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Ha-*ras*-TRANSFORMATION ALTERS THE METABOLISM OF PHOSPHATIDYLETHANOLAMINE AND PHOSPHATIDYLCHOLINE IN NIH 3T3 FIBROBLASTS

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Cultured NIH 3T3 fibroblasts were employed to investigate the changes in the phospholipid metabolism induced by Ha-*ras* transformation. All phospholipid fractions were reduced in *ras*-transformed fibroblasts except phosphatidylethanolamine (PE). The incorporation of labeled choline and ethanolamine into phosphatidylcholine (PC), PE and their corresponding metabolites were elevated in a similar manner in the transformed cells. The enhanced uptake of choline and ethanolamine correlated with the activation of choline kinase and ethanolamine kinase. Similarly, the uptake of arachidonic, oleic and palmitic acids by PC and PE was higher in *ras*-cells. Acyl-CoA synthetases, which esterify fatty acid before their incorporation into lysophospholipids, were also activated. However, both CTP:phosphocholine-cytidylyltransferase and CTP:phosphoethanolamine-chytidyltransferase were inhibited in the transformed cells. This fact, taken together with the observed activation of choline- and ethanolamine kinases, led to accumulation of phosphocholine and phosphoethanolamine, which have been presumed to participate in the processes of tumor development. PC biosynthesis seemed to be carried out through the CDP-choline pathway, which was stimulated in the oncogenic cells, whereas PE was more likely, a product of phosphatidylserine decarboxylation rather than the CDP-ethanolamine pathway.

KEYWORDS: phosphatidylcholine; phosphatidylethanolamine; phospholipid metabolism; *ras*.

INTRODUCTION

Ras genes encode proteins which regulate key cellular signaling pathways (Barbacid, 1987). Several lines of evidence have indicated that among other cellular changes the phospholipid metabolism was also altered as a result of oncogene-induced transformation (Tsai *et al.*, 1989; Yu *et al.*, 1990). Stimulated phosphatidylcholine (PC) metabolism has been observed in *Harvey-ras*-transformed mouse fibroblasts (Teegarden, 1990). The phospholipid (PL) molecules and their metabolites are also presumed to participate in the processes of oncogene-induced transformation (Lacal and Carnero, 1994). Cell transformation by oncogenes has been observed to induce elevation of DAG and phosphocholine as a result of activation of the phospholipase D/phosphatidate phosphohydrolase/choline kinase pathway (Carnero *et al.*, 1994).

Most recent investigations have been focused on the changes of PC metabolism induced by *ras*-transformation. It also seemed of interest to investigate how oncogenic transformation affected the second most abundant membrane phospholipid, phosphatidylethanolamine (PE). Our results implied that the metabolism of both PC and PE was stimulated in *ras*-transformed cells. The increase in the incorporation of labeled precursors such as arachidonic and palmitic acids was more marked into PE compared to PC in the transformed cells. The activation of choline- and

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ethanolamine kinases and the inhibition of CTP:phosphocholine- and CTP:phosphoethanolamine cytidylyltransferases could result in enhanced level of phosphocholine and phosphoethanolamine which were presumed to participate in tumor development (Cuadrado, 1993; Kiss and Crilly, 1995). The data obtained implied that the CDP:choline pathway was stimulated in *ras*-transformed cells, whereas the enhanced content of PE was more likely a result of stimulated phosphatidylserine (PS) decarboxylation rather than activated CDP:ethanolamine pathway.

MATERIALS AND METHODS

Materials

[1-^{14}C]Palmitic acid, [1-^{14}C]oleic acid, [1-^{14}C]arachidonic acid, [methyl-^{14}C]choline chloride, [2-^{14}C]ethanolamine hydrochloride, [1-^{14}C]palmitoyl-CoA, [1-^{14}C]oleoyl-CoA, were purchased from Amersham International. Cell culture reagents were from GIBCO. Phospholipids and other reagents were from Sigma.

Cell culture

NIH 3T3 fibroblasts, transfected with activated human Ha-*ras* gene (Der *et al.*, 1986, 1988), and the parental cell line were a kind gift from Professor Channing J. Der (The University of North Carolina at Chapel Hill, Chapel Hill, NC). Cells were cultured in Dulbecco's modified Eagles medium, supplemented with 9% (v/v) fetal calf serum at 37°C in a humidified 5% CO_2 atmosphere. The transfected NIH 3T3 fibroblasts were continuously grown in medium containing 400 µg/ml G 418 (Geneticin, GIBCO-BRL). For experiments, G 418 were replaced with antimycotic/antibiotic solution.

