Reversible translocation of cytidylyltransferase between cytosol and endoplasmic reticulum occurs within minutes in whole cells

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Addition of oleic acid to Krebs II cells induced a rapid incorporation of [^3H]choline into phosphatidylcholine, since 500 μM of the fatty acid stimulated choline incorporation by 5-fold over the control after 5 min of incubation. In fact, a noticeable increase in phosphatidylcholine labelling could be monitored immediately after 1 min of cell incubation with [^3H]choline, at which time 50% of cytosolic cytidylyltransferase activity (EC 2.7.7.15), the regulatory enzyme of phosphatidylcholine synthesis, was translocated on to membranes. Non-esterified [^3H]oleic acid content was also increased in the same range of time in the particulate fraction. Subcellular fractionation indicated that endoplasmic reticulum was the unique binding site for cytidylyltransferase even after 1 min of incubation. Also, [^3H]oleic acid accumulated mainly in the same internal membrane. Addition of exogenous albumin to cells prelabelled with [^3H]oleic acid induced the release of 50% of membrane-bound cytidylyltransferase activity within 1 min, together with a decrease in unesterified oleic acid in the same membrane. Although total depletion of oleic acid was obtained, total release of membrane-bound cytidylyltransferase was not. The remaining minor pool of membrane-bound cytidylyltransferase was not affected by cell incubation with dibutyl cyclic AMP, suggesting that this pool was neither regulated by fatty acid nor modulated by cyclic-AMP-dependent protein phosphorylation. Addition of [^3H]oleic acid directly to an homogenate led to a less specific accumulation of the fatty acid in the endoplasmic reticulum, but cytidylyltransferase remained exclusively associated with this membrane. We conclude that *in vivo* translocation of cytidylyltransferase provoked by oleic acid concerns one specific pool of the enzyme distinct from the enzyme firmly bound to endoplasmic reticulum, but other factor(s) than fatty acid seem to be required to explain the specificity of endoplasmic reticulum for cytidylyltransferase binding.

INTRODUCTION

Phosphatidylcholine is synthesized in many cell types through the 'de novo' pathway regulated by CTP:phosphocholine cytidylyltransferase (EC 2.7.7.15) [1–3]. A translocation process between cytosol and membranes has been pointed out to regulate the enzyme activity, following activation of cells by different stimuli [4–6]. Thus we previously demonstrated that, upon stimulation of phosphatidylcholine metabolism in Krebs II cells treated by an exogenous phospholipase C acting on plasma membrane, cytidylyltransferase was translocated specifically on to the endoplasmic reticulum and not to the plasma membrane [7,8]. A variety of processes have been described to try to explain the activation and translocation of cytidylyltransferase, including phosphorylation/dephosphorylation [9,10], a fatty acid effect [11–15], diacylglycerol action [4,7–8], hydrophobic interactions [13] or, more recently, regulation by the membrane phosphatidylcholine content [16]. Among all the stimuli, oleic acid was found to be the most potent activator of phosphatidylcholine synthesis, and we recently demonstrated that Krebs II cells stimulated by oleic acid increase phosphatidylcholine synthesis through translocation of both oleic acid and cytidylyltransferase to the endoplasmic reticulum, without increase in total cell phosphatidylcholine mass [17]. However, all the studies reported so far were related to a regulation *in vivo* of phosphatidylcholine biosynthesis by fatty acids in a time range of at least 30 min [11,13,17] to more than 24 h [18]. Only one report described direct relationship *in vivo* between the fatty acid content of whole microsomes and the amount of cytidylyltransferase bound to them [12].

