Published in final edited form as:

Prostaglandins Other Lipid Mediat. 2009 April ; 88(3-4): 73–81. doi:10.1016/j.prostaglandins.2008.10.003.

Microsomal Prostaglandin E Synthase-2 Is Not Essential For In Vivo Prostaglandin E_2 Biosynthesis

Leigh A. Jania[1], **Subhashini Chandrasekharan**[1], **Michael G. Backlund**[1], **Nicholas A. Foley**[1], **John Snouwaert**[1], **I-Ming Wang**[2], **Patsy Clark**[2], **Laurent P. Audoly**[2], and **Beverly H. Koller**[1,*]

[1]Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599, USA.

[2]Merck Frosst Centre for Therapeutic Research, Kirkland, Quebec, H9H 3L1, Canada.

Abstract

Prostaglandin E_2 (PGE_2) plays an important role in the normal physiology of many organ systems. Increased levels of this lipid mediator are associated with many disease states, and it potently regulates inflammatory responses. Three enzymes capable of *in vitro* synthesis of PGE_2 from the cyclooxygenase metabolite PGH_2 have been described. Here, we examine the contribution of one of these enzymes to PGE_2 production, *mPges-2*, which encodes microsomal prostaglandin synthase-2 (mPGES-2), by generating mice homozygous for the null allele of this gene. Loss of *mPges-2* expression did not result in a measurable decrease in PGE_2 levels in any tissue or cell type examined from healthy mice. Taken together, analysis of the mPGES-2 deficient mouse lines does not substantiate the contention that mPGES-2 is a PGE_2 synthase.

Keywords

Microsomal Prostaglandin E_2 Synthase-2; Prostaglandin E_2

1. Introduction

Prostaglandins are lipid mediators produced by all cell types and regulate multiple biological processes. The E type prostaglandins are associated with the regulation of a wide range of normal physiological functions including reproduction, smooth muscle contraction/dilation, induction of pain, sodium homeostasis and immune modulation. In addition, prostaglandin E_2 (PGE_2) is implicated in many pathological conditions such as inflammation [1] and certain epithelial cancers [2–4]. Prostaglandin E_2 biosynthesis begins with the conversion of arachidonic acid (AA) released from phospholipids to prostaglandin endoperoxide (PGH_2) by one of two cyclooxygenase enzymes, COX-1 or COX-2. PGH_2 is next isomerized to PGE_2 by terminal PGE_2 synthase enzymes. To date this activity has been ascribed to three different genes: mPGES-1 and mPGES-2, reportedly encoding microsomal prostaglandin E_2 synthases, and cPGES/p23, that was reported to encode a cytolsolic prostaglandin E_2 synthase [5].

cPGES/p23 was purified from rat brain extracts, and similar to mPGES-1, the assignment of cPGES/p23 synthase activity and PGE_2 biosynthesis was attributed principally on *in vitro* studies [6]. More recently, the generation of cPGES/p23 deficient mice has raised questions regarding the direct contribution of this protein to PGE_2 biosynthesis [5]. For example,

Address correspondence to: Dr. Beverly H. Koller, Department of Genetics, University of North Carolina at Chapel Hill, 4341 MBRB CB 7264, Chapel Hill, NC, 27599-7264, Tel. 919 962-2153, Fax. 919 843-4682; treawouns@aol.com.

analysis of cPGES/p23 *in vivo* suggests that this protein has PGE_2 independent functions. Recent data demonstrates that cPGES/p23 is essential for glucocorticoid receptor function [5, 7].

mPGES-1 is a member of the membrane-associated proteins involved in eicosanoid and glutathione metabolism (MAPEG) family of proteins, and its structure and regulation have been well characterized [8]. mPGES-1 expression is induced by various pro-inflammatory stimuli in cell populations and organs involved in immune responsiveness such as macrophages [9, 10] and the spleen and lung [11–13]. However, constitutive expression is also detected in some organs such as the stomach, spleen and kidney [11]. The role of mPGES-1 in both basal and inducible PGE_2 biosynthesis by partnering with either COX-1 or COX-2 *in vivo* has been extensively documented by recent studies using mPGES-1 deficient mice [9–11, 14].

mPGES-2 was first isolated as a microsomal protein with PGE_2 synthase activity from the bovine heart, and cDNAs encoding human and monkey homologs were subsequently identified [15]. mPGES-2 is a 41 kDa protein, which is structurally distinct from mPGES-1 and, unlike mPGES-1, does not exclusively depend on glutathione (GSH) for its catalytic activity [16]. mPGES-2 has a conserved glutaredoxin and thioredoxin like domain, although it is not similar to the GSH-S transferase family. Human *mPGES-2* expression was detected in a number of cell lines and a wide spectrum of tissues. The highest mRNA levels are detected in the heart, various regions of the brain, skeletal muscle and liver [16]. Interestingly, high levels of *mPGES-2* were detected in organs such as the intestine, heart and brain, which normally express very low levels of *mPGES-1* [12], suggesting that mPGES-2 may be a major contributor to normal PGE_2 biosynthesis in these tissues. In contrast to *mPGES-1*, expression of *mPGES-2* was not inducible by LPS treatment in organs such as the brain, heart and liver [12], again supporting a more constitutive function for mPGES-2. Also, unlike *mPGES-1*, whose expression was markedly induced in pathological human tissues, such as the heart after acute myocardial infarction, cirrhotic livers, joints with rheumatoid arthritis [12] and gastric ulcers [17], *mPGES-2* appeared to be expressed equally in both the normal and pathological samples [12]. These findings together supported a more “housekeeping” type of role for mPGES-2 *in vivo*.

