The membrane organization of leukotriene synthesis

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Cell signaling leading to the formation of leukotriene (LT)C_4 requires the localization of the four key biosynthetic enzymes on the outer nuclear membrane and endoplasmic reticulum. Whether any macromolecular organization of these proteins exists is unknown. By using fluorescence lifetime imaging microscopy and biochemical analysis, we demonstrate the presence of two distinct multimeric complexes that regulate the formation of LTs in RBL-2H3 cells. One complex consists of multimers of LTC_4 synthase and the 5-lipoxygenase activating protein (FLAP). The second complex consists of multimers of FLAP. Surprisingly, all LTC_4 synthase was found to be in association with FLAP. The results indicate that the formation of LTC_4 and LTB_4 may be determined by the compartmentalization of biosynthetic enzymes in discrete molecular complexes.

Leukotrienes (LT)s, C_4, D_4, and E_4 play a central role in the initiation and amplification of the inflammatory response, the pathogenesis of asthma, aspirin-sensitive rhinosinusitis rhinitis, and in the activation and trafficking of cells of the immune and hematopoetic systems (1–9). These products of the 5-lipoxygenase (5-LO) pathway of arachidonic acid metabolism transduce signals by means of distinct receptors for LTD_4 (CysLT1), LTC_4 (CysLT2), and LTB_4 (BLT1 and BLT2). Mast cells and macrophages play a critical role in the initiation of the inflammatory response, and these cells have the capacity to synthesize both LTC_4 and LTB_4. However, how they balance the synthesis between these two bioactive lipids is not known.

The formation of LTC_4 requires the functional interaction of at least four proteins on the outer nuclear membrane or endoplasmic reticulum. Cytoplasmic phospholipase A_2 and 5-LO are translocated to these membranes after cell activation (10–13). The 17-kDa integral membrane protein 5-LO-activating protein (FLAP), is expressed in myeloid cells, and is required for the formation of LTs. FLAP may serve as an arachidonic acid-binding protein, allowing arachidonic acid to be presented to 5-LO for conversion to both 5-HPETE and LTA_4 (14–18). To form LTC_4, LTA_4 is conjugated with reduced glutathione by LTC_4 synthase, a 17-kDa integral membrane protein that shares a high identity with FLAP (19–23). Two untested models for the membrane organization of LTC_4 synthesis can be proposed. In one model, free arachidonic acid and its products diffuse throughout cells, and the formation of LTC_4 is determined simply by substrate availability and the kinetic properties of the relevant enzymes. In the second model, an organized multiprotein complex regulates the efficient transfer of the products of one reaction to the downstream enzyme, allowing the efficient synthesis of LTC_4, and preventing the potential consequences of the free diffusion of 5-HPETE and LTA_4 (24). To search for a multiprotein complex of LT-forming enzymes, we determined the membrane interactions of LTC_4 synthase and FLAP by using fluorescence lifetime imaging microscopy (FLIM) supported by coimmunoprecipitation of endogenous FLAP and LTC_4 synthase. Our results indicate that the formation of LTC_4 and LTB_4 may be regulated by the compartmentalization of FLAP into two distinct membrane complexes. One complex, which includes LTC_4 synthase and FLAP, is likely to be dedicated to the formation of LTC_4. The other complex contains multimers of FLAP, and would provide LTA_4 for the formation of LTB_4. Our studies provide evidence for the compartmentalization of biosynthetic enzymes as a potential mechanism for regulating the balance of the synthesis of LTB_4 and LTC_4, and for the generation of other bioactive lipids.

Materials and Methods

Construction of Reporter Plasmids. To generate the pECFP-LTC_4 synthase and pEYFP-LTC_4 synthase N-terminal reporter constructs, where ECFP is enhanced cyan fluorescent protein and EYFP is enhanced yellow fluorescent protein, the human LTC_4 synthase cDNA was subcloned from pcDNA 3.1 (−) *myc*-His into pECFP-C1 and pEYFP-C1 (BD Biosciences). The human FLAP cDNA (American Type Culture Collection) was amplified by PCR. The forward primer included base pairs 26–48 preceded by the sequence CCC-CCT-GAG-G. The 3′ sequence corresponded to base pairs 496–516 preceded by the sequence CCC-GCA-TTC. The PCR conditions were: 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min for 30 cycles, followed by a 10-min extension. The PCR product was isolated from 1% agarose gels by using Gene Clean and then digested with *Xho*I and *Eco*RI, and was then cloned in-frame into the pECFP-C1 and pEYFP-C1 vectors. All constructs were sequenced before use.

