Journal of Cellular Biochemistry 96:1203–1211 (2005)

Regulating Leukotriene Synthesis: The Role of Nuclear 5-Lipoxygenase

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Abstract Leukotrienes are lipid messengers involved in autocrine and paracrine cellular signaling. They are synthesized from arachidonic acid by the 5-lipoxygenase pathway. Current models of this enzymatic pathway recognize that a key step in initiating leukotriene synthesis is the calcium-mediated movement of enzymes, including 5-lipoxygenase, to intracellular membranes. However, 5-lipoxygenase can be imported into or exported from the nucleus before calcium activation. As a result, its subcellular localization will affect its ability to be activated by calcium, as well as the membrane to which it binds and its interaction with other enzymes. This commentary focuses on the role of 5-lipoxygenase compartmentation in determining its regulation and, ultimately, leukotriene synthesis. J. Cell. Biochem. 96: 1203–1211, 2005. © 2005 Wiley-Liss, Inc.

Key words: 5-lipoxygenase; enzyme regulation; nucleus; nuclear import; leukotriene; inflammation; arachidonic acid

The enzyme 5-lipoxygenase (5-LO) initiates the synthesis of leukotrienes (LTs) from arachidonic acid (AA). The expression of 5-LO is largely restricted to certain leukocytes, including neutrophils, eosinophils, mast cells, basophils, monocytes, macrophages, and B-lymphocytes. These cells will, upon stimulation, synthesize and secrete LTs, which move short distances to target cells, bind specific receptors, and trigger cell-specific responses. The LTs include LTB_4, which promotes inflammation by attracting and activating leukocytes, and the cysteinyl LTs, LTC_4, and LTD_4, which cause edema and smooth muscle contraction. It is currently accepted that the action of 5-LO is central to immune response, as it is responsible for the generation of LTs, which are secreted and work in an autocrine or paracrine fashion to drive cellular and tissue components of innate immunity. Thus, mice that lack 5-LO cannot make LTs and have an impaired ability to clear bacterial infection [Peters-Golden et al., 2002].

While the underproduction of LTs, as occurs with malnutrition [Skerrett et al., 1990; Cederholm et al., 2000] and HIV infection [Thorsen et al., 1989; Coffey et al., 1996, 1999], results in reduced immune defense, the overproduction of LTs contributes to a wide range of diseases. For example, elevated LT levels clearly contribute to some forms of asthma [Drazen et al., 1992; Daffern et al., 1999] and pharmaceutical intervention in LT production or signaling reduce symptoms during asthma exacerbations [Dockhorn et al., 2000; Garcia Garcia et al., 2005]. The overproduction of LTs has also been associated with allergic diseases [Talbot et al., 1985; Taylor et al., 1989; Rachelefsky, 1997], pulmonary fibrosis [Wilborn et al., 1996], atherosclerosis [Spanbroek et al., 2003; Dwyer et al., 2004], hyperlipidemia-dependent inflammation of the arterial wall [Zhao et al., 2004], pulmonary hypertension [Voelkel et al., 1996], arthritis [Giffiths et al.,

Abbreviations used: AA, arachidonic acid; AKAP, A kinase anchoring protein; $cPLA_2$, cytosolic phospholipase A_2; ERK, extracellular signal-regulated kinase; FLAP, 5-lipoxygenase activating protein; GPx, glutathione peroxidase; HPETE, hydroperoxyeicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid; LO, lipoxygenase; LT, leukotriene; MK2, mitogen-activated protein kinase activated protein kinase 2; MAPK, mitogen-activated protein kinase; NLS, nuclear localization sequence; PKA, protein kinase A; PLA_2, phospholipase A2; PLC, phospholipase C.

Grant sponsor: National Institutes of Health; Grant number: RO1 AI43574.

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Received 26 August 2005; Accepted 29 August 2005

DOI 10.1002/jcb.20662

1995], and ischemia reperfusion injury [Noiri et al., 2000]. In addition, increased 5-LO expression and presumably increased LT synthesis, has been associated with lung cancer [Avis et al., 2005], pancreatic cancer [Hennig et al., 2002], bladder cancer [Yoshimura et al., 2003], breast cancer [Jiang et al., 2003], colon cancer [Nielsen et al., 2003], glioblastoma multiforme [Golubic et al., 2003], prostate cancer [Matsuyama et al., 2004], testicular cancer [Yoshimura et al., 2004], and esophageal cancer [Hoque et al., 2005]. As LTs have important roles both in normal immune defense and in disease, a better understanding of the LT biosynthetic pathway will provide insight into both health and pathogenesis.

LT SYNTHESIS: A CONCEPTUAL MODEL

As noted above, LTs are products of the enzymatic modification of AA, which is a 20 carbon omega-6 polyunsaturated fatty acid that is typically found in the sn-2 position of membrane phospholipids. Phospholipases of the class A_2 (PLA_2) mediate the liberation of AA from membrane phospholipids. An 85 kD group IV PLA_2, also known as cytosolic PLA_2 ($cPLA_2$), appears to be essential for the release of AA for LT production [Bonventre et al., 1997]. $cPLA_2$ moves from the cytoplasm to cellular membranes in a calcium-dependent fashion [Schievella et al., 1995], leading to the release of AA (Fig. 1). Binding of $cPLA_2$ to membranes is prolonged by phosphorylation [Das et al., 2003].

The first step toward LT generation is mediated by the enzyme 5-LO, which, in concert with the 5-LO activating protein (FLAP), catalyzes the insertion of molecular oxygen into AA to form 5-hydroperoxyeicosatetraenoic acid (5-HPETE) as well as its subsequent dehydration to LTA_4. The intermediate 5-HPETE is often made in significant amounts; it is rapidly modified to 5-hydroxyeicosatetraenoic acid (5-HETE). Like $cPLA_2$, 5-LO moves to cellular membranes in a calcium-dependent fashion following cell activation [Rouzer and Samuelsson, 1987]. ATP, in the presence of calcium, enhances the catalytic activity of 5-LO in cell-free assays [Ochi et al., 1983]. The membrane association or the catalytic activity of 5-LO may be augmented by phosphorylation of 5-LO on Ser271 by MAP kinase activated protein kinase 2 (MK2) [Werz et al., 2000] or MK2 and AA [Werz et al., 2002b]. Importantly, phosphorylation of 5-LO by MK2 in vitro did not significantly affect its activity [Werz et al., 2000, 2002b]. Phosphorylation on Ser663 by extracellular signal-regulated kinase (ERK) enhances 5-LO activity [Werz et al., 2002a]. The phosphorylation of 5-LO on Ser523 by protein kinase A (PKA) inhibits LT synthesis [Luo et al., 2004a]. In addition, 5-LO activity can be suppressed by nitric oxide [Brunn et al., 1997; Brock et al., 2003] and glutathione peroxidases (GPx) [Werz and Steinhilber, 1996; Straif et al., 2000].