The role of mPGES-2 as a functional terminal PGE_2 synthase has been primarily characterized *in vitro* to date [12]. Over expression of the cDNAs encoding *mPGES-2* and either *COX-1* or *COX-2* in human embryonic kidney cells revealed that mPGES-2 can partner with either COX-1 or COX-2 for increased PGE_2 production upon exposure of cells to AA. Similarly, in cells stimulated by Ca^{++} ionophore A23187 or IL1-β to release endogenous stores of AA, mPGES-2 coupled both with exogenously expressed COX-1 and COX-2 for increased PGE_2 biosynthesis. In contrast to mPGES-1, which preferentially augmented PGE_2 synthesis when co-expressed with COX-2, mPGES-2 did not display a COX-1 or COX-2 coupling selectivity. Thus, mPGES-2 was shown to participate in both acute (Ca^{++} ionophore stimulated) and delayed (IL1-β stimulated) PGE_2 biosynthesis. Similar findings in lung epithelial and rat fibroblast cell lines co-expressing mPGES-2 with COX-1 and COX-2 following stimulation with either Ca^{++} ionophore or TNF-α supported the idea that mPGES-2 non-selectively couples with COX-1 and COX-2 in multiple cell types [12]. Since mPGES-2 is co-expressed with both COX-1 and COX-2 in many organs, the findings of Murakami *et al.* further strengthened the hypothesis that mPGES-2 is non-selective in its COX isoform coupling *in vivo*.

mPGES-2 was also independently isolated from an IFN-γ stimulated macrophage cDNA library as a novel protein, GATE Binding Factor 1 (GBF-1), that bound and activated transcription from the IFN-γ responsive element GATE [18]. Sequence analysis of *GBF-1*

revealed that it was identical to *mPGES-2*, and *Gbf-1* expression was similarly detected in most tissues with highest levels in the heart, liver, kidney and brain. Hu *et al.* demonstrated that *Gbf-1/ mPges-2* expression was induced by IFN-γ in multiple organs and that GBF-1 was localized to both the nuclear and cytoplasmic compartments in MEF and HeLa cell lines. Additionally, GBF-1 was shown to bind the GATE element and stimulate gene expression of reporter constructs that were driven specifically by GATE sequences. GBF-1 over-expression in primary MEFs, upon addition of IFN-γ, also increased expression of endogenous IRF-9, an IFN-γ regulated gene with a naturally occurring GATE element in its promoter [18, 19]. Thus, Hu *et al.* proposed that GBF-1/mPGES-2 is a novel transcription factor capable of modulating IFN-γ target gene expression and speculated that the glutaredoxin domain may allow this transcription factor to be regulated via redox status.

To better define and clarify the proposed roles of mPGES-2 *in vivo* as a terminal PGE_2 synthase and/or a novel IFN-γ stimulated transcription factor, we generated mice deficient in mPGES-2 by gene-targeting. Analysis of these mice fails to support the hypothesis that mPGES-2 contributes to production of PGE_2 *in vivo*, nor does it provide evidence for a role in regulating IFN-γ gene expression.

2. Materials and methods

2.1. Animals

All animal colonies were maintained according to standard guidelines as defined by the NIH Guide for the Care and Use of Laboratory Animals and the Institutional Animal Care and Use Committee of UNC-Chapel Hill. The generation of mPGES-1 and COX-1 deficient mouse lines was described previously [9, 20]. mPGES-1 deficient mice were generated on a DBA/1lacJ genetic background and COX-1 deficient mice were bred on a C57BL/6 genetic background.

2.2. Generation of mPGES-2 deficient mice

mPges-2 cDNA sequence from *Macaca fascicularis* was used to identify the corresponding mouse cDNA and genomic sequences by homology search. The targeting construct was designed to disrupt exon 4 based on the conserved protein sequence. A neomycin resistance marker in the plasmid pJNS2 replaced 8 base pairs of exon 4 in the endogenous *mPtges-2* gene. The targeting plasmid was introduced into 129/SvEv derived ES cells by electroporation. Colonies resistant to both neomycin and gancyclovir were screened for the desired recombination event by Southern blot analysis. A 432 base pair probe was prepared from DNA downstream of the targeted locus, used to identify cells that had undergone homologous recombination, and later to genotype the mice. A targeted ES cell clone was injected into 3.5-day old C57BL/6 blastocysts and implanted in pseudopregnant females. Chimeras were crossed directly to 129/SvEv (Tatonic) to maintain the mutation on the 129 background, and thus generate co-isogenic *mPges-2* mutant lines for studies. In addition, the mutation was moved to the C57BL/6 genetic background, by 12 consecutive crosses to this laboratory strain.

2.3. Northern Blot Analysis

Total RNA was extracted from frozen kidney, lung and proximal small intestine samples using RNABee (Tel-Test). The RNA was fractionated on a 1% agarose/formaldehyde gel and subsequently transferred to a nitrocellulose membrane (Millipore). A 733 bp cDNA probe for *mPges-2* was obtained from an EST (Invitrogen). The fragment encodes for a portion of the cDNA from the middle of exon 5 to the end of exon 7, including the stop codon and the 3' UTR. The probe was labeled with ^{32}P[dCTP] (Amersham) by random

priming (Roche). The blots were stripped and re-probed for *mPges-1* and *β-actin* expression using the corresponding mouse cDNA probes.

2.4. Quantitative real-time PCR

Total RNA was extracted from tissue samples as described above. RNA was purified with Qiaprep RNeasy Kit (Qiagen) and the quality of RNA was evaluated with an Agilent 2100 Bioanalyzer. 5–10 μg RNA was reverse transcribed using the High-Capacity Reverse Transcription Kit (Applied Biosystems) according to manufacturer's instructions. cDNA was amplified with Taqman PCR Universal Master Mix (Applied Biosystems) using the Applied Biosystems 7900 HT Fast Real-Time PCR System. All samples were run in quadruplicate. Genes of interest were normalized to a housekeeping gene (*Gapdh* or *Hprt1*). All Taqman primer/probe sets were purchased from Applied Biosystems. Data was analyzed using the comparative C_T method ($\Delta\Delta C_T$).

2.5. Microarray Analysis and Experimental Design

Hearts were harvested from 4 *mPges-2* −/− mice and their wild-type controls. Total RNA was extracted and the integrity was assessed as described above. RNA was synthesized and amplified using the Agilent Low RNA Input Fluorescent Linear Amplification Kit according to the manufacturer's specifications. The RNAs were labeled with either cyanine 3-CTP (Cy-3) or cyanine 5-CTP (Cy-5) (Perkin Elmer). 750 ng of Cy-3 labeled reference and Cy-5 labeled sample cRNA were hybridized to Agilent Whole Mouse Genome microarrays overnight and scanned using an Agilent DNA microarray scanner G2565B and Feature Extraction software. Aliquots containing equal amounts of RNA were combined from 4 wild-type heart samples to serve as the reference pool. All samples, both *mPges-2* +/+ and −/−, were compared to the same reference pool. The Rosetta Resolver Gene Expression Data Analysis system was used to normalize, transform and combine the fluor-reversed duplicates. A KO-specific gene set was established based on a statistically significant up- or down-regulation only in the knock-out mice. First, the Resolver error-model was used to assign measures of confidence to each of the two experimental conditions [21]. For each probe, an error-weighted measure of the relative mRNA abundance for each of two comparisons (WT vs. WT and KO vs. WT) was calculated. Evidence of differential expression was only considered if the combined absolute fold change was greater than 1.25 and if the combined p-value was less than 0.05 only in the KO vs. WT group. 868 sequences were identified in this analysis. Second, an ANOVA analysis was applied to compare the 4 WT vs. WT and the 4 KO vs. WT experiments. 1,646 sequences with ANOVA p-value less than 0.05 were selected.

