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# Temperature Dependence of Membrane Lipid Composition in Early Blastula Embryos of *Lytechinus pictus:* Selective Sorting of Phospholipids into Nascent Plasma Membranes

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**Abstract.** *Lytechinus pictus* eggs were fertilized and incubated at 10, 16, and 23°C until the early blastula stage of embryonic development. The phospholipid composition of the embryos and control unfertilized eggs remain identical and unchanged as incubating temperatures are varied; thus, neither incubating temperature, fertilization nor membrane assembly affect their total phospholipid composition. This result agrees with metabolic studies by others, using only a single incubation temperature, and indicates that embryonic development to the early blastula stage occurs with little, if any, *de novo* phospholipid biosynthesis. However, as in all poikilotherms, the phospholipid composition of the nascent plasma membranes varies with the incubation temperature. Thus, until the blastula stage of embryonic development, the lipids of these newly formed plasma membranes are derived from lipid pools within the embryo whose phospholipid composition is static. The variation of plasma membrane composition is primarily reflected in an increase in the phosphatidylethanolamine (PE): phosphatidylcholine (PC) ratio as incubating temperatures decrease; this is achieved by an exchange of PE for PC. Several mechanisms are considered for the specificity of the selective sorting and assembly of these phospholipids into the nascent plasma membranes.

## Introduction

Although protein and nucleic acid synthesis in sea urchin eggs is initiated almost immediately after fertilization (Epel, 1967; Epel et al., 1969; Humphreys, 1971), there is no evidence of *de novo* biosynthesis of the major phospholipids (i.e., PE and PC) in the early stages of embryonic development. Thus, it appears that lipid assembly into newly formed plasma membranes of sea urchins in these early stages of development occurs from a biosynthetically static pool of phospholipids within the embryo (Schnell & Lennarz, 1974; Byrd, 1975; Pasternak, 1977; Kozhina et al., 1978). The absence of enzymes active in the biosynthesis of phospholipids (Byrd, 1975; Armant et al., 1986) is further support of this conclusion; *de novo* synthesis of membrane lipids is not observed until perhaps after the blastula stage (Kozhina et al., 1978). Similar observations have been reported for chick and toad embryo development (Siek & Newburgh, 1965; Pechen et al., 1974; Alonso et al., 1982). These studies of lipid biosynthesis considered only a single incubation temperature, but echinoderms are capable of embryonic development over a fairly broad range of temperatures (Hinegardner, 1967). For other poikilotherms, such as bacteria, plasma membrane lipid composition is generally modified by metabolism as membrane assembly in these organisms accommodates to changes in incubation temperature (Farrell & Rose, 1967; Erwin, 1973). Does a similar accommodation of the membrane lipid composition occur in sea urchins when incubation temperatures are varied? If lipid composition of the nascent plasma membranes does change with incubation temperature, how is it achieved? Does the phospholipid pool in the egg change? We have addressed these questions in fertilized eggs of *Lytechinus pictus* incubating at temperatures that span the range of viable conditions for embryonic development, 10–23°C, and measuring the phospholipid compositions of the yolk platelets and the newly formed, nascent plasma membranes at the blastula stage of development. As controls, the total phospholipid

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composition of the entire embryo and unfertilized eggs maintained at the same incubating temperatures were also measured. Our study shows that, as incubating temperatures are varied, the nascent plasma membrane phospholipid composition changes while the phospholipid composition of the unfertilized eggs, embryos, and yolk platelets remain unchanged. Thus, plasma membrane assembly in the early stages of development for these animals appears to involve a selective recruitment of phospholipids from a static pool of embryonic lipid unperturbed by lipid metabolism. A central feature of this study is the application of the critical bilayer theory of membrane assembly for identification of the newly formed membranes (Gershfeld, 1989*a*,*b;* Gershfeld & Ginsberg, 1997). The discussion which concludes this study examines several mechanisms for the lipid selection process.