Cell Culture and Transfections. RBL-2H3 cells were maintained in DMEM supplemented with 10% heat-inactivated FCS, 2 mM Gln, sodium pyruvate, nonessential amino acids, penicillin (100 units/ml), and streptomycin (0.1 mg/ml). COS-7 cells were maintained in DMEM supplemented with 10% heat-inactivated FCS, 2 mM Gln, penicillin (100 units/ml), and streptomycin (0.1 mg/ml) at 37°C with 5% CO_2. For transfections, semiconfluent (70–80% confluent) COS-7 cells were transfected in six-well culture plates. Plasmid DNA was isolated by using HiSpeed plasmid DNA isolation kits (Qiagen, Valencia, CA) as per the manufacturer's instructions. Sterile plasmid DNA was dissolved in TE buffer (10 mM Tris/1 mM EDTA, pH 8.0) and was transfected (4 μg per dish) by using the SuperFect transfection reagent (Qiagen) as per the supplier's protocol. In experiments where cells were cotransfected with two plasmids, 4 μg of each plasmid was used. After incubation for 4 h at 37°C, the transfection medium was replaced with normal growth medium and the cells were cultured for an additional 24 h. Live cells were washed three times and resuspended in PBS, pH 7.5, at room temperature before FLIM analysis.

FLIM Analysis. FLIM was used to test for fluorescence resonance energy transfer (FRET) between closely interacting fluorophores. Time-domain FLIM was used to measure the fluorescence lifetime of the donor (cyan) fluorophore, which is short-

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Abbreviations: LT, leukotriene; 5-LO, 5-lipoxygenase; FLAP, 5-LO-activating protein; FLIM, fluorescence lifetime imaging microscopy; FRET, fluoresence resonance energy transfer; ECFP, enhanced cyan fluorescent protein; EYFP, enhanced yellow fluorescent protein.

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ened when FRET occurs (25–28). COS cells expressing transfected imaging constructs were excited for FLIM analysis as follows. A femtosecond-pulsed Ti-sapphire laser (Tsunami; Spectra-Physics) mode-locked at 760 nm was used to excite ECFP. Images were acquired by using a multiphoton microscope (Radiance 2000; Bio-Rad). A high-speed microchannel plate detector (MCP5900; Hamamatsu, Ichinocho, Japan) and hardware/software from Becker & Hickl were used to record data. The fluorescence lifetimes were measured on a pixel-by-pixel basis. Lifetimes from all cells imaged were fit to two exponential decay curves with one exponential fixed at the average lifetime for the appropriate ECFP-alone fusion protein. The remaining variable exponential revealed the presence or absence of a shortened lifetime for each pixel within each image, as well as the relative amplitude compared to the amplitude of the fixed lifetime component. The lifetimes were calculated according to the formula: $t = a_1e^{-t/}\tau 1 + a_2e^{-t/}\tau 2 + a_3e^{-t/}\tau 3 + a_ne^{-t/}\tau n$, where t = the fluorescence lifetime of the cyan fluorophore measured in picoseconds after a short (100 fs) pulse of excitation light. τ is defined as the time at which the fluorescence decays to $1/e$ of the initial value. With complex decay times, the curves are fit with multiple exponentials by using multiple τs. Each exponential component has a weighted amplitude ($a1$, $a2$, . . .) that is expressed as a percentage of the sum of the amplitudes from each component. For a single exponential fit, a1 = 100%. The data were expressed in two forms. First, as bar graphs that show the mean lifetime (±SD) for analysis of all cells over multiple experiments. The second form is as a pseudocolor image from a representative experiment in which the scale ranges from blue, >2,000 ps, no interaction; to orange, 250 ps, which is representative of a strong interaction.

