Department of Medical Biochemistry and Biophysics, Division of Chemistry II, Karolinska Institutet, 171 77 Stockholm, Sweden

# Characterization of human glutathione-dependent microsomal prostaglandin E synthase-1

**Staffan Thorén**


**Stockholm 2003**

## ABSTRACT

Prostaglandins (PGs) are lipid mediators, which act as local hormones. PGs are formed in most cells and are synthesized *de novo* from membrane-released arachidonic acid (AA) upon cell activation. Prostaglandin H synthase (PGHS) –1 or 2, also referred to as COX-1 and COX-2, metabolize AA to $PGH_2$, which is subsequently converted in a cell-specific manner by downstream enzymes to biologically active prostanoids, *i.e.* $PGE_2$, $PGD_2$, $PGF_{2\alpha}$, $PGI_2$ or $TXA_2$. PGHS-1 is constitutively expressed in many cells and is mainly involved in housekeeping functions, such as vascular homeostasis, whereas PGHS-2 can be induced by proinflammatory cytokines at sites of inflammation. Prostaglandin E synthase (PGES) specifically catalyzes the conversion of $PGH_2$ to $PGE_2$, which is a biologically potent prostaglandin involved in several pathological conditions; including pain, fever, inflammation and possibly some forms of cancers and neurodegenerative diseases.

mPGES-1 was initially identified as a homologue to microsomal glutathione transferase-1 (MGST1) with 37% identity on the amino acid sequence level and referred to as MGST1-like 1 (MGST1-L1). Based on the properties of MGST1-L1, regarding size, amino acid sequence, hydropathy and membrane localization, the protein was identified as a member of the MAPEG-superfamily (**m**embrane-**a**ssociated **p**roteins in **e**icosanoid and **g**lutathione metabolism). The superfamily consists of 16-18 kDa, integral membrane proteins with typical hydropathy profiles and diverse functions. The MAPEG family comprises six human members, which in addition to mPGES-1 are; 5-lipoxygenase activating protein (FLAP), leukotriene $C_4$ synthase ($LTC_4S$), MGST1, MGST2 and MGST3. MGST1 -2 and -3 are glutathione transferases as well as glutathione-dependent peroxidases, while FLAP and $LTC_4S$ are crucial for leukotriene biosynthesis.

Human mPGES-1 was cloned and characterized as a 16 kDa, inducible GSH-dependent microsomal PGE synthase. Northern dot blot analysis of mPGES-1 mRNA demonstrated a low expression in most tissues, medium expression in reproductive organs and a high expression in two cancer cell lines (A549 and HeLa). A549 cells had been used earlier as a model system to study PGHS-2 induction by the proinflammatory cytokine IL-1$\beta$ and mPGES-1 was also induced by IL-1$\beta$ in these cells. A protein of similar size was detected in microsomes from sheep vesicular glands, which are known to contain a highly efficient microsomal PGES, indicating that mPGES-1 was the long-sought membrane bound PGES. Furthermore, a time study of PGHS-2 and mPGES-1 expression revealed a coordinate induction of these enzymes, which was correlated with increased PGES activity in the microsomal fraction. Tumor necrosis factor-$\alpha$ (TNF-$\alpha$) also induced mPGES-1 in these cells and dexamethasone was found to counteract the effect of these cytokines on mPGES-1 induction. A method based on RP-HPLC and UV-detection was developed to efficiently quantify PGES activity. A small set of potential mPGES-1 inhibitors were tested and NS-398, Sulindac sulfide and $LTC_4$ were found to inhibit PGES activity with $IC_{50}$-values of 20 µM, 80 µM and 5 µM, respectively.

The human mPGES-1 gene structure was investigated. The mPGES-1 gene span a region of approximately 15 kb, is divided into three exons and is localized on chromosome 9q34.3. A 682 bp fragment directly upstream of the translation start site exhibited promoter activity when transfected in A549 cells. The putative promoter is GC-rich, lacks a TATA box at a functional site and contains numerous potential transcription factor binding-sites. Two GC-boxes, two tandem Barbie-boxes and an aryl hydrocarbon response element were identified. The putative promoter region of mPGES-1 was transcriptionally active and reporter constructs were regulated by IL-1$\beta$ and phenobarbital.

The expression of mPGES-1 was investigated in synovial tissues from patients suffering from rheumatoid arthritis (RA). Primary synovial cells obtained from patients with RA were treated with IL-1$\beta$ or TNF-$\alpha$. Both cytokines were found to induce mPGES-1 mRNA from low basal levels to maximum levels after 24 hours and the induction by IL-1$\beta$ was inhibited by dexamethasone in a dose-dependent manner. The protein expression of mPGES-1 was also induced by IL-1$\beta$ with a linear increase up to 72 h. In contrast, the PGHS-2 induction demonstrated an earlier peak expression (4-8 h). Furthermore, the protein expression of mPGES-1 was correlated with increased microsomal PGES activity. In these biochemical experiments any significant contribution of cytosolic PGES or other cytosolic or non-inducible membrane bound PGE synthases was ruled out.

A purification protocol for mPGES-1 was developed. Human mPGES-1 was expressed with a histidine tag in *Eschericha coli*, solubilized by Triton X-100 and purified by a combination of hydroxyapatite and immobilized metal affinity chromatography. mPGES-1 catalyzed a rapid GSH-dependent conversion of $PGH_2$ to $PGE_2$ (170 µmol/min mg). The enzyme, also displayed a high GSH-dependent activity against $PGG_2$, forming 15-hydroperoxy $PGE_2$ (250 µmol/min mg). In addition, mPGES-1 possessed several other activities; glutathione-dependent peroxidase activity towards cumene hydroperoxide, 5-HpETE and 15-hydroperoxy-$PGE_2$, as well as conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) to GSH. These activities likely reflect the relationship with other MAPEG enzymes. Two-dimensional crystals of purified mPGES-1 were obtained and a 10 Å projection map was determined by electron crystallography. Hydrodynamic studies were also performed on the mPGES-1-Triton X-100 complex to investigate the oligomeric state of the protein. Electron crystallography and hydrodynamic studies independently demonstrated a trimeric organization of mPGES-1.

Together with other studies published to date, mPGES-1 has been verified biologically as a drug target and the next step in this validation process requires specific inhibitors to be tested in animal disease models.


ISBN: 91-7349-637-5

**To Gunilla**

In memory of my mother

*Imagination is more important*
*than knowledge.*

*Albert Einstein*

The present thesis is based on the following original articles.

**I** Jakobsson P-J, **Thoren S**, Morgenstern R, 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 U S A., 96(13): 7220-5.

**II** **Thoren S**, Jakobsson P-J. (2000). Coordinate up- and down-regulation of glutathione-dependent prostaglandin E synthase and cyclooxygenase-2 in A549 cells. Inhibition by NS-398 and leukotriene $C_4$. Eur J Biochem., 267(21):6428-34.

**III** Forsberg L, Leeb L, **Thoren S**, Morgenstern R, Jakobsson P-J. (2000). Human glutathione dependent prostaglandin E synthase: gene structure and regulation. FEBS Lett., 471(1): 78-82.

**IV** Stichtenoth DO, **Thoren S**, Bian H, Peters-Golden M, Jakobsson P-J, Crofford LJ. (2001). Microsomal prostaglandin E synthase is regulated by proinflammatory cytokines and glucocorticoids in primary rheumatoid synovial cells. J Immunol., 167(1): 469-74.

**V** **Thoren S**, Weinander R, Saha S, Jegerschold C, Pettersson PL, Samuelsson B, Hebert H, Hamberg M, Morgenstern R, Jakobsson P-J. (2003). Human microsomal prostaglandin E synthase-1: purification, functional characterization, and projection structure determination. J Biol Chem., 278(25): 22199-209.

These papers were reproduced with permission from the publishers.

# ABBREVIATIONS

| | |
|---|---|
| 11R-HpETE | 11-(R)-hydroperoxy-5,8,14-*cis*-12-*trans*-eicosatetraenoic acid |
| 12-HHT | 12-(S)-hydroxy-8, 10-*trans*-5-*cis*-heptadecatrienoic acid |
| 15R-HpETE | 15-(R)-hydroperoxy-5,8,11-*cis*-13-*trans*-eicosatetraenoic acid |
| 15S-HpETE | 15-(S)-hydroperoxy-5,8,11-*cis*-13-*trans*-eicosatetraenoic acid |
| 13-PGR | $\Delta^{13}$-15-ketoprostaglandin reductase |
| 15-PGDH | 15-hydroperoxyprostaglandin dehydrogenase |
| 5-HpETE | 5(S)-Hydroperoxy-8,11,14-*cis*-6-*trans*-eicosatetraenoic acid |
| 5-LO | 5-lipoxygenase |
| AA | Arachidonic acid, 5,8,11,14-*cis*-eicosatetraenoic acid |
| AD | Alzheimer's disease |
| AKR | Aldo keto reductase |
| APC | Adenomatous polyposis coli |
| ASA | Acetylsalicylic acid |
| ATP | Adenosinetriphosphate |
| cAMP | Adenosine 3',5'-cyclic monophosphate |
| cDNA | Complementary deoxyribonucleic acid |
| CDNB | 1-chloro-2,4-dinitrobenzene |
| CNS | Central nervous system |
| CoA | Coenzyme A |
| COX | Cyclooxygenase |
| cPGES | Cytosolic prostaglandin E synthase |
| $cPLA_2$ | Cytosolic $Ca^{2+}$-dependent phospholipase $A_2$ |
| CRE | cAMP response element |
| DAG | Diacylglycerol |
| DHA | Docosohexaenoic acid |
| DHPC | Diheptanoylphosphatidylcholine |
| DP | Prostaglandin D receptor |
| Egr-1 | Early growth response-1 |
| EP | Eicosanoid receptor |
| ER | Endoplasmatic reticulum |
| EST | Expressed sequence tag |
| FLAP | Five lipoxygenase activating protein |
| FP | Prostaglandin F receptor |
| GRE | Glucocorticoid response element |
| GSH | Reduced glutathione |
| GST | Glutathione S-transferase |
| $His_6$-mPGES-1 | 6-histidine tagged microsomal prostaglandin E synthase-1 |
| HSD | Hydroxystereoid dehydrogenase |
| Hsp90 | Heat shock protein 90 |
| HSPG | Heparan sulfate proteoglycan |
| HUVEC | Human umbilical vein endothelial cells |
| IC | Inhibitory concentration |
| $I\kappa\beta$ | Inhibitory kappa beta |
| IL-1 | Interleukin-1 |
| IL-1$\beta$ | Interleukin-1 beta |
| INF$\gamma$ | Interferon gamma |
| IP | Prostaglandin I receptor |
| $IP_3$ | Inositol 1,4,5-trisphosphate |
| $iPLA_2$ | Cytosolic $Ca^{2+}$-independent phospholipase $A_2$ |
| JNK/SAPK | Jun N-terminal kinase/stress activated protein kinase |
| LDL | Low-density lipoprotein |
| LPS | Lipopolysaccharide |
| LST-1 | Liver-specific organic anionic transporter-1 |
| LT | Leukotriene |
| $LTA_4$ | Leukotriene $A_4$, 5(S)-*trans*-5,6-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid |
| $LTC_4$ | Leukotriene $C_4$, 5(S)-hydroxy-6(R)-S-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid |
| $LTC_4S$ | Leukotriene $C_4$ synthase |
| $LTD_4$ | Leukotriene $D_4$, 5(S)-hydroxy-6(R)-S-cysteinylglycyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid |
| $LTE_4$ | Leukotriene $E_4$, 5(S)-hydroxy-6(R)-S-cysteinyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid |
| MAPEG | Membrane associated proteins in eicosanoid and glutathione metabolism |
| MAPK | Mitogen-activated protein kinase |
| MGST1 | Microsomal glutathione S-transferase-1 |
| MGST1-L1 | Microsomal glutathione S-transferase-1 like 1 |
| MGST2 | Microsomal glutathione S-transferase-2 |
| MGST3 | Microsomal glutathione S-transferase-3 |
| MMP | Matrix metallo protease |
| mPGES | Microsomal prostaglandin E synthase |

| | |
|---|---|
| MyD88 | Myeloid differentiation factor 88 |
| $NAD^+$ | Nicotinamid adenine dinucleotide (oxidized form) |
| NADH | Nicotinamid adenine dinucleotide (reduced form) |
| NADPH | Nicotinamid adenine dinucleotide phosphate (reduced form) |
| NEM | N-ethylmalemide |
| NF-κβ | Nuclear factor kappa beta |
| NIK | Nuclear factor kappa beta-inducing kinase |
| NSAID | Non steroidal anti-inflammatory drug |
| PA | Phosphatidic acid |
| PC | Phosphatidylcholine |
| PG | Prostaglandin |
| $PGA_2$ | Prostaglandin $A_2$, 15(S)-hydroxy-9-ketoprosta-5, 10-*cis*-13-*trans*-trienoic acid |
| $PGC_2$ | Prostaglandin $C_2$, 15(S)-hydroxy-9-ketoprosta-5, 11-*cis*-13-*trans*-trienoic acid |
| PGCS | Prostaglandin C synthase |
| $PGD_2$ | Prostaglandin $D_2$, 9α, 15(S)-dihydroxy-11-ketoprosta-5-*cis*-13-*trans*-dienoic acid |
| PGDS | Prostaglandin D synthase |
| $PGE_2$ | Prostaglandin $E_2$, 11α, 15(S)-dihydroxy-9-ketoprosta-5-*cis*-13-*trans*-dienoic acid |
| PGES | Prostaglandin E synthase |
| $PGF_{2\alpha}$ | Prostaglandin $F_{2\alpha}$, 9α,11α 15(S)-trihydroxyprosta-5-*cis*-13-*trans*-dienoic acid |
| PGFS | Prostaglandin F synthase |
| $PGG_2$ | Prostaglandin $G_2$, 15(S)-hydroxyperoxy-9α-peroxidoprosta-5-*cis*-13-*trans*-dienoic acid |
| $PGH_2$ | Prostaglandin $H_2$, 15(S)-hydroxy-9α-peroxidoprosta-5-*cis*-13-*trans*-dienoic acid |
| PGHS | Prostaglandin H synthase |
| $PGI_2$ | Prostaglandin $I_2$, 6,9α-epoxy-11α, 15(S)-dihydroxyprosta-5-*cis*-13-*trans*-dienoic acid (prostacyclin) |
| PGIS | Prostaglandin I synthase |
| $PGJ_2$ | Prostaglandin $J_2$, 15(S)-hydroxy-11-ketoprosta-5, 9-*cis*-13-*trans*-trienoic acid |
| PGT | Prostaglandin transporter |
| $PIP_2$ | Phosphatidyl inositol 4,5-bisphosphate |
| PL | Phospholipase |
| $PLA_2$ | Phospholipase $A_2$ |
| PLC | Phospholipase C |
| PLD | Phospholipase D |
| PMA | 12-myristate 13-acetate |
| POX | Peroxidase |
| PPAR-γ | Peroxisome proliferator-activated receptor gamma |
| RA | Rheumatoid arthritis |
| RP-HPLC | Reverse phase high performance liquid chromatography |
| $sPLA_2$ | Secretory phospholipase $A_2$ |
| TLR4 | Toll-like receptor 4 |
| TNF | Tumor necrosis factor |
| TNFα | Tumor necrosis factor alpha |
| TP | Thromboxane A receptor |
| TPA | Tumor-promoting phorbol esters |
| TRAF | TNF receptor associated factor |
| $TXA_2$ | Thromboxane $A_2$, 9α,11α, epoxy-15(S)-hydroxythromba-5-*cis*-13-*trans*-dienoic acid |
| TXAS | Thromboxane $A_2$ synthase |
| TXBS | Thromboxane $B_2$ synthase |
| UV | Ultraviolet |

# CONTENTS

# INTRODUCTION

## Historical background

Almost a century ago, in 1913, Battez & Boulet reported a lowered blood pressure and contraction of the urinary bladder in dogs after injections from extracts of the human prostate gland (23). In 1930, two gynecologists, Kurzrok & Lieb discovered that human semen contracted and relaxed the human uterus during artificial insemination (171). Goldblatt (95,96) and von Euler (412,413) extended this research and discovered that human semen contained a substance that lowered the blood pressure and stimulated the smooth muscle of the uterus. The substance appeared to be an amphipathic acid and was termed “prostaglandin” by von Euler (411), since its biological activity seemed to originate from the accessory genital glands. Twenty-four years later, Bergström and Sjövall isolated the prostaglandins E and F (30,31) and soon thereafter, the structures of these compounds were solved (29). Mass spectrometry was the key method for these discoveries. In 1964, Bergström and van Dorp and collaborators, independently discovered that prostaglandins originated from $C_{20}$ polyunsaturated fatty acids (28,389). They incubated homogenates of the sheep vesicular gland with [$^3$H]-labeled arachidonic acid (AA) and observed an enzymatic conversion to $PGE_2$. The biosynthetic pathway for the formation of prostaglandins was not known, but the three incorporated oxygens were found to derive from molecular oxygen (263,307) and Bengt Samuelsson postulated the involvement of a cyclic endoperoxide in the formation of these compounds (307). In 1973, Hamberg and Samuelsson with coworkers isolated two short-lived endoperoxides in the biosynthesis of prostaglandins, which subsequentially led to a series of discoveries of new metabolic products from this pathway, such as thromboxane (104,105) and prostacyclin (221,405). At that time, a high glutathione-dependent prostaglandin E synthase activity was also found in microsomes from bovine and sheep vesicular glands (223,264,363).

Other arachidonic acid metabolites were soon structurally elucidated, such as the leukotrienes (LTs), $LTC_4$ (106), $LTD_4$ and $LTE_4$ (187,230). Two separate pathways for leukotriene and prostaglandin formation were discovered, namely the 5-lipoxygenase (5-LO) pathway (39,40) and the prostaglandin H synthase (PGHS) pathway (28,389,390), respectively (Fig. 1). All metabolites that originate from arachidonic acid or other $C_{20}$ polyunsaturated fatty acids are commonly called eicosanoids due to their related structures.

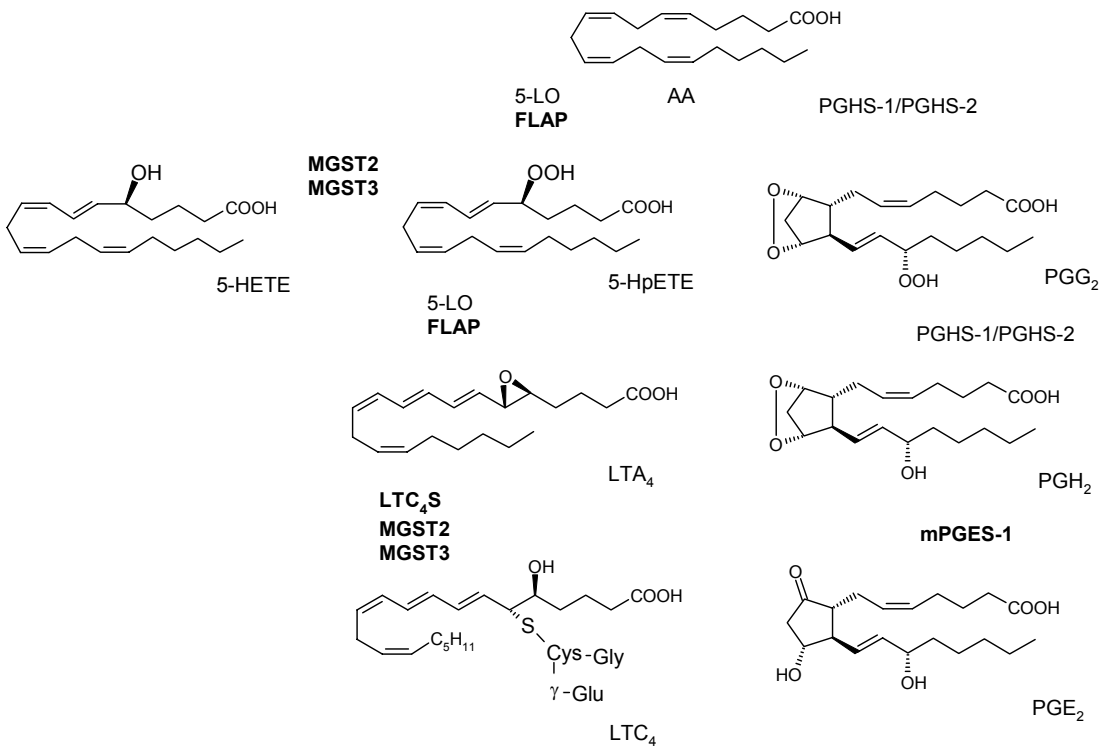


**Figure 1.** Overview of the 5-LO and PGHS pathways. $LTA_4$ and $PGH_2$ can be further metabolized by specific enzymes into various leukotrienes and prostaglandins, besides the depicted $LTC_4$ and $PGE_2$. The enzyme abbreviations shown in bold are members of the MAPEG superfamily.

# BIOSYNTHESIS OF EICOSANOIDS

## Eicosanoids

The term eicosanoid originates from the Greek word for 20 (eikosi) and implies products derived from polyunsaturated fatty acids with 20 carbon atoms, *i.e.,* prostaglandins, leukotrienes, thromboxanes, lipoxins and other related compounds. Mammalian cells do not contain the enzymes responsible for introducing double bonds beyond carbon number 9 in polyunsaturated fatty acids and therefore, linoleic acid (18:2 ω6) and linolenic acid (18:3 ω3) are essential fatty acids that need to be obtained through the diet. Linoleic acid can be elongated and desaturated to yield dihomo-γ-linolenic acid (20:3 ω6) and arachidonic acid (20:4 ω6), while linolenic acid can be converted to eicosapentaenoic acid (20:5 ω6). Of these fatty acids, arachidonic acid is the most abundant in human cells. The $C_{20}$ polyunsaturated fatty acids are metabolized into various eicosanoids (Fig. 2).

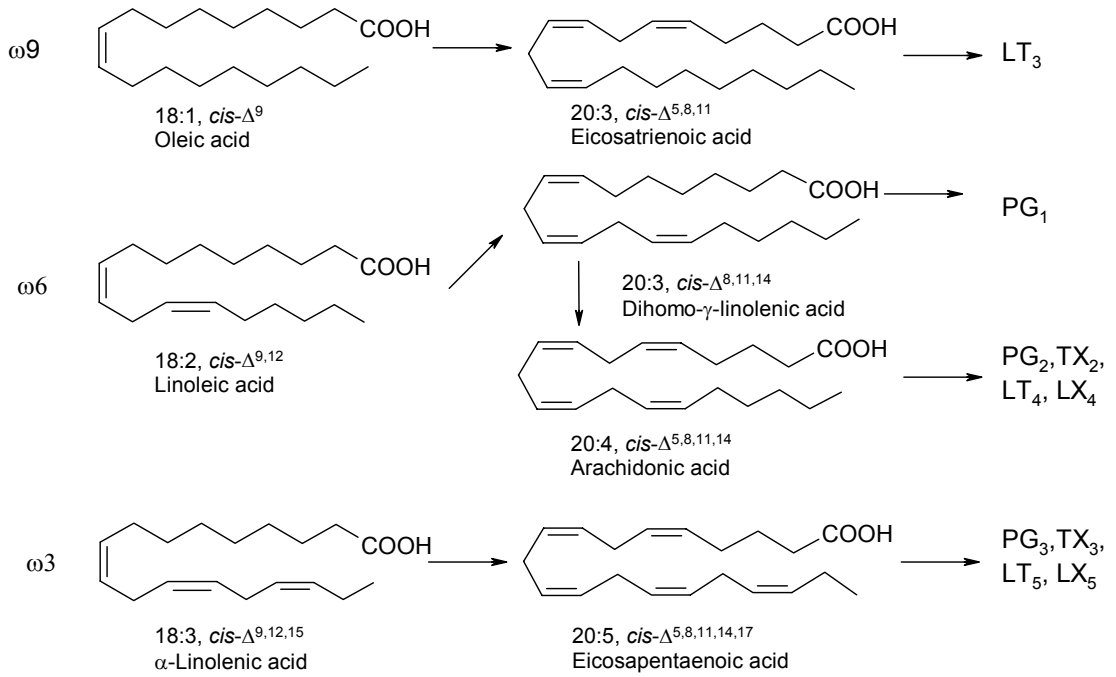


**Figure 2.** Precursors of eicosanoids.

## Release of arachidonic acid

Most arachidonic acid is stored in the cell membranes, esterified in the sn-2 position of phospholipids (137). Under normal conditions, the level of free arachidonic acid is low, but upon stimulation, arachidonic acid is released by phospholipases (PLs). Phospholipases and acyl-CoA transferases determine the concentration of free arachidonic acid through hydrolysis and re-esterification of phospholipids (97,137,335). Phosphatidylcholine (PC) may account for up to 50% of the total content of phospholipids and the inositol-containing lipids for 5-8%. Phospholipase $A_2$ and D can utilize PC as a substrate, while phospholipase C specifically hydrolyzes inositol-containing lipids (59). Many $PLA_2$ enzymes are active within the cell or in the close vicinity and have distinct, but interconnected roles in arachidonic acid release. Phospholipase activity is regulated by several mechanisms including: G-protein coupled receptors (59), phosphorylation (186) and activation by divalent cations (166). So far, 19 enzymes with $PLA_2$ activity have been identified and cloned in mammals (see reviews) (19,121,167). A classification of the $PLA_2$s that has historically been utilized, divides them into three groups: secretory ($sPLA_2$), cytosolic $Ca^{2+}$-dependent ($cPLA_2$), and cytosolic $Ca^{2+}$-independent ($iPLA_2$).

## Phospholipases

*Secreted forms of phospholipase $A_2$*

Secretory $PLA_2$s comprise the largest group of $PLA_2$ enzymes and consist of low molecular weight (14-17 kDa), disulfide-linked $Ca^{2+}$-requiring enzymes (mM range) with a highly conserved catalytic site and $Ca^{2+}$-binding loop (167). Several of the $sPLA_2$ enzymes have been reported to take part in a number of biological processes

such as inflammation and host defense. Secretory $PLA_2$-IIA is the most widely distributed isozyme in humans and rats. Secretory $PLA_2$-IIA is abundantly expressed in human tissues related to the immune response (166,322). Moreover, $sPLA_2$-IIA can be induced in a wide variety of cells by pro-inflammatory cytokines and lipopolysaccharides (LPS) (3,61,172,238,275,356). This induction is suppressed by anti-inflammatory glucocorticoids (249,313).

Secretory $PLA_2$-V is closely related to $sPLA_2$-IIA and is widely expressed in human tissues, with the highest expression in the heart (53). Secretory $PLA_2$-V is also induced by pro-inflammatory stimuli in several immune cells like mast cells, macrophages, and type 2 helper T ($T_H2$) cells (18,312), and appears to substitute for $sPLA_2$-IIA in some cells (15,287,312).

Studies with specific $sPLA_2$ inhibitors, antibodies, antisense nucleotides and transfection studies have revealed that several of the $sPLA_2$ isozymes have the capacity to regulate cellular arachidonic acid release through distinct mechanisms where arachidonic acid is provided to PGHS-1 in the immediate phase and to PGHS-2 in the delayed phase of cellular prostaglandin formation (235,236,245).

Both $sPLA_2$-IIA and $sPLA_2$-V are heparan-binding enzymes that display significant affinity for cell surface heparan sulfate proteoglycan (HSPG) (242,245). The intracellular sorting route, referred to as the HSPG-shuttling pathway, concentrates $sPLA_2$-IIA into restricted intracellular compartments, which allows colocalization with perinuclear arachidonic acid metabolizing enzymes such as PGHS and 5-LO for efficient eicosanoid production (237). However, $sPLA_2$-V can also act on the plasma membrane surface independently of HSPG (109,237).

In addition to these two $sPLA_2$s, $sPLA_2$-X has also been shown to stimulate arachidonic acid release when added exogenously to different cell lines (33,110). Although, $sPLA_2$-X has a low affinity for cell surface HSPG, it can release arachidonic acid spontaneously in the absence of stimuli (33,110). Secretory $PLA_2$-X is expressed in the intestine, colon, stomach and testis (68) and has also been detected in the immune organs (110).

*Cytosolic $Ca^{2+}$-dependent phospholipase $A_2$*

The cytosolic $PLA_2$-sub family consists of high molecular weight $PLA_2$s ($cPLA_2$s) (>60 kDa) (57,166,276,347), of which $cPLA_2\alpha$ (group IVA) has received the most attention. $Ca^{2+}$ and phosphorylation tightly regulate $cPLA_2\alpha$ activity. Cytosolic $PLA_2\alpha$ is the only known $PLA_2$ enzyme with a marked preference for arachidonic acid over other fatty acids in the sn-2 position (57,75,111,166). Cytosolic $PLA_2\alpha$ is constitutively expressed in most tissues and cells, with the exception of mature B and T lymphocytes (93,121,186). Furthermore, the expression of $cPLA_2\alpha$ is increased by pro-inflammatory cytokines and growth factors and can be prevented by glucocorticoids (128,193,313). Submicromolar concentrations of $Ca^{2+}$ is required to facilitate translocation of $cPLA_2\alpha$ from the cytosol to the nuclear membrane or endoplasmatic reticulum, rather than being necessary for catalysis (124,251,291). This translocation is essential for the initiation of arachidonic acid release (57,83,316).

There seems to be a connection between the secreted forms of $PLA_2$s and the cytosolic $PLA_2$s. In support for this, certain forms of $sPLA_2$s (IB or IIA) can

activate cPLA$_2\alpha$ and induce arachidonic acid release via the M-class sPLA$_2$ receptor-mediated pathway (87,118,135). On the other hand, in mouse macrophage-like cells and rat-fibroblastic cells, cPLA$_2\alpha$ is required for the induction and activation of sPLA$_2$-V (16,18,330) and sPLA$_2$-IIA, respectively (172,173).

*Cytosolic $Ca^{2+}$-independent phospholipase $A_2$*

A cytosolic $Ca^{2+}$-independent PLA$_2$ (iPLA$_2$) was cloned and purified in 1996 (14,180,364). iPLA$_2$ shows no strict specificity for sn-2 fatty acids and is fully active in the absence of $Ca^{2+}$, but its role in biological events is not fully elucidated (364). However, iPLA$_2$ is proposed to play function in phospholipid remodeling through deacylation of phospholipids (408).

*Other phospholipases*

Arachidonic acid can also be released through the phospholipase C (PLC)-pathway by cleavage of phosphatidyl inositol-bisphosphate (PIP$_2$) into inositol trisphosphate (IP$_3$) and diacylglycerol (DAG) (294) with subsequent metabolism of DAG by diglyceride lipase and release of arachidonic acid (17,26). Phospholipase D (PLD) can cleave phosphatidylcholine (PC) into phosphatidic acid (PA) and choline. Subsequently, PA can be metabolized by PLA$_2$ into lysoPA and arachidonic acid (364). Alternatively, PA is metabolized by phosphatidic acid phosphohydrolase (PAP) into DAG (375). DAG can then be further metabolized by diglyceride lipases into arachidonic acid (26).