Uptake studies

Subconfluent control and *ras*-transformed NIH 3T3 fibroblast cultures were trypsinized, counted and seeded in DMEM, containing 9% fetal calf serum, at a density of 2×10^5 cells in triplicates in 30-mm culture dishes. After 6 h the medium was changed with a fresh one and the radioactive precursors [1-^{14}C]arachidonic acid, [1-^{14}C]palmitic acid, [1-^{14}C]oleic acid, [methyl-^{14}C]choline chloride and [2-^{14}C]ethanolamine hydrochloride were added at a concentration of 2 µCi/ml medium. At the indicated time, the medium was aspirated, fibroblasts were washed gently three times with phosphate-buffered saline (PBS), and the cells were scraped with a rubber policeman. The cell suspension thus obtained was extracted with chloroform/methanol (Bligh and Dyer, 1959).

Lipid analysis

The organic phase obtained after the extraction was concentrated and analysed by TLC. The phospholipid fractions were separated on silica gel G 60 plates (Merck) in a solvent system containing chloroform/methanol/isopropanol/triethylamine/0.25% KCl (30:9:25:18:6 v/v) (Touchstone *et al.*, 1980). Neutral lipids were fractionated on silica gel G 60 plates in hexane/diethyl ether/acetic acid (50:50:1 v/v). The location of the radioactive metabolites was determined by autoradiography, the corresponding spots were scraped into scintillation vials and the radioactivity was measured in a Rackbeta II 1215 (LKB) scintillation counter.

Separation of aqueous metabolites

The aqueous choline-containing metabolites were separated (Yavin, 1976) using unlabeled choline, phosphocholine and CDP-choline as standards. The water-soluble ethanolamine-containing intermediates were separated by a different method (Wang and Moore, 1991).

Isolation of microsomes

Subconfluent NIH 3T3 fibroblasts (control and *ras*-transformed) were washed three times with cold PBS, scraped with a rubber policeman and disrupted by Dounce homogenizer in 50 mM Tris HCl (pH 7.5). The microsomal fraction was obtained according to a procedure described elsewhere (Koshlukova *et al.*, 1992).

Enzyme assays

Choline kinase and ethanolamine kinase were determined as described by Weinhold and Rethy (1974). Briefly, incubations contained 0.25 mM [^{14}C]choline (0.7 Ci/mol) 10 mM ATP, and 10 mM $MgCl_2$ in 100 mM Tris–HCl pH 8.0 buffer in a final volume of 1.0 ml for choline kinase and 0.5 mM [^{14}C]ethanolamine (2 Ci/mol), 3 mM ATP, 3.0 $MgCl_2$ in 60 mM glycyl glycine buffer (pH 8.5). The corresponding incubation mixture was chromatographed on an AG 1-X8 anion-exchange column and the labeled product was eluted and counted. Cytidylyltransferase assay was performed accord-

Table 1.
Phospholipid composition of control and *ras*-transformed NIH 3T3 fibroblasts. Values are means ± S.D. from two replicates in two experiments

Phospholipids	*Control*		ras	
	nmol per 10^6 cells	*%*	*nmol per 10^6 cells*	*%*
Phosphatidylcholine	24.49 ± 1.57	50.21	21.88 ± 1.32*	49.17
Phosphatidylethanolamine	11.79 ± 1.85	24.17	14.12 ± 1.07†	31.73
Phosphatidylinositol	3.72 ± 0.89	7.63	2.13 ± 0.77*	4.79
Phosphatidylserine	2.69 ± 0.74	5.52	1.52 ± 0.35†	3.42
Phosphatidylglycerol	4.33 ± 0.43	8.88	3.15 ± 0.37*	7.01
Sphingomyelin	1.75 ± 1.12	3.59	1.69 ± 0.22	3.79

*$P<0.05$, †$P<0.001$.

ing to the procedure described by Sleight and Kent (1983). The reaction mixture contained 4 mM phospho[^{14}C]choline or phospho[^{14}C]ethanolamine (0.2 Ci/mol), 6 mM $MgCl_2$, 5 mM CTP, and 40 µg of membrane protein in 20 mM Tris–succinate (pH 7.8) to a final volume of 50 µl. The reaction was terminated by the addition of 10% trichloracetic acid and the radioactive product was processed as in Sleight and Kent (1983).

