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Review

Nuclear Import of Arachidonate 5-Lipoxygenase

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Abstract. Leukotrienes are lipid messenger molecules that are secreted by leukocytes to orchestrate a rapid and prolonged immune response. The enzyme 5-lipoxygenase catalyzes the rate-limiting first two steps in the synthesis of leukotrienes from arachidonic acid. Although it has long been known that 5-lipoxygenase moves from the cytoplasm to a membrane **following** activation, it has only recently been recognized that the enzyme may shuttle into and out of the nucleus **before** activation. The regulation of this movement of soluble 5-lipoxygenase between the cytoplasm and the nucleoplasm, as well as its impact on 5-lipoxygenase action, leukotriene synthesis and cell function, is only now being elucidated. This review details the state of our understanding of the nuclear import of 5-lipoxygenase and its potential importance in immunity.

Key words: leukotrienes; 5-lipoxygenase; nuclear import.

Introduction

Leukotrienes (LTs) are intercellular messengers that play initiating roles in host defense. LTB_4 regulates inflammation, acting to recruit and activate leukocytes as well as modulate the production of pro-inflammatory cytokines. The cysteinyl LTs, LTC_4 and LTD_4, normally regulate vasoconstriction, mucus secretion and edema. However, the over-production of LTs contributes to disease, including asthma[15] and allergic hyperresponsiveness[32]. Similarly, the under-production of LTs, as occurs in neonates[8, 35] or with viral infection[10], results in reduced phagocytosis and killing of bacteria and, in this way, increases susceptibility to persistent bacterial infection[2], as may be associated with pneumonia, sepsis and meningitis.

The enzyme 5-lipoxygenase (5-LO) catalyzes the rate-limiting first two steps in the synthesis of LTs from the fatty acid substrate, arachidonic acid (AA) (Fig. 1).

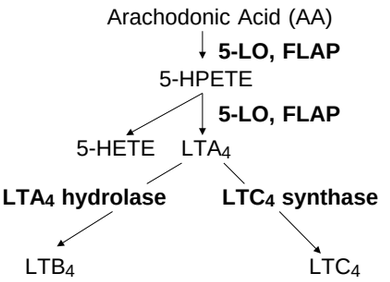

Fig. 1. Key enzymes in the synthesis of LTs from AA. 5-LO and its activating protein, FLAP, mediate the rate-limiting first steps. LTA_4 hydrolase and LTC_4 synthase convert LTA_4 to the active products LTB_4 and LTC_4, respectively

Abbreviations used: AA – arachidonic acid, FLAP – 5-lipoxygenase activating protein, 5-HPETE – 5-hydroperoxyeicosatetraenoic acid, 5-HETE – 5-hydroxyeicosatetraenoic acid, 5-LO – 5-lipoxygenase, LT – leukotriene, NIS – nuclear import sequence.

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5-LO first inserts molecular oxygen into AA to form 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and, subsequently, directs a dehydration step to produce LTA_4[28, 34]. The activity of 5-LO is greatly enhanced by the 5-LO-activating protein (FLAP)[1, 14], although FLAP is not necessary for 5-LO action. The intermediate 5-HPETE is often made in significant amounts; it is rapidly modified to 5-hydroxyeicosatetraenoic acid (5-HETE). The end-product of 5-LO action, LTA_4, can be either hydrolyzed by the enzyme LTA_4 hydrolase to give LTB_4, or 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.

Movement of 5-LO Following Cell Stimulation

The 5-LO enzyme can move in at least two distinct patterns within the cell. One pattern involves its movement from the soluble phase to a bound phase, as it becomes associated with intracellular membranes (Fig. 2). This movement, which has long been recognized[33], is taken as a hallmark of 5-LO activation and occurs following cell stimulation. The movement is commonly alluded to as „translocation" and is triggered by the transient increase in cytoplasmic calcium concentration that follows cell stimulation. Membrane association is rapid, as it is evident within seconds after cell stimulation[6], when assayed by immunofluorescent techniques.

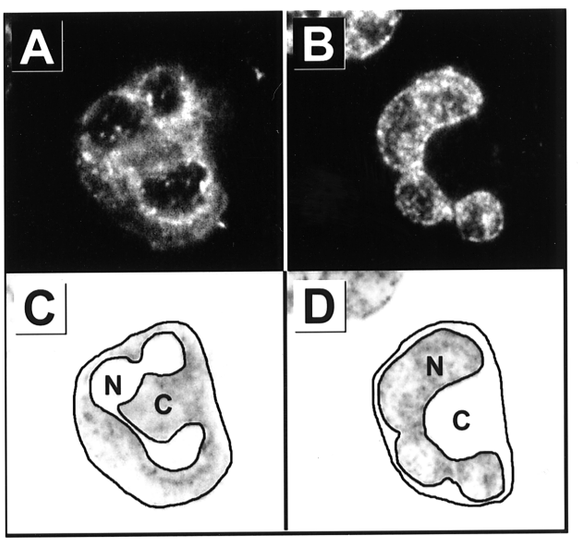

Fig. 2. The movement of cytoplasmic 5-LO to subcellular membranes. In unstimulated peripheral blood neutrophils, 5-LO is dispersed in the cytoplasm, as shown by immunofluorescent microscopy (A) and diagrammatically (C). After cell stimulation, 5-LO accumulates predominantly on the outer membrane of the nuclear envelope surrounding the multi-lobed nucleus (B, D)