The final KO-specific gene set of 171 sequences was obtained by taking the common sequences from these two analyses. These 171 sequences were further analyzed by using the Ingenuity pathway analysis tool [22].

2.6. Stimulation of Peritoneal Macrophages

Peritoneal macrophages were harvested from mice three days after an intraperitoneal (IP) injection of 0.1% solution of Zymosan A (Sigma) in PBS. The peritoneum was lavaged with 4 mL DMEM (Gibco) containing 20 mM HEPES buffer (Gibco). Cells were cultured and incubated at 37°C in a humidified incubator containing 5% CO_2 for 45 minutes, after which the media was aspirated, replaced with warmed media, and incubated for an additional 30 minutes. The supernatant was collected and replaced with media containing 5 μg/mL Ca^{++} ionophore (A23187) (Sigma) or 10^{-5}M arachidonic acid (AA) (Sigma). After 30 minutes of stimulation, the media was collected. Macrophages stimulated with lipopolysaccharide (LPS) (Sigma) were cultured in DMEM/10% FBS for 16 hours. The cell count was verified

using a β-hexosaminidase assay as previously described [23], and PGE_2 levels of pre- and post-stimulated samples were determined by ELISA (Amersham).

Peritoneal macrophages stimulated with IFN-γ were collected and cultured as described above. Macrophages were stimulated with RPMI/10%FBS containing 100 ng/mL recombinant murine IFN-γ (Sigma) for 8 and 16 hours, respectively. After IFN-γ treatment, cells were rinsed twice with PBS and lysed with RNABee for total RNA extraction.

2.7. Measurement of PGE_2

Proximal small intestine, kidney, lung, brain and heart were harvested from *mPges-2* −/−, *mPges-1* −/− and *Cox-1* −/− mice and their respective wild-type controls. All tissues were flash frozen in liquid nitrogen and stored at −80°C. The tissues were homogenized in 1X PBS/1 mM EDTA containing 10 μM indomethacin, on ice. Prostanoids were precipitated with 75% ethanol and glacial acetic acid. The supernatant was run over a C18 column (Amersham), prostaglandins were eluted in ethyl acetate, evaporated with N_2 and resuspended in PGE_2 EIA assay buffer. The quantity of PGE_2 was determined using the Prostaglandin E_2 Biotrak Enzymeimmunoassay System (Amersham) or the Correlate-EIA Prostaglandin E_2 Enzyme Immunoassay Kit (Assay Designs) according to manufacturer's specifications. Each sample was run in duplicate. The detection ranges of the kits are 50 – 6400 pg/mL and 39.1 – 2500 pg/mL, respectively; however, their cross-reactivity with isomeric PGE_2 analogs, such as 8-iso-PGE_2 has not been determined.

Measurement of PGE_2 by LC/MS was performed as described previously [24]. The lower limit of detection for PGE_2 by LC/MS is 0.002 ng/mg protein. Isomeric analogs of PGE_2 were not separated in LC/MS or ELISA, thus the amount of PGE_2 measured potentially includes the levels of these compounds.

2.8. Statistical Analysis

Statistical analysis was performed using JMP Statistical Software package v5.1 (SAS) and Prism 4 (GraphPad Software). Comparisons of the mean were made by Student's t-test or ANOVA followed by Tukey-Kramer's HSD *post hoc* test as necessary unless otherwise noted. Data are shown as mean ± SEM. Differences with $p<0.05$ were considered statistically significant.

3. Results

3.1. Generation of mice lacking expression of mPGES-2

A mutation was introduced into the mouse *mPges-2* gene, which encodes for microsomal prostaglandin E_2 synthases (mPGES-2), in ES cells by homologous recombination, using the strategy outlined in Figure 1A. The recombination event results in the replacement of 7 base pairs of exon 4, which encode for amino acids 194 to 196, with the neomycin phosphotransferase gene. ES cells in which the recombination event occurred were used to generate mice carrying the mutant *mPges-2* allele. Intercross of these heterozygous animals yielded offspring homozygous for the *mPges-2* mutation at expected frequencies. These mice are healthy, fertile, have normal growth rates and cannot be distinguished from littermates by observation alone. Morphological and histological analysis of the major organ systems failed to identify a role for mPGES-2 in normal development. Examination of thymus, spleen and lymph nodes showed normal development of T cells, B cells and macrophages (data not shown).

To verify that the mutation introduced into the *mPges-2* gene generated a null allele, RNA was prepared from tissues of *mPges-2* −/− mice and littermate controls. Three tissues

reported to express high levels of mPGES-2, the kidney, lung and intestinal tract were included in the study. For comparison, RNA was also prepared from the same tissues of *mPges-1* −/− mice, which encodes for microsomal prostaglandin E_2 synthases (mPGES-1). As expected, high levels of *mPges-2* were observed in the kidney, lung and intestinal tract of all wild type mice, as well as mice lacking *mPges-1*. In contrast, no *mPges-2* expression was detected in any of the tissues prepared from the *mPges-2* −/− animals (Fig. 1B). Similar to *mPges-2, mPges-1* expression is easily detected in the lung and kidney. However, in contrast to the high levels of *mPges-2* in the small intestine, *mPges-1* expression is barely detectable in this tissue. No obvious compensatory change in the expression of *mPges-1* was observed in the *mPges-2* −/− organs, and conversely, loss of *mPges-1* did not result in an easily detectable increase in *mPges-2* RNA levels.