## Functional coupling of PLA$_2$s and PGH synthases

Pharmacological and biochemical studies of PLA$_2$ isozymes and PGH synthases have been performed on various cells in order to identify the enzymes responsible for efficient transfer of arachidonic acid in different phases of prostaglandin biosynthesis. (172,245,252,330). PGHS-1 seems to be limited to the immediate phase of prostaglandin formation, while PGHS-2 is a prerequisite for the delayed phase, which lasts for several hours (172,235,240,252).

The importance of cPLA$_2$ for efficient PG-formation was demonstrated by the use of cPLA$_2$ inhibitors (172,239,245,252,300) and by studies using cPLA$_2$ knock-out mice (88,385). The inducible sPLA$_2$-IIA and sPLA$_2$-V have been demonstrated to participate in both immediate and delayed PG-biosynthesis by the use of antibodies, inhibitors and antisense oligonucleotides (172,252,330,358). Cotransfection studies of several PLA$_2$s with PGHS-1 or PGHS-2, demonstrated that sPLA$_2$-IIA, sPLA$_2$-V and cPLA$_2$ was functionally linked with PGHS-1 and PGHS-2 in the immediate response and predominantly with PGHS-2 in the delayed response, whereas iPLA$_2$ was preferentially linked with PGHS-1 in the immediate response (235,236,245). Furthermore, the sPLA$_2$ enzymes can act on neighboring cells to induce arachidonic acid release. Functional coupling of sPLA$_2$-V and PGHS-1 has also been seen during immediate PGD$_2$-formation (287).

# METABOLISM OF ARACHIDONIC ACID

Free arachidonic acid is metabolized through oxygenation by three enzymatic pathways in mammals. The prostaglandin H synthase (PGHS) pathway produces prostaglandins and thromboxanes via the prostaglandin endoperoxides $PGG_2$ and $PGH_2$, the lipoxygenase pathway produces leukotrienes and certain hydroperoxy acids (HpETEs) and the monooxygenase pathway (cytochrome P-450 enzymes) leads to a series of epoxy- and hydroxy-acid derivatives (see reviews) (48,334).

## Biosynthesis of prostaglandins

Prostaglandins are lipid mediators, which act as local hormones on adjacent cells. They are formed in most cells and are synthesized *de novo* from membrane-released arachidonic acid upon cell activation by mechanical stress, cytokines, growth factors or certain hormones. Both $cPLA_2$s and sPLA2s can be translocated to the endoplasmatic reticulum (ER) and Golgi apparatus after cell activation. Arachidonic acid is then released and presented to one of the forms of prostaglandin H synthase (PGHS), which metabolize AA to $PGH_2$. $PGH_2$ can subsequently be converted in a cell-specific manner by downstream enzymes to biologically active prostanoids, *i.e.* $PGE_2$, $PGD_2$, $PGF_{2\alpha}$, $PGI_2$ or $TXA_2$ (Fig. 3). In many cases, only one prostanoid is produced in a given cell type. For example, thromboxane $A_2$ synthase (TXAS) is present in platelets, prostacyclin synthase (PGIS) is present in endothelial cells, $PGF_{2\alpha}$ synthase (PGFS) is present in the uterus, two types of $PGD_2$ synthase (PGDS) are found in brain and mast cells, respectively and microsomal PGE synthase-1 (mPGES-1) is present in activated cells involved in inflammation (synoviocytes) (353).

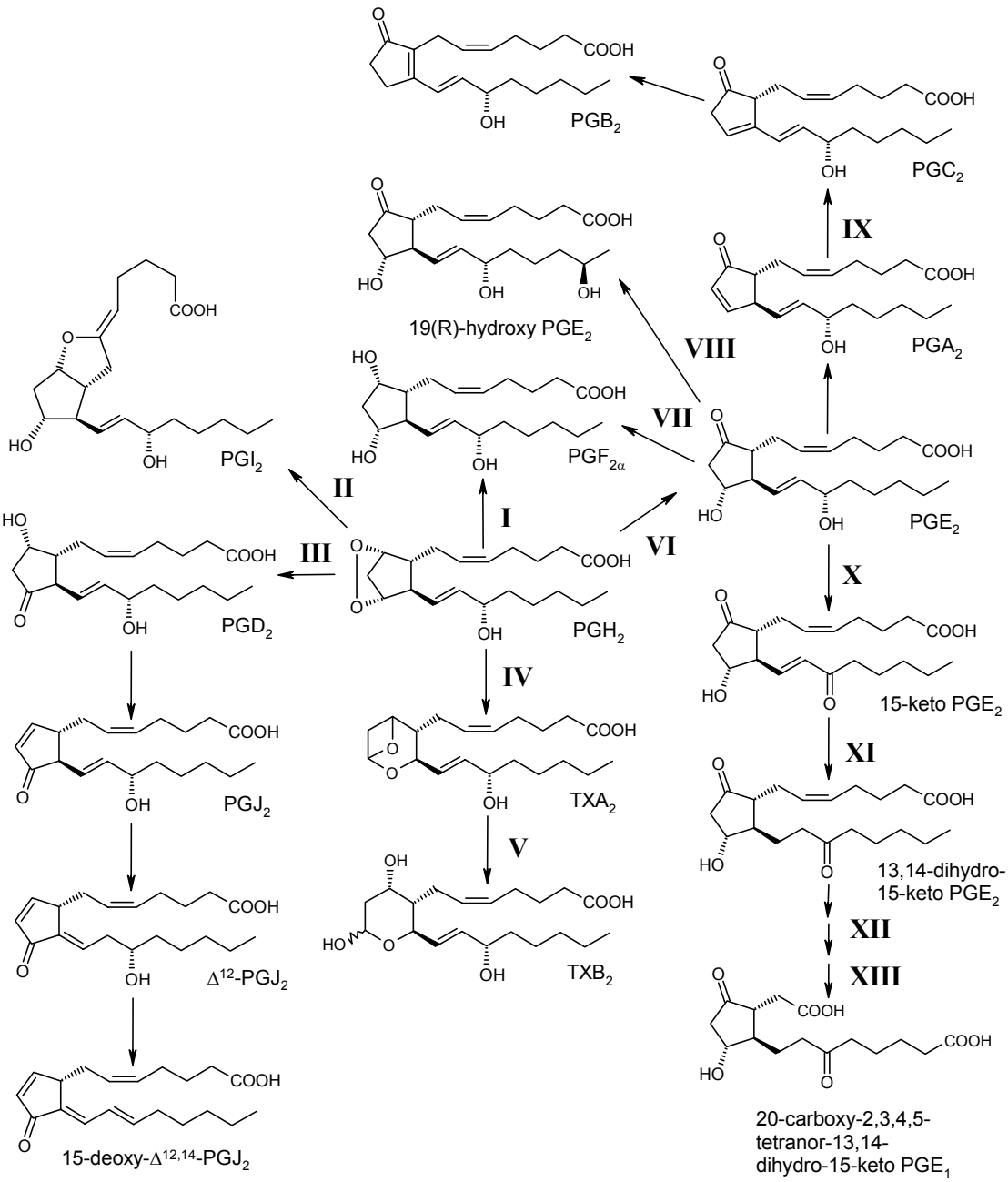


**Figure 3.** $PGH_2$-derived prostanoids. **I**, prostaglandin F synthase, **II**, prostacyclin synthase, **III**, prostaglandin D synthase, **IV**, Thromboxane $A_2$ synthase, **V**, Thromboxane $B_2$ synthase, **VI**, prostaglandin E synthase, **VII**, PGE 9-ketoreductase, **VIII**, cytochrome P450 (CYP4F), **IX**, prostaglandin C synthase, **X**, 15-hydroxyprostaglandin dehydrogenase, **XI**, $\Delta^{13}$-15-ketoprostaglandin reductase, **XII**, $\omega$-oxidation, **XIII**, $\beta$-oxidation.

## PGH-synthase

Prostaglandin H synthase (PGHS; EC 1.14.99.1), also called prostaglandin endoperoxide synthase and cyclooxygenase (COX), is a membrane bound heme-

dependent bis-oxygenase (COX) and peroxidase. PGHS catalyzes the oxidation of arachidonic acid with two molecules of $O_2$, forming the endoperoxide, $PGG_2$. The 15-hydroperoxide of $PGG_2$ is subsequentially reduced by the peroxidase (POX) activity of PGHS to $PGH_2$ (126,205,337,339).

Two isoforms of PGHS have been found in mammals, PGHS-1 (74,214,422) and PGHS-2 (168,417). PGHS-1 is constitutively expressed in most tissues and cells and is considered to be a housekeeping enzyme. In contrast, PGHS-2 is inducible by various stimuli such as hormones, cytokines and mitogens. Both PGHS-1 and PGHS-2 are glycosylated heme-containing homodimers with two catalytic sites. The enzymes are located on the luminal surfaces of the endoplasmatic reticulum (ER) and on the inner and outer membranes of the nuclear envelope (229,349). PGHS-1 and 2 are not integral membrane proteins, but rather anchored to the lipid bilayer through hydrophobic surfaces of their amphipathic helices. Considerable research has focused on the structure, function and physiology of the PGHS isoforms since they are the targets of nonsteroidal anti-inflammatory drugs (NSAIDs), which account for billions of dollars in sales for the pharmaceutical industry (392). PGHS-1 and PGHS-2 have very different expression profiles in several physiological processes (120,337). The PGHS isozymes are also involved in pathological processes. PGHS-1 is involved in thrombosis (273,274), while PGHS-2 mainly takes part in inflammation, pain and fever (64) and some forms of cancer (189,406). It has also been suggested that PGHS-2 plays a role in neurological disorders like Alzheimer's (211) and Parkinson's diseases (344).

*Gene structure and expression of PGHS-1*

The cDNA of PGHS-1 was first cloned from sheep vesicular glands, a rich source of this enzyme (74,214,422), and shortly thereafter, the human gene and primary structure was characterized (423). The human PGHS-1 gene consists of 11 exons, spans a region of approximately 22 kb and is transcribed as a 2.8 kb mRNA coding for a protein with an apparent molecular mass of 70 kDa. The protein is made up of 599 amino acids, including a glycosylation signal and a peptide of 23 amino acids, which is cleaved off in its mature form.

The PGHS-1 promoter region lacks a functional TATA or CAAT box and is GC-rich, which is consistent with a housekeeping gene. Several putative transcriptional regulatory elements are found in the promoter region, such as two sp1 motifs, two AP- sites, an NF-IL6 motif and a GATA.

*Gene structure and expression of PGHS-2*

The inducible PGHS isozyme was discovered in chicken and mouse fibroblasts in response to *src* and tumor-promoting phorbol esters (TPA) (168,417). The isozyme was named PGHS-2 and subsequent cloning work revealed that the human gene is about 8.3 kb long with 10 exons and is transcribed as 2.8, 4.0 and 4.6 kb mRNA variants (127,148). PGHS-2 is also a glycoprotein, of which the mature form contains 587 amino acids. PGHS-1 and –2 display about 60% sequence identity on the amino acid level, while sequence identity among orthologs from different species varies between 85-90% (336). A number of potential transcription regulatory elements have been identified in the 5'-flanking region of the PGHS-2 genes, including a TATA box.

Two NF-κβ, three sp1, two AP-2, one NF-IL6, one CRE and one E-box binding site have been found (136). PGHS is distantly related to a peroxidase family that split up before divergence between mammals and avians.

PGHS-2 is responsible for PG production in several physiological and pathological conditions and there are a number of studies on PGHS-2 gene expression in various tissues and cells associated with these conditions (for reviews, see) (72,119,120,337). The PGHS-2 gene expression is particularly responsive to proinflammatory mediators such as IL-1, TNFα, INFγ, LPS and 12-O-tetradecanoylphorbol 13-acetate (TPA), but also growth factors, hormones and oncogenes. PGHS-2 gene expression can be regulated by some shared signaling pathways between the NF-κβ site, NF-IL6 motif, CRE and E-box in the PGHS-2 promoter (362). Also, three mitogen-activated protein kinase (MAPK) cascades, ERK1/2, JNK/SAPK and p38 contribute to the induction of PGHS-2 either independently or in a concerted manner (354).

*Regulation of PGHS-2 expression*

Gene expression of PGHS-2 is mediated by several receptor-dependent signaling processes (Fig. 4). The Toll-like-receptor 4 (TLR4) has been shown to be the receptor responsible for LPS-mediated signaling (159,421). LPS-induced cellular activation of NF-κβ in endothelial and monocytic cells has been shown to be associated with activation of JNK, ERK1/2 and p38 (100). Interestingly, the IL-1 signaling pathway in mammals is very similar to the Toll signaling pathway in *Drosophila* and activation of these receptors induces the recruitment of an adapter molecule called myeloid differentiation factor 88 (MyD88) (113,298). MyD88 recruits IL-1 receptor-activated kinase (IRAK), which interacts with TNF receptor associated factor 6 (TRAF6). This complex can then interact with NF-κβ-inducing kinase (NIK). In turn, NIK activates the Iκβ kinase complex (IKK) that phosphorylates Iκβ. This phosphorylation leads to an ubiquitin-proteasome-mediated degradation and release of activated NF-κβ. However, the TNF-receptor signaling is not mediated by TRAF6, but instead by TRAF2. The signaling pathways of IL-1, TNF and LPS, converge after TRAF2 and TRAF6. Another adapter protein, called ECSIT, is linked to the signaling pathway from the TLR4 and IL-1 receptors to MEKK.

The MAPK cascade is a very important signaling pathway for PGHS-2 expression and consists of three different subgroups of kinases (ERK, JNK/SAPK, and p38). The ERKs are mainly activated by growth factors and oncogenes, including v-Ras and v-Src. The JNK/SAPK and p38 pathways are activated by proinflammatory cytokines, LPS and environmental stress, like ultraviolet light, ionizing radiation and oxidative stress. When the MAP kinases are activated, they phosphorylate transcription factors, which then regulate gene expression. The proinflammatory cytokine IL-1β is a well-known inducer of PGHS-2 gene expression, which signals through JNK/SAPK and p38 MAPK (99). The PGHS-2 gene has been shown to be an important Ras target since oncogenic mutations in Ras and overexpression of PGHS-2 is found in many forms of human cancers, including breast cancer and colorectal carcinoma (327,355).

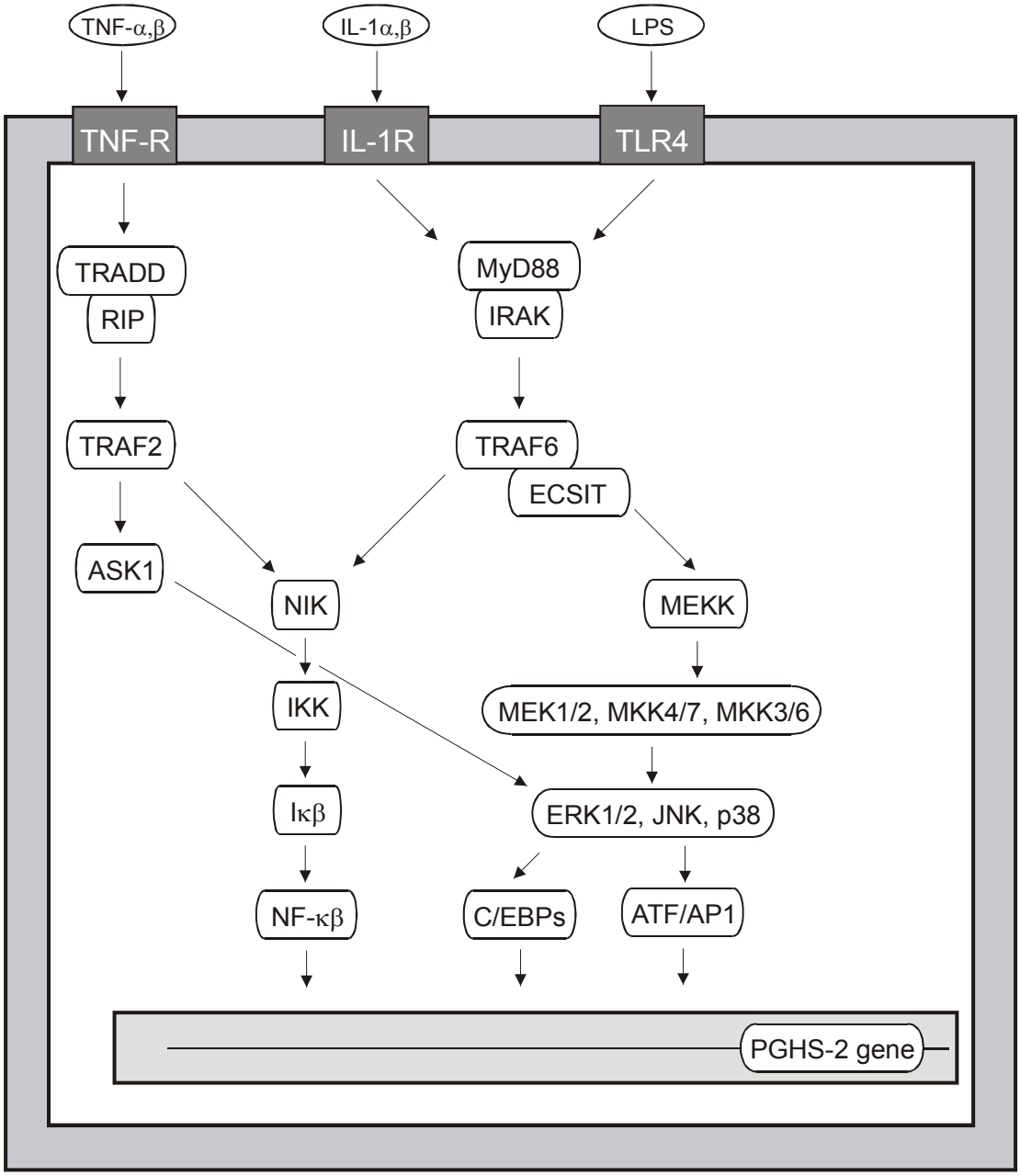


**Figure 4.** Signaling of PGHS-2 gene induction.

Dexamethasone is a common anti-inflammatory steroid, which binds to the glucocorticoid receptor (GR) and activates transcription of a number of genes via glucocorticoid response elements (GREs) (25). Dexamethasone has been found to be an efficient suppressor of inflammatory-induced PGHS-2 expression. No GREs are found in the 5'-flanking region of human PGHS-2, but the mechanism of glucocorticoid-mediated repression involves suppression of AP-1 and NF-κβ-dependent transcription (13,314). Dexamethasone can also act through other post-transcriptional mechanisms of repression and treatment of A549 cells with dexamethasone only reduced the transcription rate of PGHS-2 by 40%, while the mRNA and protein levels were completely repressed (257,258). The

dexamethasone-dependent repression involved a reduced PGHS-2 mRNA half-life with shortening of the average length of the poly(A) tails.

Activated MAPK has also been reported to regulate gene expression at the post-transcriptional level by mRNA stabilization of PGHS-2 (52,410).

*Biological functions*

PGHS-1 and PGHS-2 have striking differences in tissue expression and regulation, therefore it is postulated that they have different physiological functions. Confocal fluorescence imaging microscopy and immunohistological techniques have revealed differences in subcellular localizations of PGHS-1 and PGHS-2. Both PGHS-1 and PGHS-2 are localized in the endoplasmatic reticulum, but PGHS-2 is more concentrated in the nuclear envelope (229).

PGHS-1 is present in nearly all cells under basal conditions (62) and is expressed at high levels in specific tissues, such as vascular endothelia, monocytes, platelets, renal collecting tubules and seminal vesicles. PGHS-1 produces prostaglandins responsible for homeostatic functions, such as regulation of renal blood flow, maintenance of the gastric mucosa and platelet function. Studies of PGHS-1 (-/-) mice have revealed the biological role of PGHS-1. PGHS-1 (-/-) mice have reduced platelet aggregation, are more sensitive to radiation injury, but have no gastric pathology as would be expected, since PGHS-1 was thought to protect the gastric mucosa (132,179). However, when selective PGHS-2 inhibitors were given to PGHS-1 deleted mice, the mucosa protection was significantly decreased (231). Thus, both PGHS-1 and PGHS-2 seems to be involved in gastric mucosa protection. Furthermore, PGHS-1 seems to be important in reproduction, since PGHS-1 (-/-) pairings lead to few offspring (288).

PGHS-1 is not normally induced, but cell-lines that undergo differentiation and mimic the developmental process have increased expression of PGHS-1 (362). Shear stress has also been reported to increase PGHS-1 expression in human umbilical vein endothelial cells (HUVEC) (268) and this together with pharmacological studies (281), suggests a role for PGHS-1 in atherosclerosis.

PGHS-2 is not normally present in most tissues, but is inducible by proinflammatory cytokines, growth factors, hormones and exogenous stimuli in many cell types, like synoviocytes, endothelial cells, chondrocytes, osteoblasts and monocytes (336). However, constitutive PGHS-2 expression has been found in several tissues, like the kidney, lung epithelial cells and intact thyroid tissue (10,114,254,295). Also, tissues that are exposed to constant mechanical stress, such as bone, blood vessels and brain (electrical stimulation) express PGHS-2 constitutively (362). PGHS-2 deficient mice have more profound phenotypic changes as compared to PGHS-1 (-/-) mice. Female PGHS-2 deficient mice have deficient reproductive functions including ovulation, fertilization and implantation (76,227). Phenotype changes in PGHS-2 (-/-) mice have also been observed in peritonitis, cardiac fibrosis, renal nephropathy and failure of ductus arteriosus closure (55,178). PGHS-2 seems to play an important role in tumorigenesis. Supression of tumorigenesis in PGHS-2 (-/-) mice have confirmed epidemiological studies, demonstrating that NSAIDs suppress the incidence of colon cancer (270). However, the effect of NSAIDs on tumorigenesis cannot entirely be explained by inhibition of

PGHS-1 or PGHS-2. Studies with PGHS-1 and PGHS-2 double knockout mice have revealed novel mechanisms for NSAID activities in tumorigenesis, independent of PGHS expression (426).

*PGHS catalysis*

Each PGHS-homodimer contains one heme per subunit, which is necessary for both the COX and POX reaction (218,267). The COX and POX activities occur at separate but functionally interactive sites within the enzyme (342). The peroxidase reaction is necessary to activate the cyclooxygenase reaction. A hydroperoxide reacts with the heme-iron and initiates a two-electron oxidation, which yields an enzyme state with an oxyferryl-heme radical cation, called Compound I. The radical undergoes intramolecular migration from the heme group to Tyr385, which creates intermediate II (338,376). Already by 1967, Hamberg & Samuelsson proposed the basic steps in the PGHS mechanism (103). The postulated mechanism remains virtually the same today, however, recent structural studies of PGHS have revealed important functions in the mechanism, which can be broken down into four steps (91). The carboxyl group of AA interacts with Arg120, which leads to a proper positioning of AA in the COX channel (200,277). The tyrosyl radical (Tyr385) initiates the COX reaction by abstracting the 13*pro*S hydrogen from AA (103,377,378). This step has been shown to be the rate-limiting step of the COX reaction. Subsequently, an 11R-peroxyl radical is formed in the presence of $O_2$. The 11R-peroxyl radical then attacks carbon 9 and forms the endoperoxide with following isomerization of the radical to carbon 8. A major reconfiguration of the substrate is necessary for ring closure between carbon 8 and 12, during or immediately after formation of the endoperoxide bridge. The ω-end of the substrate will have to move much closer to the carboxyl group in this conformational change. The 11R-peroxyl radical is believed to swing “over” carbon 8 with an R-side attack on carbon 9 through the rotation of the bond between carbon 10 and 11, which brings carbon 12 closer to carbon 8 for ring closure. This would also lead to repositioning of carbon 15 for an additional attack of $O_2$, thus forming the 15S-peroxyl radical. The catalytic cycle of COX is completed by the 15S-peroxyl radical, which abstracts the hydrogen from Tyr385, thereby regenerating the tyrosyl radical and producing $PGG_2$ (91). The 15-hydroperoxyl group of $PGG_2$ is thereafter reduced to $PGH_2$ by the POX activity of PGHS. The crystal structure of PGHS does not reveal any direct pathway for $PGG_2$ to travel through the protein from the COX to the POX site. $PGG_2$ is believed to exit the COX site through the opening in the membrane binding domain and travel around the surface of the protein to get to the POX site (342).

The PGHS POX reaction occurs at a heme-containing active site, close to the protein surface, partially exposed to solvent (170,277). The POX reaction is considered to be the second step in the formation of $PGH_2$, but the peroxidase activity is absolutely required for COX activation (337,338). Initially, other peroxides, besides $PGG_2$ are needed to start the COX reaction. Neither the identity nor the source of the hydroperoxide needed for the initial heme oxidation *in vivo* is known. However, as soon as the first catalytic cycle of the COX reaction has started, it can continue independently of the POX cycle (163). Both PGHS-1 and –2 have been shown to reduce a number of peroxides with a marked preference for secondary alkyl

hydroperoxides and $PGG_2$ is probably the physiologically most important substrate (176,196).

There are some differences in the rate of POX activities between PGHS-1 and –2 with no obvious structural explanation (196). The COX activity of PGHS-2 is activated at a lower concentration of hydroperoxide than for PGHS-1, possibly related to a low peroxide state of the relevant cell (169,340).

Both the POX and COX activities of PGHS are suicide inactivated during catalysis by a breakdown of active enzyme intermediates. The exact mechanism for the suicide inactivation and protein modification associated are not known, but probably involves reaction of amino acid radicals with molecular oxygen (342).

Arachidonic acid is the best substrate for PGHS-1 and –2, but both enzymes can also oxygenate closely related fatty acids in intact cells, such as linoleic acid (18:2 w6), dihomo-γ-linolenic acid (20:3 w6) and eicosapentaenoic acid (20:5 w3). PGHS-2, but not PGHS-1 has also been shown to convert the endocannabinoids 2-arachidonylglycerol and arachidonylethanolamine into the precursors for prostaglandin glycerol esters and prostaglandin ethanolamides (164,165).

The difference in substrate specificity between PGHS-1 and –2 could explain the reason for two isozymes. Interestingly, both isozymes have been found to produce small amounts of other products, besides $PGG_2$ from AA, due to different conformers in the COX active site (11R-HpETE, 11S-HpETE, 15R-HpETE, 15S-HpETE) (372,416). However, little is known about the role of these alternative metabolites in physiology.

The first three-dimensional structure of a PGHS-enzyme was published in 1994 (ovine PGHS-1 complexed with the NSAID, flurbiprofen) (277) and soon thereafter, the crystal structure of human and murine COX-2 was elucidated (170,199). Drug interactions with PGHS have been extensively studied by the use of crystallography. The PGHS isoforms were found to be structurally homologous and quite superimposable. However, there are some structural differences between the COX site of PGHS-1 and –2. The active site of COX-1 and –2 does not share the exact same surrounding amino acids and the size of the main channel is slightly larger in PGHS-2 (~20%). These differences have made it possible to design selective drugs against PGHS-2.

*NSAID action*

PGHS-1 and –2 are the major targets for NSAIDs. Structural data of the PGHS isoforms have revealed that NSAIDs inhibit the fatty acid substrate binding at the COX site. There are two major classes of NSAIDs; the classical NSAIDs, which inhibit both PGHS-1 and –2 and the PGHS-2 selective inhibitors (73,234). Most of the NSAIDs inhibit PGHS-1 and –2 by a reversible competitive inhibition. However, acetylsalicylic acid inhibits PGHS by a rapid, reversible binding followed by a covalent modification of Ser530 (91,342,392).

Furthermore, acetylsalicylic acid has been reported to directly inhibit the NF-κβ signaling pathway through Iκβ kinase and prevent the expression of PGHS-2 (50). This might explain the anti-inflammatory properties of NSAIDs, which are independent of PGHS inhibition (419).

# PROSTAGLANDIN E SYNTHASE

Prostaglandin E synthase (PGES) (EC 5.3.99.3) specifically converts the endoperoxide moeity of prostaglandin endoperoxides into the 9-keto-11-hydroxy conformation found in $PGE_2$. $PGE_2$ is the most common prostanoid and mediates a number of biological responses through certain prostanoid receptors (see prostanoid receptors). Originally, microsomes from sheep and bovine seminal vesicles were found to contain high glutathione (GSH)-dependent PGES actvity (223,264,363). Many attempts have been made to isolate microsomal PGES, with limited success, mainly due to the instability of the enzyme. Several cytosolic glutathione transferases have also been found to possess PGES activity. Two anionic glutathione transferases with GSH-dependent PGES activity were purified from human brain (265). However, these activities were later shown to not be specific for $PGE_2$ formation, but also produced $PGD_2$ and $PGF_{2\alpha}$ from $PGH_2$ (51,384). Two other cytosolic glutathione transferases of the Mu-class have been purified from human brain cortex, which demonstrated significant GSH-dependent PGES activities (32). Another cytosolic GSH-dependent PGES (cPGES/p23) that is functionally linked with PGHS-1 was isolated from rat brain after LPS-treatment (367). There are several reports about a membrane-bound GSH-independent PGES in heart, spleen and uterus, isolated from rat and bovine (396,397). Recently, the cDNA corresponding to the microsomal GSH-independent monkey protein was cloned and purified and is now referred to as mPGES-2 (365).

This thesis is based on the characterization of human microsomal glutathione S-transferase-1-like 1 (MGST1-L1), which was cloned and identified in 1999 by Jakobsson *et al.* (143) and will thus be discussed in detail later (see discussion). Orthologs of MGST1-L1 have also been cloned from several other species (84,182,202,244). The membrane-bound PGE synthases are designated as mPGES and the cytosolic PGE-synthases as cPGES.

## mPGES-2

A membrane-bound GSH-independent PGES expressed in heart, spleen and uterus, has been isolated from rat and bovine (396,397) and the corresponding monkey protein was recently expressed, purified and referred to as mPGES-2 (365). The cDNA for monkey mPGES-2 encodes a 33-kDa protein with the consensus region of glutaredoxin and of thioredoxin. The human mPGES-2 gene was localized on chromosome 9q33-q34, close to the genes of PGHS-1, mPGES-1 and lipocalin-type PGDS. Purified mPGES-2 displayed a $K_M$ and $V_{max}$ of 28 μM and 3.3 μmol $min^{-1}$ $mg^{-1}$, respectively with a pH-optimum between 6-7 (365). Recombinant mPGES-2 was activated by several SH-reducing reagents such as dithiothreitol, GSH and β-mercaptoethanol, in order of decreasing effectiveness. Northern blot analysis demonstrated that mPGES-2 mRNA was mainly localized in various regions of the brain and heart, but not in genital organs, as compared to mPGES-1 (365). More recently, transfection studies of mPGES-2 and PGHS-1 or PGHS-2 demonstrated that mPGES-2 promoted $PGE_2$ production in the immediate and delayed responses, with modest preference to PGHS-2 (241). mPGES-2 demonstrated a constitutive

expression in many cells and was not induced during tissue inflammation. However, protein expression of mPGES-2 was elevated in human colorectal cancer.