Functionally, membrane association serves to place 5-LO close to its substrate, AA, as well as its activating protein FLAP. This facilitates the production of high levels of secretable LTs. Recent studies have demonstrated that 5-LO accumulates most abundantly at the nuclear envelope and endoplasmic reticulum[6]. Calcium-dependent membrane association of 5-LO appears to be reversible following transient stimulation[6, 19, 25]. Persistent membrane association results from chronic cell stimulation[6] and may be indicative of persistent LT synthesis linked to disease[36]. Since the 5-LO enzyme is readily inactivated by its own action[21, 26], the persistent membrane association resulting from extensive cell stimulation can also be a marker for inactivated 5-LO[6].

Movement of 5-LO Before Cell Stimulation

A second type of movement of 5-LO, which has only recently been recognized, is its import, still as a soluble protein, into the nucleus. Nuclear import of proteins is also commonly referred to as "translocation"; this overlap in terminology has undoubtedly caused confusion regarding 5-LO movement. Nuclear import is distinct from membrane association, both mechanistically and functionally. In general terms, the enzyme remains soluble during nuclear import, moving from the cytoplasm to the nucleoplasm. When visualized within the nucleoplasm by immunofluorescent techniques, soluble 5-LO distributes in a distinctive "flecked" pattern (Fig. 3). Following cell stimulation, the enzyme may be induced to move to the nuclear envelope by an increase in the calcium concentration within the nucleoplasm. Visually, 5-LO appears to be distributed across a membrane, and folds in the membrane are often apparent.

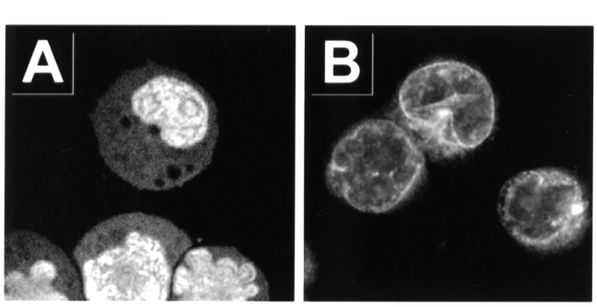

Fig. 3. The movement of intranuclear 5-LO to the nuclear envelope. In the mast cell-like rat basophilic leukemia cells, 5-LO is most abundant in the nucleoplasm before cell stimulation, as shown by immunofluorescent microscopy (A). After cell stimulation, 5-LO accumulates predominantly on the inner membrane of the nuclear envelope (B). An increase in the volume of the nucleus is a normal event immediately following cell stimulation

The mechanism of nuclear import has been the subject of several recent reviews[16, 17, 27]. Import of proteins larger than 50 kDa, such as 5-LO, occurs through a signal-mediated pathway. Typically, the protein to be imported must contain a nuclear import sequence (NIS) that mediates association with a cytoplasmic receptor protein. The best-characterized NIS consists of a cluster of basic amino acids (arginine or lysine) that are recognized by the cytoplasmic receptor, importin α. Binding to importin α allows association with importin β, docking at the nuclear pore, and entrance into the nucleus.

The mechanism by which 5-LO is imported into the nucleus has only recently received attention. Preliminary studies suggested that, although 5-LO contains three basic regions that resemble NISs, none of these were necessary for nuclear import[9]. However, a more thorough analysis of these sequences identified a bipartite NIS in the carboxyl terminus that is essential for nuclear import[18]. Mutational analysis revealed that the replacement of specific basic amino acids with uncharged residues impaired or eliminated nuclear import. Although this NIS, by itself, was able to direct the nuclear import of a heterologous protein, its import was less efficient than that of the 5-LO protein. Thus, the context in which the 5-LO NIS functions may determine its effectiveness. The cytoplasmic receptor (e.g., importin α) that mediates 5-LO import has not yet been identified.

