

Review

Prostaglandin E Synthase, a Terminal Enzyme for Prostaglandin E₂ Biosynthesis

Ichiro Kudo[†] and Makoto Murakami^{†,‡,*}

[†]Department of Health Chemistry, School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

[‡]Biomembrane Signaling Project, The Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8613, Japan

Received 4 October 2005

Biosynthesis of prostanoids is regulated by three sequential enzymatic steps, namely phospholipase A₂ enzymes, cyclooxygenase (COX) enzymes, and various lineage-specific terminal prostanoid synthases. Prostaglandin E synthase (PGES), which isomerizes COX-derived PGH₂ specifically to PGE₂, occurs in multiple forms with distinct enzymatic properties, expressions, localizations and functions. Two of them are membrane-bound enzymes and have been designated as mPGES-1 and mPGES-2. mPGES-1 is a perinuclear protein that is markedly induced by proinflammatory stimuli, is down-regulated by anti-inflammatory glucocorticoids, and is functionally coupled with COX-2 in marked preference to COX-1. Recent gene targeting studies of mPGES-1 have revealed that this enzyme represents a novel target for anti-inflammatory and anti-cancer drugs. mPGES-2 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. This enzyme is rather constitutively expressed in various cells and tissues and is functionally coupled with both COX-1 and COX-2. Cytosolic PGES (cPGES) is constitutively expressed in a wide variety of cells and is functionally linked to COX-1 to promote immediate PGE₂ production. This review highlights the latest understanding of the expression, regulation and functions of these three PGES enzymes.

Keywords: Cancer, Cyclooxygenase, Inflammation, Prostaglandin E₂, Prostaglandin E synthase

Introduction

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 A₂ (PLA₂) enzymes. The released AA is sequentially metabolized to prostaglandin (PG) G₂ and then to PGH₂ by either cyclooxygenase (COX) -1 or COX-2. PGH₂ is then converted to various bioactive PGs (thromboxane (TX) A₂, PGD₂, PGE₂, PGF₂ and PGI₂) 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 Ca²⁺ 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. 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 PLA₂ enzymes to each COX (Morita *et al.*, 1995; Reddy and Herschman, 1997) 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 (Kulmacz and Wang, 1995; Murakami *et al.*, 1999). It is therefore conceivable that COX-2-mediated

*To whom correspondence should be addressed.
Tel/Fax: 81-3-3823-5280
E-mail: mako@rinshoken.or.jp

Table 1. Properties of PGES enzymes

Enzymes	Structural properties	Expression	Subcellular localizations	COX preference	Specific characteristics	<i>In vivo</i> functions*
mPGES-1	MAPEG family	inducible glucocorticoid-sensitive	perinuclear membrane	COX-2	trimer formation	inflammation, pain, fever, cancer
mPGES-2	thioredoxin homology domain	constitutive	Golgi, cytosol	COX-1, COX-2	cleavage of N-terminal hydrophobic region dimer formation	unknown
cPGES	Hsp90 cochaperone p23	constitutive	cytosol	COX-1	complex formation with Hsp90 and CK2	unknown

**In vivo* functions revealed by analyses on gene-disrupted mice.

catalysis is preferred in particular cellular situations where AA supply is limited (Shitashige *et al.*, 1998; Murakami *et al.*, 1999). As an alternative possibility, it has been proposed that the two 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 (Tanioka *et al.*, 2000; Murakami *et al.*, 2000), and subsequently between COXs and other terminal prostanoid synthases (Ueno *et al.*, 2001).

Until now, three proteins that catalyze the conversion of PGH₂ to PGE₂ rather specifically have been identified; namely membrane-bound PGES (mPGES) -1 (Jakobsson *et al.*, 1999; Murakami *et al.*, 2000), mPGES-2 (Tanikawa *et al.*, 2002), and cytosolic PGES (cPGES) (Tanioka *et al.*, 2000). In this article, we focus on the expression and functions of these three PGES enzymes 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. The characteristic properties of three PGES enzymes (mPGES-1, mPGES-2 and cPGES) are summarized in Table 1.

mPGES-1

Preferred utilization of COX-2 over COX-1 in the PGE₂-biosynthetic pathway was initially reported in rat peritoneal macrophages, which produced TXA₂ and PGD₂ through COX-1 in the A23187-induced immediate response and PGE₂ and PGI₂ through COX-2 in the lipopolysaccharide (LPS)-induced delayed response (Naraba *et al.*, 1998; Brock *et al.*, 1999). PGE₂ production by osteoblasts occurred predominantly through COX-2 irrespective of the co-presence of COX-1 (Chen *et al.*, 1997; Murakami *et al.*, 1997). In a rat inflammatory model, COX-2-selective inhibitors reduced the accumulation of PGE₂ but not of other PGs (Harada *et al.*, 1996). These observations predicted the presence of a particular terminal PGES that is coupled with COX-2 in marked preference to COX-1.

