Translocation and Leukotriene Synthetic Capacity of Nuclear 5-Lipoxygenase in Rat Basophilic Leukemia Cells and Alveolar Macrophages*

(Received for publication, May 8, 1995, and in revised form, July 5, 1995)

Thomas G. Brock‡, Robert W. McNish, and Marc Peters-Golden§

From the Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan 48109-0652

Leukotriene (LT) synthesis involves the translocation of enzymatically active 5-lipoxygenase (5-LO) from a soluble site to a bound site, where it interacts with 5-lipoxygenase-activating protein (FLAP). In human polymorphonuclear leukocytes (PMNs), 5-LO moves from the cytosol to the nuclear envelope (NE) to interact with FLAP. However, 5-LO has recently been found within the nucleus, as well as the cytosol, of rat basophilic leukemia (RBL) cells and alveolar macrophages (AMs). To assess whether nuclear 5-LO can contribute to LT synthesis in these cells, we investigated whether this enzyme pool 1) translocates upon cell activation, 2) co-localizes with FLAP, and 3) is enzymatically active. By cell fractionation followed by immunoblotting, both cytosolic and nuclear soluble 5-LO decreased dramatically in RBL cells following activation with the calcium ionophore A23187. Concurrently, 5-LO increased in the pelletable nuclear pool, where FLAP was also detected. The loss of both cytosolic and nuclear soluble 5-LO, with concomitant increase exclusively at the NE, as well as co-localization with FLAP, were confirmed by indirect immunofluorescent and confocal microscopy. In AMs, the nuclear soluble pool of 5-LO moved to the NE, where FLAP was also found; however, the cytosolic 5-LO pool did not translocate. Application of these techniques to PMNs confirmed that cytosolic 5-LO moved to the nuclear envelope and co-localized with FLAP. By cell-free assay, nuclear soluble proteins from both RBL cells and AMs, but not PMNs, were able to generate 5-LO products from arachidonate, and this was inhibited by the direct 5-LO inhibitor zileuton. Cytosolic proteins from all cell types also showed cell-free 5-LO activity. These results demonstrate three distinct patterns of 5-LO translocation that are specific for each cell type: translocation of only a cytosolic pool in PMNs, of only a nuclear pool in AMs, and of both cytosolic and nuclear pools in RBL cells. By virtue of its enzymatic activity and ability to translocate, nuclear 5-LO has the potential to contribute to LT synthesis in RBL cells and AMs. Finally, these results provide a foundation for considering the individual functions of discrete pools of 5-LO in future studies.

Leukotrienes (LTs)1 are lipid mediators with important roles in both host defense and inflammatory disease states (1, 2). The synthesis of LTs from arachidonic acid is catalyzed by the enzyme 5-lipoxygenase (5-LO). Activation of 5-LO is thought to be initiated by the translocation of the enzyme from a soluble pool to a bound site (3, 4). This process of translocation places 5-LO in close proximity to its substrate, arachidonic acid, and to the 5-lipoxygenase-activating protein (FLAP) (5, 6), an integral membrane protein which increases the leukotriene synthetic capacity of 5-LO in intact cells (7–9).

Although translocation of 5-LO has generally been assumed to involve movement from the cytosol to a membrane (3, 10), actual identification of the soluble site in resting cells and the bound site in activated cells has only recently been attempted. Using immunoelectron microscopy, Woods et al. (11) have demonstrated that 5-LO co-localized at the nuclear envelope (NE) with FLAP in activated leukocytes. However, 5-LO could not be detected in resting cells by this technique, presumably because of loss of soluble protein during cell processing. Using indirect immunofluorescent microscopy and cell fractionation combined with immunoblotting, we recently demonstrated that 5-LO is indeed in the cytosol of resting neutrophils (12). Together, these data indicate that 5-LO moves from the cytosol to the NE in PMNs. This pattern, which we have also recognized in rat peritoneal macrophages (13), forms the basis for the current model for 5-LO translocation during cell activation.

