Phospholipid transfer activities in toad oocytes and developing embryos[1,2]

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Abstract The role of lipid transfer proteins during plasma membrane biogenesis was explored. Developing amphibia embryos were used because during their growth an active plasma membrane biosynthesis occurs together with negligible mitochondrial and endoplasmic reticulum proliferation. Sonicated vesicles, containing ^{14}C-labeled phospholipids and ^{3}H-labeled triolein, as donor particles and cross-linked erythrocyte ghosts as acceptor particles were used to measure phospholipid transfer activities in unfertilized oocytes and in developing embryos of the toad *Bufo arenarum*. Phosphatidylcholine transfer activity in pH 5.1 supernatant of unfertilized oocytes was 8-fold higher than the activity found in female toad liver supernatant, but dropped steadily after fertilization. After 20 hr of development, at the stage of late blastula, the phosphatidylcholine transfer activity had dropped 4-fold. Unfertilized oocyte supernatant exhibited phosphatidylinositol and phosphatidylethanolamine transfer activity also, but at the late blastula stage the former had dropped 18-fold and the latter was no longer detectable under our assay conditions. Our results show that fertilization does not trigger a phospholipid transport process catalyzed by lipid transfer proteins. Moreover, they imply that 75% of the phosphatidylcholine transfer activity and more than 95% of the phosphatidylinositol and phosphatidylethanolamine transfer activities present in pH 5.1 supernatants of unfertilized oocytes may not be essential for toad embryo development. Our findings do not rule out, however, that a phosphatidylcholine-specific lipid transfer protein could be required for embryo early growth. — **Rusiñol, A., R. A. Salomón, and B. Bloj.** Phospholipid transfer activities in toad oocytes and developing embryos. *J. Lipid Res.* 1987. **28:** 100–107.

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Intracellular lipid transfer proteins were described more than 25 years ago, first in eucaryotic and then in procaryotic organisms (1–5). Different transfer proteins catalyze the exchange and/or the net transfer of lipids in vitro, but evidence for their function in living cells is lacking. Based on experiments performed with purified transfer proteins, some physiological functions have been postulated such as *i*) participation in the biogenesis of precursors of plasma lipoproteins (6), *ii*) regulation of cholesterol biosynthesis and metabolism (7–10), and *iii*) intracellular net transfer and exchange of membrane polar lipids during membrane biogenesis and maintenance (11–16), among others.

We have been interested in exploring the role of lipid transfer proteins in membrane biogenesis. If these proteins participate in the net transfer of lipids to sites of membrane synthesis, developing systems may require increased lipid transfer activities. A remarkable example of development is that of the embryo. From a single germ cell, thousands of cells are produced without an overall increase in size or mass, but with membrane formation as a prominent cellular event triggered by fertilization (17). During early development, amphibia embryos show a constancy in total phospholipid content (18–21), but an active plasma membrane proliferation together with negligible mitochondrial (22) and endoplasmic reticulum (20) biogenesis. Most of the embryo phospholipids are stored in the yolk platelets (19, 20), and it has been suggested (19) that fertilization may trigger the onset of a phospholipid transport process analogous to the process catalyzed by the lipid transfer proteins. To date, however, there has been no evidence reported to support this proposal. Moreover, lipid transfer activities have been described neither in amphibia oocytes nor in developing embryos.

In the present study we have determined the PC transfer activity in amphibian oocyte supernatant and have compared it with the PC transfer activities of amphibia

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol.

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[2]A portion of this work was presented at the IV Congress of the Pan-American Association of Biochemical Societies in Buenos Aires, Argentina, November 1984.

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and rat liver. In addition, PE and PI transfer activities were measured in supernatants of unfertilized oocytes and the level of the three transfer activities was monitored during early development.

MATERIALS AND METHODS

Triolein

Tri-9,10-[^{3}H]oleoylglycerol (150 Ci/mmol) was obtained from New England Nuclear (Boston, MA) and purified by silica gel H (E. Merck, Darmstadt) thin-layer chromatography with petroleum ether (bp 55–68°C–diethyl-ether–acetic acid 80:20:1 (v/v/v). The triolein band was eluted with chloroform–methanol 80:35 (v/v) containing 0.01% butylated hydroxytoluene and stored at −20°C.