Assay of choline-phosphotransferase and ethanolamine-phosphotransferase

CDP-choline: diacylglycerol cholinephosphotransferase and CDP-ethanolamine: diacylglycerol ethanolaminephosphotransferase activities were determined as described by Niwa and Taniguchi (1986). The standard assay mixture contained 100 mM Tris–HCl (pH 8.0) 5 mM $MgCl_2$, 50 µM [^{14}C] CDP-choline (0.1 µCi) or 50 µM [^{3}H]CDP-ethanolamine (0.1 µCi) and 50 µg of microsomal protein in a total volume of 0.2 ml. The reaction was carried out at 37°C for 30 min and the radioactive phospholipid products were extracted and counted.

Acyl:CoA synthetase was assayed by a procedure described elsewhere (Koshlukova *et al.*, 1992). PS decarboxylase activity was determined according to the procedure of Trotter *et al.* (1993). All enzyme assays were performed within the range which was linear for time and protein concentration.

Protein was determined by the method of Lowry *et al.* (1951). Statistical analysis was performed using Student's *t*-test.

RESULTS

The metabolism of the two most abundant phospholipids—PC and PE—was investigated in control and Ha-*ras* transformed NIH 3T3 fibroblasts. The phospholipid composition of the parental and transformed cell lines is presented in Table 1. Apparently, the content of all phospholipids (expressed as nmol per 10^6 cells) was decreased in *ras*-fibroblasts except that of PE which was increased by about 19% compared to control cells.

In order to investigate whether the phospholipid synthesis was affected by oncogenic transformation, we monitored the time course of incorporation of several radioactive precursors into the two major membrane phospholipids—PC and PE. As evident from Fig. 1A, B arachidonic acid uptake by both phospholipids was increased in *ras*-transformed cells, this increase being more marked for PE (Fig. 1B). The uptake of oleic acid by PC and PE was increased at all time points (Fig. 1C, D). *ras*-transformation induced an almost twice as high incorporation of [^{14}C]palmitate into PE in comparison with untransformed cells (Fig. 1F). The uptake of palmitate by PC showed almost no increase during the first hour of incubation and a slight elevation was observed during the second and third hours (Fig. 1E).

Further, we investigated the uptake of two specific precursors for the PC and PE biosynthesis: choline and ethanolamine. Their distribution into the cellular choline-(respectively ethanolamine-) containing metabolites (both water- and lipid-soluble) is presented in Figures 2 and 3. Apparently, there was almost no difference in the uptake of [^{14}C]choline by the cellular choline pool between control and *ras*-fibroblasts (Fig. 2A). Most of the labeled choline was found in phosphocholine, its radioactivity being markedly elevated in the transformed cells (Fig. 2B). The incorporation of [^{14}C]choline into PC was also significantly higher in the *ras*-expressing cells (Fig. 2C). However, the