Recently, we have examined the localization of 5-LO in two other cell types that produce abundant LTs in response to activation, RBL cells and AMs. Using a variety of techniques, we have demonstrated the presence of abundant 5-LO within the nucleus, as well as in the cytosol, of both resting RBL cells (12) and AMs (14). It is unknown whether the nuclear pool of 5-LO functions in LT synthesis. Since translocation is an important early event in 5-LO action, translocation of nuclear 5-LO would support a role in LT synthesis. In previous studies, 5-LO translocation has been observed in RBL cells (15, 16), but not in AMs (17). However, in none of these studies was nuclear material examined. In fact, cells typically were disrupted using harsh techniques that disrupted nuclei, thus mixing nuclear soluble proteins with cytosol; moreover, much nuclear material was discarded in early processing steps. Thus, the fate of nuclear 5-LO has never been considered.

Efficient arachidonate processing by 5-LO requires three features: 1) translocation of 5-LO, 2) co-localization with FLAP, and 3) enzymatic activity. By cell fractionation followed by immunoblotting, we recently demonstrated that 5-LO co-localized at the nuclear envelope (NE) with FLAP in activated leukocytes. However, 5-LO could not be detected in resting cells by this technique, presumably because of loss of soluble protein during cell processing. Using indirect immunofluorescent microscopy and cell fractionation combined with immunoblotting, we recently demonstrated that 5-LO is indeed in the cytosol of resting neutrophils (12). Together, these data indicate that 5-LO moves from the cytosol to the NE in PMNs. This pattern, which we have also recognized in rat peritoneal macrophages (13), forms the basis for the current model for 5-LO translocation during cell activation.

1 The abbreviations used are: LT, leukotriene; 5-LO, 5-lipoxygenase; AM, alveolar macrophage; FLAP, 5-lipoxygenase-activating protein; NE, nuclear envelope; PMN, polymorphonuclear leukocyte; RBL, rat basophilic leukemia; PBS, phosphate-buffered saline; BSA, bovine serum albumin; HPLC, high performance liquid chromatography; HPETE, hydroperoxyeicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid.
and 3) the ability to enzymatically convert arachidonate to 5-LO products. In the present study, we have used a variety of techniques to assess the ability of nuclear 5-LO, as well as cytosolic 5-LO, to contribute to LT synthesis in RBL cells and AMs, using these criteria. For comparison, we have performed parallel experiments with PMNs.

EXPERIMENTAL PROCEDURES

Cell Lines—RBL-1 cells (American Type Culture Collection) were cultured in minimal essential medium (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal calf serum (Life Technologies, Inc.) and penicillin/streptomycin, fed 2 days later and used on the 3rd day, as described previously (12). Human neutrophils (PMNs) were isolated from venous blood drawn from normal volunteers. Purification involved the sequential steps of centrifugation through Ficol-Paque (Pharmacia Biotech Inc.), dextran sedimentation, and hypotonic lysis of erythrocytes (17). AMs were obtained from respiratory disease-free female Wistar rats (Charles River Laboratories) by bronchoalveolar lavage (18) and used immediately. Purity of both PMN and AM preparations was greater than 95%. Cell viability in all preparations was greater than 95%, as assessed by trypan blue exclusion.

Subcellular Fractionation—PBS-washed cells were resuspended in ice-cold sucrose-TKM buffer (250 mM sucrose, 50 mM Tris-HCl, pH 7.4, 25 mM KCl, 5 mM MgCl₂, 1 mM EGTA) with protease inhibitors (1 mM each of N-ethylmaleimysulfanyl fluoride, diithiothreitol, soybean trypsin inhibitor, and leupeptin) at 10⁷ cells ml⁻¹. Cells were pelleted, resuspended in 1% BSA in PBS, and mounted on slides by Cytospin (500 rpm, 1 min). Mounted cells were incubated in ice-cold sucrose-TKM buffer (250 mM sucrose, 50 mM Tris-HCl, pH 7.4, 25 mM KCl, 5 mM MgCl₂, 1 mM EGTA) supplemented with 50 mM Tris-HCl (pH 7.4), 1 mM EDTA, and 200 mM sucrose, centrifuged at 100,000 × g, 30 min, 4°C; the resulting soluble fraction was solubilized in 5% Triton X-100, and protein concentrations were determined by a modified Coomassie dye-binding assay (Pierce), using BSA as the standard. Samples were then subjected to immunoblot analysis as described previously (13). Briefly, samples containing 1–10 μg of protein were separated by SDS-polyacrylamide gel electrophoresis under reducing conditions and transferred to nitrocellulose membranes. Membranes then were blocked, washed, and probed with either rabbit polyclonal antibody raised against purified human 5-LO (a generous gift from Dr. J. Evans, Merck Forest) (19). Primary antibodies were used at titers of 1:5,000, then detected using horseradish peroxidase-conjugated goat anti-rabbit secondary (1:5,000) followed by ECL chemiluminescence (Amersham Corp.). Multiple exposure times were taken for all blots to ensure that band densities were within the linear range of exposure. Band densities were quantitated by video densitometry using image analysis software from Scion.