## Materials and Methods

### Preparation of Gametes and Embryos

Sea urchins of *Lytechinus pictus* were obtained from Marinus Biological Supply (Long Beach, CA) and kept in recirculating artificial sea water at 16°C for several months. Gametes were obtained by injection of 0.5 M KCl into the coelomic cavity. Eggs were collected in sea water; sperm were collected "dry" and stored at 5°C. To determine the range of temperatures that embryos will survive until the swimming stage of development, eggs were fertilized and incubated at various temperatures. Development was poor or nonexistent above 25°C or below 10°C, and therefore incubation was limited to within this temperature range. The following protocol was generally followed in this study. The eggs from 5–7 animals are passed through a Nitex filter (112 μm mesh) to remove debris, combined and gently mixed, and then washed with pH 8 artificial sea water (NaCl, 24.7 g/l; $CaCl_2 \cdot 2H_2O$, 1.32 g/l; $Na_2SO_4$, 3.7 g/l; HEPES, 1.3 g/l; $NaHCO_3$, 0.2 g/l; $MgCl_2 \cdot 6H_2O$, 10 g/l; pH 8.0) (ASW) containing Ampicillin (100 μg/ml) three times, using gentle aspiration to remove the ASW washes. The jelly coats of the pooled eggs are removed by suspending the eggs in pH 5 ASW containing Ampicillin for 3 minutes; this is followed by centrifugation at low speed to pack the cells and to remove by aspiration the pH 5 supernatant. The eggs are then resuspended in pH 8 ASW containing 1 mM 3-amino-1,2,4-triazole to facilitate removal of the fertilization membranes, and 100 μg/ml Ampicillin to inhibit bacterial growth. Eggs are divided into three portions: (*i*) to be fertilized and incubated until the early blastula stage at one of three temperatures 10, 16 and 23°C; (*ii*) unfertilized but to be incubated at the same temperatures and for the same duration as in (*i*); (*iii*) to be analyzed for their phospholipid content without fertilization or incubation. Eggs are fertilized by injecting "dry" sperm (50 μl), into an egg suspension (200 ml) maintained at 16°C. When >95% of the fertilization membranes have formed the membranes are removed by passing the eggs through a Nitex filter (112 μm mesh) at room temperature. The filtered cells, now free of fertilization membranes, are allowed to settle and the supernatant is removed and replaced with pH 8 ASW containing Ampicillin. The entire process: sperm injection, fertilization membrane formation (both at 16°C), and filtration (at room temperature, 20°C) takes about 45 min, and was always completed before first cell division occurs, the earliest cell division occurring after about 1–2 hr at the highest incubation temperature (23°C). The fertilized eggs are then placed into the incubation bath (10, 16, or 23°C), and the egg suspension gently stirred with a motorized paddle rotating at 5 rpm until the early blastula stage of growth is reached. This stage of development occurs after 20–24 hr at 10°C, after 15–18 hr at 16°C, and after 8–10 h at 23°C.

### Separation of Plasma Membranes and Yolk Platelets

Plasma membrane and yolk platelet separation from the embryos is attained by using sequentially the procedures of Kinsey (1986) for the membranes, and of Armant et al. (1986) for the yolk platelets. The procedure used in our study is as follows. When the early blastula stage is reached, the embryos are harvested from the incubating ASW, and resuspended at 4°C in a buffer, diluted 1:1 with distilled water, consisting of a solution of Ca-Mg-free sea water (CMF-SW) containing protease inhibitors (PI) aprotinin, leupeptin, and pepstatin, each at 1 μg/ml, (CMF-SW/PI). All the following steps are performed at 0–4°C. Following Kinsey (1986) the sample is homogenized using a size C Potter-Elvehjem homogenizer. The entire 1 ml homogenate is deposited on a discontinuous sucrose gradient consisting of 2 ml 60% (wt/vol) and 2 ml 45% sucrose made by adding sucrose to CMF-SW/PI. The gradient is centrifuged for 4 hr at 100,000 × *g* in a swinging bucket rotor (SW-50.1). Two bands form: an orange band consisting primarily of yolk platelets at the top of the 45% sucrose step covered by a thin clear CMP-SW/PI layer, and a thin colorless turbid band containing plasma membranes at the 60–45% boundary. After removal of the clear CMP-SW/PI layer, the upper yolk platelet band is removed for further centrifugation. The band containing membrane is removed to a screw-top test tube that is then filled with nitrogen, sealed with a Teflon-lined cap and stored at −20°C until its lipid components can be extracted (*see below*). Following the procedure of Armant et al. (1986), the yolk platelet band is layered on top of a discontinuous sucrose gradient consisting of 2 ml of 10% and 2 ml of 35% sucrose containing CMP-SW/PI. This is a variant of the gradient used by Armant et al., who used three steps 10, 25 and 35% sucrose for their gradient. Their results showed two broad bands of approximately equal volume, one centered at the 10/25% boundary but spread over both, and a second within the 25% sucrose solution; the first band was pure yolk platelets, and the second consisted of yolk platelets contaminated with mitochondria. We chose our 10/35% step gradient to further separate the pure yolk platelet band formerly observed at the 10/25% boundary. The gradient is then centrifuged at 100,000 × *g* in a Beckman SW-50.1 rotor for 16 hr. Two bands form, one at the top of the 10% step, and the other at the 10–35% boundary; a small amount of material is also observed as a pellet at the bottom of the 35% step. Since the densities of the two bands correspond to the ones found by Armant et al., we assume the two bands at the 10 and 35% density steps represent the two yolk platelet fractions reported previously (Armant et al., 1986); we have named the lower and high density bands YP-1 and YP-2, respectively. Each of the fractions is collected in tubes with Teflon-lined screw tops, then flushed with nitrogen, capped and stored at −20°C until processing for extraction of lipids.