## Cytosolic PGES

Cytosolic PGES (cPGES) is identical to p23, a 23 kDa, heat shock protein 90 (Hsp 90)-binding protein, originally implicated as a cofactor for the chaperone function of Hsp90 (134,147). cPGES was initially identified as a cytosolic, LPS-induced, GSH-dependent PGES with $K_M$ and $V_{max}$-values of 14 μM and 190 nmol $min^{-1}$ $mg^{-1}$, respectively (367). In a more recent study, cPGES was shown to be activated in the presence of the Hsp 90-complex, $Mg^{2+}$ and ATP (366). Unlike typical GSTs (305), cPGES not only showed negligible activities against several cytosolic GST substrates, including CDNB, but was even inhibited by them (367). These properties are similar to those of hematopoietic PGDS, which belongs to the σ-class of GSTs (151). The homology between cPGES and other cytosolic GSTs, including hematopoietic PGDS is low (~20%), but they all share a conserved tyrosine near the N-terminus (Tyr9), which serves to stabilize the GSH thiolate in many cytosolic GSTs. Mutation of this amino acid abrogated the activity of cPGES, suggesting the same function (367).

cPGES is constitutively expressed in many tissues and cells, but cannot be induced by proinflammatory stimuli, except in the brain, where LPS-treatment resulted in a three-fold induction in rat (367). cPGES is mainly localized to the cytosol, but can move to the endoplasmatic reticulum after $Ca^{2+}$-ionophore challenge (243). Cotransfection studies of cPGES and PGHS-1 in HEK293-cells have shown that these enzymes are functionally linked and associated with maintenance of tissue homeostasis (367). However, less efficient biosynthesis of $PGE_2$ has been seen in KAT-50, a well differentiated thyroid epithelial cell line, expressing PGHS-2 and cPGES (107). The role of cPGES *in vivo* is not fully understood and studies with cPGES (-/-) mice will reveal its implications in physiology and pathophysiology. Recent data indicates that cPGES may play an important role during implantation, decidualization (260) and parturition (212).

## GSTs

Several cytosolic GSTs have been reported to convert $PGH_2$ into $PGE_2$, $PGD_2$ and $PGF_{2\alpha}$, non-specifically (51,265,384). Two cytosolic glutathione transferases of the Mu-class, purified from human brain cortex demonstrated specific GSH-dependent PGES activities (32). The recombinant GSTM2-2 and 3-3 catalyzed the conversion of $PGH_2$ to $PGE_2$ at the rates of 282 and 923 nmol $min^{-1}$ $mg^{-1}$, with an apparent $K_M$ of 140 and 1500 μM, respectively, at the optimal pH of 8. The human GSTM2-2 and 3-3 are mainly expressed in the brain (301) and the rat counterpart of GSTM3-3 is localized in the thalamus and hypothalamus (146).

# CATABOLISM OF $PGE_2$ AND FORMATION OF $PGF_2$

The main pathway for catabolism of eicosanoids is initiated by oxidation of the 15(S)-hydroxyl group, catalyzed by $NAD^+$-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH) (144), followed by reduction of the $\Delta^{13}$ double bond, catalyzed by NADPH/NADH-dependent $\Delta^{13}$-15-ketoprostaglandin reductase (13-PGR) (Fig. 3) (8). These enzymes are ubiquitously expressed in mammalian tissues (359) and the degradation products of these enzymes (15-ketoprostaglandins and 13,14-dihydro-15-ketoprostaglandins) have significantly reduced biological activities (7). There are two types of 15-PGDHs, type I is $NAD^+$-dependent and selective towards eicosanoids (144), while type II can use both $NAD^+$ and $NADP^+$ and has a broader substrate specificity (194). Since the type II enzyme has much higher $K_M$ for prostaglandins, compared to type I, it is not believed to play an important role in the catabolism of eicosanoids (359). The eicosanoids can be further metabolized, in several steps by $\beta$- and $\omega$-oxidation of the side chains into various dinor- and tetranor products (Fig. 3) (308). For example, $\beta$-oxidation shortens the $\alpha$-chain of various prostaglandins into the corresponding C18-homologues (102) and $\omega$-oxidation of prostaglandins is performed by cytochrome P-450 enzymes (45,46,269). Actually the most abundant prostaglandins in the semen are 19R-hydroxy-$PGE_1$ and 19R-hydroxy-$PGE_2$ (368).

$PGE_2$ can also be metabolized non-enzymatically to $PGA_2$, which can be further isomerized enzymatically at $\Delta^{10}$ of $PGA_2$ to $\Delta^{11}$, by PGCS, thus forming $PGC_2$ (Fig. 3) (149). PGCS has been found in serum or plasma from human, rabbit, pig, dog, rat and cat (149) and the cat enzyme has also been partially purified.

## PGF synthases

$PGF_2$ is formed via three pathways from $PGH_2$, $PGE_2$ or $PGD_2$ by PGH 9-, 11-endoperoxide reductase, PGE 9-ketoreductase, or by PGD 11-ketoreductase, respectively (Fig. 3) (393). These activities are dependent on NADH or NADPH. Furthermore, a 16.5 kDa, microsomal GSH-dependent $PGF_{2\alpha}$ synthase was partially purified from sheep vesicular glands (43) and recently, a novel GSH-activated, LPS-inducible $PGF_{2\alpha}$ synthase was detected in various cells, with the highest activity found in lung (250). These two $PGF_{2\alpha}$ synthases specifically converted $PGH_2$ to $PGF_{2\alpha}$. Several GSH-(S) transferases also have the capacity to produce $PGF_{2\alpha}$ from $PGH_2$ (51).

PGE 9–ketoreductase can convert $PGE_2$ specifically to $PGF_{2\alpha}$ in the presence of NADH or NADPH. PGE 9–ketoreductase is a cytosolic enzyme, purified from chicken heart (184), human (404) and bovine placenta (153). The enzyme is a member of the aldo-keto reductase (AKR) superfamily (409), based on the broad substrate specificity, size, cofactors and sequence identities. Furthermore, a carbonyl reductase, detected in human brain (403) and a 20$\alpha$-hydroxysteroid dehydrogenase (HSD), were found to be identical with PGE 9–ketoreductase (11,315).

# PROSTAGLANDIN TRANSPORT

$PGH_2$ is synthesized in the lumen of the ER by PGHS-1 or PGHS-2 (229) and is then believed to diffuse through the membrane, where it is further metabolized by terminal enzymes (302,363). The synthesized prostaglandins diffuse across the plasma membrane into the extracellular compartment, driven by pH and the membrane potential (319). However, metabolic clearance of prostaglandins requires energy-dependent uptake across the plasma membrane, with following catabolism. Local signal termination of prostaglandins must be achieved to prevent undesired effects. Several types of prostaglandin transporters that are involved in the re-uptake of prostaglandins have been characterized. PGT is a lactate/prostaglandin exchanger and is expressed in PGHS-containing cells (197). Another prostaglandin transporter, called organic anionic transporter (OATP), has lower affinity and specificity for prostaglandins (319). Also, a liver-specific OAT (LST-1) has been cloned from human (1).

# PROSTANOID RECEPTORS

The biological activities of prostaglandins are mediated through the binding of specific G-protein coupled rhodopsin-type receptors (GPCR) with seven transmembrane domains (255). These receptors originate from different genes and have been cloned from various species. The receptors have been classified into 8 types with several subtypes, based on their responsiveness to various agonists/antagonists (256) and include: the PGD receptor (DP) (37), four subtypes of the PGE receptor ($EP_{1-4}$) (22,89,290,420), the PGF receptor (FP) (2), the PGI receptor (IP) (36) and the TXA receptor (TP) (123) (Table 1). There are several splice variants of the $EP_3$, FP and TP receptors (278,285,289), which differ only in their C-terminal ends. Furthermore, a novel DP receptor was characterized as CRTH2 (Chemoattractant Receptor-homologous molecule expressed on T-Helper type 2 cells) and reported to be involved in allergic inflammation and rhinitis (122). Functionally, the prostanoid receptors can be divided into three groups: the relaxant, contractile and inhibitory receptors. The $DP_1$, $EP_2$, $EP_4$ and IP receptors are called the “relaxant” receptors, since they signal through a $G_s$-mediated intracellular increase of cyclic adenosine monophosphate (cAMP). The $EP_1$, FP and TP receptors signal through a $G_q$-mediated increase in intracellular calcium and are thus called the “contractile” receptors and the $EP_3$ and $DP_2$ receptors are “inhibitory” receptors that couple to $G_i$ and decreases cAMP-formation. Most of the prostanoid receptors are located at the plasma membrane, but some have also been found at the nuclear envelope (34,35). The roles of prostaglandins in various physiological and pathophysiological events have been investigated in mice, deficient in each of the prostanoid receptors. A summary of the results from gene-disruption studies is given in table 2. The $EP_{1-4}$ receptors are clearly involved in many pathological conditions.

**Table 1**: Properties of prostanoid receptor subtypes.

| Receptor type | G protein | Signaling |
|---|---|---|
| $DP_1$ | $G_s$ | cAMP↑ |
| $DP_2$ (CRTH2) | $G_{\alpha i/o}$ | cAMP↓ |
| $EP_1$ | Unidentified | $[Ca^{2+}]$↑ |
| $EP_2$ | $G_s$ | cAMP↑ |
| $EP_3$ | $G_i$, $G_s$, $G_q$ | cAMP↓ |
| | | $[Ca^{2+}]$↑ |
| | | cAMP↑ |
| $EP_4$ | $G_s$ | cAMP↑ |
| FP | $G_q$ | $[Ca^{2+}]$↑ |
| IP | $G_s$, $G_q$ | cAMP↑ |
| | | $[Ca^{2+}]$↑ |
| TP | $G_q$, $G_i$, $G_s$ | $[Ca^{2+}]$↑ |
| | | cAMP↓ |
| | | cAMP↑ |

**Table 2**: Major phenotypes of prostanoid receptor deficient mice.

| Disrupted gene | Phenotypes | Reference |
|---|---|---|
| DP | Decreased allergic response in bronchial asthma | (208) |
| $EP_1$ | Decreased tumor formation in colon | (395) |
| $EP_2$ | Impaired ovulation and fertilization | (125,158,373) |
| | Impaired regulation of blood pressure | (12,158,373,427) |
| | Loss of bronchodilation | (325) |
| | Impaired bone metabolism | (191) |
| $EP_3$ | Impaired febrile response to pyrogens | (387) |
| | Impaired gastric mucosal integrity | (361) |
| | Impaired regulation of blood pressure | (12) |
| | Impaired kidney functions | (86) |
| | Decreased pain sensitization | (382) |
| $EP_4$ | Impaired closure of ductus arteriosus | (259,321) |
| | Impaired regulation of blood pressure | (12) |
| | Impaired bone resorption | (219,304) |
| FP | Loss of parturition | (357) |
| IP | Thrombotic tendency | (246) |
| | Decreased inflammatory pain and swelling | (246,383) |
| | Decreased pain sensitization | (246,266,382) |
| TP | Bleeding tendency | (369) |

# THE MAPEG-SUPERFAMILY

The MAPEG (**m**embrane **a**ssociated **p**roteins in **e**icosanoid and **g**lutathione metabolism)-superfamily was defined according to enzymatic activities, sequence motifs and structural properties (141,142). The MAPEG-members are 16-18 kDa membrane-bound proteins with similar hydropathy profiles, which indicate that they have 3-4 membrane-spanning regions and a similar topology (Fig. 5A) (142). Multiple sequence alignment of the human MAPEG members demonstrates six strictly conserved amino acids (Fig. 5B). The family was found to consist of six human proteins, including 5-lipoxygenase activating protein (FLAP), leukotriene $C_4$ synthase ($LTC_4S$), MGST1, MGST2, MGST3 and MGST1-L1. Several members have also been identified in plant, *i.e.* (*Arabidopsis thaliana, Oryza sativa* and *Ricinus communis*), fungi (*Aspergillus nidulans*) and bacteria (*Synechosystis, Escherichia coli* and *Vibrio cholerae*).

Based on multiple sequence alignments, the MAPEG-family can be divided into four subgroups. FLAP, $LTC_4S$ and MGST2 belong to one group and are crucial for leukotriene biosynthesis. MGST3, together with members in plant and fungi make up one group, while *Escherichia coli* and *Vibrio cholerae* belong to yet another group and MGST1 and MGST1-L1 make up the last group. MGST1, 2- and -3 all have glutathione transferase and glutathione –dependent peroxidase activities, possibly related to detoxification and protection against oxidative stress.

FLAP is hypothesized to act as a substrate provider for 5-lipoxygenase (5-LO) and is necessary for efficient 5-LO activity (77,201,215). $LTC_4S$ specifically catalyzes the conjugation of $LTA_4$ (the 5-LO product) with GSH, thus forming $LTC_4$ (174,261,402). MGST2 and MGST3 have also been found to possess $LTC_4S$ activities (139,140), but the activity of MGST3 was modest. Human MGST2 expression was localized in the liver, endothelial cells and lung membranes (320), while $LTC_4S$ was mainly localized in lung membranes, platelets, eosinophils and mast cells. $LTC_4S$ is specific for $LTA_4$, but MGST2 and MGST3 have broader substrate specificity. MGST2 and MGST3 can also catalyze GSH-dependent peroxidase activity against 5-HpETE, with apparent $K_M$ of 7 μM and 21 μM, respectively (140). Furthermore, MGST2, but not MGST3 can catalyze conjugation of GSH to CDNB, implying that MGST2 and MGST3 are involved in detoxification and oxidative stress. MGST1 has wide substrate specificity and is broadly expressed, with the highest concentration in the liver (225). The substrates for MGST1 include halogenated arenes, like CDNB, various polyhalogenated hydrocarbons (5) and lipid hydroperoxides (224,233), suggesting a role in protection against oxidative stress (232). $LTA_4$ and other epoxides are poor substrates for MGST1 (226,345). Interestingly, $LTC_4$ is a tight-binding inhibitor of MGST1, but the function for this is not known (21). An overview of the MAPEG members in eicosanoid metabolism is presented in figure 1.

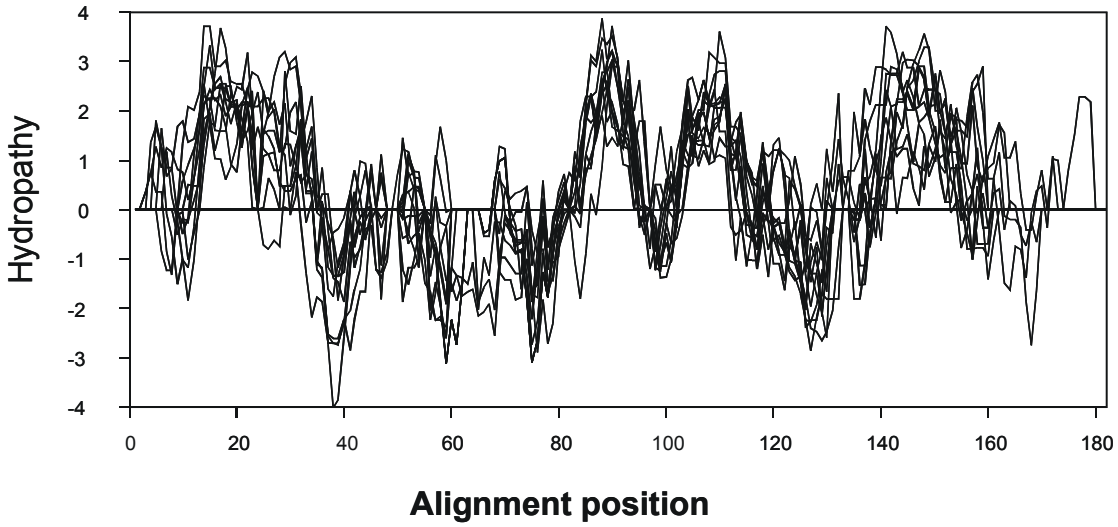


**Figure 5A.** Hydropathy plots of the human MAPEG members, based on the alignments in 5B.

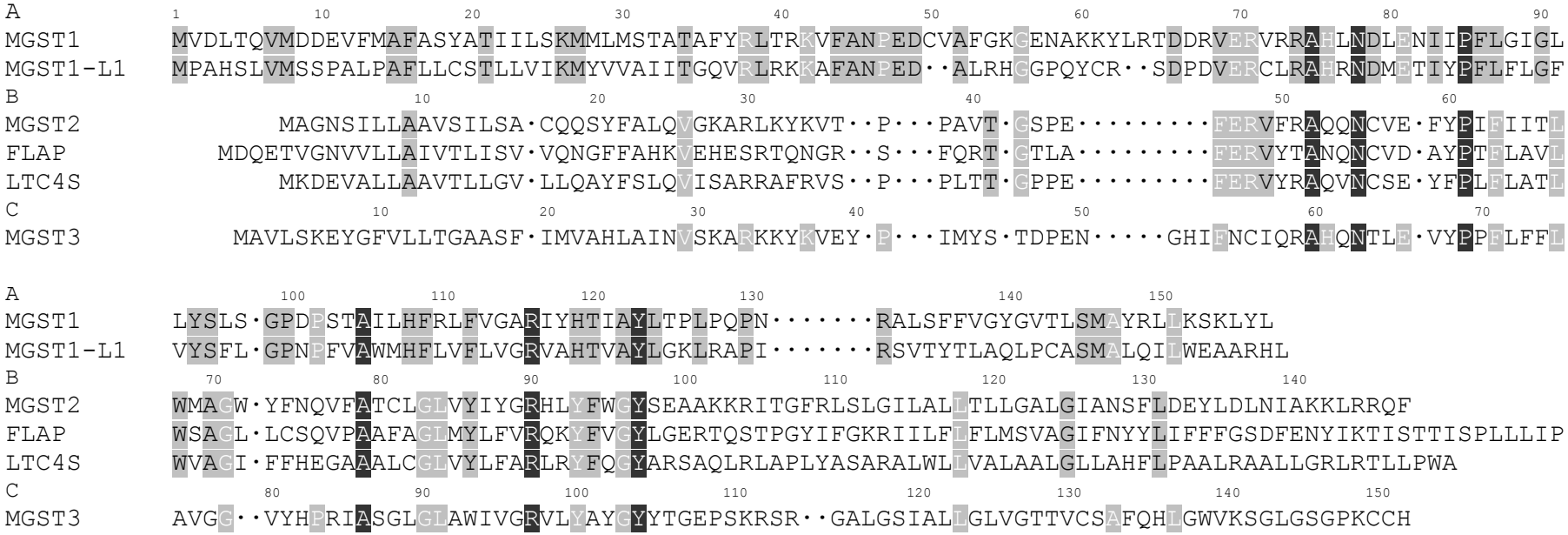


**Figure 5B.** Multiple sequence alignments of the human MAPEG members. The groups are based on sequence similarity derived from a larger multiple sequence analysis made by Jakobsson *et al*. (141).

M :Same within a group
M :Same in all
M :Same in two groups

# AIMS OF THE PRESENT INVESTIGATION

mPGES-1 was initially identified as a homologue to microsomal glutathione transferase-1 (MGST1) with 37% identity on the amino acid sequence level. Before the function of mPGES-1 was known, it was referred to as MGST1-like 1 (MGST1-L1). Based on primary structure alignment and hydropathy plot studies, mPGES-1 was discovered to belong to the MAPEG-superfamily. mPGES-1 was found to specifically catalyze the conversion of $PGH_2$ to $PGE_2$ in the presence of glutathione. The aim of this study was to biochemically characterize human mPGES-1 and explore its relation to PGHS-2 in various pathological conditions.

# METHODOLOGY

## Common methods

I performed the following methods in the original articles; cell culture (**paper I, II, III**), subcellular fractionation (**paper I, II, III, IV**), gel electrophoresis and Western blotting (**paper I, II, III, IV**), Northern blotting (**paper I**), reverse phase high performance liquid chromatography (RP-HPLC), (**paper I, II, IV, V**), UV-spectroscopy (**paper V**), protein determination (**paper I, II, III, IV, V**) and ultracentrifugation (**paper I, II, III, IV, V**).

## Assay development

In **paper I**, we used [$^3$H]-$PGH_2$ to measure PGES activity, however, with unsatisfactory quantitative results. Due to the unstable nature of $PGH_2$, a quantitative and reproducible PGES assay was needed. Therefore, we developed a PGES assay, based on RP-HPLC and UV detection (**paper II**). Several compounds were tested as potential internal standards and 11β-$PGE_2$ was found to be the most suitable candidate. 11β-$PGE_2$ eluted with almost baseline separation from $PGE_2$ and did not interfere with non-enzymatically formed $PGF_{2\alpha}$ or $PGD_2$ (**paper II, fig. 1**). 11β-$PGE_2$ and $PGE_2$ demonstrated identical UV absorbance properties at 195 nm. Equal amounts of 11β-$PGE_2$ and $PGE_2$ (as determined by GC/MS) were compared before and after solid phase extraction to test the recovery of the PGES assay (~90 %). To optimize the quantitative analysis and minimize interference by non-enzymatic degradation products, a stop-solution containing $FeCl_2$ was added. $FeCl_2$ terminates the reaction and converts $PGH_2$ into mainly 12-HHT and malondialdehyde. The substrate ($PGH_2$) was kept on $CO_2$-ice until use to keep the non-enzymatic activity low. Solid phase extraction was performed immediately after terminating the reaction, since prostaglandins are more stable in organic solvent. Analysis was then performed, using RP-HPLC and UV-detection at 195 nm.

A purification protocol for recombinant human 6-histidine tagged ($His_6$) mPGES-1 was developed. Human $His_6$-mPGES-1, expressed in *E. coli* BL21(DE3) was purified in two steps by hydroxyapatite and immobilized metal affinity chromatography (**Paper V**). First, $His_6$-mPGES-1 was completely solubilized in 4% Triton X-100, with preserved enzymatic activity in the solubilized extract. The solubilized extract was mixed with hydroxyapatite and after a 10-min incubation on ice, was subjected to a short centrifugation pulse. The supernatant, containing the unbound fraction was cleared by centrifugation and filtration. The cleared fraction was then loaded on a 1-ml HiTrap 10 chelating column (Amersham Biosciences), charged with $Ni^{2+}$. The remaining unspecifically bound proteins were removed by a wash step of 60 mM imidazole and the purified histidine-tagged protein was finally eluted with 350 mM imidazole as a single peak. The eluted protein was immediately desalted on a HiPrep 26/10 desalting column (Amersham Biosciences). Human $His_6$-mPGES-1 was purified to apparent homogeneity with a yield of 1.0-3.5 mg per liter of BL21(DE3) expression culture, when purifying from

whole cell lysate (**Paper V, fig. 4**). An important finding for successful purification was that GSH and glycerol had a stabilizing function for solubilized mPGES-1 (therefore included in all the buffers used for purification).

### Hydrodynamic studies

To determine the molecular mass of mPGES-1, we performed hydrodynamic studies in presence of Triton X-100 (**Paper V**). The sedimentation coefficient of the mPGES-Triton X-100 complex was determined by the use of sucrose gradients. Purified mPGES-1 and marker proteins with known sedimentation coefficients were added on top of a 5-20 % sucrose gradient containing GSH and Triton X-100. The samples were then subjected to ultracentrifugation at 160,000 x $g_{av}$ for 45 h at 20ºC. Fractions were collected and PGES activity, refractive index and protein content were measured (**Paper V, fig. 9A**). After plotting the activities and protein content, the sedimentation coefficient of mPGES-1 could be calculated by linear regression. The partial specific volume of the mPGES-Triton X-100 complex was determined by density equilibrium centrifugation. Purified mPGES-1 was added to a 20-50 % sucrose gradient containing GSH and Triton X-100. The tubes were centrifuged at 246,000 x $g_{av}$ at 20ºC until equilibrium had been reached (72 h). Fractions were collected and the refractive index, *i.e*. sucrose content, was plotted against PGES activity (**Paper V, fig. 9B**). The sucrose content of the fraction with the highest PGES activity corresponded to the density of the mPGES-Triton X-100 complex and the density was inverted to yield the partial specific volume. The Stokes radius was determined by the use of gel exclusion chromatography. Purified mPGES-1 was loaded on a Sephacryl S-300 HR column together with marker enzymes (high molecular weight gel filtration calibration kit). The samples were eluted, collected and analyzed by measuring PGES activity and absorbance at 280 and 405 nm (**Paper V, fig. 9C**). The square root of –log $K_{av}$ values were plotted against the known Stokes radii of the marker enzymes and the Stokes radius of the mPGES-Triton X-100 complex was obtained.

The sedimentation coefficient, partial specific volume and Stokes radius were then substituted into the Svedberg equation (**Paper V, Eq. 1**) and the molecular weight of the mPGES-1-Triton X-100 complex was calculated. To determine the amount of bound detergent in the complex, protein content and UV-absorbance was measured on the eluted fractions from the immobilized metal ion affinity column. The resulting weight of the mPGES-1-Triton X-100 complex agreed with a trimeric quaternary structure.

# RESULTS

## Paper I

Human mPGES-1 was cloned and characterized as a 16 kDa, inducible, GSH-dependent, microsomal PGE synthase. The tissue distribution of mPGES-1 was analyzed by Northern blot and high expression was found in two cancer cell lines, A549 and HeLa cells. mPGES-1 was also expressed at intermediate levels in reproductive organs. A rabbit anti-human antiserum was raised against the amino acids 59-74 of mPGES-1 and Western blot analysis specifically recognized a 15-to 16-kDa protein in the membrane fraction of bacteria expressed mPGES-1. PGES activity was measured after incubation with [$^3$H]-$PGH_2$ and analyzed by RP-HPLC with radioactivity detection. The membrane fraction contained a high GSH-dependent PGES-activity (0.25 µmol $min^{-1}$ $mg^{-1}$). A549 cells had been used earlier as a model system to study PGHS-2 induction by the proinflammatory cytokine IL-1$\beta$ and mPGES-1 was also induced by IL-1$\beta$ in these cells. Western blot analysis also detected a 16-kDa protein in sheep vesicular glands.

## Paper II

In the following study, protein expression of PGHS-2 and mPGES-1 was further investigated in A549 cells after IL-1$\beta$ treatment. A time study of PGHS-2 and mPGES-1 expression revealed a coordinate induction of the enzymes. The observed induction was correlated with increased PGES activity in the microsomal fraction. Tumor necrosis factor-$\alpha$ (TNF-$\alpha$) also induced mPGES-1 in these cells and dexamethasone was found to completely suppress the effect of both cytokines on mPGES-1 induction. A method based on RP-HPLC and UV-detection was developed to measure PGES activity. This method was used to screen for potential inhibitors of mPGES-1, including a small set of NSAIDs, stable $PGH_2$ analogues and cysteinyl leukotrienes. NS-398, Sulindac sulfide and $LTC_4$ were found to inhibit PGES activity with $IC_{50}$-values of 20 µM, 80 µM and 5 µM, respectively.

## Paper III

In order to investigate the gene structure of mPGES-1, a P1 clone containing the gene for mPGES-1 was isolated and characterized. The mPGES-1 gene was localized on chromosome 9q34.3, spanning a region of approximately 15 kb and divided into three exons. The transcription start of the PGES gene has not been identified, but a 682 bp fragment directly upstream of the translation start site exhibited promoter activity when transfected in A549 cells. The putative promoter is GC-rich, lacks a TATA box at a functional site and contains numerous potential transcription factor binding-sites. Two GC-boxes, two tandem Barbie-boxes and an aryl hydrocarbon response element (AHR) were identified. The putative promoter region of mPGES-1 was transcriptionally active and reporter constructs were regulated by IL-1$\beta$ and phenobarbital.

## Paper IV

Since $PGE_2$ had been demonstrated to play an important role in several pathological conditions like rheumatoid arthritis (RA), it was important to study the role of mPGES-1 in these conditions. Primary synovial cells were obtained from synovial tissues derived from patients suffering from RA. These cells were treated with IL-1$\beta$ or TNF-$\alpha$ and the expression of mPGES-1 and PGHS-2 were investigated by Northern and Western blot. Both cytokines induced mPGES-1 mRNA from a low basal level to a maximum level at 24 h. Moreover, the IL-1$\beta$ induction was inhibited by dexamethasone in a dose-dependent manner. The protein expression of mPGES-1 was induced by IL-1$\beta$ with a linear increase up to 72 h. PGHS-2 was also induced, however with an earlier peak expression (4-8 h). The protein expression of mPGES-1 correlated with PGES activity and demonstrated a 3-5 -fold increase after IL-1$\beta$ treatment. No PGES activities were found in the cytosolic fractions nor in the absence of GSH, ruling out any contribution of cytosolic PGE synthases and GSH-independent PGE synthases.

## Paper V

In order to study the structure and biochemical function of mPGES-1, the protein was overexpressed as an N-terminal 6-histidine tag fusion protein in *E.coli* BL21(DE3). Bacterial recombinant histidine-tagged mPGES-1 was solubilized with Triton X-100 and purified by a combination of hydroxyapatite- and immobilized metal affinity chromatography. mPGES-1 catalyzed a rapid GSH-dependent conversion of $PGH_2$ to $PGE_2$ and demonstrated a high $k_{cat}/K_M$ (**paper V, Table I**). mPGES-1 could also catalyze several other activities; GSH-dependent conversion of $PGG_2$ to 15-hydroperoxy $PGE_2$, glutathione-dependent peroxidase activity towards cumene hydroperoxide, 5-HpETE and 15-hydroperoxy-$PGE_2$, as well as conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) to GSH (**paper V, Table II**).

Two-dimensional crystals of purified mPGES-1 were prepared and a projection map was determined by electron crystallography. Furthermore, hydrodynamic studies were performed on the solubilized mPGES-1 detergent complex (Triton X-100) to investigate the oligomerization of the protein. These two methods independently demonstrated a trimeric organization of mPGES-1.

# DISCUSSION

## Identification of mPGES-1

Human, microsomal, GSH-dependent prostaglandin E synthase (mPGES-1) was initially discovered as an EST-tag by database searches of enzymes involved in eicosanoid and glutathione metabolism. The EST-clone was found to code for a protein with 37% sequence homology to microsomal glutathione S-transferase-1 (MGST1), thus it was referred to as MGST1-like 1 (L1). The gene coding for MGST1-L1 was also independently identified as a p53 induced gene (PIG12), but no function was described (279). Based on the properties of MGST1-L1 regarding size, amino acid sequence, hydropathy and membrane localization, it was identified as a member of the MAPEG superfamily. In 1997, Burgess and Reddy reported on a 16.5 kDa, microsomal GSH-dependent $PGF_{2\alpha}$ synthase in sheep vesicular glands (43) and since the $PGF_{2\alpha}$ synthase possessed certain properties similar to other MAPEG members, $PGH_2$ was tested as a substrate for several of the members, including MGST1-L1. MGST1-L1 did not produce any $PGF_{2\alpha}$, but showed a specific formation of $PGE_2$. Hence, MGST1-L1 was referred to as microsomal PGES-1 (**paper I**). An antiserum raised against an internal peptide of mPGES-1 also recognized a protein of similar size in microsomes from sheep vesicular glands, which are known to contain a highly efficient microsomal PGES, indicating that mPGES-1 was the long-sought membrane bound PGES (**Paper I**).