The Regulation of Nuclear Import of 5-LO

Recent studies have demonstrated that the nuclear import of 5-LO can be activated by adherence[4, 5] and cytokine stimulation[12], indicating that this process is regulated. The signaling pathways that mediate the activation of 5-LO nuclear import are as yet unknown. Phosphorylation of a protein can modulate its import into the nucleus, presumably by altering its capacity to bind to importin α. Recently, Fitzpatrick and colleagues presented evidence that 5-LO could be directly phosphorylated and that all phosphorylated 5-LO was associated with a DNA-containing nuclear fraction[23]. This suggests that direct phosphorylation might play a role in the nuclear import of 5-LO. Nuclear import may also be regulated through intermolecular interactions. Coactosin-like protein, a protein found to interact with 5-LO in a yeast two-hybrid screen[31], is a homologue of the actin-binding protein coactosin. This protein could function to anchor 5-LO to the cytoskeleton and thus retain it in the cytoplasm. In this scenario, phosphorylation of 5-LO might serve to release it from the cytoskeleton and allow its subsequent import. Alternatively, nuclear import of 5-LO could be constitutive and 5-LO subcellular distribution regulated at the level of nuclear export. 5-LO contains several regions that resemble nuclear export sequences; the functional importance of each of these sequences has yet to be evaluated.

The Impact of Nuclear Import of 5-LO

Effect on LT secretion

The discovery that 5-LO can migrate into the nucleus in unstimulated cells immediately raised the question: Why? If the sole function of 5-LO is to make LTs for secretion, then why should 5-LO move from the cytoplasm into the nucleus? The most obvious answer is that nuclear import is necessary for LT synthesis. This possibility was supported by the finding that mutations that eliminated nuclear import of 5-LO also inhibited LT synthesis[9]. However, when nuclear import is induced in eosinophils, LT synthesis actually declines[4]. Thus, 5-LO import is not necessary for LT synthesis, at least in eosinophils.

A related purpose of nuclear import may be to alter 5-LO function and, in this way, modulate LT synthesis. This effect has been found. In neutrophils, import is induced by either adherence or recruitment into sites of inflammation. Blood neutrophils, which have cytoplasmic 5-LO, secrete abundant LTB_4. However, adherent neutrophils, which have intranuclear 5-LO, require a greater stimulus to activate LT synthesis but secrete 3 to 5 times as much LTB_4 as blood neutrophils[5]. Similarly, blood monocytes have cytoplasmic 5-LO and make low amounts of LTs. Differentiation of monocytes to macrophages can have two outcomes. If differentiation occurs in the peritoneum, then 5-LO distribution is not altered and there is little change in LT generation. On the other hand, if differentiation occurs in the alveolus, then 5-LO accumulates in the nucleus and LT synthesis increases dramatically[3, 30]. This suggests that factors in the alveolus can drive both nuclear import and an increase in LTB_4 production. Supporting this conclusion is the finding that the removal of macrophages from the alveolus into culture results in the loss of both the nuclear 5-LO and the increased LTB_4 synthetic capacity[11]. While these findings suggest that nuclear localization of 5-LO is associated with an increase in secretable LT synthesis, this correlation is not always the case. As noted above, the nuclear import of

5-LO in eosinophils, following adherence, leads to a dramatic decrease in LTC_4 secretion following cell stimulation[4]. It is not clear whether this reversal in the impact of nuclear import is related to inherent differences between cell types or relates more to differences in the LTB_4 vs. LTC_4 synthetic pathways.

There are several possible ways that a change in the subcellular distribution of 5-LO could lead to a change in the amount of secreted LT. One relates to the subcellular distribution of other enzymes linked in the metabolism of arachidonic acid to each type of LT. For example, the positioning of LTC_4 synthase on perinuclear membranes[29] may place it close to 5-LO moving to membranes from the cytoplasm, but distant from 5-LO moving from the nucleoplasm. The nuclear import of 5-LO, in effect, may result in an uncoupling of the enzymes in the LTC_4 synthetic pathway. Alternatively, the microenvironments of the nucleus and cytoplasm may differ subtly, perhaps in pH or reducing capacity, producing significant changes in the biochemical characteristics of the 5-LO enzyme. Or, the availability or regulation of essential co-factors, like calcium and ATP, may differ between the two compartments. The regulation of calcium signaling within the nucleus appears to vary with cell type and stimulus, so that in some cases nuclear calcium levels change independently from cytosolic calcium levels and in other cases the changes in the two compartments are linked. As a result, the import of 5-LO into the nucleus may serve to buffer it from activation by calcium signaling that occurs in the cytoplasm. These possibilities are yet to be clarified.

