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Generation, modulation and maintenance of the plasma membrane asymmetric phospholipid composition in yeast cells during growth: their relation to surface potential and membrane protein activity

Jorge Cerbón *, Víctor Calderón

Department of Biochemistry, Centro de Investigación y de Estudios Avanzados del I.P.N. México, Apartado Postal 14-740, 07000 Mexico, D.F., Mexico

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Abstract

During growth a cyclic exposure of anionic phospholipids to the external surface of the plasma membrane was found. The surface charge density (σ) increased gradually reaching a maximum in the first 5 h of growth and returned gradually to their initial value at the end of the logarithmic phase of growth (10–12 h). Phosphatidylinositol, that determines to a large extent the magnitude of the σ, increased 83% in the yeast cells during the first 4 h of growth and returned gradually to their initial level at 10–12 h. During the stationary phase (12–24 h), both σ and the anionic/zwitterionic phospholipid ratio, remained without any significant variation. The high-affinity H-linked glutamate transport system that behaves as a sensor of the changes in the membrane surface potential (φ) increased its activity in the first 5 h and then decreased it, following with great accuracy the σ variations and remained without changes during the stationary phase of growth. The phosphatidylserine (PS) relative concentration in the cells (9.0%) did not significantly change during the whole growth curve, but their asymmetric distribution varied, contributing to the changes in σ. PS facing the outer membrane surface increased 2.45-times during the first 5 h of growth and then returned to their original value at the end of the log phase (12 h). Phosphatidylcholine (PC) remained constant during the whole growth curve (50%), while phosphatidylethanolamine (PE) decreased 3-fold in the first 4 h and then increased to its original value at 10 h. Interestingly, PE at the outer membrane surface remained constant (3% of the total phospholipids) during the whole growth curve. During growth yeast cells change their phospholipid composition originating altered patterns of the plasma membrane phospholipid composition and IN-OUT distribution. This dynamic asymmetry is involved in the regulation of the surface potential and membrane protein activity.

Keywords: Phospholipid composition; Surface potential; Plasma membrane; (Yeast)

1. Introduction

In the study of the lipid composition during membrane biogenesis several steps should be considered: lipid biosynthesis, lipid transport from their site of synthesis to the cellular membranes, transmembrane movements required as a consequence of the asymmetric synthesis of most lipid products and lipid degradation and transformation. Yeast cells synthesize the major glycerophospholipids found in other eukaryotic organisms [1] and in addition inositolphosphate ceramides (IPCs) [2]. Glycerophospholipid biosynthesis is regulated at several levels. In the absence of exogenous inositol and choline, maximum activities of CDP-diacylglycerol synthase, phosphatidylserine synthase, and the phospholipid *N*-methyltransferases were observed during the exponential phase of growth. These activities were reduced when inositol or inositol plus choline were added. A complex regulatory cascade responding to the presence of inositol and choline exerts control over the transcription of a number of structural genes and only phosphatidylinositol synthase (activity and subunit) was not affected by growth in medium supplemented with or without inositol-choline or the growth phase of culture [3]. In addition, the activities of phospholipid biosynthetic enzymes (in vitro) are influenced by the phospholipid composition of their local environment [4]. Contact and perhaps fusion (microsomes-plasma membrane) is the more probable mechanism for PI and PC transfer, since it was demonstrated that PI transfer protein does not participate

* Corresponding author. Fax: +52 754 6804.

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[5]. For the transmembrane movements (IN-OUT translocation) of PI and PS a charge driven ($\Delta\varphi$) transbilayer transport mechanism has been proposed and tested experimentally in vivo [6]. A model for the φ regulation of the anionic phospholipid composition and IN-OUT translocation of yeast membranes was proposed [7]. The present study was performed to know if the changes in phospholipid synthesis during growth originate alterations in the plasma membrane phospholipid composition and their asymmetric distribution, which may influence membrane protein activity. During the whole growth curve the cell's phospholipid composition was determined measuring the relative proportion of each phospholipid in steady-state labelling experiments. Also, the magnitude of the external surface charge density (σ) was characterized to estimate the transfer of the anionic phospholipids (PI and PS) from their site of synthesis to the plasma membrane, including IN-OUT translocation [7]. In addition, the dynamics of PE and PS were detected during this process by determining the accessibility of aminophospholipids to the impermeant reagent 2,4,6-trinitrobenzenesulfonic acid (TNBS) [6]. The activity of the high-affinity glutamate transport system (surface potential sensor) was also studied to determine if there were alterations on the membrane protein activity [8]. It was found that during growth the relative phospholipid composition in the cells varies considerably, originating changes in the plasma membrane PI and PS IN-OUT translocation. These changes altered membrane surface potential, modulating the activity of membrane proteins. These and previous results [6–9] contribute to better understand the mechanisms of generation, modulation and maintenance of the asymmetric phospholipid composition of the yeast plasma membrane.