## Biochemical characterization of mPGES-1

*Basal expression of mPGES-1*

We examined the expression of mPGES-1 in human tissues, and Northern dot blot analysis of mPGES-1 mRNA demonstrated a low expression in most tissues, medium expression in reproductive organs and a high expression in two cancer cell lines (A549 and HeLa) (**Paper I**). A low basal expression of mPGES-1 has also been observed in several rat tissues, but high constitutive expression was seen in the stomach (202). A high expression of mPGES-1 was also found in the thymus of rat as well as epithelial cells of the human thymus (297). The highest constitutive expression of mPGES-1 in mouse was observed in the ovary, urinary bladder and kidney (98). Furthermore, mPGES-1 has been detected in conjunction with ovulation and fertilization (84,183). Impaired fertility was seen in mice lacking the $EP_2$ receptor (125,158) and PGHS-2 (76,227). Furthermore, mPGES-1 has been implicated in bone metabolism as well as certain metabolic bone diseases like osteoporosis (54,244,303). mPGES-1 may therefore participate in normal physiology, especially in renal function, bone metabolism, reproduction and maintenance of the gastrointestinal mucosa. However, it should be noted that mPGES-1 null mice do not demonstrate any phenotypic changes in these organs and functions, suggesting that other non-inducible PGE synthases can substitute for mPGES-1 in normal physiology (374,381).

*Regulation of mPGES-1 expression*

A549 cells were reported to produce more $PGE_2$ after cytokine treatment (133,217) and fibroblasts treated with LPS or platelet derived growth factor (PDGF) contained high microsomal PGES activity (157,207). Since A549 cells were earlier used as a model system for PGHS-2 induction by IL-1β and mPGES-1 mRNA was highly expressed in these cells, it was logical to study mPGES-1 protein expression in this model.

Non-treated A549 cells had a low expression of mPGES-1, but the protein expression was induced significantly by IL-1β (**Paper I**). The basal expression of mPGES-1 in rat was also very low, but could be dramatically induced by LPS in various organs (202,244,418). These data suggested that the induction of PGHS-2 and mPGES-1 could be co-regulated. We examined this possibility in a time study of PGHS-2 and mPGES-1 expression in A549 cells. A coordinate induction of PGHS-2 and mPGES-1 protein expression was observed in these cells after treatment with IL-1β with a linear increase up to 72h (**Paper II**). Various proinflammatory stimuli have been shown to co-ordinately induce mPGES-1 and PGHS-2 in several types of cultured cells, often associated with increased $PGE_2$ production (**Paper II and IV**) (202,244,346). Furthermore, induction of mPGES-1 was seen after β-amyloid treatment of astrocytes (311) and hormonal stimulation of ovarian follicles (84). A summary of mPGES-1 induction in various tissues and cells is presented in table 3.

Glucocorticoids are known to reverse inflammatory processes, and cytokine-induced mPGES-1 expression was abolished by dexamethasone in A549 cells (**Paper II**). Inhibition of mPGES-1-induction by dexamethasone has also been reported in other cell types, such as macrophages and synoviocytes (**Paper IV**) (244). Even though mPGES-1 and PGHS-2 appear to be co-regulated in many cases, their rate of induction differ in some cell systems, indicating separate regulatory mechanisms of induction (**Paper IV**). Co-expression of PGHS-2 and mPGES-1 have been observed in various pathological conditions and diseases; including pain, fever, inflammation, cancer and Alzheimer's disease, suggesting a functional link between these enzymes for efficient prostaglandin production (**Paper II and IV**), (78,202,244,374,381,418).

**Table 3**: Induction of mPGES-1 in various tissues and cells.

| Source | Stimulus | Reference |
|---|---|---|
| *In vivo* | | |
| Rat tissues | LPS | (202,244) |
| Rat, lung and hind paw | Adjuvant | (202) |
| Rat, brain endothelial cells | IL-1β, LPS | (78,418) |
| Bovine, follicular granulosa cells | Gonadotropin | (84) |
| *In vitro* | | |
| Human, lung adenocarcinoma cells | IL-1β, TNFα | (143,370) |
| Human, vascular smooth muscle cells | IL-1β, TNFα, LPS, PMA | (346) |
| Human, rheumatoid synovial cells | IL-1β, TNFα | (353) |
| Human, orbital fibroblasts | IL-1β | (108) |
| Rat, peritoneal macrophages | LPS | (244) |
| Rat, calvaria osteoblasts | IL-1β | (244) |
| Rat, astrocytes | β-amyloid | (311) |
| Mouse, osteoblastic cells | IL-1β, TNFα | (244) |

*Functional coupling of mPGES-1 and PGHS-2*

The reports about coordinate induction of mPGES-1 and PGHS-2 implicated that they were functionally linked (**Paper II and IV**), (244). In support for this, AA-treated HEK293 cells, co-transfected with mPGES-1 and PGHS-2 produced several times more $PGE_2$, compared to when mPGES-1 or PGHS-2 were expressed alone (244). Furthermore, *in vivo* studies of rats demonstrated that PGHS-2 inhibitors reduced formation of $PGE_2$ more efficiently compared to other prostaglandins (112). Also, several reports have demonstrated co-localization of mPGES-1 and PGHS-2 in the endoplasmatic reticulum and perinuclear membrane (182,244,418). This is in agreement with our studies of IL-1β induced A549 cells, where the only PGES activity was found in microsomes and only in the presence of GSH (**paper II**). Recently, mPGES-1 was also shown to specifically convert $PGG_2$ to 15-hydroperoxy $PGE_2$, suggesting an alternative pathway for $PGE_2$ biosynthesis (**paper V**). Although this has not been observed *in vivo*, it implies yet another functional link between PGHS-2 and mPGES-1.

However, our study on synoviocytes isolated from patients with RA, suggest that mPGES-1 and PGHS-2 expression is regulated by different mechanisms (**paper IV**). IL-1β increased the expression of mPGES-1 in a linear manner up to 72h, while the expression of PGHS-2 reached a maximum already after 4-8h. The IL-1β induced PGHS-2 expression seems to involve NF-κβ, but the mPGES-1 promoter does not contain any such NF-κβ site. Furthermore, the 3'-region of mPGES-1 lacks the AUUUA instability sequences found in the PGHS-2 gene. Also, cytokine-induced expression of mPGES-1 was recently demonstrated to be regulated by Egr-1 (**e**arly **g**rowth **r**esponse-1) (253), mediated by the p38 mitogen–activated protein kinase pathway (299), implying

that PGHS-2 and mPGES-1 can be induced by the same kind of stimuli, but use different signaling pathways.

*Gene structure and expression of mPGES-1*

The primary structures of human, mouse and rat mPGES-1 demonstrate a high degree of amino acid sequence homology (>80%). The gene organization of human mPGES-1 is similar to MGST1, but differs from other MAPEG members that contain additional exons (**paper III**) (156). The putative promoters of both mPGES-1 and MGST1 are GC-rich, lacks a TATA-box at a functional site and contain several potential transcription binding sites. A number of potential transcription factor binding-sites were identified in the promoter of mPGES-1, *i.e*. two GC-boxes, two tandem Barbie-boxes and an aryl hydrocarbon response element (AHR) (**paper III**). The putative promoter region of mPGES-1 was shown to be transcriptionally active and could be induced by IL-1$\beta$ and down-regulated by phenobarbital. If physiologically relevant, the mPGES-1 gene is the first gene known to be repressed by phenobarbital.

However, the tandem Barbie-boxes were recently shown not to be involved in the down-regulation of mPGES-1 by phenobarbital (79). Phenobarbital is believed to act on a *cis*-acting PB-Responsive Enhancer Module (PBREM) element (130), but the mechanism behind this down-regulation has not been elucidated. Also, Sp1 and Sp3 were shown to be responsible for basal expression of mPGES-1, but only Sp1 was needed for basal MGST1 expression (79).

There are several reports that indicate that both $PGE_2$ and $PGD_2$ participate in regulation of sleep and wakefulness. $PGD_2$ has been shown to be the most potent endogenous sleep-promoting substance (386), while $PGE_2$ has been suggested to counteract the effect of $PGD_2$ (115,116). $PGE_2$ is also present at higher levels in the brain during wakefulness (92). Phenobarbital is an effective sedative and anticonvulsant and since we found that it reduced mPGES-1 promoter activity, it may indicate that mPGES-1 is involved in sleep and wakefulness (**paper III**). However, this remains to be investigated and mPGES-1 null-mice will be a useful tool to study this hypothesis.

The gene and primary structure of mouse mPGES-1 were recently characterized (253). The organization of the mouse mPGES-1 gene was similar to the human mPGES-1 gene, regarding exons/introns and lack of a functional TATA box. Several consensus *cis*-acting elements were found within 1.8 kb upstream of the mouse mPGES-1 gene, including C/EBP$\alpha$ and –$\beta$, AP-1, two tandem GC-boxes, three putative glucocorticoid response elements and two progesterone response elements (253). The tandem GC-boxes in the mPGES-1 promoter were shown to play a major role in regulating its inducible transcription. Electro mobility shift assay (EMSA) studies of the mouse mPGES-1 promoter region indicated that the GC-boxes did not bind Sp1 or Sp3, but bound an inducible zinc finger protein, called Egr-1, instead. Egr-1 was demonstrated to be a key transcription factor in regulating the inducible expression of mPGES-1. Cytokine-induced mPGES-1 expression was demonstrated to be regulated by Egr-1, mediated by the p38 mitogen–activated protein kinase pathway (299). Interestingly, no binding site for NF-$\kappa\beta$, CRE or E-box has been found in the mPGES-1 promoter, as seen

in PGHS-2 induction. Thus, it indicates that the mechanisms for induction of mPGES-1 and PGHS-2 are different.

*Steady state kinetics of mPGES-1*

mPGES-1 specifically converts $PGH_2$ to $PGE_2$, but only in the presence of GSH, which is absolutely required for activity and GSH can not be substituted by other thiols (**paper V**), (271). Purified, bacteria expressed, mPGES-1 demonstrated an efficient catalytic conversion of $PGH_2$ with a $V_{max}$ value of 170 µmol $min^{-1}$ $mg^{-1}$ at 37ºC (**paper V, Table I and II**). The apparent $K_M$ for mPGES-1 was 0.16 mM and is comparable to $K_M$ for other PG-synthases (**paper V, Table IV**). Furthermore, in line with our data, Lazarus *et al.* observed a $K_M$ of 130 µM for mouse mPGES-1, expressed in *Escherichia coli* (182). Also, our preliminary data suggest a high $K_M$ for $PGH_2$ in IL-1β-induced A549 cells (unpublished). The apparent $K_M$ for GSH was 0.7 mM, determined at a concentration of 400 µM $PGH_2$ (**paper V, Table I**).

In 1974, Samuelsson *et al.* proposed that $PGE_2$ could be formed through isomerization of $PGH_2$ or by isomerization of $PGG_2$ to 15-hydroperoxy-$PGE_2$, with subsequent reduction to $PGE_2$ (309). In the search for additional activities, we investigated whether the unstable intermediate $PGG_2$ could be isomerized by mPGES-1. Incubation with $PGG_2$ actually demonstrated an even more efficient conversion of $PGG_2$ to 15-hydroperoxy-$PGE_2$ ($V_{max}$: 250 µmol $min^{-1}$ $mg^{-1}$) in the presence of GSH, as compared to $PGH_2$ (**paper V, Table I and II**). The importance of $PGG_2$ as an alternative substrate for mPGES-1 will be further discussed below.

Independently from our study, Ouellet *et al.* reported on the purification of mPGES-1 expressed in a baculovirus system (271). They also observed a high PGES-activity, but a lower $K_M$ compared to us, in the presence of dodecylmaltoside ($V_{max}$: 38 µmol $min^{-1}$ $mg^{-1}$, $K_M$: 14 µM at 0ºC). The discrepancies in activity and $K_M$ for mPGES-1 may depend on differences in the enzyme activity assay, the temperature and the type of detergent used. Other explanations can be differences in post-transcriptional modifications and lipid composition between prokaryotic and eukaryotic cells. There are actually several putative phosphorylation sites in the mPGES-1 sequence that might be important for modulation of mPGES-1 (unpublished).

*Glutathione as cofactor*

Reduced glutathione (GSH) is a γ-glutamylcysteinylglycine-tripeptide, which is quite abundant within cells (~ 5 mM). GSH is essential for maintaining the structure of red blood cells and for keeping hemoglobin in the ferrous state, but is also involved in detoxification reactions with hydrogen peroxide and organic peroxides. GSH is absolutely required for mPGES-1 activity (**paper I**), with an apparent $K_M$ of 0.7 mM (**paper V, Table I**), but also has a stabilizing effect on solubilized mPGES-1. Furthermore, GSH could not be substituted by other SH-reducing agents for mPGES-1 activity (**paper V**) and does not seem to be oxidized during mPGES-1 catalysis (271).

*Speculations on $PGE_2$ biosynthesis*

mPGES-1 efficiently catalyzed a GSH-dependent conversion of $PGG_2$ to 15-hydroperoxy-$PGE_2$ (15-Hp$PGE_2$) **(paper V, Table II)**. This activity suggests an alternative pathway for the synthesis of $PGE_2$ (**paper V, fig 10**). Either, $PGG_2$ is converted into 15-hydroperoxy-$PGE_2$ by mPGES-1 with subsequent reduction to $PGE_2$, non-enzymatically or enzymatically by glutathione-dependent peroxidases (9,262) or alternatively by the POX activity of PGHS-1/-2.

Since the two active sites of PGHS are spatially well-separated, $PGG_2$ has to diffuse from the COX site to the other side of the enzyme to reach the POX pocket (Fig. 6A) (337). This may not be the most efficient route for the unstable $PGG_2$ metabolite. An alternative pathway for $PGE_2$ production could occur through shuttling of the intermediate between PGHS and mPGES-1. Possibly, $PGG_2$ is transferred to mPGES-1 instead of diffusing to the POX pocket. mPGES-1 could then convert $PGG_2$ to 15-hydroperoxy-$PGE_2$, which is subsequently shuttled back to PGHS-2, where the reduction to $PGE_2$ occurs (Fig 6B).

Biochemical topology studies on MGST1 have demonstrated that the active site is located on the cytoplasmic side of the ER (6). According to the “positive-inside rule” and topology predictions of prokaryotic MAPEG members (unpublished), the N- and C-terminals are located on the periplasmic side of prokaryotic cells, which corresponds to the luminal side of the ER in eukaryotic cells (67,333,414). In line with this, hydropathy plots of the MAPEG members indicate that the N- and C-terminals of each protein are located on the same side of the membrane (Fig. 5A) (141). Furthermore, mutational work on the hydrophilic loops of $LTC_4S$ (175) and affinity studies of MK-886 on FLAP (203) indicate that the loops responsible for activity are located on the same side of the membrane. Together, these data implies that the active site of mPGES-1 could be located on the cytoplasmic side of the ER.

FLAP is involved in the transfer of AA to 5-LO and is necessary for efficient 5-LO activity (77,201,215). In analogy with FLAP, interaction between PGHS-2 and mPGES-1 could hypothetically facilitate more efficient $PGE_2$ biosynthesis. Transfection of FLAP into certain cell systems has been reported to increase PGHS-2 expression and $PGE_2$ biosynthesis (24). However, FLAP has no $PGH_2$ metabolizing capacity (unpublished results).

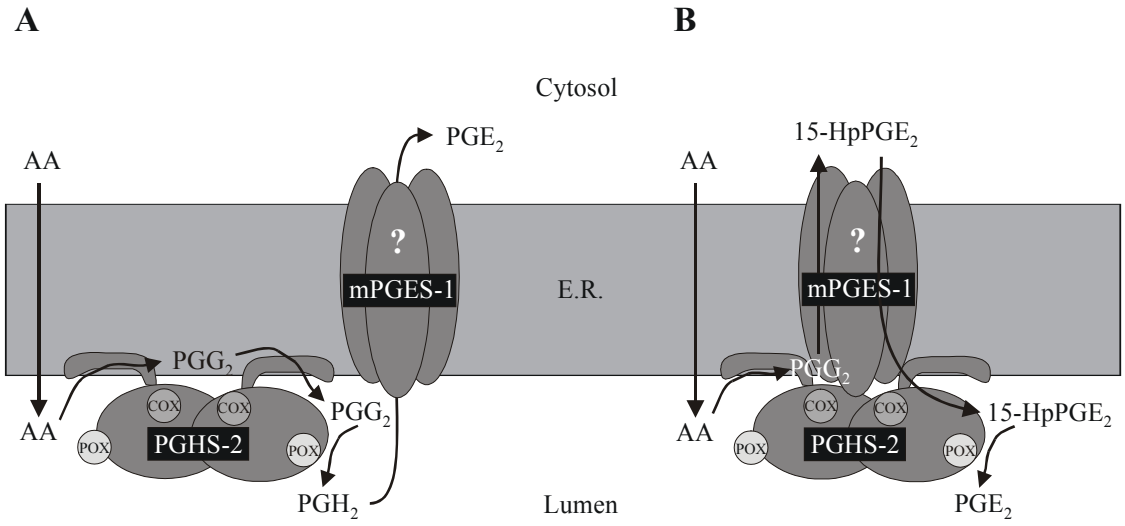


**Figure 6.** Hypothetical pathways for $PGE_2$-biosynthesis.

*Speculations on mPGES-1 catalysis*

Several mechanisms have been proposed for the GSH-dependent conversion of $PGH_2$ to $PGE_2$. These mechanisms include nucleophilic attack by the thiolate anion of glutathione. In 1971, Lands *et al.* proposed a mechanism with a GSH-assisted hydride shift (177). However, this reaction involves the unstable intermediate, thiohemiketal. More likely, the nucleophilic thiolate anion will attack the peroxide oxygen on C-9, thus forming an adduct of GSH (or enzyme cysteine thiol) and $PGH_2$, with subsequent enzyme-assisted deprotonation of C-9, producing $PGE_2$ and thiolate anion (Fig. 7A). Possibly, another $GS^-$ (or enzyme cysteine thiolate) in solution could act as a base in the second step. This is in agreement with the proposed mechanism of hematopoietic PGDS (151). The mechanism for mPGES-1 could also proceed in a concerted manner by abstraction of the proton on C-9 by a glutathione or enzyme cysteine thiolate, followed by isomerization into $PGE_2$ (Fig. 7B). These mechanisms of mPGES-1 involve activation of reduced GSH, which is in line with our observation of a Meisenheimer complex with mPGES-1 (unpublished results). A tyrosine has been suggested to activate GSH in several cytosolic GSTs (305), hematopoietic PGDS (151) (σ-class cytosolic GST), cPGES/p23 (367) and also in $LTC_4S$ (Tyr93) (175). However, mutation of the highly conserved Tyr117 in mPGES-1 did not affect catalytic activity (244) and neither did mutations of tyrosines in MGST1 inhibit catalysis (399). The amino acids responsible for GSH-binding and catalytic activity in mPGES-1 and MGST1 have not yet been identified. However, N-ethylmalemide (NEM) activates MGST1 and inhibits mPGES-1, implying that a Cys or Ser participates in the mechanism of MGST1 and mPGES-1 (**Paper V**), (399). Recently, Watanabe *et al.* demonstrated that Cys110 is essential in the active site of mPGES-2 (398). A mechanism involving Cys110 was proposed, similar to the one in

figure 7A where Cys110 attacks the peroxide linkage at C-9, instead of activated GSH. Arg110 is strictly conserved in all MAPEG members and when it was replaced with Ser in mPGES-1, the catalytic function was abrogated, implying an essential role (244). In $LTC_4S$, Arg51 has been proposed to function as a proton donor in the opening of the $LTA_4$ epoxide for conjugation with GSH (175). However, mutation of the corresponding amino acid in mPGES-1 (Arg70) did not affect the PGES-activity.

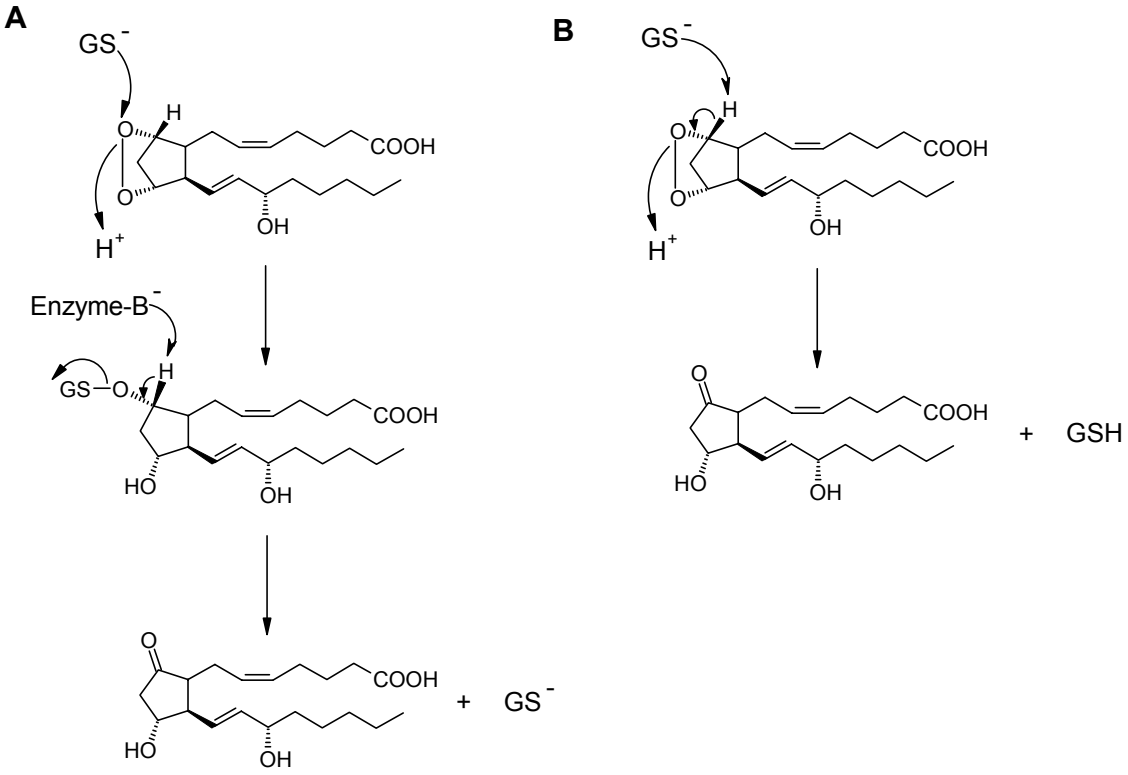


**Figure 7.** Proposed mechanisms for $PGE_2$ formation by mPGES-1.

*Inhibition of mPGES-1*

We tested some common NSAIDs, stable $PGH_2$-analogues and cysteinyl leukotrienes *in vitro* as putative inhibitors of mPGES-1. The mPGES-1 activity was inhibited by $LTC_4$, NS-398 (a specific PGHS-2 inhibitor) and sulindac sulfide with $IC_{50}$-values of 5, 20 and 80 μM, respectively (**Paper V**). mPGES-1 was also inhibited by MK-886, a FLAP inhibitor with an $IC_{50}$-value of 3 μM (202). MK-886 binds to the AA-binding region of FLAP, which is highly conserved in $LTC_4S$ and mPGES-1 and could possibly be involved in the binding of eicosanoids (203). The motif ERXXXAXXNXXD/E might represent a consensus sequence for interaction with AA and/or other eicosanoids (202). Furthermore, 15-deoxy-$\Delta^{12,14}$-$PGJ_2$, arachidonic acid, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were recently reported to inhibit mPGES-1 with similar $IC_{50}$-values (0.3 μM) (282). This observation suggests a novel mechanism of action for

the anti-inflammatory effects of DHA, EPA and 15-deoxy-$\Delta^{12,14}$-$PGJ_2$. The main target for 15-deoxy-$\Delta^{12,14}$-$PGJ_2$ is believed to be the peroxisome proliferator-activated receptor-$\gamma$ (PPAR-$\gamma$) (145,292), but can also inhibit IL-1$\beta$ -induced $PGE_2$ formation by a PPAR$\gamma$-independent way in rheumatoid synoviocytes (379). The PPAR$\gamma$-independent anti-inflammatory effect of 15-deoxy-$\Delta^{12,14}$-$PGJ_2$ could at least partially be explained by inhibition of mPGES-1.

15-deoxy-$\Delta^{12,14}$-$PGJ_2$ is formed from PGHS-2 derived $PGD_2$ through a series of dehydration reactions (Fig. 3). The presence of 15-deoxy-$\Delta^{12,14}$-$PGJ_2$ *in vivo* has been demonstrated by immunohistochemistry on LPS-treated macrophages and macrophages in atherosclerotic plaques (329). Formation of 15-deoxy-$\Delta^{12,14}$-$PGJ_2$ has also been reported during resolution of inflammation in carrageenin-induced pleurisy in rats (94).

Furthermore, the activity of mPGES-1 was not inhibited by CDNB, a common substrate for GSTs (244), in contrast to cPGES (367).

## Purification of mPGES-1

Previous attempts to isolate GSH-dependent membrane-bound PGES were only partially successful (223,264,363). The main obstacle was the instability of the protein, which lost activity rapidly after purification. Recently, we managed to purify human mPGES-1 by a combination of hydroxyapatite and immobilized metal affinity chromatography (**Paper V**). The expression system of histidine-tagged mPGES-1 was quite efficient for a membrane-bound protein, as seen by the amount of pure enzyme recovered and purification factor (up to 2% of the total membrane protein).

Earlier purification attempts of mPGES-1 demonstrated that Triton X-100 was a suitable detergent for solubilization, but higher concentrations (>1.5%) led to a decreased recovery of activity (363). However, recombinant histidine-tagged mPGES-1 was completely solubilized by 4% Triton X-100, with preserved enzymatic activity in the solubilized extract when GSH and glycerol were included in the buffer (**Paper V, fig. 2A**).

Another purification protocol of mPGES-1, based on hydroxyapatite column chromatography, has been described (271). One percent diheptanoylphosphatidylcholine (DHPC) was used for solubilization of Sf-9 cell membranes and gave 72% recovery of the mPGES-1 activity. Since DHPC is rather expensive, the purification of mPGES-1 was conducted in the presence of 1% octylglucoside instead. These expression systems and purification protocols for mPGES-1 provide useful tools for future crystallization and mechanistic studies.

## Quaternary structure of mPGES-1

2-D crystallization attempts on MGST1 have previously been successful (117) with increasingly higher resolutions of the projection structure (317,318). Purified $his_6$-mPGES-1 in 1% Triton X-100, was successfully crystallized by adding phospholipids prior to reduction of the detergent content (**Paper V**). Triton X-100 was used for solubilization of bacteria expressed mPGES-1 and fortunately, the same detergent was suitable for 2-D crystallization. Another important factor for successful crystallization of

mPGES-1 was the use of crystallization conditions similar to those successful for MGST1. A 10 Å projection structure of mPGES-1 was obtained after several steps of image processing (**Paper V, fig. 8**). The molecular weight of $his_6$-mPGES-1 in relation to the unit cell size implied that mPGES-1 forms a trimer in the crystal. Optimization of the 2-D crystallization conditions will hopefully lead to a high resolution structure of mPGES-1 in the future.

We have performed hydrodynamic studies on the mPGES-1-Triton X-100 complex (**Paper V**). To determine the molecular mass of the complex, the sedimentation coefficient, partial specific volume and Stokes radius were measured. A sedimentation coefficient of 4.1 S, partial specific volume of 0.891 $cm^3/g$ and a Stokes radius of 5.09 nm were obtained and the Svedberg equation was then used to calculate the molecular mass of the mPGES-1-Triton X-100 complex, which was found to be 215 000. The detergent content of the mPGES-1-Triton X-100 complex was 2.8 g Triton X-100 /g protein and after subtracting the values for the detergent content our calculations match with a trimeric quaternary structure (**Paper V**). Thus, two independent methods indicate that mPGES-1 has a trimeric quaternary structure. This is in line with studies on the closely related MGST1, which also has been demonstrated to be a trimer (41,117,198,400). The quaternary structure of FLAP and $LTC_4S$ remains to be determined.

## Additional activities of mPGES-1

*Glutathione dependent peroxidase activity*

Since MGST1, -2 and -3 are GSH–dependent peroxidases (142), mPGES-1 was tested with several peroxide substrates. mPGES-1 was found to reduce 15-hydroperoxy-$PGE_2$ to $PGE_2$ in presence of GSH, albeit at a low catalytic rate (0.04 µmol $min^{-1}$ $mg^{-1}$) (**Paper V, Table II**). Non-enzymatic production of $PGE_2$ from 15-hydroperoxy-$PGE_2$ was significant. GSH is present intracellulary at mM concentrations under normal physiological conditions and might be sufficient for non-enzymatic reduction of 15-hydroperoxy-$PGE_2$ (341). Alternatively, GSH-dependent peroxidases or PGHS-1/-2 could reduce 15-hydroperoxy-$PGE_2$ to $PGE_2$. In addition, mPGES-1 showed a modest GSH-dependent activity against 5-HpETE (0.04 µmol $min^{-1}$ $mg^{-1}$), but catalyzed the GSH-dependent peroxidase activity towards cumene hydroperoxide more efficiently (0.17 µmol $min^{-1}$ $mg^{-1}$) (**Paper V, Table II**). In fact, this activity was in the same range as with recombinant rat MGST1 (401). These data indicate that mPGES-1 might be involved in protection against oxidative stress, but since the peroxidase activities of mPGES-1 are low compared to the PGES activity, they probably reflect the relationship with other MAPEG enzymes.

*Glutathione transferase activity*

Several proteins with PGES-activity also have GST-activity towards CDNB, like the two anionic forms of cytosolic PGH-E isomerases (265) and the cytosolic Mu-class glutathione transferases M2-2 and M3-3 (32). Since several of the MAPEG members

also possess glutathione transferase activity (141,142,225), it was of interest to study GST-activity on mPGES-1. mPGES-1 catalyzed a small, but significant CDNB-GSH conjugating activity (0.8 μmol $min^{-1}$ $mg^{-1}$) (**Paper V, Table II**). However, no GST-activity against $LTA_4$ was detected with mPGES-1 (unpublished). The GST-activity of mPGES-1 might also reflect the relationship with other MAPEG enzymes.