Indirect Immunofluorescent Microscopy—As described previously (12), cells were pelleted, resuspended in 1% BSA in PBS, and cytocentrifuged on slides by Cytospin (500 rpm, 1 min). Mounted cells were incubated in PBS with 1 mM CaCl₂, and in some cases subsequently they were transferred to the same solution containing the calcium ionophore A23187. Cells were fixed in 20°C methanol for 30 min, permeabilized in 20°C acetone, and blocked with 1% BSA in PBS containing non-immune goat serum. Primary antibodies were separated in 1% BSA-PBS (5-LO, 1:100; FLAP, 1:50) and applied for 1 h, 37°C. Mounts were washed with 1% BSA-PBS, and incubated with rhodamine-conjugated goat anti-rabbit antibody (1:200; Sigma) for 1 h, 37°C, then washed extensively and placed on coverslips. For each antibody, similar results were obtained if cells were instead fixed with 2% paraformaldehyde, 30 min, 22°C, and permeabilized with 0.1% Triton X-200 in 3% BSA-PBS, or if cells were fixed prior to mounting. Fluorescence was imaged using a Nikon Labophot 2 microscope equipped for epifluorescence. Confocal imaging of identically stained samples was performed on a Bio-Rad MRC-600 laser confocal microscope, with pinhole aperture adjusted to give the minimal optical slice thickness (approximately 0.3 μm).

5-Lipoxygenase Activity—Cell-free 5-LO activity was determined using a modification of the method of Percival et al. (20). Cytosol or nuclear soluble fractions (100 μL, approximately 100 μg) were mixed with 900 μL of reaction buffer (50 mM Tris-HCl, pH 7.3, 0.1 mM EDTA, 1 mM ATP, 13.3 μg/ml phosphatidylcholine (Avanti Polar Lipids) containing unlabeled arachidonic acid (20 μM; Nu-Chek) plus [5,6,8,9,11,12,14,15-³H]arachidonic acid (approximately 4 × 10⁶ dpm; specific activity, 100 mCi/mmole; DuPont NEN). In some cases, the specific, direct 5-LO inhibitor zileuton was included (final concentration, 3 μM, which blocked 5-LO product formation >90%, but reduced prostaglandin formation by <10% in preliminary experiments on whole cells). After 15 min at room temperature the reaction was stopped by adding 4 volumes of ether, MeOH, 1 mM citric acid (30:4:1 (v:v:v)) and vortexing. Following brief centrifugation the ether phase was separated into solubilized (S) and pelletable (P) fractions. Fractions were then probed for S-LO and FLAP by immunoblot analysis.
Statistical Analyses—All results are representative of at least three independent experiments. Statistical significance was evaluated by a paired Student’s t test, using a p value of 0.05.

RESULTS

Translocation of 5-LO in Activated RBL Cells—5-LO was found in all fractions of resting RBL cells by immunoblot analysis (Fig. 1A), as described previously (12). That is, soluble pools of 5-LO were found in both cytosolic and nuclear fractions, and bound 5-LO was evident in both membrane and nuclear pelletable fractions. Following stimulation of RBL cells with ionophore for 15 min, 5-LO decreased dramatically in both soluble pools. 5-LO increased predominantly in the nuclear pelletable pool; increases in 5-LO in the membrane fraction were not statistically significant (Fig. 1A and B). As noted above, the nuclear pellet should include NE, DNA and associated proteins, and nuclear structural proteins. In resting RBL cells, the 5-LO associated with this nuclear pelletable fraction has previously been characterized as tightly bound and not membrane-associated (12). Consistent with this, very little 5-LO was solubilized from this fraction by detergent extraction (Fig. 1C, left). In contrast to this situation in resting cells, abundant 5-LO could be solubilized from the nuclear pellet of stimulated cells (Fig. 1C, right), suggesting that, following stimulation, 5-LO had become associated with the NE.