### Extraction and Quantitative Analysis of Lipids

The method of Rose and Oklander (1965) was adapted to extract the lipids from eggs and from each of the embryo fractions. To each of the fractions, approximately 1–2 ml, 11 ml isopropyl alcohol is added, and the mixture is vortexed intermittently for a minimum of 30 min; 7 ml of chloroform is then added, and the resulting mixture again vortexed intermittently for 30 min. The final mixture will normally form a

single, clear solution phase of the lipid with water from buffer or tissue that may be present, and a precipitate of protein that can be pelleted by a low speed, bench-top centrifuge. However, the presence of relatively large concentrations of sucrose from the gradients in each of the fractions altered the phase relations of the isopropyl-chloroform-water extraction system; two liquid phases appear, a lower phase consisting primarily of chloroform, and an upper phase of water, with isopropyl alcohol partitioned between the two phases. Under these conditions a quantitative separation of the lipid and protein components is problematic. These conditions may be reversed by the stepwise addition of isopropyl alcohol to the mixture until only a single liquid phase forms. The protein is then sedimented by low speed centrifugation leaving a clear solution phase containing lipid; this phase is easily decanted. The solution is evaporated under a stream of nitrogen and the dried lipid is redissolved in 1 ml chloroform, the tube filled with nitrogen, sealed and wrapped with Al foil to shield the solution from light, and stored at −20°C.

Two methods are used for quantitative phospholipid analysis. (*i*) In the first method we used two-dimensional thin layer chromatography with silica gel G 20 cm × 20 cm plates (Alltech, Deerfield, IL). The two solvent systems used in the 1st dimension chloroform/methanol/$NH_4OH$/water in the volume ratios 45/22/6/1, and in the 2nd dimension chloroform/methanol/water in the volume ratios 65/25/4. After the chromatograms are developed the plates are sprayed with an aqueous solution of rhodamine G and the lipid spots identified by their fluorescence under UV. The spots are scraped, collected and their phosphorus contents assayed by the method of Rouser, Fleischer & Yamamoto (1970). (*ii*) In the second method we used HPLC with detection by an evaporative light scattering detector (ELSD) (Sedex model 50, Richard Scientific, Novato, CA). A gradient flow system is formed using two solvent systems consisting of A: chloroform/methanol/water/$NH_4OH$ (30%) in the ratio (vol) 60/34/5.5/2 and B: chloroform/methanol/$NH_4OH$ (30%) in the ratio (vol) 80/19.5/0.5; a flow rate of 1 ml/min with a varying gradient consisting of 0 to 100% (A) in 16 min concluding with 100% (A) in 4 min. The silica column used is 3 μm (100 × 4.6 mm) (Advantage Si). The ELSD detector requires calibration of each phospholipid by measuring the scattered light signal as a function of lipid concentration. Since the signal intensity depends on the type of fatty acids on each phospholipid class, we use a mixture of the *L. pictus* egg lipids (1–10 μg/10 μl per injection) whose concentration has been determined by thin layer chromatography as a standard for calibration. For the unknown samples agreement betweeen the two methods is within the experimental error of each method (±5%). The ELSD method requires much smaller samples (<1%) for quantitative analysis of lipids than thin layer chromatography; it is invaluable for measuring the lipids in membranes because only a small amount of material is available.