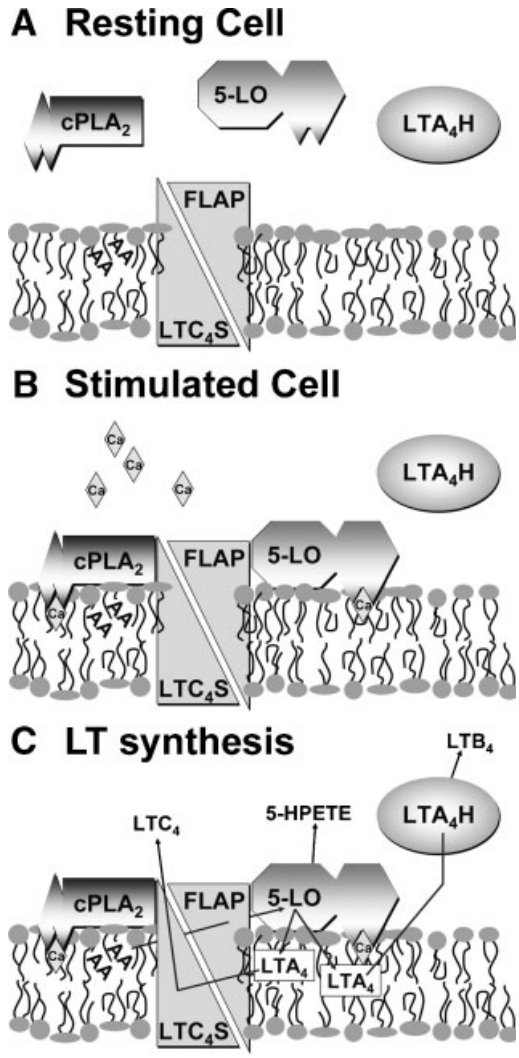

Fig. 1. A simple model for LT synthesis. (**A**) Resting cell. (**B**) In stimulated cells, elevation of cytoplasmic calcium causes both $cPLA_2$ and 5-LO to move to cellular membranes, where $cPLA_2$ releases AA from membrane phospholipids. (**C**) Free AA is delivered to 5-LO by FLAP, leading to the synthesis of LTA_4, which is further processed by LTA_4 hydrolase or LTC_4 synthase to produce LTB_4 or LTC_4, respectively. The activity of 5-LO may be enhanced by ATP, phosphorylation by MK2, or AA, or inhibited by the action of GPx or by phosphorylation by PKA.

The end product of 5-LO action, LTA_4, can be hydrolyzed by the enzyme LTA_4 hydrolase to give LTB_4. Alternatively, LTA_4 can be conjugated with glutathione by the enzyme LTC_4 synthase to produce LTC_4. LTC_4 may be further metabolized to LTD_4 and LTE_4. In resting leukocytes, 5-LO is predominantly soluble. FLAP and LTC_4 synthase are structurally related integral membrane proteins that may exist as monomers or form homo- or heterodimers or trimers [Mandal et al., 2004]. LTA_4 hydrolase is a soluble protein that appears to remain soluble whether the cell is activated or resting. The ratio of LTB_4:LTC_4 produced by a given cell may depend on the ratio of LTA_4 hydrolase:LTC_4 synthase; alternatively, it has been proposed that the ratio of LTB_4:LTC_4 may be determined by the ratio of FLAP/FLAP homomers to FLAP/LTC_4 synthase heteromers [Mandal et al., 2004].

Taken together, these findings have led to a simple conceptual model for LT synthesis (Fig. 1). The stimulation of leukocytes in a way that produces a rise in intracellular calcium concentration triggers the movement of both $cPLA_2$ and 5-LO from a soluble phase to intracellular membranes. $cPLA_2$ releases AA from membrane phospholipids and this free AA is transferred by FLAP to 5-LO to produce LTA_4, which in turn is metabolized to LTB_4 by LTA_4 hydrolase or LTC_4 by LTC_4 synthase. As $cPLA_2$, FLAP, 5-LO, and LTC_4 synthase all act at the membrane surface, it seems likely that some or all of these enzymes assemble to form a single catalytic structure, or metabolon [Peters-Golden and Brock, 2001].

5-LO CAN BE IMPORTED INTO THE NUCLEUS

In 1993, Peters-Golden and McNish used cell fractionation followed by immunoblotting to demonstrate that both $cPLA_2$ and 5-LO associate with nuclear membranes [Peters-Golden and McNish, 1993]. In the same year, Woods and colleagues provided immunoelectron microscopic evidence that 5-LO and FLAP protein were associated with the inner membrane of the nuclear envelope of activated leukocytes [Woods et al., 1993]. In addition to pointing to the nuclear envelope as the site of AA release and metabolism to LTs, these pivotal studies indicated that a portion of the synthetic process occurs at the inner membrane inside the nucleus.

Subsequent studies by our group and others demonstrated that soluble 5-LO could be found predominantly in the cytoplasm of some cell types, or strongly accumulated in the nucleus of other types of cells [Brock et al., 1994, 1995, 1999; Chen et al., 1995; Woods et al., 1995; Healy et al., 1999]. For example, 5-LO is cytoplasmic in peripheral blood neutrophils or eosinophils, but predominantly intranuclear in alveolar macrophages or cultured mast cells. In all cell types, stimulation with calcium ionophore to increase intracellular calcium and activate 5-LO resulted in movement of 5-LO to the nuclear envelope and perinuclear membranes as well as LT synthesis [Brock et al., 1995, 1998, 1999]. These results indicated that 5-LO in either the cytoplasmic or nuclear compartment of the cell could be activated to produce LTs.