2. Materials and methods

2.1. Yeast strain and culture conditions

The yeast strain *Saccharomyces carlsbergensis* ATCC 9080 was utilized. Cells were grown at 37° C on a rotary shaker. In 0.5% (w/v) nutrient broth containing 1.5% (w/v), yeast extract and 1% (w/v) maltose, 53.25 mM K^+, 25.25 mM Na^+, 6.25 mM Mg^{2+} and 80 μM Ca^{2+} [7].

2.2. Steady-state labelling using $[^{32}P]P_i$

In all experiments the media were supplemented with $[^{32}P]P_i$ 1 μCi/ml in order to have uniformly labelled phospholipids. The same specific activity was used in the cells overnight preculture to be sure of steady-state labelling. Growth of cultures were monitored by optical density measurements at $\lambda = 600$ nm. Also, total cell numbers were determined by microscopic examination with a hematocytomer.

2.3. Phospholipid composition analysis

At the indicated times, phospholipids were extracted from yeast cultures uniformly labelled with $[^{32}P]P_i$, by chloroform/methanol (2:1) and separated by thin-layer chromatography, as described previously [7]. Phospholipid composition was expressed as percentage distribution of ^{32}P-labelled lipids.

2.4. Transport of phospholipids between the endoplasmic reticulum or from internal membranes to the plasma membrane

By monitoring the changes in σ during growth, it is possible to know how much of the anionic phospholipids synthesized were transferred to the plasma membrane and were IN-OUT translocated. Binding of 9-AA allowed to follow the changes in σ and its release with monovalent and divalent cations was used to calculate the surface charge density. From the surface charge density values, the percentage of anionic phospholipids at the exofacial side of the plasma membrane were estimated as in Cerbón and Calderón, 1994 [7].

2.5. Localization of phosphatidylethanolamine (PE) and phosphatidylserine (PS) by reaction with trinitrobenzenesulfonic acid (TNBS)

PE and PS on the external surface of the plasma membrane react with TNBS at 4° C under the conditions described by Cerbón and Calderón [6], while, the internal aminophospholipids were derivatized at 25° C. At the indicated times during growth, the cells were treated with TNBS (at 4° C) and PE-TNBS and PS-TNBS were separated from PE and PS by extraction and thin-layer chromatography. Amounts of PE and PS in the external layer were determined by analysis of the kinetics of formation of the trinitrophenyl derivates.

2.6. High-affinity glutamate transport assay (φ biosensor)

As previously described, at the indicated times the yeast were collected water washed and used at once as follows. The uptake of glutamate was assayed at 23° C. The cells were resuspended (1.0 mg dry wt./ml) in prewarmed 20 mM Tris-citrate solution (23° C) containing 200 μg/ml cycloheximide, adjusted to pH 5.0 (optimum pH value for amino acids transport [9]). Cells were incubated for 5 min before the glutamate was added. The uptake of $[^3H]$glutamate by the cells was determined as a function of time. At the desired times the cells were separated from the suspension medium by centrifugation through dibutyl phthalate in a Beckman E microfuge. Radioactivity in the cell pellet was determined by scintillation counting in a Beckman LS 6000TA scintillation counter. The K_m and V_{max} values were obtained from double reciprocal plots

optimized by linear regression analysis. For the determination of K_m the reaction was terminated after 30 s.

2.7. Chemicals

[^{32}P]P_i as orthophosphoric acid, L-[2,3-^{3}H]glutamic acid were purchased from New England Research Products. The other reagents used: 9-aminoacridine, methyl viologen, 1,1'-dimethyl 4,4'-bipyridinium dichloride $(MV)Cl_2$, solvents, salts, etc. were purchased from Sigma, J.T. Baker, Mallinckrodt and E. Merck, dibutyl phthalate was purchased from Eastman Kodak.