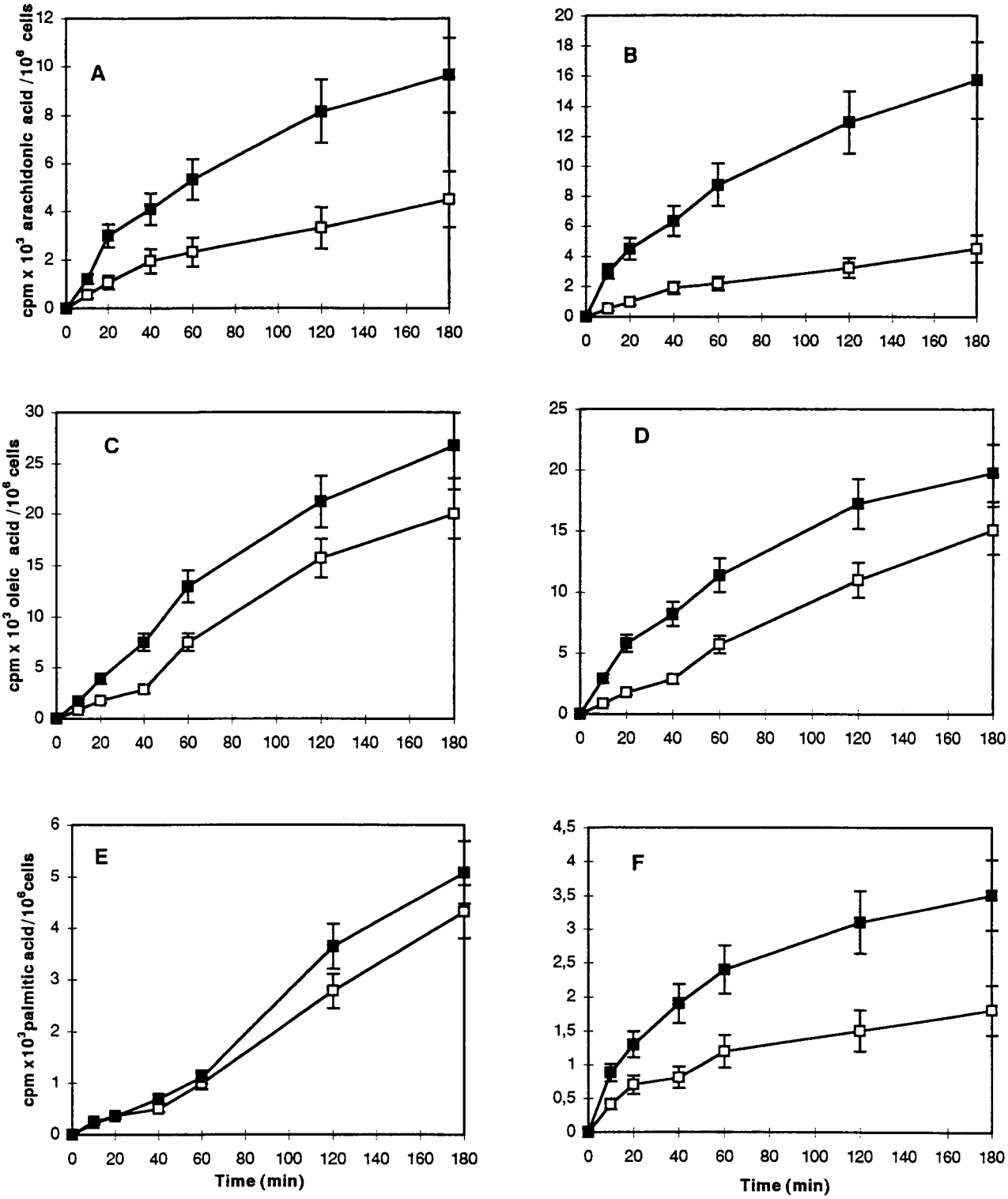

Fig. 1. Time course of incorporation of radiolabeled arachidonic acid (A and B), oleic acid (C and D) and palmitic acid (E and F) into phosphatidylcholine (A, C and E) and into phosphatidylethanolamine (B, D and F) in control (□) and *ras*-transformed (■) NIH 3T3 fibroblasts. Values of uptake are given as mean cpm/10^6 cells ± S.D. from two replicates in two separate experiments.