3.2. PGE_2 levels in mPges-2 −/− mice

Basal production of PGE_2 by the intestinal tissue of *mPges-2* −/− mice and wild-type littermate controls was determined by ELISA and compared to levels measured in similar samples obtained from *Cox-1* −/− and *mPges-1* −/− mice and appropriate controls. PGE_2 can be easily detected in all wild-type samples, albeit at different levels, likely reflecting differences between mouse strains in basal PGE_2 production. As expected, the production of PGE_2 in healthy intestine is largely dependent on *Cox-1* expression (Fig. 2C, $p<0.001$). In mice lacking the COX-1 enzyme, PGE_2 levels are close to the lower limit of detection. Surprisingly, loss of *mPges-2* expression did not reduce PGE_2 levels in the small intestine (Fig. 2A). In contrast, *mPges-1* mRNA levels, as assessed by Northern analysis, are very low in the intestinal tract; however, basal PGE_2 production appears to be almost completely dependant on this enzyme, as PGE_2 levels are significantly diminished in *mPges-1* −/− mice (Fig. 2B, $p<0.05$). PGE_2 levels were also measured in additional tissues from *mPges-2* −/− mice and co-isogenic controls. Basal PGE_2 levels in lung, kidney, heart and brain of *mPges-2* −/− mice did not differ significantly from those of controls (Fig. 3).

In addition to examining PGE_2 levels in mPGES-2 deficient mice on a 129/SvEv genetic background, the *mPges-2* mutation was also backcrossed onto the C57BL/6 genetic background for twelve consecutive generations. Mice homozygous for the *mPges-2* mutation were generated by intercross of these animals, generating congenic C57BL/6 *mPges-2* −/− animals. Examination of tissues from these animals also failed to identify a contribution of mPGES-2 to basal levels of PGE_2 in these tissues (data not shown).

To further verify these findings we examined levels of PGE_2 and other prostanoids in extracts prepared from the proximal small intestine and the brain of 129/SvEv *mPges-2* −/− and co-isogenic controls by LC/MS. Both the brain and small intestine express high levels of *mPges-2*, while *mPges-1* expression is barely detectable in these organs in basal conditions (Fig. 1B) [11]. As can be seen in Figure 4A, the level of PGE_2 in intestinal tissue is not reduced by loss of *mPges-2* expression. In fact, PGE_2 levels were slightly elevated in the *mPges-2* −/− tissue compared to co-isogenic controls, although this difference did not achieve statistical significance ($p=0.0565$, $n=3$). No significant differences were measured in PGE_2 levels in either the brain (Fig. 4B) or kidney (data not shown). Taken together, these studies failed to support a role for *mPges-2* in basal production of PGE_2.

3.3. Role of mPGES-2 in production of COX-1 and COX-2 dependent PGE_2 by macrophages

The role of mPGES-1 in the production of PGE_2 by macrophages has been established; however, these studies also showed that macrophages can produce some PGE_2 independent of expression of this enzyme [9]. To determine if mPGES-2 contributed to PGE_2 production by macrophages, peritoneal macrophages were isolated from 129/SvEv *mPges-2* −/− mice and their co-isogenic controls. After peritoneal lavage, macrophages were enriched by

adherence to plastic, and PGE_2 levels in the culture supernatant were determined before and at various times after stimulation of the cells with A23187, AA or LPS. In some cases, parallel cultures were treated with indomethacin, a potent COX-1 and COX-2 inhibitor, prior to stimulation. As expected, both Ca^{++} ionophore and AA result in a rapid increase in PGE_2 production (Fig. 5A). Pre-treatment with indomethacin inhibited PGE_2 synthesis to background levels detected in unstimulated cell supernatants (data not shown). However, no difference was observed in the production of PGE_2 by the *mPges-2* −/− and control macrophages in response to either stimulus (Fig. 5A). As expected, PGE_2 synthesis was significantly reduced, but not completely abolished, in *mPges-1* −/− peritoneal macrophages in response to both AA and A23187 (Fig. 5B).

Treatment of macrophages with LPS has been demonstrated to induce COX-2 expression and increase production of PGE_2 [25]. A functional role for mPGES-1 in LPS stimulated PGE_2 biosynthesis has been independently described [9, 10]. In accordance with these data, LPS treatment resulted in high levels of PGE_2 in the supernatant of both *mPges-2* +/+ and −/− macrophages in a dose dependent manner. However, loss of *mPges-2* did not alter the ability of these cells to produce PGE_2 (Fig. 5C). These experiments, taken together, do not support a role for mPGES-2 in the production of PGE_2 by peritoneal macrophages.

3.4. mPGES-2/GBF-1 and IFN-γ mediated IRF-9 expression

Given the failure to measure a role for mPGES-2 in PGE_2 synthesis, it was of interest to determine whether other functions attributed to this gene could be supported by studies of the mPGES-2 deficient mice. *mPGES-2* expression was reported to be inducible by IFN-γ and contribute to IFN-γ mediated regulation of *IRF-9* gene expression. To assess the role of mPGES-2 as GBF-1, we examined induction of *Irf9* expression after treatment of macrophages with IFN-γ. Peritoneal macrophages were harvested by lavage of the peritoneal cavity 72 hours after induction of acute peritonitis with Zymosan A, and enriched by adherence to plastic. Cells were then stimulated with 100 ng/mL IFN-γ for 8 or 16 hours. *mPges-2* expression levels were unchanged compared to untreated macrophages at 8 and 16 hours (Fig. 6A). As expected, IFN-γ treatment significantly increases endogenous *Irf9* expression after 16 hours of stimulation (Fig. 6B). However, *Irf9* expression is not significantly diminished in IFN-γ treated, mPGES-2 deficient macrophages compared to wild-type levels at both time points. Therefore, while *Irf9* expression was induced by IFN-γ treatment, this data indicates that mPGES-2 is not essential for IRF-9 induction.

3.5. Expression profiling of mPges-2 −/− tissue

Survey of RNA prepared from various mouse organs by quantitative real-time PCR indicates that moderate levels of *mPges-2* are detected in the heart under basal conditions (Fig. 7). The heart is a less complex organ with fewer cell types than the lung and intestine and is also less sensitive to metabolic variations (compared to liver or intestine). Additionally, because low basal levels of PGE_2 are produced, despite moderate expression levels of *mPges-2* in this organ, it seemed ideal for the identification of functions of mPGES-2 independent of PGE_2 synthesis. RNA was prepared from the hearts of four age, sex, and weight matched 129/SvEv *mPges-2* −/− mice and their co-isogenic controls for microarray analysis. Analysis revealed surprisingly few differences between the wild-type and *mPges-2* −/− hearts. The top genes that increased or decreased in expression in the *mPges-2* −/− heart are shown in Table 2 in order of fold change. The expression of only one gene increased, while the expression of two genes decreased significantly with a change greater than 2.0 fold. As anticipated, this analysis showed a significant decrease of *mPges-2* mRNA levels in the heart of *mPges-2* −/− animals. We chose to verify the change in gene expression for those candidates with commercially available primer/probe sets by quantitative real-time PCR analysis. PCR was carried out both with the samples used for

microarray analysis and from independent RNA samples prepared from *mPges-2* −/− and control animals. Of these candidates only changes in the expression level of *Trim13* were confirmed by quantitative real-time PCR in the heart. To further explore the effect of mPGES-2 on *Trim13* expression in *mPges-2* −/− mice, kidney and intestinal tract samples were also evaluated for *Trim13* mRNA levels by quantitative real-time PCR. *Trim13* expression was significantly decreased in mPGES-2 deficient heart, kidney and jejunum to nearly half of wild-type levels (Fig. 8, $p<0.005$). While, in general, the impact of loss of mPGES-2 was surprisingly modest, these results demonstrate that mPGES-2 loss can directly or indirectly affect the expression of other genes.

