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Review

Coupling between cyclooxygenases and terminal prostanoid synthases

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Abstract

Biosynthesis of prostanoids is regulated by three sequential enzymatic steps, namely phospholipase A_2, cyclooxygenase (COX), and terminal prostanoid synthase. Recent evidence suggests that lineage-specific terminal prostanoid synthases, including prostaglandin (PG) E_2, PGD_2, $PGF_{2\alpha}$, PGI_2, and thromboxane synthases, show distinct functional coupling with upstream COX isozymes, COX-1 and COX-2. This can account, at least in part, for segregated utilization of the two COX isozymes in distinct phases of PG-biosynthetic responses. In terms of their localization and COX preference, terminal prostanoid synthases are classified into three categories: (i) the perinuclear enzymes that prefer COX-2, (ii) the cytosolic enzyme that prefers COX-1, and (iii) the translocating enzyme that utilizes both COXs depending on the stimulus. Additionally, altered supply of arachidonic acid by phospholipase A_2s significantly affects the efficiency of COX-terminal prostanoid synthase coupling. In this review, we summarize our recent understanding of the coupling profiles between the two COXs and various terminal prostanoid synthases.

Keywords: Cyclooxygenase; Terminal prostanoid synthase; Prostaglandin E synthase; Prostaglandin D synthase; Prostaglandin F synthase; Prostaglandin I synthase; Thromboxane synthase; Phospholipase A_2; Arachidonic acid

Biosynthesis of prostanoids involves oxidation and subsequent isomerization of membrane-derived arachidonic acid (AA) via three sequential enzymatic reactions. The initial step of this metabolic pathway is the stimulus-induced liberation of AA from membrane glycerophospholipids by phospholipase A_2 (PLA_2) enzymes. The released AA is sequentially metabolized to prostaglandin (PG) G_2 and then to PGH_2 by either cyclooxygenase (COX)-1 or COX-2. PGH_2 is then converted to various bioactive PGs (TXA_2, PGD_2, PGE_2, $PGF_{2\alpha}$, and PGI_2) by the respective terminal prostanoid synthases, which have different structures and exhibit cell- and tissue-specific distributions.

It is now believed that segregated utilization of COX-1 and COX-2 occurs in the distinct PG-biosynthetic pathways, even when they co-exist in the same cell. Generally, the constitutive COX-1 is mainly utilized in the immediate PG biosynthesis, which occurs within several minutes after stimulation with Ca^{2+} mobilizers, whereas the inducible COX-2 is an absolute requirement for delayed PG biosynthesis, which lasts for several hours following various stimuli. Although the precise molecular mechanisms underlying the functional segregation between the two COXs have still remained obscure, subtle differences in their subcellular location and enzymatic properties can account for this event [1–6]. It has been shown that COX-1 is located in the endoplasmic reticulum (ER) and perinuclear membranes, whereas COX-2 resides predominantly in the perinuclear envelope: this difference may influence the availability of the substrate AA released by distinct PLA_2 enzymes to each COX [1,2]. In addition, in vitro enzymatic analyses have pointed that effective catalysis by COX-2 can proceed at low hydroperoxide levels that are insufficient to sustain catalysis by COX-1 [3,4]. It is therefore conceivable that COX-2-mediated catalysis is preferred in particular cellular situations where AA supply is limited [4,5]. As an alternative possibility, it has been proposed that the two

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COX isozymes display distinct functional coupling with individual terminal prostanoid synthases. This concept was initially suggested for coupling between COXs and PGE synthase (PGES) enzymes [6,7], and subsequently between COXs and other terminal prostanoid synthases [8]. In this article, we focus on the properties and regulatory functions of PGES and other terminal prostanoid synthases in the context of COX coupling, which have been further supported by several in vivo studies using COX isozyme-selective inhibitors and gene-manipulated mice.

Coupling between COXs and PGESs

Preferred utilization of COX-2 over COX-1 in the PGE_2-biosynthetic pathway was initially reported in rat peritoneal macrophages, which produced thromboxane (TX) A_2 and PGD_2 through COX-1 in the A23187-induced immediate response, and PGE_2 and PGI_2 through COX-2 in the lipopolysaccharide (LPS)-induced delayed response [9,10]. PGE_2 production by osteoblasts occurred predominantly through COX-2, irrespective of the co-presence of COX-1 [11,12]. In a rat inflammatory model, COX-2-selective inhibitors reduced the accumulation of PGE_2 but not of other PGs [13]. These observations predicted the presence of a particular terminal PGES that is coupled with COX-2 in marked preference to COX-1.

Until now, three proteins that catalyze the conversion of PGH_2 to PGE_2 rather specifically have been identified; namely membrane-bound PGES (mPGES)-1 [7,14], mPGES-2 [15], and cytosolic PGES (cPGES) [6]. It is now apparent that, of these three enzymes, mPGES-1 represents a long sought, COX-2-preferential PGES. mPGES-1 is a glutathione (GSH)-requiring perinuclear protein belonging to the MAPEG (for membrane-associated proteins involved in eicosanoid and GSH metabolism) family [7,14]. As in the case of COX-2, expression of mPGES-1 is induced by proinflammatory stimuli and is down-regulated by anti-inflammatory glucocorticoid. Induction of mPGES-1 expression has also been observed in various systems in which COX-2-derived PGE_2 has been implicated to play a critical role, such as inflammation, fever, pain, female reproduction, tissue repair, and cancer [16]. Stimulus-inducible expression of mPGES-1 is in part regulated by the mitogen-activated protein kinase pathways [17], where the kinases may switch on the inducible transcription factor Egr-1 that in turn binds to the proximal GC box in the *mPGES-1* promoter, leading to mPGES-1 transcription [18]. Importantly, studies employing cotransfection of mPGES-1 and either COX isozyme, as well as those with small interfering RNA or antisense technology to knock down mPGES-1, have revealed that mPGES-1 is functionally coupled with COX-2 in marked preference to COX-1 [7,19]. Colocalization of COX-2 and mPGES-1 in the same perinuclear membrane may allow their efficient functional coupling. Nonetheless, COX-1-mPGES-1 coupling can also occur if AA is abundantly supplied by explosive activation of cytosolic $PLA_2\alpha$ ($cPLA_2\alpha$) [7].

