layer in small petri dishes. Before the measurement, the embryos were visually inspected in a fluorescence microscope (JENALUMAR, Carl Zeiss Jena) to check their integrity. It was found that embryos with *leaky* membranes, which internalized FITC, showed anomalous diffusion characteristics (not presented). All such embryos were excluded from further measurements.

FRAP Measurements. Lateral diffusion measurements were performed using the FRAP instrument described elsewhere [15]. Briefly, an argon laser beam was focused on a cell membrane by a 12.5/0.35 dry objective into a Gaussian spot with an e^{-2} radius of 2.6 μm. The illumination intensity was controlled by an acoustooptic modulator. Fluorescence was detected by photoncounting electronics. An image plane diaphragm was used to reduce the depth of the layer, from which light was collected. Diffusion coefficients (D) and mobile fractions (R) were obtained from recovery curves by a nonlinear curve-fitting procedure in a manner similar to that described in Ref. 16.

RESULTS AND DISCUSSION

Fluorescence Labeling. The eggs exhibit a weak autofluorescence, which was in most cases 10–5 times lower than that excited from a labeled membrane. Moreover, the bleaching efficiency was found to be two to three times lower for autofluorescence than for bound FITC. Apparently, autofluorescence did not contribute significantly to fluorescence recovery data and was not considered in the analysis. We have found that a small portion of free FITC in the vicinity of the cytoplasmic membrane cannot be removed even by repeated rinsing. This is analogous to findings of Peters and Richter [6,13]. Simple calculations (not presented) indicate, however, that the detected small amounts of FITC give negligible contribution to fluorescence recoveries (less than 2%), because the fluorescence from the solution was substantially eliminated by the depth of field-limiting image plane diaphragm of the microscope. Moreover, the recovery curves were found to correspond in all cases to one-component diffusion, with no fast-diffusing component detected.

Distribution of FITC Within the Plasma Membrane. The distribution of bound FITC in the plasma membrane was nonuniform, as shown in Table I. The nonhomogeneity increases with the time of incubation after labeling—the fluorophore concentrates in the membranes of blastomeres. This can be attributed to the much more microvillous surface of the animal part of the embryo, with the degree of microvillation increasing in the course of early development [4].

Regional Differences in Lateral Mobility. Figure 1 shows significant differences in the lateral mobility at the animal and vegetal poles. It is also apparent that these differences are preserved during the period of ob-

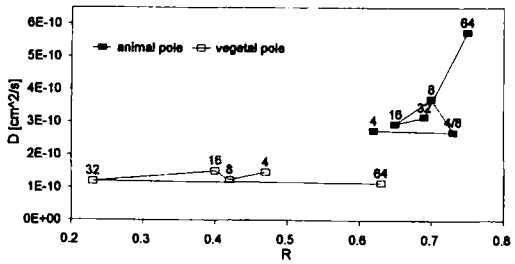

Fig. 1. Animal–vegetal differences in lateral mobility of proteins in the embryonal plasma membrane. Each point is an average of 10–20 measurements on three to five embryos. Numbers indicate the number of blastomeres at the corresponding stage of development.

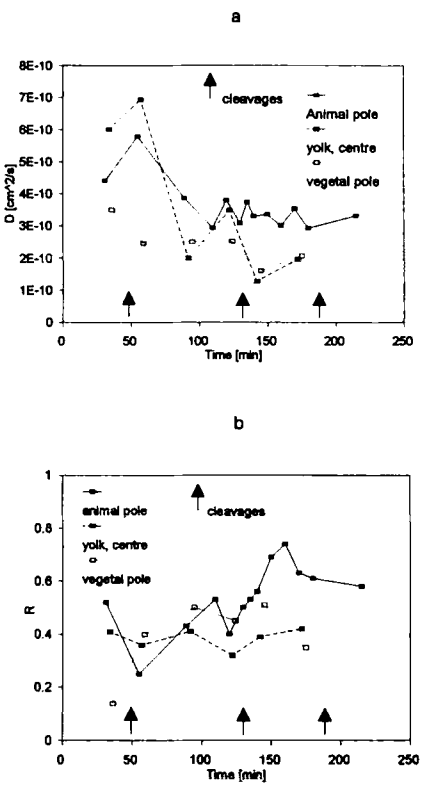

Fig. 2. Time dependences of the diffusion coefficient D (a) and mobile fraction R (b) of proteins in different regions of the embryonal plasma membrane: animal pole (■), vegetal pole (□), and central yolk region (☒). The three arrows indicate moments of the second, third, and fourth cleavages. Each point is the average for three embryos.

