Abstract  Eicosanoids have been implicated in a vast number of devastating inflammatory conditions, including arthritis, atherosclerosis, pain, and cancer. Currently, over a hundred different eicosanoids have been identified, with many having potent bioactive signaling capacity. These lipid metabolites are synthesized de novo by at least 50 unique enzymes, many of which have been cloned and characterized. Due to the extensive characterization of eicosanoid biosynthetic pathways, this field provides a unique framework for integrating genomics, proteomics, and metabolomics toward the investigation of disease pathology. To facilitate a concerted systems biology approach, this review outlines the proteins implicated in eicosanoid biosynthesis and signaling in human, mouse, and rat. Applications of the extensive genomic and lipidomic research to date illustrate the questions in eicosanoid signaling that could be uniquely addressed by a thorough analysis of the entire eicosanoid proteome. — Buczynski, M. W., D. S. Dumlao, and E. A. Dennis. An integrated omics analysis of eicosanoid biology. J. Lipid Res. 2009. 50: 1015–1038.

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SYSTEMS BIOLOGY AND EICOSANOID “OMICS”

Biological processes are comprised of numerous converging signals that concertedly create a coherent effect. Any individual signaling element may elicit a physiological response, yet its loss is not fatal to the organism. In fact, a wide range of genetic variation can be observed within individual members of a given species without leading to a dramatic loss of function. As these signals have redundant and emergent properties, it can be difficult to explain how a biological process works using a limited number of molecular indicators. For this reason, systems biology has emerged to address the question of how molecular biology works as an integrated process (1).

Systems biology has advanced exponentially during the past two decades, with transcriptomics, proteomics, and metabolomics each playing an integral role. Each of these platforms brings its own unique advantages and limitations in facilitating the investigation of disease pathology. A transcriptomic approach can detect the upregulation and downregulation of important biosynthetic and signaling genes; however, gene changes often don’t directly correlate with changes in protein levels (2). Proteomic analyses can identify enzymes and posttranslational protein changes in a cell or tissue, but fall short of determining which particular enzymes actively produce metabolites under disease conditions; likewise, metabolic approaches successfully identify bioactive signaling molecules, but therapeutic intervention generally requires definitive protein targets.

The eicosanoid class of signaling molecules highlights many of the issues facing comprehensive systems biology...
analyses. Eicosanoids comprise a class of bioactive lipid mediators derived from the metabolism of polyunsaturated fatty acids by cyclooxygenases (3–5), lipoxygenases (3, 6), cytochrome P450s, or nonenzymatic pathways (Fig. 1). Technically, “eicosanoids” refers to fatty acids containing 20 carbons (eicosa), but the field generally uses the term eicosanoids more broadly to also include similar metabolites of other polyunsaturated fatty acids. This lipid class has been intensely studied over the past 30 years because of its contribution to the inflammatory response in diseases such as arthritis and asthma, yet the majority of this work has focused on a select few genes, proteins, or metabolites within it. In many instances, the regulation of one arm has important regulatory implications for another arm of the eicosanoid biosynthetic pathway. Many of these enzymes produce multiple lipid products; likewise, many lipid products can be formed by a number of different enzymes acting in parallel or in concert (Fig. 2). For these reasons, a comprehensive systems biology approach would be invaluable for understanding and treating diseases implicating eicosanoid signaling.

Transcriptomic and lipidomic analyses of eicosanoid biosynthesis can be performed using existing methodologies. With the completion of the major mammalian genome projects, commercial gene array technology has rapidly progressed and allowed the generation of whole-genome data sets of mRNA changes to become a commonplace tool at many research institutions. This proliferation allowed academic research groups to focus on the more challenging task of interpreting this data and made transcriptomic analysis the gold standard in systems biology research. Due to its immense scope, metabolomics has not yet achieved this level of standardization in scientific practice. However, as a part of their goal to create the infrastructure necessary to identify and quantify all lipid molecular species, the Lipid Metabolites and Pathways Strategies (LIPID MAPS) consortium has developed liquid chromatography tandem mass spectrometry methodology that comprehensively covers nearly all the known metabolites of this class (7, 8). Specifically, a complete lipidomic analysis of eicosanoids is now available (9), along with corresponding gene microarray data (www.lipidmaps.org). Thus, it is timely to consider a proteomic analysis that could complete an integrated picture of the entire eicosanoid signaling network.

A quantitative proteomic analysis of the eicosanoid pathway has thus far lagged behind concomitant transcriptomic and lipidomic studies. These tandem mass spectrometry techniques can be divided into two philosophical approaches (10). The classically practiced MS1 and MS2 proteomic scanning techniques have demonstrated the potential to rapidly identify thousands of proteins from a single sample, yet these often represent only a fraction of the total protein content of a cell or tissue, and the investigator has little control over which proteins make the cut (11). On the other hand, by measuring the levels of a selected population of proteins using multiple reaction monitoring, significantly lower limits of detection for can be achieved (12). However, to create a method to investigate a selected proteome, one must first have a fundamental understanding of what proteins could be involved and how these proteins fit into the larger scheme.

In the book Functional Lipidomics, Bowers-Gentry et al. (7) give a broad outline for using a lipidomic approach to classify and measure eicosanoids. To facilitate a concerted systems biology approach, this review provides a systematic overview of the proteins involved in eicosanoid biosynthesis and signaling. The genes and proteins responsible for these activities are listed in supplementary Tables I–VII online, provided one of the following pieces of evidence: 1) the gene has been expressed and characterized in vitro; 2) the purified protein has been sequenced and characterized in vitro; 3) the gene has been overexpressed (or for transcription factors, expressed with a reporter gene) and characterized in a cellular system; 4) loss of activity has been demonstrated in cells or tissue from gene knockout animals. Furthermore, as studies of human biology and disease commonly employ mouse or rat models as a surrogate, important differences between these species are highlighted.

PHOSPHOLIPASE A2

Arachidonic acid and other polyunsaturated fatty acids serve as the metabolic precursors for eicosanoid synthesis (13, 14). Biologically, these molecules are generally not available in large quantities in the free acid form, but are stored at the sn-2 position on the glycerol backbone of membrane phospholipids. To be used for biosynthesis, phospholipase A2 (PLA2) liberates sn-2 fatty acids from phospholipids at the membrane interface.

Phospholipase A2 represents a superfamily of at least 15 groups that have wide-ranging roles in biological processes. These enzymes can be considered as five types: cytosolic PLA2 (cPLA2), secreted PLA2 (sPLA2), calcium-independent PLA2, platelet-activating factor acetylhydrolase, and lysosomal PLA2; their classification and biological functions have been extensively reviewed (13–15).

Current literature strongly implicates the Group IVA cPLA2 as the chief enzyme involved in polyunsaturated fatty acid release for eicosanoid biosynthesis (16, 17), with...
Fig. 2. Major eicosanoid biosynthetic pathways. The metabolites of the major pathways are indicated in color: COX (purple), 5-LOX (orange), 15-LOX (green), 12-LOX (yellow), CYP epoxygenase (red), CYP ω-hydroxylase (cyan), and nonenzymatic oxidation (gray). The products of arachidonic acid metabolism are illustrated, but similar products can be formed from other fatty acids (e.g., linoleic acid, eicosapentenoic acid, and docosahexaenoic acid).
sPLA2 potentially playing a supplementary role (see supplementary Table I). Bonventre et al. (18) and Uozumi et al. (19) independently demonstrated that peritoneal macrophages from Group IVA PLA2 knockout mice were incapable of producing eicosanoids. More recently, Adler et al. (20) identified a patient with inherited Group IVA deficiency and determined that nearly all arachidonic acid used for eicosanoid production by platelets and circulating leukocytes was attributable to this enzyme. The Group IVA cPLA2 uses a catalytic Ser/Asp dyad to hydrolyze fatty acids and contains a C2 domain that facilitates calcium-dependent translocation from the cytosol to the membrane surface where it encounters phospholipid substrate. Overall, while cPLA2s have a broad range of homology between human, mouse, and rat, the Group IVA cPLA2 has 95% identity between these species. Other cPLA2s show between 54 and 82% identity in these species and have been less well-characterized to date (15), yet may still play a supplementary role in eicosanoid biosynthesis.

While Group IVA cytosolic PLA2 is the primary enzyme involved in eicosanoid biosynthesis, sPLA2s have been demonstrated to play a supplementary role, reviewed extensively by Lambeau and Gelb (21). In particular, the Group IIA, Group V, and Group X PLA2s have all been shown to modulate eicosanoid levels in mammalian cellular systems. However, the majority of these studies have been performed by either overexpression or exogenous supplementation of sPLA2, which complicates the investigation of its role in pathology. Future work using sPLA2 knockout mice may help further elucidate the role these enzymes play in eicosanoid biosynthesis during an inflammatory response.