## mPGES-1 in pathology

The collective data from knock-out and pharmacological studies of PGHS-2 (76,190,192,227,231,270) and EP-receptors (125,158,216,303,321,387,394) clearly suggest involvement of $PGE_2$ in various pathological conditions. Several enzymes with PGES activity have been identified and cloned, but mPGES-1 is the most active, inducible PGES enzyme reported thus far (**paper V, Table IV**), (271) and constitutes a highly probable candidate for $PGE_2$ production in pathology. mPGES-1 null mice demonstrated a normal phenotype regarding fertility and number of offspring (374,381), unlike the PGHS-2 null mice, which showed deficient renal and reproductive functions (76,227). Likely, other proteins with PGES activity are involved in fertilization and development. Importantly, the mPGES-1-deficient mice completely lost the capability to produce $PGE_2$ over baseline levels after LPS-treatment *in vivo* (381). Recently, Trebino *et al.* studied mPGES-1 null mice in several pathological models and observed a marked reduction of inflammatory responses and pain, as compared with wild-type mice (374).

*Inflammation*

The classical signs of acute inflammation are pain, swelling, heat generation and local reddening and loss of function (*dolor, tumor, calor, rubor and functio laesa*).

NSAIDs diminish pathological symptoms related to inflammation by inhibition of prostaglandin formation (206,228,391). $PGE_2$ is formed in large quantities at sites of inflammation and can mediate several pathological features of inflammation (181). $PGE_2$ is a potent vasodilator (407) and acts synergistically with histamine and bradykinin to increase microvascular permeability with edema as a consequence (70,284). These vascular changes also result in local warmth, erythema and inflammatory pain.

Trials with PGHS-2 specific inhibitors have confirmed the dominant role of PGHS-2 in producing proinflammatory prostaglandins (64). Furthermore, genetic deletion of PGHS-2 prevents the development of autoimmune arthritis (248). Both PGHS-2 and mPGES-1 are induced by proinflammatory stimuli, like IL-1$\beta$, TNF$\alpha$ and LPS both *in vitro* (**Paper I, II and IV**) (244,346) and *in vivo* (78,202,244,374,381,418), implying an important role for these enzymes in inflammatory conditions. Also, induced mPGES-1 expression was inhibited by the anti-inflammatory glucocorticoid dexamethasone (**Paper II and IV**), (202,244). mPGES-1 was recently demonstrated to be overexpressed in synovial tissues from patients with RA (Westman, 2003, submitted). Furthermore, Trebino *et al.* studied the pathogenesis of collagen-induced arthritis in mPGES-1 deficient mice. They demonstrated the importance of mPGES-1 in both acute and chronic $PGE_2$-dependent inflammation *in vivo* (374). These data clearly strengthen

the importance of mPGES-1 in inflammatory processes. mPGES-1 represents an important, “overlooked” drug target for the treatment of inflammatory disease. This is probably due to the seemingly opposed modes of action by $PGE_2$. For instance, $PGE_2$ is both proinflammatory and promotes resolution of inflammation (20). Furthermore, PGHS-2 derived prostanoids, like 15-epi-lipoxins and resolvins have been demonstrated to promote resolution of inflammation (94,188,323). Inhibition of mPGES-1 will thus theoretically spare the beneficial prostanoids, including physiologically important $PGE_2$.

*Pain*

Prostaglandins are involved in pain as shown by the antinocieceptive effect of NSAIDs and studies with exogenously added prostaglandins, which are able to induce hyperalgesia and allodynia (161). The importance of $PGE_2$ in inflammatory pain has also been demonstrated using selective anti-$PGE_2$ antibodies that inhibit pain sensitization, edema and hyperalgesia in rats (220,280). Peripheral nocieceptor terminals are sensitized by PGHS-2 derived $PGE_2$ and produce localized pain hypersensitivity (210). Peripheral inflammation also affects the neighboring tissue and causes pain hypersensitivity (secondary hyperalgesia) and can also cause diffuse muscle and joint pain, fever, fatigue and anorexia (69). These illness symptoms were thought to occur by a brain-regulating mechanism involving nerve impulses from the injured region through the spinal cord to the brain (415). However, Samad *et al.* (306) and Ek *et al.* (78) independently proposed that nerve impulses are not involved, but it is the proinflammatory cytokine, IL-1$\beta$ that signals the brain about local inflammation. PGHS-2 seems to be involved in these central nervous system (CNS) responses, since it is induced by IL-1$\beta$ in the spinal cord neurons, elevating $PGE_2$ levels in the cerebrospinal fluid (306). Knock-out studies of prostanoid receptors have shown that IP and $EP_3$ are the major prostaglandin receptors, mediating enhanced acetic acid-induced writhing response in LPS pre-treated mice, *i.e.* in endotoxin-enhanced inflammatory nocieception (382). Furthermore, spinal $EP_1$ and $EP_3$ receptors have been demonstrated to mediate $PGE_2$-induced allodynia and hyperalgesia, respectively (216). Recently, knock-out studies demonstrated that mPGES-1 is involved in mediating acute pain in inflammatory processes (374). Acetic acid-induced pain was reduced by 50% in mPGES-1-null mice, similar to NSAID-treated control mice. These data implicate an important role for mPGES-1 in inflammatory pain.

*Fever*

Fever is a CNS-controlled rise of body temperature in response to infection or inflammation (310). At the site of inflammation, activated immune cells produce proinflammatory cytokines, including IL-1, IL-6, and TNF-$\alpha$, which reach the bloodstream and target the brain (80). Fever can be suppressed by NSAIDs, implying that prostaglandins are involved in fever generation. Indeed, $PGE_2$ has been shown to play a critical role in the CNS where it acts on $EP_3$ receptors (387). Studies using selective PGHS-2 inhibitors and knock-out mice have shown that PGHS-2, but not PGHS-1, is involved in suppression of $PGE_2$-formation, associated with a decrease in fever (47,190).

The role of mPGES-1 in fever was recently elucidated (78,418). Intravenous injection of rats with IL-1β or LPS induced mPGES-1 messenger RNA in vascular cells throughout the brain (78,418). PGHS-2 was also induced with a more transient up-regulation of mRNA. Furthermore, *in situ* hybridization and immunohistochemistry revealed a co-localization of mPGES-1 and PGHS-2 after IL-1β treatment, indicating an efficient $PGE_2$ production. mPGES-1 mRNA was also co-expressed with mRNA coding for the interleukin-1 receptor (IL-1 R) in blood brain barrier endothelial cells (78). Furthermore, recent data on mPGES-1 (-/-) mice have confirmed that this enzyme is critical for fever generation (Engblom *et al.*, 2003, submitted). The current mechanism of $PGE_2$ synthesis in fever-mediation is believed to proceed through a series of steps; circulating IL-1β binds to IL-1 receptors on the luminal surface of endothelial cells, resulting in PGHS-2 and mPGES-1 expression, followed by $PGE_2$ synthesis. Due to the amphipathic properties of $PGE_2$, it can then diffuse into the parenchyma where it binds to prostaglandin-sensitive neurons in the ventromedial preoptic area (VMPO), leading to fever (82).

*Rheumatoid arthritis*

Rheumatoid arthritis (RA) is a common chronic inflammatory and destructive joint disease with a prevalence of 0.5-1%. Typical symptoms are joint swelling and pain caused by the inflammatory process, eventually leading to destruction of the joint (343). The cause of RA is not entirely understood, but it has been speculated whether the disease can be triggered by infectious agents (332). RA is regarded as an autoimmune disease and there is a strong association to a number of autoantibodies (351). Proinflammatory cytokines, like TNF-α, IL-1 and IL-6 play a central role in pathogenesis of RA (71,85). Several proinflammatory cytokines like IL-1β and TNF-α are known to induce the production of $PGE_2$ in RA at sites of inflammation (**Paper IV**) (296). There is evidence for $PGE_2$ as a mediator of inflammation in arthritis (63). $PGE_2$ affects tissue remodeling at sites of chronic inflammation and can also modulate the immune system. $PGE_2$ is also involved in inflammatory angiogenesis (27), bone destruction (296) and induction of matrix metalloproteinases (MMPs) in RA (213,324).

During the last couple of years, a number of reports have described the importance of PGHS-2 and mPGES-1 in the pathology of RA and models of RA. High expression of PGHS-2 has been seen in RA (66,152,185,331) and PGHS-2-specific inhibitors clearly have a great impact on pain and inflammation (64,155). Induced co-expression of PGHS-2 and mPGES-1 was recently seen in human synovial cells after treatment with proinflammatory cytokines (**Paper IV**) (162). Several models of RA have implicated the importance of mPGES-1. For instance, induction of mPGES-1 was seen in a rat-adjuvant induced arthritis model (58,202). This arthritis model was also demonstrated to have a systemic effect, inducing mPGES-1 in the endothelial cells along the blood-brain barrier and in the parenchyma (81). The unambiguous involvement of mPGES-1 in RA was demonstrated in a collagen-induced arthritis model, using mPGES-1 (-/-) mice (374). Recently, another report demonstrated that mPGES-1 is

overexpressed in synovial tissues from patients with RA (Westman *et al.*, 2003, submitted).

*Cancer*

There is clinical, biochemical and genetic evidence for the importance of PGHS-2-derived $PGE_2$ in the development of colorectal cancer and also possibly other cancers (406). A number of epidemiological studies indicate that chronic use of NSAIDs lowers the incidence and mortality rate for colorectal cancer in humans and in animal models (204,283,326). PGHS-2 is highly expressed in various transformed cell lines and tumor tissues (154,355) and treatment with selective PGHS-2 inhibitors reduces the adenoma burden in both humans (350) and animals (286). Also, overexpression and antisense suppression have demonstrated that PGHS-2 is involved in the progression of several cancer forms (195,380).

More direct evidence for the importance of PGHS-2 and $PGE_2$ in colorectal tumorigenesis was found in gene targeting studies. In a human model for familial adenomatous polyposis, gene disruption of PGHS-2 (270) or the $EP_2$ (348) receptor resulted in reduction of the number of intestinal polyps. In another model, disruption of the genes for $EP_1$ (395) or $EP_4$ (247) suppressed the development of carcinogen-induced colorectal cancer. Furthermore, gene disruption of cytosolic $PLA_{2\alpha}$ also lead to reduced polyposis in *Apc* mutant mice (129,360). Angiogenesis, important for tumor progression, was markedly suppressed in $EP_3$ (-/-) mice, in a model that mimics tumor-stromal angiogenesis (4).

Both PGHS-2 and mPGES-1 are needed for efficient $PGE_2$-biosynthesis. This has been shown in a human embryonic kidney cell line (HEK293) and when co-transfected with mPGES-1 and PGHS-2, but not PGHS-1, showed an aggressive growth and aberrant morphology (244). In a following study, co-expression of mPGES-1 and PGHS-2 resulted in colony formation in soft agar culture and tumor formation when implanted into nude mice (150).

However, when HEK293 cells were incubated with $PGE_2$ for at least two weeks, no change in morphology was seen (244). This is contradictive to what has been seen in human colorectal carcinomas where $PGE_2$ treatment led to increased growth, motility and change in morphology, possibly mediated through the $EP_4$ receptor (327,328) and must be further investigated.

Since mPGES-1 was found to possess other activities besides PGES activity, other metabolites produced by mPGES-1 could possibly contribute to procarcinogenic effects (**Paper V**). For example, the biological function of 15-hydroperoxy-$PGE_2$ is not known and is a possible candidate.

mPGES-1 was also overexpressed in colon cancer, lung cancer and endometrial carcinoma (138,424,425). mPGES-1 was demonstrated to be overexpressed in >80% of the human colon and lung tumors and adenomas, but there were differences in the degree of expression between mPGES-1 and PGHS-2 and some tumors expressed very little of these enzymes (424,425). Recently, mPGES-1 was found to be overexpressed in >50% of human gastric cancer cells (van Rees *et al.*, 2003, in press). However, in

contrast to PGHS-2, mPGES-1 was not induced by phorbol 12-myristate 13-acetate (PMA) or IL-1β, indicating different regulatory mechanisms for PGHS-2 and mPGES-1 expression. Further studies on mPGES-1 are necessary to elucidate its implications in carcinogenesis.

*Alzheimer's disease*

There are three major pathologies that characterize Alzheimer's disease (AD): senile plaques, neurofibrillary tangles and inflammation (101). One of the most critical events for the onset of Alzheimer's disease is the deposition of β-amyloid in the brain. In the search for β-amyloid-induced genes in rat astrocytes, mPGES-1 mRNA was identified by a cDNA subtraction technique, suggesting a potential role in the development of Alzheimer's disease (311). Many epidemiological studies indicate that chronic use of NSAIDs delays the onset of Alzheimer's disease (42,101). This is probably due to inhibition of the increased PGHS-2 expression and $PGE_2$ production found in patients with Alzheimer's disease (160,222,272). Also, IL-1β-induced PGHS-2 expression and $PGE_2$-secretion was inhibited by dexamethasone in human neuroblastoma cells, suggesting a coupling of glial derived IL-1β and increased PGHS-2 expression in neuronal cells in chronic degenerative diseases, like Alzheimer's disease (131).

*Atherosclerosis*

Inflammation has been found to play a central role in the events that leads to erosion of atherosclerotic plaques (388). In fact, markers of inflammation are related to increased risk of cardiovascular disease (293). Macrophages participate in the inflammatory process and synthesize MMPs, which are capable of degrading plaque constituents. Increased expression of MMP-2 and MMP-9 has been found in human plaques in association with macrophages (90). Induction of MMP-2 and MMP-9 is activated by a $PGE_2$/cAMP-dependent pathway (60). In agreement, colocalization of PGHS-2 and mPGES-1 was recently seen in symptomatic atherosclerotic plaques, coupled with induction of metalloproteinases, resulting in plaque rupture and clinical symptoms (56). Also, a specific PGHS-2 inhibitor (NS-398) decreased production of MMPs, which was reversed by $PGE_2$. Furthermore, the involvement of PGHS-2 in early atherogenesis was recently confirmed in low-density lipoprotein (LDL)-receptor deficient mice (44). Another report describes the importance of PGHS-1-derived prostaglandins in acceleration of atherogenesis in LDL-receptor knockout mice (281). Thus, both PGHS-1 and –2 seem to be involved in atherosclerosis and an evaluation of the effects of various selective PGHS-2 inhibitors on plaque progression in humans should follow. The beneficial effects of a selective mPGES-1 remain to be studied.

## mPGES-1 as a drug target

PGHS-2 specific inhibitors have reduced gastrointestinal toxicity compared to less selective PGHS-2 inhibitors, but other unwanted side effects exist (64). Specific PGHS-2 inhibition can lead to edema and elevated blood pressure due to altered excretion of

sodium (352). Specific PGHS-2 inhibition also leads to inhibition of renal and systemic $PGI_2$ (49,209), which may alter the balance between platelet-derived thromboxane $A_2$ and endothelial-derived $PGI_2$, leading to altered vascular haemostasis and perhaps to an increased risk of thrombosis (38,65). Thus, a specific mPGES-1 inhibitor is desirable, sparing beneficial prostanoids that participate in resolution of inflammation and important physiological systems in which other prostaglandins participate.

Other effects of a specific mPGES-1 inhibitor in inflammatory conditions may include conversion of $PGH_2$ (shunting) to other enzymatically or non-enzymatically produced prostanoids (Fig. 3) or novel anti-inflammatory products (94). For example, cells that express PGDS could "shunt" $PGH_2$ into $PGD_2$, which can be non-enzymatically metabolized into 15-deoxy-$\Delta^{12,14}$-$PGJ_2$. This shunting could thus lead to an even more efficient anti-inflammatory effect through inhibition of mPGES-1, since 15-deoxy-$\Delta^{12,14}$-$PGJ_2$ is an inhibitor of mPGES-1 and also has anti-inflammatory properties. Studies with specific inhibitors of mPGES-1 on various cell systems and mPGES-1 (-/-) mice are needed to further elucidate any shunting effects. Shunting may also be a disadvantage if large amounts of $TXA_2$ are produced.

Another advantage with specific inhibition of mPGES-1 was seen in mPGES-1 (-/-) mice, which demonstrated a normal phenotype, suggesting that other PGE synthases substitute for mPGES-1 in normal physiology (374,381).

## Conclusions

Our data cover characterization of human mPGES-1 from initial identification to purification, 2–D crystallization and kinetic characterization, including discovery of new activities. Human mPGES-1 was cloned and characterized as a 16 kDa, inducible, GSH-dependent, microsomal PGE synthase and was identified as a member of the MAPEG superfamily. mPGES-1 possessed several activities besides PGES-activity, like GSH-dependent peroxidase activities and GSH-transferase activities, probably representing the evolutionary relationship to other MAPEG members. We observed that mPGES-1 was induced by proinflammatory cytokines and this induction was prevented by dexamethasone, suggesting a role in inflammatory processes. mPGES-1 was found to play an important role in RA where it was induced by IL-1$\beta$ and TNF-$\alpha$ in an experimental setting. Several studies of mPGES-1 and PGHS-2 indicate that they are functionally linked and can be induced by the same kind of stimuli, but with different signaling pathways. PGHS-2 and mPGES-1 play an important role in a number of pathological conditions and diseases like inflammation, pain, fever, RA, cancer, atherosclerosis and neurodegenerative diseases.

mPGES-1 is a very interesting, novel drug target due to its seemingly specific involvement in pathophysiology. Continued research is required, especially on the structure of the protein and on the catalytic mechanism. The aim is to produce specific and effective inhibitors to be tested in various clinical trials.

# ACKNOWLEDGEMENTS

***I would like to express my sincere gratitude to a number of people:***

I thank Bengt Samuelsson for the opportunity to start my PhD at MBB and for providing excellent working facilities.

I would also like to thank my supervisor Per-Johan Jakobsson for great enthusiasm, guidance and support throughout my period as a PhD-student. You have been a true “driving force” in the lab ☺.

I would also like to thank all our collaborators for excellent cooperation and fruitful discussions. I especially would like to thank Ralf Morgenstern for educational discussions over lunch and also Mats Hamberg, Hans Hebert, Richard Svensson, Lena Ekström, Louise Lyrenäs, Caroline Jegerschöld and Karina Iliescu for great assistance.

I thank Jesper Haeggström for support, guidance and nice evening chats, keeping up the good spirit in the lab.

I would also like to thank Hans-Erik Claesson, Olof Rådmark and Jan-Åke Lindgren for nice discussions and excellent scientific advice.

***Many thanks to everyone in the lab on chemistry II:***

I especially would like to thank Sipra Saha for a great deal of help in the lab and very nice discussions over the years. You have truly been wonderful to me.

A special thanks to my roommate, Fredrik Tholander for tremendous support and endless scientific discussions, of course with the best solutions on the Wednesday pubs.

A very big thanks to the “helylle” guy, Pelle Pettersson who has helped me with almost everything the last year, including some parts of my thesis.

Also, a very big thanks to our former postdoc, Rolf Weinander for tremendous help and support during his time here.

I would also like to thank my former roommates, Peter Rudberg and Filippa Kull for interesting discussions and nice company.

A special thanks to Matthew Hall for proofreading my thesis. I would also like to thank our ambitious student, Sven Pawelzik for nice evening discussions.

A very big thanks to everyone else in the lab for help and interesting chats in the coffee room; Tove Hammarberg, David Dishart, Marija Rakonjac, Mikael Sjölinder, Héléne Ax:son Johnson Martina Blomster, Pontus Larsson-Forsell, Stina Feltermark, Susanne Tornhamre, Cecilia Roos, Yilmas Mahshid, Åsa Brunnström, Erik Andersson, Yasmin Huque-Andersson, Ylva Tryselius, Märta Svedberg, Sofia, Hong Qiu, Mattias Sjöström, Eva Ohlson, Agneta Nordberg, Gunvor Hamberg, Anders Wetterholm and all the students and postdocs; Veronika, Erik and Lars...plus everyone else not mentioned here.

Furthermore, a big thanks to the rest of the persons working at MBB that have helped me during this time.

Last, but not least I would like to thank my darling Gunilla for support, love and understanding during my period as a PhD-student.

# REFERENCES


1. Abe, T., Kakyo, M., Tokui, T., Nakagomi, R., Nishio, T., Nakai, D., Nomura, H., Unno, M., Suzuki, M., Naitoh, T., Matsuno, S., and Yawo, H. (1999) Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. *J Biol Chem* 274, 17159-17163
2. Abramovitz, M., Boie, Y., Nguyen, T., Rushmore, T. H., Bayne, M. A., Metters, K. M., Slipetz, D. M., and Grygorczyk, R. (1994) Cloning and expression of a cDNA for the human prostanoid FP receptor. *J Biol Chem* 269, 2632-2636
3. Akiba, S., Hatazawa, R., Ono, K., Kitatani, K., Hayama, M., and Sato, T. (2001) Secretory phospholipase A2 mediates cooperative prostaglandin generation by growth factor and cytokine independently of preceding cytosolic phospholipase A2 expression in rat gastric epithelial cells. *J Biol Chem* 276, 21854-21862
4. Amano, H., Hayashi, I., Endo, H., Kitasato, H., Yamashina, S., Maruyama, T., Kobayashi, M., Satoh, K., Narita, M., Sugimoto, Y., Murata, T., Yoshimura, H., Narumiya, S., and Majima, M. (2003) Host prostaglandin E(2)-EP3 signaling regulates tumor-associated angiogenesis and tumor growth. *J Exp Med* 197, 221-232
5. Andersson, C., Mosialou, E., Weinander, R., and Morgenstern, R. (1994) Enzymology of microsomal glutathione S-transferase. *Adv Pharmacol* 27, 19-35
6. Andersson, C., Weinander, R., Lundqvist, G., DePierre, J. W., and Morgenstern, R. (1994) Functional and structural membrane topology of rat liver microsomal glutathione transferase. *Biochim Biophys Acta* 1204, 298-304
7. Anggard, E. (1966) The biological activities of three metabolites of prostaglandin E 1. *Acta Physiol Scand* 66, 509-510
8. Anggard, E., Larsson, C., and Samuelsson, B. (1971) The distribution of 15-hydroxy prostaglandin dehydrogenase and prostaglandin-delta 13-reductase in tissues of the swine. *Acta Physiol Scand* 81, 396-404
9. Arthur, J. R. (2000) The glutathione peroxidases. *Cell Mol Life Sci* 57, 1825-1835
10. Asano, K., Lilly, C. M., and Drazen, J. M. (1996) Prostaglandin G/H synthase-2 is the constitutive and dominant isoform in cultured human lung epithelial cells. *Am J Physiol* 271, L126-131
11. Asselin, E., and Fortier, M. A. (2000) Detection and regulation of the messenger for a putative bovine endometrial 9-keto-prostaglandin E(2) reductase: effect of oxytocin and interferon-tau. *Biol Reprod* 62, 125-131
12. Audoly, L. P., Tilley, S. L., Goulet, J., Key, M., Nguyen, M., Stock, J. L., McNeish, J. D., Koller, B. H., and Coffman, T. M. (1999) Identification of specific EP receptors responsible for the hemodynamic effects of PGE2. *Am J Physiol* 277, H924-930
13. Auphan, N., DiDonato, J. A., Rosette, C., Helmberg, A., and Karin, M. (1995) Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science* 270, 286-290
14. Balboa, M. A., Balsinde, J., Jones, S. S., and Dennis, E. A. (1997) Identity between the Ca2+-independent phospholipase A2 enzymes from P388D1 macrophages and Chinese hamster ovary cells. *J Biol Chem* 272, 8576-8580
15. Balboa, M. A., Balsinde, J., Winstead, M. V., Tischfield, J. A., and Dennis, E. A. (1996) Novel group V phospholipase A2 involved in arachidonic acid mobilization in murine P388D1 macrophages. *J Biol Chem* 271, 32381-32384
16. Balsinde, J., Balboa, M. A., and Dennis, E. A. (1998) Functional coupling between secretory phospholipase A2 and cyclooxygenase-2 and its regulation by cytosolic group IV phospholipase A2. *Proc Natl Acad Sci U S A* 95, 7951-7956
17. Balsinde, J., Diez, E., and Mollinedo, F. (1991) Arachidonic acid release from diacylglycerol in human neutrophils. Translocation of diacylglycerol-deacylating enzyme activities from an intracellular pool to plasma membrane upon cell activation. *J Biol Chem* 266, 15638-15643
18. Balsinde, J., Shinohara, H., Lefkowitz, L. J., Johnson, C. A., Balboa, M. A., and Dennis, E. A. (1999) Group V phospholipase A(2)-dependent induction of cyclooxygenase-2 in macrophages. *J Biol Chem* 274, 25967-25970

19. Balsinde, J., Winstead, M. V., and Dennis, E. A. (2002) Phospholipase A(2) regulation of arachidonic acid mobilization. *FEBS Lett* 531, 2-6
20. Bandeira-Melo, C., Serra, M. F., Diaz, B. L., Cordeiro, R. S., Silva, P. M., Lenzi, H. L., Bakhle, Y. S., Serhan, C. N., and Martins, M. A. (2000) Cyclooxygenase-2-derived prostaglandin E2 and lipoxin A4 accelerate resolution of allergic edema in Angiostrongylus costaricensis-infected rats: relationship with concurrent eosinophilia. *J Immunol* 164, 1029-1036
21. Bannenberg, G., Dahlen, S. E., Luijerink, M., Lundqvist, G., and Morgenstern, R. (1999) Leukotriene C4 is a tight-binding inhibitor of microsomal glutathione transferase-1. Effects of leukotriene pathway modifiers. *J Biol Chem* 274, 1994-1999
22. Bastien, L., Sawyer, N., Grygorczyk, R., Metters, K. M., and Adam, M. (1994) Cloning, functional expression, and characterization of the human prostaglandin E2 receptor EP2 subtype. *J Biol Chem* 269, 11873-11877
23. Battez, G., and Boulet, L. (1913) Action de l'extrait de prostate humaine sur la vessie et sur la pression artérielle. *CR Soc Biol Paris* 74, 8
24. Battu, S., Beneytout, J. L., Pairet, M., and Rigaud, M. (1998) Cyclooxygenase-2 up-regulation after FLAP transfection in human adenocarcinoma cell line HT29 cl.19A. *FEBS Lett* 437, 49-55
25. Beato, M., Truss, M., and Chavez, S. (1996) Control of transcription by steroid hormones. *Ann N Y Acad Sci* 784, 93-123
26. Bell, R. L., Kennerly, D. A., Stanford, N., and Majerus, P. W. (1979) Diglyceride lipase: a pathway for arachidonate release from human platelets. *Proc Natl Acad Sci U S A* 76, 3238-3241
27. Ben-Av, P., Crofford, L. J., Wilder, R. L., and Hla, T. (1995) Induction of vascular endothelial growth factor expression in synovial fibroblasts by prostaglandin E and interleukin-1: a potential mechanism for inflammatory angiogenesis. *FEBS Lett* 372, 83-87
28. Bergström, S., Danielsson, H., and Samuelsson, B. (1964) The enzymatic formation of prostaglandin $E_2$ from arachidonic acid. Prostaglandin and related factors 32. *Biochim Biophys Acta* 90, 207-210
29. Bergström, S., Ryhage, R., Samuelsson, B., and Sjövall, J. (1963) Prostaglandins and related factors. 15. The structures of prostaglandin $E_1$, $F_{1\alpha}$ and $F_{1\beta}$. *J Biol Chem* 238, 3555-3564
30. Bergström, S., and Sjövall, J. (1960) The isolation of prostaglandin E from sheep prostate glands. *Acta Chem Scand* 14, 1701-1705
31. Bergström, S., and Sjövall, J. (1960) The isolation of prostaglandin F from sheep prostate glands. *Acta Physiol Scand* 14, 1693-1700
32. Beuckmann, C. T., Fujimori, K., Urade, Y., and Hayaishi, O. (2000) Identification of mu-class glutathione transferases M2-2 and M3-3 as cytosolic prostaglandin E synthases in the human brain. *Neurochem Res* 25, 733-738
33. Bezzine, S., Koduri, R. S., Valentin, E., Murakami, M., Kudo, I., Ghomashchi, F., Sadilek, M., Lambeau, G., and Gelb, M. H. (2000) Exogenously added human group X secreted phospholipase A(2) but not the group IB, IIA, and V enzymes efficiently release arachidonic acid from adherent mammalian cells. *J Biol Chem* 275, 3179-3191
34. Bhattacharya, M., Peri, K., Ribeiro-da-Silva, A., Almazan, G., Shichi, H., Hou, X., Varma, D. R., and Chemtob, S. (1999) Localization of functional prostaglandin E2 receptors EP3 and EP4 in the nuclear envelope. *J Biol Chem* 274, 15719-15724
35. Bhattacharya, M., Peri, K. G., Almazan, G., Ribeiro-da-Silva, A., Shichi, H., Durocher, Y., Abramovitz, M., Hou, X., Varma, D. R., and Chemtob, S. (1998) Nuclear localization of prostaglandin E2 receptors. *Proc Natl Acad Sci U S A* 95, 15792-15797
36. Boie, Y., Rushmore, T. H., Darmon-Goodwin, A., Grygorczyk, R., Slipetz, D. M., Metters, K. M., and Abramovitz, M. (1994) Cloning and expression of a cDNA for the human prostanoid IP receptor. *J Biol Chem* 269, 12173-12178
37. Boie, Y., Sawyer, N., Slipetz, D. M., Metters, K. M., and Abramovitz, M. (1995) Molecular cloning and characterization of the human prostanoid DP receptor. *J Biol Chem* 270, 18910-18916
38. 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) Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. *N Engl J Med* 343, 1520-1528, 1522 p following 1528
39. Borgeat, P., Hamberg, M., and Samuelsson, B. (1976) Transformation of arachidonic acid and homo-gamma-linolenic acid by rabbit polymorphonuclear leukocytes. Monohydroxy acids from novel lipoxygenases. *J Biol Chem* 251, 7816-7820

