The distribution and metabolism of arachidonate-containing phospholipids in cellular nuclei°

Marc E. SURETTE†1 and Floyd H. CHILTON†

INTRODUCTION

The cell nucleus has recently generated much interest as the subcellular location of many enzymes involved in arachidonic acid (AA) metabolism. For example, cyclooxygenase II and 5-lipooxygenase activating protein have been localized to nuclear membranes while the 85 kDa phospholipase A2, and 5-lipogenase are found in the cytosol of a number of cells and have the capacity to translocate to the nuclear envelope during cell stimulation [1–5]. These findings have raised important questions regarding the composition and control of levels of arachidonate-containing phospholipids which reside around the nucleus.

Mammalian cells generally contain as many as 20 arachidonate-containing phospholipid molecular species and arachidonate is moved through different phospholipids in a sequential fashion [6]. For example, AA as a free acid in circulation is initially incorporated in glycerolipids around the nuclear envelope and then is moved into other cellular compartments [25,26]. Moreover, newly incorporated cellular arachidonate appears to move between cellular compartments in the same time frame as it is remodelled between phospholipid molecular species [25,27]. These observations raised the possibility that the nucleus is an important initial site of control for the incorporation and redistribution of arachidonate into releasable phospholipid pools during stimulation of inflammatory cells [18,23,24]. Although the pathways of arachidonate uptake and remodelling have been studied in several cells, little is currently known about the subcellular location of the arachidonate-containing phospholipids or enzymes that remodel these phospholipids.

This pathway of remodelling is selective for 20-carbon fatty acids and is believed to be important not only in the maintenance of homeostatic arachidonate levels within cellular phospholipid species throughout the cell, but also in the rapid redistribution of arachidonate into releasable phospholipid pools during stimulation of inflammatory cells [18,23,24]. Although the pathways of arachidonate uptake and remodelling have been studied in several cells, little is currently known about the subcellular location of the arachidonate-containing phospholipids or enzymes that remodel these phospholipids.

Previous studies in fibroblasts suggest that arachidonic acid is initially incorporated in glycerolipids around the nuclear envelope and then is moved into other cellular compartments [25,26]. Moreover, newly incorporated cellular arachidonate appears to move between cellular compartments in the same time frame as it is remodelled between phospholipid molecular species [25,27]. These observations raised the possibility that the nucleus is an important initial site of control for the incorporation and redistribution of arachidonate in phospholipids. Given the lack of information on arachidonate–phospholipid metabolism in the nucleus, the goal of the current study was to determine the distribution of arachidonate in nuclear phospholipids and the capacity of the nucleus to remodel arachidonate between phospholipid molecular species.

Abbreviations used: CoA-IT, CoA-independent transacylase; AA, arachidonic acid; GPC, glycerol-3-phosphocholine; GPE, glycerol-3-phosphoethanolamine; GPL, glycerol-3-phosphoinositol; PLC, phosphatidylinositol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PLα, phospholipase A2; PS, phosphatidylserine; NL, neutral lipids; HSA, human serum albumin; FBS, fetal bovine serum; HBSS, Hanks Balanced Salt Solution; TLC, thin-layer chromatography; BMMC, murine bone marrow-derived mast cells; NICI-GC/MS, negative ion chemical ionization gas chromatography/mass spectrometry. *Section on Pulmonary and Critical Care Medicine, and †Department of Biochemistry, Bowman Gray School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1054, U.S.A.

1 To whom correspondence should be addressed, at the present address: Centre de Recherche en Rhumatologie et Immunologie, Centre Hospitalier de Québec, local T 1-49 Pavillon CHUL, Ste. Foy, Québec G1V 4G2, Canada.
METHODS

Materials

Phospholipid standards phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylethanolamine (PE) were obtained from Avanti Polar Lipids, Inc. (Birmingham, AL, U.S.A.). Fatty acid standards were obtained from Cayman Chemical Co. (Ann Arbor, MI, U.S.A.). Pyridine and acetic anhydride were purchased from Altech Associates Inc. (Deerfield, IL, U.S.A.). Pentafluorobenzyl bromide (20% in ethanol) was purchased from Fisher Scientific (Silver Spring, MD, U.S.A.). All solvents (HPLC grade) were obtained from Fischer Scientific (Columbus, OH, U.S.A.). Pentafluorobenzyl esters of fatty acids were prepared and the quantities of fatty acids were determined by negative ion chemical ionization gas chromatography/mass spectrometry (NICI-GC/MS) as previously described [29].