3. Results and discussion

3.1. Yeast phospholipid composition at lag, exponential and stationary phases of growth

Steady-state labelling of *Saccharomyces carlsbergensis* cells with $^{32}P_i$ was performed to analyze phospholipid composition (Table 1) during the growth curve (Fig. 1). The growth periods were defined as follows: lag phase (1–2 h) early log phase (2–6 h), late log phase (6–12 h) and stationary phase (12–24 h). The first 2 h were considered lag phase since the optical density at 650 nm remained without significant variations and the cell number increases 8–10% (of 90 min to 120 min).

In the lag phase phosphatidylinositol (PI) started increasing and phosphatidylethanolamine decreasing. These two processes continued during the early log phase. PI increased 2.7-times and PE decreased 3.5-times. Both phospholipids returned gradually to their initial relative concentration, reaching it at the late exponential phase. The cellular content of phosphatidylcholine (PC) 50.31 ± 1.0% and phosphatidylserine (PS) 9.25 ± 0.6% remained without any appreciable change during the whole growth

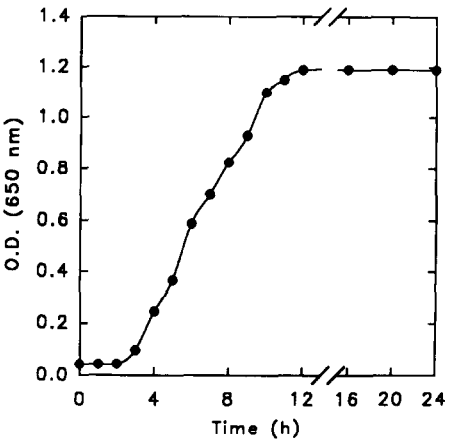

Fig. 1. Yeast cells were cultured on nutrient broth containing 1.5% (w/v) yeast extract and 1% (w/v) maltose. Cell density was expressed as optical density at 650 nm.

curve. In the stationary phase no changes in the yeast phospholipid composition were observed.

The increase in PI was presumably due to an increase in PI synthesis, given the readily available exogenous inositol and PI reduction can be related to an increase in degradation and or transformation. It is known that PI becomes degraded and glycerophosphorylinositol is released to the culture medium during growth and PI is transformed to inositolphosphate containing ceramides (IPCs) [2,10]. Taking into account that PS decarboxylation is considered the main route of PE formation, PE reduction could be related to a limited PS synthesis. It is known that in the presence of inositol PS synthase, PS decarboxylase and the *N*-methyl PE transferases are repressed [11]. Also, PS synthase activity in vitro decreases its activity 2- to 3-fold when the ratio PI/PS increases [12]. Our results in vivo showed (Table 1) that when the ratio PI/PS increases of 1.16 to 2.34 (lag phase), the PE relative concentration decreases of 27.7 to 19% and vice versa, in the late log phase when the PI/PS

Table 1
Changes in the yeast phospholipid composition during growth

Growth phase	Time (h)	PI	PS	PE	PC	A/Z ratio
Lag	0	12.1 ± 0.62	10.3 ± 0.61	27.7 ± 0.58	49.9 ± 0.93	0.29 ± 0.02
	1	18.0 ± 0.46	9.2 ± 0.50	23.3 ± 0.50	49.5 ± 0.61	0.38 ± 0.02
	2	21.8 ± 1.31	9.3 ± 0.56	19.0 ± 1.9	49.9 ± 0.82	0.45 ± 0.04
Early log	3	28.9 ± 1.27	9.8 ± 0.53	9.5 ± 1.2	51.8 ± 0.45	0.63 ± 0.02
	4	32.8 ± 0.84	9.8 ± 0.24	7.9 ± 1.0	49.5 ± 0.76	0.74 ± 0.03
	5	31.0 ± 0.6	8.5 ± 0.33	9.7 ± 1.39	50.9 ± 1.54	0.66 ± 0.02
Late log	6	21.9 ± 1.62	9.0 ± 0.74	18.0 ± 1.0	51.1 ± 1.72	0.44 ± 0.02
	8	15.4 ± 0.67	9.1 ± 0.7	25.7 ± 2.5	49.8 ± 2.29	0.32 ± 0.01
	10	14.4 ± 1.65	8.8 ± 0.65	25.2 ± 2.4	51.6 ± 1.37	0.30 ± 0.02
	12	12.8 ± 0.7	9.2 ± 0.19	27.7 ± 0.81	50.4 ± 0.6	0.28 ± 0.01
Stationary	18	12.4 ± 0.59	9.0 ± 0.94	29.8 ± 1.83	48.8 ± 1.17	0.27 ± 0.02
	24	12.9 ± 0.45	9.0 ± 0.69	27.5 ± 1.96	50.6 ± 2.13	0.28 ± 0.01