CYCLOOXYGENASE METABOLITES

Cyclooxygenases

Prostaglandins (PGs) are bioactive signaling molecules derived from cyclooxygenase (COX) and subsequent PG synthase activity on arachidonic acid (4, 5, 22) (see supplementary Table I). COXs contain two distinct active sites, a COX and peroxidase site, both of which use the same tyrosyl radical and heme-iron for catalysis. The COX site incorporates molecular O2 at the 11- and 15-carbon on arachidonic acid to form PGG2, which contains the following moieties: a five-member ring linked at C-8 and C-12, an endoperoxide bridge across C-9 and C-11, and a peroxide at C-15. The peroxidase site reduces the peroxide to a hydroxyl to form PGH2, the substrate for the various PG synthases. In addition to forming PGH2, arachidonic acid can situate in the active site pocket in a limited number of suboptimal conformations that incorporate a single molecular O2, forming trace amounts of either 11R-hydroxyeicosatetraenoic acid (HETE) or 15(S)-HETE. COX activity does not exhibit long-term stability, as the catalytic tyrosyl radical can be transferred to a nearby tyrosyl residue and cause “suicide inactivation” after ~300 turnovers. Because the enzyme can perform only a limited number of reactions, it must be constantly re-expressed to generate metabolites. The details of these mechanisms have been reviewed extensively (5).

COXs constitute two distinct genes, COX-1 and COX-2. These two isoforms exhibit ~60% identity and have nearly identical active site residues (4). The most significant structural difference between these enzymes is an isoleucine to valine substitution, which results in a larger COX-2 active site pocket. This allows COX-2 to be more permissive in selecting substrates, and unlike COX-1, it can metabolize dihomo-γ-linolenic and eicosapentaenoic acid in addition to arachidonic acid. Pharmaceutical companies have also taken advantage of the larger COX-2 active site, creating isoform selective inhibitors such as Celecoxib (Celebrex™). While COX-1 and COX-2 have similar structural and catalytic features, these isozymes exhibit different expression patterns. COX-1 is constitutively expressed by most cell types and has been implicated in a number of homeostatic processes, including stomach acidity control, endometrial cycling, and renal function. In contrast, COX-2 expression is controlled by the pro-inflammatory transcription factor NF-κB and highly upregulated in response to infection, atherosclerosis, and a number of cancers.

Prostaglandins

PGH2 can form a number of different bioactive products through the action of PG synthases. This includes a number of important signaling molecules, including PGl2 (also known as prostacyclin), thromboxane A2, PGE2 (also known as dinoprostone), PGD2, and PGF2α. Following the PG abbreviation (Fig. 3), the nomenclature highlights the two important structural features: the components of the five-member prostanate ring, as denoted by a letter A–K, and the number of double bonds, denoted by a subscript number (23). The lone exception is thromboxane, which has a six-member oxane ring, and is abbreviated TX.

Prostaglandin I2 and thromboxane A2

PGl2 was first identified in 1976 (24) and is formed by the prostacyclin synthase, a member of the cytochrome P450 monoxygenase superfamily (25). Structural elucidation of the human prostacyclin synthase was not obtained until 2006 (26), confirming the mechanism of PGH2 endoperoxide bridge rearrangement proposed by Hecker and Ullrich in 1989 (27). The active site heme-iron interacts with the C-11 oxygen, promoting the hemolytic cleavage of the endoperoxide bond and the formation of an ether linkage between C-9 and C-5. The PGI2 ring is highly labile and rapidly hydrolyzed to form the stable but biologically inactive 6-keto PGF1α, and because of this, PGI2 and 6-keto PGF1α are often used interchangeably in the literature. PGI2 binds the G-coupled protein receptor IP (28, 29) as well as the transcription factors peroxisome proliferator-activated receptor (PPAR) α, PPARβ, and PPARγ (30, 31).

Identified in 1975 (32), thromboxane A2 (TXA2) is the physiological counterbalancing signal to PGI2. Similar to PGI2, its biosynthesis is catalyzed by a member of the cytochrome P450 superfamily member, thromboxane A synthase (27, 33). This enzyme facilitates rearrangement
of the PGH₂ endoperoxide bridge by a complimentary mechanism to prostacyclin synthase, interacting with the C-9 oxygen to promote endoperoxide bond cleavage. The C-11 oxygen radical initiates intramolecular rearrangement, resulting in either the formation of TXA₂ or 12-hydroxyheptadecatrienoic acid. TXA₂ contains an unstable ether linkage that is rapidly hydrolyzed in vivo under aqueous conditions to form the biologically inert TXB₂. It also binds a specific G protein-coupled receptor (GPCR), termed the TP receptor, which culminates in increasing intracellular calcium concentrations (33).

Prostaglandin E₂

PGE₂ may be the best characterized signaling molecule within the eicosanoid class. A PubMed search for “PGE₂” reveals that, since the identification and isolation of PGE₂ from human seminal plasma in 1963 (34), well over 27,000 articles have been published studying this molecule. The effects of PGE₂ have been implicated in innumerable biological processes and disease pathologies, including parturition, bronchodilation, pain signaling, innate and adaptive immune responses, cancer, arthritis, and atherosclerosis (4, 35). The wide spectrum of PGE₂ signaling can be partially attributed to differences in expression and function of its four known receptors, EP₁₄. The EP receptors are all GPCRs, and their most potent natural ligand is PGE₂. Functionally, EP₁ and EP₃ mediate their responses through calcium signaling, whereas EP₂ and EP₄ do so by increasing cAMP levels. Likewise, PGE₂ synthesis occurs through three unique enzymes, named the cytosolic PGE synthase (cPGES), microsomal PGE synthase-1 (mPGES-1) and mPGES-2 (35).

Cytosolic PGE₂ synthase was identified by Tanioka et al. (36) in 2000 from rat brain and determined it to be identical to human p23. Previously, this protein was identified as a chaperone protein that interacts with heat shock protein-90, which upregulates cPGES activity in vitro. The catalytic activity of cPGES requires glutathione and the conserved Tyr-9 residue for activity, similar to other cytosolic glutathione S-transferases, including hematopoietic PGD synthase (35, 37). In this case, the glutathione thiol is expected to interact with the C-9 oxygen of PGH₂, facilitating cleavage of the endoperoxide bridge and the formation of PGE₂.

mPGES-1 expression is coregulated with COX-2, and both proteins colocalize to the endoplasmic reticulum.

Fig. 3. PG nomenclature and structure. Arachidonic acid carbons are numbered 1–20, starting from the carboxylate. The prostaglandin letter indicates composition of the prostane ring, with PGH₂ as an example.
the endoperoxide ring. Exogenous sulfhydryl compounds glutathione via Tyr-8, such that the glutathione thiol functioned estimated a 5 Å distance between the Cys-65 thiol and the C-11 oxygen, consistent with this model. Following crystallization in 1997, Kanaoka et al. proposed a mechanism for l-PGDS-2 catalysis. A hydrogen bonding network between Phe-112, Cys-110, Cys-113, and Tyr-107 lowers the Pka of Cys-110 such that it can donate its thiol hydrogen to the C-11 oxygen. This leads to cleavage of the endoperoxide bridge and the creation of a ketone by the C-9 oxygen, forming PGE2. The final step is facilitated by free thiols, activated by Tyr-107; however, this does not appear to be necessary for productive catalysis.

Prostaglandin D2

PGD2 is a structural isomer of PGE2, and early studies of D-series PGs regarded them as side-products of E-series biosynthesis (44). Whereas the prostane ring on PGE2 has a 9-keto and 11-hydroxy moiety, the positions of these substituents are reversed on PGD2. In the late 1970s, PGD2 was identified as a major product in rat brain homogenates (45) and activated mast cells (46). PGD2 is formed by two evolutionarily distinct, but functionally convergent, prostaglandin D synthases (PGDS): the lipocalin-type (l-PGDS) and hematopoietic-type (h-PGDS). One critical difference between the PGDSs is the requirement for glutathione; l-PGDS can function without this cofactor, while h-PGDS uses it for catalysis. Urade et al. (47) proposed a mechanism for l-PGDS, whereby the thiol from Cys-65 forms a transient bond with the C-11 oxygen of PGH2, opening the endoperoxide ring. Exogenous sulfhydryl compounds then remove the C-11 hydrogen, catalyzing in PGD2 production and release. NMR structural studies and molecular modeling estimated a 5 Å distance between the Cys-65 thiol and the C-11 oxygen, consistent with this model (48). Following crystallization in 1997, Kanaoka et al. (49) proposed that h-PGDS contains a pocket that activates glutathione by Tyr-8, such that the glutathione thiol functions analogous to Cys-65 of l-PGDS.

PGD2 has two known receptors, DP1 and chemoattractant receptor-homologous, molecule expressed on TH2 cells (CRTH2) (50). DP1 belongs to the family of GPCR that includes TP, EP1,4, and FP, and ligand binding leads to intracellular increases of cAMP. While exhibiting similar PGD2 binding, CRTH2 shares little homology with DP1 and appears more closely related to the leukotriene B4 receptors BLT1 and BLT2. CRTH2 activation leads to elevation of intracellular calcium and decreased cAMP levels (51).