Crucial involvement of mPGES-1 in various pathological events has been clarified by studies using mPGES-1 knockout mice [19–23]. mPGES-1 accounted for the majority of inducible PGES activity in various tissues of LPS-treated mice [19,22], and peritoneal macrophages isolated from mPGES-1-null mice produced minimal PGE_2 in response to LPS [19,20]. Two studies have provided unequivocal evidence for the involvement of mPGES-1 in synovial inflammation. In the collagen-induced arthritis (CIA) model, mPGES-1-deficient mice developed no or little arthritis [21]. Similar CIA phenotypes have been observed in mice lacking $cPLA_2\alpha$ [24], COX-2 [25] or the PGE receptor EP4 [26], thus revealing a metabolic flow of the $cPLA_2$/COX-2/mPGES-1/EP4 pathway leading to the development of inflammatory arthritis. The results seen in the CIA model might be influenced in part by inadequate proximal lymphocyte-mediated responses, since the anti-collagen antibody formation in COX-2-deficient mice revealed a remarkable decrease in this humoral response [25] and since COX-2-deficient mice exhibited altered helper T cell development, a process reversed by PGE_2 [27]. In another study using the collagen antibody-induced arthritis (CAIA) model, in which the influence of lymphocyte-mediated humoral responses was postulated to be minimal, the severity of synovial inflammation, including bone destruction and juxta-articular bone loss, was mild in mPGES-1-deficient mice as compared with replicate wild-type mice, although the incidence of the inflammation was unaffected [19].

Antigen-induced paw edema was markedly reduced in mPGES-1-deficient mice as compared with replicate wild-type mice [21]. This deficit in edema was accompanied by a marked reduction in the number of white blood cells infiltrating the injection site. Likewise, migration of macrophages following peritoneal injection of thioglycollate was strikingly reduced in mPGES-1-null mice relative to replicate wild-type mice (Fig. 1A). This event may be associated with reduced expression of the chemokine receptors CCR2 and CCR3 on macrophages (Fig. 1B). Formation of inflammatory granulation tissue and attendant angiogenesis in the dorsum induced by subcutaneous implantation of a cotton thread was significantly reduced in mPGES-1 knockout mice as compared with wild-type mice [19]. The main cell type expressing mPGES-1 in the granulation tissues of wild-type mice was ascribed to macrophages. In mPGES-1 knockout mice, infiltration of macrophages into the granulation tissue was again mitigated, which was accompanied by a concomitant reduction in the expression of the monocyte/macrophage-directed chemokines. mPGES-1 deficiency was also associated with reduced induction of vascular endothelial cell growth factor (VEGF) in the granulation tissue. Thus, mPGES-1-derived PGE_2, in cooperation with VEGF, may play a critical role in the development of inflammatory granulation and angiogenesis.

As assessed by the acetic acid writhing test, pain nociception was significantly reduced in mPGES-1-deficient

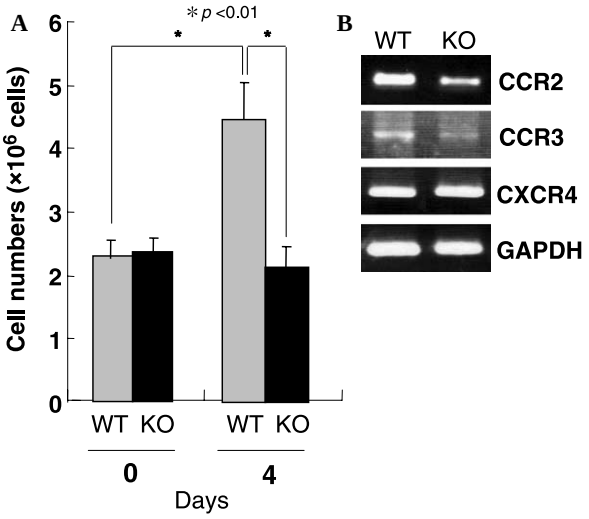

Fig. 1. mPGES-1 is involved in the migration of macrophages into the inflamed site. (A) Numbers of macrophages recovered from the peritoneal cavities of thioglycollate-treated (Day 4) or -nontreated (Day 0) wild-type (WT) and knockout (KO) mice. Values are means ± SE of six independent experiments. Although the numbers of resident macrophages (Day 0) were comparable between WT and KO mice, increase in macrophages after thioglycollate treatment, an event that was evident in WT mice, did not occur appreciably in mPGES-1 KO mice. (B) Thioglycollate-elicited macrophages from WT and KO mice were subjected to RT-PCR analysis to assess the expression of several chemokine receptors. The expression of the receptors for two macrophage-directed chemokines, CCR2 and CCR3, was reduced in macrophages from KO mice relative to those from WT mice. The expression of another chemokine receptor, CXCR4, was similar between WT and KO mice. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used as a control for equal sample loading into each lane.

mice relative to wild-type mice [19,21]. This phenotype was particularly evident when these mice were primed with LPS, where the stretching behavior and the peritoneal PGE_2 level of knockout mice were far less than those of wild-type mice [19], being consistent with elevated expression of COX-2 and mPGES-1 in response to LPS. The basal (i.e., LPS-nonprimed) writhing response was also partially reduced in mPGES-1-null mice [19,21], where COX-1-mPGES-1 coupling takes place.