For uniform labelling the cells were first grown for 24 h in the presence of [^{32}P]P_i (1 μCi/ml) and then transplanted to an identical culture medium. At the indicated times aliquots were taken, lipids extracted and separated by thin-layer chromatography. The results are expressed as % of the total glycerophospholipids counts. A/Z is the anionic/zwitterionic phospholipid ratio.

ratio decreases of 2.43 to 1.45 the relative PE concentration increases of 18 to 25.2%. Therefore, the synthesis of PC during the first hours of growth is probably the result of the CDP-choline pathway.

The changes in the phospholipid composition of yeast cells during growth may be attributed to a combination of the repressive effects of inositol on the regulated enzymes in conjunction with the effect of the membrane phospholipid composition.

3.2. Changes in the membrane phospholipid asymmetry during growth as detected by surface charge density (σ) measurements and trinitrobenzenesulfonic acid (TNBS) derivatization

9-Aminoacridine binding (9-AA)

Having found that changes in the composition and asymmetric distribution of PI and PS are associated to changes in the membrane (σ) in yeast cells [6] and that the fluorescent probe 9-AA provides quantitative information on the surface potential of yeast membranes [8] (i.e., on the amount of anionic phospholipids), the binding of 9-AA was used for detecting acidic phospholipids facing the outer membrane surface of the plasma membrane during growth. Fig. 2 shows that each increment in the cell PI concentration (first 4 h) was followed by an increase in the binding of 9-AA, indicating that anionic phospholipids were translocated from their site of synthesis (cytoplasmic) toward the external leaflet of the plasma membrane (IN-OUT translocation). When the PI concentration in the cells was reduced (5–10 h) the 9-AA binding decreases also. These results are in agreement with the proposal of degradation of PI at the external membrane surface by an specific phospholipase B, which becomes activated during growth [10].

Surface charge density measurements

Surface charge density measurements (Table 2) showed that σ increased 2.38-times from 0 to 4 h and then returned gradually to their initial value at 12 h, remaining without change up to 24 h. The calculated percentage of anionic phospholipids facing the outer surface of the plasma membrane from the σ values, was always smaller than the percentage in the cells. Considering that: only a 6.5% of the total PS, 9.2% of total PE and 15% of total PI are facing the external plasma membrane leaflet [6], and that in purified yeast plasma membranes glycerophospholipids represent 42–48% of the total phospholipids, and the relative concentration of PI + PS oscillates between 31% [13] and 51% [14] (2–3-times more than in the inner membranes), an asymmetric distribution of anionic phospholipids with more facing the cytoplasmic leaflet occurs in yeast and seems to be maintained during growth.

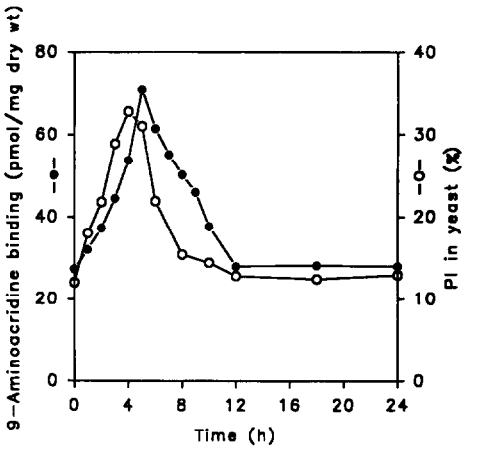

Fig. 2. Time-dependent variations of the surface charge density ●—● (9-aminoacridine-binding 9-AA) and the phosphatidylinositol concentration (○—○ PI) in yeast cells during growth. At the indicated times, cells were washed and resuspended to 1 mg dry wt./ml in 20 μM Tris-citrate buffer (pH 6.0), containing 2 μM 9-AA and the fluorescence in the supernatant measured. The amount of dye bound to the cells, due to surface charge effects, was calculated from the decrease of fluorescence in the supernatant and the recovery of fluorescence observed after the addition of 100 mM $MgCl_2$. PI relative concentration was expressed as in Table 1.