More interestingly, the subcellular localization of soluble 5-LO in resting leukocytes was found to be regulated. Thus, 5-LO was found in the cytoplasm of blood neutrophils, either immediately after purification from peripheral blood [Brock et al., 1997] or when evaluated in fixed tissue by immunohistochemistry [Brock et al., 2001]. However, 5-LO rapidly moved into the nucleus of neutrophils following adherence in vitro or after migration into inflammatory sites [Brock et al., 1997, 2001]. Similarly, 5-LO is cytoplasmic in freshly purified eosinophils and migrates into the nucleus following adherence or recruitment [Brock et al., 1999]. Also, the subcellular distribution of 5-LO can be regulated during differentiation: when human cord blood mononuclear cells are differentiated to become mast cells over 5 days in the presence of either interleukin-3 or interleukin-5, 5-LO is found in the nucleus [Hsieh et al., 2001]. While these studies demonstrated that the nuclear import of 5-LO could be activated, evidence that nuclear export can be regulated is limited. Culturing alveolar macrophages in vitro over 3 days produced a shift in 5-LO from the nucleus to the cytoplasm [Woods et al., 1995], but this could have been due to turnover of nuclear 5-LO protein with failure to import newly synthesized 5-LO. It remains to be determined if 5-LO has a nuclear export sequence or can be exported through some other mechanism, and whether this is a regulated process.

Examination of the primary sequence of 5-LO revealed excellent candidates for nuclear localization sequences (NLS), including a bipartite

NLS near the carboxyl terminus, as well as two potential monopartite NLSs. Initial studies found that these sites were not functional NLSs [Chen et al., 1998], although mutation of Arg651, within the potential bipartite pattern, stopped nuclear import [Healy et al., 1999]. Further evaluation of this site revealed that this arginine is conserved across all lipoxygenase proteins and serves a critical role in maintaining structural integrity [Jones et al., 2002], suggesting that mutation of this residue prevented nuclear import by causing improper protein folding. A more complete analysis indicated that the 5-LO protein has three distinct NLSs [Jones et al., 2002, 2003]. Each sequence is independently capable of directing the nuclear accumulation of a different protein. More interestingly, mutation of any one of the three NLSs completely eliminated nuclear import in a portion of cells expressing the mutant, with reduced but still active import occurring in other cells within the same population of cells. These results were interpreted to mean that each NLS could be regulated: when one NLS was inactivated by mutation, the lack of import in some cells indicated that the other NLSs were "off" in those cells, whereas some degree of import occurred when at least one of the alternative NLSs was "on." The presence of three NLSs that could each be independently activated allowed different levels of import of 5-LO, with the greatest nuclear accumulation when all NLSs were activated [Luo et al., 2004b]. As a result, the ratio of cytoplasmic to nuclear 5-LO, and possible LT synthesis, could be exquisitely controlled, presumably by extracellular signals.

The other soluble proteins involved in LT synthesis, $cPLA_2$ and LTA_4 hydrolase, can also move from the cytoplasm into the nucleus. $cPLA_2$ has been described to accumulate in the nucleus of sub-confluent human and bovine endothelial cells, whereas it was cytoplasmic in confluent cells [Sierra-Honigmann et al., 1996]. The subcellular localization of $cPLA_2$ was not thought to be associated with the cell cycle, as growth arrest left the protein within the nucleus. In this study, $cPLA_2$ was also found to be intranuclear in sub-confluent MDCK and HeLa cells, although others have shown $cPLA_2$ to be cytoplasmic in MDCK cells [Evans et al., 2001]. LTA_4 hydrolase has been found in the nucleus of alveolar macrophage and the mast cell-like rat basophilic leukemia cell and in the cytoplasm of neutrophils [Brock et al., 2001]. More recently, LTA_4 hydrolase was reported to be accumulated in the nucleus of lung epithelial cells under certain conditions but in the cytoplasm during other conditions, with nuclear accumulation appearing to correlate with higher growth rates [Brock et al., 2005]. The molecular factors that control the subcellular distributions of $cPLA_2$ and LTA_4 hydrolase remain to be determined.

EFFECT OF NUCLEAR LOCALIZATION OF 5-LO ON LT SYNTHESIS

Alveolar macrophages accumulate 5-LO within the nucleus, whereas peripheral blood monocytes have cytoplasmic 5-LO. Alveolar macrophages can produce much more LTB_4 than monocytes, correlating nuclear localization of 5-LO with increased LTB_4 synthesis. Similarly, adherent neutrophils, which have nuclear 5-LO, can synthesize much more LTB_4 than non-adherent neutrophils, which have cytoplasmic 5-LO [Brock et al., 1997]. Also, human mast cells primed for 5 days with IL-3 or IL-5 accumulate 5-LO within the nucleus and make more cysteinyl LTs when given IL-4 and then activated with IgE cross-linking [Hsieh et al., 2001]. In each of these examples, nuclear localization of 5-LO before cell stimulation correlates with increased LT generation with subsequent cell activation. However, the differences in LT synthesis in each case could be due to other factors than 5-LO localization. To more clearly assess the effect of the nuclear localization of 5-LO on LT synthesis, we created a collection of 5-LO mutants that had different subcellular distributions but comparable intrinsic (cell-free) catalytic activities. NIH 3T3 cells over-expressing these different mutants were stimulated with calcium ionophore plus arachidonic acid and the amount of synthesized LTB_4 was determined and adjusted for 5-LO protein expression. In these cells, which differed only in the subcellular distribution of 5-LO before cell stimulation, the amount of LTB_4 produced in response to stimulation with calcium ionophore was proportional to the amount of 5-LO accumulated in the nucleus [Luo et al., 2003]. These results indicate that the subcellular localization of 5-LO can be an important determinant of the amount of LT produced upon cell stimulation, with nuclear localization of 5-LO correlating with increased LT synthesis. An interesting

exception has been reported for eosinophils, which rapidly import 5-LO into the nucleus upon adherence to fibronectin, coinciding with a strong decrease in stimulated LTC_4 synthesis [Brock et al., 1999]. Whether this is a cell-specific or matrix-specific effect, or is due to other factors, is unknown.