### Identifying Membrane Lipids by the Shear-Stress Method

As an independent check that the membrane lipids are from plasma membranes that form during embryonic development, we utilize a property of phospholipids that is exhibited only at the physiological growth temperature where membranes assemble, i.e., the incubation temperature. Bilayers formed in aqueous dispersions from the total lipid extracts of cell membranes are in a unique physical, critical state that exists only at a critical temperature T*; for membrane lipids T* equals the growth temperature, T*g*, of the cell from which the lipids have been extracted (Gershfeld, 1986; Ginsberg, Gilbert & Gershfeld, 1991; Gershfeld & Ginsberg, 1997; Jin et al., 1998). A number of techniques have been employed to demonstrate the existence of this critical lipid state (Tajima & Gershfeld, 1985; Gershfeld et al., 1993; Jin et al., 1998), and for the present study we have utilized the shear-stress method (Gershfeld & Ginsberg, 1997). In this method a constant shearing stress is applied to a suspension of multilamellar vesicles composed of membrane lipids and encapsulating a water soluble fluorescent probe 5(6)-carboxyfluorescene, (Molecular Probes, Eugene, OR) at self-quenching concentrations; the sample fluorescence is scanned as a function of temperature. In a narrow interval approaching the physiological growth temperature T*g* of the cellular source of the lipid the bilayers weaken, are ruptured by a threshold shearing stress, and fluorescent dye from the vesicles enters the external aqueous phase, is diluted and fluoresces. When temperatures exceed T*g*, leakage of fluorescent probe from the vesicles decreases. T*g* is thus readily identified and indicates that the lipid in the dispersion has been obtained from membranes that have been assembled at the incubation temperature of the embryos. Typical results for samples of the multilamellar vesicles formed from the nascent plasma membrane lipids of *L. pictus* are shown in Fig. 1.

### Abbreviations

| | |
|---|---|
| CL | cardiolipin |
| LPC | lysophosphatidylcholine |
| PA | phosphatidic acid |
| PC | phosphatidylcholine |
| PE | phosphatidylethanolamine |
| PG | phosphatidylglycerol |
| PI | phosphatidylinositol |
| PS | phosphatidylserine |
| YP | yolk platelet |
| T* | temperature of critical bilayer assembly; T*g*: growth temperature = $T_{INC}$, incubation temperature |

## Results

### Phospholipid Composition of Eggs, Embryos and Nascent Plasma Membranes as a Function of Temperature

To monitor the influence of temperature on the phospholipid composition of each of the embryo components we use the molar ratio of PE to PC. Since these lipids are the major phospholipid components of these tissues and represent more than 80% of the total phospholipid content of embryos (Kozhina et al., 1978), their ratio will be a sensitive indicator of whether the composition has been affected by temperature. Table 1 shows this ratio for eggs, for embryos and the two yolk platelet fractions YP-1, pure yolk platelet and YP-2, yolk platelet contaminated with mitochondria, and for the plasma membranes at incubation temperatures of 10, 16 and 23°C. At each temperature the ratio of PE:PC is identical for the embryos and for the control incubated unfertilized eggs. Thus, neither fertilization nor incubation temperature affects the relative amounts of PE and PC in the eggs and in the embryos. Although the ratio of PE to PC differs between the two yolk platelet fractions of the embryo, the incubating temperature has no effect on these ratios. The average of the two yolk platelet fractions is approximately the same as the ratio in the eggs and embryos.