40. Borgeat, P., and Samuelsson, B. (1979) Transformation of arachidonic acid by rabbit polymorphonuclear leukocytes. Formation of a novel dihydroxyeicosatetraenoic acid. *J Biol Chem* 254, 2643-2646
41. Boyer, T. D., Vessey, D. A., and Kempner, E. (1986) Radiation inactivation of microsomal glutathione S-transferase. *J Biol Chem* 261, 16963-16968
42. Breitner, J. C., and Zandi, P. P. (2001) Do nonsteroidal antiinflammatory drugs reduce the risk of Alzheimer's disease? *N Engl J Med* 345, 1567-1568
43. Burgess, J. R., and Reddy, C. C. (1997) Isolation and characterization of an enzyme from sheep seminal vesicles that catalyzes the glutathione-dependent reduction of prostaglandin H2 to prostaglandin F2 alpha. *Biochem Mol Biol Int* 41, 217-226
44. Burleigh, M. E., Babaev, V. R., Oates, J. A., Harris, R. C., Gautam, S., Riendeau, D., Marnett, L. J., Morrow, J. D., Fazio, S., and Linton, M. F. (2002) Cyclooxygenase-2 promotes early atherosclerotic lesion formation in LDL receptor-deficient mice. *Circulation* 105, 1816-1823
45. Bylund, J., Hidestrand, M., Ingelman-Sundberg, M., and Oliw, E. H. (2000) Identification of CYP4F8 in human seminal vesicles as a prominent 19-hydroxylase of prostaglandin endoperoxides. *J Biol Chem* 275, 21844-21849
46. Bylund, J., and Oliw, E. H. (2001) Cloning and characterization of CYP4F21: a prostaglandin E2 20-hydroxylase of ram seminal vesicles. *Arch Biochem Biophys* 389, 123-129
47. Cao, C., Matsumura, K., Ozaki, M., and Watanabe, Y. (1999) Lipopolysaccharide injected into the cerebral ventricle evokes fever through induction of cyclooxygenase-2 in brain endothelial cells. *J Neurosci* 19, 716-725
48. Capdevila, J. H., and Falck, J. R. (2002) Biochemical and molecular properties of the cytochrome P450 arachidonic acid monooxygenases. *Prostaglandins Other Lipid Mediat* 68-69, 325-344
49. Catella-Lawson, F., McAdam, B., Morrison, B. W., Kapoor, S., Kujubu, D., Antes, L., Lasseter, K. C., Quan, H., Gertz, B. J., and FitzGerald, G. A. (1999) Effects of specific inhibition of cyclooxygenase-2 on sodium balance, hemodynamics, and vasoactive eicosanoids. *J Pharmacol Exp Ther* 289, 735-741
50. Cavallini, L., Francesconi, M. A., Zoccarato, F., and Alexandre, A. (2001) Involvement of nuclear factor-kappa B (NF-kappaB) activation in mitogen-induced lymphocyte proliferation: inhibitory effects of lymphoproliferation by salicylates acting as NF-kappaB inhibitors. *Biochem Pharmacol* 62, 141-147
51. Chang, M., Hong, Y., Burgess, J. R., Tu, C. P., and Reddy, C. C. (1987) Isozyme specificity of rat liver glutathione S-transferases in the formation of PGF2 alpha and PGE2 from PGH2. *Arch Biochem Biophys* 259, 548-557
52. Chen, C. Y., Del Gatto-Konczak, F., Wu, Z., and Karin, M. (1998) Stabilization of interleukin-2 mRNA by the c-Jun NH2-terminal kinase pathway. *Science* 280, 1945-1949
53. Chen, J., Engle, S. J., Seilhamer, J. J., and Tischfield, J. A. (1994) Cloning and recombinant expression of a novel human low molecular weight Ca(2+)-dependent phospholipase A2. *J Biol Chem* 269, 2365-2368
54. 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, 5952-5958
55. Cheng, H. F., Wang, J. L., Zhang, M. Z., Wang, S. W., McKanna, J. A., and Harris, R. C. (2001) Genetic deletion of COX-2 prevents increased renin expression in response to ACE inhibition. *Am J Physiol Renal Physiol* 280, F449-456
56. Cipollone, F., Prontera, C., Pini, B., Marini, M., Fazia, M., De Cesare, D., Iezzi, A., Ucchino, S., Boccoli, G., Saba, V., Chiarelli, F., Cuccurullo, F., and Mezzetti, A. (2001) Overexpression of functionally coupled cyclooxygenase-2 and prostaglandin E synthase in symptomatic atherosclerotic plaques as a basis of prostaglandin E(2)-dependent plaque instability. *Circulation* 104, 921-927
57. Clark, J. D., Lin, L. L., Kriz, R. W., Ramesha, C. S., Sultzman, L. A., Lin, A. Y., Milona, N., and Knopf, J. L. (1991) A novel arachidonic acid-selective cytosolic PLA2 contains a Ca(2+)-dependent translocation domain with homology to PKC and GAP. *Cell* 65, 1043-1051
58. Claveau, D., Sirinyan, M., Guay, J., Gordon, R., Chan, C. C., Bureau, Y., Riendeau, D., and Mancini, J. A. (2003) Microsomal prostaglandin e synthase-1 is a major terminal synthase that is selectively up-regulated during cyclooxygenase-2-dependent prostaglandin e(2) production in the rat adjuvant-induced arthritis model. *J Immunol* 170, 4738-4744

59. Cockcroft, S. (1992) G-protein-regulated phospholipases C, D and A2-mediated signalling in neutrophils. *Biochim Biophys Acta* 1113, 135-160
60. Corcoran, M. L., Stetler-Stevenson, W. G., DeWitt, D. L., and Wahl, L. M. (1994) Effect of cholera toxin and pertussis toxin on prostaglandin H synthase-2, prostaglandin E2, and matrix metalloproteinase production by human monocytes. *Arch Biochem Biophys* 310, 481-488
61. Couturier, C., Brouillet, A., Couriaud, C., Koumanov, K., Bereziat, G., and Andreani, M. (1999) Interleukin 1beta induces type II-secreted phospholipase A(2) gene in vascular smooth muscle cells by a nuclear factor kappaB and peroxisome proliferator-activated receptor-mediated process. *J Biol Chem* 274, 23085-23093
62. Crofford, L. J. (1997) COX-1 and COX-2 tissue expression: implications and predictions. *J Rheumatol* 24 Suppl 49, 15-19
63. Crofford, L. J. (1996) in *Improved Non-steroid Anti-Inflammatory Drugs* (Vane, J. R., Botting, J., and Botting, R. M., eds), pp. 133, Kluwer, Hingham
64. 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
65. 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 cyclooxygenase 2 inhibitors. A report of four cases. *Arthritis Rheum* 43, 1891-1896
66. Crofford, L. J., Wilder, R. L., Ristimaki, A. P., Sano, H., Remmers, E. F., Epps, H. R., and Hla, T. (1994) Cyclooxygenase-1 and -2 expression in rheumatoid synovial tissues. Effects of interleukin-1 beta, phorbol ester, and corticosteroids. *J Clin Invest* 93, 1095-1101
67. Cserzo, M., Wallin, E., Simon, I., von Heijne, G., and Elofsson, A. (1997) Prediction of transmembrane alpha-helices in prokaryotic membrane proteins: the dense alignment surface method. *Protein Eng* 10, 673-676
68. Cupillard, L., Koumanov, K., Mattei, M. G., Lazdunski, M., and Lambeau, G. (1997) Cloning, chromosomal mapping, and expression of a novel human secretory phospholipase A2. *J Biol Chem* 272, 15745-15752
69. Dantzer, R., Bluthe, R. M., Gheusi, G., Cremona, S., Laye, S., Parnet, P., and Kelley, K. W. (1998) Molecular basis of sickness behavior. *Ann N Y Acad Sci* 856, 132-138
70. Davies, P., Bailey, P. J., Goldenberg, M. M., and Ford-Hutchinson, A. W. (1984) The role of arachidonic acid oxygenation products in pain and inflammation. *Annu Rev Immunol* 2, 335-357
71. Dayer, J. M., Graham, R., Russell, G., and Krane, S. M. (1977) Collagenase production by rheumatoid synovial cells: stimulation by a human lymphocyte factor. *Science* 195, 181-183
72. DeWitt, D., and Smith, W. L. (1995) Yes, but do they still get headaches? *Cell* 83, 345-348
73. DeWitt, D. L. (1999) Cox-2-selective inhibitors: the new super aspirins. *Mol Pharmacol* 55, 625-631
74. DeWitt, D. L., and Smith, W. L. (1988) Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary DNA sequence. *Proc Natl Acad Sci U S A* 85, 1412-1416
75. Diez, E., Louis-Flamberg, P., Hall, R. H., and Mayer, R. J. (1992) Substrate specificities and properties of human phospholipases A2 in a mixed vesicle model. *J Biol Chem* 267, 18342-18348
76. Dinchuk, J. E., Car, B. D., Focht, R. J., Johnston, J. J., Jaffee, B. D., Covington, M. B., Contel, N. R., Eng, V. M., Collins, R. J., Czerniak, P. M., and et al. (1995) Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. *Nature* 378, 406-409
77. Dixon, R. A., Diehl, R. E., Opas, E., Rands, E., Vickers, P. J., Evans, J. F., Gillard, J. W., and Miller, D. K. (1990) Requirement of a 5-lipoxygenase-activating protein for leukotriene synthesis. *Nature* 343, 282-284
78. Ek, M., Engblom, D., Saha, S., Blomqvist, A., Jakobsson, P. J., and Ericsson-Dahlstrand, A. (2001) Inflammatory response: pathway across the blood-brain barrier. *Nature* 410, 430-431
79. Ekstrom, L., Lyrenas, L., Jakobsson, P. J., Morgenstern, R., and Kelner, M. J. (2003) Basal expression of the human MAPEG members microsomal glutathione transferase 1 and prostaglandin E synthase genes is mediated by Sp1 and Sp3. *Biochim Biophys Acta* 1627, 79-84
80. Elmquist, J. K., Scammell, T. E., and Saper, C. B. (1997) Mechanisms of CNS response to systemic immune challenge: the febrile response. *Trends Neurosci* 20, 565-570

81. Engblom, D., Ek, M., Andersson, I. M., Saha, S., Dahlstrom, M., Jakobsson, P. J., Ericsson-Dahlstrand, A., and Blomqvist, A. (2002) Induction of microsomal prostaglandin E synthase in the rat brain endothelium and parenchyma in adjuvant-induced arthritis. *J Comp Neurol* 452, 205-214
82. Engblom, D., Ek, M., Saha, S., Ericsson-Dahlstrand, A., Jakobsson, P. J., and Blomqvist, A. (2002) Prostaglandins as inflammatory messengers across the blood-brain barrier. *J Mol Med* 80, 5-15
83. Evans, J. H., Spencer, D. M., Zweifach, A., and Leslie, C. C. (2001) Intracellular calcium signals regulating cytosolic phospholipase A2 translocation to internal membranes. *J Biol Chem* 276, 30150-30160
84. Filion, F., Bouchard, N., Goff, A. K., Lussier, J. G., and Sirois, J. (2001) Molecular cloning and induction of bovine prostaglandin E synthase by gonadotropins in ovarian follicles prior to ovulation in vivo. *J Biol Chem* 276, 34323-34330
85. Firestein, G. S., Alvaro-Gracia, J. M., Maki, R., and Alvaro-Garcia, J. M. (1990) Quantitative analysis of cytokine gene expression in rheumatoid arthritis. *J Immunol* 144, 3347-3353
86. Fleming, E. F., Athirakul, K., Oliverio, M. I., Key, M., Goulet, J., Koller, B. H., and Coffman, T. M. (1998) Urinary concentrating function in mice lacking EP3 receptors for prostaglandin E2. *Am J Physiol* 275, F955-961
87. Fonteh, A. N., Atsumi, G., LaPorte, T., and Chilton, F. H. (2000) Secretory phospholipase A2 receptor-mediated activation of cytosolic phospholipase A2 in murine bone marrow-derived mast cells. *J Immunol* 165, 2773-2782
88. Fujishima, H., Sanchez Mejia, R. O., Bingham, C. O., 3rd, Lam, B. K., Sapirstein, A., Bonventre, J. V., Austen, K. F., and Arm, J. P. (1999) Cytosolic phospholipase A2 is essential for both the immediate and the delayed phases of eicosanoid generation in mouse bone marrow-derived mast cells. *Proc Natl Acad Sci U S A* 96, 4803-4807
89. Funk, C. D., Furci, L., FitzGerald, G. A., Grygorczyk, R., Rochette, C., Bayne, M. A., Abramovitz, M., Adam, M., and Metters, K. M. (1993) Cloning and expression of a cDNA for the human prostaglandin E receptor EP1 subtype. *J Biol Chem* 268, 26767-26772
90. Galis, Z. S., Sukhova, G. K., Lark, M. W., and Libby, P. (1994) Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest* 94, 2493-2503
91. Garavito, R. M., and Mulichak, A. M. (2003) The Structure of Mammalian Cyclooxygenases. *Annu Rev Biophys Biomol Struct*
92. Gerozissis, K., de Saint-Hilaire, Z., Python, A., Rouch, C., Orosco, M., and Nicolaidis, S. (1998) Microdialysis and EEG in rats reveal cortical PGE2 changes during sleep and wakefulness. *Neuroreport* 9, 1327-1330
93. Gilbert, J. J., Stewart, A., Courtney, C. A., Fleming, M. C., Reid, P., Jackson, C. G., Wise, A., Wakelam, M. J., and Harnett, M. M. (1996) Antigen receptors on immature, but not mature, B and T cells are coupled to cytosolic phospholipase A2 activation: expression and activation of cytosolic phospholipase A2 correlate with lymphocyte maturation. *J Immunol* 156, 2054-2061
94. Gilroy, D. W., Colville-Nash, P. R., Willis, D., Chivers, J., Paul-Clark, M. J., and Willoughby, D. A. (1999) Inducible cyclooxygenase may have anti-inflammatory properties. *Nat Med* 5, 698-701
95. Goldblatt, M. W. (1933) A depressor substance in seminal fluid. *Chem Ind* 52, 1056-1057
96. Goldblatt, M. W. (1935) Properties of human seminal plasma. *J Physiol (London)* 84, 208-218
97. Goppelt-Struebe, M., Koerner, C. F., Hausmann, G., Gemsa, D., and Resch, K. (1986) Control of prostanoid synthesis: role of reincorporation of released precursor fatty acids. *Prostaglandins* 32, 373-385
98. Guan, Y., Zhang, Y., Schneider, A., Riendeau, D., Mancini, J. A., Davis, L., Komhoff, M., Breyer, R. M., and Breyer, M. D. (2001) Urogenital distribution of a mouse membrane-associated prostaglandin E(2) synthase. *Am J Physiol Renal Physiol* 281, F1173-1177
99. Guan, Z., Buckman, S. Y., Miller, B. W., Springer, L. D., and Morrison, A. R. (1998) Interleukin-1beta-induced cyclooxygenase-2 expression requires activation of both c-Jun NH2-terminal kinase and p38 MAPK signal pathways in rat renal mesangial cells. *J Biol Chem* 273, 28670-28676
100. Haeffner, A., Thieblemont, N., Deas, O., Marelli, O., Charpentier, B., Senik, A., Wright, S. D., Haeffner-Cavaillon, N., and Hirsch, F. (1997) Inhibitory effect of growth hormone on TNF-alpha secretion and nuclear factor-kappaB translocation in lipopolysaccharide-stimulated human monocytes. *J Immunol* 158, 1310-1314

101. Halliday, G., Robinson, S. R., Shepherd, C., and Kril, J. (2000) Alzheimer's disease and inflammation: a review of cellular and therapeutic mechanisms. *Clin Exp Pharmacol Physiol* 27, 1-8
102. Hamberg, M. (1968) Metabolism of prostaglandins in rat liver mitochondria. *Eur J Biochem* 6, 135-146
103. Hamberg, M., and Samuelsson, B. (1967) On the mechanism of the biosynthesis of prostaglandins E-1 and F-1-alpha. *J Biol Chem* 242, 5336-5343
104. Hamberg, M., Svensson, J., and Samuelsson, B. (1975) Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc Natl Acad Sci U S A* 72, 2994-2998
105. Hamberg, M., Svensson, J., Wakabayashi, T., and Samuelsson, B. (1974) Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. *Proc Natl Acad Sci U S A* 71, 345-349
106. Hammarstrom, S., Murphy, R. C., Samuelsson, B., Clark, D. A., Mioskowski, C., and Corey, E. J. (1979) Structure of leukotriene C. Identification of the amino acid part. *Biochem Biophys Res Commun* 91, 1266-1272
107. Han, R., and Smith, T. J. (2002) Cytoplasmic prostaglandin E2 synthase is dominantly expressed in cultured KAT-50 thyrocytes, cells that express constitutive prostaglandin-endoperoxide H synthase-2. Basis for low protaglandin E2 production. *J Biol Chem* 277, 36897-36903
108. Han, R., Tsui, S., and Smith, T. J. (2002) Up-regulation of prostaglandin E2 synthesis by interleukin-1beta in human orbital fibroblasts involves coordinate induction of prostaglandin-endoperoxide H synthase-2 and glutathione-dependent prostaglandin E2 synthase expression. *J Biol Chem* 277, 16355-16364
109. Han, S. K., Kim, K. P., Koduri, R., Bittova, L., Munoz, N. M., Leff, A. R., Wilton, D. C., Gelb, M. H., and Cho, W. (1999) Roles of Trp31 in high membrane binding and proinflammatory activity of human group V phospholipase A2. *J Biol Chem* 274, 11881-11888
110. Hanasaki, K., Ono, T., Saiga, A., Morioka, Y., Ikeda, M., Kawamoto, K., Higashino, K., Nakano, K., Yamada, K., Ishizaki, J., and Arita, H. (1999) Purified group X secretory phospholipase A(2) induced prominent release of arachidonic acid from human myeloid leukemia cells. *J Biol Chem* 274, 34203-34211
111. Hanel, A. M., Schuttel, S., and Gelb, M. H. (1993) Processive interfacial catalysis by mammalian 85-kilodalton phospholipase A2 enzymes on product-containing vesicles: application to the determination of substrate preferences. *Biochemistry* 32, 5949-5958
112. Harada, Y., Kawamura, M., Hatanaka, K., Saito, M., Ogino, M., Ohno, T., Ogino, K., and Yang, Q. (1998) Differing profiles of prostaglandin formation inhibition between selective prostaglandin H synthase-2 inhibitors and conventional NSAIDs in inflammatory and non-inflammatory sites of the rat. *Prostaglandins Other Lipid Mediat* 55, 345-358
113. Hardiman, G., Rock, F. L., Balasubramanian, S., Kastelein, R. A., and Bazan, J. F. (1996) Molecular characterization and modular analysis of human MyD88. *Oncogene* 13, 2467-2475
114. Harris, R. C., McKanna, J. A., Akai, Y., Jacobson, H. R., Dubois, R. N., and Breyer, M. D. (1994) Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction. *J Clin Invest* 94, 2504-2510
115. Hayaishi, O. (1991) Molecular mechanisms of sleep-wake regulation: roles of prostaglandins D2 and E2. *Faseb J* 5, 2575-2581
116. Hayaishi, O., Matsumura, H., Onoe, H., Koyama, Y., and Watanabe, Y. (1991) Sleep-wake regulation by PGD2 and E2. *Adv Prostaglandin Thromboxane Leukot Res* 21B, 723-726
117. Hebert, H., Schmidt-Krey, I., Morgenstern, R., Murata, K., Hirai, T., Mitsuoka, K., and Fujiyoshi, Y. (1997) The 3.0 A projection structure of microsomal glutathione transferase as determined by electron crystallography of p 21212 two-dimensional crystals. *J Mol Biol* 271, 751-758
118. Hernandez, M., Burillo, S. L., Crespo, M. S., and Nieto, M. L. (1998) Secretory phospholipase A2 activates the cascade of mitogen-activated protein kinases and cytosolic phospholipase A2 in the human astrocytoma cell line 1321N1. *J Biol Chem* 273, 606-612
119. Herschman, H. R. (1999) Function and regulation of prostaglandin synthase 2. *Adv Exp Med Biol* 469, 3-8
120. Herschman, H. R. (1996) Prostaglandin synthase 2. *Biochim Biophys Acta* 1299, 125-140
121. Hirabayashi, T., and Shimizu, T. (2000) Localization and regulation of cytosolic phospholipase A(2). *Biochim Biophys Acta* 1488, 124-138

122. Hirai, H., Tanaka, K., Yoshie, O., Ogawa, K., Kenmotsu, K., Takamori, Y., Ichimasa, M., Sugamura, K., Nakamura, M., Takano, S., and Nagata, K. (2001) Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J Exp Med* 193, 255-261
123. Hirata, M., Hayashi, Y., Ushikubi, F., Yokota, Y., Kageyama, R., Nakanishi, S., and Narumiya, S. (1991) Cloning and expression of cDNA for a human thromboxane A2 receptor. *Nature* 349, 617-620
124. Hixon, M. S., Ball, A., and Gelb, M. H. (1998) Calcium-dependent and -independent interfacial binding and catalysis of cytosolic group IV phospholipase A2. *Biochemistry* 37, 8516-8526
125. 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 EP(2). *Proc Natl Acad Sci U S A* 96, 10501-10506
126. Hla, T., Bishop-Bailey, D., Liu, C. H., Schaefers, H. J., and Trifan, O. C. (1999) Cyclooxygenase-1 and -2 isoenzymes. *Int J Biochem Cell Biol* 31, 551-557
127. Hla, T., and Neilson, K. (1992) Human cyclooxygenase-2 cDNA. *Proc Natl Acad Sci U S A* 89, 7384-7388
128. Hoeck, W. G., Ramesha, C. S., Chang, D. J., Fan, N., and Heller, R. A. (1993) Cytoplasmic phospholipase A2 activity and gene expression are stimulated by tumor necrosis factor: dexamethasone blocks the induced synthesis. *Proc Natl Acad Sci U S A* 90, 4475-4479
129. Hong, K. H., Bonventre, J. C., O'Leary, E., Bonventre, J. V., and Lander, E. S. (2001) Deletion of cytosolic phospholipase A(2) suppresses Apc(Min)-induced tumorigenesis. *Proc Natl Acad Sci U S A* 98, 3935-3939
130. Honkakoski, P., Zelko, I., Sueyoshi, T., and Negishi, M. (1998) The nuclear orphan receptor CAR-retinoid X receptor heterodimer activates the phenobarbital-responsive enhancer module of the CYP2B gene. *Mol Cell Biol* 18, 5652-5658
131. Hoozemans, J. J., Veerhuis, R., Janssen, I., Rozemuller, A. J., and Eikelenboom, P. (2001) Interleukin-1beta induced cyclooxygenase 2 expression and prostaglandin E2 secretion by human neuroblastoma cells: implications for Alzheimer's disease. *Exp Gerontol* 36, 559-570
132. Houchen, C. W., Stenson, W. F., and Cohn, S. M. (2000) Disruption of cyclooxygenase-1 gene results in an impaired response to radiation injury. *Am J Physiol Gastrointest Liver Physiol* 279, G858-865
133. Huang, M., Stolina, M., Sharma, S., Mao, J. T., Zhu, L., Miller, P. W., Wollman, J., Herschman, H., and Dubinett, S. M. (1998) Non-small cell lung cancer cyclooxygenase-2-dependent regulation of cytokine balance in lymphocytes and macrophages: up-regulation of interleukin 10 and down-regulation of interleukin 12 production. *Cancer Res* 58, 1208-1216
134. Hutchison, K. A., Stancato, L. F., Owens-Grillo, J. K., Johnson, J. L., Krishna, P., Toft, D. O., and Pratt, W. B. (1995) The 23-kDa acidic protein in reticulocyte lysate is the weakly bound component of the hsp foldosome that is required for assembly of the glucocorticoid receptor into a functional heterocomplex with hsp90. *J Biol Chem* 270, 18841-18847
135. Huwiler, A., Staudt, G., Kramer, R. M., and Pfeilschifter, J. (1997) Cross-talk between secretory phospholipase A2 and cytosolic phospholipase A2 in rat renal mesangial cells. *Biochim Biophys Acta* 1348, 257-272
136. Inoue, H., Yokoyama, C., Hara, S., Tone, Y., and Tanabe, T. (1995) Transcriptional regulation of human prostaglandin-endoperoxide synthase-2 gene by lipopolysaccharide and phorbol ester in vascular endothelial cells. Involvement of both nuclear factor for interleukin-6 expression site and cAMP response element. *J Biol Chem* 270, 24965-24971
137. Irvine, R. F. (1982) How is the level of free arachidonic acid controlled in mammalian cells? *Biochem J* 204, 3-16
138. Jabbour, H. N., Milne, S. A., Williams, A. R., Anderson, R. A., and Boddy, S. C. (2001) Expression of COX-2 and PGE synthase and synthesis of PGE(2)in endometrial adenocarcinoma: a possible autocrine/paracrine regulation of neoplastic cell function via EP2/EP4 receptors. *Br J Cancer* 85, 1023-1031
139. Jakobsson, P. J., Mancini, J. A., and Ford-Hutchinson, A. W. (1996) Identification and characterization of a novel human microsomal glutathione S-transferase with leukotriene C4 synthase activity and significant sequence identity to 5-lipoxygenase-activating protein and leukotriene C4 synthase. *J Biol Chem* 271, 22203-22210

140. Jakobsson, P. J., Mancini, J. A., Riendeau, D., and Ford-Hutchinson, A. W. (1997) Identification and characterization of a novel microsomal enzyme with glutathione-dependent transferase and peroxidase activities. *J Biol Chem* 272, 22934-22939
141. Jakobsson, P. J., Morgenstern, R., Mancini, J., Ford-Hutchinson, A., and Persson, B. (1999) Common structural features of MAPEG -- a widespread superfamily of membrane associated proteins with highly divergent functions in eicosanoid and glutathione metabolism. *Protein Sci* 8, 689-692
142. Jakobsson, P. J., Morgenstern, R., Mancini, J., Ford-Hutchinson, A., and Persson, B. (2000) Membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG). A widespread protein superfamily. *Am J Respir Crit Care Med* 161, S20-24
143. 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 U S A* 96, 7220-7225
144. Jarabak, J., and Fried, J. (1979) Comparison of substrate specificities of the human placental NAD- and NADP-linked 15-hydroxyprostaglandin dehydrogenases. *Prostaglandins* 18, 241-246
145. Jiang, C., Ting, A. T., and Seed, B. (1998) PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature* 391, 82-86
146. Johnson, J. A., el Barbary, A., Kornguth, S. E., Brugge, J. F., and Siegel, F. L. (1993) Glutathione S-transferase isoenzymes in rat brain neurons and glia. *J Neurosci* 13, 2013-2023
147. Johnson, J. L., Beito, T. G., Krco, C. J., and Toft, D. O. (1994) Characterization of a novel 23-kilodalton protein of unactive progesterone receptor complexes. *Mol Cell Biol* 14, 1956-1963
148. Jones, D. A., Carlton, D. P., McIntyre, T. M., Zimmerman, G. A., and Prescott, S. M. (1993) Molecular cloning of human prostaglandin endoperoxide synthase type II and demonstration of expression in response to cytokines. *J Biol Chem* 268, 9049-9054
149. Jones, R. L., Cammock, S., and Horton, E. W. (1972) Partial purification and properties of cat plasma prostaglandin A isomerase. *Biochim Biophys Acta* 280, 588-601
150. 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*
151. Kanaoka, Y., Ago, H., Inagaki, E., Nanayama, T., Miyano, M., Kikuno, R., Fujii, Y., Eguchi, N., Toh, H., Urade, Y., and Hayaishi, O. (1997) Cloning and crystal structure of hematopoietic prostaglandin D synthase. *Cell* 90, 1085-1095
152. Kang, R. Y., Freire-Moar, J., Sigal, E., and Chu, C. Q. (1996) Expression of cyclooxygenase-2 in human and an animal model of rheumatoid arthritis. *Br J Rheumatol* 35, 711-718
153. Kankofer, M., and Wiercinski, J. (1999) Prostaglandin E2 9-keto reductase from bovine term placenta. *Prostaglandins Leukot Essent Fatty Acids* 61, 29-32
154. Kargman, S. L., O'Neill, G. P., Vickers, P. J., Evans, J. F., Mancini, J. A., and Jothy, S. (1995) Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. *Cancer Res* 55, 2556-2559
155. Kawai, S. (2003) Current drug therapy for rheumatoid arthritis. *J Orthop Sci* 8, 259-263
156. Kelner, M. J., Stokely, M. N., Stovall, N. E., and Montoya, M. A. (1996) Structural organization of the human microsomal glutathione S-transferase gene (GST12). *Genomics* 36, 100-103
157. Kelner, M. J., and Uglik, S. F. (1994) PDGF-induces the glutathione-dependent enzyme PGH2/PGE2 isomerase in NIH3T3 and pEJ transformed fibroblasts. *Biochem Biophys Res Commun* 198, 298-303
158. Kennedy, C. R., Zhang, Y., Brandon, S., Guan, Y., Coffee, K., Funk, C. D., Magnuson, M. A., Oates, J. A., Breyer, M. D., and Breyer, R. M. (1999) Salt-sensitive hypertension and reduced fertility in mice lacking the prostaglandin EP2 receptor. *Nat Med* 5, 217-220
159. Kirschning, C. J., Wesche, H., Merrill Ayres, T., and Rothe, M. (1998) Human toll-like receptor 2 confers responsiveness to bacterial lipopolysaccharide. *J Exp Med* 188, 2091-2097
160. Kitamura, Y., Shimohama, S., Koike, H., Kakimura, J., Matsuoka, Y., Nomura, Y., Gebicke-Haerter, P. J., and Taniguchi, T. (1999) Increased expression of cyclooxygenases and peroxisome proliferator-activated receptor-gamma in Alzheimer's disease brains. *Biochem Biophys Res Commun* 254, 582-586
161. Kobayashi, T., and Narumiya, S. (2002) Function of prostanoid receptors: studies on knockout mice. *Prostaglandins Other Lipid Mediat* 68-69, 557-573
162. Kojima, F., Naraba, H., Sasaki, Y., Okamoto, R., Koshino, T., and Kawai, S. (2002) Coexpression of microsomal prostaglandin E synthase with cyclooxygenase-2 in human rheumatoid synovial cells. *J Rheumatol* 29, 1836-1842