FLAP protein was localized primarily in the nuclear pelletable fraction of resting RBL cells, with some detectable protein also in the membrane fraction (Fig. 1D). As mentioned above, the membrane fraction should include endoplasmic reticulum, which has previously been identified as being a minor site for FLAP protein (11). Cell activation with ionophore did not alter the distribution of FLAP (Fig. 1D). Detergent treatment extracted FLAP from the nuclear pelletable fractions of either resting or activated cells (data not shown). Similar results, for 5-LO and FLAP, were obtained when cells were initially disrupted using Dounce homogenization, rather than nitrogen cavitation (data not shown).

These results using immunoblot analysis indicated that, upon activation, 5-LO, originating from two distinct soluble pools, translocated to the NE, where it co-localized with FLAP. To confirm this interpretation, the localization of 5-LO in in-
When longer activation times, a second change in 5-LO distribution became evident, cytosolic staining for 5-LO had diminished, and within the nucleus, the pattern of 5-LO staining changed from granular to diffuse (Fig. 2B). Residual cytosolic staining, particularly around the nucleus, as well as some persistent granular staining within the nucleus, was apparent in a minority of cells (<20%) at this early time point. By 5 min, essentially all cytosolic 5-LO was depleted, and some 5-LO appeared to reside at the NE, as evidenced by the appearance of membrane folds in some cells (Fig. 2C). This pattern was consistently seen in most cells examined at 7.5 and 10 min after ionophore addition (not shown).

With longer activation times, a second change in 5-LO distribution became apparent. By 15 min after ionophore addition, 5-LO was no longer diffusely dispersed throughout the nucleus. Instead, larger amounts of 5-LO were concentrated at the NE, as indicated by a pronounced nuclear “rimming” pattern of fluorescence (Fig. 2D). This pattern of 5-LO distribution was quite similar to that of FLAP in RBL cells (Fig. 2E). In contrast to the staining for 5-LO or FLAP, control staining with nonimmune serum was minimal (Fig. 2F).

The change in 5-LO pattern between 5 and 15 min of activation could result from the translocation of a dispersed intranuclear pool to the nuclear envelope during that interval. To address this possibility, we used confocal microscopy to obtain thin optical sections through the nucleus 5 and 15 min after activation. As compared with resting cells (Fig. 3A), thin sections of nuclei from RBL cells activated for 5 min (Fig. 3B) were characterized by diffuse fluorescence across the nucleus, with no pronounced signal at the nuclear envelope. Scans of nuclei from cells activated for 15 min showed much less 5-LO inside the nucleus and much more at the NE (Fig. 3C). Thus, translocation of nuclear 5-LO involved two steps, the first being the rapid dispersion of 5-LO throughout the nucleus, followed by a slower movement to the NE.

Translocation of 5-LO in AMs and PMNs—Having determined that 5-LO is translocated from soluble pools within the cytosol and nucleus to the NE in RBL cells, similar experiments were performed using AMs and PMNs. As in RBL cells, 5-LO in resting AMs was found to occur in both cytosolic and nuclear soluble pools (Fig. 4A). However, when AMs were stimulated, the cytosolic pool did not translocate, whereas most of the nuclear soluble pool moved to the nuclear pellet. Also as in RBL cells, FLAP protein was detected in both the membrane and nuclear pellet from resting AMs; this pattern, likewise, was unaffected by cell activation. Consistent with previous reports (11), 5-LO in human PMNs translocated from the cytosol to the nuclear pellet upon cell activation, and thus co-localized with FLAP (Fig. 4B).