It is now apparent that 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 (Jakobsson *et al.*, 1999; Murakami *et al.*, 2000). mPGES-1 constitutes a trimer in the crystal (Murakami and Kudo, 2004). 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 PGE₂ has been implicated to play a critical role, such as inflammation, fever, pain, female reproduction, tissue repair, and cancer (Thoren *et al.*, 2003). Stimulus-inducible expression of mPGES-1 is controlled by the transcription factor Egr-1, which binds to the proximal GC box in the *mPGES-1* promoter leading to mPGES-1 transcription (Naraba *et al.*, 2002). Studies employing cotransfection of mPGES-1 and either COX isozyme, as well as those with small interfering RNA or antisense technology to knockdown mPGES-1, have revealed that mPGES-1 is functionally coupled with COX-2 in marked preference to COX-1 (Murakami *et al.*, 2000; Kamei *et al.*, 2004). Colocalization of COX-2 and mPGES-1 in the same perinuclear membrane may allow their efficient functional coupling. Nonetheless, Coupling between COX-1 and mPGES-1 can also occur if AA is abundantly supplied by explosive activation of cytosolic PLA₂ (cPLA₂) (Murakami *et al.*, 2000).

Crucial involvement of mPGES-1 in various pathophysiological events has been clarified by studies using mPGES-1 knockout mice (Uematsu *et al.*, 2002; Trebino *et al.*, 2003; Engblom *et al.*, 2003; Boulet *et al.*, 2004; Kamei *et al.*, 2004). mPGES-1 accounted for the majority of inducible PGES activity in various tissues of LPS-treated mice (Boulet *et al.*, 2004; Kamei *et al.*, 2004), and peritoneal macrophages isolated from mPGES-1-null mice produced minimal PGE₂ in response to LPS (Uematsu *et al.*, 2002; Kamei *et al.*, 2004). In the collagen- or the collagen antibody-induced arthritis model, mPGES-1-deficient mice developed milder arthritis than did wild-type mice (Trebino *et al.*, 2003; Kamei *et al.*, 2004). Similar arthritic phenotypes have been observed in mice lacking cPLA₂ (Hegen *et al.*, 2003), COX-2 (Myers *et al.*, 2001) or the PGE receptor EP4 (Rocca *et al.*, 1999), thus revealing a metabolic flow of the cPLA₂/COX-2/mPGES-1/EP4 pathway leading to the development of inflammatory

arthritis. The results seen in the collagen-induced arthritis model might be influenced in part by inadequate proximal lymphocyte-mediated responses, since COX-2-deficient mice exhibited altered helper T cell development, a process reversed by PGE₂ (Rocca *et al.*, 1999).

Antigen-induced paw edema was markedly reduced in mPGES-1-deficient mice as compared with replicate wild-type mice (Trebino *et al.*, 2003). This deficit in edema was accompanied by a marked reduction in the infiltration of white blood cells into the inflamed site. Likewise, migration of macrophages following peritoneal injection of thioglycollate was strikingly reduced in mPGES-1-null mice relative to replicate wild-type mice (Kamei *et al.*, 2004). 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 (Kamei *et al.*, 2004). In this model, mPGES-1 deficiency was also associated with reduced induction of vascular endothelial cell growth factor (VEGF) in the granulation tissue. Thus, mPGES-1-derived PGE₂, in cooperation with VEGF, may play a critical role in the development of inflammatory granulation and angiogenesis.