**Table 1.** Molar ratio PE:PC (±SD) in incubated unfertilized eggs, embryos, yolk platelet fractions YP-1 and YP-2 of embryos, and plasma membranes as a function of incubation temperature $T_{INC}$ for *L. pictus* at the early blastula stage of development

| $T_{INC}$ °C | Incubated, unfertilized eggs | Embryos | YP-1 | YP-2 | Plasma membranes |
|---|---|---|---|---|---|
| 10 | 0.42 ± .04 (10)[a] | 0.41 ± .04 (5) | 0.29 ± .03 (13) | 0.54 ± .05 (17) | 0.70 ± .04 (8) |
| 16 | 0.43 ± .03 (4) | 0.43 ± .07 (6) | 0.29 ± .01 (4) | 0.54 ± .03 (13) | 0.65 ± .01 (2) |
| 23 | 0.40 ± .05 (11) | 0.40 ± .05 (6) | 0.29 ± .05 (16) | 0.52 ± .05 (16) | 0.51 ± .06 (10) |

[a] Number of measurements

The total phospholipid composition of each of these preparations is given in Table 2. Because we could not discern any statistically significant temperature dependence for each of the phospholipid components, these data were calculated by pooling all of the temperature results for each of the preparations. This also permitted a better estimate of the composition contributed by the minor components of PI, PS and PG. The sum of PE and PC is remarkably constant at about 85% in each of the samples of egg, embryo and YP fractions, and this value does not change with incubating temperature. This confirms for all incubation temperatures what previous studies have demonstrated for a single temperature, that no *de novo* biosynthesis of these phospholipids occurs in the development of the embryo to the blastula stage. The concentrations of PI and PS are equal and approximately 5%; PG content is 3–4%. These phospholipids constitute more than 96% of the total phospholipid content of these preparations. The remaining lipids are composed of small amounts of LPC, PA and CL; the experimental errors do not allow an assessment of whether the concentrations of these minor components change with incubating temperature. However, if their concentration does vary with temperature, these changes must be small. The phospholipid composition of the eggs and embryos are similar to those reported for the sea urchin *S. intermedius* (Kozhina et al., 1978).

In contrast to the eggs and embryos, the incubation temperature exerts a significant effect on the PE:PC ratio of the nascent plasma membranes (Table 1). This ratio decreases as the incubating temperature increases. Since these membranes contain only about 2–4% of the phospholipid of the embryo (as measured by the relative amount of membrane phosphorus in the embryo), this change in composition is not reflected in the temperature dependence of the overall composition of the yolk platelets. Although the ratio of PE:PC in the plasma membranes changes with temperature, we do not find any significant temperature dependence for the *sum* of their contributions, or for the composition of the other phospholipid components. As in the case of the eggs and embryos, the analysis for all the membrane preparations at the various temperatures are combined, and they are listed in Table 2. The sum of PE and PC and the composition of PI, PS and PG in the nascent membranes of the blastula are similar to the corresponding fractions in eggs, embryos and yolk platelet fractions.

**Table 2.** Phospholipid composition (mole % ± SD) of eggs, embryos, yolk platelet fractions YP-1 and YP-2 of embryos, and plasma membranes of *L. pictus* at the early blastula stage of development

| | PE + PC | PI ≈ PS | PG | Total[a] |
|---|---|---|---|---|
| Eggs | 82 ± 5 (18)[b] | 6 ± 3 (18) | 3 ± 1 (18) | 97% |
| Embryos | 85 ± 4 (11) | 4 ± 2 (11) | 3 ± 1 (11) | 96% |
| YP-1 | 86 ± 5 (9) | 4 ± 3 (9) | 4 ± 1 (9) | 98% |
| YP-2 | 84 ± 5 (9) | 6 ± 3 (9) | 3 ± 1 (9) | 99% |
| Plasma membranes | 88 ± 4 (8) | 3 ± 1 (8) | 4 ± 1 (8) | 98% |

[a] Remaining phospholipids consist of LPC, PA, and CL
[b] Number of measurements

## Verification that the Membrane Lipids are from Nascent Plasma Membranes; the Shear-Stress Method

Although the composition of the lipids obtained from the 45–60% sucrose gradient interface varies with temperature, as might be expected for membranes of poikilotherms, an independent verification that these are membrane lipids is necessary. The shear-stress method (*see* Materials and Methods) provides a direct test of whether these lipids are from nascent membranes of the embryo. The method is based on the concept that membrane lipids at the temperature of membrane assembly, the growth temperature T*g*, exhibits unique physical properties characteristic of a critical state (Gershfeld et al., 1993; Jin et al., 1998). The shear-stress method probes the structure of the lipid vesicles in vitro as temperature is varied. In general, the critical temperature for unilamellar vesicles formed by membrane lipids (T*) equals the growth temperature T*g* (Gershfeld, 1986; Ginsberg et al., 1991; Jin et al., 1998). Figure 1 shows typical results of the method for dispersions of plasma membrane lipids obtained at each of the three incubation temperatures 10, 16 and 23°C; the critical temperature T* is indicated for