Another fraction of the extracted lipids was used to separate glycerolipid classes by HPLC using a silica column (UltraspHERE, 4.6 x 250 mm; Rainin Instrument Inc., Woburn, MA, U.S.A.) with a hexane:2-propanol:ethanol:50 mM phosphate buffer (pH 7.4):acetic acid (490:367:100:30:6, by vol.) mobile phase [30]. After 5 min, the composition of phosphate buffer was increased from 5 to 6% over a 5 min period and maintained at 6% for the remainder of the run. Fractions containing neutral lipids (NL), PE, PI, PS and PC were collected. The arachidonate content of each fraction was determined by NICI-GC/MS as described above.

For phospholipid subclass analysis, the PE and PC fraction was isolated by normal phase HPLC and solvents were removed under a stream of nitrogen. One millilitre of 100 mM Tris buffer (pH 7.4) was added to the dried lipids. Phospholipase C, 40 U and 20 U was added to the PE and PC fractions, respectively, followed by 2 ml of ethyl ether and the solutions were incubated for 6 h at 37°C in a shaking water bath. The resulting diglycerides were extracted with 2 ml hexane and then again with 2 ml hexane:ether (1:1, v/v). Solvents were removed from extracts under a stream of nitrogen and diglycerides were acetylated by incubating overnight in pyridine:acetic anhydride (1:5, v/v) at 37°C in a shaking water bath. The solvents were then dried under N2 and the lipids were extracted twice with ether:hexane (1:1, v/v). The extract was washed once with 1 ml water and the subclasses were separated by TLC using a benzene:hexane:ether:acetic acid (50:45:4, by vol.) mobile phase. The areas containing 1-acyl, 1-alkyl and 1-alk-1-enyl-linked lipids were identified by co-migration with standards which were visualized with iodine. The TLC scrapings were extracted with ether:methyl alcohol (9:1, v/v) and were exposed to base hydrolysis as described above. Fatty acids were then derivatized and analysed by NICI-GC/MS as described above.

Total lipid phosphorus was determined colorimetrically [31]. In some experiments, phosphorus was assayed directly on TLC scrapings following the separation of lipid classes by TLC using a chloroform:methanol:acetic acid:water (50:25:8:3, by vol.) mobile phase.

Labelling of nuclei with [3H]arachidonic acid

In experiments where cells were labelled with [3H]AA (200 Ci/mmol), cells were washed by centrifugation once with HBSS and resuspended at 2 x 10^7 cells per ml in Ca<sup>2+</sup>-free HBSS. [3H]AA (2 µCi/2 x 10^7 cells) was added in 200 µl of HBSS containing HSA (250 µg/ml) and the cells were incubated for 10 s at 37°C. The cells were then washed once by centrifugation at 4°C with ice-cold HBSS containing 250 µg HSA/ml, and were then resuspended in HBSS at 37°C for the indicated incubation times. In parallel experiments, nuclei were prepared as described above and resuspended in 250 µl HBSS containing 5 mM MgCl<sub>2</sub> or in 200 µl HBSS with 5 µM MgCl<sub>2</sub> supplemented with 50 µl of cellular cytosol (1 x 10<sup>6</sup> cell equivalents). To this solution [3H]AA (5 µCi) was then added in 50 µl of HBSS containing HSA (250 µg/ml) and the suspension was allowed to incubate for
15 min at 37 °C. The nuclei were then washed by centrifugation at 4 °C with HBSS containing 5 mM MgCl₂ and HSA (250 µg/ml) (×2), were resuspended in HBSS containing 5 mM MgCl₂ and were incubated in the presence or absence of 25 µM CoA-IT inhibitor SK & F98625 at 37 °C. At the indicated times, aliquots of the incubations were removed, the lipids were extracted, and the phospholipid classes were separated by HPLC as described above. The radioactivity associated with each phospholipid class was measured by liquid scintillation spectrometry. In some experiments subclasses of PE and PC species were then separated by TLC as described above, and the radioactivity associated with each subclass was measured by liquid scintillation spectrometry.

CoA-IT activity

CoA-IT activity was measured as described previously [32]. Nuclear preparations or whole cell homogenates (5–20 µg protein) were diluted in PBS with 1 mM EGTA to the desired protein concentration. Reactions were initiated by the addition of [1-²H]alkyl-2-lyso-GPC (0.1 µCi/tube) and unlabelled 1-alkyl-2-lyso-GPC (1 µM final concentration) in assay buffer (with 250 µg/ml BSA). The reaction mixture was incubated for 10 min at 37 °C. The reaction was stopped, the lipids were extracted [28] and separated by TLC in chloroform/methanol/acetic acid/water (50:25:8:2.5, v/v). The radioactive profile of products was visualized by radioscanning (BioScan), the products were scraped and were then quantified by liquid scintillation spectrometry.