3.6. Expression of enzymes involved in PGE_2 metabolism or catabolism

Given the observed ability of loss of mPGES-2 to alter *Trim13* expression, we considered the possibility that the identification of mPGES-2 as a synthase might reflect its ability to regulate expression of other enzymes involved in either PGE_2 synthesis or catabolism, as had been observed for cPGES/p23 [5]. This possibility was heightened by the observation that slightly elevated levels of PGE_2 levels were observed occasionally in some tissues lacking *mPges-2*. To test this hypothesis, we examined the expression of *Cox-1*, *Cox-2* and *mPges-1*, enzymes critical in PGE_2 production in *mPges-2* +/+ and −/− small intestine. Expression of the cytosolic prostaglandin E_2 synthase, *cPges*, a gene also purported to convert PGH_2 to PGE_2 was examined, as well. PGE_2 is rapidly metabolized to 15-PGE_2 by 15-PGDH (encoded by *15-Pgdh*), the primary enzyme involved in PGE_2 catabolism. Thus, expression analysis of *15-Pgdh* was also incorporated in the study. As seen in Figure 9, there was no change in the expression of *Cox-1*, *Cox-2*, *mPges-1* and *cPges* in the *mPges-2* −/− small intestine. A significant reduction in expression of *15-Pgdh* was initially observed in some samples from the 129 co-isogenic animals (Fig. 9); however, this reduction was not consistently observed in small intestine samples from *mPges-2* −/− mice on a C57BL/6 genetic background (data not shown). Taken together, the loss of mPGES-2 expression did not consistently alter either the PGE_2 levels or expression of enzymes required for PGE_2 synthesis or metabolism.

4. Discussion

mPGES-1 was the first enzyme identified and characterized as capable of catalyzing the conversion of PGH_2 to PGE_2 [26]. The generation of mice lacking this enzyme verified the role of mPGES-1 in PGE_2 synthesis, both in its contribution to basal PGE_2 levels and to increased PGE_2 production during inflammatory responses [9–11].

A number of observations made during the study of these mice suggested the existence of mPGES-1 independent PGE_2 production. The expression levels of mPGES-1 in various tissues did not correspond well with the basal levels of PGE_2 produced by these tissues. Furthermore, while mPGES-1 was responsible for the majority of PGE_2 biosynthesis, measurable amounts of PGE_2 could still be detected in mPGES-1 deficient cells and tissues. Moreover, contrary to expectation, some phenotypes of mice lacking COX-2 and the EP receptors were not evident in mPGES-1 deficient animals. Notably, the patent ductus observed in COX-2 deficient [27] and EP4 deficient [28] mice, was not recapitulated in mPGES-1 deficient mice [29].

A possible explanation for these observations was suggested by the cloning of an enzyme, designated mPGES-2, initially purified from microsomal fractions of bovine heart [15]. Over-expression of mPGES-2 in cell lines resulted in increased PGE_2 levels [12]. Several subsequent publications furthered the supposition that mPGES-2 was a PGE_2 synthase. Interestingly, *mPges-2* is located in a cluster of genes, including *Cox-1* and *Cox-2*, on mouse chromosome 2. To define the *in vivo* role of *mPges-2* in PGE_2 biosynthesis, we have

generated a mouse carrying a null allele of this gene. No *mPges-2* mRNA can be detected in any of the tissues examined to date. However, surprisingly, analysis of PGE_2 levels in these mice fails to support the classification and designation of *mPges-2* as a prostaglandin E_2 synthase. Basal PGE_2 levels were not decreased in any of the mPGES-2 deficient tissues examined in this study. *Ex vivo* studies of macrophages isolated from *mPges-2* −/− mice also failed to support a role for mPGES-2 in PGE_2 production. *mPges-2* −/− macrophages produced amounts of PGE_2 similar to wild-type controls in response to AA, Ca^{++} ionophore and LPS. Under identical conditions, mPGES-1 independent PGE_2 production was measured as reported previously [9, 10]. These data suggest that mPGES-2 is not responsible for residual PGE_2 production observed in mPGES-1 deficient mice.

These findings are in contrast with studies published by Tanikawa *et al.* and Murakami *et al.* [12, 16], which demonstrate that mPGES-2 enzymatically promotes PGE_2 biosynthesis *in vitro*. In studies by Murakami *et al.*, over-expression of mPGES-2 in cell lines, which express mPGES-1 at very low levels, resulted in a measurable increase in PGE_2 production. The basis for the apparent discrepancy between these observations and our results is not obvious. It is possible that the ability of transfected cells to increase PGE_2 was the indirect consequence of increased levels of this protein and not its function as a synthase. For instance, increases in PGE_2 levels may potentially result from facilitating the non-enzymatic conversion of PGH_2 to PGE_2 or by alterations in the catabolism of PGE_2. It is possible that the ability of mPGES-2 to facilitate PGE_2 production requires very high and non-physiological levels of expression, which are not observed in most healthy, normal tissues. However, at this time, we also cannot rule out the possibility that populations of cells or tissues not examined in this study can utilize this protein in the production of PGE_2.