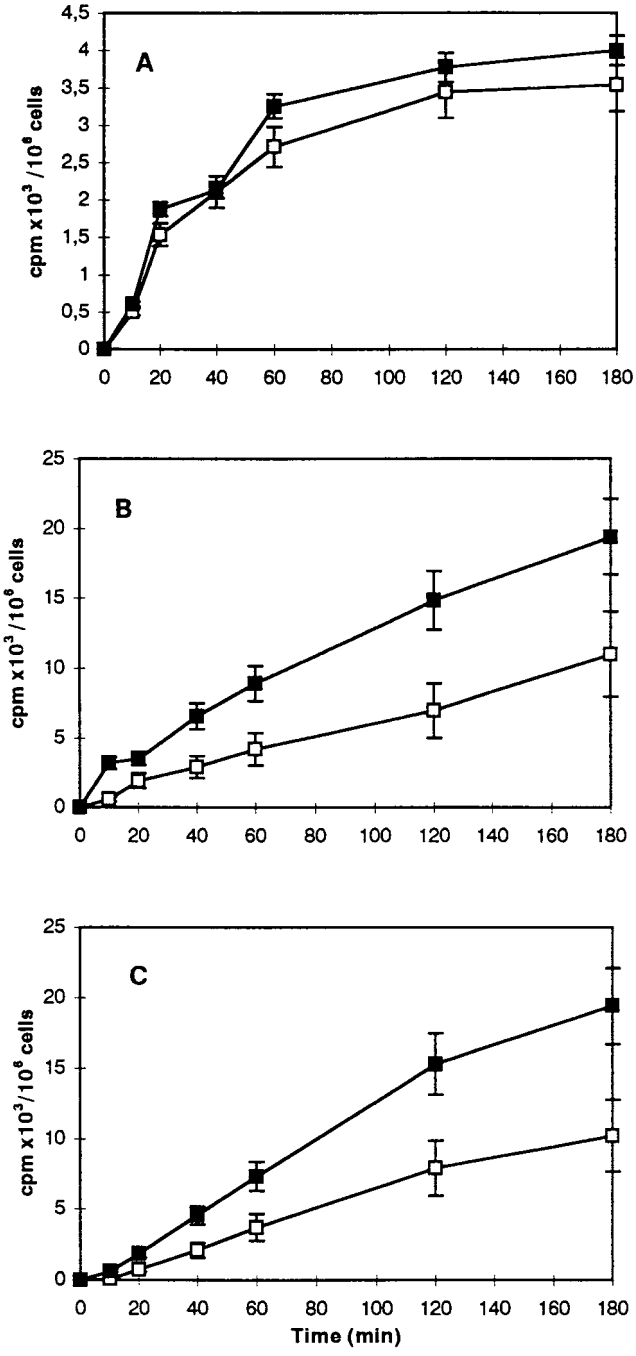

Fig. 2. Time course of incorporation of radiolabeled choline into the cellular choline (A), phosphocholine (B) and phosphatidylcholine (C) in control (□) and *ras*-transformed fibroblasts (■). Values are given as mean cpm/10^6 cells ± S.D. from two replicates in two experiments.

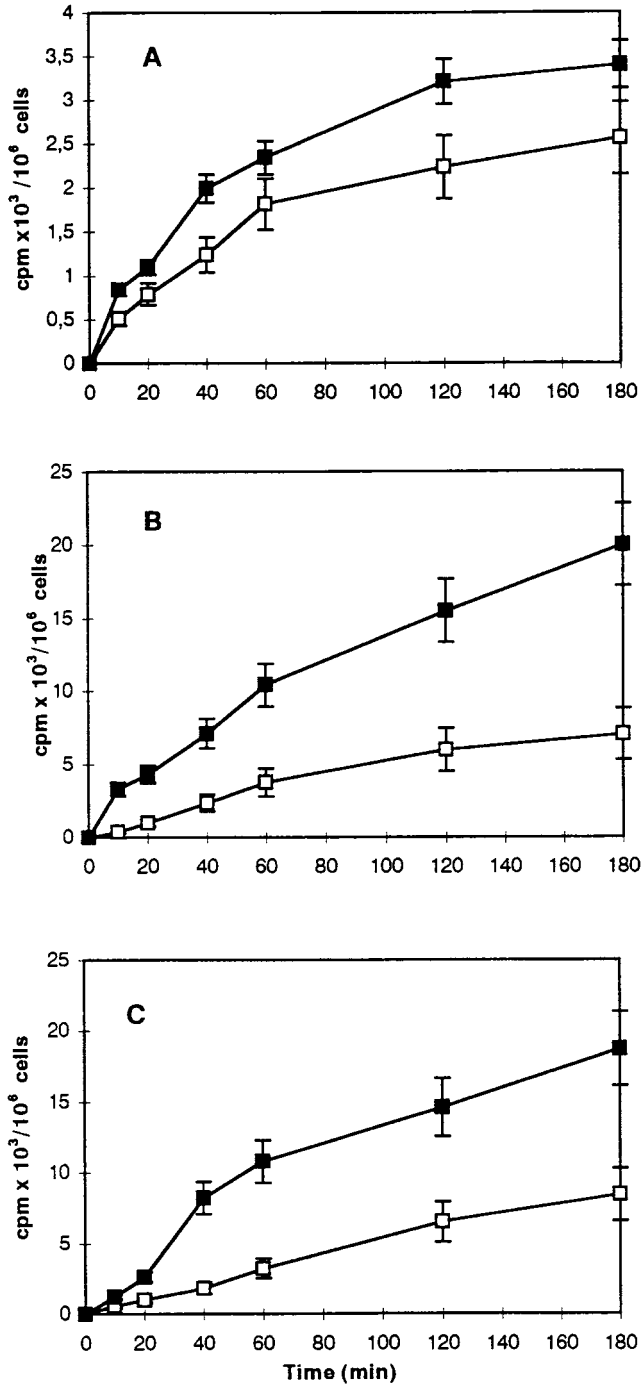

Fig. 3. Time course of incorporation of radiolabeled ethanolamine into the cellular ethanolamine (A), phosphoethanolamine (B) and phosphatidylethanolamine (C) in control (□) and *ras*-transformed fibroblasts (■). Values are given as mean cpm/10^6 cells ± S.D. from two replicates in two experiments.