Genetic inactivation of COX-2, but not COX-1, resulted in reduction of PGE_2 levels in the central nervous system (CNS), in association with impaired LPS-induced febrile response [28]. Likewise, mPGES-1 knockout mice showed no fever and no central PGE_2 synthesis after peripheral injection of LPS [23]. Treatment of rats with LPS led to an increase in the expression of mPGES-1 in blood vessels, especially in veins and venules, in the whole brain [29]. Thus, cerebral vascular endothelial cells express components enabling blood-borne cytokines to stimulate the synthesis of PGE_2, whose small size and lipophilic property allow it to pass across the blood–brain barrier and to diffuse into the CNS neurons, thereby evoking the febrile response.

COX-2 plays a critical role in the development of colorectal cancer and likely other types of cancer. Pharmacological or genetic inactivation of COX-2 led to suppression of cell growth and survival as well as reduction of tumor size, invasion, and metastasis [30,31]. Disruption of $cPLA_2\alpha$ [32] or the PGE receptor EP2 [33] also resulted in a reduced incidence of gastrointestinal polyps, providing strong evidence for a link between PGE_2 signaling and oncogenesis. Tumorigenic potential of mPGES-1 has been suggested by the observations that transfection of mPGES-1 in combination with COX-2, but not with COX-1, into HEK293 cells led to cellular transformation with a concomitant increase in PGE_2 [7], that the COX-2/mPGES-1-cotransfected cells formed a number of large colonies in soft agar culture and were tumorigenic when implanted into nude mice [34], and that transgenic mice overexpressing both COX-2 and mPGES-1 developed metaplasia, hyperplasia, and tumorous growth in the glandular stomach with heavy macrophage infiltration [35]. Moreover, tumor development, metastasis, and associated angiogenesis following implantation of lung carcinoma cells were significantly reduced in mPGES-1-null mice as compared with those in wild-type mice (D. Kamei et al., unpublished observation).

It can be thus concluded that mPGES-1 is involved in various types of pathology including inflammation, pain hyperalgesia, fever, and cancer. On the other hand, the absence of gross abnormalities in ductus arteriosus closure immediately after birth, which is markedly impaired in COX-1/COX-2-double [36] and EP4 [37] knockout mice, and in female reproduction, where EP2 is involved in the ovulation step [38], implies the compensatory participation of other PGESs in these physiological events. These facts, together with its inducible property during inflammation and other pathogenesis, agree well with the proposal that mPGES-1 represents a target for the treatment of various inflammatory diseases that will spare important physiological systems in which other PGs are involved.

The second membrane-associated form of PGES, mPGES-2, has a catalytic glutaredoxin/thioredoxin-like domain and is activated by various thiol reagents [15]. This enzyme is synthesized as a Golgi membrane-associated protein, and the proteolytic removal of the N-terminal hydrophobic domain leads to the formation of a mature cytosolic enzyme [15,39]. Cotransfection of mPGES-2 with either COX isozyme demonstrated that mPGES-2 could be coupled with both COX-1 and COX-2 [39]. Expression of mPGES-2 is rather constitutive in various cells and tissues, and is not elevated appreciably during inflammation or tissue damage. However, a considerable increase of mPGES-2 expression is observed in human colorectal cancer, in which mPGES-1 is also overexpressed [39]. Furthermore, we recently found that LPS stimulation of mouse bone marrow stromal cells resulted in marked elevation of mPGES-2 expression (Fig. 2A). Since stromal cells from mPGES-1-deficient mice produced small but substantial amounts of PGE_2 in response to LPS (Fig. 2B), inducible mPGES-2 may contribute partly to the delayed PGE_2-synthetic response in this case.