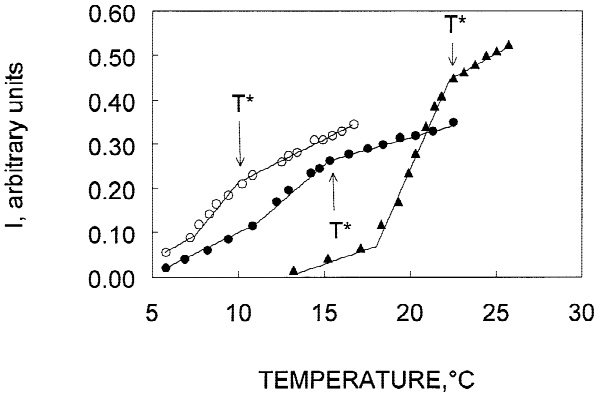


**Fig. 1.** Shear-stress method for measuring T*, the temperature of formation of the critical unilamellar state. The fluorescence intensity I of dispersions of nascent membrane lipids from *L. pictus* nascent membranes as a function of temperature. Multilamellar vesicles of lipid in water are formed containing 5,6-carboxyfluorescence at self-quenching concentrations (0.1 M). A brief, constant shearing stress is applied to the dispersion at each temperature, and the fluorescence intensity is monitored. The critical temperature T* = T*g* is indicated for lipids extracted from plasma membranes of embryos. Each of the results shown are typical of the temperature behavior of dispersions of membrane lipids to an applied shear stress (Gershfeld & Ginsberg, 1997). The values of T* reported in Table 3 were obtained from the average of three independent runs for each lipid sample. Incubation temperatures: ○ 10°C; ● 16°C; ▲ 23°C.

each of the measurements. In each example there is a low temperature and a high temperature interval where the temperature dependence of the inherent leakage of the vesicles is unaffected by the applied shear. At intermediate temperatures the applied shear causes an enhancement of dye leakage which ceases at the critical temperature T* = T*g* of each sample. A summary of the results comparing T* with the incubation temperatures for the *L. pictus* lipids is given in Table 3. The agreement between T* and the incubation temperatures confirms that these lipids are from the nascent plasma membranes.

## Discussion

The results of our study demonstrate that in the temperature range where embryonic development can occur, between 10 and 23°C, the phospholipid composition of *L. pictus* embryos at the blastula stage is unaffected by the incubation temperature. However, the nascent plasma membranes of the developing embryos accommodate to each incubating temperature by changing their phospholipid composition. Since the phospholipid composition of the embryo does not change, and in the absence of *de novo* phospholipid biosynthesis (Byrd, 1975; Kozhina et al., 1978; Armant et al., 1986), the accommodation of the membrane composition to each incubation temperature

**Table 3.** Comparison of the critical temperature T* for vesicles formed by lipids extracted from plasma membranes of blastula stage embryos, with the incubation temperatures, $T_{INC}$[a]

| $T_{INC}$, °C | T*, °C (±SD) |
|---|---|
| 10 | 10.2 ± 0.6 (3)[b] |
| 16 | 15.8 ± 0.2 (3) |
| 23 | 22.8 ± 0.4 (3) |

[a] See Fig. 1 and Materials and Methods
[b] Number of measurements

must be achieved by a redistribution of the phospholipid components within the embryo as new membranes assemble. Tables 1 and 2 indicate that as incubation temperatures increase membrane PE concentrations decrease and PC concentrations increase, while the sum of these contributions (PC + PE) and those of the remaining lipids (PS, PI, and PG) remain constant. Thus, the newly formed plasma membranes appear to accommodate to the temperature by a simple exchange of PE and PC.