Biochemical Analysis of Interacting Proteins. RBL-2H3 cells from 50–60% confluent cultures were washed twice at room temperature with PBS, harvested by centrifugation, and the cell pellet was resuspended in ice-cold lysis buffer (20 mM Hepes buffer, pH 7.4, containing 100 mM NaCl, 1% Triton X-100, 2 mM EGTA, 50 mM β-glycerophosphate, 1 mM Na_3VO_4, 2 mM leupeptin, 10 units/ml aprotinin, 2 mM pepstatin, and 400 mM PMSF) for whole-cell extract preparation. After suspension in lysis buffer, cells were lysed by passing the cell suspension through a 25G5/8 needle five times. Protein was then solubilized by allowing the cell lysates to remain on ice for 1 h, with mixing every 10 min. The cell lysates were then centrifuged in an Eppendorf microcentrifuge at 14,000 rpm for 20 min. The supernatants (extracts) were removed and were then used for further analysis. For preclearing of cell extracts, 3 μl (1.0 mg) of normal rabbit IgG and 30 μl of prewashed, BSA-blocked protein-A agarose bead slurry (25%, Santa Cruz Biotechnology) were added to 500 μl of the solubilized extract (≈1500 mg of total protein), incubated at 4°C for 30 min with continuous rocking, and then centrifuged at 1000 × g for 1 min at 4°C. To 500 μl of the precleared cell lysate, 500 μl of the lysis buffer and rabbit polyclonal anti-LTC_4 synthase antisera directed against the amino acids 36–51 (loop 1) were added and were then incubated at 4°C with continuous rocking. After 3 h of incubation, 40 μl of protein-A agarose bead slurry was added to the lysate and was incubated at 4°C with continuous rocking for 1 h and was then centrifuged at 1000 × g for 1 min at 4°C. The antibody-conjugated protein-A agarose bead pellet was gently washed four times with ice-cold lysis buffer. The pellet was resuspended in 50 μl of 2× Laemmli buffer with/without 2-mercaptoethanol (as indicated), heated at 90°C for 5 min, and centrifuged. The immunoprecipitated proteins in the supernatants were resolved by electrophoresis using 12% SDS/Tris-glycine polyacrylamide gels. Proteins were then electrotransferred to polyvinylidene difluoride membrane (Bio-Rad) and the membrane was blocked overnight in PBS containing 0.1% Tween-20, 0.1% Triton X-100, and 5% fat-free milk. Immunoblot analysis was performed by using rabbit anti-LTC_4 synthase antisera (1:100) or rabbit anti-FLAP antibody (1:150). After incubation with primary antibodies, the blots were washed with 1x PBS containing 0.1% Tween-20 and 0.1% Triton X-100. This procedure was followed by incubation with horseradish peroxidase-conjugated anti-rabbit IgG (1:3,000, Cell Signaling, Beverly, MA), and chemiluminescent detection was performed by using the ECL system (Amersham Pharmacia-Pharmacia Biotech, Piscataway, NJ) according to the manufacturer's instructions.

For crosslinking, RBL-2H3 cells from 50–60% confluent cultures were washed twice at room temperature with PBS and then treated with dimethyl-3–3′-dithiobispropionimidate·2 HCl (Pierce) in 4 mM of 20 mM sodium phosphate buffer, pH 7.5 containing 150 mM NaCl) for 1 h at room temperature. To quench the crosslinking reaction, 1 M Tris·HCl, pH 7.5 was then added to achieve a final Tris concentration of 50 mM. The cells were kept at room temperature for 10 min before extraction and analysis.

RNA Isolation and Northern Blot Analysis. Total RNA was isolated from RBL-2H3 cells by using the Qiagen RNeasy minikit. A total of 40 μg of RNA was resolved in each lane by electrophoresis on 1.3% formaldehyde/formamide-agarose gels. After electrophoresis, RNAs were transferred to the Hybond N^+ (Amersham Pharmacia-Pharmacia Biotech) and UV-crosslinked by using the Stratagene UV Stratalinker. The RNA blots were hybridized at 65°C in ExpressHyb solution (Clontech) with ^{32}P-labeled cDNA probes corresponding to the coding region of human LTC_4 synthase, human FLAP, or mouse 5-LO. The blots were washed twice with 2 × SSC containing 0.1% SDS at room temperature, and three times with 0.1 × SSC containing 0.1% SDS at 50°C for 40 min, and were analyzed by autoradiography. The size of the transcript was estimated from RNA molecular size markers run in a parallel lane. The probes were then stripped from membranes with 0.5% SDS solution. The membranes were tested by autoradiography to confirm the removal of the previous probe, and were then probed with a ^{32}P-labeled cDNA corresponding to the coding region of mouse GAPDH. Each of the cDNAs have 86–93% identity with the corresponding rat sequence.

Expression of FLAP and LTC_4 Synthase in Sf9 Cells. The C-terminal epitope constructs of FLAP (Avi-tag/FLAG) and LTC_4 synthase (His_6) were expressed in Sf9 cell membranes, and were solubilized (ref. 18, and *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site). In certain instances, they were purified to homogeneity by affinity chromatography.