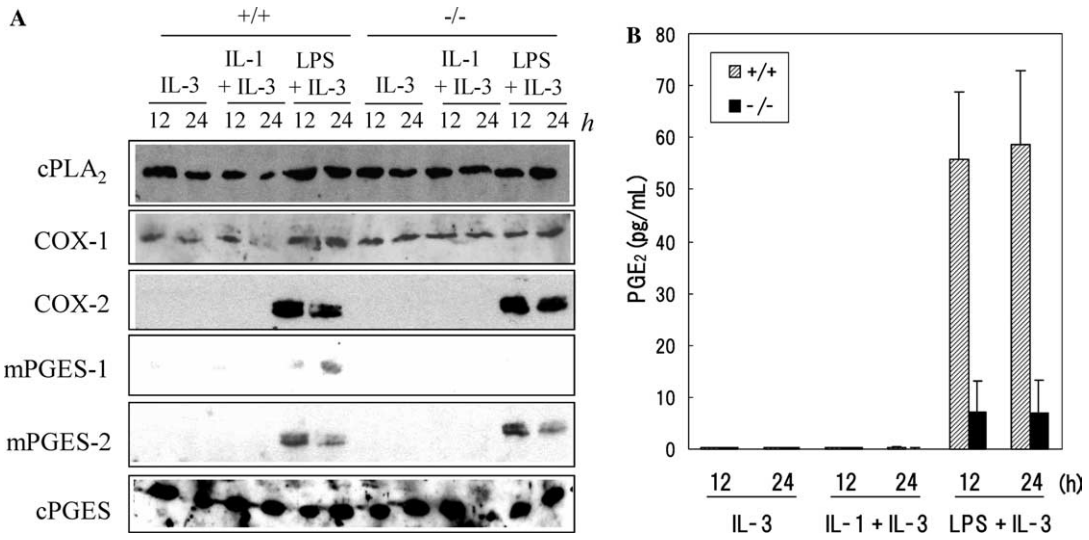

Fig. 2. PGE_2-biosynthetic response in mouse bone marrow stromal cells. Bone marrow cells from wild-type (+/+) and mPGES-1 knockout (−/−) mice were cultured for 2 weeks with IL-3, and adherent cells were used as stromal cells. These cells were then treated for 12 or 24 h with the indicated combinations of cytokines and LPS. The cells were harvested and subjected to Western blotting with antibodies for various enzymes in the PGE_2-biosynthetic pathway (A), and the culture supernatants were taken for PGE_2 enzyme immunoassay (B). Of the enzymes tested, the expressions of COX-2, mPGES-1 (absent in −/−), and mPGES-2 were markedly induced in stromal cells following LPS stimulation, whereas those of $cPLA_2\alpha$, COX-1, and cPGES were unchanged under all conditions (A). PGE_2 was markedly elevated in +/+ stromal cells after 12–24 h of LPS stimulation, whereas PGE_2 generation was reduced by ~80% in −/− stromal cells relative to that in +/+ stromal cells (B). These results suggest that the majority of LPS-stimulated PGE_2 production is attributable to the COX-2-mPGES-1 pathway in +/+ stromal cells and that substantial PGE_2 production observed in −/− stromal cells occurs through the mPGES-1-independent, likely through the inducible mPGES-2-dependent, pathway.

Cytosolic PGES (cPGES) is a GSH-requiring enzyme constitutively expressed in a wide variety of cells and is identical to p23, a co-chaperone of heat shock protein 90 (Hsp90) [6]. Cotransfection and antisense experiments indicated that cPGES is capable of converting COX-1-, but not COX-2-, derived PGH_2 to PGE_2 in cells, particularly during the immediate PGE_2-biosynthetic response elicited by Ca^{2+}-evoked stimuli [6]. Localization of cPGES in the cytosol may allow coupling with proximal COX-1 in the ER in preference to distal COX-2 in the perinuclear envelope. Although the expression of cPGES is constitutive and is unaffected by proinflammatory stimuli in most cases, some exceptions have been reported. Administration of IL-1 into the mouse cortex via intraparenchymal microinjection led to an increase in PGE_2, which was accompanied by elevated expression of cPGES as well as those of COX-2 and mPGES-1 with different kinetics [40]. In pregnant female mice, cPGES was strongly detected in the stroma underlying the luminal epithelium surrounding the implanting blastocyst at the implantation site and in decidualized cells under artificial decidualization [41].

cPGES is directly associated with and phosphorylated by casein kinase 2 (CK2), resulting in marked reduction of K_m for the substrate PGH_2 [42]. In activated cells, CK2-directed phosphorylation of cPGES occurs in parallel with increased cPGES enzymatic activity and PGE_2 production, and these processes are facilitated by interaction with Hsp90 [42]. cPGES, CK2, and Hsp90 form a stoichiometric complex of 1:1:1 immediately after cell activation. In this context, Hsp90 may act as an essential scaffold protein that brings cPGES and CK2 in proximity, thereby spatially allowing their efficient functional interaction under physiological condition. Pharmacologic inhibition of CK2 or Hsp90 or mutation of two CK2-directed phosphorylation sites on cPGES results in poor activation of cPGES, indicating that the tertiary complex formation and attendant phosphorylation are essential for cPGES to act in cells. These observations provide the first evidence that the cellular function of the eicosanoid-biosynthetic enzyme is under the control of a molecular chaperone and its client protein kinase.

Coupling between COXs and other terminal prostanoid synthases

Conversion of PGH_2 to TXA_2 is catalyzed by TX synthase (TXS), which belongs to the cytochrome P-450 family [43]. This enzyme is localized in the ER and perinuclear membranes. Beneficial effect of low dose aspirin on the incidence of cardiovascular diseases is due to suppression of the synthesis of prothrombotic TXA_2 by platelets, in which COX-1/TXS coupling occurs because COX-1 is the dominant COX isozyme in this cell type [44]. Macrophages produce TXA_2 via constitutive COX-1 after Ca^{2+} ionophore stimulation and via inducible COX-2 after LPS stimulation [9], indicating that TXS can be functionally linked with both COX enzymes. Cotransfection experiments using HEK293 cells revealed that TXS is coupled with COX-2 in preference to COX-1, yet high state of $cPLA_2\alpha$ activation (i.e., supply of high concentration of AA) allows TXS to be coupled with COX-1 [8]. These observations suggest that TXA_2 generation profoundly depends on the

substrate availability and that COX-1-dependent production of TXA_2 can occur in cells intrinsically expressing a high level of cPLA$_2\alpha$ under physiological conditions, exemplified by events occurring in platelets [44] and macrophages [9]. Overall, the COX-2 selectivity of TXS is similar to that of mPGES-1.

Conversion of PGH_2 to PGI_2 is catalyzed by PGI synthase (PGIS), which also belongs to the cytochrome P-450 family [45]. The observations that PGI_2 was preferentially produced via COX-2 in macrophages [10] and endothelial cells [46] and that serum PGI_2 level was blunted after in vivo administration of COX-2-selective inhibitors [47] suggest that PGIS is coupled with COX-2 rather strictly. In agreement with this standpoint, cotransfection of PGIS with either COX isozyme into HEK293 cells revealed more strict COX-2 preference of PGIS than that of TXS [8]. This could be explained in part by restricted localization of PGIS in the perinuclear membrane, where COX-2 is located [8]. This view is further supported by recent clinical trials warranting that successive application of COX-2-selective inhibitors may increase the risk of cardiovascular diseases, most likely because they abrogate the synthesis of anti-thrombotic PGI_2 by endothelial cells without affecting the synthesis of prothrombotic TXA_2 by platelets [48].