Radioactive phospholipids

Sodium [1-^{14}C]acetate (3 mCi/mmol) was obtained from the Comisión Nacional de Energía Atómica (Buenos Aires, Argentina). Five g of soybean seeds was moistened with 100 μCi of radioactive acetate in 5 ml of tap water and, when the solution was completely absorbed, the seeds were allowed to germinate in the dark at 30°C for 55 hr. They were placed in 5 ml of hot (85°C) 0.25% aqueous acetic acid and heated for 30 min to inactivate lipases (23). The seeds were then ground in a glass mortar with 5 ml of water in the presence of acid-washed sand, extracted with 120 ml of chloroform–methanol 1:1 (v/v) for 15 min, and filtered through Whatman No. 1 filter paper. The extract was partitioned with 0.5 vol of 40 mM $CaCl_2$ (24) and the lower chloroform phase was washed twice with the same volume of 0.25% acetic acid containing 1 mM Na_2EDTA. The washed extract was dried under N_2 in a rotatory evaporator, dissolved in 5 ml of petroleum ether (bp 55–68°C), and the phospholipids were precipitated by the addition of 100 ml of cold acetone. After several hours at −10°C, the phospholipids were collected by centrifugation. Of the 24.8×10^6 ^{14}C dpm present in the washed lipid extract, 13.4×10^6 was recovered in the crude phospholipid mixture. Phospholipids were separated by CM-cellulose column chromatography (25). A microgranular (pre-swollen) CM-cellulose (CM52, Whatman Ltd., Springfield Mill, Kent) column (1 × 20 cm) was packed in chloroform and the crude phospholipid mixture was applied in the same solvent. The column was eluted (flow rate ≅ 100 ml/hr) with 200-ml portions of 2, 4, 6, 10, 23, 30, and 50% (v/v) methanol in chloroform. One hundred-ml fractions were collected and 1-ml aliquots were analyzed by silica gel H thin-layer chromatography with chloroform–methanol–water 65:25:4 (v/v/v) as solvent. PC, PE, and PI were eluted with 4%, 10%, and 30% of methanol in chloroform, respectively. PC and PE were separated from contaminating glycolipids by column chromatography on silicic acid columns (2.5 × 4 cm) packed in chloroform. Following elution of glycolipids with 400 ml of acetone, the phospholipid was eluted with 200 ml of methanol. The radiopurity of the three phospholipids, checked by thin-layer chromatography (as described above) was greater than 97%. Specific activities ranged between 0.15 and 0.30 μCi/μmol. The phospholipids, to which 0.1 mol % butylated hydroxytoluene was added, were stored in chloroform at −20°C.

Egg yolk PC

Egg yolk PC was purified on neutral alumina columns (type WN-3, Sigma Chemical Co., St. Louis, MO) by a modification of the procedure of Luthra and Sheltawy (24). Yolk lipids from a medium-size egg were extracted with 10 volumes of chloroform–methanol 1:1 (v/v) and, after filtration, the extract was partitioned with 40 mM $CaCl_2$, dried in a rotatory evaporator under reduced pressure, and taken up in 10 ml of chloroform. The crude extract was applied to a 3-cm diameter column packed with a chloroform slurry of 25 g of alumina activated overnight at 110°C. After eluting the neutral lipids and some PC with 100 ml of chloroform–methanol 50:1 (v/v), pure PC was eluted with 250 ml of chloroform–methanol 19:1 (v/v). Two hundred and fifty μmol exhibited a single spot by thin-layer chromatography on silica gel H with either chloroform–methanol–water 65:25:4 (v/v/v) or chloroform–methanol–conc.NH_4 65:35:5 (v/v/v) as solvents. After adding 0.1 mol % of butylated hydroxytoluene, the PC was stored in chloroform at −20°C.