PGE₂ is known to be a mediator of inflammatory pain. Peripheral pain nociception, as assessed by acetic acid writhing test, was significantly reduced in mPGES-1-deficient mice relative to wild-type mice (Trebino *et al.*, 2003; Kamei *et al.*, 2004). This phenotype was particularly evident when these mice were primed with LPS, where the stretching behavior and the peritoneal PGE₂ level of knockout mice were far less than those of wild-type mice (Kamei *et al.*, 2004), 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 (Trebino *et al.*, 2003; Kamei *et al.*, 2004), where COX-1-mPGES-1 coupling takes place. In a neuropathic pain model prepared by spinal nerve transection, mPGES-1-null mice failed to exhibit mechanical allodynia and thermal hyperalgesia (Mabuchi *et al.*, 2004).

Genetic inactivation of COX-2, but not COX-1, resulted in reduction of PGE₂ levels in the central nervous system (CNS), in association with impaired LPS-induced febrile response (Li *et al.*, 1999). Likewise, mPGES-1 knockout mice showed no fever and no central PGE₂ synthesis after peripheral injection of LPS (Engblom *et al.*, 2003). 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 (Yamagata *et al.*, 2001). Thus, cerebral vascular endothelial cells express components enabling blood-borne cytokines to stimulate the synthesis of PGE₂, 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. In contrast, wild-type and mPGES-1 mutant mice showed similar psychogenic stress-induced hyperthermia, diurnal temperature variations, and reduced motor activity following injection of turpentine (an aseptic cytokine-induced pyresis inducer) (Saha

et al., 2005). This indicates that mPGES-1 is functionally dissociated from the stress-induced hyperthermia, circadian temperature regulation, and the inflammation-induced activity depression.

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 (Oshima *et al.*, 1996). Disruption of cPLA₂ (Takaku *et al.*, 2002) or the PGE receptor EP2 (Sonoshita *et al.*, 2001) also resulted in a reduced incidence of gastrointestinal polyps, providing strong evidence for a link between PGE₂ 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 PGE₂ (Murakami *et al.*, 2000), 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 (Kamei *et al.*, 2003), 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 (Oshima *et al.*, 2004).

It can be thus concluded that mPGES-1 is involved in various types of pathology including inflammation, pain hyperalgesia, fever, and cancer. Notably, the absence of gross abnormalities in ductus arteriosus closure immediately after birth, which is markedly impaired in COX-1/COX-2-double (Loftin *et al.*, 2001) and EP4 (Segi *et al.*, 1998) knockout mice, and in female reproduction, where EP2 is involved in the ovulation step (Hizaki *et al.*, 1999), 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.

mPGES-2

The second membrane-associated form of PGES, mPGES-2, has a catalytic glutaredoxin/thioredoxin-like domain and is activated by various thiol reagents (Tanikawa *et al.*, 2002). 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 (Tanikawa *et al.*, 2002; Murakami *et al.*, 2003). Cotransfection of mPGES-2 with either COX isozyme demonstrated that mPGES-2 could be coupled with both COX-1 and COX-2 (Murakami *et al.*, 2003). 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 (Murakami *et al.*, 2003).

Crystallization of mPGES-2 reveals that it forms a dimer and is attached to lipid membrane by anchoring the N-terminal section (Yamada *et al.*, 2005). Two hydrophobic pockets connected to form a V shape are located in the bottom of a large cavity. The geometry suggests that the SH of Cys¹¹⁰ in the glutaredoxin/thioredoxin-like domain is most likely the catalytic site of mPGES-2. PGH₂ fits well into the V-shaped pockets and its endoperoxide moiety interacts with the SH of Cys¹¹⁰. The fold of mPGES-2 is quite similar to those of GSH-dependent hematopoietic PGD synthase, except for the two large loop sections.

cPGES

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) (Tanioka *et al.*, 2000). Cotransfection and antisense experiments indicated that cPGES is capable of converting COX-1-, but not COX-2-, derived PGH₂ to PGE₂ in cells, particularly during the immediate PGE₂-biosynthetic response elicited by Ca²⁺-evoked stimuli (Tanioka *et al.*, 20). 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 PGE₂, which was accompanied by elevated expression of cPGES as well as that of COX-2 and mPGES-1 with different kinetics (Moore *et al.*, 2004). 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 (Ni *et al.*, 2003).

cPGES is directly associated with and phosphorylated by casein kinase 2 (CK2), resulting in marked reduction of K_m for the substrate PGH₂ (Kobayashi *et al.*, 2004). In activated cells, CK2-directed phosphorylation of cPGES occurs in parallel with increased cPGES enzymatic activity and PGE₂ production, and these processes are facilitated by interaction with Hsp90 (Kobayashi *et al.*, 2004). 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 results 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.