differences in choline uptake by CDP-choline were not statistically significant in the parental and transformed cells, which is the reason why these results were not presented graphically among the other PC intermediates in Figure 2. The incorporation of [^{14}C]ethanolamine into the cellular ethanolamine pool and enhanced although not dramatically at all time points in the transformed fibroblasts (Fig. 3A). Most of the label was found in phosphoethanolamine (Fig. 3B). and PE (Fig. 3C), which was quite similar to the distri-

Table 2.
Specific activity of enzymes participating in the phospholipid biosynthesis (nmol/min/mg protein)

Enzyme	*Control*	ras
Choline kinase	1.64 ± 0.12	2.48 ± 0.27*
Ethanolamine kinase	1.47 ± 0.15	2.37 ± 0.23*
CTP: phosphocholine cytidylyltransferase	1.56 ± 0.17	0.94 ± 0.08*
CTP: phosphoethanolamine cytidylyltransferase	1.21 ± 0.14	0.78 ± 0.09*
Choline phosphotransferase	1.37 ± 0.17	1.79 ± 0.21*
Ethanolamine phosphotransferase	1.07 ± 0.11	0.69 ± 0.07*
Phosphatidylserine decarboxylase	0.57 ± 0.06	1.29 ± 0.11*

*$P<0.001$.

bution of [^{14}C]choline in the choline-containing metabolites. Similarly, the uptake of ethanolamine by CDP-ethanolamine was not significantly different in control and *ras*-cells although a slight increase was observed after the first 30 min of incubation (data not shown). We also studied the rate of ethanolamine incorporation into PC and its two methylated intermediates—phosphatidyl-*N*-monomethylethanolamine and phosphatidyl-*N*,*N*-dimethylethanolamine—in order to follow the eventual changes in the alternative pathway for PC synthesis through PE methylation in *ras*-transformed fibroblasts. The radioactivity in both of them was either unchanged or slightly reduced, which ruled out the possibility that part of the PE molecules became transformed to PC through enhanced methylation in the transfected cells.

Since the uptake of lipid precursors seemed stimulated in the oncogene-expressing cells, we investigated the activities of some enzymes, participating in the phospholipid biosynthesis such as choline/ethanolamine kinase, CTP: phosphocholine/phosphoethanolamine cytidylyltransferase, choline/ethanolamine phosphotransferase. The results showed that both choline kinase and ethanolamine kinase were stimulated in the oncogene-expressing cells (Table 2). On the contrary, both CTP:phosphocholine- and CTP: phosphoethanolamine cytidylyltransferase activities were reduced. Interestingly, diacylglycerol: cholinephosphotransferase was activated, whereas diacylglycerol: ethanolaminephosphotransferase was inhibited in microsomes from *ras*-cells (Table 2). As evident from Table 2 phosphatidylserine decarboxylase was activated about twice in the transformed cells. The activities of acyl-CoA-synthetases in the oncogene-expressing cells were changed in the following manner: oleoyl-CoA-synthetase was activated by 66% (specific activity 1.04 vs 1.73 nmol/h/mg protein, $P<0.01$) and palmitoyl-CoA-synthetase—by 45% (0.97 vs 1.41 nmol/h/mg protein, $P<0.05$).

DISCUSSION

The analysis of the phospholipid composition showed that PE was the only fraction which was increased in *ras*-transformed NIH 3T3 fibroblasts, whereas the content of the other major membrane phospholipid PC was slightly reduced. As mentioned above, PC metabolism has been reported to be stimulated during *ras*-transformation (Teegarden *et al.*, 1990). However, to our knowledge, the metabolism of PE has not been investigated in detail in *ras*-cells. In order to evaluate how oncogenic transformation affected the metabolism of the two major phospholipid fractions, we studied the uptake of several radiolabeled lipid precursors in control and transformed cells. It should be noted that the uptake of all used fatty acids (arachidonic, palmitic and oleic) was significantly higher in *ras*-transformed cells except palmitate uptake by PC, which was increased insignificantly. Oleic acid was incorporated more actively into PC. Oleoyl-CoA-synthetase, which esterifies unsaturated long-chain fatty acids to give the corresponding CoA-esters (a process preceding the acylation of lysophospholipids to form phospholipid molecules), was also activated in the *ras*-transfected cells.