Prostaglandin F2α

PGF2α was isolated and structurally characterized in 1963 by Samuelsson (34) from human seminal fluid and demonstrably stimulated smooth muscles from rabbit duodenum. Since then, it has been implicated in a number of physiological processes and disease states (52), including endometrial cycling, embryo development, parturition, vasconstriction, as well as acute inflammation, oxygen-depravation injury, and atherosclerosis. Only one PGF2α-specific receptor has been cloned (53), a GPCR termed FP, which upon binding ligand results in an elevation of intracellular calcium. To date, three PGF2α biosynthetic enzymes have been cloned and characterized (54): prostaglandin F synthase (PGFS) (55), prostamide/PGFS (56), and 9-keto prostaglandin reductase (9K-PGR).

The human PGFS was cloned in 1999 by Suzuki-Yamamoto et al. (55) and determined to be identical to aldol-keto reductase 1C3 (AKR1C3). Similar to mPGES-2 and h-PGDS, PGFS evolved from the thioredoxin protein family and contains the putative C-x-x-C motif. Crystal structures of PGFS were published in 2004 (57) and 2006 (58) by Komoto et al. and used to propose a mechanism for PGF2α catalysis. In this model, no specific amino acids coordinate with the PGH2 endoperoxide; however, the reactive hydrogen of NADPH interacts with the C-9 oxygen of PGH2. This leads to a hydride shift and cleavage of the endoperoxide bond, whereby the C-11 oxygen can be protonated by H2O to complete the reaction. Interestingly, PGFS can also take PGD2 as a substrate. They suggest that His-117 and Tyr-55 form hydrogen bonds with the carbonyl at C-9, promoting an analogous hydride shift from NADPH onto C-9. This forms a stable hydroxyl moiety and the creation of 11β-PGF2α.

Structural comparisons show that PGFS is nearly identical to AKR1C1 and AKR1C2, with only seven amino acid differences, and their structures superimposable (57); however, these substitutions all incorporate smaller amino acids to form a larger active site cavity and mitigate potential steric hindrance with PG substrates predicted in AKR1C1 and AKR1C2. An in vitro analysis of purified AKR-1C1, AKR-1C2, and AKR-1C4 confirms they have no significant activity toward PG substrate (59). To date, neither the mouse or rat homologs of PGFS have been conclusively cloned and identified.

Prostamide/PGFS was identified in mouse and swine brain in 2008 (56), and the recombinant murine protein was expressed and characterized. Like PGFS, prostamide/PGFS is classified as a thioredoxin protein, and its C-x-x-C motif is required for productive catalysis. This protein facilitates the reduction of PGH2 to PGF2α by a similar mechanism to PGFS, involving a hydride transfer from NADPH; in contrast to PGFS, it does not appear to productively reduce either PGE2 or PGD2 but instead takes PGH2-ethanolamide as a substrate. A comparison between the swine and murine protein demonstrated similar catalytic parameters, and based on sequence homology, it is predicted that its function is well conserved in mammalian species, including human and rat.

In addition to using PGH2 and PGD2 as substrates for the formation of PGF2α, it has been shown that PGF2 can be reduced by 9K-PGR activity to form this molecule (60). In 2000, Asselin and Fortier (61) identified and char-
characterized bovine AKR1C5 as the putative 9K-PGR but have yet to definitively characterize a functional homolog in human, mouse, or rat. Since previous work has shown that only a few substitutions can radically alter the substrate specificity of enzymes in the AKR family, homology modeling in absence of in vitro data must be viewed with caution.

Carbonyl reductase-1 (CBR-1) has also been demonstrated to reduce a number of exogenous and endogenous metabolic substrates, including 9-keto reductase activity on PGE₂ to form PGF₂α (62, 63). Its high $K_v$ value compared with other substrates indicates that PGE₂ may not be an endogenous substrate; however, a number of studies have implicated murine Carbonyl reductase-1 in vivo with lower PGE₂ levels (64) and alterations in the PGE₂/PGF₂α ratio (65), warranting more thorough investigation into its role in eicosanoid metabolism.

Cyclopentenone prostaglandins

Cyclopentenone PGs comprise a family of molecules that are formed by dehydration of hydroxyl moieties PGE₂ and PGD₂ (66). Dehydration of PGE₂ leads to PGD₂, which has been shown to isomerize and form PGC₂. This highly unstable molecule rapidly undergoes a secondary isomerization, culminating in PGB₂. Analogous to PGE₂, PGD₂ dehydration of the prostane ring forms PGL₂. When PGL₂ isomerizes to form Δ12-PGJ₂, it promotes a secondary dehydration of the C-15 hydroxyl culminating in 15d-PGJ₂. Cyclopentenone PGs contain highly electrophilic α,β-un saturated carbonyls that have been shown to react with the thiol moiety on cysteinyl residues, either on glutathione or cellular proteins. PGₐ₂ and PGₐ₂ contain a single α,β-un saturated carbonyl, while both Δ12-PGJ₂ and 15d-PGJ₂ contain two of these electrophilic centers.

Cyclopentenones can exert their effects through both receptor-mediated signaling and covalent protein interaction. Dehydration dramatically lowers the affinity of a cyclopentenone for the PG receptor of its precursor PG molecule. However, 15d-PGJ₂ has been identified as a high affinity ligand for the transcription factor PPARα as well as a less potent activator of PPARβ and PPARδ. It is unclear whether the levels of 15d-PGJ₂ necessary to activate PPARs are high enough to elicit this response in vivo (67). Evidence suggests that nonreceptor-mediated effects of cyclopentenones can be attributed to their electrophilic α,β-un saturated carbonyl centers. Many of these biological actions can be recapitulated by cyclopentenone, while structurally related molecules lacking a reactive center, such as PGB₂, are unable to elicit these effects.

5-Lipoxygenase Metabolites

5-Lipoxygenase and 5-lipoxygenase activating protein

Metabolites from the 5-lipoxygenase (5-LOX) pathway were first structurally characterized in 1979, when Murphy, Hammarstrom, and Samuelsson (68) identified leukotriene C₄ as a component of the slow-reacting substance of anaphylaxis. Leukotrienes comprise a family of bioactive signaling molecules formed by the activity of 5-LOX on arachidonic acid (see supplementary Table III). 5-LOX contains a nonheme iron bound by three histidine residues, which it uses to catalyze the addition of molecular oxygen into arachidonic acid. 5-LOX was cloned in 1988 (69), but a three-dimensional structure has not yet been determined. However, attempts have been made to model the human 5-LOX sequence onto the rabbit 15-LOX crystal structure, which has 57% sequence identity. Similar to cPLA₂, 5-LOX has a catalytic domain linked to a C2 domain that facilitates translocation to the membrane surface. Phosphorylation of Ser²⁷¹ and Ser⁶⁶³ increase activity, whereas Ser⁵²³ phosphorilation inactivates this enzyme. 5-LOX also appears to have an ATP binding site that does not require hydrolysis, but instead stabilizes its structure.

Unlike other lipoxygenases, 5-LOX requires the presence of 5-LOX activating protein (FLAP) for productive leukotriene synthesis in vivo. FLAP is a member of the MAPEG superfamily, localizing to the nuclear envelope. Recently, a group at Merck Research Laboratories led by Joseph W. Becker succeeded in crystallizing FLAP bound either both MK-886 and MK-591, inhibitors of 5-LOX metabolism (70). They suggest FLAP exists as a trimer, creating a 3200 Å binding pocket that allows arachidonic acid to laterally diffuse into the protein complex from the membrane. The cytosolic loops of FLAP interact with the 5-LOX catalytic domain and transfer arachidonic acid into the 5-LOX active site. The rat 5-LOX and FLAP have not been expressed for specific characterization by in vitro systems; however, both the 5-LOX and FLAP have been studied using chemical inhibitors, their sequences are highly conserved (>90% identity with their human and mouse homologs) and are generally accepted to play a role in leukotriene biosynthesis.

5-LOX performs the initial enzymatic step in leukotriene synthesis (71), creating 5-hydroperoxy-eicosatetraenoic acid (5-HpETE) by incorporating one molecular oxygen at the C-5 position of arachidonic acid. Depending on cellular conditions, 5-HpETE has a number of potential metabolic fates. It can be secreted in its peroxide form, reduced to 5-HETE, or undergo a catalytic rearrangement in the 5-LOX active site to form leukotriene (LT) A₄ (Fig. 4). When produced in vivo, LTA₄ can be acted on by LTA₄ hydrolase (LTAH) to generate the stereospecific LTB₄ or used as a substrate by LTC₄ synthase (LTCS) to catalyze the conjugation of the glutathione to form LTG₄. Additionally, LTA₄ serves as a precursor for lipoxin biosynthesis, described in greater detail in subsequent sections. Nonenzymatic hydrolysis of the LTA₄ epoxide creates all four potential stereoisomers of LTB₄, including Δ6-trans LTB₄, 12-epi LTB₄, and Δ6-trans, 12-epi LTB₄. The enzymes involved in leukotriene metabolism are described here, and a thorough review of the subject has been published by Murphy and Gijon (71).