The role of phosphatidylcholine as precursor for second messengers in signal transduction was suggested to be via the stimulation of phospholipase(s) C [19] or phospholipase(s) D [20] in different cell lines. We recently demonstrated that the stimulation of a phospholipase D specific for phosphatidylcholine in human neutrophils was complete within a time range of 1 min for *N*-formylmethionyl-leucyl phenylalanine to 5 min for phorbol 12-myristate 13-acetate, indicating that hydrolysis can be a rapid process [21]. Since the existence of a phosphatidylcholine cycle was recently proposed [22], it seemed conceivable that, for an efficient regulation of cell metabolism, resynthesis should be regulated in the same range of time as hydrolysis. Therefore we have studied the cytidylyltransferase translocation process at short times after oleic acid addition.

In this paper we demonstrate activation *in vivo* of phosphatidylcholine synthesis by oleic acid in Krebs II cells is detectable within 1 min and follows a reversible translocation of cytidylyltransferase specifically on to the endoplasmic reticulum.

MATERIALS AND METHODS

Chemicals and products

[methyl- ^3H]Choline chloride [2.89 TBq (78 Ci)/mmol], phospho[methyl- ^{14}C]choline, ammonium salt [2.22 GBq (60 mCi)/mmol] and [9,10(n)- ^3H]oleic acid [185 GBq (5 Ci)/mol] were purchased from The Radiochemical Centre (Amersham, Bucks., U.K.). Eagle's minimum essential medium and Hepes were obtained from Seromed (Lille, France) and Percoll was from Pharmacia (Uppsala, Sweden). Oleic acid, CTP, phosphocholine, choline and dibutyl cyclic AMP were purchased from

Abbreviation used: TKM, Tirs/KCl/MgCl₂ buffer.

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Sigma (St Louis, MO, U.S.A.). A stock solution of 100 mM-oleic acid was prepared as described [11] by dissolving the fatty acid in 0.12 M-KOH in 95% (v/v) ethanol and stored at -20°C . Before experiments, oleic acid from stock solution was dried under nitrogen and resuspended at the required concentration in Eagle's medium by stirring vigorously and by sonication.

Krebs II cell preparation

Cells were obtained as described [23] by collecting ascitic fluid by puncture from Swiss mice, infected 1 week previously and pelleted by centrifugation (200 g for 10 min). The pellet was washed twice in 100 mM-KCl/5 mM-MgCl₂/25 mM-Tris/HCl, pH 7.4 (TKM buffer), and resuspended in Eagle's medium containing 40 mM-Hepes, pH 7.4, to a final concentration between 2×10^6 and 2×10^7 cells/ml, depending on the experiment.

Determination of [³H]choline incorporation

Oleic acid solution was added to cells previously resuspended in Eagle's medium (2×10^6 cells/ml) containing [³H]choline, giving a final concentration of 1 $\mu\text{Ci/ml}$ and a specific radioactivity of 140 $\mu\text{Ci/mmol}$ (taking into account the choline concentration in Eagle's medium). At each incubation time, 0.5 ml of cell suspension was harvested and pelleted (2800 g for 1 min) by using an MSE Microfuge (Kontron Instruments). The cell pellet was extracted as described by Bligh & Dyer [24] and radioactivity from the organic phase was determined. In experiments performed in a time range of seconds, cells were prelabelled for 1 h with [³H]choline; then oleic acid was added and 0.5 ml of cell suspension was extracted directly from the incubation medium.

Cellular fractionation

Cells were incubated in Eagle's medium (4×10^6 cells/ml) in the absence or the presence of oleic acid (500 μM). At each incubation time, cells were pelleted at 4°C (600 g for 5 min) and washed twice in cold TKM buffer, then resuspended to 10^7 cells/ml in cold lysis buffer (TKM buffer containing 1 mM-ATP and adjusted to pH 9.6). All the procedures for cell disruption, differential centrifugation and density-gradient centrifugation were performed at 4°C as previously described [7,17]. This procedure included a first step of separation between cytosol and particulate fraction before the Percoll gradient, avoiding contamination of the particulate fraction by cytosolic cytidyltransferase activity. Fractions (2 ml) were collected from the top of the gradient and stored at 4°C for up to a maximum of 1 h.