Results and Discussion

FLIM Analysis of the Interaction of FLAP and LTC_4 Synthase. To explore the interactions of FLAP and LTC_4 synthase, we initially used a two-stage process. The first stage used a live-cell imaging approach to identify potential interactions, and then a biochemical approach with endogenous proteins was used to confirm the results. To determine whether FLAP monomers could form homotypic interactions, COS cells were cotransfected with ECFP-FLAP and EYFP-FLAP in six-well culture dishes and live-cell FLIM was performed in PBS. The fluorescence lifetime of ECFP-FLAP in the presence of EYFP-FLAP was 427.4 ± 94.3 ps (n = 16, Fig. 1). In contrast, the lifetime measured in cells transfected with ECFP-FLAP alone was 2192 ± 71.5 ps (n = 5, Fig. 1). The shortened lifetime of ECFP-FLAP in the presence of EYFP-FLAP indicates FRET between a homomeric assembly of FLAP proteins. The lifetime for the ECFP protein when coexpressed with EYFP (Fig. 1) was 2,356 ps (n = 2), indicating no FRET or interaction. Similarly, EYFP transfected alone showed negligible contribution to fluorescence intensity and

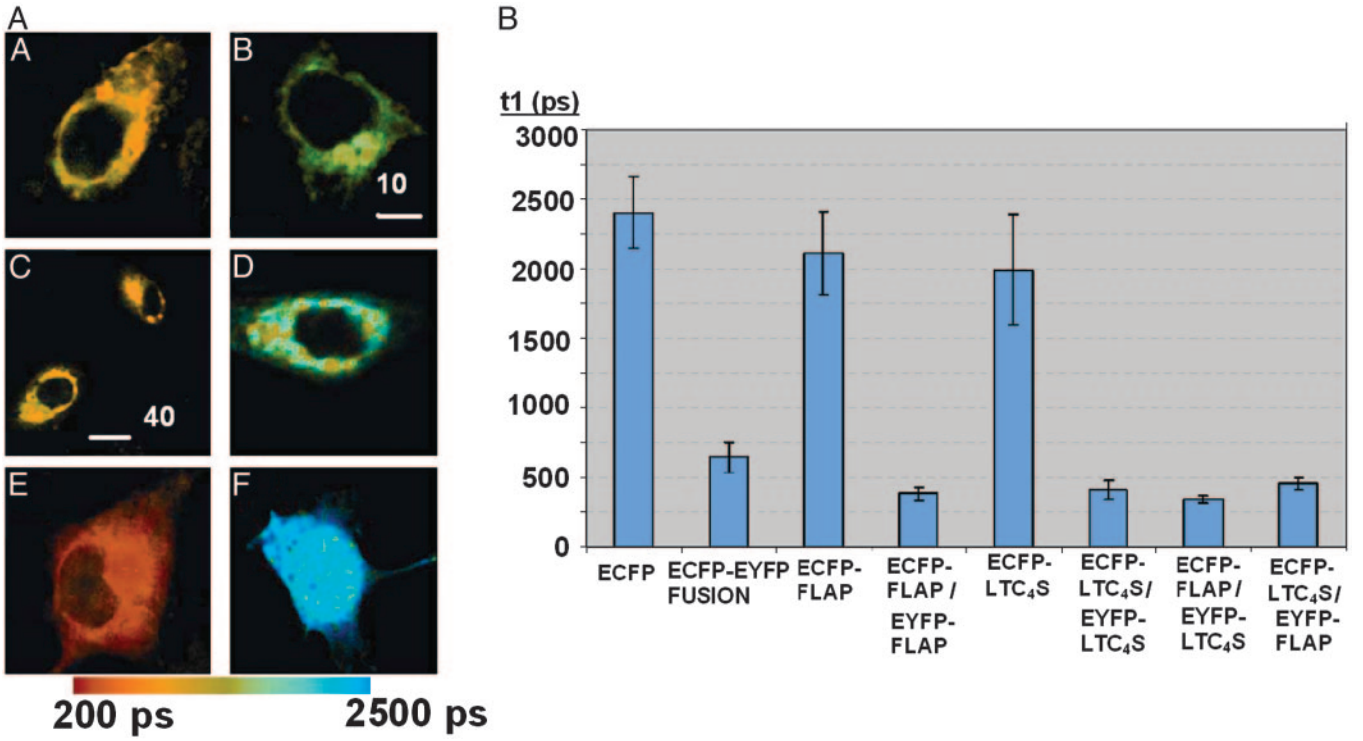

Fig. 1. FLIM analysis of the interactions of FLAP and LTC_4 synthase. (*A*) Pseudocolor imaging of the interaction of LTC_4 synthase and FLAP analyzed by live-cell FLIM. COS cells were transfected with 4 μg of individual plasmids in six-well culture dishes and were analyzed 24 h later. Transfected or cotransfected COS cells were analyzed by time-domain FLIM in PBS by using double-exponential decay algorithms. The data are from a representative experiment. Pseudocolor imaging represents the weighted average lifetime for two lifetime values per pixel. The lifetime pseudocolor scale on the bottom depicts the fluorescence lifetime over a scale of 2,500 ps (blue) to 250 ps (orange), which depict increasingly strong interactions. (*AA*) FLIM of ECFP-FLAP and EYFP-FLAP. (*AB*) FLIM of ECFP-FLAP alone. (*AC*) FLIM of ECFP-LTC_4 synthase and EYFP-LTC_4 synthase. (*AD*) FLIM of ECFP-LTC_4 synthase alone. (*AE*) FLIM of ECFP-FLAP and EYFP-LTC_4 synthase. (*AF*) FLIM of soluble ECFP and EYFP proteins coexpressed in a single cell. (Scale bars, 10 μm in *AB*; 40 μm in *AC*.) (*B*) Bar graph analysis of FLIM data. The data represent the mean ± SD for 5–17 separate cells taken from four to six experiments, with the exception of the ECFP-EYFP fusion protein where the data represent the mean of two cells from two experiments. t_1, the fast-lifetime component of the average lifetime; LTC_4S, LTC_4 synthase.

lifetime measurements. As a positive control for FRET between closely apposed fluorophores, an ECFP-EYFP fusion protein was transfected into cells resulting in a lifetime of 641 ± 108 ps (mean, $n = 2$, Fig. 1*B*). The observation that the decrease in the lifetime of FLAP vectors was as great as the control suggested that the interaction between these two monomers were relatively strong.