Since these are static results only for the blastula we do not have dynamic evidence of the mechanism utilized by the embryo to effect the redistribution of the phospholipids into the newly formed plasma membranes. However, some general characteristics of the process may be deduced. A stoichiometric transfer of the lipid from the yolk platelet fractions, a major pool of these lipids that contains large amounts of glycoproteins with indistinct structures (Armant et al., 1986), is ruled out because their composition and temperature independence do not match the requirements of the plasma membranes. Another source of phospholipids is the endoplasmic reticulum; a direct transfer of phospholipids to plasma membranes from ER does not provide a mechanism for the selection process, but simply imposes the selection process to another pool of lipid. Clearly, a selection process of phospholipids from a source, e.g., yolk platelet or ER, that is temperature dependent must be involved in their transport to the newly forming plasma membranes. Phospholipid transfer proteins, found in all eukaryotic cells including oocytes (Rusinol et al., 1987), are a major means for transporting phospholipids between lipoproteins, organelles, and membranes. There are at least three classes of proteins that catalyze the transfer of lipids: one tranfers PC, another transfers both PI and PC, and a third transfers all the common phospholipids (Dowhan, 1991). In principle, all three classes of proteins may be involved in the transfer of phospholipids to yield the appropriate membrane composition at each incubation temperature. However, some temperature dependent property of their selectivity would have to exist to account for the changing membrane compositions. Although a temperature-sensitive allele in the gene encoding the PI/PC transfer protein has been reported (PattonVogt et al., 1997), at present little is known of the

temperature dependence of the relative phospholipid transfer rates of the three types of transfer proteins.

Perhaps the simplest mechanism for membrane assembly in the embryo is the spontaneous process associated with the formation of the critical bilayer state at T*. Since there is no *de novo* biosynthesis, and no evidence of phospholipid breakdown (i.e., only small amounts of LPC, the principal breakdown product, are present), the amount of the lipid in the embryo as well as its phospholipid composition are static. These static conditions allow us to consider the redistribution of the phospholipids as controlled by an underlying equilibrium that depends only on the ambient temperature and the phospholipid chemical potentials in the three coexisting phases: plasma membrane, cytosol and phospholipid chemical pool (yolk platelets and ER). The equilibrium between the vesicles formed by membrane lipids and the aqueous suspending medium is analogous to the putative equilibrium in the cytosol-membrane system. Consistent with this analogy is the agreement between T*, the temperature of formation for the critical state in vesicles, and T*g;* the equality of the two temperatures indicates that the same state is present in the vesicles and in the cell membrane at this temperature (Gershfeld, 1986, 1989*a,b*). A corollary of the thermodynamic principles which govern the formation of the critical state at T* is the requirement that the phospholipid composition of the bathing solution (the cytosol in the case of the embryo) must be identical to that of the assembling membrane (Tajima & Gershfeld, 1986; Gershfeld, 1989*b*). According to this mechanism the assembly of phospholipid into membranes is a spontaneous process of the lipid partitioning between cytosol and membranes. The assembly process is driven principally by the membrane lipid composition, and the requirement that membranes can form only if the phospholipid composition is matched to the incubating temperature. According to this equilibrium mechanism for membrane assembly the selection of the required lipids takes place at the phospholipid pool-cytosol boundary where phospholipid transfer proteins accelerate the transfer of phospholipid to the cytosol. Because the spontaneous assembly of the critical bilayer state at T* is fundamentally a lipid phenomenon, the presence of phospholipid transfer proteins will accelerate the redistribution process from the lipid pools, and membrane proteins will define the locus of the lipid sink, but the proteins do not affect the basic equilibrium between membrane and cytosol. The mechanism of selection by the phospholipid transfer proteins is, therefore, likely to depend on the relative rates of lipid transfer, from the phospholipid pool (yolk platelets or ER) to cytosol, among the three classes of proteins and their response to different incubation temperatures.

The equilibrium model does not provide any details of the assembly mechanism; indeed, all assembly processes (e.g., by adsorption, by accretion, or by insertion) are equivalent. However, this model provides a rationale for delineating some of the processes that are likely to be involved in the selective transport of the phospholipid for membrane assembly. They have been presented as a guide to those aspects of the lipid transport processes in membrane assembly that should be emphasized in future studies. In summary, we have established that *Lytechinus pictus* embryos accommodate to a range of incubating temperatures by modifying the PE:PC ratio in the nascent plasma membranes. Until the blastula stage of embryonic development this compositional accommodation occurs from a metabolically static pool of yolk lipids by transfer of the appropriate lipids to the membranes. We have invoked a class of phospholipid transfer proteins as the medium for the transfer of the lipids.

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