Enzyme assay

CTP:phosphocholine cytidyltransferase activity was assayed as previously described [7,25]. The incubation mixture contained 20 mM-Tris/succinate, pH 7.8, 6 mM-MgCl₂, 8 mM-CTP, 4 mM-phospho[methyl-¹⁴C]choline (0.5 Ci/mol) and up to 300 μg of protein. A sonicated suspension of total lipid extract from Krebs II cells was added to the assay for cytosolic enzyme at a final concentration of 1 mM lipid P as previously described [7]. Incubations were carried out at 37°C for 30 min, stopped in boiling water in the presence of non-labelled phosphocholine (200 mM final concn.), and CDP-choline was separated and measured as described [26].

Subcellular distribution of [³H]oleic acid

Before incubation, [³H]oleic acid (13.5 mCi/mmol) was dried under nitrogen and suspended in Eagle's medium as described under 'Chemicals and products'. This solution was added to cells at a final concentration of 500 μM and incubated for up to 15 min. All the fractionation procedures were carried out as reported above. Lipids were directly extracted from gradient

fractions as described [17,21]. Organic phases were concentrated under nitrogen and a sample was counted for total radioactivity. Lipids were then separated on silica gel G with hexane/diethyl ether/formic acid (55:45:1, by vol.) as solvent, as radioactivity was determined by scanning plates with an automatic t.l.c. linear analyser (Berthold LB 2842) before radioactivity counting.

Distribution of cytidyltransferase and [³H]oleic acid in a cell-free system

Cells were suspended at 2×10^7 cells/ml in TKM buffer and lysed by nitrogen cavitation. The homogenate was centrifuged (1000 g for 5 min) and the post-nuclear supernatant was incubated for 5 min at 37°C with 400 μM -oleic acid previously resuspended in TKM buffer. The mixture was then centrifuged (120000 g for 45 min) to remove cytosol, and the particulate material was then fractionated through a Percoll gradient. All procedures for cytidyltransferase assay and lipid extraction were performed as mentioned above.

Miscellaneous determinations

Protein was determined by the method of Lowry *et al.* [27] in the presence of SDS (0.07%, w/v), with BSA as a standard. Radioactivity was counted with a Kontron analytical Inter-technique counter (type SL4000) with automatic quenching correction, by using Picofluor 15 for aqueous samples or Instafluor for organic samples (Packard Instrument Co.) as scintillation fluids.

RESULTS

Effect of short-time cell treatment with oleic acid on the incorporation of [³H]choline into phosphatidylcholine

We measured the incorporation of [³H]choline into phosphatidylcholine after incubation of cells up to 15 min in the presence of 500 μM -oleic acid (Fig. 1). This fatty acid concentration was found to be the most efficient without any noticeable cell lysis [17]. We observed a rapid increase in phosphatidylcholine labelling, since a 5 min incubation was sufficient to increase

Fig. 1. Short-time-dependent incorporation of [³H]choline into phosphatidylcholine in the presence of oleic acid

Cells were incubated with [³H]choline (1 $\mu\text{Ci/ml}$) as described in the Materials and methods section in the absence (○) or in the presence of 500 μM -oleic acid (△), -palmitic acid (●) or -stearic acid (▼), and radioactivity incorporated into phosphatidylcholine was measured. Inset: cells were prelabelled with [³H]choline, then incubated in the presence of oleic acid; extraction was directly performed on the incubation medium as described in the Materials and methods section. Results are expressed as percentages of the control phosphatidylcholine labelling at zero time. Results are means $\pm$ S.E.M. of three determinations.