163. Koshkin, V., and Dunford, H. B. (1999) Coupling of the peroxidase and cyclooxygenase reactions of prostaglandin H synthase. *Biochim Biophys Acta* 1430, 341-348
164. Kozak, K. R., Crews, B. C., Ray, J. L., Tai, H. H., Morrow, J. D., and Marnett, L. J. (2001) Metabolism of prostaglandin glycerol esters and prostaglandin ethanolamides in vitro and in vivo. *J Biol Chem* 276, 36993-36998
165. Kozak, K. R., Rowlinson, S. W., and Marnett, L. J. (2000) Oxygenation of the endocannabinoid, 2-arachidonylglycerol, to glyceryl prostaglandins by cyclooxygenase-2. *J Biol Chem* 275, 33744-33749
166. Kramer, R. M., Roberts, E. F., Manetta, J., and Putnam, J. E. (1991) The Ca2(+)-sensitive cytosolic phospholipase A2 is a 100-kDa protein in human monoblast U937 cells. *J Biol Chem* 266, 5268-5272
167. Kudo, I., and Murakami, M. (2002) Phospholipase A2 enzymes. *Prostaglandins Other Lipid Mediat* 68-69, 3-58
168. Kujubu, D. A., Fletcher, B. S., Varnum, B. C., Lim, R. W., and Herschman, H. R. (1991) TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. *J Biol Chem* 266, 12866-12872
169. 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
170. Kurumbail, R. G., Stevens, A. M., Gierse, J. K., McDonald, J. J., Stegeman, R. A., Pak, J. Y., Gildehaus, D., Miyashiro, J. M., Penning, T. D., Seibert, K., Isakson, P. C., and Stallings, W. C. (1996) Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature* 384, 644-648
171. Kurzrok, R., and Lieb, C. C. (1930) Biochemical studies of human semen. The biological action of semen on the human uterus. *Proc Soc Exp Biol Med* 28, 268-272
172. Kuwata, H., Nakatani, Y., Murakami, M., and Kudo, I. (1998) Cytosolic phospholipase A2 is required for cytokine-induced expression of type IIA secretory phospholipase A2 that mediates optimal cyclooxygenase-2-dependent delayed prostaglandin E2 generation in rat 3Y1 fibroblasts. *J Biol Chem* 273, 1733-1740
173. Kuwata, H., Yamamoto, S., Miyazaki, Y., Shimbara, S., Nakatani, Y., Suzuki, H., Ueda, N., Murakami, M., and Kudo, I. (2000) Studies on a mechanism by which cytosolic phospholipase A2 regulates the expression and function of type IIA secretory phospholipase A2. *J Immunol* 165, 4024-4031
174. Lam, B. K., Penrose, J. F., Freeman, G. J., and Austen, K. F. (1994) Expression cloning of a cDNA for human leukotriene C4 synthase, an integral membrane protein conjugating reduced glutathione to leukotriene A4. *Proc Natl Acad Sci U S A* 91, 7663-7667
175. Lam, B. K., Penrose, J. F., Xu, K., Baldasaro, M. H., and Austen, K. F. (1997) Site-directed mutagenesis of human leukotriene C4 synthase. *J Biol Chem* 272, 13923-13928
176. Landino, L. M., Crews, B. C., Gierse, J. K., Hauser, S. D., and Marnett, L. J. (1997) Mutational analysis of the role of the distal histidine and glutamine residues of prostaglandin-endoperoxide synthase-2 in peroxidase catalysis, hydroperoxide reduction, and cyclooxygenase activation. *J Biol Chem* 272, 21565-21574
177. Lands, W., Lee, R., and Smith, W. (1971) Factors regulating the biosynthesis of various prostaglandins. *Ann N Y Acad Sci* 180, 107-122
178. Langenbach, R., Loftin, C. D., Lee, C., and Tiano, H. (1999) Cyclooxygenase-deficient mice. A summary of their characteristics and susceptibilities to inflammation and carcinogenesis. *Ann N Y Acad Sci* 889, 52-61
179. Langenbach, R., Morham, S. G., Tiano, H. F., Loftin, C. D., Ghanayem, B. I., Chulada, P. C., Mahler, J. F., Lee, C. A., Goulding, E. H., Kluckman, K. D., and et al. (1995) Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell* 83, 483-492
180. Larsson, P. K., Claesson, H. E., and Kennedy, B. P. (1998) Multiple splice variants of the human calcium-independent phospholipase A2 and their effect on enzyme activity. *J Biol Chem* 273, 207-214
181. Lawrence, T., Willoughby, D. A., and Gilroy, D. W. (2002) Anti-inflammatory lipid mediators and insights into the resolution of inflammation. *Nat Rev Immunol* 2, 787-795
182. Lazarus, M., Kubata, B. K., Eguchi, N., Fujitani, Y., Urade, Y., and Hayaishi, O. (2002) Biochemical characterization of mouse microsomal prostaglandin E synthase-1 and its

colocalization with cyclooxygenase-2 in peritoneal macrophages. *Arch Biochem Biophys* 397, 336-341
183. Lazarus, M., Munday, C. J., Eguchi, N., Matsumoto, S., Killian, G. J., Kubata, B. K., and Urade, Y. (2002) Immunohistochemical localization of microsomal PGE synthase-1 and cyclooxygenases in male mouse reproductive organs. *Endocrinology* 143, 2410-2419
184. Lee, S. C., and Levine, L. (1975) Purification and regulatory properties of chicken heart prostaglandin E 9-ketoreductase. *J Biol Chem* 250, 4549-4555
185. Lee, Y. H., Choi, S. J., Kim, A., Kim, C. H., Ji, J. D., and Song, G. G. (2000) Expression of cyclooxygenase-1 and -2 in rheumatoid arthritis synovium. *J Korean Med Sci* 15, 88-92
186. Leslie, C. C. (1997) Properties and regulation of cytosolic phospholipase A2. *J Biol Chem* 272, 16709-16712
187. Lewis, R. A., Drazen, J. M., Austen, K. F., Clark, D. A., and Corey, E. J. (1980) Identification of the C(6)-S-conjugate of leukotriene A with cysteine as a naturally occurring slow reacting substance of anaphylaxis (SRS-A). Importance of the 11-cis-geometry for biological activity. *Biochem Biophys Res Commun* 96, 271-277
188. Levy, B. D., Clish, C. B., Schmidt, B., Gronert, K., and Serhan, C. N. (2001) Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol* 2, 612-619
189. Levy, G. N. (1997) Prostaglandin H synthases, nonsteroidal anti-inflammatory drugs, and colon cancer. *Faseb J* 11, 234-247
190. 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
191. Li, X., Okada, Y., Pilbeam, C. C., Lorenzo, J. A., Kennedy, C. R., Breyer, R. M., and Raisz, L. G. (2000) Knockout of the murine prostaglandin EP2 receptor impairs osteoclastogenesis in vitro. *Endocrinology* 141, 2054-2061
192. Lim, H., Paria, B. C., Das, S. K., Dinchuk, J. E., Langenbach, R., Trzaskos, J. M., and Dey, S. K. (1997) Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* 91, 197-208
193. Lin, L. L., Lin, A. Y., and DeWitt, D. L. (1992) Interleukin-1 alpha induces the accumulation of cytosolic phospholipase A2 and the release of prostaglandin E2 in human fibroblasts. *J Biol Chem* 267, 23451-23454
194. Lin, Y. M., and Jarabak, J. (1978) Isolation of two proteins with 9-ketoprostaglandin reductase and NADP-linked 15-hydroxyprostaglandin dehydrogenase activities and studies on their inhibition. *Biochem Biophys Res Commun* 81, 1227-1234
195. Liu, C. H., Chang, S. H., Narko, K., Trifan, O. C., Wu, M. T., Smith, E., Haudenschild, C., Lane, T. F., and Hla, T. (2001) Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. *J Biol Chem* 276, 18563-18569
196. Lu, G., Tsai, A. L., Van Wart, H. E., and Kulmacz, R. J. (1999) Comparison of the peroxidase reaction kinetics of prostaglandin H synthase-1 and -2. *J Biol Chem* 274, 16162-16167
197. Lu, R., Kanai, N., Bao, Y., and Schuster, V. L. (1996) Cloning, in vitro expression, and tissue distribution of a human prostaglandin transporter cDNA(hPGT). *J Clin Invest* 98, 1142-1149
198. Lundqvist, G., Yucel-Lindberg, T., and Morgenstern, R. (1992) The oligomeric structure of rat liver microsomal glutathione transferase studied by chemical cross-linking. *Biochim Biophys Acta* 1159, 103-108
199. Luong, C., Miller, A., Barnett, J., Chow, J., Ramesha, C., and Browner, M. F. (1996) Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2. *Nat Struct Biol* 3, 927-933
200. Malkowski, M. G., Ginell, S. L., Smith, W. L., and Garavito, R. M. (2000) The productive conformation of arachidonic acid bound to prostaglandin synthase. *Science* 289, 1933-1937
201. Mancini, J. A., Abramovitz, M., Cox, M. E., Wong, E., Charleson, S., Perrier, H., Wang, Z., Prasit, P., and Vickers, P. J. (1993) 5-lipoxygenase-activating protein is an arachidonate binding protein. *FEBS Lett* 318, 277-281
202. Mancini, J. A., Blood, K., Guay, J., Gordon, R., Claveau, D., Chan, C. C., and Riendeau, D. (2001) Cloning, expression, and up-regulation of inducible rat prostaglandin e synthase during lipopolysaccharide-induced pyresis and adjuvant-induced arthritis. *J Biol Chem* 276, 4469-4475
203. Mancini, J. A., Waterman, H., and Riendeau, D. (1998) Cellular oxygenation of 12-hydroxyeicosatetraenoic acid and 15-hydroxyeicosatetraenoic acid by 5-lipoxygenase is stimulated by 5-lipoxygenase-activating protein. *J Biol Chem* 273, 32842-32847
204. Marnett, L. J. (1992) Aspirin and the potential role of prostaglandins in colon cancer. *Cancer Res* 52, 5575-5589

205. Marnett, L. J., Rowlinson, S. W., Goodwin, D. C., Kalgutkar, A. S., and Lanzo, C. A. (1999) Arachidonic acid oxygenation by COX-1 and COX-2. Mechanisms of catalysis and inhibition. *J Biol Chem* 274, 22903-22906
206. Masferrer, J. L., Zweifel, B. S., Manning, P. T., Hauser, S. D., Leahy, K. M., Smith, W. G., Isakson, P. C., and Seibert, K. (1994) Selective inhibition of inducible cyclooxygenase 2 in vivo is antiinflammatory and nonulcerogenic. *Proc Natl Acad Sci U S A* 91, 3228-3232
207. Matsumoto, H., Naraba, H., Murakami, M., Kudo, I., Yamaki, K., Ueno, A., and Oh-ishi, S. (1997) Concordant induction of prostaglandin E2 synthase with cyclooxygenase-2 leads to preferred production of prostaglandin E2 over thromboxane and prostaglandin D2 in lipopolysaccharide-stimulated rat peritoneal macrophages. *Biochem Biophys Res Commun* 230, 110-114
208. Matsuoka, T., Hirata, M., Tanaka, H., Takahashi, Y., Murata, T., Kabashima, K., Sugimoto, Y., Kobayashi, T., Ushikubi, F., Aze, Y., Eguchi, N., Urade, Y., Yoshida, N., Kimura, K., Mizoguchi, A., Honda, Y., Nagai, H., and Narumiya, S. (2000) Prostaglandin D2 as a mediator of allergic asthma. *Science* 287, 2013-2017
209. 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 U S A* 96, 272-277
210. McCleskey, E. W., and Gold, M. S. (1999) Ion channels of nociception. *Annu Rev Physiol* 61, 835-856
211. McGeer, P. L., and McGeer, E. G. (1999) Inflammation of the brain in Alzheimer's disease: implications for therapy. *J Leukoc Biol* 65, 409-415
212. Meadows, J. W., Eis, A. L., Brockman, D. E., and Myatt, L. (2003) Expression and localization of prostaglandin E synthase isoforms in human fetal membranes in term and preterm labor. *J Clin Endocrinol Metab* 88, 433-439
213. Mehindate, K., al-Daccak, R., Dayer, J. M., Kennedy, B. P., Kris, C., Borgeat, P., Poubelle, P. E., and Mourad, W. (1995) Superantigen-induced collagenase gene expression in human IFN-gamma-treated fibroblast-like synoviocytes involves prostaglandin E2. Evidence for a role of cyclooxygenase-2 and cytosolic phospholipase A2. *J Immunol* 155, 3570-3577
214. Merlie, J. P., Fagan, D., Mudd, J., and Needleman, P. (1988) Isolation and characterization of the complementary DNA for sheep seminal vesicle prostaglandin endoperoxide synthase (cyclooxygenase). *J Biol Chem* 263, 3550-3553
215. Miller, D. K., Gillard, J. W., Vickers, P. J., Sadowski, S., Leveille, C., Mancini, J. A., Charleson, P., Dixon, R. A., Ford-Hutchinson, A. W., Fortin, R., and et al. (1990) Identification and isolation of a membrane protein necessary for leukotriene production. *Nature* 343, 278-281
216. Minami, T., Nakano, H., Kobayashi, T., Sugimoto, Y., Ushikubi, F., Ichikawa, A., Narumiya, S., and Ito, S. (2001) Characterization of EP receptor subtypes responsible for prostaglandin E2-induced pain responses by use of EP1 and EP3 receptor knockout mice. *Br J Pharmacol* 133, 438-444
217. Mitchell, J. A., Belvisi, M. G., Akarasereenont, P., Robbins, R. A., Kwon, O. J., Croxtall, J., Barnes, P. J., and Vane, J. R. (1994) Induction of cyclo-oxygenase-2 by cytokines in human pulmonary epithelial cells: regulation by dexamethasone. *Br J Pharmacol* 113, 1008-1014
218. Miyamoto, T., Ogino, N., Yamamoto, S., and Hayaishi, O. (1976) Purification of prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes. *J Biol Chem* 251, 2629-2636
219. Miyaura, C., Inada, M., Suzawa, T., Sugimoto, Y., Ushikubi, F., Ichikawa, A., Narumiya, S., and Suda, T. (2000) Impaired bone resorption to prostaglandin E2 in prostaglandin E receptor EP4-knockout mice. *J Biol Chem* 275, 19819-19823
220. Mnich, S. J., Veenhuizen, A. W., Monahan, J. B., Sheehan, K. C., Lynch, K. R., Isakson, P. C., and Portanova, J. P. (1995) Characterization of a monoclonal antibody that neutralizes the activity of prostaglandin E2. *J Immunol* 155, 4437-4444
221. Moncada, S., Gryglewski, R., Bunting, S., and Vane, J. R. (1976) An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature* 263, 663-665
222. Montine, T. J., Sidell, K. R., Crews, B. C., Markesbery, W. R., Marnett, L. J., Roberts, L. J., 2nd, and Morrow, J. D. (1999) Elevated CSF prostaglandin E2 levels in patients with probable AD. *Neurology* 53, 1495-1498
223. Moonen, P., Buytenhek, M., and Nugteren, D. H. (1982) Purification of PGH-PGE isomerase from sheep vesicular glands. *Methods Enzymol* 86, 84-91

224. Morgenstern, R., and DePierre, J. W. (1983) Microsomal glutathione transferase. Purification in unactivated form and further characterization of the activation process, substrate specificity and amino acid composition. *Eur J Biochem* 134, 591-597
225. Morgenstern, R., Guthenberg, C., and Depierre, J. W. (1982) Microsomal glutathione S-transferase. Purification, initial characterization and demonstration that it is not identical to the cytosolic glutathione S-transferases A, B and C. *Eur J Biochem* 128, 243-248
226. Morgenstern, R., Lundqvist, G., Hancock, V., and DePierre, J. W. (1988) Studies on the activity and activation of rat liver microsomal glutathione transferase, in particular with a substrate analogue series. *J Biol Chem* 263, 6671-6675
227. Morham, S. G., Langenbach, R., Loftin, C. D., Tiano, H. F., Vouloumanos, N., Jennette, J. C., Mahler, J. F., Kluckman, K. D., Ledford, A., Lee, C. A., and et al. (1995) Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. *Cell* 83, 473-482
228. Morita, I. (2002) Distinct functions of COX-1 and COX-2. *Prostaglandins Other Lipid Mediat* 68-69, 165-175
229. Morita, I., Schindler, M., Regier, 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
230. Morris, H. R., Taylor, G. W., Piper, P. J., and Tippins, J. R. (1980) Structure of slow-reacting substance of anaphylaxis from guinea-pig lung. *Nature* 285, 104-106
231. Morteau, O., Morham, S. G., Sellon, R., Dieleman, L. A., Langenbach, R., Smithies, O., and Sartor, R. B. (2000) Impaired mucosal defense to acute colonic injury in mice lacking cyclooxygenase-1 or cyclooxygenase-2. *J Clin Invest* 105, 469-478
232. Mosialou, E., Ekstrom, G., Adang, A. E., and Morgenstern, R. (1993) Evidence that rat liver microsomal glutathione transferase is responsible for glutathione-dependent protection against lipid peroxidation. *Biochem Pharmacol* 45, 1645-1651
233. Mosialou, E., Piemonte, F., Andersson, C., Vos, R. M., van Bladeren, P. J., and Morgenstern, R. (1995) Microsomal glutathione transferase: lipid-derived substrates and lipid dependence. *Arch Biochem Biophys* 320, 210-216
234. Munroe, D. G., and Lau, C. Y. (1995) Turning down the heat: new routes to inhibition of inflammatory signaling by prostaglandin H2 synthases. *Chem Biol* 2, 343-350
235. 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
236. Murakami, M., Kambe, T., Shimbara, S., Yamamoto, S., Kuwata, H., and Kudo, I. (1999) Functional association of type IIA secretory phospholipase A(2) with the glycosylphosphatidylinositol-anchored heparan sulfate proteoglycan in the cyclooxygenase-2-mediated delayed prostanoid-biosynthetic pathway. *J Biol Chem* 274, 29927-29936
237. Murakami, M., Koduri, R. S., Enomoto, A., Shimbara, S., Seki, M., Yoshihara, K., Singer, A., Valentin, E., Ghomashchi, F., Lambeau, G., Gelb, M. H., and Kudo, I. (2001) Distinct arachidonate-releasing functions of mammalian secreted phospholipase A2s in human embryonic kidney 293 and rat mastocytoma RBL-2H3 cells through heparan sulfate shuttling and external plasma membrane mechanisms. *J Biol Chem* 276, 10083-10096
238. Murakami, M., Kudo, I., and Inoue, K. (1993) Molecular nature of phospholipases A2 involved in prostaglandin I2 synthesis in human umbilical vein endothelial cells. Possible participation of cytosolic and extracellular type II phospholipases A2. *J Biol Chem* 268, 839-844
239. 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
240. Murakami, M., Matsumoto, R., Austen, K. F., and Arm, J. P. (1994) Prostaglandin endoperoxide synthase-1 and -2 couple to different transmembrane stimuli to generate prostaglandin D2 in mouse bone marrow-derived mast cells. *J Biol Chem* 269, 22269-22275
241. Murakami, M., Nakashima, K., Kamei, D., 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*
242. Murakami, M., Nakatani, Y., and Kudo, I. (1996) Type II secretory phospholipase A2 associated with cell surfaces via C-terminal heparin-binding lysine residues augments stimulus-initiated delayed prostaglandin generation. *J Biol Chem* 271, 30041-30051

243. Murakami, M., Nakatani, Y., Tanioka, T., and Kudo, I. (2002) Prostaglandin E synthase. *Prostaglandins Other Lipid Mediat* 68-69, 383-399
244. Murakami, M., Naraba, H., Tanioka, T., Semmyo, N., Nakatani, Y., Kojima, F., Ikeda, T., Fueki, M., Ueno, A., Oh, 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
245. Murakami, M., Shimbara, S., Kambe, T., Kuwata, H., Winstead, M. V., Tischfield, J. A., and Kudo, I. (1998) The functions of five distinct mammalian phospholipase A2S in regulating arachidonic acid release. Type IIa and type V secretory phospholipase A2S are functionally redundant and act in concert with cytosolic phospholipase A2. *J Biol Chem* 273, 14411-14423
246. Murata, T., Ushikubi, F., Matsuoka, T., Hirata, M., Yamasaki, A., Sugimoto, Y., Ichikawa, A., Aze, Y., Tanaka, T., Yoshida, N., Ueno, A., Oh-ishi, S., and Narumiya, S. (1997) Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. *Nature* 388, 678-682
247. Mutoh, M., Watanabe, K., Kitamura, T., Shoji, Y., Takahashi, M., Kawamori, T., Tani, K., Kobayashi, M., Maruyama, T., Kobayashi, K., Ohuchida, S., Sugimoto, Y., Narumiya, S., Sugimura, T., and Wakabayashi, K. (2002) Involvement of prostaglandin E receptor subtype EP(4) in colon carcinogenesis. *Cancer Res* 62, 28-32
248. Myers, L. K., Kang, A. H., Postlethwaite, A. E., Rosloniec, E. F., Morham, S. G., Shlopov, B. V., Goorha, S., and Ballou, L. R. (2000) The genetic ablation of cyclooxygenase 2 prevents the development of autoimmune arthritis. *Arthritis Rheum* 43, 2687-2693
249. Nakano, T., Ohara, O., Teraoka, H., and Arita, H. (1990) Glucocorticoids suppress group II phospholipase A2 production by blocking mRNA synthesis and post-transcriptional expression. *J Biol Chem* 265, 12745-12748
250. Nakashima, K., Ueno, N., Kamei, D., Tanioka, T., Nakatani, Y., Murakami, M., and Kudo, I. (2003) Coupling between cyclooxygenases and prostaglandin F(2alpha) synthase. Detection of an inducible, glutathione-activated, membrane-bound prostaglandin F(2alpha)-synthetic activity. *Biochim Biophys Acta* 1633, 96-105
251. Nalefski, E. A., Sultzman, L. A., Martin, D. M., Kriz, R. W., Towler, P. S., Knopf, J. L., and Clark, J. D. (1994) Delineation of two functionally distinct domains of cytosolic phospholipase A2, a regulatory Ca(2+)-dependent lipid-binding domain and a Ca(2+)-independent catalytic domain. *J Biol Chem* 269, 18239-18249
252. 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
253. 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
254. Narko, K., Ritvos, O., and Ristimaki, A. (1997) Induction of cyclooxygenase-2 and prostaglandin F2alpha receptor expression by interleukin-1beta in cultured human granulosa-luteal cells. *Endocrinology* 138, 3638-3644
255. Narumiya, S., and FitzGerald, G. A. (2001) Genetic and pharmacological analysis of prostanoid receptor function. *J Clin Invest* 108, 25-30
256. Negishi, M., Sugimoto, Y., and Ichikawa, A. (1995) Molecular mechanisms of diverse actions of prostanoid receptors. *Biochim Biophys Acta* 1259, 109-119
257. Newton, R., Hart, L. A., Stevens, D. A., Bergmann, M., Donnelly, L. E., Adcock, I. M., and Barnes, P. J. (1998) Effect of dexamethasone on interleukin-1beta-(IL-1beta)-induced nuclear factor-kappaB (NF-kappaB) and kappaB-dependent transcription in epithelial cells. *Eur J Biochem* 254, 81-89
258. Newton, R., Seybold, J., Kuitert, L. M., Bergmann, M., and Barnes, P. J. (1998) Repression of cyclooxygenase-2 and prostaglandin E2 release by dexamethasone occurs by transcriptional and post-transcriptional mechanisms involving loss of polyadenylated mRNA. *J Biol Chem* 273, 32312-32321
259. Nguyen, M., Camenisch, T., Snouwaert, J. N., Hicks, E., Coffman, T. M., Anderson, P. A., Malouf, N. N., and Koller, B. H. (1997) The prostaglandin receptor EP4 triggers remodelling of the cardiovascular system at birth. *Nature* 390, 78-81

260. 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
261. Nicholson, D. W., Ali, A., Vaillancourt, J. P., Calaycay, J. R., Mumford, R. A., Zamboni, R. J., and Ford-Hutchinson, A. W. (1993) Purification to homogeneity and the N-terminal sequence of human leukotriene C4 synthase: a homodimeric glutathione S-transferase composed of 18-kDa subunits. *Proc Natl Acad Sci U S A* 90, 2015-2019
262. Nugteren, D. H., and Hazelhof, E. (1973) Isolation and properties of intermediates in prostaglandin biosynthesis. *Biochim Biophys Acta* 326, 448-461
263. Nugteren, D. H., and van Dorp, D. A. (1965) The participation of molecular oxygen in the biosynthesis of prostaglandins. *Biochim Biophys Acta* 98, 654-656
264. Ogino, N., Miyamoto, T., Yamamoto, S., and Hayaishi, O. (1977) Prostaglandin endoperoxide E isomerase from bovine vesicular gland microsomes, a glutathione-requiring enzyme. *J Biol Chem* 252, 890-895
265. Ogorochi, T., Ujihara, M., and Narumiya, S. (1987) Purification and properties of prostaglandin H-E isomerase from the cytosol of human brain: identification as anionic forms of glutathione S-transferase. *J Neurochem* 48, 900-909
266. Ohishi, S., Ueno, A., Matsumoto, H., Murata, T., Ushikubi, F., and Narumiya, S. (1999) Evidence for involvement of prostaglandin I2 as a major nociceptive mediator in acetic acid-induced writhing reaction: a study using IP-receptor disrupted mice. *Adv Exp Med Biol* 469, 265-268
267. Ohki, S., Ogino, N., Yamamoto, S., and Hayaishi, O. (1979) Prostaglandin hydroperoxidase, an integral part of prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes. *J Biol Chem* 254, 829-836
268. Okahara, K., Sun, B., and Kambayashi, J. (1998) Upregulation of prostacyclin synthesis-related gene expression by shear stress in vascular endothelial cells. *Arterioscler Thromb Vasc Biol* 18, 1922-1926
269. Oliw, E. H., Stark, K., and Bylund, J. (2001) Oxidation of prostaglandin H(2) and prostaglandin H(2) analogues by human cytochromes P450: analysis of omega-side chain hydroxy metabolites and four steroisomers of 5-hydroxyprostaglandin I(1) by mass spectrometry. *Biochem Pharmacol* 62, 407-415
270. 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 Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* 87, 803-809
271. Ouellet, M., Falgueyret, J. P., Hien Ear, P., Pen, A., Mancini, J. A., Riendeau, D., and Percival, M. D. (2002) Purification and characterization of recombinant microsomal prostaglandin E synthase-1. *Protein Expr Purif* 26, 489-495
272. Pasinetti, G. M., and Aisen, P. S. (1998) Cyclooxygenase-2 expression is increased in frontal cortex of Alzheimer's disease brain. *Neuroscience* 87, 319-324
273. Patrignani, P., Panara, M. R., Greco, A., Fusco, O., Natoli, C., Iacobelli, S., Cipollone, F., Ganci, A., Creminon, C., Maclouf, J., and et al. (1994) Biochemical and pharmacological characterization of the cyclooxygenase activity of human blood prostaglandin endoperoxide synthases. *J Pharmacol Exp Ther* 271, 1705-1712
274. Patrono, C. (1994) Aspirin as an antiplatelet drug. *N Engl J Med* 330, 1287-1294
275. Peilot, H., Rosengren, B., Bondjers, G., and Hurt-Camejo, E. (2000) Interferon-gamma induces secretory group IIA phospholipase A2 in human arterial smooth muscle cells. Involvement of cell differentiation, STAT-3 activation, and modulation by other cytokines. *J Biol Chem* 275, 22895-22904
276. Pickard, R. T., Strifler, B. A., Kramer, R. M., and Sharp, J. D. (1999) Molecular cloning of two new human paralogs of 85-kDa cytosolic phospholipase A2. *J Biol Chem* 274, 8823-8831
277. Picot, D., Loll, P. J., and Garavito, R. M. (1994) The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. *Nature* 367, 243-249
278. Pierce, K. L., Bailey, T. J., Hoyer, P. B., Gil, D. W., Woodward, D. F., and Regan, J. W. (1997) Cloning of a carboxyl-terminal isoform of the prostanoid FP receptor. *J Biol Chem* 272, 883-887
279. Polyak, K., Xia, Y., Zweier, J. L., Kinzler, K. W., and Vogelstein, B. (1997) A model for p53-induced apoptosis. *Nature* 389, 300-305
280. Portanova, J. P., Zhang, Y., Anderson, G. D., Hauser, S. D., Masferrer, J. L., Seibert, K., Gregory, S. A., and Isakson, P. C. (1996) Selective neutralization of prostaglandin E2 blocks inflammation, hyperalgesia, and interleukin 6 production in vivo. *J Exp Med* 184, 883-891

281. Pratico, D., Tillmann, C., Zhang, Z. B., Li, H., and FitzGerald, G. A. (2001) Acceleration of atherogenesis by COX-1-dependent prostanoid formation in low density lipoprotein receptor knockout mice. *Proc Natl Acad Sci U S A* 98, 3358-3363
282. Quraishi, O., Mancini, J. A., and Riendeau, D. (2002) Inhibition of inducible prostaglandin E(2) synthase by 15-deoxy-Delta(12,14)-prostaglandin J(2) and polyunsaturated fatty acids. *Biochem Pharmacol* 63, 1183-1189
283. Rao, C. V., Rivenson, A., Simi, B., Zang, E., Kelloff, G., Steele, V., and Reddy, B. S. (1995) Chemoprevention of colon carcinogenesis by sulindac, a nonsteroidal anti-inflammatory agent. *Cancer Res* 55, 1464-1472
284. Raud, J., Dahlen, S. E., Sydbom, A., Lindbom, L., and Hedqvist, P. (1988) Enhancement of acute allergic inflammation by indomethacin is reversed by prostaglandin E2: apparent correlation with in vivo modulation of mediator release. *Proc Natl Acad Sci U S A* 85, 2315-2319
285. Raychowdhury, M. K., Yukawa, M., Collins, L. J., McGrail, S. H., Kent, K. C., and Ware, J. A. (1995) Alternative splicing produces a divergent cytoplasmic tail in the human endothelial thromboxane A2 receptor. *J Biol Chem* 270, 7011
286. Reddy, B. S., Hirose, Y., Lubet, R., Steele, V., Kelloff, G., Paulson, S., Seibert, K., and Rao, C. V. (2000) Chemoprevention of colon cancer by specific cyclooxygenase-2 inhibitor, celecoxib, administered during different stages of carcinogenesis. *Cancer Res* 60, 293-297
287. Reddy, S. T., Winstead, M. V., Tischfield, J. A., and Herschman, H. R. (1997) Analysis of the secretory phospholipase A2 that mediates prostaglandin production in mast cells. *J Biol Chem* 272, 13591-13596
288. Reese, J., Zhao, X., Ma, W. G., Brown, N., Maziasz, T. J., and Dey, S. K. (2001) Comparative analysis of pharmacologic and/or genetic disruption of cyclooxygenase-1 and cyclooxygenase-2 function in female reproduction in mice. *Endocrinology* 142, 3198-3206
289. Regan, J. W., Bailey, T. J., Donello, J. E., Pierce, K. L., Pepperl, D. J., Zhang, D., Kedzie, K. M., Fairbairn, C. E., Bogardus, A. M., Woodward, D. F., and et al. (1994) Molecular cloning and expression of human EP3 receptors: evidence of three variants with differing carboxyl termini. *Br J Pharmacol* 112, 377-385
290. Regan, J. W., Bailey, T. J., Pepperl, D. J., Pierce, K. L., Bogardus, A. M., Donello, J. E., Fairbairn, C. E., Kedzie, K. M., Woodward, D. F., and Gil, D. W. (1994) Cloning of a novel human prostaglandin receptor with characteristics of the pharmacologically defined EP2 subtype. *Mol Pharmacol* 46, 213-220
291. Reynolds, L. J., Hughes, L. L., Louis, A. I., Kramer, R. M., and Dennis, E. A. (1993) Metal ion and salt effects on the phospholipase A2, lysophospholipase, and transacylase activities of human cytosolic phospholipase A2. *Biochim Biophys Acta* 1167, 272-280
292. Ricote, M., Li, A. C., Willson, T. M., Kelly, C. J., and Glass, C. K. (1998) The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 391, 79-82
293. Ridker, P. M., Hennekens, C. H., Buring, J. E., and Rifai, N. (2000) C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 342, 836-843
294. Rittenhouse-Simmons, S. (1979) Production of diglyceride from phosphatidylinositol in activated human platelets. *J Clin Invest* 63, 580-587
295. Robertson, R. P. (1998) Dominance of cyclooxygenase-2 in the regulation of pancreatic islet prostaglandin synthesis. *Diabetes* 47, 1379-1383
296. Robinson, D. R., Smith, H., McGuire, M. B., and Levine, L. (1975) Prostaglandin synthesis by rheumatoid synovium and its stimulation by colchicine. *Prostaglandins* 10, 67-85
297. Rocca, B., Maggiano, N., Habib, A., Petrucci, G., Gessi, M., Fattorossi, A., Lauriola, L., Landolfi, R., and Ranelletti, F. O. (2002) Distinct expression of cyclooxygenase-1 and -2 in the human thymus. *Eur J Immunol* 32, 1482-1492
298. Rock, F. L., Hardiman, G., Timans, J. C., Kastelein, R. A., and Bazan, J. F. (1998) A family of human receptors structurally related to Drosophila Toll. *Proc Natl Acad Sci U S A* 95, 588-593
299. Rolli, M., Kotlyarov, A., Sakamoto, K. M., Gaestel, M., and Neininger, A. (1999) Stress-induced stimulation of early growth response gene-1 by p38/stress-activated protein kinase 2 is mediated by a cAMP-responsive promoter element in a MAPKAP kinase 2-independent manner. *J Biol Chem* 274, 19559-19564