THE REGULATION OF CYTOPLASMIC VERSUS NUCLEAR 5-LO

The majority of studies into the regulation of cellular 5-LO, as opposed to purified recombinant 5-LO or 5-LO in cell lysates, have been interpreted using some version of the simplified conceptual model presented in Figure 1. Recognizing that the subcellular distribution of 5-LO is variable, it becomes important to consider how signaling pathways might have distinct effects on the activity of nuclear versus cytoplasmic 5-LO. Furthermore, these pathways should be considered for their capacity to drive nuclear import or export, as well as (or instead of) enzymatic activity.

Calcium and ATP Effects

Early studies on the source of calcium for 5-LO activation stressed the importance of extracellular calcium, rather than calcium released from internal stores [Schatz-Munding et al., 1991; Wong et al., 1991]. In these and similar studies, extracellular calcium was thought to move into the cytoplasm, where it activated 5-LO and $cPLA_2$, consequently leading to LT synthesis. Freshly isolated peripheral blood neutrophils or eosinophils would certainly fit this model and, indeed, these were the cells being examined. However, it becomes relevant to now ask if this applies to cells with nuclear 5-LO. That is, will imported calcium move through nuclear pores and stimulate nuclear 5-LO directly, and, if so, will a greater influx of calcium be required to activate nuclear 5-LO than cytoplasmic 5-LO? Consistent with this, cells with nuclear 5-LO (alveolar macrophages, elicited neutrophils) have higher activation thresholds for LT synthesis than do cells with cytoplasmic 5-LO (peritoneal macrophages, peripheral blood neutrophils) [Peters-Golden et al., 1990; Brock et al., 1997], when treated with calcium ionophore. Once stimulated, however, cells with nuclear 5-LO made more LTs than cells with cytoplasmic 5-LO.

Activation of phospholipase C (PLC) leads to the release of inositol 1,4,5-trisphosphate, which triggers the release of calcium from intracellular stores. In addition, there are both cytoplasmic and nuclear isoforms of PLC [Manzoli et al., 2005]; an intranuclear isoform, PLC-β1, is activated by ERK [Xu et al., 2001]. Inositol 1,4,5-trisphospate, generated by the action of cytoplasmic or intranuclear PLC, may increase intranuclear calcium levels. This may lead to activation of intranuclear 5-LO via a PLC-dependent mechanism. Consistent with this model, the addition of extracellular PLC can trigger LT synthesis [Meyers and Berk, 1990] and the inhibition of phosphoinositide generation also inhibits LT synthesis in RBL-2H3 cells [Lin et al., 1991], cells which have predominantly intranuclear 5-LO. Interestingly, activation of cells with LTs can stimulate PLC activity, so it's possible that inositide metabolism may serve as a positive feedback mechanism for LT generation in cells with predominantly nuclear 5-LO. Additional work is needed to understand the regulation of calcium levels within the nucleus and how this determines 5-LO function in various types of leukocytes.

The subcellular positioning of 5-LO may also affect the ability of ATP to modulate the catalytic activity of 5-LO. The availability of ATP may be greater in the cytoplasm close to mitochondria, suggesting that this co-factor might be most important in cells with cytoplasmic 5-LO. However, ATP certainly modulates the activity of enzymes within the nucleus (e.g., [Calapez et al., 2002; Wagner et al., 2004]). This suggests that ATP should normally be available to stimulate 5-LO activity within the nucleus. It remains possible that under certain conditions, nuclear ATP can become depleted in a way that impacts on the action of nuclear 5-LO and, consequently, LT synthesis.

MK2 and ERK Effects

MK2, which phosphorylates and activates 5-LO [Werz et al., 2000, 2002b], is activated by p38 MAP kinase. Before activation, MK2 resides within the nucleus. Activation of p38 MAP kinase leads to phosphorylation, activation, and rapid export of MK2 from the nucleus [Engel et al., 1998; Neininger et al., 2001]. Targets of MK2 can act be found in the cytoplasm (e.g., HSP25/27, hnRNP A0, tristetraprolin) or nucleus (e.g., CREB, serum response factor,

ER81) [Roux and Blenis, 2004]. Whether MK2 can phosphorylate nuclear 5-LO with the same efficiency as cytoplasmic 5-LO is unknown. Also, the effect of MK2 phosphorylation of Ser271 of 5-LO on the subcellular localization of 5-LO is not known. Like MK2, ERKs phosphorylate targets in both the cytoplasm and the nucleus, as well as on membranes and cytoskeletal elements [Roux and Blenis, 2004]. Also as for MK2, the subcellular pool of 5-LO best targeted by ERKs and the effect of phosphorylation on Ser663 on 5-LO localization has not been solved.

PKA and GPXs

The catalytic subunit of PKA, while most commonly recognized as phosphorylating targets in the cytoplasm, can also move into the nucleus and act there. Whether PKA more efficiently or more quickly phosphorylates and inactivates 5-LO when it is localized within the cytoplasm, or inactivates nuclear 5-LO as well as cytoplasmic 5-LO, remains to be determined. In addition, PKA can be sequestered in different subcellular sites by A kinase anchoring proteins (AKAPs), with substrates as well as other kinases or phosphatases assembling as a unit. It is not known if phosphorylation of 5-LO by PKA requires interaction at an AKAP.

The GPxs, which suppress 5-LO activity, appear to be predominantly cytoplasmic. As a result, their activity may at least partially explain why more LTs are made by cells with nuclear, rather than cytoplasmic, 5-LO. Factors that regulate the function of the GPxs, then, may be most effective in modulating LT synthesis in cells with predominantly cytoplasmic 5-LO.