Fig. 2. Time-dependent distribution of cytidylyltransferase activity and unesterified $[^3\text{H}]$ oleic acid between cytosol and particulate fraction

Cells (1.65×10^8) were incubated in the presence of $500 \mu\text{M}$ -oleic acid. At each incubation time, cells were lysed by nitrogen cavitation and total cytidylyltransferase activity (a) and unesterified oleic acid content (b) were measured in the particulate (P, ●) and cytosolic (C, ○) fractions obtained from a post-nuclear supernatant. Results are means $\pm$ S.E.M. from three determinations.

Fig. 3. Time-dependent subcellular localization of cytidylyltransferase and unesterified $[^3\text{H}]$ oleic acid

Cells were incubated up to 15 min in the presence of $500 \mu\text{M}$ - $[^3\text{H}]$ oleic acid ($3.4 \mu\text{Ci}/\mu\text{mol}$), and the particulate cell fraction was fractionated on a Percoll gradient. (a) Cytidylyltransferase activity was measured in each gradient fraction from control cells (○) or from cells incubated for 1 min (●), 5 min (△) or 15 min (▲) with the fatty acid; results are means $\pm$ S.E.M. from three experiments. (b) Unesterified $[^3\text{H}]$ oleic acid distribution was analysed after extraction of gradient fractions and lipid separation as described in the Materials and methods section.

$[^3\text{H}]$ choline incorporation by 5-fold over the control. However, because of the possible interference of choline uptake at incubation times below 5 min, we performed other experiments with cells prelabelled at equilibrium with $[^3\text{H}]$ choline (Fig. 1, inset). Under these conditions, we were able to demonstrate an

increase in phosphatidylcholine synthesis already after 1 min of cell challenge with oleic acid.

Cytidylyltransferase translocation and $[^3\text{H}]$ oleic acid distribution in cells incubated with exogenous fatty acid

Cells incubated for up to 15 min in the presence of $500 \mu\text{M}$ - $[^3\text{H}]$ oleic acid were lysed, and cytidylyltransferase was measured in the particulate and cytosolic fractions (Fig. 2a). Total activity in resting cells was $7.2 \text{ nmol/min per } 10^7 \text{ cells}$, and 10.6% was located in the particulate fraction. Results showed a 3.2-fold increase in particulate total activity immediately after 1 min of incubation, concomitant with a decrease in the cytosolic activity. The particulate fraction then accounted for 34% of total cell activity, which indicated that 23% of cellular cytidylyltransferase had been translocated within 1 min. Oleic acid was much more potent than phospholipase C treatment, since in this latter case particulate activity was increased only by 15% after 3 h of treatment [7]. In addition, unesterified $[^3\text{H}]$ oleic acid (Fig. 2b) was accumulated in the particulate cell fraction already after 1 min of incubation, whereas only traces of the fatty acid were detectable in the cytosol. At that time, 86% of the fatty acid was present in unesterified form in the cell. Thus the early translocation of cytidylyltransferase occurred simultaneously with the presence of oleic acid in the membranes.

Determination of the target cell membrane for cytidylyltransferase and $[^3\text{H}]$ oleic acid

The particulate fraction from cells incubated in the presence of $[^3\text{H}]$ oleic acid was further fractionated on a Percoll gradient as previously described [7]. When cytidylyltransferase activity was assayed on gradient fractions from control cells (Fig. 3a), we detected a low activity in the endoplasmic reticulum, as previously reported [7]. In oleic acid-treated cells, a net increase in cytidylyltransferase activity was observed only in the endoplasmic reticulum, immediately after 1 min of incubation. The increase in activity was 4.3-, 6.8- and 9.9-fold respectively after 1, 5 and 15 min of incubation, demonstrating the rapid and selective binding of cytidylyltransferase on the internal membrane.

When the distribution of unesterified $[^3\text{H}]$ oleic acid was analysed under the same incubation conditions (Fig. 3b), we found a peak of radioactivity in the dense gradient fractions, increasing with time. A smaller peak was also present in the light fractions (plasma membrane), but remained constant with time, indicating that the unesterified fatty acid content in plasma membrane reached a steady state already after 1 min of incubation. Thus both cytidylyltransferase and its translocator agent, unesterified oleic acid, were targeted to the endoplasmic reticulum. Plasma membrane never accounted for any cytidylyltransferase activity, although some unesterified oleic acid was present in this fraction.