5-HETE and 5-oxoETE

Current literature suggests that 5-HETE itself does not appear to play a significant role in biological signal-
ing. However, it can be further reduced by 5-hydroxy-
eicosatetraenoic acid dehydrogenase (5-HEDH) to form
the bioactive 5-oxo-eicosatetraenoic acid (5-oxoETE). While
the gene has not yet been cloned, the biophysical properties
of the human enzyme have been well characterized by Powell
and Rokach (72). It localizes to the microsomal fraction of
a number of cell types, including neutrophils, monocytes,
eosinophils, and platelets. It is highly selective for 5-hydroxyl
lipids, as HETEs with hydroxyl groups at the 8 through 15 po-
sition are not significantly metabolized. It requires NADP
as
a cofactor, which regulates activity levels in vivo. When neu-
trophils activate NADPH oxidase, responsible for super-
oxide formation during the respiratory burst, it generates
NADP+ and leads to full activity of 5-hydroxy-eicosatetraeu-
noic acid dehydrogenase in vivo. Activation of the human
5-hydroxy-eicosatetraenoic acid receptor (OXE, previously
known as orphan G-coupled protein receptor R527) leads
to increased intracellular calcium concentrations and in-
hibition of cAMP production (73). This receptor is highly
expressed in neutrophils, eosinophils, and alveolar macro-
phages and appears to plays a role in chemotaxis. The
mouse and rat homologs have not yet been identified.

**Leukotriene B₄**

Leukotriene B₄ was characterized as a 5-LOX metabolite produced by polymorphonuclear leukocytes (74) and demonstrates potent autocrine activation of chemotaxis and aggregation (75). Leukotriene B₄ signals through two GPCR receptors, BLT₁ and BLT₂, which lead to increased intracellular calcium levels (76). BLT₁ has ~20-fold higher affinity for LTB₄ compared with BLT₂ and is highly expressed in leukocytes. Studies using BLT₁ knockout mice have primarily attributed a number of LTB₄-dependant signaling responses to this receptor, including neutrophil chemotaxis (77), neutrophil recruitment in vivo (78), and LTB₄-induced leukocyte rolling to firm adhesion (77). BLT₂ has broader substrate specificity (76), with 12-HETE, 12-HpETE, and 15-HETE demonstrating dose-dependant binding properties; furthermore, it displays a more ubiquitous expression pattern in humans, where in addition to leukocytes it has been detected in the spleen, liver, ovary, pancreas, heart, prostate, lung, and colon. In 2008, Okuno et al. (79) demonstrated that 12-hydroxyheptadecaenoic acid showed 10-fold higher affinity for human BLT₂ compared with LTB₄, leading to reconsideration of LTB₄ binding as this receptor’s primary function.

**LTAH**

LTAH is zinc-dependant metalloprotein that leads to the stereospecific hydrolysis of the LTA₄ to form the dihydroxy
LTB₄ (80, 81). Its activity was characterized from plasma samples in a wide range of mammalian species (82) prior to being sequenced from human lung cDNA libraries in 1987 (83). Homology studies revealed that, in addition to other zinc hydrolases, this enzyme shares sequence similarity with a number of zinc-dependant aminopeptidases (71). LTAH exhibits aminopeptidase activity, and LTB₄ biosynthesis can be blocked by peptidase inhibitors (81),
suggesting a potential evolutionary origin. The first high-resolution crystal structure of LTAH was published in 2001 (84) and used to propose its catalytic mechanism. The active-site zinc ion and Glu-271 coordinate with a free water molecule and position it for deprotonation by the LTA4 epoxide oxygen. The delocalized carbocation created between C-6 and C-12 is attacked by a second water molecule, directed to C-12 by Asp-375 in a stereospecific manner forming LTE4.

Cysteinyl leukotrienes

Cysteinyl leukotrienes LTC4, LTD4, and LTE4 together make up the slow-acting substance of anaphylaxis and play an important signaling role in inflammation (85). They can be synthesized by a number of different cell types, including mast cells, eosinophils, neutrophils, basophils, and macrophages. Two receptors have been implicated in cysteinyl leukotriene signaling, cysLT1 and cysLT2. The expression of cysLT1 appears to be highest in cell types that produced these metabolites, whereas cysLT2 is more broadly expressed and can be found in the heart, brain, and vasculature (86). These receptors are both GPCRs that culminates in intracellular calcium mobilization and have similar binding affinities; cysLT1 has a rank order of that culminates in intracellular calcium mobilization and vasculature (86). These receptors are both GPCRs broadly expressed and can be found in the heart, brain, and vasculature (86). These receptors are both GPCRs that culminates in intracellular calcium mobilization and have similar binding affinities; cysLT1 has a rank order of

Leukotriene C4

In 1979, it was demonstrated that LTA4 could be conjugated to glutathione to form leukotriene C4, the first identified component of the slow-reacting substance of anaphylaxis (68). The enzyme mainly responsible for LTC4 production is LTCS, identified as a transmembrane protein from the MAPAG superfamily that includes FLAP and mPGES-1 (87, 88). Other MAPEG enzymes, mGST-2 and mGST-3, also exhibit LTC4 synthase activity and appear to play a role in LTC4 biosynthesis in epithelial cells and the testis (89, 90). However, LTCS knockout mice demonstrate its role as the primary contributor to LTC4 biosynthesis in vivo.

It wasn’t until 2007 that the LTCS structure was solved using X-ray crystallography (91, 92), suggesting the following mechanism of catalysis. Leukotriene C4 synthase forms a homotrimer, with each monomer containing four transmembrane α-helices. The LTCS active site forms at the interface between two adjacent monomers. One monomer binds glutathione in a U-shaped conformation that positions the thiol for deprotonation by Arg-104 and subsequent nucleophilic attack of C-6. The Arg-31 of the second monomer donates a proton to form a hydroxyl group at C-5. The α-terminus of LTA4 binds a hydrophobic cavity in the interface that is capped by Trp-116, serving as a molecular “ruler” to facilitate LTCS selectivity for 20-carbon polyunsaturated fatty acids.

Leukotriene D4

Soon after its discovery, it was recognized that the glutathione amino acids of LTC4 could be cleaved by endogenous peptidases. The first enzyme identified to work on LTC4 was γ-glutamyl transpeptidase (GGT) (93), which removes glutamic acid to form LTD4. In addition to LTD4, it can hydrolyze a broad range of glutathione-containing metabolites proceeding through a modified ping-pong mechanism thought to involve Arg-107 and Asp-423 in the human isoform (94, 95). GGT resides on the extracellular membrane surface, and its activity can be detected in a number of tissues and fluids, including blood plasma (93, 94).

Interestingly, separate studies using GGT knockout mice and human subjects deficient in GGT both demonstrated residual activity, suggesting GGT was not solely responsible for LTD4 production. Using a human cDNA library, Heisterkamp et al. (96) identified related GGT gene, originally termed GGT-rel, with 40% amino acid similarity. This enzyme effectively hydrolyzed LTC4 while having low activity toward other glutathione substrates for GGT, suggesting it could act specifically on cysteinyl leukotrienes. In the GGT-knockout mouse study, the residual peptidase activity toward LTC4 was purified from intestinal tissue homogenates (95). This activity was originally termed γ-glutamyl leukotrienase (GGL) due to its selectivity for LTC4 as a substrate. Subsequent cloning and sequencing confirmed that murine GGL was a homolog to human GGT-rel gene (97). Both GGT and GGL appear to play a functional role in LTC4 metabolism, as demonstrated by metabolism studies using single and double knockout mice (98). Neither of the rat homologs have been characterized.

Leukotriene E4

Leukotriene D4 can be further metabolized by dipeptidases. The first such enzyme characterized was the human membrane-bound peptidase-1 (MBD-1) (99), which facilitates the hydrolysis of a broad range of cysteinyl-glycine adducts. MBD-1 is a metalloproteinase that uses an active-site zinc ion to facilitate the hydrolysis of the amide between dipeptides; in leukotriene biochemistry, it catalyzes the metabolism of LTD4 to LTE4. Using an MBD-knockout mouse, overall LTE4 levels decreases, but significant levels of LTE4 were detectable; furthermore, the changes in activity varied between tissues, with some losing >50% of their activity with others remaining unaffected (100), suggesting the presence of additional genes in LTD4 metabolism. Two additional MBD genes, MBD-2 and MBD-3, were identified from a BLAST search of the mouse genome (101); expression of MBD-2 determined it was capable of hydrolyzing LTD4 into LTE4, whereas MBD-3 did not productively metabolize LTD4. The rat homologs have not been studied in vitro to date.