Phosphocholine which incorporated intensively labeled choline (Fig. 2B) is known to be a product of either choline kinase-triggered phosphorylation of the cellular choline or of phospholipase C-induced hydrolysis of PC which was activated in *ras*-cells according to our previous studies (Momchilova and Markovska, 1999). Choline kinase has been reported to be stimulated in oncogene-transformed cells (Teegarden *et al.*, 1990;

Macara, 1989; Lacal, 1990) which was confirmed by the present studies. In contrast, Geilen *et al.* (1996) observed activated PC metabolism but no alterations in the choline kinase activities in *ras*-expressing keratinocytes, which might indicate that the precise mechanisms mediating the stimulation of PC biosynthesis in oncogene-transformed cells depend on the cell type. The activation of choline kinase, taken together with the inhibition of CTP:phosphocholine cytidylyltransferase lead to accumulation of phosphocholine which has been presumed to act as a positive regulator of cell growth (Cuadrado, 1993). The lack of significant increase in the CDP-choline radioactivity which we observed in the transformed fibroblasts (data not shown), was probably also a result of the reduced activity of CTP:phosphocholine cytidylyltransferase, taken together with the activation of phosphocholinetransferase, which used both CDP-choline and DAG to produce PC. The uptake of radiolabeled ethanolamine by ethanolamine-containing metabolites revealed some specific characteristics. Ethanolamine uptake by the ethanolamine cellular pool was enhanced in the *ras*-cells at all time points, in contrast to choline uptake by the choline pool. In addition, the rate of ethanolamine uptake by phosphoethanolamine was elevated about 3 fold at the third hour of incubation, while for phosphocholine this elevation was about 80%. Some human cancers have been reported to contain increased levels of phosphoethanolamine, but not phosphocholine (Kiss and Crilly, 1995). This makes the differences in the uptake and metabolism of these two phospholipid intermediates an important issue in the understanding of the mechanisms underlying oncogenic transformation of cells. Ethanolamine and choline are presumably phosphorylated by the same kinase activity (Porter and Kent, 1990), whereas their transport across the cell membrane maybe involves different mechanisms (Lipton *et al.*, 1990). Our results imply that the synthesis of PC was stimulated as a result of activation of the CDP:choline pathway in transformed fibroblasts. The fact that the content of PC was not elevated in *ras*-cells was most probably due to its intensified hydrolysis by phospholipase A2 (Momchilova *et al.*, 1998) and phospholipases C and D (Momchilova and Markovska, 1999). The synthesis of PE also seemed to be stimulated which resulted in elevation of its content in *ras*-cells. However, it seems likely that PE was accumulated not so much through the CDT:ethanolamine pathway, but more likely through phosphatidylserine decarboxylation. Thus, in spite of the stimulated degradation of PE (Momchilova and Markovska, 1999) its level was augmented in the transformed cells.

In conclusion, the activation of PC and PE metabolism in *ras*-transformed cells makes these two phospholipids potent sources of intermediates which play active metabolic and maybe regulatory role in the process of oncogenic transformation such as phosphocholine, phosphoethanolamine and diacylglycerols which act as second messengers in the signal transduction pathways (Nishizuka, 1992).

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REFERENCES

Barbacid M, 1987. Ras genes. *Annu Rev Biochem* **56:** 779–827.

Bligh E, Dyer W, 1959. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37:** 911–917.

Carnero A, Cuadrado A, Del Peso L, Lacal J, 1994. Activation of typeD phospholipase by serum stimulation and ras-induced transformation in NIH3T3 cells. *Oncogene* **9:** 1387–1395.

Cuadrado A, Carnero A, Dolfi F, Jimenez B, Lacal J, 1993. Phosphorylcholine: a novel second messenger essential for mitogenic activity of growth factors. *Oncogene* **8:** 2959–2968.

Der C, Fimkel T, Cooper M, 1986. Biological and biochemical properties of Human ras-H gene mutated at codon 61. *Cell* **4:** 167–176.

Der C, Weissman B, MacDonald M, 1988. Altered guanine nucleotide binding and Ha-ras transforming and differentiating activities. *Oncogene* **3:** 105–112.