Reversibility of cytidylyltransferase translocation to the endoplasmic reticulum is observed in whole cells

We then investigated the time course of cytidylyltransferase release from the endoplasmic reticulum, using BSA (Fig. 4). We demonstrated that about 50% of the membrane-bound enzyme could be released within 1 min with exogenous BSA. After 15 min of incubation with BSA, the endoplasmic reticulum was virtually depleted of oleic acid, whereas a noticeable amount of enzyme was still bound to this membrane (Fig. 4).

The correlation between endoplasmic-reticulum content (fractions 9–11 from the gradient) of oleic acid and the binding of cytidylyltransferase has been analysed in Fig. 5. Linear-regression parameters clearly demonstrated that the amount of oleic acid controlled the amount of cytidylyltransferase binding in a reversible manner. As shown by the ordinate intercept of

Fig. 4. Short-time release of cytidylyltransferase from cells incubated in the presence of albumin

Cells were incubated as in Fig. 3 in the presence of oleic acid up to 30 min, but BSA (20 mg/ml) was directly added to the incubation medium 15 min, 5 min and 1 min before the end of the incubation. Particulate material was fractionated on a Percoll gradient as described in the Materials and methods section. Results are expressed as the amount of free oleate (○) and cytidylyltransferase activity (●) remaining on the endoplasmic reticulum (fractions 9–11).

Fig. 5. Correlation between cytidylyltransferase activity and unesterified [³H]oleic acid distribution in endoplasmic reticulum

Free oleic acid content in fractions 9–11 were plotted versus cytidylyltransferase activity in the same fractions, from experiments performed as in Fig. 3 during pulse experiments with oleic acid (●), or as in Fig. 4, by treating cells (challenged for 30 min by oleic acid) with BSA (○). Each point represents an experiment performed at a specific time. Linear regression coefficient is 0.981 and the ordinate intercept is 0.67 nmol/min. Results are from Figs 3 and 4 and other experiments (not shown).

curves in Fig. 5, the remaining membrane-bound enzyme activity after oleic acid depletion corresponded to the initial membrane activity of cytidylyltransferase in resting cells (0.67 nmol/min). We checked whether this enzyme pool could be controlled by phosphorylation, through cyclic-AMP-dependent protein kinase [9,10]. However, incorporation of [³H]choline was not affected by either dibutyl cyclic AMP or okadaic acid (Fig. 6a). In addition, cell incubation with dibutyl cyclic AMP for up to 1 h had no effect on the basal membrane cytidylyltransferase activity (Fig. 6b). Therefore we suggested the existence in Krebs II cells of a membrane pool of cytidylyltransferase firmly bound to the endoplasmic reticulum. Instead, about 50% of the enzyme from the 'dynamic' pool was either translocated to (Fig. 3a) or released (Fig. 4) from the endoplasmic reticulum within minutes.

Cytidylyltransferase translocation induced by oleic acid in a cell-free system

Another approach to analyse the translocation process was performed on a cell-free system, to by-pass the uptake of oleic acid. Thus the fatty acid was added directly to a post-nuclear supernatant of non-treated cells, and further incubated for 5 min

Fig. 6. Effect of dibutyl cyclic AMP and okadaic acid on phosphatidylcholine synthesis

(a) Time course of [³H]choline incorporation in cells treated with either 500 μ M-dibutyl cyclic AMP (■) or 0.5 μ M-okadaic acid (△), compared with controls (□). (b) Cytidylyltransferase activity across gradient from control cells (○) or from cells incubated for 1 min (●), 15 min (△), and 30 min (▲) with dibutyl cyclic AMP.