Electron microscopy

Nuclei were fixed with 2.5% glutaraldehyde in 100 mM phosphate buffer, pH 7.3. Samples were then prepared for transmission electron microscopy and samples to be sectioned were embedded in Spurr Resin (Polysciences, Warrington, PA). Thin sections (0.1 µm) were visualized at 80 keV in a Phillips EM-400.

DNA measurement

In all experiments, the amount of an arachidonate-containing glycerolipid was normalized to DNA content. DNA was measured as previously described by fluorometry using the Hoechst 33258 dye [33].

RESULTS

Nuclear preparations

In order to investigate the distribution of arachidonate in the nucleus it was important to ensure that pure nuclear preparations were obtained. Nuclei were prepared from several myeloid cell lines, utilizing a technique in which cells were disrupted by homogenization in a hypotonic solution and nuclei were isolated by centrifugation through a density gradient. This method requires minimal sample processing, thus reducing the potential for the mixing of lipids from different cellular compartments following cell disruption. Figure 1 shows electron micrographs of nuclear preparations from three cell types, THP-1, CFTL-15 and BMMC. Nuclear preparations from both THP-1 cells and CFTL-
Table 1  The distribution of arachidonate in lipid classes extracted from THP-1 nuclear preparations and of non-nuclear lipids

Lipids were extracted from total cell homogenates or from nuclear preparations. The lipid classes were separated by normal phase HPLC and the arachidonate content of NL, PE, PI/PS and PC was determined by NICI/GC-MS analysis as described in the Methods section. The non-nuclear values of arachidonate were obtained by measuring the arachidonate mass per µg DNA for each phospholipid class in nuclear preparations and subtracting this value from arachidonate mass per µg DNA for each phospholipid class in the total cellular extracts. DNA was measured as described in the Methods section. The data are the mean ± S.E.M. of five separate experiments.

<table>
<thead>
<tr>
<th>Lipid phosphorus (%)</th>
<th>PC</th>
<th>PI/PS</th>
<th>PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell</td>
<td>48 ± 1.7</td>
<td>18 ± 2.1</td>
<td>34 ± 1.1</td>
</tr>
<tr>
<td>Nucleus</td>
<td>45 ± 3.6</td>
<td>25 ± 2.9</td>
<td>30 ± 1.8</td>
</tr>
</tbody>
</table>

Table 2  The phospholipid composition of THP-1 cells and nuclei

Lipids were extracted, phospholipid classes were separated by TLC and the lipid phosphorus content of each class was measured as described in the Methods section. Values are expressed as the means ± S.E.M. (n = 3). There were no significant differences (P ≤ 0.05) between values in different rows as determined by a two-tailed paired Student’s t-test analyses.

<table>
<thead>
<tr>
<th>Arachidonate (ng/µg DNA)</th>
<th>NL</th>
<th>PC</th>
<th>PI/PS</th>
<th>PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear</td>
<td>1.0 ± 0.4</td>
<td>4.3 ± 0.6</td>
<td>5.8 ± 1.3</td>
<td>12.5 ± 4.1</td>
</tr>
<tr>
<td>Non-nuclear</td>
<td>2.7 ± 0.6</td>
<td>12.0 ± 2.0</td>
<td>14.7 ± 2.6</td>
<td>51.2 ± 7.9</td>
</tr>
</tbody>
</table>

15 routinely contained intact nuclei that were separated from most cellular debris. Nuclear preparations from BMMCC cells and human neutrophils (not shown), however, yielded nuclei that were not completely dissociated from other cellular structures. THP-1 cells were therefore utilized for all further experiments. The decrease in the specific activities of marker enzymes for Golgi, endoplasmic reticulum, mitochondria and plasma membrane in nuclear preparations from THP-1 cells compared to whole cell homogenates confirmed the purity of the nuclear preparations (data not shown).