OTHER LIPOXYGENASE METABOLITES

Lipoxygenases

Lipoxygenases are nonheme iron metalloproteins that catalyze the stereoselective insertion of molecular oxygen into arachidonic acid (71, 102). While the three-dimensional
structures of most mammalian lipoxigenases have not been solved, the structure of soybean LOX (103, 104) and rabbit leukocyte-type 12/15-LOX (105) have been reported and used as the basis for understanding other lipoxigenases through homology modeling. The LOX reaction mechanism involves an iron-catalyzed hydrogen abstraction from arachidonic acid at C-7, C-10, or C-13, forming a conjugated radical reaching two carbons in either direction (106). The structure of a given LOX enzyme generally conforms tightly around the fatty acid, and small channels within the protein direct molecular oxygen toward selected carbons, facilitating the formation of the following HpETEs and corresponding HETEs: 8-HETE, 12-HETE, and 15-HETE (see supplementary Table III). With the exception of 12R-LOX, mammalian LOXs typically direct the oxygen to attack the pro-S face of arachidonic acid. The positional specificity by which a LOX incorporates oxygen onto arachidonic acid is determined by how deeply it penetrates the cavity, as well as the orientation (either C terminus or ω terminus) it enters the active site. Work by Jisaka et al. suggests that two amino acid positions opposite of the catalytic iron ion determine the entry orientation. When a histidine and a bulky aromatic amino acid fill this position, arachidonic acid C terminus enters the active side and promotes 5-LOX and 8-LOX activity. Substitution of these amino acids with either a glutamine or aspartate alongside an aliphatic residue promotes the entry of the ω terminus and facilitates 12-LOX and 15-LOX activity.

Hydroxyeicosatetraenoic acids

In mammalian systems, lipoxigenase activities have been implicated in the biosynthesis of 5-HETE (see above), 8-HETE, 12-HETE, and 15-HETE (71, 106). The HETEs have been implicated as potential ligands for PPARα (107) and PPARγ (108). They exert numerous biological effects, including, but not limited to, mitogen-activated protein kinase signaling (109), monocyte chemoattractant protein-1 expression (110), angiogenesis (111), cancer growth and metastasis (112, 113), and neuronal apoptosis (114); however, the precise molecular mechanisms behind these effects remain unclear. Interestingly, 12- and 15-HpETE, but not the corresponding HETEs, have been shown to directly activate the capsaicin-sensitive vanilloid receptor (VR1) (115), which is involved in inflammatory pain signaling.

Hepoxilins

Hepoxilins are biologically relevant signaling molecules produced by certain 12-LOXs (116) (Fig. 5). Two hepoxilins have been identified, HXA₃ and HXB₃, both of which incorporate an epoxide across the C-11 and C-12 double bond, as well as an additional hydroxyl moiety (117); HXA₃ has a C-8 hydroxyl, whereas the HXB₃ hydroxyl occurs at C-10. The epoxy moiety is labile and can be hydrolyzed either by a hepoxilin specific epoxide hydrolase (HXEH) (118) or in acidic aqueous solution to form the corresponding diol metabolites trioxilin A₃ and B₃ (117). Most of the known bioactive effects can be attributed to HXA₃, which include intracellular calcium release (119), neutrophil migration (120), insulin secretion (121), ichthyosis (122), and modulation of neuronal signaling (123). Current evidence suggests the existence of a HXA₃ receptor (124), but to date one has not been identified.

Lipoxins

Early studies of arachidonic acid metabolism demonstrated that multiple lipoxigenases can act on the same substrate to generate a new class of lipid mediators termed lipoxins (LX) (125). Lipoxins A₄ and B₄, structurally characterized from human neutrophils incubated with 15-HpETE, each contain three hydroxyl moieties and a conjugated tetraene. The third hydroxyl of LXA₄ is positioned at C-6, and of LXB₄ at C-14.

The action of 5-LOX, in concert with a 12-LOX or 15-LOX activity, has been shown to produce lipoxins by three distinct pathways (126), which often occur between heterogeneous transcellular interactions in vivo. Neutrophil 5-LOX can produce and secrete LTA₄ that is taken up by platelets, where it is acted upon by 12-LOX-p to form lipoxins (127). Likewise, 15-LOXs can generate either 15-HpETE or

![Fig. 5. Structures of 12-lipoxigenase metabolites. 12-Lipoxigenase creates 12-HpETE, which can further isomerize to form HXA₃.](http://www.jlr.org/content/suppl/2009/09/16/R900004-JLR20/0.DC1.html)
15-HETE that can be taken up by monocytes and neutrophils, where highly expressed 5-LOX uses it to generate lipoxins (125). Finally, aspirin acetylated COX-2, rendered unable to synthesize PGs, can act as a 15-LOX. This leads to the formation of 15R-HETE and culminates in creation of epi-lipoxins (128), which have altered stereochemistry at the C-15 hydroxyl but similar biological potency.

Lipoxins are the first lipid mediators discovered that demonstrated anti-inflammatory activity as well as the capacity to promote the resolution of inflammation and return to tissue homeostasis (6, 126, 129). Their biological activity has been shown to be mediated through the lipoxin A4 receptor (ALX), cysLT1, and the aryl hydrocarbon receptor, a nuclear transcription factor (129). The ALX receptor is a GPCR that upon binding ligand can lead to intracellular calcium increases, PLD activation, and decreased NF-κB activity. Additionally, ALX cross-talks with vascular endothelial growth factor receptor, connective tissue growth factor receptor, and platelet-derived growth factor receptor to downregulate their activity. This receptor can also bind a number of small peptides; some dock the LXA4 binding site, while others appear to function at a different site and lead to distinct downstream signaling events. LXA4 binds to recombinant cysLT1 with approximately equal affinity as LTD4 (130) and has been suggested to function as a competitive inhibitor. Lipoxin binding to the aryl hydrocarbon receptor transcription factor leads to the expression of suppressor of cytokine signaling-2 in dendritic cells (131, 132). On a pathological level, the lipoxin signaling promotes inhibition of neutrophil migration, the recruitment of nonphlogistic macrophages, and increased phagocytosis among the countless documented effects (6, 126). The complex nature of lipoxin receptor signaling has been reviewed by Chiang et al. (129) in greater detail.

**Eoxins**

Analogous to 5-LOX biosynthesis of LTA4, 12/15-LOX-1 can form an epoxide across C-14 and C-15 to form 14,15-LTA4 (133). Feltenmark et al. (134) demonstrated that murine eosinophils and mast cells can use 14,15-LTA4 to generate structural analogs of the cysteinyl leukotrienes, which they termed eoxins (EX). The enzymatic mechanism of eoxin biosynthesis has not been definitively elucidated, but likely occurs by a similar pathway as the cysteinyl leukotrienes (Fig. 6). Eoxins had weaker contractile activity on guinea pig pulmonary parenchyma or ileum (155, 136); however, in a cell culture model of epithelial barrier function EXC4, EXD4, and EXE4 demonstrably effected epithelial permeability at a lower dose than histamine (134).

**Phylogenetic classification of LOX enzymes**

Historically, lipoxygenases have been classified according to their activity (102). This system works well for the 5-LOX, where the human isoform catalyzes 5-HETE formation in a similar manner to the mouse and rat homologs. However, it becomes more confusing when applied to other lipoxygenases, whose positional specificities are not as clearly defined or tightly conserved between mammalian homologs. For example, the leukocyte 12/15-LOX (12/15-LOX-4) from mouse produces significant amounts of both 12-HETE and 15-HETE (137). The human isoform of 15-LOX-2 produces 15-HETE, whereas the mouse homolog forms 8-HETE (138). For these reasons, an alternative classification scheme based on phylogeny has been proposed (102), describing the following 8-LOXs, 12-LOXs, and 15-LOXs: platelet-type 12-LOX (12-LOX-p), epidermis-type 12-LOXs (12-LOX-e, 12R-LOX, and e-LOX-3), leukocyte-type 12/15-LOX (12/15-LOX-l), and 8/15-lipoxygenase (8/15-LOX-2).

**Platelet-type 12-LOX**

In 1974, Hamberg and Samuelsson (139) described the activity of a lipoxygenase in platelets that converted arachidonic acid into 12-HETE. The human enzyme was concurrently cloned by Funk, Furci, and FitzGerald (140) and Isumi et al. (141) in 1990, and due to its high level of expression in these cells has been termed platelet-type 12-LOX (12-LOX-p). Epidermis-type 12-LOXs (12-LOX-e, 12R-LOX, and e-LOX-3), leukocyte-type 12/15-LOX (12/15-LOX-l), and 8/15-lipoxygenase (8/15-LOX-2).

**Fig. 6.** Structure of eoxin C4. Eoxins are the 15-LOX analogs of the cysteinyl leukotrienes, where the thiol attachment occurs at C-14.
firmed the native gene’s positional specificity for producing 12-HETE (140, 145). While mutations designed to knock 12-lipoxygenase activity into the rabbit leukocyte-type 12/15-LOX (primarily a 15-LOX) succeeded (146, 147), the reverse mutagenesis experiments failed to convert 12-LOX-p into an effective 15-lipoxygenase (145). Other studies indicate that 12-LOX-p can function as a hepoxilin synthase on 12-HpETE (148), as well as a lipoxin synthase on LTA₄ (149).