Immunofluorescent microscopy confirmed that 5-LO translocated to the NE upon cell activation in both AMs and PMNs. Resting AMs showed the same pattern of 5-LO distribution as RBL cells: diffuse cytosolic staining combined with granular nuclear staining (Fig. 5A). Following activation, the nuclear 5-LO rapidly dispersed in over 90% of all cells, as had been observed in RBL cells; however, unlike RBL cells, cytosolic 5-LO did not change (Fig. 5B). The image presented in Fig. 5B was selected because it contains two cells which stain exactly like resting AMs and thus can be compared with the single activated cell to demonstrate that there is no change in the cytosolic 5-LO pool. With longer incubations, the dispersed 5-LO within the nucleus moved to the NE (Fig. 5C), resulting in a pattern of distribution similar to that seen for FLAP (Fig. 5D). In PMNs, 5-LO moved from the cytosol in resting cells (Fig. 5E) to the NE of the multilobed nucleus following cell activation (Fig. 5F), again co-localizing with FLAP (Fig. 5G).

Cell-free 5-LO Activity of Cytosolic and Nuclear Soluble Fractions—To demonstrate functional capacity of these various 5-LO pools, soluble fractions from both the cytosol and the...
nucleus were tested for their ability to metabolize arachidonate in a cell-free assay. Both cytosolic and nuclear soluble fractions from RBL cells were capable of converting arachidonate to products which co-elute with LTs (LTA₄ metabolites/LTB₄) and 5-HETE (Fig. 6). Generation of these products was effectively blocked by zileuton, a specific 5-LO inhibitor, while the amount of unmetabolized arachidonic acid increased. The specific FLAP inhibitor MK-886 did not block product formation by either fraction in the cell-free 5-LO assay. In parallel studies, both pools in AMs also were found to demonstrate zileuton-inhibitable 5-LO activity in the cell-free assay (Table I). As expected, the cytosolic pool, but not the nuclear pool, of PMNs had significant 5-LO activity.

DISCUSSION

The current paradigm for 5-LO action is that cell activation causes the translocation of 5-LO from a soluble pool to a bound site, where it can interact with FLAP to efficiently produce LTA₄ from free arachidonate. Recent work with neutrophils has indicated that the target of translocation is the NE (11). In this study, we have extended our understanding of this process. First and foremost, our data establish that there is no single model for 5-LO translocation. Instead, we have described three patterns of translocation, in three different cells types. Second, we have shown that some pools are able to translocate, and that others do not under the conditions tested. Third, we have demonstrated that, despite this heterogeneity in patterns of translocation, the NE appears to be the target of translocation in all cases. Fourth, we have confirmed the localization of FLAP to the NE and contiguous endoplasmic reticulum. Fifth, we have shown that nuclear 5-LO is enzymatically active and can be inhibited by the specific 5-LO inhibitor zileuton. Finally, we have demonstrated that, although all translocating pools of 5-LO may possess the capacity for activity, enzymatically active 5-LO may not always translocate.

Of the three patterns of translocation, neutrophils fit the simplest model, in that the single cytosolic pool of 5-LO moves to the NE following activation. In AMs, which have both cytosolic and intranuclear 5-LO pools, only the nuclear pool moves following cell activation. RBL cells, like AMs, have both cytosolic and nuclear pools; unlike AMs, both pools translocate. In all cases, the primary target of translocation is the NE. Interestingly, the response in RBL cells appears to involve two steps. Both the translocation of cytosolic 5-LO and the change in nuclear 5-LO, from a granular to dispersed distribution, are immediate and therefore may be a direct response to elevated calcium levels. These changes may represent a phase of translocation which is reversible upon removal of agonist, described previously (21, 22). On the other hand, movement of the dispersed intranuclear 5-LO to the NE is a slower event, requiring several minutes. This delay suggests that steps subsequent to
the calcium flux are necessary to drive translocation of the nuclear pool. These steps remain to be elucidated, but may include binding of 5-LO to a chaperone or docking protein, or cytoskeletal elements, via its Src homology 3 (SH3) binding motif (23).