Studies of mPGES-2 deficient mice also failed to support reports that this gene could act as GBF-1, a novel transactivator of IFN-γ dependent gene expression. Hu *et al.* [18] demonstrated that mPGES-2/GBF-1 activated transcription of ISGF3g/p48/IRF-9 upon IFN-γ treatment of RAW264.7 murine macrophage cells. While a robust induction of *Irf9* was measured, both in wild-type and *mPges-2* −/− peritoneal macrophages stimulated with IFN-γ, *Irf9* levels measured in mPGES-2 deficient macrophages were not significantly different from the wild-type macrophages (Fig. 6A). Hu *et al.* also demonstrate that *Gbf-1/mPges-2* mRNA expression was induced in RAW cells after 8 hours of IFN-γ stimulation. In contrast, *Gbf-1/mPges-2* mRNA levels are not up-regulated in peritoneal macrophages isolated from wild-type mice (Fig. 6B) after 8 or 16 hours of IFN-γ stimulation. In the context of these data, it is not surprising that *Irf9* levels were not significantly reduced in *mPges-2* −/− peritoneal macrophages. It is possible that peritoneal macrophages, unlike, RAW264.7 murine macrophage cells may not be dependent on GBF-1/mPGES-2, and these cells may have a different transcriptional program resulting from their immortalization.

Meng *et al.* [30] recently reported that IFN-γ induces the interaction of GBF-1/mPGES-2 with CEBP-β, and that recruitment of GBF-1 to the GATE/ CEBP-β element is CEBP-β dependent. It is therefore possible that IRF-9 levels are not significantly decreased in the absence of mPGES-2 because *IRF9* transcription is predominantly dependent on transcription factors such as CEBP-β in peritoneal macrophages. Alternately, it remains possible that steady state mRNA levels may not accurately represent changes in the rate of transcription of the *IRF9* gene.

Because of the report that mPGES-2/GBF-1 may play a role in regulation of gene expression we carried out an additional line of inquiry into this potential function, using genome wide expression analysis of the heart. Surprisingly, few differences were found in the gene expression pattern of hearts lacking *mPges-2*. Of the genes with statistically significant differences in expression levels, only one of the five candidates tested could be validated by

quantitative real-time PCR. We verified that expression levels of *Trim13/RFP2/Leu5* (tripartite motif 13, Ret Finger Protein 2), were significantly down regulated in the heart, and interestingly, in the kidney and proximal small intestine, as well.

Trim13/ Leu5/ RFP2, a putative transcription factor, is a member of the tripartite motif family proteins containing RING finger, B-box type and coiled-coil domains [31]. The mouse *Trim13/RFP2/Leu5* gene locus located on chromosome 14 is not linked to *mPges-2* and potentially encodes for two proteins, RFP2/Leu5 and DLTET, of unknown function [32, 33]. Loss of RFP2/Leu5 appears to be an early event in human B-cell CLL suggesting that RFP2/Leu5 is a tumor suppressor gene [34]. To date, we have not observed any lymphoid tumors in *mPges-2* −/− mice that are over 12 months old. Furthermore, FACS analysis of cells isolated from the spleen, thymus and lymph nodes has not revealed an abnormal increase in B-cells or any other lymphoid population. It is unclear at this time if the changes in *Trim13* expression are directly caused by loss of *mPges-2* dependent transcription or are indirect and potentially compensatory for the loss of mPGES-2 function. Thus, while a role for mPGES-2 in the regulation of IRF-9 is not supported by these studies, the gene expression profiles leave open the possibility that mPGES-2 can act as a transcription factor.

It was therefore of interest to determine if the increased basal levels of PGE_2 observed in some tissues lacking mPGES-2 reflected altered expression of genes involved in PGE_2 synthesis or metabolism. No differences were observed in expression levels of *Cox-1*, *Cox-2* or *mPges-1* mRNA, and while in some cases a decrease in expression of *15-Pgdh* was observed, we failed to consistently observe increases in PGE_2 levels. Furthermore, the potential increase in PGE_2 levels was not observed after backcrossing of the *mPges-2* mutation to the C57BL/6 genetic background.

In summary, our data indicates that, similar to cPGES, mPGES-2 does not encode a prostaglandin sythase. Thus mPGES-1 mediated conversion of cyclooxygenase-derived PGH_2 to PGE_2 may represent the only enzymatic pathway by which PGE_2 is generated *in vivo*, with other non-enzymatic sources of production accounting for the differences in the phenotypes of the prostaglandin receptor-deficient mice and the mice lacking this enzyme.

Abbreviations

AA	arachidonic acid
COX	cycloxygenase
15-PGDH	15-hydroxyprostaglandin dehydrogenase
PG	prostaglandin
mPGES-1	microsomal prostaglandin E_2 synthase-1
mPGES-2	microsomal prostaglandin E_2 synthase-2
cPGES	cytosolic prostaglandin E_2 synthase

Acknowledgments

We thank Anne Latour for assistance in tissue culture. This work was supported by National Institutes of Health Grants DK069896 (B.H.K.), DK38108, and HL068141 (B.H.K.) and the Department of Defense DAMD17-03-1-0453 (S.C.)