The same approach was used to test for homomeric interactions of LTC_4 synthase. When ECFP-LTC_4 synthase and EYFP-LTC_4 synthase were coexpressed (Fig. 1), the lifetime of ECFP-LTC_4 synthase was 371 ± 66.3 ps ($n = 16$), which was much shorter than the values of 2142 ± 45.5 ps ($n = 5$) in cells expressing ECFP-LTC_4 synthase alone (Fig. 1). These results demonstrate the close proximity and likely interaction of LTC_4 synthase with itself. Because FLAP and LTC_4 synthase are closely related membrane-associated proteins in eicosanoid and glutathione metabolism enzymes, we tested the ability of these proteins to form heterodimers. We therefore coexpressed ECFP-FLAP and EYFP-LTC_4 synthase in COS cells. The lifetime for the enhanced cyan protein was 304.8 ± 46.7 ps ($n = 16$, Fig. 1), suggesting very strong FRET and interaction between the two proteins. As shown in Fig. 1*B*, when the fluorescent labels were reversed and ECFP-LTC_4 synthase was coexpressed with EYFP-FLAP, the fluorescence lifetime of ECFP was also shortened (538.3 ± 166.8 ps, $n = 11$), confirming these results and indicating the potential for heteromeric assemblies of FLAP and LTC_4 synthase.

FLAP and LTC_4 Synthase Have Strong Interactions. To confirm and expand the results obtained with live-cell imaging, we determined the interactions between endogenous FLAP and LTC_4 synthase in RBL-2H3 cells, which are known to contain each of these proteins and to make LTC_4 and LTB_4. The cells were extracted and immunoprecipitated using antisera to LTC_4 synthase (22), and were analyzed by Western blotting using antibodies to either FLAP (13, 18) or LTC_4 synthase. As shown in Fig. 2*A*, both FLAP and LTC_4 synthase were detected as the corresponding 17-kDa bands by the relevant antibodies. Although additional bands were detected at higher molecular weights, these bands were seen by using control nonimmune IgG. They were shown to be derived from an IgG directed at rabbit IgG present in all rabbit serum. The bands observed at 17 kDa were therefore determined to be specific. To further confirm these results, LTC_4 synthase (His_6) and FLAP (Avi-tag/FLAG) were coexpressed and solubilized from Sf9 cell microsomes, immunoprecipitated with either anti-FLAG or anti-His_6 antibody, or anti-LTC_4 synthase or anti-FLAP antibody, and the immunoprecipitates analyzed by Western blotting. As is shown in Fig. 2*B*, analysis of Western blots with anti-FLAP or anti-LTC_4 synthase antibody detected coimmunoprecipitation of proteins with antibody to either the native protein or to either epitope tag. These results indicated a tight association between FLAP and LTC_4 synthase. Of note, antibodies to the His_6 and FLAG epitopes coimmunoprecipitated relatively equal amounts of proteins, whereas antibodies to the native protein precipitated their target proteins in