PGD synthase (PGDS), which isomerizes PGH_2 to PGD_2, occurs in two distinct forms: lipocalin-type PGDS (L-PGDS), a secreted enzyme known as β-trace that is abundantly present in the central nervous system [49], and hematopoietic PGDS (H-PGDS), which represents the σ class of the cytosolic GST family [50]. Although coupling between COXs and L-PGDS has remained unclear, H-PGDS has been shown to exhibit unique COX coupling profiles among various tPGSs. Preferred utilization of COX-1 by H-PGDS is manifested by the observations that A23187 stimulation of macrophages resulted in the production of PGD_2 in preference to those of PGE_2 and PGI_2 [9] and that IgE/antigen-dependent immediate production of PGD_2 by mast cells entirely depended on COX-1 [51]. Additionally, H-PGDS also produced PGD_2 via COX-2 in mast cells during the cytokine-stimulated delayed response [52], revealing dual COX selectivity of H-PGDS in the same cell following distinct stimuli. Consistently, reconstitution experiments in HEK293 cells confirmed that H-PGDS preferentially utilizes COX-1 in the immediate response and COX-2 in the delayed response [8]. This unique, bidirectional COX coupling of H-PGDS in different phases of cell activation may rely on altered subcellular distribution of this enzyme following distinct stimuli. H-PGDS translocates from the cytosol to the perinuclear area outside the nuclear envelope (a part of the ER) during the immediate response, thereby being colocalized with ER-associated COX-1, whereas it moves to the perinuclear envelope, where COX-2 resides, during the delayed response [8]. Thus, spatiotemporal compartmentalization of COXs and H-PGDS may crucially influence their functional coupling to occur properly.

$PGF_{2\alpha}$ is synthesized from PGH_2 by PGF synthase (PGFS; PGH 9,11-endoperoxide reductase) and from PGE_2 by PGE 11-ketoreductase, which belongs to the aldo-keto reductase (AKR) family [53]. Two PGFS enzymes (PGFS-I and -II), purified and cloned from lung (lung-type) [54] and liver (liver-type) [55], respectively, are highly homologous, NADPH-requiring proteins. These enzymes also display more potent PGD 11-ketoreductase activity to produce 9α,11β-PGF_2 and are also capable of reducing various xenobiotic carbonyl compounds [53]. In a COX/PGFS-I coexpression study, PGFS-I failed to be coupled with both COX isoforms, even though a modest increase in $PGF_{2\alpha}$ production was observed if rather high concentrations of exogenous AA were supplied to the cells [56]. Thus, it remains uncertain whether PGFS-I or -II indeed acts as a $PGF_{2\alpha}$-synthesizing enzyme in physiological circumstances. Recently, it has been shown that 20α-hydroxysteroid dehydrogenase (another member of the AKR family), rather than other AKR enzymes including PGFS-I and -II, may be responsible for $PGF_{2\alpha}$ production in the endometrium at the time of luteolysis [57]. In addition, the presence of a stimulus-inducible, GSH-dependent, membrane-associated form of PGFS, which prefers COX-2 to COX-1, has been suggested [58], although the molecular identity of this enzyme is unknown.

Concluding remarks

This review summarizes the nature of the regulatory mechanisms of functional coupling between COXs and terminal prostanoid synthases. The amounts of AA released by PLA_2 enzymes and the subcellular localization of COXs and terminal prostanoid synthases significantly affect the coupling efficiency between the enzymes in the biosynthetic pathway. In terms of their localization and COX preference, terminal prostanoid synthases are classified into three categories: (i) the perinuclear enzymes that prefer COX-2 (mPGES-1, TXS, and PGIS), (ii) the cytosolic enzyme that prefers COX-1 (cPGES), and (iii) the translocating enzyme that utilizes both COXs depending on the stimulus (H-PGDS). In addition, the kinetic parameters of each terminal prostanoid synthase may also represent a crucial determinant of the ratio of PGs produced [8]. Since each tPGS has different K_m and V_{max} values for PGH_2, the ratios of the terminal products can vary according to the amounts of PGH_2 produced as a result of PLA_2/COX coupling at the moment when PG generation takes place. Moreover, the COX-1 versus COX-2 pathways can also be differently affected by other cellular factors, such as nitric oxide [59,60]. Whether other cofactor(s) that might facilitate selective linkage between each COX and terminal prostanoid synthase under particular conditions exist in cells remains to be elucidated.

References

[1] I. Morita, M. Schindler, M.K. Reiger, J.C. Otto, T. Hori, D.L. DeWitt, W.L. Smith, Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2, J. Biol. Chem. 270 (1995) 10902–10908.