Fig. 7. Comparative subcellular localization of cytidylyltransferase and unesterified [³H]oleic acid in a cell-free system

Cells were lysed and the post-nuclear supernatant was incubated for 5 min in the absence or in the presence of [³H]oleic acid (400 μ M, 3.4 μ Ci/ μ mol). Particulate material was prepared as described in the Materials and methods section and further fractionated on a Percoll gradient, and cytidylyltransferase and unesterified oleic acid were measured in each gradient fraction. (a) Cytidylyltransferase activity in control cell-free system (○) and after addition of oleic acid (●). (b) Unesterified oleic acid distribution expressed as percentage of total radioactivity across gradient (□).

(Fig. 7). Under these conditions, cytidylyltransferase activity (Fig. 7a) was increased by about 10-fold over the control in the endoplasmic reticulum, whereas unesterified oleic acid (Fig. 7b) displayed a bimodal distribution, both in plasma membrane and

Table 1. Comparative distribution of unesterified oleic acid and phospholipids in whole cells and cell-free system

Abbreviations: PM, plasma membrane; ER, endoplasmic reticulum.
^aTaken from data of Fig. 3(b) (incubation time 5 min). ^bTaken from data of Fig. 7(b) (incubation time 5 min). ^cData from ref. [17].

	Percentage of total amount across gradient		
	PM	ER	PM/ER
[³ H]Oleic acid in whole cells ^a	15.5	63.6	0.24
[³ H]Oleic acid in cell-free system ^b	24.0	50.0	0.57
Total phospholipids ^c	19.3	45.8	0.42

Table 2. Free oleic acid/lipid phosphorus ratio in plasma membrane and endoplasmic reticulum

Values were calculated from data in Fig. 3(b) for oleic acid and from ref. [17] for lipid P content for plasma membrane (fractions 3+4) and endoplasmic reticulum (fractions 9-11).

Membrane fraction	Time...	Ratio		
		1 min	5 min	15 min
Plasma membrane		0.186	0.173	0.195
Endoplasmic reticulum		0.180	0.224	0.352

in endoplasmic reticulum. At variance with experiments performed on total cells (Fig. 3), the localization of unesterified oleic acid was no longer specific for the endoplasmic reticulum, since plasma membrane and endoplasmic reticulum accounted for 24% and 50% respectively of total radioactivity across the gradient. This distribution is similar to the total phospholipid content of plasma membrane and endoplasmic reticulum (Table 1), whereas the fatty acid was accumulated more specifically in the endoplasmic reticulum when added to intact cells. In the latter case, the ratio of unesterified oleic acid to phospholipids increased with time in the endoplasmic reticulum, whereas it remained constant for plasma membrane (Table 2).

DISCUSSION

The growing interest in the involvement of phosphatidylcholine hydrolysis by phospholipase C or D in signal transduction [22,28] shows that a number of cells (including transformed cell lines [29]) activate phosphatidylcholine hydrolysis in less than 1 min [21,30-31]. This suggests that the pathways of phosphatidylcholine synthesis could be activatable in the same range of time in order to compensate for phosphatidylcholine degradation. Moreover, oleic acid, which was found to activate *in vitro* a phospholipase D specific for phosphatidylcholine [32,33], is also the major activator for phosphatidylcholine synthesis [1]. We have therefore investigated the synthesis of phosphatidylcholine *de novo* at the possibly shortest time, upon cell challenge with oleic acid.