Analysis of arachidonate in nuclei from THP-1 cells

The amount of cellular arachidonate associated with nuclear preparations varied in the different cell types. When arachidonate mass was normalized to DNA content 41 ± 7, 22 ± 3 and 48 ± 10 % of the cellular arachidonate was associated with the nucleus in BMMCC, THP-1 and CFTL cells, respectively. The distribution of the nuclear arachidonate in phospholipid classes was then compared to that of the rest of the cell. Table 1 shows the mass distribution of both nuclear and non-nuclear arachidonate in phospholipid classes from THP-1 cells. The values for the non-nuclear arachidonate were obtained by measuring the arachidonate mass per µg DNA for each phospholipid class in nuclear preparations and subtracting this value from arachidonate mass per µg DNA for each phospholipid class in the total cellular extracts. While PE was the most abundant arachidonate-containing class in both nuclear and non-nuclear compartments, PE contained 1.8-fold more arachidonate than all other glycerolipid pools combined in the non-nuclear fractions, whereas in nuclear preparations, PE contained roughly the same quantity of arachidonate as all other glycerolipids combined. The difference in the distribution of arachidonate in the nucleus and non-nuclear fractions was not due to a greater content of total PE (containing all fatty acids) in the non-nuclear fractions since there were no differences in the distribution of total lipid phosphorus in nuclei and whole cells (Table 2).

While the experiments described above showed some differences in the distribution of arachidonate in phospholipid classes, it was not until these classes were further separated into subclasses that pronounced differences were observed. These experiments revealed that the enrichment of the non-nuclear compartment in arachidonate-containing PE is largely due to the abundance of 1-alk-1-ethyl-2-arachidonoyl-PE (Table 3). This molecular species accounts for approx. 70 % of the arachidonate-containing PE in the non-nuclear fraction and was more than twice as abundant as any other arachidonate-containing species in the non-nuclear compartment. In contrast, 1-acyl-linked molecular species are the predominant arachidonate-containing phospholipids in the nucleus with 1-acyl-2-arachidonoyl-GPI being the most abundant arachidonate-containing species. In fact, approximately equivalent amounts of 1-acyl-2-arachidonoyl-GPC, 1-acyl-2-arachidonoyl-GPE, 1-acyl-2-arachidonoyl-GPI and 1-alk-1-ethyl-2-arachidonoyl-GPE were found in the nuclear lipid fraction. Very little 1-alkyl-2-arachidonoyl-GPC, the major cellular precursor for platelet activating factor, was found in the nucleus. The predominance of 1-acyl-linked molecular species in the nucleus was further emphasized when the ratio of 1-ester to 1-ether-linked phospholipids was compared in the nuclear and non-nuclear fractions. The nucleus contained a 1.5:1 ratio of arachidonate in 1-ester-linked species relative to 1-ether-linked species (contains both 1-alkyl and 1-alk-1-ethyl species), while non-nuclear membranes had a 1-ester to 1-ether ratio of 0.6:1.

CoA-IT activity and remodelling of arachidonate in nuclear preparations

1-Acyl-linked phospholipids are proposed to be the major donors of arachidonate in arachidonate-phospholipid remodelling (orchestrated by the CoA-IT reaction) and 1-ether-linked phospholipids are proposed to be the major acceptor phospholipids of arachidonate in this reaction. The observation that the nucleus is enriched in the major natural donor substrates of CoA-IT (1-acyl-2-arachidonoyl-linked phospholipids) in association with previous reports in the literature which show that newly incorporated AA is associated with the nucleus and is subsequently shuttled to other cellular locations [25,26], lead to the hypothesis that CoA-IT may orchestrate not only the remodelling of arachidonate between phospholipid subclasses, but also the subcellular redistribution of cellular arachidonate. Therefore, subsequent experiments were designed to determine whether nuclear preparations contained CoA-IT activity and whether arachidonate-labelled nuclei had the capacity to remodel arachidonate. Since nuclei had never been reported to exhibit CoA-IT activity, nuclear preparations were assayed for CoA-IT activity using [3H]-alkyl-2-lyso-GPC as the acceptor substrate. The nuclear preparations contained CoA-IT activity and were able to acylate the [3H]-alkyl-2-lyso-GPC substrate at a rate of 0.23 ± 0.02 µmol·µg DNA⁻¹·min⁻¹ while whole cell preparations had a specific activity of 1.01 ± 0.26 µmol·µg DNA⁻¹·min⁻¹.