Table 2
Changes in the outer surface charge density (σ) for yeast cells during growth

Growth (h)	Surface charge density (C/m^2)	Anionic phospholipids				
		area ($1/nm^2$)	percentage			φ^{out} (mV)
			CUT		IN cells	
			a	b		
0	0.0169 ± 0.001	1/9.47	7.6	6.33	22.4	−25.0
4	0.0402 ± 0.0021	1/3.98	18.6	15.07	42.6	−52.19
6	0.0351 ± 0.0020	1/4.56	17.78	13.15	30.9	−47.0
12	0.0167 ± 0.0013	1/9.58	7.51	6.26	22.0	−24.7
24	0.0170 ± 0.0012	1/9.41	7.65	6.37	21.9	−25.13

Surface charge density was calculated from the dependence of 9-aminoacridine fluorescence on the presence of monovalent (KCl) and divalent (methyl viologen) cations in yeast cell suspensions as in [8]. By expressing the σ as electronic charges/surface area and considering 0.6 nm^2/lipid molecule (a) or 0.72 nm^2/lipid molecule (b), the percentage of anionic phospholipids OUT was calculated. Surface potential φ^{out} was calculated considering a 78 mM concentration of monovalent cations in the cells culture medium.

Trinitrobenzenesulfonic acid accessibility of the aminophospholipids at the outer membrane surface

Having found that PS facing the outer leaflet of the yeast plasma membrane increases when the content of PI in the cells increases (normal cells vs. PI-rich cells) [7], and the PI relative concentration varies during growth (Table 1), the aminophospholipids were determined by TNBS-derivatization at 4° C (non penetrating conditions). It can be seen (Table 3) that PS-TNBS increased 2.45-times from 0 to 5 h and then returned gradually to their initial value at 10 h, following the changes in 9-AA binding (Fig. 2). On the other hand PE-TNBS remained without any significant variation during the whole growth curve (around 3% of the total phospholipids). A direct correlation between anionic/zwitterionic phospholipid ratio, surface charge density and PS facing the outer membrane surface was found during the whole growth curve. A dynamic lipid asymmetry was observed during the lag-log phases of growth against a static asymmetry during the stationary phase of growth.

3.3. Time-dependent modulation of the glutamate transporter. and its relation to changes in surface charge density during growth

Having found that H^+-linked transport systems behave as sensors of the changes in surface potential [8], the activity of the high-affinity glutamate transporter was determined in yeast cells collected at different times during growth. It can be seen (Fig. 3) that the maximum transport activity was observed in cells grown for 5 h and decreased gradually to their initial activity after 12 h. The increase in

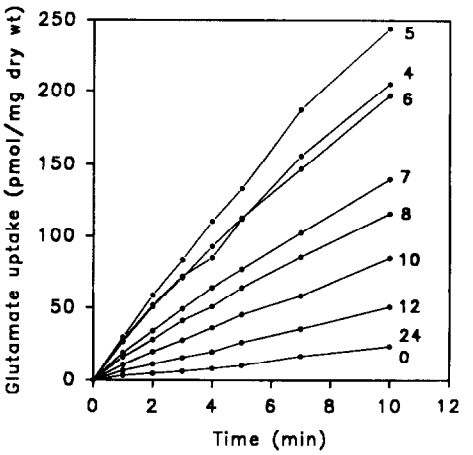

Fig. 3. Time-course of glutamate uptake in cells collected at different times (0–24 h) during growth. The cells were water washed twice and resuspended in 20 mM Tris-citrate (pH 5.0), after 5 min at 23° C the substrate was added (10 μM), the uptake was stopped at the desired times (1–10 min) by centrifugation through dibutyl phthalate in a microfuge and radioactivity on the cell pellet determined. Data are expressed as pmol/mg dry wt. of cells.