Preparation of sonicated vesicles

Appropriate volumes of the stock lipid solutions were mixed and dried at 37°C under a stream of N_2. The final mixture contained 350 μmol of egg yolk PC, 350 μmol of [^{14}C]PC, [^{14}C]PE, or [^{14}C]PI, and a trace of [^{3}H]triolein. The lipids were redissolved in a few ml of diethylether and dried again under N_2. After the addition of 2 ml of 25 mM Tris-HCl, 150 mM NaCl, 1 mM Na_2EDTA, 5 mM β-mercaptoethanol, 3 mM NaN_3, pH 7.4 (Tris buffer), the suspension was briefly shaken on a vortex mixer and sonicated in a sonicating water bath (Laboratory Supplies Co., Hicksville, NY) for 20 to 30 min below 20°C. The sonicated preparations were centrifuged at 12,800 *g* for 10 min before use. When not used immediately, they were stored at 4°C in the dark under N_2 for no longer than a week and resonicated for 15 min before use.

Cross-linked erythrocyte ghosts

Cross-linked erythrocyte ghosts were prepared by a modification of the procedure of Cuppoletti et al. (26). White ghosts were prepared from fresh human blood according to Steck (27). The packed ghosts, in 20 mM sodium phosphate, pH 8, were cross-linked by the addi-

tion of glutaraldehyde to a final concentration of 100 mM. After 5 min at room temperature, they were pelleted by centrifugation (10^4 *g* for 5 min in a Sorvall RC-5B refrigerated centrifuge). After several washes, the cross-linked ghosts were suspended in Tris buffer and kept in 5-ml portions at −60°C.

Assay of phospholipid transfer activities

Phospholipid transfer activities were determined by measuring the transfer of ^{14}C-labeled phospholipids from sonicated vesicles to cross-linked erythrocyte ghosts. Sonicated vesicles (40 nmol of phospholipids) were incubated with cross-linked ghosts (200 nmol of phospholipid phosphorus) and an appropriate aliquot of pH 5.1 supernatant in a final volume of 1 ml. Incubations were carried out for up to 2 hr at 37°C with periodic agitation. Transfer was terminated by sedimenting the ghosts (2 min at 12,800 *g*) in an Eppendorf micro-centrifuge model 5412. The supernatants were discarded and the pellets were washed twice with 1 ml of Tris buffer. They were then dissolved in 1 ml of scintillation medium (Triton X-100, 333 ml; toluene, 667 ml; PPO, 5 g; dimethyl POPOP, 0.5 g) and quantitatively transferred to counting vials. The centrifuge tubes were washed twice with 1-ml aliquots of scintillation medium and 7 additional ml was then added to the vials. Radioactivity was counted in a Beckman LS-100 C scintillation counter and appropriate overlap and efficiency corrections were made. Blank incubations without active protein were performed simultaneously and all the determinations were done in duplicate. The amount of labeled phospholipid transferred from the vesicles was calculated from the ^{14}C counts found in the pellets and expressed as percentage of the total amount of label in the incubation. Apparent transfer due to co-sedimentation of sonicated vesicles with cross-linked ghosts was monitored by the inclusion of the nontransferable marker [^{3}H]triolein.

Toad oocytes and embryos

Adult, *Bufo arenarum,* Hensel toads captured in the surroundings of the city of Tucumán were used. They were kept in a humidified container without food for 3 to 6 weeks prior to the experiments. To induce ovulation, homogenates of homologous pituitary glands made in amphibian Holtfreter solution were injected into one of the dorsal lymphatic sacs. The toads began to eliminate oocytes through the cloaca 14 to 18 hr later (28). At this point, they were demedulated and the oocytes were collected from the ovisac in a Petri dish. Artificial fertilization was accomplished by adding homogenates of two toad testicles to 8,000–10,000 oocytes. Under these conditions, more than 95% of the oocytes became fertilized and underwent normal development to tadpoles. Development was allowed to proceed at 20–25°C and was followed using as a reference the morphological characteristics described by Del Conte and Sirlin (29). Unfertilized oocytes and embryos at different stages of development were sampled after removal of the jelly-coat with neutralized 2% thioglycolic acid.