The capacity of the nucleus to remodel arachidonate was also assessed using two other approaches. Firstly, isolated nuclei were incubated with [3H]AA in an attempt to label nuclear phospholipids and subsequently determine whether the incorporated arachidonate would be remodelled following an incubation
Table 3 The distribution of arachidonate in phospholipid subclasses of nuclear and non-nuclear fractions from THP-1 cells

The lipid classes were separated by normal phase HPLC and the PC and PE subclasses (1-acyl-, 1-alkyl- and 1-alk-1-enyl-) were further isolated by TLC as described in Methods. The arachidonate content of each subclass was determined by GC-MS analysis. The non-nuclear values of arachidonate were obtained by measuring the arachidonate mass per µg DNA for each subclass in nuclear preparations and subtracting this value from arachidonate mass per µg DNA for each phospholipid subclass in the total cellular extracts. DNA was measured as described in the Methods. The values are the mean±S.E. of three or five separate experiments.

<table>
<thead>
<tr>
<th>Arachidonate (ng/µg DNA)</th>
<th>Fraction</th>
<th>PC</th>
<th>PE</th>
<th>PI/PS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1-acyl</td>
<td>1-alkyl</td>
<td>1-alk-1-enyl</td>
</tr>
<tr>
<td>Nuclear</td>
<td></td>
<td>3.2±1.1</td>
<td>1.0±0.1</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>Non-nuclear</td>
<td></td>
<td>4.9±1.0</td>
<td>3.8±0.3</td>
<td>1.0±0.6</td>
</tr>
</tbody>
</table>

DISCUSSION

It is not apparent why mammalian cells have a unique set of enzymes that incorporate and remodel arachidonate, but not other more abundant fatty acids, through multiple phospholipid pools. Since recent studies have revealed the nucleus as a key subcellular site for enzymes involved in the release and metabolism of arachidonic acid [1–5,34], the current study focused on the distribution and metabolism of arachidonate-containing phospholipids at this subcellular location. The differential distribution of arachidonate-containing phospholipids among nuclear and non-nuclear membranes reported here sheds new light on a number of previous observations. Neufeld and colleagues [26] showed by quantitative electron microscope autoradiography that following a pulse-label of murine fibrosarcoma cells with [3H]AA, nuclear membranes were initially enriched in radio-labelled arachidonate and that over 24 h this label was redistributed to other cellular membranes. A similar finding was later reported by Capriotti and colleagues in human fibroblasts [25]. The present finding that the nucleus is enriched in 1-acyl-linked arachidonate-containing phospholipids together with the observation that arachidonic acid taken up by mammalian cells is initially incorporated into 1-acyl-linked phospholipids by a 1-acyl-2-lyso-sn-glycero-phospholipid:arachidonoyl-CoA trans-ferase(s) shown to prefer 1-acyl-linked (versus 1-ether-linked) phospholipids [13], suggests that the movement of arachidonate observed by Neufeld may be associated with the CoA-IT-driven remodelling of 1-acyl-linked phospholipids to ether-linked phospholipids. These results are thus consistent with the hypothesis that the remodelling of arachidonate may involve not only the movement of arachidonate between phospholipid molecular species but also the movement of arachidonate from the nucleus into other cellular membranes (Scheme 1).

The current study also demonstrates that the isolated nucleus contains a relatively large amount of CoA-IT activity as well as the capacity to remodel arachidonate between nuclear phospholipids. Although most of the potential arachidonate acceptor phospholipids for the CoA-IT reaction (1-ether linked phospholipids) appear to reside in non-nuclear membranes, nuclear preparations exhibited the capacity to remodel [3H]arachidonate between phospholipid classes and subclasses which was inhibited by the CoA-IT inhibitor SK & F98625. The CoA-IT reaction requires the generation of lyso-phospholipids to act as acceptors for arachidonate and, although not proven, this reaction has been assumed to be catalysed by a calcium-independent phospholipase A2 in resting cells [35]. Therefore, the capability of isolated nuclei to remodel [3H]arachidonate also indicates that these preparations likely possess the phospholipase A2 (PLA2) activity required to generate lyso-phospholipid acceptors.