AA Availability to Nuclear 5-LO

Perhaps one of the most perplexing issues regarding the effect of subcellular localization on 5-LO action relates to its ability to access its substrate, AA. The current model for AA release by $cPLA_2$ positions this event near the cytoplasm, at the Golgi, endoplasmic reticulum, and outer membrane of the nuclear envelope [Evans et al., 2001]. Calcium-triggered membrane association of 5-LO from the cytoplasm should place 5-LO and free AA together at the same site; 5-LO from the nucleoplasm, on the other hand, should move to the inner membrane of the nuclear envelope and be separated from the site of AA release by at least the lumen of the nuclear envelope. This would suggest that more AA might be metabolized by cytoplasmic 5-LO than nuclear 5-LO. However, the opposite appears to be the most common case. One possible explanation is that liberated AA may be rapidly carried into the nucleus, through the nuclear pore, by fatty acid-binding proteins [Huang et al., 2002]. Also, exogenous AA is known to rapidly accumulate at the nucleus [Neufeld et al., 1985; Capriotti et al., 1988]. It seems possible that in leukocytes the trafficking of free AA, from either endogenous or exogenous sources, is at least in part regulated by fatty acid-binding proteins, with short term targeting into the nucleus. This, then, may represent an important point of regulation of LT synthesis, by controlling the interaction of 5-LO and AA.

SUMMARY

It is becoming clear that there are numerous complex mechanisms for regulating 5-LO activity. It is well established that calcium causes membrane association and activates 5-LO, and that ATP augments the calcium effect on activity. Recent data have demonstrated that 5-LO can be phosphorylated by MK-2 in vitro and by PKA in vitro and in cells. Phosphorylation by MK-2 in vitro has no effect on 5-LO activity, whereas phosphorylation by PKA significantly reduces 5-LO activity. Also, the subcellular positioning of 5-LO appears to be a dynamic process; a variety of factors can alter the ratio of cytoplasmic to nuclear 5-LO. Finally, the subcellular localization of 5-LO before cell stimulation can significantly affect LT production. In most cases, localization of 5-LO within the nucleus, as opposed to the cytoplasm, will predict a significant increase in LT synthesis upon cell stimulation.

A more complete model regarding the regulation of 5-LO is given in Figure 2. When 5-LO is in the cytoplasm, it may be acted upon by MK2, PKA, GPx, or ERK; the same kinases may phosphorylate 5-LO within the nucleus, but GPx does not appear to enter the nucleus. Following cell stimulation, 5-LO in the cytoplasm will move to the endoplasmic reticulum and extranuclear membranes, potentially forming a multi-protein complex to generate LTs. Positioning of 5-LO within the nucleus, on the other hand, leads to membrane association of 5-LO with the inner membrane of the nuclear

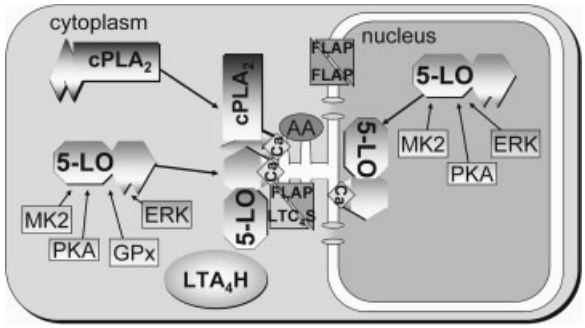

Fig. 2. The regulation of 5-LO. When in the cytoplasm, 5-LO can be affected by MK2, PKA, GPx, or ERK. MK2, PKA, and ERK may also phosphorylate 5-LO within the nucleus. Upon cell stimulation and a rise in intracellular calcium, cytosolic 5-LO moves to perinuclear membranes, which also include activated $cPLA_2$, FLAP–FLAP homodimers, and FLAP-LTC_4 synthase heterodimers. Nuclear 5-LO, when activated with calcium, moves to the inner nuclear membrane, where it can interact with FLAP–FLAP homodimers. LTA_4 hydrolase (LTA_4H) is soluble and does not associate with membranes.

envelope. This may maximize its interaction with FLAP–FLAP homodimers and minimize its ability to interact with other proteins, like $cPLA_2$ and LTC_4 synthase.

In summary, studies on the regulation of 5-LO have revealed that it is intriguingly complex. The abundance of ways to modulate 5-LO localization and activity suggest that this is both an interesting and important step in the regulation of LT synthesis. A more complete understanding of the regulation of 5-LO should provide important clues into mechanisms of dysregulation that contribute to several diseases, including asthma, atherosclerosis, and fibrosis.

REFERENCES

Avis I, Martinez A, Tauler J, Zudaire E, Mayburd A, Abu-Ghazaleh R, Ondrey F, Mulshine JL. 2005. Inhibitors of the arachidonic acid pathway and peroxisome proliferator-activated receptor ligands have superadditive effects on lung cancer growth inhibition. Cancer Res 65:4181–4190.

Bonventre JV, Huang Z, Taheri MR, O'Leary E, Li E, Moskowitz MA, Sapirstein A. 1997. Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A_2. Nature 390:622–625.

Brock TG, Paine RI, Peters-Golden M. 1994. Localization of 5-lipoxygenase to the nucleus of unstimulated rat basophilic leukemia cells. J Biol Chem 269:22059–22066.

Brock TG, McNish RW, Peters-Golden M. 1995. Translocation and leukotriene synthetic capacity of nuclear 5-lipoxygenase in rat basophilic leukemia cells and alveolar macrophages. J Biol Chem 270:21652–21658.

Brock TG, McNish RW, Bailie MB, Peters-Golden M. 1997. Rapid import of cytosolic 5-lipoxygenase into the nucleus of neutrophils after in vivo recruitment and in vitro adherence. J Biol Chem 272:8276–8280.

Brock TG, McNish RW, Peters-Golden M. 1998. Capacity for repeatable leukotriene generation following transient stimulation of mast cells and macrophages. Biochem J 329:519–525.

Brock TG, Anderson JA, Fries FP, Peters-Golden M, Sporn PHS. 1999. Decreased leukotriene C_4 synthesis accompanies nuclear import of 5-lipoxygenase in human blood eosinophils. J Immunol 162:1669–1676.

Brock TG, Maydanski E, McNish RW, Peters-Golden M. 2001. Co-localization of leukotriene A_4 hydrolase with 5-lipoxygenase in nuclei of alveolar macrophages and rat basophilic leukemia cells but not neutrophils. J Biol Chem 276:35071–35077.