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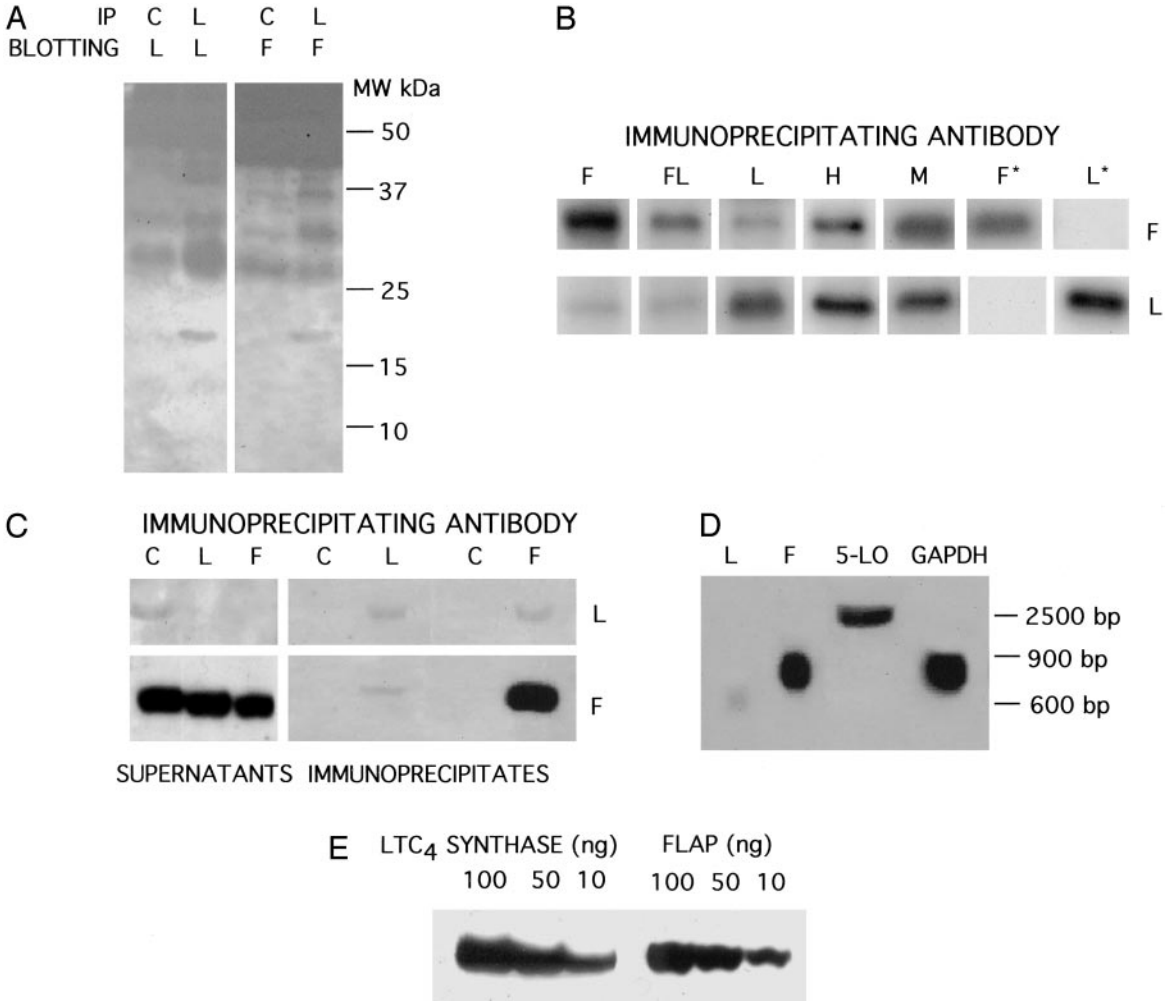