Epidermal-type 12-LOXs (12-LOX-e, 12R-LOX, and e-LOX-3)

The epidermal type 12-lipoxygenase (12-LOX-e) was separately cloned from mice by Funk et al. in 1996 (150) and Kinzig et al. in 1997 (151). The gene was the same size as the platelet-type and leukocyte-type 12-LOX and had 60% identity to these enzymes. Expression of the murine 12-LOX-e gene confirmed its lipoxygenase activity, specifically producing 12(S)-HETE. By analogy, 12-LOX-e likely functions by a similar catalytic mechanism as other 12-LOX. Using in situ hybridization, 12-LOX-e expression was demonstrated in keratinocytes and targeted areas of the hair follicle. The human isoform of 12-LOX-e was identified as a nonfunctional pseudogene (152), while in rat its activity has not been investigated.

As early as 1975, scaly lesions caused by psoriasis, an inflammatory disorder that affects the skin and joints, were known to contain increased levels of stereochemically pure 12-HETE (153). Distinct from other lipoxygenase metabolites, the hydroxyl moiety of 12-HETE found in these lesions was in the R-configuration (154). In 1998, Boeglin, Kim, and Brash (155) discovered the source of the 12R-HETE originated from 12R-lipoxygenase (12-LOX), the first known mammalian R-lipoxygenase. Expression of 12R-LOX demonstrated that it produced 12R-HETE to the exclusion of significant amounts of 12S-HETE, as well as not making 5-HETE, 8-HETE, 9-HETE, 11-HETE, and 15-HETE. Using mutants of two R-lipoxygenases (human 12R-LOX and coral 8R-LOX) and two S-lipoxygenases (human and mouse 8/15-LOX-2 isoforms), Confal and Brash (156) identified a conserved amino acid, corresponding with 12R-LOX Gly-441, that confers a substantial amount of stereochemical control to these enzymes. At this position, an alanine-to-glycine mutation in S-LOXs reversed the chirality of lipid metabolites. However, while mutating the glycid residue of coral 8R-LOX fully converted it to a 12S-LOX, the 12R-LOX mutant had mixed activity. Meruvu et al. (157) identified Val631 as another important residue in facilitating the positional and stereochemical specificity of this enzyme.

Another epidermal-type LOX, e-LOX-3, has been cloned from both mouse (158) and human (159). While it contained the putative lipoxygenases’ structural features and had >50% homology with 12R-LOX, gene expression in a number of cell lines indicated it had no activity toward arachidonic acid (158). However, mutations in both 12R-LOX and e-LOX-3 were identified in patients afflicted with psoriasis (160), and in 2003, Yu et al. (161) identified e-LOX-3 as a hepoxilin synthase that specifically used 12R-HpETE as a substrate. This suggests that impairment of hepoxilin production by a 12R-LOX/e-LOX-3 pathway leads to psoriasis in human patients.

Leukocyte-type 12/15-LOX (12/15-LOX-1)

The human 12/15-LOX-l was originally cloned as a 15-LOX from a cDNA library in 1988 (162), followed by rabbit (163), rat (164), and mouse (165). The first structural elucidation of a mammalian lipoxygenase was reported using X-ray crystallography on the rabbit 12/15-LOX-1 homolog (105). The substrate binding pocket is lined by hydrophobic residues, and four residues comprise its base: Phe-353, Ile-418, Met-419, and Ile-593 (147). These residues are conserved in the human isomorph and are considered critical for promoting 15-LOX activity. By comparison, the mouse and rat 12/15-LOX-1 have amino acid substitutions at each of these positions that could explain their predilection toward 12-LOX activity. The rat 12/15-LOX-1 also has intrinsic hepoxilin synthase activity (148), whereas human homolog does not. Based on the evidence that human 12-LOX-p can synthesize hepxoxilins, Nigam et al. (116) surmised that lipoxygenase positional specificity, and not genetic homology, confer an enzyme with hepoxilin synthase activity. This would implicate the mouse 12/15-LOX-1 as a hepoxilin synthase, but to date it has not been experimentally examined. The human 12/15-LOX-1 exhibits oxygenase activity that generates 14,15-LTs (134); the rabbit (166), pig (133), and sheep (167) homologs also demonstrate this activity, suggesting that mouse and rat enzymes retain this capacity.

From even preliminary investigations (168), 12/15-LOX-1 has demonstrated positional promiscuity when oxidizing arachidonic acid, forming 15-HETE, 12-HETE, and 8,15-diHETE. The production of 12-HETE by 12/15-LOX-1 has been shown to increase expression of monocyte chemo-attractant protein-1, which increases vascular adhesion and the recruitment of macrophages (168). In addition to action on free arachidonic acid, 12/15-LOX-1 can function at the membrane surface and on LDL to oxidize phospholipid and cholesterol ester fatty acids, leading to the development of atherosclerosis (169). In 2008, Harkewicz et al. (170) identified 12/15-LOX-1 cholesterol ester peroxidases as the active components of minimally oxidized LDL. In contrast to producing pro-inflammatory molecules, 12/15-LOX-1 can take LTA₄ as a substrate and produce the anti-inflammatory lipoxins (6, 129). Using both knockout and overexpression transgenic mouse models, Merched et al. (171) propose that 12/15-LOX-1 helps suppress atherosclerosis. Undoubtedly, the capacity of 12/15-LOX-1 to make pro-inflammatory as well anti-inflammatory metabolites complicates the study of this enzyme at the whole-animal level, and future research will help elucidate its role in signaling.

8/15-lipoxygenase-2

In 1997, Brash, Boeglin, and Chang (172) reported the discovery of a second 15-LOX gene in humans. The murine homolog has primarily 8-LOX activity (173), while the rat
The homolog has not been characterized to date. Mutational analyses of these enzymes determined that substitution of Tyr-603 and His-604 to the corresponding residues in the human isoform (aspartate and valine) confers 15-LOX activity (174). Likewise, mutation of the human enzyme to the murine residues confers 8-LOX activity. Though these enzymes appear to have different metabolic output, overexpression of either isoform leads to similar biological consequences in murine keratinocytes (138). In this model, 8/15-LOX-2 expression, as well as exogenous addition of 15-HETE and 8-HETE, blocked premalignant cell growth in a manner attributed to the inhibition of DNA synthesis. Interestingly, both human and murine 8/15-LOX-2 have been shown to produce 8,15-diHETE in vitro (175), a more potent agonist of PPARα than 8-HETE. In these studies, the murine enzyme was capable of both reactions and sequentially oxygenates the C-8 and C-15 position. The human homolog could only perform the oxygenation of C-15 but was able to use 8-HETE as a substrate. Future investigations may yet elucidate other novel metabolites produced by 8/15-LOX-2 that have important bioactivities.

CYTOCHROME P450 METABOLITES

Cytochrome P450

Cytochrome P450 (CYP) enzymes comprise a diverse superfamily of enzymes present in bacteria, fungi, protists, plants, and animals (176, 177). The name was coined in 1962 to describe the first characterized cytochrome P450 based on the unusual absorbance peak of 450 nm by its carbon monoxide-bound form (178). CYP facilitate the addition of oxygen on a wide range of substrates. To date, 7232 genes in 781 families have been categorized, and the list is constantly growing as more complete genome sequences become available; the generally accepted nomenclature system and current list of known CYPs is curated by Dr. David R. Nelson (http://drnelson.utmem.edu/CytochromeP450.html). They play an important role in the metabolism of toxins in the liver as well as signaling throughout the body. In eicosanoid biosynthesis, CYP activities are classified by hydroxylase and epoxygenase activities.

Similar to LOXs, CYPs catalyze the hydroxylation and epoxygenation of arachidonic acid (Fig. 7). However, whereas LOX uses an active nonheme iron to abstract hydrogen directly from arachidonic acid, CYPs contain a heme-iron active site that oxidizes its substrate by a different mechanism (179, 180). Using NADPH as a cofactor, CYP performs a stepwise reduction of the catalytic iron and molecular oxygen to form a highly reactive oxo-iron species. Depending on the relative position of arachidonic acid, the reactive oxygen can perform an abstraction of an sp³ hybridized hydrogen or removal of an electron from an sp² hybridized carbon. In both cases, the transfer of oxygen to the unstable arachidonic acid intermediate terminates the reaction by forming HETE or epoxy-eicosatrienoic acid, respectively.

Each species has its own unique set of CYPs (181). In 2004, Nelson et al. (182) performed an extensive analysis of the human and mouse genomes, identifying 57 human and 102 mouse functional CYPs. They identified the same CYP families in human and mouse; however, four subfamilies have been evolutionarily discarded in humans, and a significant level of gene cluster size and organization has occurred. These gene expansions and mutations have significant impact on function, with subtle changes in the size and shape of the binding pocket capable of imparting dramatic changes in substrate selectivity and alignment, as well as product formation. In many cases, a single amino acid mutation can drastically alter the function of a CYP. For example, an S473V substitution on CYP2C2 allows a lauric acid hydroxylase to accept the steroid progesterone as a substrate (183). Thus, CYP homology modeling in the absence of experimental data may inaccurately represent in vivo function. For these reasons, we did not attempt to match CYP homologs between species (see supplementary Tables IV and V).