The rapid incorporation of [³H]choline into phosphatidylcholine observed in Fig. 1 was well correlated both with the incorporation of unesterified oleic acid into the particulate fraction (Fig. 2b) and the increased cytidylyltransferase activity

in the same fraction (Fig. 2a). In addition, cytidylyltransferase activity was at any time exclusively associated with the endoplasmic reticulum (Fig. 3a), as we previously observed after long-time phospholipase C treatment [7]. Since the ratio of unesterified oleic acid to phospholipids content at 1 min (Table 2) is similar for plasma membrane and endoplasmic reticulum (although a portion of cytidylyltransferase has already been translocated to the endoplasmic reticulum), the amount of unesterified oleic acid present in membranes cannot explain by itself the targeting of cytidylyltransferase to the endoplasmic reticulum. In addition, this ratio (Table 2) is always above the critical value of 0.1 necessary to induce cytidylyltransferase translocation on to liposomal membranes [34]. Also, when [³H]oleic acid was added to a cell-free system, the proportion of unesterified oleic acid present in the plasma membrane was significantly higher than in whole cells treated with the fatty acid. As shown in Table 1, when added to a cell-free system, [³H]oleic acid distributed homogeneously between the various membranes, according to their respective total phospholipid content, with no subsequent effect on cytidylyltransferase translocation to plasma membrane. In contrast, there is evidently a rapid flux of unesterified oleic acid towards the endoplasmic reticulum in whole cells (Fig. 3b and Table 2). It remains to be shown by which mechanism [³H]oleic acid is accumulated in the endoplasmic reticulum. Our results emphasize that only oleic acid present on the endoplasmic reticulum is effective for cytidylyltransferase binding.

The observed reversibility of the process *in vivo* (through addition of BSA; Fig. 4), strengthens the relationship between the amount of unesterified oleic acid present in endoplasmic reticulum and the binding of cytidylyltransferase. This reversibility has been observed previously for longer incubation times (hours) upon treatment of CHO cells with exogenous phospholipase C [35], but no correlation could be established between diacylglycerols generated by phospholipase C treatment and localization of cytidylyltransferase. In our work, we demonstrate a good correlation at the level of endoplasmic reticulum between unesterified oleic acid and cytidylyltransferase activity (Fig. 5).

We also demonstrate *in vivo* the existence of a specific pool of the enzyme independent of the presence of fatty acid. The existence of a pool of cytidylyltransferase associated with microsomes independently of fatty acid was previously observed in a cell-free system [13]. We also demonstrate that this pool is not released from the membrane through phosphorylation by cyclic-AMP-dependent protein kinase (Fig. 6), another process involved in the regulation of cytidylyltransferase translocation [10,36]. Thus we can suggest the existence of two different pools of cytidylyltransferase: a minor pool permanently associated with the endoplasmic reticulum, which can serve to maintain a basal level of phosphatidylcholine synthesis; and a major pool, mainly cytosolic in resting cells, which can become available to enhance phosphatidylcholine synthesis upon cell activation.

A striking observation is that in cell-free systems cytidylyltransferase is again exclusively translocated to the endoplasmic reticulum, although unesterified oleic acid is homogeneously distributed between the various cell membranes. This was unexpected, since artificial membranes such as phosphatidylcholine/oleic acid liposomes are able to bind and to activate soluble cytidylyltransferase [34]. This suggests that additional factors other than unesterified oleic acid are required to explain the specificity of endoplasmic reticulum as a target membrane. Recent observations underline the role of diacylglycerols in the cytidylyltransferase translocation process [37]. However, the translocation of protein kinase C to monolayers was recently found to be regulated by membrane surface pressure [38], and the binding to membranes of a myristoylated tyrosine kinase [39] or

5-lipoxygenase [40] is regulated by additional proteins, 32 kDa or FLAP proteins ('Five Lipoxygenase Activating Protein') respectively. Seeking such proteins would be the next step of investigation to understand the targeting mechanism of cytidylyl-transferase to the endoplasmic reticulum.

Altogether, our results demonstrate for the first time that the 'de novo' pathway of phosphatidylcholine synthesis is activatable within minutes. Assessment of this rapid stimulation of the 'de novo' pathway in other cell systems which hydrolyse phosphatidylcholine after agonist-induced cell activation would establish the relevance of the phosphatidylcholine cycle [22].

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