Concluding Remarks

It has become apparent that there are multiple PGES enzymes in mammalian cells and that they display distinct functional coupling with upstream COX enzymes. Distinct PGES enzymes may control the spatial and temporal production of PGE₂ in different aspects of pathophysiology in particular tissues and cells. Therefore, understanding the regulatory mechanisms for each PGES is of considerable importance. Although COX-2 inhibitors have reduced gastrointestinal toxicity as compared with traditional NSAIDs, there are also some adverse effects associated with this new group of drugs (Crofford *et al.*, 2000). For instance, specific inhibition of COX-2 blocks the production of renal and systemic PGI₂ (McAdam *et al.*, 1999), thereby causing altered excretion of sodium, edema, and elevated blood pressure (Stichtenoth and Frolich, 2000). In addition, specific inhibition of COX-2 alters the balance between platelet-derived thromboxane A₂ and endothelium-derived PGI₂, leading to increases in the risk of thrombosis due to altered vascular tone (Crofford *et al.*, 2000; Bombardier *et al.*, 2000). Thus, more selective modulation of the prostanoid pathway appears to be desirable. From this standpoint, mPGES-1 represents an attractive novel target for therapeutic intervention for patients with various inflammatory diseases and cancer.

References

- Bombardier, C., Laine, L., Reicin, A., Shapiro, D., Burgos-Vargas, R., Davis, B., Day, R., Ferraz, M. B., Hawkey, C. J., Hochberg, M. C., Kvien, T. K. and Schnitzer, T. J. (2000) VIGOR study group. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. *N. Engl. J. Med.* **343**, 1520-1528.
- Boulet, L., Ouellet, M., Bateman, K. P., Ethier, D., Percival, M. D., Riendeau, D., Mancini, J. A. and Methot, N. (2004) Deletion of microsomal prostaglandin E₂ synthase-1 reduces both inducible and basal PGE₂ production and alters the gastric prostanoid profile. *J. Biol. Chem.* **279**, 23229-23237.
- Brock, T. G., McNish, R. W. and Peters-Golden, M. (1999) Arachidonic acid is preferentially metabolized by cyclooxygenase-2 to prostacyclin and prostaglandin E₂. *J. Biol. Chem.* **274**, 11660-11666.
- Chen, Q-R., Miyaura, C., Higashi, S., Murakami, M., Kudo, I., Saito, S., Hiraide, T., Shibasaki, Y. and Suda, T. (1997) Activation of cytosolic phospholipase A₂ by platelet-derived growth factor is essential for cyclooxygenase-2-dependent prostaglandin E₂ synthesis in mouse osteoblasts cultured with interleukin-1. *J. Biol. Chem.* **272**, 5952-5968.
- Crofford, L. J., Lipsky, P. E., Brooks, P., Abramson, S. B., Simon, L. S. and van de Putte, L. B. (2000) Basic biology and clinical