The failure of the cytosolic 5-LO pool to translocate upon activation in AMs (Figs. 4 and 5) has been suggested previously from cruider studies (17). The finding that this pool is nonetheless enzymatically active suggests that its function may be different from the translocating nuclear 5-LO pool that co-localizes with FLAP. 5-LO can metabolize arachidonate derived from extracellular sources even when FLAP is effectively inhibited (9, 24, 25); thus, FLAP activity and, conceivably, co-localization with FLAP may not be necessary for 5-LO to process exogenously derived arachidonate. We suggest that the nontranslocating cytosolic pool of 5-LO in AMs could therefore play a role in processing arachidonate from such exogenous sources. This hypothesis would be consistent with the observation that AMs can efficiently use free arachidonate released by adjacent lung epithelial cells to generate 5-LO products (26). On the other hand, utilization of arachidonate from intracellular (endogenous) sources may depend on FLAP-mediated delivery of the fatty acid to translocated 5-LO for efficient LT synthesis, and the intranuclear 5-LO pool may be preferentially involved in this process.

Translocation of both cytosolic and intranuclear 5-LO to the NE underscores the role of this membrane system in arachidonate metabolism. Previous studies have demonstrated that arachidonate released following agonist activation originates from NE phospholipids (27) and that an arachidonate-selective phospholipase A2 binds to the nuclear fraction following cell activation (13). It is thus possible that the substrate for 5-LO comes from NE phospholipids. Furthermore, FLAP is at the NE. Using immunoelectron microscopy, Woods et al. (11) found FLAP on both the inner and outer NE membranes in leukocytes, as well as the endoplasmic reticulum. Thus FLAP should be able to utilize its arachidonate binding capacity (28) to deliver arachidonate to, and thus “activate,” 5-LO on either membrane of the NE.

The results presented here support roles for both nuclear and cytosolic pools of 5-LO in LT synthesis. Although the advantages of 5-LO compartmentation are unclear, there are several possibilities relating to both known catalytic functions as well as possible noncatalytic functions. In terms of catalytic functions, distinct 5-LO pools could generate different amounts or profiles of products. Likewise, pools may be differentially regulated, having unique rates or magnitudes of response to different agonists. Or, 5-LO pools may have unique catalytic functions within their specific compartments. Since free arachidonate is a potent intracellular effector (29–31), pools of 5-LO may scavenge arachidonate within a given compartment. Similarly, 5-LO-generated products may act directly at the point of synthesis, as has recently been suggested (32, 33).

Novel noncatalytic functions of particular intracellular pools of 5-LO could be mediated by direct association of the protein with specific molecular targets, perhaps via its SH3-binding domain. 5-LO interacts specifically with SH3 sites on select cell signaling proteins and cytoskeletal elements in vitro (23), suggesting a role for cytosolic 5-LO protein in related processes. Similarly, within the nucleus, 5-LO may interact with signaling or structural proteins, or nucleic acids within the euchromatin region, where 5-LO protein has been observed (14). These possibilities provide the basis for considering the specific functions subserved by different 5-LO pools in future studies.

Acknowledgment—The invaluable contribution of Dr. Robert Paine III, through helpful comments and immunofluorescence expertise, is gratefully acknowledged.