Fig. 2. FLAP and LTC_4 synthase interact. (*A*) Extracts of RBL-2H3 cells were immunoprecipitated (IP) with antibody to LTC_4 synthase (L) or control nonimmune IgG (C). Immunoprecipitates were resolved by using SDS/12% gels, and were then analyzed by Western blotting with antibodies to LTC_4 synthase or FLAP (F). (*B*) Avi-tag/FLAG-FLAP and His_6-tagged LTC_4 synthase were immunoprecipitated from extracts of Sf9 cell microsomes by using antibodies to FLAP, LTC_4 synthase, FLAG (FL), or the His_6 epitope (H). The immunoprecipitates were resolved on two identical gels, which were analyzed by Western blotting using antibodies to LTC_4 synthase or FLAP (*Right*). Microsomes (M) and 100 ng of pure protein (F* and L*) were also probed as controls. (*C*) Two populations of FLAP exist. Extracts of RBL-2H3 cells were immunoprecipitated with control IgG, antibody to LTC_4 synthase, or antibody to FLAP. The resulting supernatants and pellets were resolved by SDS/12% gels and were then analyzed by Western blotting using the antibodies shown at *Right*. (*D*) Northern blot analysis of mRNA for LTC_4 synthase, FLAP, 5-LO, and a representative GAPDH control. mRNA sizes (kb) are shown at *Right*. A representative lane of GAPDH analysis is shown; all lanes were indistinguishable. (*E*) Anti-LTC_4 synthase and anti-FLAP antibodies have equal titers. Identical concentrations of homogeneous purified His-epitope-tagged LTC_4 synthase or avitag-FLAP were separated by SDS/12% gels, were electroblotted, and were probed with antisera to LTC_4 synthase or FLAP at dilutions of 1:100 or 1:200, respectively. Immunoprecipitates using antisera against FLAP, LTC_4 synthase, FLAG, or His_6 were analyzed with antibodies to FLAP or LTC_4 synthase.

significant excess, suggesting that these antibodies were potentially disrupting some of the interactions.

All or Most LTC_4 Synthase Is Associated with FLAP. We next analyzed the supernatants and immunoprecipitates to explore the efficiency of the immunoprecipitation of LTC_4 synthase and FLAP. As shown in Fig. 2*C*, immunoprecipitation with anti-LTC_4 synthase antibody removed all detectable LTC_4 synthase from the supernatant, but only a small percentage of the FLAP protein. When the converse experiment was performed, immunoprecipitation by using anti-FLAP antibody removed ≈50% of total FLAP and all LTC_4 synthase protein and activity. These results are most likely related to the large amount of cellular FLAP relative to the amount LTC_4 synthase. Supporting this difference in protein levels are the results of Northern blotting experiments that showed significantly more mRNA coding for FLAP than for LTC_4 synthase (Fig. 2*D*) and the observation that the antibodies to FLAP and LTC_4 synthase used in these experiments recognized their respective protein antigens with essentially the same affinity (Fig. 2*E*).

The extent to which different size complexes are active in catalysis is not known. The formation of trimers is particularly conceptually appealing because it is consistent with the projection structure determined for the closely related membrane-associated proteins in eicosanoid and glutathione metabolism, prostaglandin E_2 synthase-1, and microsomal glutathione transferase 1 (29–31). We therefore sought to identify higher, multimeric forms of FLAP and LTC_4 synthase within RBL-2H3 cells. We performed *in situ* crosslinking followed by direct analysis by SDS gels. As shown in Fig. 3, we identified three different species of FLAP in cells that were crosslinked but not reduced. These species corresponded to monomers, dimers, and trimers. The relatively small amounts of FLAP trimer observed may be due to the inefficiency of crosslinking. However, until the