Preparation of pH 5.1 supernatants

The entire procedure was carried out at 0–4°C. Oocytes and developing embryos were washed with Tris buffer to remove the thioglycolic acid and 30% (w/v) homogenates were prepared in the same buffer. They were centrifuged for 15 min at 15,000 *g* and the supernatants were adjusted to pH 5.1 with 1 N HCl. After 15 min, the samples were centrifuged for 20 min at 15,000 *g* to yield clear supernatants that were adjusted to pH 7.4 with 2 N NaOH, and the transfer activities were measured immediately. Aliquots were also kept frozen at −60°C, but this procedure resulted in a severe loss of biological activity. A similar procedure was carried out to prepare toad and rat liver supernatants but 20% homogenates (w/v) were used.

Analytical procedures

Protein concentration was determined by the method of Bradford (30) with bovine serum albumin as a standard. Lipid phosphorus was determined by the method of Ames and Dubin (31).

RESULTS

Cross-linked erythrocyte ghosts as acceptors in phospholipid transfer assays

It has been shown that erythrocyte ghosts are poor acceptor particles for lipid transfer assays. Low recovery of sonicated vesicles used as donor particles (32) as well as fragmentation of the ghosts during the assay (33) have been demonstrated. Glutaraldehyde treatment of the ghosts yields an excellent particle whose advantages can be summarized as follows. *a*) They are easy to prepare and can be stored frozen for a very long time. *b*) The particles are very stable and sediment quantitatively at relatively low *g* forces. *c*) They exhibit low levels of nonspecific interactions with sonicated vesicles, as revealed by the high recovery of the nonexchangeable marker (see Fig. 1); vesicle recovery is excellent even when the vesicles are made of 50% acidic phospholipid (see Table 3). *d*) Blank incubations exhibited very low spontaneous transfer of radioactive phospholipids (as shown in Fig. 1 and in Table 3); this improved the precision of the measurements.

Fig. 1C shows that there was a nearly linear relationship between the amount of [^{14}C]PC transferred and the amount of oocyte pH 5.1 supernatant protein, up to 5 mg. The system appeared suitable for the assay of PC transfer activity. The linearity of transfer up to 25% of [^{14}C]PC transferred was confirmed in separate assays (not shown).

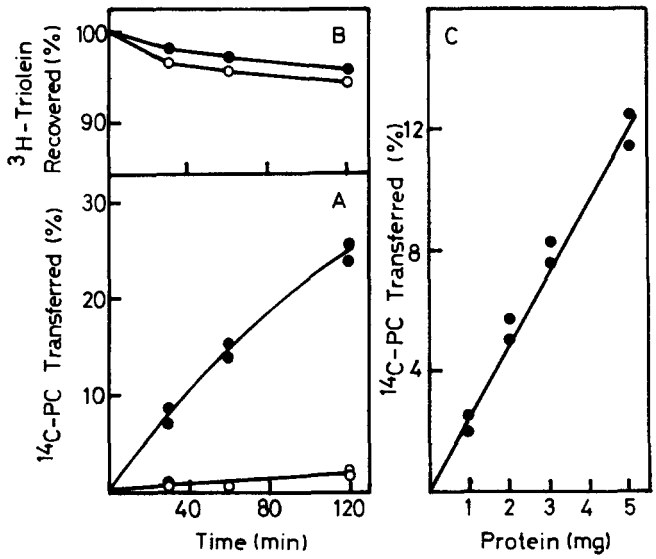

Fig. 1. Assay for phosphatidylcholine transfer. Sonicated vesicles of egg yolk phosphatidylcholine and ^{14}C-labeled phosphatidylcholine (molar ratio 1:1; 40 nmol of phospholipid phosphorus) containing a trace of [^{3}H]triolein were incubated with unlabeled cross-linked erythrocyte ghosts (200 nmol of phospholipid phosphorus) in a final volume of 1 ml of 25 mM Tris-HCl, 150 mM NaCl, 1 mM Na_2EDTA, 5 mM β-mercaptoethanol, 3 mM NaN_3, pH 7.4, at 37°C. After the incubations, the ghosts were sedimented by centrifugation, washed twice, and transferred to counting vials as described under Materials and Methods. A, Incubations in absence (○ — ○) and in presence (● — ●) of 0.98 mg of protein of pH 5.1 oocyte supernatant. B, Recovery of [^{3}H]triolein in the supernatant. C, Incubations with increasing amount of pH 5.1 supernatant were carried out for 20 min. A blank transfer of 1.8% was subtracted.