servation (second–sixth cleavage). The differences are significant at individual stages of development, both pairwise (for individual eggs) and unpaired (averaged for a group of eggs), with t-test statistics corresponding to $P < 3\%$. This difference is opposite to that which could have been deduced from the presence of microvilli in the animal region: The presence of microvilli would lead to a decrease in the observed mobility, whereas we have found larger D and R values in the animal region than in the vegetal, where the plasma membrane is much smoother.

Lateral Mobility during Early Embryogenesis. Figure 2 shows time dependencies (averaged for three eggs) of the diffusion coefficient (a) and mobile fraction (b) in three regions of the embryonal cytoplasmic membrane. Apparently, there is a remarkable increase in the diffusion coefficient, a decrease in the mobile fraction, and (not shown) an increase in their fluctuations at the beginning of each cleavage. The origin of fluctuations in the measured values is due mainly to a decrease in the synchronicity of cell cycles of individual blastomeres. After the sixth cleavage it was no longer possible to determine the common stage of the cell cycle. Intensive movements of the embryo in the process of cleavage also contribute to the fluctuations, but they take place on a time scale which is sufficiently long compared to the speed of the fluorescence signal accumulation during measurement so that this effect could have been substantially eliminated in the process of measurement and data analysis.

There have been only a few results on lateral mobilities of proteins in embryonal cell membranes. Dictus *et al.* [10] report animal–vegetal differences opposite to our in eggs of *Xenopus laevis*; their values of R and D are much lower at the animal than at the vegetal pole. The results presented here indicate that, while the lateral mobility of lipids in the animal region has been found to be either lower than [10,11,14] or equal to [13,8] that in the vegetal region, the mobility of proteins in our embryos is higher in the animal region than in the vegetal. Our results concerning changes of D and R in the cell cycle are compatible with those obtained by de Laat *et al.* [5] on neuroblastoma cells.

REFERENCES

1. G. L. Nicolson (1976) *Biochim. Biophys. Acta* **457**, 57–108.
2. G. L. Nicolson (1976) *Biochim. Biophys. Acta* **458**, 1–72.
3. J. Campisi and C. Scandella (1980) *Nature* **286**, 185–186.
4. V. P. Bozhkova *et al.* (1992) *Int. J. Dev. Biol.* **36**, 579–582.
5. S. de Laat *et al.* (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1526–1528.
6. R. Peters and H.-P. Richter (1981) *Dev. Biol.* **86**, 285–293.
7. D. E. Wolf *et al.* (1981) *Dev. Biol.* **81**, 133–138.
8. D. E. Wolf, A. Handyside, and M. Edidin (1982) *Biophys. J.* **38**, 295–297.
9. D. E. Wolf and C. A. Ziomek (1983) *J. Cell Biol.* **96**, 1786–1780.
10. W. Dictus *et al.* (1984) *Dev. Biol.* **101**, 201–211.
11. P. A. T. Tetteroo *et al.* (1984) *Dev. Biol.* **104**, 210–218.
12. R. Peters, C. Sardet, and J.-P. Richter (1984) *Dev. Growth Differ.* **26**(1), 105–110.
13. R. Peters, C. Sardet, and H.-P. Richter (1984) *Dev. Biol.* **86**, 105–110.

14. J. Speeksnijder *et al.* (1985) *Dev. Biol.* **110**, 207–216.
15. D. Chorvat and P. Kvasnicka (1993) in J. E. I. Korppi-Tommola (ed.), *Laser Study of Macroscopic Biosystems*, Proc. SPIE 1922, pp. 116–125.
16. B. G. Barisas and M. D. Leuther (1979) *Biophys. Chem.* **10**, 221–229.
17. B.M. Aizenbud and N. D. Gershon (1982) *Biophys. J.* **38**, 287–293.