[2] S.T. Reddy, H.R. Herschman, Prostaglandin synthase-1 and prostaglandin synthase-2 are coupled to distinct phospholipases for the generation of prostaglandin D_2 in activated mast cells, J. Biol. Chem. 272 (1997) 3231–3237.
[3] R.J. Kulmacz, L.-H. Wang, Comparison of hydroperoxide initiator requirements for the cyclooxygenase activities of prostaglandin H synthase-1 and -2, J. Biol. Chem. 270 (1995) 24019–24023.
[4] M. Murakami, T. Kambe, S. Shimbara, I. Kudo, Functional coupling between various phospholipase A_2s and cyclooxygenases in immediate and delayed prostanoid biosynthetic pathways, J. Biol. Chem. 274 (1999) 3103–3115.
[5] M. Shitashige, I. Morita, S. Murota, Different substrate utilization between prostaglandin endoperoxide H synthase-1 and -2 in NIH3T3 fibroblasts, Biochim. Biophys. Acta 1389 (1998) 57–66.
[6] T. Tanioka, Y. Nakatani, N. Semmyo, M. Murakami, I. Kudo, Molecular identification of cytosolic prostaglandin E_2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E_2 biosynthesis, J. Biol. Chem. 275 (2000) 32775–32782.
[7] M. Murakami, H. Naraba, T. Tanioka, N. Semmyo, Y. Nakatani, F. Kojima, T. Ikeda, M. Fueki, A. Ueno, S. Oh-Ishi, I. Kudo, Regulation of prostaglandin E_2 biosynthesis by inducible membrane-associated prostaglandin E_2 synthase that acts in concert with cyclooxygenase-2, J. Biol. Chem. 275 (2000) 32783–32792.
[8] N. Ueno, M. Murakami, T. Tanioka, K. Fujimori, Y. Urade, I. Kudo, Coupling between cyclooxygenases, terminal prostanoid synthases and phospholipase A_2s, J. Biol. Chem. 276 (2001) 34918–34927.
[9] H. Naraba, M. Murakami, H. Matsumoto, S Shimbara, A. Ueno, I. Kudo, S. Oh-ishi, Segregated coupling of phospholipases A_2, cyclooxygenases, and terminal prostanoid synthases in different phases of prostanoid biosynthesis in rat peritoneal macrophages, J. Immunol. 160 (1998) 2974–2982.
[10] T.G. Brock, R.W. McNish, M. Peters-Golden, Arachidonic acid is preferentially metabolized by cyclooxygenase-2 to prostacyclin and prostaglandin E_2, J. Biol. Chem. 274 (1999) 11660–11666.
[11] Q.-R. Chen, C. Miyaura, S. Higashi, M. Murakami, I. Kudo, S. Saito, T. Hiraide, Y. Shibasaki, T. Suda, Activation of cytosolic phospholipase A_2 by platelet-derived growth factor is essential for cyclooxygenase-2-dependent prostaglandin E_2 synthesis in mouse osteoblasts cultured with interleukin-1, J. Biol. Chem. 272 (1997) 5952–5968.
[12] M. Murakami, H. Kuwata, Y. Amakasu, S. Shimbara, Y. Nakatani, G. Atsumi, I. Kudo, Prostaglandin E_2 amplifies cytosolic phospholipase A_2 and cyclooxygenase-2- dependent delayed prostaglandin E_2 generation in mouse osteoblastic cells: enhancement by secretory phospholipase A_2, J. Biol. Chem. 272 (1997) 19891–19897.
[13] Y. Harada, K. Hatanaka, M. Kawamura, M. Saito, M. Ogino, M. Majima, T. Ohno, K. Ogino, K. Yamamoto, Y. Taketani, S. Yamamoto, M. Katori, Role of prostaglandin H synthase-2 in prostaglandin E_2 formation in rat carrageenin-induced pleurisy, Prostaglandins 51 (1996) 19–33.
[14] P.-J. Jakobsson, S. Thoren, R. Morgenstern, B. Samuelsson, Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target, Proc. Natl. Acad. Sci. USA 96 (1999) 7220–7225.
[15] N. Tanikawa, Y. Ohmiya, H. Ohkubo, K. Hashimoto, K. Kangawa, M. Kojima, S. Ito, K. Watanabe, Identification and characterization of a novel type of membrane-associated prostaglandin E synthase, Biochem. Biophys. Res. Commun. 291 (2002) 884–889.
[16] M. Murakami, I. Kudo, Recent advances in molecular biology and physiology of the prostaglandin E_2-biosynthetic pathway, Prog. Lipid Res. 43 (2004) 3–35.
[17] R. Han, S. Tsui, T.J. Smith, Up-regulation of prostaglandin E_2 synthesis by interleukin-1β in human orbital fibroblasts involves coordinate induction of prostaglandin-endoperoxide H synthase-2 and glutathione-dependent prostaglandin E_2 synthase expression, J. Biol. Chem. 277 (2002) 16355–16364.