- application of specific cyclooxygenase-2 inhibitors. *Arthritis Rheum.* **43**, 4-13.
- Crofford, L. J., Oates, J. C., McCune, W. J., Gupta, S., Kaplan, M. J., Catella-Lawson, F., Morrow, J. D., McDonagh, K. T. and Schmaier, A. H. (2000) Thrombosis in patients with connective tissue diseases treated with specific COX-2 inhibitors: a report of four cases. *Arthritis Rheum.* **43**, 1891-1896.
- Engblom, D., Saha, S., Engstrom, L., Westman, M., Audoly, L. P., Jakobsson, P. J. and Blomqvist, A. (2003) Microsomal prostaglandin E synthase-1 is the central switch during immune-induced pyresis. *Nat. Neurosci.* **6**, 1137-1138.
- Harada, Y., Hatanaka, K., Kawamura, M., Saito, M., Ogino, M., Majima, M., Ohno, T., Ogino, K., Yamamoto, K., Taketani, Y., Yamamoto, S. and Katori, M. (1996) Role of prostaglandin H synthase-2 in prostaglandin E₂ formation in rat carrageenin-induced pleurisy. *Prostaglandins* **51**, 19-33.
- Hegen, M., Sun, L., Uozumi, N., Kume, K., Goad, M. E., Nickerson-Nutter, C. L., Shimizu, T. and Clark, J. D. (2003) Cytosolic phospholipase A₂-deficient mice are resistant to collagen-induced arthritis. *J. Exp. Med.* **197**, 1297-1302.
- Hizaki, H., Segi, E., Sugimoto, Y., Hirose, M., Saji, T., Ushikubi, F., Matsuoka, T., Noda, Y., Tanaka, T., Yoshida, N., Narumiya, S. and Ichikawa, A. (1999) Abortive expansion of the cumulus and impaired fertility in mice lacking the prostaglandin E receptor subtype EP2. *Proc. Natl. Acad. Sci. USA* **96**, 10501-10506.
- Jakobsson, P.-J., Thoren, S., Morgenstern, R. and Samuelsson, B. (1999) Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. *Proc. Natl. Acad. Sci. USA* **96**, 7220-7225.
- Kamei, D., Murakami, M., Nakatani, Y., Ishikawa, Y., Ishii, T. and Kudo, I. (2003) Potential role of microsomal prostaglandin E synthase-1 in tumorigenesis. *J. Biol. Chem.* **278**, 19396-19405.
- Kamei, D., Yamakawa, K., Takegoshi, Y., Mikami-Nakanishi, M., Nakatani, Y., Oh-Ishi, S., Yasui, H., Azuma, Y., Hirasawa, N., Ohuchi, K., Kawaguchi, H., Ishikawa, Y., Ishii, T., Uematsu, S., Akira, S., Murakami, M., and Kudo, I. (2004) Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin E synthase-1. *J. Biol. Chem.* **279**, 33684-33695.
- Kobayashi, T., Nakatani, Y., Tanioka, T., Tsujimoto, M., Nakajo, S., Nakaya, K., Murakami, M. and Kudo, I. (2004) Regulation of cytosolic prostaglandin E synthase by phosphorylation. *Biochem. J.* **381**, 59-69.
- Kulmacz, R. J. and Wang, L.-H. (1995) Comparison of hydroperoxide initiator requirements for the cyclooxygenase activities of prostaglandin H synthase-1 and -2. *J. Biol. Chem.* **270**, 24019-24023.
- Li, S., Wang, Y., Matsumura, K., Ballou, L. R., Morham, S. G. and Blatteis, C. M. (1999) The febrile response to lipopolysaccharide is blocked in cyclooxygenase-2 (-/-), but not in cyclooxygenase-1 (-/-) mice. *Brain Res.* **825**, 86-94.
- Loftin, C. D., Trivedi, D. B., Tiano, H. F., Clark, J. A., Lee, C. A., Epstein, J. A., Morham, S. G., Breyer, M. D., Nguyen, M., Hawkins, B. M., Goulet, J. L., Smithies, O., Koller, B. H. and Langenbach, R. (2001) Failure of ductus arteriosus closure and remodeling in neonatal mice deficient in cyclooxygenase-1 and cyclooxygenase-2. *Proc. Natl. Acad. Sci. USA* **98**, 1059-1064.