ω-Hydrolase HETEs

In addition to hydroxylation between C-5 and C-15 to produce LOX-like HETEs, the CYP enzyme
can add a hydroxyl moiety to the sp³-hybridized ω-carbons to form a unique class of HETEs (177). Historically, ω-hydrolase activity has been attributed to CYP4A and 4F in mammalian systems, though CYP from other families have demonstrable activity as well (see supplementary Table IV). The most well characterized of these is 20-HETE, hypothesized to play an important role in hypertension (184). 20-HETE has been shown to promote systemic vasconstriction by inhibiting Kca channel activity (185, 186), and in the kidney it blocks sodium reabsorption by inhibiting Na+/K+-ATPase activity (187). Other ω-HETEs demonstrate considerable bioactivity that often act in opposition to 20-HETE. For example, 18-HETE and 19-HETE dose-dependently induce vasodilatation by inhibiting the effects of 20-HETE (188). They also, in addition to 16-HETE and 17-HETE, have been shown to induce sodium reuptake in the kidney (188). More recently, 16-HETE has demonstrated the unique capacity among ω-HETEs to inhibit neutrophil adhesion, suggesting an important role in inflammation (189). Though it has been suggested that ω-HETEs signal through a putative receptor, neither the receptor nor its active second-messenger component has been identified (177).

Epoxyeicosatrienoic acids

The epoxidation of arachidonic acid by CYPs results in the formation of unique bioactive lipid mediators termed epoxyeicosatrienoic acids (EETs) (190). Each double bond has been shown to be susceptible to oxidation, resulting in 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET. In mammals, the most well documented CYP epoxigenases include the 2C and 2F families, although currently numerous others have been implicated in EET biosynthesis (see supplementary Table V). The CYP epoxidases display significant promiscuity, with most enzymes making more than one EET, as well as retaining a significant level of ω-hydrolase activity. EETs have been implicated in a number of important biological processes, including vascular tone, renal function, leukocyte adhesion, neuronal signaling, and angiogenesis. However, the effect of specific EET isomers often remains uncertain. In 1999, Node et al. (191) determined that 11,12-EET specifically block the expression of vascular cell adhesion molecule-1 by inhibiting NF-κB, an important mediator of leukocyte recruitment during inflammation. Using a model of vascular laminar flow, Liu et al. (192) suggest NF-κB inhibition can be achieved by any of the four EET stereoisomers.

Current evidence suggests that EET signaling occurs through a putative GPCR, though to date it has not been identified. Membranes from human U937 monocyte cell line were discovered by Wong et al. (193) to contain a high affinity binding site for 11,12-EET and 14,15-EET, which has recently been characterized as a GPCR that induces PKA activation and increases in cAMP (194). The EET-induced GPCR activity leads to the opening of BKca channels through the Gαg-protein (195, 196). Falck et al. (197) synthesized a library of 11,12-EET analogs and, using vascular cell adhesion molecule-1 expression in human endothelial cells, identified a number of structural elements required for functional recognition by the putative GPCR binding site. A corresponding study measuring BKca activity induced by a series of 14,15-EET analogs identified similar structural elements (198), suggesting these two EETs may bind the same receptor in vivo.

In addition to GPCR signaling, EETs have been identified as bioactive ligands for cation channels and PPARs. Watanabe et al. (199) identified 5,6-EET, and to a lesser degree 8,9-EET, as an endogenous ligand for the vanilloid type 4 receptor (TRPV4). When activated, TRPV4 acts as a cation channel to allow the influx of extracellular calcium, and its activity has been implicated in rodent models of inflammatory hyperalgesia (200–202). Downstream activation of BKca channels also result from TRPV4 (203). While these studies implicate EETs in the induction of hyperalgesia, Inceoglu et al. (204) demonstrate that inhibiting soluble epoxy hydrolase, which raises endogenous levels of EETs, blocks inflammatory hyperalgesia in the rat model. Receptor binding assays identified micrometer EET affinity for cannabinoid CB2, neurokinin NK1, and dopamine D3 receptors. In rat cardiomyocytes, EETs activate KATP channels by reducing their sensitivity to endogenous ATP inhibition (205). Studies have also implicated EETs in the direct activation of transcription factors PPARα and PPARγ (192, 206). However, the selectivity of these receptors for specific EET regioisomers as well as the downstream biological consequences have remained unclear, and undoubtedly further work will characterize the role that EETs play in biological signaling (207, 208).

Soluble epoxy hydrolase and dihydroxyeicosatrienoic acids

The majority of the EET biological activities are diminished by the hydrolysis to the corresponding dihydroxyeicosatrienoic acids (DHET). In mammals, this process is primarily catalyzed by the enzyme soluble epoxy hydrolase (sEH). The murine and human isoforms have been cloned and structurally characterized by X-ray crystallography (209–211), allowing for a detailed mechanistic description of its catalytic function using the human sequence (212). The sEH active site forms a deep, L-shaped hydrophobic pocket with a catalytic aspartyl at the L-turn accessible to both openings. Upon entering the active site, the EET epoxide moiety interacts with Tyr-382 and Tyr-463. His-523 positions and activates Asp-334 for a nucleophilic attack on an epoxide carbon, opening the ring and facilitating water hydrolysis to the corresponding diol. Unlike other eicosanoid biosynthetic enzymes, sEH does not require any metal cofactor for catalysis. However, its activity can be inhibited by the presence of zinc, suggesting a straightforward mechanism for regulating EET metabolism during inflammation (213). Due to the loss of EET bioactivity, sEH formation of DHETs has been considered the metabolic inactivation step of EET signaling. However, recent work has demonstrated that DHETs can retain certain EET signaling capacities, effectively activating BKca channels in smooth muscle cells (214), as well as PPARα and PPARγ (192, 215).
Nonenzymatic lipid metabolites

Free radicals generated by oxidative stress have long been believed to participate in the development of a number of neurodegenerative and inflammatory diseases by causing damage to DNA, proteins, and lipids (216). In 1990, Morrow et al. (217) described a class of PG-like compounds formed in vitro by free radical catalyzed peroxidation. Abstraction of hydrogen from C-13 forms a five carbon delocalized radical that can form a prostane ring when followed by the addition of molecular oxygen at the activated C-11. Due to the lack of enzymatic control in the reaction, these PG-like compounds comprise a series of stereoisomers termed 15-series due to the location of the nonprostane hydroxyl moiety. Furthermore, the reaction can also be initiated at C-7, leading to the 5-series, as well as at C-10, leading to both the 8- and 12-series of isoprostanes. In total, 64 possible isoprostane isomers can be generated by radical initiated peroxidation (216). To date, no specific isoprostane receptors have been identified. However, isoprostanes have demonstrated binding affinity for PG receptors, including the FP and TP receptor (218, 219).

Lipid peroxidation can also proceed forward in a lipoxygenase-like activity. Following the abstraction of hydrogen, the peroxide formed by oxygenation of the radical can simply be reduced to a corresponding HETE. By this mechanism, these PG-like compounds comprise a series of stereoisomers termed 15-series due to the location of the nonprostane hydroxyl moiety. Furthermore, the reaction can also be initiated at C-7, leading to the 5-series, as well as at C-10, leading to both the 8- and 12-series of isoprostanes. In total, 64 possible isoprostane isomers can be generated by radical initiated peroxidation (216). To date, no specific isoprostane receptors have been identified. However, isoprostanes have demonstrated binding affinity for PG receptors, including the FP and TP receptor (218, 219).

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11-Hydroxythromboxane B2 reductase

Studies investigating thromboxane clearance indicate that a significant portion undergoes dehydrogenation at C-11 to form 11dh-TXB2. This degradation metabolite is readily detected in both human blood plasma and urine (223, 224). The enzyme responsible for catalysis in vivo has been termed 11-dehydrothromboxane B2 dehydrogenase (11-TXDH) and has two distinct isofoms. Using amino acid composition analysis, the liver-type isofrom from swine has been classified as a cytosolic aldehyde dehydrogenase family member (225). The second enzyme, the kidney-type 11-TXDH, can catalyze the dehydrogenation but not the reverse reductions, distinguishing it from the liver type. While the human, mouse, and rat 11-TXDH isofoms have not been identified, 11dh-TXB2 has been detected in urine samples from these species. Historically, TXB2 and 11dh-TXB2 have been considered biologically inert due to their inability to activate the thromboxane TP receptor (226), but work by Böhm et al. (227) demonstrates that 11dh-TXB2 is an agonist of the CRTH2 receptor.