Some properties of the PC transfer activity of oocyte cytosol

Because this is (to our knowledge) the first publication demonstrating PC transfer activity in cellular extracts of amphibian oocytes, preliminary studies on its properties have been carried out. We performed parallel extracts and transfer assays with Tris buffer containing either 150 mM or 20 mM NaCl. Transfer activities measured as percent of PC transferred per hr per mg of protein were 9.1 and 6.6, respectively. When the samples were dialyzed overnight at 4°C against 100 vol of the 20 mM NaCl buffer before the assay, the transfer activities dropped to 6.9 and 5.3 for the high-salt and the low-salt extracts, respectively. These results showed that buffer containing 150 mM NaCl yielded supernatants with higher transfer activities and that, contrary to what was shown with some mammalian transfer proteins (34, 35), PC transfer activities of oocyte cytosol were not inhibited by increasing concentration of NaCl. On the other hand, the transfer activity was sensitive to heat: incubation at 62°C for 10 min resulted in the loss of 80% of the transfer activity. Very similar PC transfer activity was obtained when the assays were performed with post-microsomal supernatant (105,000 *g* for 1 hr) instead of pH 5.1 supernatant. In view of this result, pH 5.1 supernatants were used in all the experiments reported here.

PC transfer activity in toad oocytes and in developing embryos

Rat liver homogenates provided the first conclusive evidence for the existence of lipid transfer proteins, and also proved to be a valuable starting material for the purification and characterization of several of these proteins (1-5). In **Table 1,** the PC transfer activity of rat liver is compared with the transfer activity exhibited by toad liver and oocytes. Oocyte pH 5.1 supernatant exhibited the highest PC transfer activity: 8-15 times higher than toad liver and 3 times higher than rat liver.

In order to check the level of PC transfer activity during early embryo development, the activity was measured in supernatants obtained from embryos after 8 and 20 hr of development. After 8 hr at 20-25°C, the embryos are at the level of 6th cleavage, meaning that the original cell has given rise to 64 cells. After 20 hr, the embryos are at the stage of late blastula, being formed by about 10^4 cells. As shown in **Table 2** (first column), the process of cell proliferation was accompanied by a decrease of the transfer activity expressed per mg of protein. This decrease could simply mean a constant level of PC transfer activity but increasing amounts of other soluble proteins. In the second column of Table 2, the transfer activity is expressed per embryo. A 4-fold decrease in PC transfer activity is seen when the embryos reach the stage of late blastula.

PE and PI transfer activities during early amphibian development

PE transfer activity has been detected in pH 5.1 supernatant of rat liver but not in supernatant from rat brain. Brain, on the other hand, has served as a major source of PI transfer activity (1-5). It was therefore of interest to look for PE and PI transfer activities in toad oocytes.

TABLE 1. Phosphatidylcholine transfer activity of pH 5.1 supernatant from liver and oocytes

Source of Supernatant	Transfer Activity[a]
	% transfer · hr^{-1} · mg^{-1}
Toad oocytes	12.0 (10.0-13.2)
Male toad liver	0.8 (0.8-0.8)
Female toad liver	1.5 (1.5-1.6)
Rat liver	3.7 (2.9-4.6)

Transfer activities were assayed using sonicated vesicles and cross-linked erythrocyte ghosts as described under Materials and Methods. Final values were obtained from the linear regression analysis of measurements made in duplicate for 30 and 60 min.

[a]Values are averages of two to four separate experiments with the range in parentheses.

TABLE 2. Phosphatidylcholine transfer activity during early amphibia development

Embryological Stage	Hours of Development at 20-25°C	Transfer Activity[a]	
	hr	*% hr^{-1} per mg $protein^{-1}$*	*% hr^{-1} per 10^2 embryos*
Oocyte	0	12.0 (10.0-13.2)	25.6 (22.9-30.6)
6th Cleavage	8	5.8 (4.4-6.5)	16.6 (14.5-19.3)
Late blastula	20	2.3 (2.2-2.4)	6.5 (4.9-8.0)

[a] Values are the average of three to four separate experiments with the range in parentheses. Transfer activities were measured as described in Table 1.