- Mabuchi, T., Kojima, H., Abe, T., Takagi, K., Sakurai, M., Ohmiya, Y., Uematsu, S., Akira, S., Watanabe, K. and Ito, S. (2004) Membrane-associated prostaglandin E synthase-1 is required for neuropathic pain. *Neuroreport* **15**, 1395-1398.
- McAdam, B. F., Catella-Lawson, F., Mardini, I. A., Kapoor, S., Lawson, J. A. and FitzGerald, G. A. (1999) Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. *Proc. Natl. Acad. Sci. USA* **96**, 272-277.
- Moore, A. H., Olschowka, J. A. and O'Banion, M. K. (2004) Intraparenchymal administration of interleukin-1 induces cyclooxygenase-2-mediated expression of membrane- and cytosolic-associated prostaglandin E synthases in mouse brain. *J. Neuroimmunol.* **148**, 32-40.
- Morita, I., Schindler, M., Reiger, M. K., Otto, J. C., Hori, T., DeWitt, D. L. and Smith, W. L. (1995) Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2. *J. Biol. Chem.* **270**, 10902-10908.
- Murakami, M., Kambe, T., Shimbara, S. and Kudo, I. (1999) Functional coupling between various phospholipase A₂s and cyclooxygenases in immediate and delayed prostanoid biosynthetic pathways. *J. Biol. Chem.* **274**, 3103-3115.
- Murakami, M. and Kudo, I. (2004) Recent advances in molecular biology and physiology of the prostaglandin E₂-biosynthetic pathway. *Prog. Lipid Res.* **43**, 3-35.
- Murakami, M., Kuwata, H., Amakasu, Y., Shimbara, S., Nakatani, Y., Atsumi, G. and Kudo, I. (1997) Prostaglandin E₂ amplifies cytosolic phospholipase A₂ and cyclooxygenase-2- dependent delayed prostaglandin E₂ generation in mouse osteoblastic cells: enhancement by secretory phospholipase A₂. *J. Biol. Chem.* **272**, 19891-19897.
- Murakami, M., Nakashima, K., Kamei, K., Masuda, S., Ishikawa, Y., Ishii, T., Ohmiya, Y., Watanabe, K., and Kudo, I. (2003) Cellular prostaglandin E₂ production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and -2. *J. Biol. Chem.* **278**, 37937-37947.
- Murakami, M., Naraba, H., Tanioka, T., Semmyo, N., Nakatani, Y., Kojima, F., Ikeda, T., Fueki, M., Ueno, A., Oh-Ishi, S. and Kudo, I. (2000) Regulation of prostaglandin E₂ biosynthesis by inducible membrane-associated prostaglandin E₂ synthase that acts in concert with cyclooxygenase-2. *J. Biol. Chem.* **275**, 32783-32792.
- Myers, L. K., Higgins, G. C., Finkel, T. H., Reed, A. M., Thompson, J. W., Walton, R. C., Hendrickson, J., Kerr, N. C., Pandya-Lipman, R. K., Shlopov, B. V., Stastny, P., Postlethwaite, A. E. and Kang, A. H. (2001) Juvenile arthritis and autoimmunity to type II collagen. *Arthritis Rheum.* **44**, 1775-1781.
- Naraba, H., Murakami, M., Matsumoto, H., Shimbara, S., Ueno, A., Kudo, I. and Oh-ishi, S. (1998) Segregated coupling of phospholipases A₂, cyclooxygenases, and terminal prostanoid synthases in different phases of prostanoid biosynthesis in rat peritoneal macrophages. *J. Immunol.* **160**, 2974-2982.
- Naraba, H., Yokoyama, C., Tago, N., Murakami, M., Kudo, I., Fueki, M., Oh-Ishi, S. and Tanabe, T. (2002) Transcriptional regulation of the membrane-associated prostaglandin E₂ synthase gene. Essential role of the transcription factor Egr-1. *J. Biol. Chem.* **277**, 28601-28608.
- Ni, H., Sun, T., Ma, X. H. and Yang, Z. M. (2003) Expression and regulation of cytosolic prostaglandin E synthase in mouse