[18] H. Naraba, C. Yokoyama, N. Tago, M. Murakami, I. Kudo, M. Fueki, S. Oh-Ishi, T. Tanabe, Transcriptional regulation of the membrane-associated prostaglandin E_2 synthase gene. Essential role of the transcription factor Egr-1, J. Biol. Chem. 277 (2002) 28601–28608.
[19] D. Kamei, K. Yamakawa, Y. Takegoshi, M. Mikami-Nakanishi, Y. Nakatani, S. Oh-Ishi, H. Yasui, Y. Azuma, N. Hirasawa, K. Ohuchi, H. Kawaguchi, Y. Ishikawa, T. Ishii, S. Uematsu, S. Akira, M. Murakami, I. Kudo, Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin E synthase-1, J. Biol. Chem. 279 (2004) 33684–33695.
[20] S. Uematsu, M. Matsumoto, K. Takeda, S. Akira, Lipopolysaccharide-dependent prostaglandin E_2 production is regulated by the glutathione-dependent prostaglandin E_2synthase gene induced by the Toll-like receptor 4/MyD88/NF-IL6 pathway, J. Immunol. 168 (2002) 5811–5816.
[21] C.E. Trebino, J.L. Stock, C.P. Gibbons, B.M. Naiman, T.S. Wachtmann, J.P. Umland, K. Pandher, J.M. Lapointe, S. Saha, M.L. Roach, D. Carter, N.A. Thomas, B.A. Durtschi, J.D. McNeish, J.E. Hambor, P.J. Jakobsson, T.J. Carty, J.R. Perez, L.P. Audoly, Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase, Proc. Natl. Acad. Sci. USA 100 (2003) 9044–9049.
[22] L. Boulet, M. Ouellet, K.P. Bateman, D. Ethier, M.D. Percival, D. Riendeau, J.A. Mancini, N. Methot, Deletion of microsomal prostaglandin E_2 synthase-1 reduces both inducible and basal PGE_2 production and alters the gastric prostanoid profile, J. Biol. Chem. 279 (2004) 23229–23237.
[23] D. Engblom, S. Saha, L. Engstrom, M. Westman, L.P. Audoly, P.J. Jakobsson, A. Blomqvist, Microsomal prostaglandin E synthase-1 is the central switch during immune-induced pyresis, Nat. Neurosci. 6 (2003) 1137–1138.
[24] M. Hegen, L. Sun, N. Uozumi, K. Kume, M.E. Goad, C.L. Nickerson-Nutter, T. Shimizu, J.D. Clark, Cytosolic phospholipase $A_2\alpha$-deficient mice are resistant to collagen-induced arthritis, J. Exp. Med. 197 (2003) 1297–1302.
[25] L.K. Myers, G.C. Higgins, T.H. Finkel, A.M. Reed, J.W. Thompson, R.C. Walton, J. Hendrickson, N.C. Kerr, R.K. Pandya-Lipman, B.V. Shlopov, P. Stastny, A.E. Postlethwaite, A.H. Kang, Juvenile arthritis and autoimmunity to type II collagen, Arthritis Rheum. 44 (2001) 1775–1781.
[26] J.M. McCoy, J.R. Wicks, L.P. Audoly, The role of prostaglandin E_2 receptors in the pathogenesis of rheumatoid arthritis, J. Clin. Invest. 110 (2002) 651–658.
[27] B. Rocca, L.M. Spain, E. Pure, R. Langenbach, C. Patrono, G.A. FitzGerald, Distinct roles of prostaglandin H synthases 1 and 2 in T-cell development, J. Clin. Invest. 103 (1999) 1469–1477.
[28] S. Li, Y. Wang, K. Matsumura, L.R. Ballou, S.G. Morham, C.M. Blatteis, The febrile response to lipopolysaccharide is blocked in cyclooxygenase-2 (−/−), but not in cyclooxygenase-1 (−/−) mice, Brain Res. 825 (1999) 86–94.
[29] K. Yamagata, K. Matsumura, W. Inoue, T. Shiraki, K. Suzuki, S. Yasuda, H. Sugiura, C. Cao, Y. Watanabe, S. Kobayashi, Coexpression of microsomal-type prostaglandin E synthase with cyclooxygenase-2 in brain endothelial cells of rats during endotoxin-induced fever, J. Neurosci. 21 (2001) 2669–2677.
[30] M. Oshima, J.E. Dinchuk, S.L. Kargman, H. Oshima, B. Hancock, E. Kwong, J.M. Trzaskos, J.F. Evans, M.M. Taketo, Suppression of intestinal polyposis in $Apc^{\Delta 716}$ knockout mice by inhibition of cyclooxygenase 2 (COX-2), Cell 87 (1996) 803–809.
[31] M. Tsujii, S. Kawano, S. Tsuji, H. Sawaoka, M. Hori, R.N. DuBois, Cyclooxygenase regulates angiogenesis induced by colon cancer cells, Cell 93 (1998) 705–716.
[32] K. Takaku, M. Sonoshita, N. Sasaki, N. Uozumi, Y. Doi, T. Shimizu, M.M. Taketo, Suppression of intestinal polyposis in $Apc^{\Delta 716}$ knockout mice by an additional mutation in the cytosolic phospholipase A_2 gene, J. Biol. Chem. 275 (2002) 3406–34013.
[33] M. Sonoshita, K. Takaku, N. Sasaki, Y. Sugimoto, F. Ushikubi, S. Narumiya, M. Oshima, M.M. Taketo, Acceleration of intestinal polyposis through prostaglandin receptor EP2 in $Apc^{\Delta 716}$ knockout mice, Nat. Med. 7 (2001) 1048–1051.