References

1. Tilley SL, Coffman TM, Koller BH. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. The Journal of clinical investigation. 2001; 108(1): 15–23. [PubMed: 11435451]
2. Matsuo M, Ensor CM, Tai HH. Characterization of the genomic structure and promoter of the mouse NAD+-dependent 15-hydroxyprostaglandin dehydrogenase gene. Biochemical and biophysical research communications. 1997; 235(3):582–586. [PubMed: 9207200]
3. Backlund MG, Mann JR, Holla VR, et al. 15-Hydroxyprostaglandin dehydrogenase is down-regulated in colorectal cancer. The Journal of biological chemistry. 2005; 280(5):3217–3223. [PubMed: 15542609]
4. Wang D, Dubois RN. Cyclooxygenase-2: a potential target in breast cancer. Seminars in oncology. 2004; 31(1 Suppl 3):64–73. [PubMed: 15052544]
5. Lovgren AK, Kovarova M, Koller BH. cPGES/p23 is required for glucocorticoid receptor function and embryonic growth but not prostaglandin E2 synthesis. Molecular and cellular biology. 2007; 27(12):4416–4430. [PubMed: 17438133]
6. Tanioka T, Nakatani Y, Semmyo N, Murakami M, Kudo I. Molecular identification of cytosolic prostaglandin E2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E2 biosynthesis. The Journal of biological chemistry. 2000; 275(42):32775–32782. [PubMed: 10922363]
7. Grad I, McKee TA, Ludwig SM, et al. The Hsp90 cochaperone p23 is essential for perinatal survival. Molecular and cellular biology. 2006; 26(23):8976–8983. [PubMed: 17000766]
8. Murakami M, Nakatani Y, Tanioka T, Kudo I. Prostaglandin E synthase. Prostaglandins & other lipid mediators. 2002:68–69. 383–399.
9. Trebino CE, Stock JL, Gibbons CP, et al. Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100(15):9044–9049. [PubMed: 12835414]
10. Uematsu S, Matsumoto M, Takeda K, Akira S. 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. 2002; 168(11):5811–5816. [PubMed: 12023384]
11. Boulet L, Ouellet M, Bateman KP, et al. Deletion of microsomal prostaglandin E2 (PGE2) synthase-1 reduces inducible and basal PGE2 production and alters the gastric prostanoid profile. The Journal of biological chemistry. 2004; 279(22):23229–23237. [PubMed: 15016822]
12. Murakami M, Nakashima K, Kamei D, et al. Cellular prostaglandin E2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and-2. The Journal of biological chemistry. 2003; 278(39):37937–37947. [PubMed: 12835322]
13. Murakami M, Naraba H, Tanioka T, et al. Regulation of prostaglandin E2 biosynthesis by inducible membrane-associated prostaglandin E2 synthase that acts in concert with cyclooxygenase-2. The Journal of biological chemistry. 2000; 275(42):32783–32792. [PubMed: 10869354]
14. Chandrasekharan S, Foley NA, Jania L, et al. Coupling of COX-1 to mPGES1 for prostaglandin E2 biosynthesis in the murine mammary gland. Journal of lipid research. 2005; 46(12):2636–2648. [PubMed: 16204198]
15. Watanabe K, Kurihara K, Suzuki T. Purification and characterization of membrane-bound prostaglandin E synthase from bovine heart. Biochimica et biophysica acta. 1999; 1439(3):406–414. [PubMed: 10446427]
16. Tanikawa N, Ohmiya Y, Ohkubo H, et al. Identification and characterization of a novel type of membrane-associated prostaglandin E synthase. Biochemical and biophysical research communications. 2002; 291(4):884–889. [PubMed: 11866447]
17. Gudis K, Tatsuguchi A, Wada K, et al. Microsomal prostaglandin E synthase (mPGES)-1, mPGES-2 and cytosolic PGES expression in human gastritis and gastric ulcer tissue. Laboratory investigation; a journal of technical methods and pathology. 2005; 85(2):225–236.

18. Hu J, Meng Q, Roy SK, et al. A novel transactivating factor that regulates interferon-gamma-dependent gene expression. The Journal of biological chemistry. 2002; 277(33):30253–30263. [PubMed: 12050152]
19. Weihua X, Kolla V, Kalvakolanu DV. Interferon gamma-induced transcription of the murine ISGF3gamma (p48) gene is mediated by novel factors. Proceedings of the National Academy of Sciences of the United States of America. 1997; 94(1):103–108. [PubMed: 8990168]
20. Langenbach R, Morham SG, Tiano HF, et al. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. Cell. 1995; 83(3):483–492. [PubMed: 8521478]
21. Hughes TR, Marton MJ, Jones AR, et al. Functional discovery via a compendium of expression profiles. Cell. 2000; 102(1):109–126. [PubMed: 10929718]
22. Calvano SE, Xiao W, Richards DR, et al. A network-based analysis of systemic inflammation in humans. Nature. 2005; 437(7061):1032–1037. [PubMed: 16136080]
23. Hooper M, Hardy K, Handyside A, Hunter S, Monk M. HPRT-deficient (Lesch-Nyhan) mouse embryos derived from germline colonization by cultured cells. Nature. 1987; 326(6110):292–295. [PubMed: 3821905]
24. Guay J, Bateman K, Gordon R, Mancini J, Riendeau D. Carrageenan-induced paw edema in rat elicits a predominant prostaglandin E2 (PGE2) response in the central nervous system associated with the induction of microsomal PGE2 synthase-1. The Journal of biological chemistry. 2004; 279(23):24866–24872. [PubMed: 15044444]
25. Hempel SL, Monick MM, Hunninghake GW. Lipopolysaccharide induces prostaglandin H synthase-2 protein and mRNA in human alveolar macrophages and blood monocytes. The Journal of clinical investigation. 1994; 93(1):391–396. [PubMed: 8282809]
26. Jakobsson PJ, Thoren S, Morgenstern R, Samuelsson B. Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96(13):7220–7225. [PubMed: 10377395]
27. Loftin CD, Tiano HF, Langenbach R. Phenotypes of the COX-deficient mice indicate physiological and pathophysiological roles for COX-1 and COX-2. Prostaglandins & other lipid mediators. 2002:68–69. 177–185.
28. Nguyen M, Camenisch T, Snouwaert JN, et al. The prostaglandin receptor EP4 triggers remodelling of the cardiovascular system at birth. Nature. 1997; 390(6655):78–81. [PubMed: 9363893]
29. Narumiya S, Sugimoto Y, Ushikubi F. Prostanoid receptors: structures, properties, and functions. Physiological reviews. 1999; 79(4):1193–1226. [PubMed: 10508233]
30. Meng Q, Raha A, Roy S, Hu J, Kalvakolanu DV. IFN-gamma-stimulated transcriptional activation by IFN-gamma-activated transcriptional element-binding factor 1 occurs via an inducible interaction with CAAAT/enhancer-binding protein-beta. J Immunol. 2005; 174(10):6203–6211. [PubMed: 15879117]
31. Reymond A, Meroni G, Fantozzi A, et al. The tripartite motif family identifies cell compartments. The EMBO journal. 2001; 20(9):2140–2151. [PubMed: 11331580]
32. Corcoran MM, Hammarsund M, Zhu C, et al. DLEU2 encodes an antisense RNA for the putative bicistronic RFP2/LEU5 gene in humans and mouse. Genes, chromosomes & cancer. 2004; 40(4): 285–297. [PubMed: 15188451]
33. Baranova A, Hammarsund M, Ivanov D, et al. Distinct organization of the candidate tumor suppressor gene RFP2 in human and mouse: multiple mRNA isoforms in both species- and human-specific antisense transcript RFP2OS. Gene. 2003; 321:103–112. [PubMed: 14636997]
34. Mertens D, Wolf S, Schroeter P, et al. Down-regulation of candidate tumor suppressor genes within chromosome band 13q14.3 is independent of the DNA methylation pattern in B-cell chronic lymphocytic leukemia. Blood. 2002; 99(11):4116–4121. [PubMed: 12010815]