300. Roshak, A., Sathe, G., and Marshall, L. A. (1994) Suppression of monocyte 85-kDa phospholipase A2 by antisense and effects on endotoxin-induced prostaglandin biosynthesis. *J Biol Chem* 269, 25999-26005
301. Rowe, J. D., Nieves, E., and Listowsky, I. (1997) Subunit diversity and tissue distribution of human glutathione S-transferases: interpretations based on electrospray ionization-MS and peptide sequence-specific antisera. *Biochem J* 325 ( Pt 2), 481-486
302. Ruan, K. H., Wang, L. H., Wu, K. K., and Kulmacz, R. J. (1993) Amino-terminal topology of thromboxane synthase in the endoplasmic reticulum. *J Biol Chem* 268, 19483-19490
303. Sakuma, Y., Tanaka, K., Suda, M., Komatsu, Y., Yasoda, A., Miura, M., Ozasa, A., Narumiya, S., Sugimoto, Y., Ichikawa, A., Ushikubi, F., and Nakao, K. (2000) Impaired bone resorption by lipopolysaccharide in vivo in mice deficient in the prostaglandin E receptor EP4 subtype. *Infect Immun* 68, 6819-6825
304. Sakuma, Y., Tanaka, K., Suda, M., Yasoda, A., Natsui, K., Tanaka, I., Ushikubi, F., Narumiya, S., Segi, E., Sugimoto, Y., Ichikawa, A., and Nakao, K. (2000) Crucial involvement of the EP4 subtype of prostaglandin E receptor in osteoclast formation by proinflammatory cytokines and lipopolysaccharide. *J Bone Miner Res* 15, 218-227
305. Salinas, A. E., and Wong, M. G. (1999) Glutathione S-transferases--a review. *Curr Med Chem* 6, 279-309
306. Samad, T. A., Moore, K. A., Sapirstein, A., Billet, S., Allchorne, A., Poole, S., Bonventre, J. V., and Woolf, C. J. (2001) Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. *Nature* 410, 471-475
307. Samuelsson, B. (1965) On the incorporation of oxygen in the conversion of 8, 11, 14-eicosatrienoic acid to prostaglandin $E_1$. *J Am Chem Soc* 87, 3011-3013
308. Samuelsson, B., Granstrom, E., Green, K., Hamberg, M., and Hammarstrom, S. (1975) Prostaglandins. *Annu Rev Biochem* 44, 669-695
309. Samuelsson, B., and Hamberg, M. (1974) in *Proceedings of an International Symposium on Prostaglandin Synthetase Inhibitors* (Robinson, H. J., and Vane, J. R., eds), pp. 107-119, Raven Press, New York
310. Saper, C. B., and Breder, C. D. (1994) The neurologic basis of fever. *N Engl J Med* 330, 1880-1886
311. Satoh, K., Nagano, Y., Shimomura, C., Suzuki, N., Saeki, Y., and Yokota, H. (2000) Expression of prostaglandin E synthase mRNA is induced in beta-amyloid treated rat astrocytes. *Neurosci Lett* 283, 221-223
312. Sawada, H., Murakami, M., Enomoto, A., Shimbara, S., and Kudo, I. (1999) Regulation of type V phospholipase A2 expression and function by proinflammatory stimuli. *Eur J Biochem* 263, 826-835
313. Schalkwijk, C. G., Vervoordeldonk, M., Pfeilschifter, J., and van den Bosch, H. (1993) Interleukin-1 beta-induced cytosolic phospholipase A2 activity and protein synthesis is blocked by dexamethasone in rat mesangial cells. *FEBS Lett* 333, 339-343
314. Scheinman, R. I., Cogswell, P. C., Lofquist, A. K., and Baldwin, A. S., Jr. (1995) Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. *Science* 270, 283-286
315. Schieber, A., Frank, R. W., and Ghisla, S. (1992) Purification and properties of prostaglandin 9-ketoreductase from pig and human kidney. Identity with human carbonyl reductase. *Eur J Biochem* 206, 491-502
316. Schievella, A. R., Regier, M. K., Smith, W. L., and Lin, L. L. (1995) Calcium-mediated translocation of cytosolic phospholipase A2 to the nuclear envelope and endoplasmic reticulum. *J Biol Chem* 270, 30749-30754
317. Schmidt-Krey, I., Mitsuoka, K., Hirai, T., Murata, K., Cheng, Y., Fujiyoshi, Y., Morgenstern, R., and Hebert, H. (2000) The three-dimensional map of microsomal glutathione transferase 1 at 6 A resolution. *Embo J* 19, 6311-6316
318. Schmidt-Krey, I., Murata, K., Hirai, T., Mitsuoka, K., Cheng, Y., Morgenstern, R., Fujiyoshi, Y., and Hebert, H. (1999) The projection structure of the membrane protein microsomal glutathione transferase at 3 A resolution as determined from two-dimensional hexagonal crystals. *J Mol Biol* 288, 243-253
319. Schuster, V. L. (2002) Prostaglandin transport. *Prostaglandins Other Lipid Mediat* 68-69, 633-647

320. Scoggan, K. A., Jakobsson, P. J., and Ford-Hutchinson, A. W. (1997) Production of leukotriene C4 in different human tissues is attributable to distinct membrane bound biosynthetic enzymes. *J Biol Chem* 272, 10182-10187
321. 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. (1998) Patent ductus arteriosus and neonatal death in prostaglandin receptor EP4-deficient mice. *Biochem Biophys Res Commun* 246, 7-12
322. Seilhamer, J. J., Pruzanski, W., Vadas, P., Plant, S., Miller, J. A., Kloss, J., and Johnson, L. K. (1989) Cloning and recombinant expression of phospholipase A2 present in rheumatoid arthritic synovial fluid. *J Biol Chem* 264, 5335-5338
323. Serhan, C. N., Hong, S., Gronert, K., Colgan, S. P., Devchand, P. R., Mirick, G., and Moussignac, R. L. (2002) Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J Exp Med* 196, 1025-1037
324. Shankavaram, U. T., Lai, W. C., Netzel-Arnett, S., Mangan, P. R., Ardans, J. A., Caterina, N., Stetler-Stevenson, W. G., Birkedal-Hansen, H., and Wahl, L. M. (2001) Monocyte membrane type 1-matrix metalloproteinase. Prostaglandin-dependent regulation and role in metalloproteinase-2 activation. *J Biol Chem* 276, 19027-19032
325. Sheller, J. R., Mitchell, D., Meyrick, B., Oates, J., and Breyer, R. (2000) EP(2) receptor mediates bronchodilation by PGE(2) in mice. *J Appl Physiol* 88, 2214-2218
326. Sheng, H., Shao, J., Kirkland, S. C., Isakson, P., Coffey, R. J., Morrow, J., Beauchamp, R. D., and DuBois, R. N. (1997) Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. *J Clin Invest* 99, 2254-2259
327. Sheng, H., Shao, J., Morrow, J. D., Beauchamp, R. D., and DuBois, R. N. (1998) Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. *Cancer Res* 58, 362-366
328. Sheng, H., Shao, J., Washington, M. K., and DuBois, R. N. (2001) Prostaglandin E2 increases growth and motility of colorectal carcinoma cells. *J Biol Chem* 276, 18075-18081
329. Shibata, T., Kondo, M., Osawa, T., Shibata, N., Kobayashi, M., and Uchida, K. (2002) 15-deoxy-delta 12,14-prostaglandin J2. A prostaglandin D2 metabolite generated during inflammatory processes. *J Biol Chem* 277, 10459-10466
330. Shinohara, H., Balboa, M. A., Johnson, C. A., Balsinde, J., and Dennis, E. A. (1999) Regulation of delayed prostaglandin production in activated P388D1 macrophages by group IV cytosolic and group V secretory phospholipase A2s. *J Biol Chem* 274, 12263-12268
331. Siegle, I., Klein, T., Backman, J. T., Saal, J. G., Nusing, R. M., and Fritz, P. (1998) Expression of cyclooxygenase 1 and cyclooxygenase 2 in human synovial tissue: differential elevation of cyclooxygenase 2 in inflammatory joint diseases. *Arthritis Rheum* 41, 122-129
332. Silman, A. J., and Pearson, J. E. (2002) Epidemiology and genetics of rheumatoid arthritis. *Arthritis Res* 4 Suppl 3, S265-272
333. Sipos, L., and von Heijne, G. (1993) Predicting the topology of eukaryotic membrane proteins. *Eur J Biochem* 213, 1333-1340
334. Smith, W. L. (1989) The eicosanoids and their biochemical mechanisms of action. *Biochem J* 259, 315-324
335. Smith, W. L. (1992) Prostanoid biosynthesis and mechanisms of action. *Am J Physiol* 263, F181-191
336. Smith, W. L., and Dewitt, D. L. (1996) Prostaglandin endoperoxide H synthases-1 and -2. *Adv Immunol* 62, 167-215
337. Smith, W. L., DeWitt, D. L., and Garavito, R. M. (2000) Cyclooxygenases: structural, cellular, and molecular biology. *Annu Rev Biochem* 69, 145-182
338. Smith, W. L., Eling, T. E., Kulmacz, R. J., Marnett, L. J., and Tsai, A. (1992) Tyrosyl radicals and their role in hydroperoxide-dependent activation and inactivation of prostaglandin endoperoxide synthase. *Biochemistry* 31, 3-7
339. Smith, W. L., Garavito, R. M., and DeWitt, D. L. (1996) Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. *J Biol Chem* 271, 33157-33160
340. Smith, W. L., and Langenbach, R. (2001) Why there are two cyclooxygenase isozymes. *J Clin Invest* 107, 1491-1495
341. Smith, W. L., and Marnett, L. J. (1991) Prostaglandin endoperoxide synthase: structure and catalysis. *Biochim Biophys Acta* 1083, 1-17

342. Smith, W. L., and Song, I. (2002) The enzymology of prostaglandin endoperoxide H synthases-1 and -2. *Prostaglandins Other Lipid Mediat* 68-69, 115-128
343. Smolen, J. S., and Steiner, G. (2003) Therapeutic strategies for rheumatoid arthritis. *Nat Rev Drug Discov* 2, 473-488
344. Smythies, J. (1996) On the functional of neuromelanin. *Proc R Soc Lond B Biol Sci* 263, 487-489
345. Soderstrom, M., Hammarstrom, S., and Mannervik, B. (1988) Leukotriene C synthase in mouse mastocytoma cells. An enzyme distinct from cytosolic and microsomal glutathione transferases. *Biochem J* 250, 713-718
346. Soler, M., Camacho, M., Escudero, J. R., Iniguez, M. A., and Vila, L. (2000) Human vascular smooth muscle cells but not endothelial cells express prostaglandin E synthase. *Circ Res* 87, 504-507
347. Song, C., Chang, X. J., Bean, K. M., Proia, M. S., Knopf, J. L., and Kriz, R. W. (1999) Molecular characterization of cytosolic phospholipase A2-beta. *J Biol Chem* 274, 17063-17067
348. 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 Apc(Delta 716) knockout mice. *Nat Med* 7, 1048-1051
349. Spencer, A. G., Thuresson, E., Otto, J. C., Song, I., Smith, T., DeWitt, D. L., Garavito, R. M., and Smith, W. L. (1999) The membrane binding domains of prostaglandin endoperoxide H synthases 1 and 2. Peptide mapping and mutational analysis. *J Biol Chem* 274, 32936-32942
350. Steinbach, G., Lynch, P. M., Phillips, R. K., Wallace, M. H., Hawk, E., Gordon, G. B., Wakabayashi, N., Saunders, B., Shen, Y., Fujimura, T., Su, L. K., and Levin, B. (2000) The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N Engl J Med* 342, 1946-1952
351. Steiner, G., and Smolen, J. (2002) Autoantibodies in rheumatoid arthritis and their clinical significance. *Arthritis Res* 4 Suppl 2, S1-5
352. Stichtenoth, D. O., and Frolich, J. C. (2000) COX-2 and the kidneys. *Curr Pharm Des* 6, 1737-1753
353. Stichtenoth, D. O., Thoren, S., Bian, H., Peters-Golden, M., Jakobsson, P. J., and Crofford, L. J. (2001) Microsomal prostaglandin E synthase is regulated by proinflammatory cytokines and glucocorticoids in primary rheumatoid synovial cells. *J Immunol* 167, 469-474
354. Su, B., and Karin, M. (1996) Mitogen-activated protein kinase cascades and regulation of gene expression. *Curr Opin Immunol* 8, 402-411
355. Subbaramaiah, K., Telang, N., Ramonetti, J. T., Araki, R., DeVito, B., Weksler, B. B., and Dannenberg, A. J. (1996) Transcription of cyclooxygenase-2 is enhanced in transformed mammary epithelial cells. *Cancer Res* 56, 4424-4429
356. Suga, H., Murakami, M., Kudo, I., and Inoue, K. (1993) Participation in cellular prostaglandin synthesis of type-II phospholipase A2 secreted and anchored on cell-surface heparan sulfate proteoglycan. *Eur J Biochem* 218, 807-813
357. Sugimoto, Y., Yamasaki, A., Segi, E., Tsuboi, K., Aze, Y., Nishimura, T., Oida, H., Yoshida, N., Tanaka, T., Katsuyama, M., Hasumoto, K., Murata, T., Hirata, M., Ushikubi, F., Negishi, M., Ichikawa, A., and Narumiya, S. (1997) Failure of parturition in mice lacking the prostaglandin F receptor. *Science* 277, 681-683
358. Tada, K., Murakami, M., Kambe, T., and Kudo, I. (1998) Induction of cyclooxygenase-2 by secretory phospholipases A2 in nerve growth factor-stimulated rat serosal mast cells is facilitated by interaction with fibroblasts and mediated by a mechanism independent of their enzymatic functions. *J Immunol* 161, 5008-5015
359. Tai, H. H., Ensor, C. M., Tong, M., Zhou, H., and Yan, F. (2002) Prostaglandin catabolizing enzymes. *Prostaglandins Other Lipid Mediat* 68-69, 483-493
360. Takaku, K., Sonoshita, M., Sasaki, N., Uozumi, N., Doi, Y., Shimizu, T., and Taketo, M. M. (2000) 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, 34013-34016
361. Takeuchi, K., Ukawa, H., Kato, S., Furukawa, O., Araki, H., Sugimoto, Y., Ichikawa, A., Ushikubi, F., and Narumiya, S. (1999) Impaired duodenal bicarbonate secretion and mucosal integrity in mice lacking prostaglandin E-receptor subtype EP(3). *Gastroenterology* 117, 1128-1135
362. Tanabe, T., and Tohnai, N. (2002) Cyclooxygenase isozymes and their gene structures and expression. *Prostaglandins Other Lipid Mediat* 68-69, 95-114

363. Tanaka, Y., Ward, S. L., and Smith, W. L. (1987) Immunochemical and kinetic evidence for two different prostaglandin H-prostaglandin E isomerases in sheep vesicular gland microsomes. *J Biol Chem* 262, 1374-1381
364. Tang, J., Kriz, R. W., Wolfman, N., Shaffer, M., Seehra, J., and Jones, S. S. (1997) A novel cytosolic calcium-independent phospholipase A2 contains eight ankyrin motifs. *J Biol Chem* 272, 8567-8575
365. 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
366. Tanioka, T., Nakatani, Y., Kobayashi, T., Tsujimoto, M., Oh-ishi, S., Murakami, M., and Kudo, I. (2003) Regulation of cytosolic prostaglandin E(2) synthase by 90-kDa heat shock protein. *Biochem Biophys Res Commun* 303, 1018-1023
367. 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
368. Taylor, P. L., and Kelly, R. W. (1974) 19-Hydroxylated E prostaglandins as the major prostaglandins of human semen. *Nature* 250, 665-667
369. Thomas, D. W., Mannon, R. B., Mannon, P. J., Latour, A., Oliver, J. A., Hoffman, M., Smithies, O., Koller, B. H., and Coffman, T. M. (1998) Coagulation defects and altered hemodynamic responses in mice lacking receptors for thromboxane A2. *J Clin Invest* 102, 1994-2001
370. Thoren, S., and Jakobsson, P. J. (2000) Coordinate up- and down-regulation of glutathione-dependent prostaglandin E synthase and cyclooxygenase-2 in A549 cells. Inhibition by NS-398 and leukotriene C4. *Eur J Biochem* 267, 6428-6434
371. Thoren, S., Weinander, R., Saha, S., Jegerschold, C., Pettersson, P. L., Samuelsson, B., Hebert, H., Hamberg, M., Morgenstern, R., and Jakobsson, P. J. (2003) Human microsomal prostaglandin E synthase-1: purification, functional characterization, and projection structure determination. *J Biol Chem* 278, 22199-22209
372. Thuresson, E. D., Lakkides, K. M., and Smith, W. L. (2000) Different catalytically competent arrangements of arachidonic acid within the cyclooxygenase active site of prostaglandin endoperoxide H synthase-1 lead to the formation of different oxygenated products. *J Biol Chem* 275, 8501-8507
373. Tilley, S. L., Audoly, L. P., Hicks, E. H., Kim, H. S., Flannery, P. J., Coffman, T. M., and Koller, B. H. (1999) Reproductive failure and reduced blood pressure in mice lacking the EP2 prostaglandin E2 receptor. *J Clin Invest* 103, 1539-1545
374. 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 U S A* 100, 9044-9049
375. Tronchere, H., Record, M., Terce, F., and Chap, H. (1994) Phosphatidylcholine cycle and regulation of phosphatidylcholine biosynthesis by enzyme translocation. *Biochim Biophys Acta* 1212, 137-151
376. Tsai, A., and Kulmacz, R. J. (2000) Tyrosyl radicals in prostaglandin H synthase-1 and -2. *Prostaglandins Other Lipid Mediat* 62, 231-254
377. Tsai, A., Kulmacz, R. J., and Palmer, G. (1995) Spectroscopic evidence for reaction of prostaglandin H synthase-1 tyrosyl radical with arachidonic acid. *J Biol Chem* 270, 10503-10508
378. Tsai, A., Palmer, G., Xiao, G., Swinney, D. C., and Kulmacz, R. J. (1998) Structural characterization of arachidonyl radicals formed by prostaglandin H synthase-2 and prostaglandin H synthase-1 reconstituted with mangano protoporphyrin IX. *J Biol Chem* 273, 3888-3894
379. Tsubouchi, Y., Kawahito, Y., Kohno, M., Inoue, K., Hla, T., and Sano, H. (2001) Feedback control of the arachidonate cascade in rheumatoid synoviocytes by 15-deoxy-Delta(12,14)-prostaglandin J2. *Biochem Biophys Res Commun* 283, 750-755
380. Tsujii, M., and DuBois, R. N. (1995) Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell* 83, 493-501
381. Uematsu, S., Matsumoto, M., Takeda, K., and Akira, S. (2002) Lipopolysaccharide-dependent prostaglandin E(2) production is regulated by the glutathione-dependent prostaglandin E(2) synthase gene induced by the Toll-like receptor 4/MyD88/NF-IL6 pathway. *J Immunol* 168, 5811-5816

382. Ueno, A., Matsumoto, H., Naraba, H., Ikeda, Y., Ushikubi, F., Matsuoka, T., Narumiya, S., Sugimoto, Y., Ichikawa, A., and Oh-ishi, S. (2001) Major roles of prostanoid receptors IP and EP(3) in endotoxin-induced enhancement of pain perception. *Biochem Pharmacol* 62, 157-160
383. Ueno, A., Naraba, H., Ikeda, Y., Ushikubi, F., Murata, T., Narumiya, S., and Oh-ishi, S. (2000) Intrinsic prostacyclin contributes to exudation induced by bradykinin or carrageenin: a study on the paw edema induced in IP-receptor-deficient mice. *Life Sci* 66, PL155-160
384. Ujihara, M., Tsuchida, S., Satoh, K., Sato, K., and Urade, Y. (1988) Biochemical and immunological demonstration of prostaglandin D2, E2, and F2 alpha formation from prostaglandin H2 by various rat glutathione S-transferase isozymes. *Arch Biochem Biophys* 264, 428-437
385. Uozumi, N., Kume, K., Nagase, T., Nakatani, N., Ishii, S., Tashiro, F., Komagata, Y., Maki, K., Ikuta, K., Ouchi, Y., Miyazaki, J., and Shimizu, T. (1997) Role of cytosolic phospholipase A2 in allergic response and parturition. *Nature* 390, 618-622
386. Urade, Y., and Hayaishi, O. (1999) Prostaglandin D2 and sleep regulation. *Biochim Biophys Acta* 1436, 606-615
387. Ushikubi, F., Segi, E., Sugimoto, Y., Murata, T., Matsuoka, T., Kobayashi, T., Hizaki, H., Tuboi, K., Katsuyama, M., Ichikawa, A., Tanaka, T., Yoshida, N., and Narumiya, S. (1998) Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. *Nature* 395, 281-284
388. van der Wal, A. C., Becker, A. E., van der Loos, C. M., and Das, P. K. (1994) Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation* 89, 36-44
389. van Dorp, D. A., Beerthuis, R. K., Nugteren, D. H., and Vonkeman, H. (1964) The biosynthesis of prostaglandins. *Biochim Biophys Acta*, 204-207
390. van Dorp, D. A., Beerthuis, R. K., Nugteren, D. H., and Vonkeman, H. (1964) Enzymatic conversion of all-*cis*-polyunsaturated fatty acids into prostaglandins. *Nature*, 839-841
391. Vane, J. R. (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol* 231, 232-235
392. Vane, J. R., and Botting, R. M. (1995) New insights into the mode of action of anti-inflammatory drugs. *Inflamm Res* 44, 1-10
393. Watanabe, K. (2002) Prostaglandin F synthase. *Prostaglandins Other Lipid Mediat* 68-69, 401-407
394. Watanabe, K., Kawamori, T., Nakatsugi, S., Ohta, T., Ohuchida, S., Yamamoto, H., Maruyama, T., Kondo, K., Narumiya, S., Sugimura, T., and Wakabayashi, K. (2000) Inhibitory effect of a prostaglandin E receptor subtype EP(1) selective antagonist, ONO-8713, on development of azoxymethane-induced aberrant crypt foci in mice. *Cancer Lett* 156, 57-61
395. Watanabe, K., Kawamori, T., Nakatsugi, S., Ohta, T., Ohuchida, S., Yamamoto, H., Maruyama, T., Kondo, K., Ushikubi, F., Narumiya, S., Sugimura, T., and Wakabayashi, K. (1999) Role of the prostaglandin E receptor subtype EP1 in colon carcinogenesis. *Cancer Res* 59, 5093-5096
396. Watanabe, K., Kurihara, K., and Suzuki, T. (1999) Purification and characterization of membrane-bound prostaglandin E synthase from bovine heart. *Biochim Biophys Acta* 1439, 406-414
397. Watanabe, K., Kurihara, K., Tokunaga, Y., and Hayaishi, O. (1997) Two types of microsomal prostaglandin E synthase: glutathione-dependent and -independent prostaglandin E synthases. *Biochem Biophys Res Commun* 235, 148-152
398. Watanabe, K., Ohkubo, H., Niwa, H., Tanikawa, N., Koda, N., Ito, S., and Ohmiya, Y. (2003) Essential 110Cys in active site of membrane-associated prostaglandin E synthase-2. *Biochem Biophys Res Commun* 306, 577-581
399. Weinander, R., Ekstrom, L., Andersson, C., Raza, H., Bergman, T., and Morgenstern, R. (1997) Structural and functional aspects of rat microsomal glutathione transferase. The roles of cysteine 49, arginine 107, lysine 67, histidine, and tyrosine residues. *J Biol Chem* 272, 8871-8877
400. Weinander, R., Ekstrom, L., Raza, H., Lindqvist, G., Sun, T. H., Hebert, H., Schmidt-Krey, I., and Morgenstern, R. (1996) in *Glutathione-S transferases: Structure, function and clinical inplications* (Vermeulen, N., Mulder, G., Nieuwenhuyse, H., Peters, W., and van Bladeren, P., eds), pp. 49-56, Taylor and Francis, London
401. Weinander, R., Mosialou, E., DeJong, J., Tu, C. P., Dypbukt, J., Bergman, T., Barnes, H. J., Hoog, J. O., and Morgenstern, R. (1995) Heterologous expression of rat liver microsomal glutathione transferase in simian COS cells and Escherichia coli. *Biochem J* 311 ( Pt 3), 861-866

402. Welsch, D. J., Creely, D. P., Hauser, S. D., Mathis, K. J., Krivi, G. G., and Isakson, P. C. (1994) Molecular cloning and expression of human leukotriene-C4 synthase. *Proc Natl Acad Sci U S A* 91, 9745-9749
403. Wermuth, B. (1981) Purification and properties of an NADPH-dependent carbonyl reductase from human brain. Relationship to prostaglandin 9-ketoreductase and xenobiotic ketone reductase. *J Biol Chem* 256, 1206-1213
404. Westbrook, C., and Jarabak, J. (1975) Purification and partial characterization of an NADH-linked delta13-15-ketoprostaglandin reductase from human placenta. *Biochem Biophys Res Commun* 66, 541-546
405. Whittaker, N., Bunting, S., Salmon, J., Moncada, S., Vane, J. R., Johnson, R. A., Morton, D. R., Kinner, J. H., Gorman, R. R., McGuire, J. C., and Sun, F. F. (1976) The chemical structure of prostaglandin X (prostacyclin). *Prostaglandins* 12, 915-928
406. Williams, C. S., Mann, M., and DuBois, R. N. (1999) The role of cyclooxygenases in inflammation, cancer, and development. *Oncogene* 18, 7908-7916
407. Williams, T. J., and Peck, M. J. (1977) Role of prostaglandin-mediated vasodilatation in inflammation. *Nature* 270, 530-532
408. Winstead, M. V., Balsinde, J., and Dennis, E. A. (2000) Calcium-independent phospholipase A(2): structure and function. *Biochim Biophys Acta* 1488, 28-39
409. Wintergalen, N., Thole, H. H., Galla, H. J., and Schlegel, W. (1995) Prostaglandin-E2 9-reductase from corpus luteum of pseudopregnant rabbit is a member of the aldo-keto reductase superfamily featuring 20 alpha-hydroxysteroid dehydrogenase activity. *Eur J Biochem* 234, 264-270
410. Winzen, R., Kracht, M., Ritter, B., Wilhelm, A., Chen, C. Y., Shyu, A. B., Muller, M., Gaestel, M., Resch, K., and Holtmann, H. (1999) The p38 MAP kinase pathway signals for cytokine-induced mRNA stabilization via MAP kinase-activated protein kinase 2 and an AU-rich region-targeted mechanism. *Embo J* 18, 4969-4980
411. von Euler, U. S. (1936) On the specific vaso-dilating and plain muscle stimulating substances from accessory genital glands in man and certain animals (prostaglandin and vesiglandin). *J Physiol* 88, 213-234
412. von Euler, U. S. (1935) Über die spezifische blutdrucksenkende Substanz des menschlichen Prostata- und Samenblasensekretes. *Klinische Wochenschrift* 14, 1182-1183
413. von Euler, U. S. (1934) Zur Kenntnis der pharmakologischen Wirkungen von Nativsekreten und Extrakten männlicher accessorischer Geslechtsdrüsen. *Arch Exp Path Pharmak* 175, 78-84
414. von Heijne, G. (1992) Membrane Protein Structure Prediction: Hydrophobicity Analysis and the 'Positive Inside' Rule. *J Mol Biol* 225, 487-494
415. Woolf, C. J., and Salter, M. W. (2000) Neuronal plasticity: increasing the gain in pain. *Science* 288, 1765-1769
416. Xiao, G., Tsai, A. L., Palmer, G., Boyar, W. C., Marshall, P. J., and Kulmacz, R. J. (1997) Analysis of hydroperoxide-induced tyrosyl radicals and lipoxygenase activity in aspirin-treated human prostaglandin H synthase-2. *Biochemistry* 36, 1836-1845
417. Xie, W. L., Chipman, J. G., Robertson, D. L., Erikson, R. L., and Simmons, D. L. (1991) Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc Natl Acad Sci U S A* 88, 2692-2696
418. 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
419. Yamamoto, Y., and Gaynor, R. B. (2001) Therapeutic potential of inhibition of the NF-kappaB pathway in the treatment of inflammation and cancer. *J Clin Invest* 107, 135-142
420. Yang, J., Xia, M., Goetzl, E. J., and An, S. (1994) Cloning and expression of the EP3-subtype of human receptors for prostaglandin E2. *Biochem Biophys Res Commun* 198, 999-1006
421. Yang, R. B., Mark, M. R., Gray, A., Huang, A., Xie, M. H., Zhang, M., Goddard, A., Wood, W. I., Gurney, A. L., and Godowski, P. J. (1998) Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling. *Nature* 395, 284-288
422. Yokoyama, C., Takai, T., and Tanabe, T. (1988) Primary structure of sheep prostaglandin endoperoxide synthase deduced from cDNA sequence. *FEBS Lett* 231, 347-351
423. Yokoyama, C., and Tanabe, T. (1989) Cloning of human gene encoding prostaglandin endoperoxide synthase and primary structure of the enzyme. *Biochem Biophys Res Commun* 165, 888-894

424. Yoshimatsu, K., Altorki, N. K., Golijanin, D., Zhang, F., Jakobsson, P. J., Dannenberg, A. J., and Subbaramaiah, K. (2001) Inducible prostaglandin E synthase is overexpressed in non-small cell lung cancer. *Clin Cancer Res* 7, 2669-2674
425. Yoshimatsu, K., Golijanin, D., Paty, P. B., Soslow, R. A., Jakobsson, P. J., DeLellis, R. A., Subbaramaiah, K., and Dannenberg, A. J. (2001) Inducible microsomal prostaglandin E synthase is overexpressed in colorectal adenomas and cancer. *Clin Cancer Res* 7, 3971-3976
426. Zhang, X., Morham, S. G., Langenbach, R., and Young, D. A. (1999) Malignant transformation and antineoplastic actions of nonsteroidal antiinflammatory drugs (NSAIDs) on cyclooxygenase-null embryo fibroblasts. *J Exp Med* 190, 451-459
427. Zhang, Y., Guan, Y., Schneider, A., Brandon, S., Breyer, R. M., and Breyer, M. D. (2000) Characterization of murine vasopressor and vasodepressor prostaglandin E(2) receptors. *Hypertension* 35, 1129-1134