This differential distribution of arachidonate in nuclear and non-nuclear membranes is also consistent with the subcellular location and proposed substrate specificity of different PLA2 enzymes. For example, the high molecular mass, cytosolic...
phospholipase A₂ (cPLA₂) is localized to the cytosol of most resting cells and then translocates to the nuclear envelope after addition of an appropriate agonist [1,2] where it is hypothesized to release arachidonate from a phospholipid pool whose specific activity in labelling experiments matches that of 1-acyl-2-arachidonoyl-GPE and GPI [36]. In contrast, low molecular mass, secretory phospholipase A₂ moves from the inside to the outside of cells during stimulation and can then hydrolyse arachidonic acid from outer membranes of the cell [37–41] from a pool that has a specific activity in labelling experiments that strongly suggests it is released from 1-alk-1-etyl-2-arachidonoyl-GPE [36]. Similar results have recently been reported in macrophages [42]. The current results strongly support the hypothesis that the different PLₐ enzymes act on different AA-containing phospholipid species which reside at different subcellular locations by showing that the appropriate subcellular locations are enriched with the phospholipid substrates proposed to be hydrolysed by secretory and cytosolic phospholipases A₂. This is also consistent with results obtained in calcium ionophore-stimulated rat alveolar epithelial cells where the release of AA that is inhibited by the cPLA₂ inhibitor, arachidonoyl trifluoromethyl ketone, is predominantly derived from nuclear phospholipids [34].

The content of arachidonate in nuclear membranes has also been speculated to have an impact on rates of cell proliferation. This concept was first postulated in liver regeneration experiments and in rat ascites hepatoma cells where the amount of arachidonate associated with the nuclei of proliferating cells is greatly diminished compared to non-proliferating cells [43].

Table 4 The distribution and remodelling of [¹³C]arachidonate in cellular and nuclear phospholipid subclasses

<table>
<thead>
<tr>
<th></th>
<th>Cell</th>
<th>Nucleus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-acyl</td>
<td>1-alkyl</td>
</tr>
<tr>
<td>PE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T = 0 h</td>
<td>17.2 ± 2.7</td>
<td>4.6 ± 0.5</td>
</tr>
<tr>
<td>T = 4 h</td>
<td>14.3 ± 2.8</td>
<td>7.2 ± 1.0</td>
</tr>
<tr>
<td>PC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T = 0 h</td>
<td>21.4 ± 5.8</td>
<td>4.2 ± 0.7</td>
</tr>
<tr>
<td>T = 4 h</td>
<td>10.9 ± 0.3</td>
<td>5.1 ± 0.1</td>
</tr>
</tbody>
</table>

* Denotes statistically different (P ≤ 0.05) from T = 0 value as determined by two-tailed paired Student’s t test analyses.

Figure 2 The distribution of [¹³C]arachidonate in nuclear and cellular phospholipids

(A) Nuclei isolated from THP-1 cells and (B) THP-1 cells were pulse-labelled with [¹³C]AA, washed and incubated for various times at 37 °C as described in the Methods. At the indicated times, aliquots from the incubations were removed, lipids were extracted, separated by HPLC and TLC, respectively, and the radioactivity associated with the different phospholipid classes was measured. Values for whole cells are the percentage of total [¹³C]arachidonate in cellular phospholipids associated with each subclass and are expressed as the average ± S.E. of four separate experiments.

Scheme 1 Proposed pathway for the movement of arachidonate between nuclear and non-nuclear phospholipids
Proliferating tumour cell lines, including THP-1 cells in the present study, have been observed to remodel arachidonate at extremely rapid rates when compared to similar non-neoplastic cells [44]. The current study suggests that this rapid remodelling could contribute to the removal of arachidonate from the nuclear membrane resulting in the elevated quantities of arachidonate in non-nuclear 1-alk-1-enyl-2-arachidonoyl-GPE [15,18]. Consistent with this hypothesis, the inhibition of CoA-IT in HL-60 cells results in the accumulation of arachidonate in 1-acyl-linked phospholipids and is associated with an inhibition of cell proliferation [45].

In conclusion, the differential distribution of arachidonate in nuclear and non-nuclear phospholipids described here provides important clues into the role of arachidonate–phospholipid remodelling in controlling arachidonate availability and in maintaining the subcellular distribution of arachidonate in resting and stimulated as well as normal and neoplastic cells. Any perturbation of this pathway resulting in an altered subcellular distribution of arachidonate may impact on the capacity of cells to mobilize arachidonate and undergo cell division.

The authors acknowledge the technical assistance of Dennis Swan (CG-MS) and Ken Grant (Electron Microscopy). M.E.S. was the recipient of a Centennial Fellowship awarded by the Medical Research Council of Canada. This work was supported in part by National Institutes of Health Grant (Electron Microscopy). M.E.S. was the recipient of a Centennial Fellowship awarded by the Medical Research Council of Canada. This work was supported in part by National Institutes of Health Grant AI24985 (to F.H.C.).

REFERENCES


Received 23 June 1997/22 October 1997; accepted 11 November 1997

Arachidonate–phospholipid metabolism in nuclei 921