- uterus during the peri-implantation period. *Biol. Reprod.* **68**, 744-750.
- Oshima, H., Oshima, M., Inaba, K. and Taketo, M. M. (2004) Hyperplastic gastric tumors induced by activated macrophages in COX-2/mPGES-1 transgenic mice. *EMBO J.* **23**, 1669-1678.
- Oshima, M., Dinchuk, J. E., Kargman, S. L., Oshima, H., Hancock, B., Kwong, E., Trzaskos, J. M., Evans, J. F. and Taketo, M. M. (1996) Suppression of intestinal polyposis in *Apc⁷¹⁶* knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* **87**, 803-809.
- Reddy, S. T. and Herschman, H. R. (1997) Prostaglandin synthase-1 and prostaglandin synthase-2 are coupled to distinct phospholipases for the generation of prostaglandin D₂ in activated mast cells. *J. Biol. Chem.* **272**, 3231-3237.
- Rocca, B., Spain, L. M., Pure, E., Langenbach, R., Patrono, C. and FitzGerald, G. A. (1999) Distinct roles of prostaglandin H synthases 1 and 2 in T-cell development. *J. Clin. Invest.* **103**, 1469-1477.
- Saha, S., Engstrom, L., Mackerlova, L., Jakobsson, P. J. and Blomqvist, A. (2005) Impaired febrile responses to immune challenge in mice deficient in microsomal prostaglandin E synthase-1. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **288**, 1100-1107.
- Segi, E., Sugimoto, Y., Yamasaki, A., Aze, Y., Oida, H., Nishimura, T., Murata, T., Matsuoka, T., Ushikubi, F., Hirose, M., Tanaka, T., Yoshida, N., Narumiya, S., and Ichikawa, A. Patent ductus arteriosus and neonatal death in prostaglandin receptor EP4-deficient mice. *Biochem. Biophys. Res. Commun.* **246**, 7-12.
- Shitashige M, Morita I, Murota S. (1998) Different substrate utilization between prostaglandin endoperoxide H synthase-1 and -2 in NIH3T3 fibroblasts. *Biochim. Biophys. Acta* **1389**, 57-66.
- Sonoshita, M., Takaku, K., Sasaki, N., Sugimoto, Y., Ushikubi, F., Narumiya, S., Oshima, M., and Taketo, M. M. (2001) Acceleration of intestinal polyposis through prostaglandin receptor EP2 in *Apc⁷¹⁶* knockout mice. *Nat. Med.* **7**, 1048-1051.
- Stichtenoth, D. O. and Frolich, J. C. (2000) COX-2 and the kidneys. *Curr. Pharm. Des.* **6**, 1737-1753.
- Takaku, K., Sonoshita, M., Sasaki, N., Uozumi, N., Doi, Y., Shimizu, T. and Taketo, M. M. (2002) Suppression of intestinal polyposis in *Apc⁷¹⁶* knockout mice by an additional mutation in the cytosolic phospholipase A₂ gene. *J. Biol. Chem.* **275**, 34013-34016.
- Tanikawa, N., Ohmiya, Y., Ohkubo, H., Hashimoto, K., Kangawa, K., Kojima, M., Ito, S. and Watanabe, K. (2002) Identification and characterization of a novel type of membrane-associated prostaglandin E synthase. *Biochem. Biophys. Res. Commun.* **291**, 884-889.
- Tanioka, T., Nakatani, Y., Semmyo, N., Murakami, M. and Kudo, I. (2000) Molecular identification of cytosolic prostaglandin E₂ synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E₂ biosynthesis. *J. Biol. Chem.* **275**, 32775-32782.
- Thoren, S., Weinander, R., Saha, S., Jegerschold, C., Pettersson, P. L., Samuelsson, B., Hebert, H., Hamberg, M., Morgenstern, R. J. and Jakobsson, P. J. (2003) Human microsomal prostaglandin E synthase-1: purification, functional characterization, and projection structure determination. *J. Biol. Chem.* **278**, 22199-22209.
- Trebino, C. E., Stock, J. L., Gibbons, C. P., Naiman, B. M., Wachtmann, T. S., Umland, J. P., Pandher, K., Lapointe, J. M., Saha, S., Roach, M. L., Carter, D., Thomas, N. A., Durtschi, B. A., McNeish, J. D., Hambor, J. E., Jakobsson, P. J., Carty, T. J., Perez, J. R. and Audoly, L. P. (2003) Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. *Proc. Natl. Acad. Sci. USA* **100**, 9044-9049.
- Uematsu, S., Matsumoto, M., Takeda, K. and Akira, S. (2002) Lipopolysaccharide-dependent prostaglandin E₂ production is regulated by the glutathione-dependent prostaglandin E₂ synthase gene induced by the Toll-like receptor 4/MyD88/NF-IL6 pathway. *J. Immunol.* **168**, 5811-5816.
- Ueno, N., Murakami, M., Tanioka, T., Fujimori, K., Urade, Y. and Kudo, I. (2001) Coupling between cyclooxygenases, terminal prostanoid synthases and phospholipase A₂. *J. Biol. Chem.* **276**, 34918-34927.
- Yamada, T., Komoto, J., Watanabe, K., Ohmiya, Y. and Takusagawa, F. (2005) Crystal structure and possible catalytic mechanism of microsomal prostaglandin E synthase type 2 (mPGES-2). *J. Mol. Biol.* **348**, 1163-1176.
- Yamagata, K., Matsumura, K., Inoue, W., Shiraki, T., Suzuki, K., Yasuda, S., Sugiura, H., Cao, C., Watanabe, Y., and Kobayashi, S. (2001) Coexpression of microsomal-type prostaglandin E synthase with cyclooxygenase-2 in brain endothelial cells of rats during endotoxin-induced fever. *J. Neurosci.* **21**, 2669-2677.