Table 3
Aminophospholipids at the outer membrane surface of yeast cells during growth as detected by trinitrobenzylation (TNBS) under non penetrating conditions (4° C)

Growth phase	Time (h)	Aminophospholipid (cpm × 10^{-3})			
		PS	TNBS-PS	PE	TNBS-PE
Lag	0	18.54	1.45	51.45	5.74
		(9.27)	(0.73)	(25.7)	(2.87)
	2	17.0	1.89	33.4	5.99
		(8.0)	(0.95)	(16.7)	(2.99)
Early log	3	17.6	2.59	15.3	6.11
		(8.8)	(1.29)	(7.64)	(3.0)
	4	16.8	2.93	13.14	5.86
		(8.4)	(1.46)	(6.57)	(2.92)
	5	13.4	3.58	12.16	6.2
		(6.7)	(1.79)	(6.0)	(3.11)
Late log	6	15.2	3.14	33.9	5.9
		(7.6)	(1.57)	(16.9)	(2.93)
	8	14.98	2.0	44.1	6.3
		(7.5)	(1.0)	(22.0)	(3.15)
	10	15.58	1.13	39.33	5.6
		(8.67)	(0.63)	(21.9)	(3.11)
	12	16.47	1.3	47.60	6.4
		(8.24)	(0.66)	(23.8)	(3.2)
Stationary	18	15.27	1.13	54.13	5.67
		(7.63)	(0.56)	(27.0)	(2.83)
	24	16.0	1.38	51.68	6.17
		(8.0)	(0.69)	(25.8)	(3.0)

The data are expressed as cpm × 10^{-3} of phospholipids/200000 cpm in PC which was taken as a standard since its composition was 50.36 ± 1.34% in all cases. Within brackets is the amount, expressed as a percentage of total phospholipids. The data represent the average of three separate experiments in duplicate with S.D. < 10%. The TNBS concentration was 5.0 mM, pH 8.2, 4° C darkness (non penetrating conditions); 95–97% of the aminophospholipids were derivatized at 25° C in penetrating conditions.

Table 4
Kinetic constants of the high-affinity glutamate transport system at different times of growth

Growth (h)	K_m (M)	V_{max} (mmol/kg per min)
0	$1.81 \pm 0.17 \cdot 10^{-4}$	0.67 ± 0.07
5	$7.10 \pm 0.8 \cdot 10^{-5}$	0.64 ± 0.05
12	$1.70 \pm 0.20 \cdot 10^{-4}$	0.64 ± 0.08
24	$1.80 \pm 0.1 \cdot 10^{-4}$	0.65 ± 0.05

The K_m (M) and V_{max} (mmol/kg dry wt. of cells) values were calculated from Lineweaver-Burk plots optimized by linear regression analysis. The substrate concentrations used were 1 μM to 500 μM. Values are the average of three separate experiments by duplicate. The correlation coefficients for regression were around 0.97.

activity was due to a decrease in K_m without significant alteration in V_{max} (Table 4), in agreement with the proposed mechanism necessarily ordered protons-before anions [8]. A difference of −24.3 mV was calculated for φ from the K_m values of cells grown 5 h vs. cells grown 24 h or 0 h, according to Katchalsky et al. [15] using the formula $\Delta\varphi = \frac{k_B T}{ze} \ln \frac{K_m^1}{K_m}$; were k_B is the Boltzman constant, T is the temperature (K), e is the charge on electron and z is (−1). This value is in good agreement with the $\Delta\varphi$ values calculated using the φ values of Table 2 that were obtained from the σ data (−27 mV (0 h vs. 4 h) and −22 mV (0 h vs. 6 h)).

3.4. How the asymmetric distribution of phospholipids of the plasma membrane is generated, modulated and maintained during the yeast cells growth

The cyclic exposure of anionic phospholipids to the external membrane surface can be clearly seen by plotting the PI concentration or the anionic/zwitterionic phospholipid ratio vs. the σ (Fig. 4). During the lag-early log phase of growth (wide line) the results indicate that the steps of synthesis (I), transfer and IN-OUT translocation (II) of PI predominate over degradation (III) and transformation (IV), while the reverse seems to occur during the late log phase (thin line). Considering a membrane surface potential regulation of the cells phospholipid composition and IN-OUT translocation, which may modulate the anionic phospholipids synthesis proposed before [7], the results can be interpreted as follows: The existence of anionic phospholipids on both the inner (IN) and the outer (OUT) membrane surfaces, generates a transmembrane surface potential ($\Delta\varphi = \varphi^{in} - \varphi^{out}$). During membrane biogenesis, anionic phospholipids synthesis in (cytoplasmic side) increases the φ^{in} and anionic phospholipids are IN-OUT translocated.