References
1. Samuelsson, B. (1983) Science 220, 568–575
2. Lewis, R., Austen, K., and Soberman, R. (1990) N. Engl. J. Med. 323, 645–655
3. Rouzer, C. A., and Kargman, S. (1988) J. Biol. Chem. 263, 10980–10986
4. Kargman, S., Prialt, P., and Evans, J. (1991) J. Biol. Chem. 266, 23740–23752
5. Dixon, R. A. F., Diehl, R. E., Opas, E., Rands, E., Vickers, P. J., Evans, J. F., Gillard, J. W., and Miller, D. K. (1990) Nature 343, 282–284
6. Miller, D. K., Gillard, J. W., Vickers, P. J., Sadowski, S., Leveille, C., Mancini, J. A., Charleson, P., Dixon, R. A. F., Ford-Hutchinson, A. W., Fortin, R., Gauthier, J. Y., Rodkey, J., Rosen, R., Rouzer, C., Sigal, I. S., strader, C. D., and Evans, J. F. (1990) Nature 343, 278–281
7. Reid, G. K., Kargman, S., Vickers, P. J., Mancini, J. A., Leveille, C., Ether, D., Miller, D. K., Gillard, J. W., Dixon, R. A. F., and Evans, J. F. (1990) J. Biol. Chem. 265, 19818–19823
8. Kargman, S., Vickers, P. J., and Evans, J. F. (1991) J. Biol. Chem. 266, 1701–1702
9. Abramovitz, M., Wong, E., Cox, M., Richardson, C., Li, C., and Vickers, P. (1993) Eur. J. Biochem. 215, 105–111
10. Rouzer, C. A., Ford-Hutchinson, A. W., Morton, H. E., and Gillard, J. W. (1990) J. Biol. Chem. 265, 1436–1442
11. Woods, J., Evans, J., Ether, D., Scott, S., Vickers, P., Hearn, L., Charleson, S., Hrebink, J., and Singer, I. (1993) J. Exp. Med. 178, 1935–1946
12. Brock, T. G., Paine, R. J., and Petersen-Golden, M. (1994) J. Biol. Chem. 269, 20209–20206
13. Petersen-Golden, M., and Mcnichol, R. H. (1993) Biochem. Biophys. Res. Commun. 196, 147–153
14. Woods, J., Coffey, M., Brock, T., Singer, I., and Petersen-Golden, M. (1995) J. Clin. Invest. 95, 2035–2046
15. Wang, A., Hwang, S., M., Cook, M. N., Hobgloom, G. K., and Cooke, S. T. (1988) Biochemistry 27, 6763–6769
 Functional Significance of Nuclear 5-Lipoxygenase

21658

16. Wong, A., Cook, M., Foley, J., Sarau, H., Marshall, P., and Hwang, S. (1991) Biochemistry 30, 9346–9354
17. Coffey, M., Peters-Golden, M., Fantone, J., III, and Sporn, P. (1992) J. Biol. Chem. 267, 570–576
18. Peters-Golden, M., McNish, R. W., Hyzy, R., Shelly, C., and Toews, G. B. (1990) J. Immunol. 144, 263–270
19. Mancini, J., Prasit, P., Coppolino, M., Charleson, P., Leger, S., Evans, J., Gillard, J., and Vickers, P. (1992) Mol. Pharmacol. 41, 267–272
20. Perdval, M., Denis, D., Riendeau, D., and Gressler, M. (1992) Eur. J. Biochem. 210, 109–117
21. Rouzer, C. A., and Samuelsson, B. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 7393–7397
22. Malaviya, R., Malaviya, R., and Jakschik, B. (1993) J. Biol. Chem. 268, 4039–4044
23. Lepley, R. A., and Fitzpatrick, F. (1994) J. Biol. Chem. 269, 24163–24168
24. Coffey, M., Wilcoxen, S., and Peters-Golden, M. (1994) Am. J. Respir. Cell Mol. Biol. 11, 153–158
25. Steinhilber, D., Hoshiko, S., Grunewald, J., Radmark, O., and Samuelsson, B. (1993) Biochim. Biophys. Acta 1178, 1–8
26. Peters-Golden, M., and Feyssa, A. (1993) Am. J. Physiol. 264, L438–L447
27. Capriotti, A., Furth, E., Arrasmith, M., and Laposata, M. (1988) J. Biol. Chem. 263, 10029–10034
28. Mancini, J., Abramovitz, M., Cox, M., Wong, E., Charleson, S., Perrier, H., Wang, Z., Prasit, P., and Vickers, P. (1993) FEBS Lett. 318, 277–281
29. Jurivich, D., Sistonen, L., Sarge, K., and Morimoto, R. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 2280–2284
30. Soliven, G., Takeda, M., Shandy, T., and Nelson, D. (1993) Am. J. Physiol. 264, C632–C640
31. Rotman, E., Brostrom, M., and Brostrom, C. (1992) Biochem. J. 282, 487–494
32. Hatzelmann, A., Fruchtmann, R., Mohrs, K.-H., Raddatz, S., and Muller-Peddinghaus, R. (1994) Biochem. Pharmacol. 48, 31–39
33. Guidot, D. M., Repine, M., Westcott, J., and Repine, J. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 8156–8159