Geilen C, Wieder T, Boremski S, Marcus W, Orfanos C, 1996. c-Has-ras oncogene expression increases choline uptake, CTP:phosphocholine cytidylyl transferase activity and phosphatidylcholine biosynthesis in the immortalized human keratinocyte cell line HaCaT. *Biochim Biophys Acta* **1299:** 299–305.

Kiss Z, Crilly K, 1995. Ha-ras stimulates uptake and phosphorylation of ethanolamine:inhibition by wortmanin. *FEBS Lett* **357:** 279–282.

Koshlukova S, Momchilova-Pankova A, Markovska T, Koumanov K, 1992. Phospholipid dependence of rat liver microsomal acyl-CoA synthetase and acyl-CoA:lysophospholipid acyltransferase. *J Membr Biol* **127:** 113–119.

Lacal J, 1990. Diacylglycerol production in Xenopus laevis oocytes after microinjection of p21ras proteins is a consequence of activation of phosphatidylcholine metabolism. *Mol Cell Biol* **10:** 333–340.

Lacal J, Carnero A, 1994. Regulation of ras proteins and their involvement in signal transduction pathways. *Oncol Rep* **1:** 677–693.

Lipton B, Davidson E, Ginsberg B, Yorek M, 1990. Ethanolamine metabolism in cultured bovine aortic endothelial cells. *J Biol Chem* **265:** 7195–7201.

Lowry O, Rozenbrough N, Farr A, Randall R, 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275.

Macara I, 1989. Elevated phosphocholine concentration in ras-transformed NIH 3T3 cells arises from increased choline kinase activity, not from phosphatidylcholine breakdown. *Mol Cell Biol* **9:** 325–328.

Momchilova A, Markovska T, 1999. Phosphatidylethanolamine and phosphatidylcholine are sources of diacylglycerol in *ras*-transformed NIH 3T3 fibroblasts. *Int J Biochem Cell Biol* **31:** 311–318.

Momchilova A, Markovska T, Pankov R, 1998. Phospholipid dependence of membrane-bound phospholipase A_2 in *ras*-transformed NIH 3T3 fibroblasts. *Biochimie* **80:** 1055–1062.

Nishizuka Y, 1992. Intracellular signalling by hydrolysis of phospholipids and activation of protein kinase C. *Science* **258:** 607–614.

Niwa Y, Taniguchi S, 1986. Phospholipid base exchange in human leukocyte membranes: Quantitation and correlation with other phospholipid biosynthetic pathways. *Arch Biochem Biophys* **250:** 345–357.

Porter T, Kent C, 1990. Purification and characterization of choline/ethanolamine kinase from rat liver. *J Biol Chem* **265:** 414–422.

Sleight T, Kent C, 1983. Regulation of phosphatidylcholine biosynthesis in mammalian cells. *J Biol Chem* **258:** 831–835.

Teegarden D, Taparowsky E, Kent C, 1990. Altered phosphatidylcholine metabolism in C3H10T1/2 cells transfected with the Harvey-ras oncogene. *J Biol Chem* **265:** 6042–6047.

Touchstone J, Chen J, Beaver K, 1980. Improved separation of phospholipids by thin layer chromatography. *Lipids* **15:** 61–62.

Trotter P, Pedretti J, Voelker D, 1993. Phosphatidylserine decarboxylase from *Saccharomyces cerevisiae*. *J Biol Chem* **268:** 21,416–21,424.

Tsai M, Yu C, Stacey D, 1989. The effect of GTPase activating protein upon ras is inhibited by mitogenically responsive lipids. *Science* **243:** 522–526.

Wang X, Moore T, 1991. Phosphatidylethanolamine synthesis by castor endosperm. *J Biol Chem* **266:** 19,981–19,987.

Weinhold P, Rethy V, 1974. The separation, purification, and characterization of ethanolamine kinase and choline kinase from rat liver. *Biochemistry* **25:** 5135–5140.

Yavin E, 1976. Regulation of phospholipid metabolism in differentiating cells from rat brain cerebral hemispheres in culture. *J Biol Chem* **251:** 1392–1397.

Yu C, Tsay M, Stacey D, 1990. Serum stimulation of NIH 3T3 cells induces the production of lipids able to inhibit GTPase activating protein activity. *Mol Cell Biol* **10:** 6683–6689.