Table 3 shows an experiment in which the transfer activities were measured. Several points of the assay are noteworthy: very low transfer of labeled phospholipids when the supernatant is omitted; excellent recovery of donor particles; and a nearly linear increase of transfer with the time of incubation. These results demonstrate that oocyte supernatant is capable of catalyzing the transfer of radioactive PE and PI in vitro. **Table 4** shows the transfer of both phospholipids as a function of embryo development. The transfer activities of the embryo supernatant decreased as development proceeded. At the stage of late blastula, PE transfer activity was no longer detectable under our experimental conditions, while PI transfer activity dropped to about 5% of the level exhibited by unfertilized oocytes.

DISCUSSION

The outstanding feature of eggs of almost all nonmammalian vertebrates is their large size. Echinoderms and amphibia have been extensively studied as experimental models for fertilization, growth, and development. These eggs can be easily fertilized in vitro and rapid, highly synchronous development ensues. In relation to lipid changes during growth and development, it was found that the rate of introduction of fatty acyl groups into glycoproteins increases markedly prior to gastrulation in sea urchin (36). Studies on *Bufo arenarum* have established that PC, PE, and PI are the main phospholipids of toad oocytes and developing embryos, and that their content remains constant until late gastrula (18, 21). Subcellular distribution studies showed that the major portions of the three phospholipids are located in the yolk platelets, from there they have to be mobilized to support the requirements of the active membranogenesis triggered by fertilization (20). In spite of the constancy in the phospholipid content, active de novo lipid biosynthesis and polar head group turnover have been demonstrated during early embryonic development (21). It is therefore possible that lipid transfer to the sites of membrane biogenesis occurs simultaneously with the chemical modification of the phospholipid molecules.

There are at least three likely mechanisms by which phospholipids can move from the yolk platelets to the sites of membrane biogenesis: *1*) diffusion through the cytoplasm; *2*) vesicular transport; and *3*) lipid transfer protein-mediated transport. In the present work we have started to investigate the presence of the third of these mechanisms. Our results demonstrate that oocyte supernatant is capable of catalyzing the transfer of PC, PE, and PI. In the case of PC, which comprises more than 60% of its phospholipids, the *Bufo arenarum* oocyte exhibits a specific transfer activity 8-fold higher than the transfer activity of the female toad liver from which the oocytes are obtained. This relatively high level of transfer activity drops steadily during the first 20 hr of embryo development. It has been suggesteed (18, 19) that fertilization may trigger a transport mechanism for carrying phospholipids from the yolk platelets. Our results strongly suggest that fertilization does not activate protein-mediated phospholipid transfer.

After 20 hr of development, the embryo is at the stage of late blastula, and its cells continue to duplicate every 200 min on average (37) in spite of the fact that PI transfer activity has dropped 18-fold and PE transfer activity is no longer detectable. If the measurements, carried out on cell-free supernatants and using artificial donor and acceptor particles, reflect the level of transfer activities present in intact embryos, our results would indicate that

TABLE 3. Transfer of phosphatidylethanolamine (PE) and phosphatidylinositol (PI) catalyzed by oocyte pH 5.1 supernatant

Phospholipid Assayed	pH 5.1 Supernatant	Transfer Activity			Vesicle Recovery		
		Time (min)			Time (min)		
		30	60	120	30	60	120
		% per mg $protein^{-1}$			*%*		
PE	–	0	0.2	0	100	93	90
PE	+[a]	1.6	3.2	6.0	98	97	94
PI	–	0	0.5	1.0	95	90	83
PI	+[a]	1.4	4.0	8.9	93	88	90

Transfer activities were assayed using sonicated vesicles of egg yolk phosphatidylcholine and radioactive phosphatidylethanolamine or phosphatidylinositol as described under Material and Methods.
[a] Two mg of protein per ml.

TABLE 4. Phosphatidylethanolamine and phosphatidylinositol transfer activities during early amphibian development

Embryological Stage	Transfer Activity[a]	
	Phosphatidylethanolamine	Phosphatidylinositol
	% · hr⁻¹ per 10² embryos	
Oocyte	5.6 (4.6-6.4)	11.1 (9.6-13.4)
6th Cleavage	3.0 (2.7-3.2)	5.0 (4.7-5.3)
Late blastula	n.d.	0.6 (0.3-0.9)

Transfer activities were assayed as described under Materials and Methods. Final values were obtained from the linear regression analysis of measurements made in duplicate for 30 and 60 min; n.d., not detectable.