Intracellular actions of LTs

An extremely attractive explanation for the subcellular redistribution of 5-LO is that its products have intracellular effects, close to the site of synthesis. Thus, LTs synthesized by cytosolic 5-LO are available to modulate cytosolic processes and LTs synthesized by nuclear 5-LO are available to modulate nuclear processes (Fig. 4). LTs have been reported to influence a variety of cellular processes, although the specific mechanisms of action have not been elucidated. Secreted LTs are known to initiate their effects on target cells by binding to specific LT receptors on the cell surface[24, 38]. In addition, LTB_4 has been shown to bind to the intracellular protein PPARα, a nuclear receptor, and modulate its activity[13]. These results demonstrate that LTs are capable of binding to proteins and influencing their activity. Thus, LTs could potentially affect other intracellular functions by binding to intracellular proteins and acting as activators, cofactors or inhibitors. Cytoplasmic processes that may be directly regulated by LTs include phagocytosis and bacterial killing, the activity of cytosolic enzymes, and cytoskeletal rearrangement. Nucleoplasmic events that may be regulated by LTs include gene transcription, apoptosis and DNA replication and repair. The autocrine action of LTs is a new field of study that should bear interesting fruit in the near future.

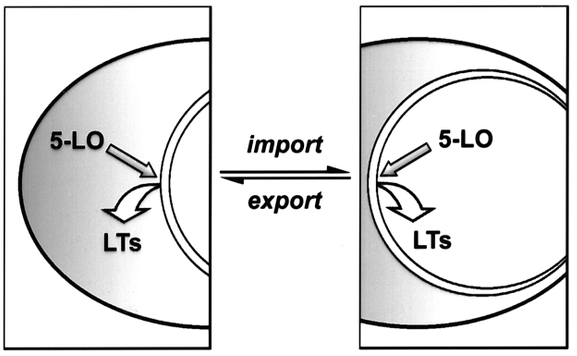

Fig. 4. Schematic diagram of the compartmentalization of 5-LO action. The two regulated processes of nuclear import and export will place 5-LO in either the nucleoplasm or the cytoplasm. This difference in localization will result in membrane association of 5-LO, after cell stimulation, on distinct membranes of the nuclear envelope. The synthesized LTs may then be generated in distinct compartments, producing different effects on leukocyte function

In the search for subcellular actions of 5-LO products, initial interest may focus on the effects of LTB_4, if only because it is the best recognized product. However, the more immediate products of 5-LO action, 5-HPETE, 5-HETE and LTA_4, may also be important. It should be noted that the amounts of these mediators that may be necessary to alter intracellular functions may be very small relative to the amounts normally made for secretion. As a result, the level of 5-LO activity that is required may also be very low. This suggests that the roles of FLAP and membrane association may be reduced. It is even possible that the residual activity of 5-LO in the absence of calcium activation may be sufficient to produce enough lipid mediator for intracellular effects. In short, most of our assumptions regarding the regulation of 5-LO may not apply to the metabolism of arachidonic acid for intracellular signaling.

Direct effects of 5-LO

A surprising implication for nuclear import of 5-LO involves the enzyme itself. Early immunoelectron microscopy analysis found 5-LO to be particularly abundant in the euchromatin region of nuclei of alveo-

lar macrophages[37]. The euchromatin is the site of active DNA transcription. This suggests that the 5-LO protein may associate with active DNA and directly affect transcription. A portion of the 5-LO protein in the nuclear pellet from rat basophilic leukemia cells was found to be resistant to extraction with high salt concentrations, consistent with a tight association with chromatin[7]. LEPLEY and FITZPATRICK[22] supported this possibility with evidence that tyrosine phosphorylated 5-LO was associated with DNA from activated HL-60 cells. The significance of the association of 5-LO with DNA remains to be elucidated.

Alternatively, 5-LO may interact with other proteins. LEPLEY and FITZPATRICK[20] demonstrated that 5-LO contains a functional Src homology 3-binding motif, which is a domain recognized by adapter proteins that mediate protein-protein linkages. Direct association of 5-LO with other proteins has also been identified by yeast two-hybrid studies[31]. These studies indicate that a variety of direct interactions between 5-LO and other proteins can be found, but the functional significance of each interaction has yet to be determined. It seems likely that changes in the nuclear import of 5-LO will affect these interactions, or, alternatively, these interactions may affect changes in the subcellular distribution of 5-LO.

Conclusion

The recent finding that 5-LO can move into the nucleoplasm of resting myeloid cells has potentially far-reaching consequences for the role of LTs in immunity. The simple possibility that nuclear import may serve to modulate the level of secretable LT has obvious significance: because nuclear import is regulated, dysfunction in that regulation becomes a tenable basis for diseases featuring aberrant LT generation, such as asthma. At a more complex level, the subcellular redistribution of 5-LO may impact on autocrine actions of 5-LO products. Finally, evidence that 5-LO directly interacts with DNA and other proteins indicates that the 5-LO protein may have additional roles, beyond its AA-processing function, in regulating myeloid cells.

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