[34] D. Kamei, M. Murakami, Y. Nakatani, Y. Ishikawa, T. Ishii, I. Kudo, Potential role of microsomal prostaglandin E synthase-1 in tumorigenesis, J. Biol. Chem. 278 (2003) 19396–19405.
[35] H. Oshima, M. Oshima, K. Inaba, M.M. Taketo, Hyperplastic gastric tumors induced by activated macrophages in COX-2/mPGES-1 transgenic mice, EMBO J. 23 (2004) 1669–1678.
[36] C.D. Loftin, D.B. Trivedi, H.F. Tiano, J.A. Clark, C.A. Lee, J.A. Epstein, S.G. Morham, M.D. Breyer, M. Nguyen, B.M. Hawkins, J.L. Goulet, O. Smithies, B.H. Koller, R. Langenbach, Failure of ductus arteriosus closure and remodeling in neonatal mice deficient in cyclooxygenase-1 and cyclooxygenase-2, Proc. Natl. Acad. Sci. USA 98 (2001) 1059–1064.
[37] E. Segi, Y. Sugimoto, A. Yamasaki, Y. Aze, H. Oida, T. Nishimura, T. Murata, T. Matsuoka, F. Ushikubi, M. Hirose, T. Tanaka, N. Yoshida, S. Narumiya, A. Ichikawa, Patent ductus arteriosus and neonatal death in prostaglandin receptor EP4-deficient mice, Biochem. Biophys. Res. Commun. 246 (1998) 7–12.
[38] H. Hizaki, E. Segi, Y. Sugimoto, M. Hirose, T. Saji, F. Ushikubi, T. Matsuoka, Y. Noda, T. Tanaka, N. Yoshida, S. Narumiya, A. Ichikawa, Abortive expansion of the cumulus and impaired fertility in mice lacking the prostaglandin E receptor subtype EP2, Proc. Natl. Acad. Sci. USA 96 (1999) 10501–10596.
[39] M. Murakami, K. Nakashima, K. Kamei, S. Masuda, Y. Ishikawa, T. Ishii, Y. Ohmiya, K. Watanabe, I. Kudo, Cellular prostaglandin E_2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and -2, J. Biol. Chem. 278 (2003) 37937–37947.
[40] A.H. Moore, J.A. Olschowka, M.K. O'Banion, Intraparenchymal administration of interleukin-1β induces cyclooxygenase-2-mediated expression of membrane- and cytosolic-associated prostaglandin E synthases in mouse brain, J. Neuroimmunol. 148 (2004) 32–40.
[41] H. Ni, T. Sun, X.H. Ma, Z.M. Yang, Expression and regulation of cytosolic prostaglandin E synthase in mouse uterus during the periimplantation period, Biol. Reprod. 68 (2003) 744–750.
[42] T. Kobayashi, Y. Nakatani, T. Tanioka, M. Tsujimoto, S. Nakajo, K. Nakaya, M. Murakami, I. Kudo, Regulation of cytosolic prostaglandin E synthase by phosphorylation, Biochem. J. 381 (2004) 59–69.
[43] R.F. Shen, H.H. Tai, Thromboxanes: synthase and receptors, J. Biomed. Sci. 5 (1998) 153–172.
[44] R. Langenbach, S.G. Morham, H.F. Tiano, C.D. Loftin, B.I. Ghanayem, P.C. Chulada, J.F. Mahler, C.A. Lee, E.H. Goulding, K.D. Kluckman, H.S. Kim, O. Smithies, Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration, Cell 83 (1995) 483–492.
[45] S. Hara, A. Miyata, C. Yokoyama, H. Inoue, R. Brugger, F. Lottspeich, V. Ullrich, T. Tanabe, Isolation and molecular cloning of prostacyclin synthase from bovine endothelial cells, J. Biol. Chem. 269 (1994) 19897–19903.
[46] G.E. Caughey, L.G. Cleland, P.S. Penglis, J.R. Gamble, M.J. James, Roles of cyclooxygenase (COX)-1 and COX-2 in prostanoid production by human endothelial cells: selective up-regulation of prostacyclin synthesis by COX-2, J. Immunol. 167 (2001) 2831–2838.
[47] B.F. McAdam, F. Catella-Lawson, I.A. Mardini, S. Kapoor, J.A. Lawson, G.A. FitzGerald, Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2, Proc. Natl. Acad. Sci. USA 96 (1999) 272–277.
[48] G.A. Fitzgerald, Coxibs and cardiovascular disease, N. Engl. J. Med. 351 (2004) 1709–1711.
[49] A. Nagata, Y. Suzuki, M. Igarashi, N. Eguchi, H. Toh, Y. Urade, O. Hayaishi, Human brain prostaglandin D synthase has been evolutionarily differentiated from lipophilic-ligand carrier proteins, Proc. Natl. Acad. Sci. USA 88 (1991) 4020–4024.
[50] Y. Kanaoka, H. Ago, E. Inagaki, T. Nanayama, M. Miyano, R. Kikuno, Y. Fujii, N. Eguchi, H. Toh, Y. Urade, O. Hayaishi, Cloning and crystal structure of hematopoietic prostaglandin D synthase, Cell 90 (1997) 1085–1095.
[51] M. Murakami, R. Matsumoto, Y. Urade, K.F. Austen, J.P. Arm, c-*Kit* ligand mediates increased expression of cytosolic phospholipase A_2, prostaglandin endoperoxide synthase-1, and hematopoietic prostaglandin D_2 synthase and increased IgE-dependent prostaglandin D_2 generation in immature mouse mast cells, J. Biol. Chem. 270 (1995) 3239–3246.
[52] M. Murakami, R. Matsumoto, K.F. Austen, J.P. Arm, Prostaglandin endoperoxide synthase-1 and -2 couple to different transmembrane stimuli to generate prostaglandin D_2 in mouse bone marrow-derived mast cells, J. Biol. Chem. 269 (1994) 22269–22275.
[53] K. Watanabe, Prostaglandin F synthase, Prostaglandins Other Lipid Mediat. 68-69 (2002) 401–407.
[54] K. Watanabe, Y. Fujii, K. Nakayama, H. Ohkubo, S. Kuramitsu, H. Kagamiyama, S. Nakanishi, O. Hayaishi, Structural similarity of bovine lung prostaglandin F synthase to lens epsilon-crystallin of the European common frog, Proc. Natl. Acad. Sci. USA 85 (1988) 11–15.
[55] T. Suzuki, Y. Fujii, M. Miyano, L.Y. Chen, T. Takahashi, K. Watanabe, cDNA cloning, expression, and mutagenesis study of liver-type prostaglandin F synthase, J. Biol. Chem. 274 (1999) 241–248.
[56] K. Nakashima, N. Ueno, D. Kamei, T. Tanioka, Y. Nakatani, M. Murakami, I. Kudo, Coupling between cyclooxygenases and prostaglandin $F_{2\alpha}$ synthase: detection of an inducible, glutathione-activated, membrane-bound prostaglandin $F_{2\alpha}$-synthetic activity, Biochim. Biophys. Acta 1633 (2003) 96–105.
[57] E. Madore, N. Harvey, J. Parent, P. Chapdelaine, J.A. Arosh, M.A. Fortier, An aldose reductase with 20 alpha-hydroxysteroid dehydrogenase activity is most likely the enzyme responsible for the production of prostaglandin $F_{2\alpha}$ in the bovine endometrium, J. Biol. Chem. 278 (2003) 11205–11212.
[58] J.R. Burgess, C.C. Reddy, Isolation and characterization of an enzyme from sheep seminal vesicles that catalyzes the glutathione-dependent reduction of prostaglandin H_2 to prostaglandin $F_{2\alpha}$, Biochem. Mol. Biol. Int. 41 (1997) 217–226.
[59] R. Clancy, B. Varenika, W. Huang, L. Ballou, M. Attur, A.R. Amin, S.B. Abramson, Nitric oxide synthase/COX cross-talk: nitric oxide activates COX-1 but inhibits COX-2-derived prostaglandin production, J. Immunol. 165 (2000) 1582–1587.
[60] L.J. Marnett, T.L. Wright, B.C. Crews, S.R. Tannenbaum, J.D. Morrow, Regulation of prostaglandin biosynthesis by nitric oxide is revealed by targeted deletion of inducible nitric-oxide synthase, J. Biol. Chem. 275 (2000) 13427–13430.