[a]Values are the average of two or three separate experiments with the range in parentheses.

75% of the PC transfer activity and more than 95% of the PE and PI transfer activities present in the pH 5.1 supernatant of unfertilized oocyte are not essential for embryo development. It seems appropriate to point out here that the yolk platelet fraction comprises more than 80% of the oocyte phospholipids and that this distribution remains unchanged at least to the late gastrula stage (20).

Although the data demonstrate an overall decrease in the cytosol lipid transfer activities as development proceeds, they do not rule out the possibility that some PC-specific phospholipid transfer protein was required for cell duplication. It has been shown that pH 5.1 supernatants from liver, brain, and heart contain distinct lipid transfer proteins that can be differentiated, among other properties, by their relative specificity toward the phospholipids whose transfer they catalyze (1-5). It is likely that the oocyte supernatant also contains a mixture of lipid transfer proteins, and this possibility is supported by the differential drop of the three transfer activities during the early stages of development. Further investigations must be carried out to separate the different lipid transfer proteins present in embryo supernatant and to follow their activities during development.

It is somewhat puzzling that most of the phospholipid transfer activities present in pH 5.1 supernatants of oocytes do not seem to be required for embryo development. A plausible explanation to this paradox could be found when oogenesis is considered (38). Ovarian sequestration of vitellogenin, a lipoglycophosphoprotein secreted by the liver, is predominantly responsible for oocyte growth. At the same time, the oocyte performs the nonspecific sequestration of serum albumin and other macromolecules present in plasma. Several lipid transfer proteins have been described in plasma (39-41) and very recently the nonspecific lipid transfer protein, which is regarded as the major source of PE transfer activity in cell cytoplasm, was shown to be a secretory product of cultured hepatoma cells (42). Also monocyte-derived macrophages secrete lipid transfer proteins (43). It is therefore possible that most of the lipid transfer activity present in oocyte supernatant is, in fact, due to proteins taken up nonspecifically from plasma during vitellogenesis. If, in addition, it is taken into account that 80 to 90% of the total protein in fully grown oocytes is found in yolk platelets as insoluble crystalline structures (38), the relatively high specific activity for PC transfer in oocytes could be explained. On the other had, if all the lipid transfer proteins present in oocytes are in fact of "endogenous" origin, future investigations will have to provide explanations for the marked decrease of their activities as development proceeds. It might be relevant in this regard, that inhibitors for some lipid transfer proteins have been described (44). Experiments are being performed to elucidate the origin of the lipid transfer activities found in amphibia oocytes and to understand the mechanism of their decrease during early development. Preliminary experiments have shown that when the 15,000 *g* pellet, that contains the yolk platelets, is resuspended and subjected to some disruptive treatments, additional PC transfer activity is released.

As far as we are aware, this is the first study of lipid transfer activities in amphibia embryos and also the first to show a developing system in which the lipid transfer activities exhibit a very marked decrease as development proceeds. Similar studies on other developing systems are scarce. Some studies have been made in mouse lung, where PC transfer activity has been shown to correlate with fetal maturation of the surfactant system (45). However, mammalian lung is a complex organ, made up of over 40 different cell types which do not contribute equally to the lipid metabolism of the organ (46). Carey and Foster (47) have studied phospholipid transfer activities in the supernatant fraction of developing rat brain and found that the overall phospholipid transfer activity increased postnatally. PC transfer increased from 9 days after birth to reach maximum values in the mature brain, while PI and PE transfer activities increased postnatally to a maximum at 9 days of age, with the lowest values in adult brain. It is known that rat brain contains at least four different lipid transfer proteins, differing in specificity and probably also in function. Besides, brain, like lung, is a complex multicompartmental tissue with different cell types (48). Compared with these two mammalian systems and in view of the results presented in this report, amphibia embryos at early stages of development (that is, before differentiation can be observed) seem to offer distinct advantages as an experimental model for the investigation of the role of lipid transfer proteins in plasma membrane biogenesis.

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