15-Prostaglandin dehydrogenase

The catabolism of selected PGs, leukotrienes, and HETEs is initiated by oxidation by 15-prostaglandin dehydrogenase (15-PGDH). Two distinct enzymes have been identified with this activity, 15-PGDH-I and 15-PGDH-II. However, the high Km values of 15-PGDH-II for most PGs suggest that the majority of the in vivo activity can be attributed to 15-PGDH-I (222). The 15-PGDH-I is NAD+ dependent and metabolizes E-series PGs, lipoxins, 15-HETE, 5,15-diHETE, and 8,15-diHETE, while other PGs appear to be less susceptible substrates (228). In all likelihood, 15-PGDH can metabolize a larger array of eicosanoids than those which have been tested.

The 15-PGDH-I genes have been cloned in human, mouse, and rat, but a crystal structure of the enzyme has not yet been determined. In 2006, Cho et al. (229) used the molecular modeling to develop a three-dimensional structure of the human protein and propose a catalytic mechanism. Following the sequential binding of NAD+ and PGE2, the 15-hydroxy is coordinated with Tyr-151, Ser-138, and Gln-148. Tyr-151 deprotonates the 15-hydroxyl, facilitating a hydride transfer from C-15 and the release of 15k-PGE2. The oxidation to a 15-ketone reduces the biological activity of most eicosanoid substrates, and loss of function has been implicated in the development of certain cancers (230). However, in 2007, Chou et al. (231) identified 15k-PGE2 as a ligand for PPARγ and an active component of murine 3T3-L1 fibroblast differentiation into adipocytes.

13-Prostaglandin reductase

The 13-prostaglandin reductases (13-PGR) metabolize eicosanoids by catalyzing NADH/NADPH-dependent double bond reduction. The gene was originally cloned from human and pig sources as leukotriene B4 12-hydroxydehydogenase (LTB4DH) (232), but later discovered to have dual functionality as a PG reductase (13-PGR-I) (233). In addition to reducing PGs, 13-PGR has been shown to use lipoxins as substrates (234). The resulting metabolites from 13-PGR activity show reduced biological activity, and PGE2 metabolites from this pathway are readily detected in urinary excretion (235). The rat protein has LTB4DH and 13-PGR activities, suggesting that these func...
tions are retained in the murine homolog (see supplementary Table VI).

Structural determinations of human 13-PGR-1 in complex with NADP$^+$ and 15k-PGE$_2$ were solved in 2004 and used to propose the following mechanism of catalysis (236). 13-PGR-1 forms a homodimer, with an active site pocket formed at the dimer interface. The 2'-hydroxyl group of NADPH forms a hydrogen bond with the C-15 oxygen on PGE$_2$, promoting the stabilization of an enolate intermediate. The NADPH hydride attacks the carbocation at C-13, followed by ketone formation and the release of the reduced metabolite 13,14-dihydro,15-keto-PGE$_2$ (dhk-PGE$_2$). LTB$_4$ oxidation likely proceeds by a similar mechanism as 15-PGDH, with a conserved tyrosine serving as the catalytic residue. In 2007, Chou et al. (231) reported the discovery of a second PG reductase gene (13-PGR-2) in mice that they demonstrate works by a similar reductive mechanism on 15k-PGE$_2$ but have not exhaustively studied its substrate selectivity or potential LTB$_4$DH activity.

Further metabolism for urinary excretion

As part of a Phase I clinical trial for LTB$_4$ administration, Berry et al. (237) performed an exhaustive study of its catabolism. In this study, urine samples from subjects injected with LTB$_4$ were analyzed, and 11 LTB$_4$ metabolites were identified. Based on the qualitative and quantitative analysis of these metabolites, they proposed a pathway for LTB$_4$ metabolism that, in addition to LTB$_4$-specific enzymes, proceeds through three main pathways of catabolism: β-oxidation, CYP ω-hydrolases, and glucuronidation (Fig. 8).

β-Oxidation

In addition to working on fatty acids, the β-oxidation metabolic pathway can capably act on the carboxyl moiety of PGs, leukotrienes, and HETEs, reviewed in detail by Diczfalusy (238). Studies in patients with Zellweger syndrome, a congenital disorder characterized by the absence of intact peroxisomes, determined that the majority of eicosanoid chain shortening occurs though peroxisomal β-oxidation pathways. Peroxisomal β-oxidation requires acyl-CoA oxidase, multifunctional enzyme, and acetyl-CoA acyltransferase activities (239); however, the specific isoforms implicated in eicosanoid degradation have not been conclusively identified. The subsequent losses of two (dinor) or four (tetranor) carbons significantly increases the hydrophilicity of these molecules for urinary excretion. With thromboxane as an exception, the majority of PG metabolites detected in urine have undergone β-oxidation of the C terminus, determined by Diczfalusy (238). Pharmacokinetic analysis of first generation lipoxin analogs suggests that this class also undergoes β-oxidation (240). The HETEs also undergo β-oxidation from the C terminus, with the exception of 5-HETE.

Likewise, leukotrienes also appear to only be β-oxidized from the ω-terminus following CYP ω-hydroxylation and subsequent carboxylate formation by further oxidation, suggesting that C-5 hydroxyl moieties modulate the efficacy of this degradation pathway. Consistent with this, LTB$_4$ injections in human patients lead to β-oxidation from the ω terminus (237).

CYP ω-hydrolases

The CYP ω-hydroxylated metabolites have a number of important bioactivities, as previously described. In addition, CYP hydrolases facilitate the catabolism of PGs, leukotrienes, and DHETs for urinary excretion (177). Hydroxylation of eicosanoids both reduces their bioactivity and increases their water solubility. Some ω-hydroxyl eicosanoids can be further metabolized by CYPs to form carboxylic acids (241, 242), allowing for β-oxidation of this end of the molecule. Following LTB$_4$ administration, LTB$_4$ metabolites were found to be hydroxylated at C-17. 
Fig. 9. Temporal genomic and lipidomic changes in eicosanoid biosynthesis in RAW264.7 macrophages in response to Kdo2-Lipid A stimulation. Greater intensity of red indicates increasing levels, greater intensity of green indicates decreasing levels, and gray represents no change in levels relative to unstimulated cells. Changes are represented as a function of time (left to right), where rectangles indicate mRNA data and circles indicate lipid data. When enzyme activities can result from multiple genes, each is represented as a separate line. The transcriptomic data will be reported by S. Subramaniam and S. Gupta (unpublished observations), and the lipidomic data will be reported by Gupta et al. (unpublished observations); both data sets are also publicly available at www.lipidmaps.org.
though C-20; however, the majority of these compounds had undergone further catabolism to form glucuronides at these sites (237).

**Glucuronidation**

In addition to action by CYPs, lipophilic molecules are further catabolized into water-soluble metabolites through the addition of the sugar glucuronide (243). In mammals, this reaction is catalyzed by members of the UDP-glucuronosyltransferase enzymes. To date, 15 UDP enzymes have been found in humans, and a study by Turgeon et al. (244) in 2003 using a HEK293 expression system found that most of them were capable of metabolizing LTB₄ and LOX-derived HETEs. Glucuronidation also plays a significant role in the catabolism of DHETs in humans, but rats excrete the diol form directly (245). In the human trials of LTB₄ metabolism, nearly all metabolites with a hydroxyl moiety were found to be glucuronidated, suggesting nonspecific catalysis of eicosanoids by UDPs plays a significant role in their removal from the body (237).

**APPLICATIONS OF A SYSTEMS APPROACH TO EICOSANOID BIOLOGY**

The LIPID MAPS consortium has produced preliminary studies comparing comprehensive lipidomic and transcriptomic changes in macrophages (7–9). In response to the lipopolysaccharide Kdo₂-Lipid A, RAW264.7 murine macrophage cells were analyzed for eicosanoids by LC/MS/MS, as well as transcriptomic changes using Affimetrix gene arrays. Using publicly available data (www.lipidmaps.org), these changes can be mapped together to investigate the correlation between genes and metabolites (Fig. 9). This stimulatory pathway causes upregulation of the numerous COX genes and downregulation of the 5-LOX pathway, causing primarily increases in COX metabolites (9); PGDH, which metabolically inactivates PGE₂, was downregulated, causing primarily increases in COX metabolites (9); PGDH, COX genes and downregulation of the 5-LOX pathway, stimulatory pathway causes upregulation of the numerous proteins from the entire activated macrophage proteome, the stable isotope labeling with amino acids in cell culture relative and absolute quantitation chemical tagging or of the eicosanoid proteins to give a fully integrated bio-system. Thus, it is timely to carry out a proteomic analysis of some samples. Provided purified protein standards are available, this methodology could be readily expanded to investigate the entire eicosanoid proteome.

The information contained herein provides the basis necessary to decipher the eicosanoid proteome and complete the integrated pathway map. Microarray analysis of gene regulation and mass spectrometry methodology for lipid analysis capable of comprehensively analyzing these pathways now exist. This review provides the tools necessary to create a complimentary proteomic methodology. The sequential flow of information from gene to protein to metabolite remains a central focus in biological research. However, the integration of eicosanoid transcriptomic, proteomic, and lipidomic platforms, if carried out, would represent the first time such an analysis was performed on any biological system. This would undoubtedly have enormous implications for our understanding of biology and disease pathology and serve as a model for future systems biology endeavors.

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