Brock TG, McNish RW, Mancuso P, Coffey MJ, Peters-Golden M. 2003. Prolonged lipopolysaccharide inhibits leukotriene synthesis in peritoneal macrophages: Mediation by nitric oxide and prostaglandins. Prostaglandins Other Lipid Mediat 71:131–145.

Brock TG, Lee YJ, Maydanski E, Marburger TL, Luo M, Paine RI, Peters-Golden M. 2005. Nuclear localization of leukotriene A_4 hydrolase in type II alveolar epithelial cells in normal and fibrotic lung. Am J Physiol Lung Cell Mol Physiol 289:L224–L232.

Brunn G, Hey C, Wessler I, Racke K. 1997. Endogenous nitric oxide inhibits leukotriene B_4 release from rat alveolar macrophages. Eur J Pharmacol 326:53–60.

Calapez A, Pereira HM, Calado A, Braga J, Rino J, Carvalho C, Tavanez JP, Wahle E, Rosa AC, Carmo-Fonseca M. 2002. The intranuclear mobility of messenger RNA binding proteins is ATP dependent and temperature sensitive. J Cell Biol 159:795–805.

Capriotti A, Furth E, Arrasmith M, Laposata M. 1988. Arachidonate released upon agonist stimulation preferentially originates from arachidonate most recently incorporated into nuclear membrane phospholipids. J Biol Chem 263:10029–10034.

Cederholm T, Lindgren JA, Palmblad J. 2000. Impaired leukotriene C_4 generation in granulocytes from protein-energy malnourished chronically ill elderly. J Intern Med 247:715–722.

Chen X-S, Naumann TA, Kurre U, Jenkins NA, Copeland NG, Funk CD. 1995. cDNA cloning, expression, mutagenesis, intracellular localization, and gene chromosomal assignment of mouse 5-lipoxygenase. J Biol Chem 270: 17993–17999.

Chen X-S, Zhang Y-Y, Funk C. 1998. Determinants of 5-lipoxygenase nuclear localization using green fluorescent protein-5-lipoxygenase fusion proteins. J Biol Chem 273: 31237–31244.

Coffey M, Phare SM, Kazanjian PH, Peters-Golden M. 1996. 5-Lipoxygenase metabolism in alveolar macrophages from subjects infected with the human immunodeficiency virus. J Immunol 157:393–399.

Coffey MJ, Phare SM, Cinti S, Peters-Golden M, Kazanjian PH. 1999. Granulocyte-macrophage colony-stimulating factor upregulates reduced 5-lipoxygenase metabolism in peripheral blood monocytes and neutrophils in acquired immunodeficiency syndrome. Blood 94:3897–3905.

Daffern PJ, Muilenburg D, Hugli TE, Stevenson DD. 1999. Association of urinary leukotriene E_4 excretion during

aspirin challenges with severity of respiratory responses. J Allergy Clin Immunol 104:559–564.

Das S, Rafter JD, Kim KP, Gygi SP, Cho W. 2003. Mechanism of group IVA cytosolic phospholipase A_2 activation by phosphorylation. J Biol Chem 278:41431–41442.

Dockhorn RJ, Baumgartner RA, Leff JA, Noonan M, Vandormael K, Stricker W, Weinland DE, Reiss TF. 2000. Comparison of the effects of intravenous and oral montelukast on airway function: A double blind, placebo controlled, three period, crossover study in asthmatic patients. Thorax 55:260–265.

Drazen J, O'Brien J, Sparrow D, Weiss S, Martins M, Israel E. 1992. Recovery of leukotriene E_4 from the urine of patients with airway obstruction. Am Rev Respir Dis 146: 104–108.

Dwyer JH, Allayee H, Dwyer KM, Fan J, Wu H, Mar R, Lusis AJ, Mehrabian M. 2004. Arachidonate 5-lipoxygenase promoter genotype, dietary arachidonic acid, and atherosclerosis. N Engl J Med 350:29–37.

Engel K, Kotlyarov A, Gaestel M. 1998. Leptomycin B-sensitive nuclear export of MAPKAP kinase 2 is regulated by phosphorylation. EMBO J 17:3363–3371.

Evans JH, Spencer DM, Zweifach A, Leslie CC. 2001. Intracellular calcium signals regulating cytosolic phospholipase A_2 translocation to internal membranes. J Biol Chem 276:30150–30160.

Garcia Garcia M, Wahn U, Gilles L, Swern A, Tozzi C, Polos P. 2005. Montelukast, compared with fluticasone, for control of asthma among 6- to 14-year-old patients with mild asthma: The MOSAIC study. Pediatrics 116:360–369.

Giffiths RJ, Pettipher ER, Koch K, Farrell CA, Breslow R, Conklyn MJ, Smith MA, Hackman BC, Wimberely DJ, Milici AJ, Scampoli DN, Cheng JB, Pillar J, Paazoles C, Doherty N, Melvin L, Reiter L, Biggars M, Falkner F, Mitchell D, Liston T, Showell H. 1995. Leukotriene B_4 plays a critical role in the progression of collagen-induced arthritis. Proc Natl Acad Sci USA 92:517–521.

Golubic M, Prayson RA, Vargo L, Bondar J, Barnett GH. 2003. Increased expression of 5-lipoxygenase in glioblastoma multiforme. Adv Exp Med Biol 525:205–208.

Healy AM, Peters-Golden M, Yao JP, Brock TG. 1999. Identification of a bipartite nuclear localization sequence necessary for nuclear import of 5-lipoxygenase. J Biol Chem 274:29812–29818.

Hennig R, Ding XZ, Tong WG, Schneider MB, Standop J, Friess H, Buchler MW, Pour PM, Adrian TE. 2002. 5-Lipoxygenase and leukotriene B_4 receptor are expressed in human pancreatic cancers but not in pancreatic ducts in normal tissue. Am J Pathol 161:421–428.

Hoque A, Lippman SM, Wu TT, Xu Y, Liang ZD, Swisher S, Zhang H, Cao L, Ajani JA, Xu XC. 2005. Increased 5-lipoxygenase expression and induction of apoptosis by its inhibitors in esophageal cancer: A potential target for prevention. Carcinogenesis 26:785–791.