Both, the driving force for translocation ($\Delta\varphi$) and the external surface charge σ increased (0–5 h) in spite of the fact that PI is being degraded at the outer membrane leaflet [10]. At the same time, PI is being transformed to inositol phosphate ceramides (IPCs) in the endoplasmic reticulum (ER) and Golgi apparatus [16]. These processes might originate a decrease in PI at the cytoplasmic side of the plasma membrane and in consequence φ^{in} and $\Delta\varphi$ are reduced, IN-OUT translocation becomes limited and σ decreases (5–12 h) because PI continues to be degraded at the outer membrane leaflet during growth. It is known that when growth is arrested (lack of glucose in the medium), the release of glycerophosphorylinositol (GPI) ceases also. This is consistent with σ remaining constant during the stationary phase (12–24 h). When glucose was added, growth and GPI release were restored and the surface phospholipase B was activated by growth or by energy derived of glucose metabolism [10]. It is plausible that the surface PL-B could have a high turnover rate and the presence or absence of glucose indirectly affects the relative rate of enzyme synthesis and breakdown. Alternatively, the lipid breakdown may reflect the regulation of the IN-OUT translocation of PI to the sites of breakdown. Considering that phospholipid acylhydrolases from the plasma membrane of *S. cerevisiae* are activated by the presence of anionic detergents and inhibited by cations [17], their regulation by the increase in σ is likely. If so, steps I, II and III are coordinated and regulated by the electrical properties of the membrane. A detailed study of the inositol phosphate ceramides dynamics during yeast growth is being performed in order to know when and how much PI is transformed. Also, the participation of IPCs in the σ is suspected, since MIPC and $M(IP)_2C$ were found mainly in purified plasma membrane fractions of *S. cerevisiae* [13].

The study of mutants and double mutants of *S. cerevisiae* showed that zwitterionic phospholipids can substi-

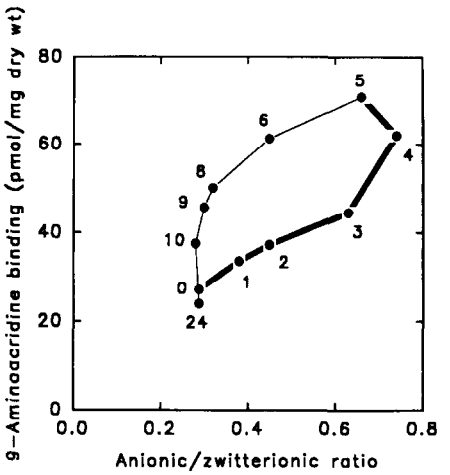

Fig. 4. Cyclic exposure of anionic phospholipids to the external surface of the plasma membrane during growth. 9-AA binding was estimated as in Fig. 2 and the anionic/zwitterionic phospholipid ratio as in Table 1. The wide line represents lag early log phases of growth (0–5 h) and the thin line represents the late log phase (5–12 h) and the stationary phase (12–24 h).

tute each other [1]; the role of PC in the membranes can be played by PE and vice versa. PI can substitute PS but PS can not substitute PI. Therefore, the role of PI is unique in the generation, modulation and maintenance of the asymmetric composition of the yeast plasma membrane.

PI is generated by synthesis in the (ER) [14], from there it is transferred to the inner side of the plasma membrane by contact [5] (ER-plasma membrane) and IN-OUT translocated by a mechanism $\Delta\varphi$ dependent [6]. PI asymmetry is modulated and maintained by PI breakdown at the outer membrane leaflet [10] and by its transformation to IPCs in the ER and Golgi compartments.

In addition to modulate the activity of enzymes and transport proteins, other processes can be regulated by the membrane lipid composition and asymmetric distribution. The Ca^{2+} dependent binding of protein kinase C to negatively charged lipids essential for the enzyme activation provides a good example [18]. The requirement of negatively charged phospholipids for the translocation of prephoE across the inner-membrane of *Escherichia coli* [19], plus the fact that negatively charged lipids are essential for the α helix formation of the signal peptide [20] and for the conformational changes of membrane proteins [21]; are just a few examples of the possible function and roles for the dynamic lipid asymmetry observed during growth. A similar cyclic variation of the σ and the A/Z phospholipid ratio has been observed in *Escherichia coli* (data to be reported). It will be interesting to know whether a similar anionic phospholipid dynamic asymmetry occurs during growth in other eukaryote (mammalian) cells.

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