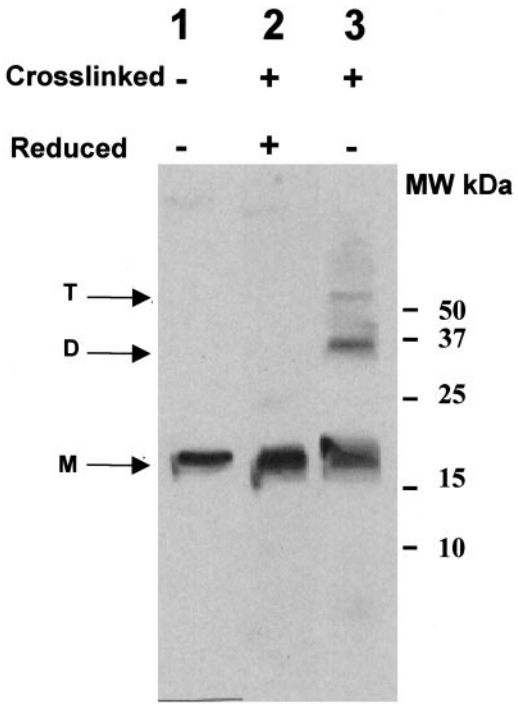

Fig. 3. Endogenous FLAP exists as multimers. RBL cells were analyzed directly (lane 1) or after crosslinking *in situ* (lanes 2 and 3) with the reducible, membrane permeant crosslinker dimethyl-3–3′-dithiobispropionimidate·2 HCl. Cell extracts were analyzed directly (lane 3) or were resolved after reduction with 2-mercaptoethanol by using SDS/12% gels (lane 2). They were then analyzed by Western blotting with antibody to FLAP. M, monomers; D, dimers; T, trimers.

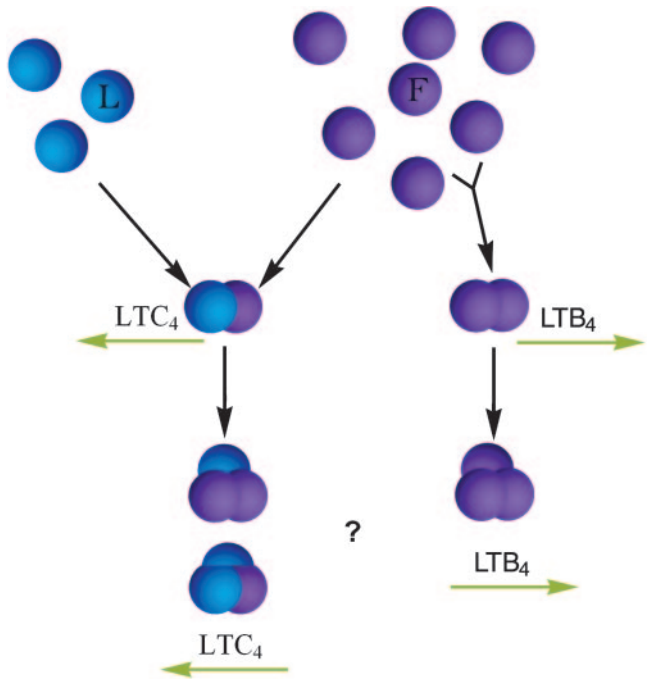

Fig. 4. The potential membrane interactions of FLAP and LTC_4 synthase. Because FLAP (F, dark blue) is in excess to LTC_4 synthase (L, light blue), there is no free LTC_4 synthase. Two populations of FLAP are predicted to exist within membranes, one that is dedicated to the formation of LTC_4, and one that is dedicated to the formation of LTB_4. The possibility of both dimers and trimers contributing to the formation of leukotrienes is shown. ?, the unknown composition of trimers.

crystal structures of FLAP and LTC_4 synthase are determined, the possibility that dimers may also be functionally active exists. To test the possibility that LTC_4 synthase forms multimers, we took a parallel approach. We were not able to detect LTC_4 synthase in Western blots without prior immunoprecipitation. As described above, for technical reasons, we were not able to analyze the portion of the gel that would include trimers of LTC_4 synthase after immunoprecipitation, leaving this question open. Based on our mRNA and antibody analysis, the most likely reason for our inability to detect LTC_4 synthase in crude blots is the relatively small amounts of LTC_4 synthase expressed in these cells.

A direct implication of this work is that the synthesis of LTC_4 and LTB_4 is likely to be coordinated by two different populations of FLAP (Fig. 4). The formation of LTC_4 would likely be regulated by a heterodimer or possibly a heterotrimer composed of FLAP and LTC_4 synthase. The tight association of FLAP and LTC_4 synthase would ensure the efficient conversion of LTA_4 to LTC_4. The homotrimers or homodimers of FLAP would be responsible for the generation of a pool of LTA_4 that would be available for conversion to LTB_4 by LTA_4 hydrolase. The composition of trimeric complexes that include FLAP, and their relative significance, remains to be determined. FLAP-LTC_4 synthase heterodimers or trimers may represent a multisubunit enzyme with one active site, as has been suggested for microsomal glutathione transferase 1, which binds one molecule of glutathione per trimer (29–32). How 5-LO is incorporated into a complex remains unknown, but it is possible that its active site is either incorporated into this complex or is directly abutting it. Finally, the results establish the principle that compartmentalization of proteins has the potential to organize eicosanoid biosynthesis at the membrane level.

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