Hsieh FH, Lam BK, Penrose JF, Austen KF, Boyce JA. 2001. T helper cell type 2 cytokines coordinately regulate immunoglobulin E-dependent cysteinyl leukotriene production by human cord blood-derived mast cells: Profound induction of leukotriene C4) synthase expression by interleukin 4. J Exp Med 193:123–133.

Huang H, Starodub O, McIntosh A, Kier AB, Schroeder F. 2002. Liver fatty acid-binding protein targets fatty acids to the nucleus. Real time confocal and multiphoton fluorescence imaging in living cells. J Biol Chem 277: 29139–23151.

Jiang WG, Douglas-Jones A, Mansel RE. 2003. Levels of expression of lipoxygenases and cyclooxygenase-2 in human breast cancer. Prostaglandins Leukot Essent Fatty Acids 69:275–281.

Jones SM, Luo M, Healy AM, Peters-Golden M, Brock TG. 2002. Structural and functional criteria reveal a new nuclear import sequence on the 5-lipoxygenase protein. J Biol Chem 277:38550–38556.

Jones SM, Luo M, Peters-Golden M, Brock TG. 2003. Identification of two novel nuclear import sequences on the 5-lipoxygenase protein. J Biol Chem 278:10257–10263.

Lin PY, Wiggan GA, Welton AF, Gilfillan AM. 1991. Differential effects of propranolol on the IgE-dependent, or calcium ionophore-stimulated, phosphoinositide hydrolysis and calcium mobilization in a mast (RBL 2H3) cell line. Biochem Pharmacol 41:1941–1948.

Luo M, Jones SM, Peters-Golden M, Brock TG. 2003. Nuclear localization of 5-lipoxygenase as a determinant of leukotriene B_4 synthetic capacity. Proc Natl Acad Sci USA 100:12165–12170.

Luo M, Jones SM, Coffey MJ, Peters-Golden M, Brock TG. 2004a. Protein kinase A inhibits leukotriene synthesis by phosphorylation of 5-lipoxygenase on serine 523. J Biol Chem 279:41512–41520.

Luo M, Pang CWM, Gerken AE, Brock TG. 2004b. Multiple nuclear localization sequences allow fine control of 5-lipoxygenase nuclear import Traffic 5:847–854.

Mandal AK, Skoch J, Bacskai BJ, Hyman BT, Christmas P, Miller D, Yamin TT, Xu S, Wisniewski D, Evans JF, Soberman RJ. 2004. The membrane organization of leukotriene synthesis. Proc Natl Acad Sci USA 101:6587–6592.

Manzoli L, Martelli AM, Billi AM, Faenza I, Fiume R, Cocco L. 2005. Nuclear phospholipase C: Involvement in signal transduction. Prog Lipid Res 44:185–206.

Matsuyama M, Yoshimura R, Mitsuhashi M, Hase T, Tsuchida K, Takemoto Y, Kawahito Y, Sano H, Nakatani T. 2004. Expression of lipoxygenase in human prostate cancer and growth reduction by its inhibitors. Int J Oncol 24:821–827.

Meyers DJ, Berk RS. 1990. Characterization of phospholipase C from *Pseudomonas aeruginosa* as a potent inflammatory agent. Infect Immun Infect Immun 58: 659–666.

Neininger A, Thielemann H, Gaestel M. 2001. FRET-based detection of different conformations of MK2. EMBO Rep 2:703–708.

Neufeld EJ, Majerus PW, Krueger CM, Saffitz JE. 1985. Uptake and subcellular distribution of [^{3}H] arachidonic acid in murine fibrosarcoma cells measured by electron microscope autoradiography. J Cell Biol 101:573–581.

Nielsen CK, Ohd JF, Wikstrom K, Massoumi R, Paruchuri S, Juhas M, Sjolander A. 2003. The leukotriene receptor CysLT1 and 5-lipoxygenase are upregulated in colon cancer. Adv Exp Med Biol 525:201–204.

Noiri E, Yokomizo T, Nakao A, Izumi T, Fujita T, Kimura S, Shimizu T. 2000. An in vivo approach showing the chemotactic activity of leukotriene B_4 in acute renal ischemic-reperfusion injury. Proc Natl Acad Sci USA 18: 823–828.

Ochi K, Yoshimoto T, Yamamoto S, Taniguchi K, Miyamoto T. 1983. Arachidonate 5-lipoxygenase of guinea pig

peritoneal polymorphonuclear leukocytes: Activation by adenosine 5′-triphosphate. J Biol Chem 258:5754–5758.
Peters-Golden M, Brock TG. 2001. Intracellular compartmentalization of leukotriene synthesis: Unexpected nuclear secrets. FEBS Lett 487:323–326.
Peters-Golden M, McNish R. 1993. Redistribution of 5-lipoxygenase and cytosolic phospholipase A_2 to the nuclear fraction upon macrophage activation. Biochem Biophys Res Commun 196:147–153.
Peters-Golden M, McNish RW, Hyzy R, Shelly C, Toews GB. 1990. Alterations in the pattern of arachidonate metabolism accompany rat macrophage differentiation in the lung. J Immunol 144:263–270.
Peters-Golden M, Bailie M, Marshall T, Wilke C, Phan SH, Toews GB, Moore BB. 2002. Protection from pulmonary fibrosis in leukotriene-deficient mice. Am J Respir Crit Care Med 165:229–235.
Rachelefsky G. 1997. Childhood asthma and allergic rhinitis: The role of leukotrienes. J Pediatrics 131:348–355.
Roux PP, Blenis J. 2004. ERK and p38 MAPK-activated protein kinases: A family of protein kinases with diverse biological functions. Microbiol Mol Biol Rev 68:320–344.
Rouzer CA, Samuelsson B. 1987. Reversible, calcium-dependent membrane association of human leukocyte 5-lipoxygenase. Proc Natl Acad Sci USA 84:7393–7397.
Schatz-Munding M, Hatzelmann A, Ullrich V. 1991. The involvement of extracellular calcium in the formation of 5-lipoxygenase metabolites by human polymorphonuclear leukocytes. Eur J Biochem 197:487–493.
Schievella A, Regier M, Smith W, Lin L. 1995. Calcium-mediated translocation of cytosolic phospholipase A_2 to the nuclear envelope and endoplasmic reticulum. J Biol Chem 270:30749–30754.
Sierra-Honigmann M, Bradley J, Pober J. 1996. "Cytosolic" phospholipase A_2 is in the nucleus of subconfluent endothelial cells but confined to the cytoplasm of confluent endothelial cells and redistributes to the nuclear envelope and cell junctions upon histamine stimulation. Lab Invest 74:684–695.
Skerrett SJ, Henderson WR, Martin TR. 1990. Alveolar macrophage function in rats with severe protein calorie malnutrition: Arachidonic acid metabolism, cytokine release, and antimicrobial activity. J Immunol 144:1052–1061.
Spanbroek R, Grabner R, Lotzer K, Hildner M, Urbach A, Ruhling K, Moos MP, Kaiser B, Cohnert TU, Wahlers T, Zieske A, Plenz G, Robenek H, Salbach P, Kuhn H, Radmark O, Samuelsson B, Habenicht AJ. 2003. Expanding expression of the 5-lipoxygenase pathway within the arterial wall during human atherogenesis. Proc Natl Acad Sci USA 100:1238–1243.
Straif D, Werz O, Kellner R, Bahr U, Steinhilber D. 2000. Glutathione peroxidase-1 but not-4 is involved in the regulation of cellular 5-lipoxygenase activity in monocytic cells. Biochem J 349:455–461.
Talbot SF, Atkins PC, Goetzl EJ, Zweiman B. 1985. Accumulation of leukotriene C_4 and histamine in human allergic skin reactions. J Clin Invest 76:650–656.
Taylor G, Black P, Turner N, Taylor I, Maltby N, Fuller R, Dollery C. 1989. Urinary leukotriene E_4 after antigen challenge and in acute asthma and allergic rhinitis. Lancet 1:584–588.
Thorsen S, Busch-Sorensen M, Sondergaard J. 1989. Reduced neutrophil production of leukotriene B4 associated with AIDS. AIDS 3:651–653.
Voelkel NF, Tuder RM, Wade K, Hoper M, Lepley RA, Goulet JL, Koller BH, Fitzpatrick F. 1996. Inhibition of 5-lipoxygenase-activating protein (FLAP) reduces pulmonary vascular reactivity and pulmonary hypertension in hypoxic rats. J Clin Invest 97:2491–2498.
Wagner S, Chiosea S, Ivshina M, Nickerson JA. 2004. In vitro FRAP reveals the ATP-dependent nuclear mobilization of the exon junction complex protein SRm160. J Cell Biol 164:843–850.
Werz O, Steinhilber D. 1996. Selenium-dependent peroxidases suppress 5-lipoxygenase activity in B-lymphocytes and immature myeloid cells. The presence of peroxidase-insensitive 5-lipoxygenase activity in differentiated myeloid cells. Eur J Biochem 242:90–97.
Werz O, Klemm J, Samuelsson B, Radmark O. 2000. 5-Lipoxygenase is phosphorylated by p38 kinase-dependent MAPKAP kinases. Proc Natl Acad Sci USA 97: 5261–5266.
Werz O, Burkert E, Fischer L, Szellas D, Dishart D, Samuelsson B, Radmark O, Steinhilber D. 2002a. Extracellular signal-regulated kinases phosphorylate 5-lipoxygenase and stimulate 5-lipoxygenase product formation in leukocytes. FASEB J 16:1441–1443.
Werz O, Szellas D, Steinhilber D, Radmark O. 2002b. Arachidonic acid promotes phosphorylation of 5-lipoxygenase at Ser-271 by MAPK-activated protein kinase 2 (MK2). J Biol Chem 277:14793–14800.
Wilborn J, Bailie M, Coffey M, Burdick M, Strieter R, Peters-Golden M. 1996. Constitutive activation of 5-lipoxygenase in the lungs of patients with idiopathic pulmonary fibrosis. J Clin Invest 97:1827–1836.
Wong A, Cook M, Foley J, Sarau H, Marshall P, Hwang S. 1991. Influx of extracellular calcium is required for the membrane translocation of 5-lioxygenase and leukotriene synthesis. Biochemistry 30:9346–9354.
Woods JW, Evans JF, Ethier D, Scott S, Vickers PJ, Hearn L, Charleson S, Heibein JA, Singer II. 1993. 5-Lipoxygenase and 5-lipoxygenase activating protein are localized in the nuclear envelope of activated human leukocytes. J Exp Med 178:1935–1946.
Woods JW, Coffey MJ, Brock TG, Singer II, Peters-Golden M. 1995. 5-Lipoxygenase is located in the euchromatin of the nucleus in resting human alveolar macrophages and translocates to the nuclear envelope upon cell activation. J Clin Invest 95:2035–2040.
Xu A, Suh PG, Marmy-Conus N, Pearson RB, Seok OY, Cocco L, Gilmour RS. 2001. Phosphorylation of nuclear phospholipase C beta1 by extracellular signal-regulated kinase mediates the mitogenic action of insulin-like growth factor I. Mol Cell Biol 21:2981–2990.
Yoshimura R, Matsuyama M, Tsuchida K, Kawahito Y, Sano H, Nakatani T. 2003. Expression of lipoxygenase in human bladder carcinoma and growth inhibition by its inhibitors. J Urol 170:1994–1999.
Yoshimura R, Matsuyama M, Mitsuhashi M, Takemoto Y, Tsuchida K, Kawahito Y, Sano H, Nakatani T. 2004. Relationship between lipoxygenase and human testicular cancer. Int J Mol Med 13:389–393.
Zhao L, Moos MP, Grabner R, Pedrono F, Fan J, Kaiser B, John N, Schmidt S, Spanbroek R, Lotzer K, Huang L, Cui J, Rader DJ, Evans JF, Habenicht AJ, Funk CD. 2004. The 5-lipoxygenase pathway promotes pathogenesis of hyperlipidemia-dependent aortic aneurysm. Nat Med 10: 966–973.