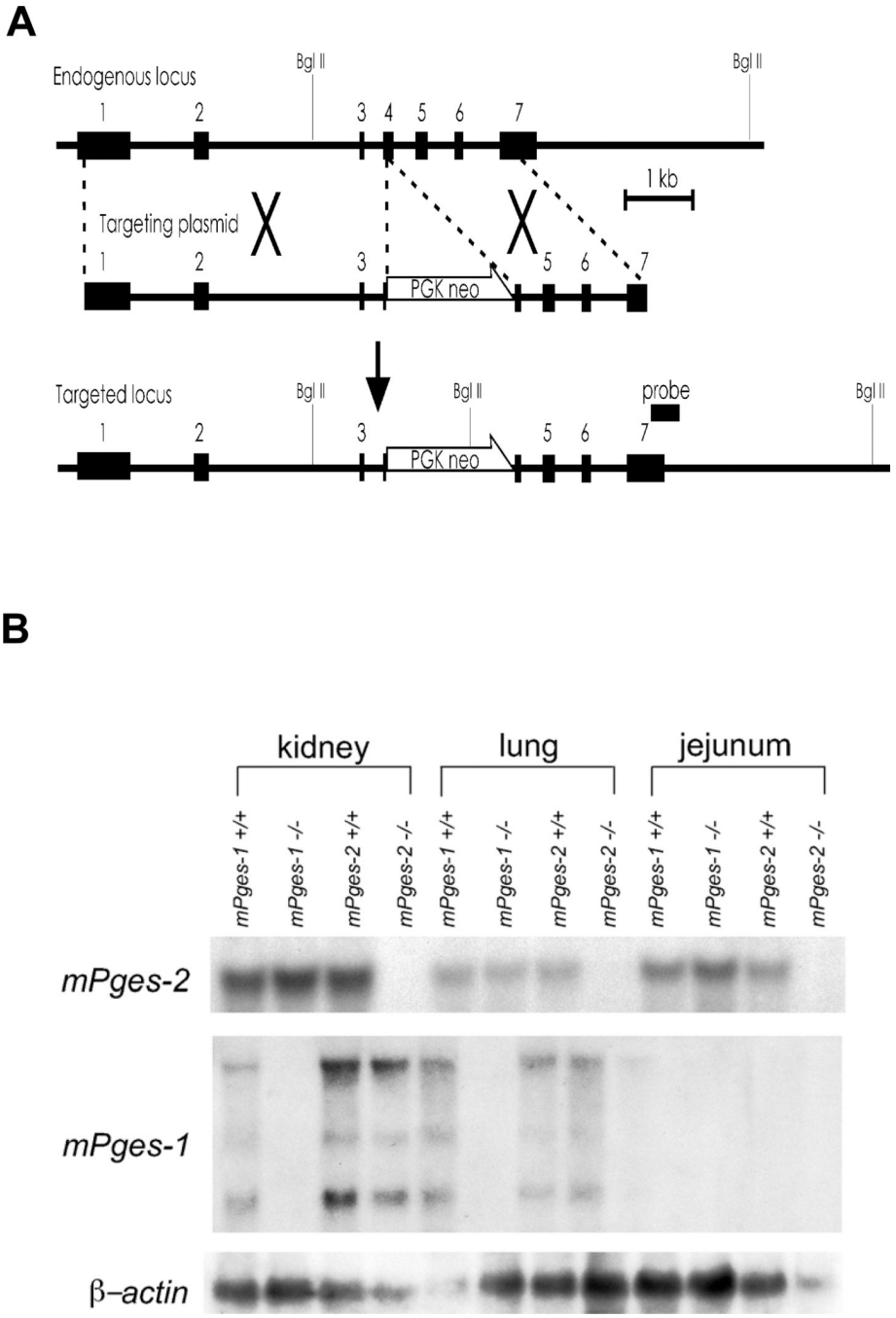

Fig. 1.

Targeting of the *mPges-2* gene. (A) Schematic of the targeting strategy to disrupt exon 4 of the *mPges-2* gene representing the endogenous locus (top), the targeting plasmid (middle), and the targeted *mPges-2* locus (bottom). The probe used to screen genomic DNA, digested with *Bgl* II, for homologous recombination is indicated. (B) Northern blot analysis of total RNA isolated from kidney, lung and jejunum of *mPges-2* −/− and *mPges-1* −/− animals and their respective wild-type controls. *mPges-2* and *mPges-1* cDNA probes were labeled with ^{32}P[dCTP] and hybridized to the blot. β-*actin* was used as a loading control.

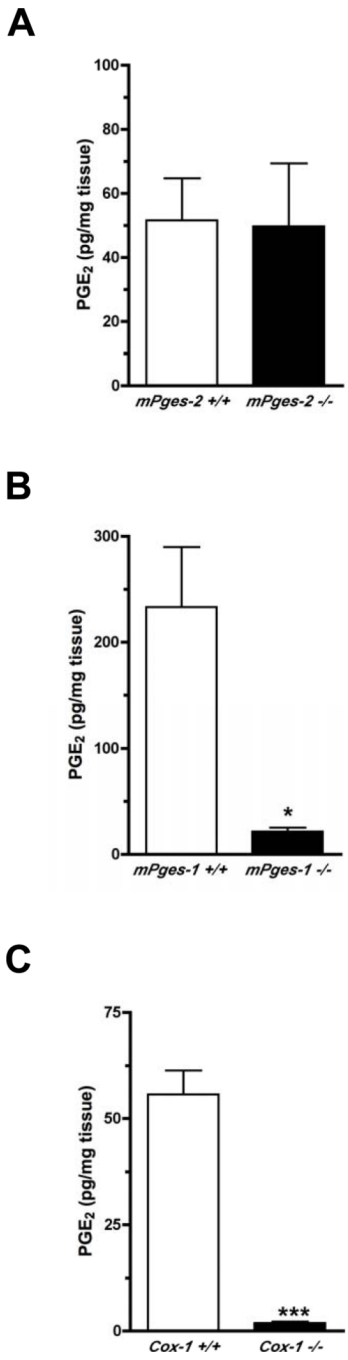

Fig. 2.

PGE_2 levels in jejunum. Basal PGE_2 production was measured in the jejunum of (A) *mPges-2* −/−, (B) *mPges-1* −/− and (C) *Cox-1* −/− deficient mice and their respective wild-type controls. PGE_2 levels were significantly diminished in both *mPges-1* and *Cox-1* −/− animals relative to their wild-type controls. *, $p<0.05$; ***, $p<0.0001$. (n=4–7 for each group).

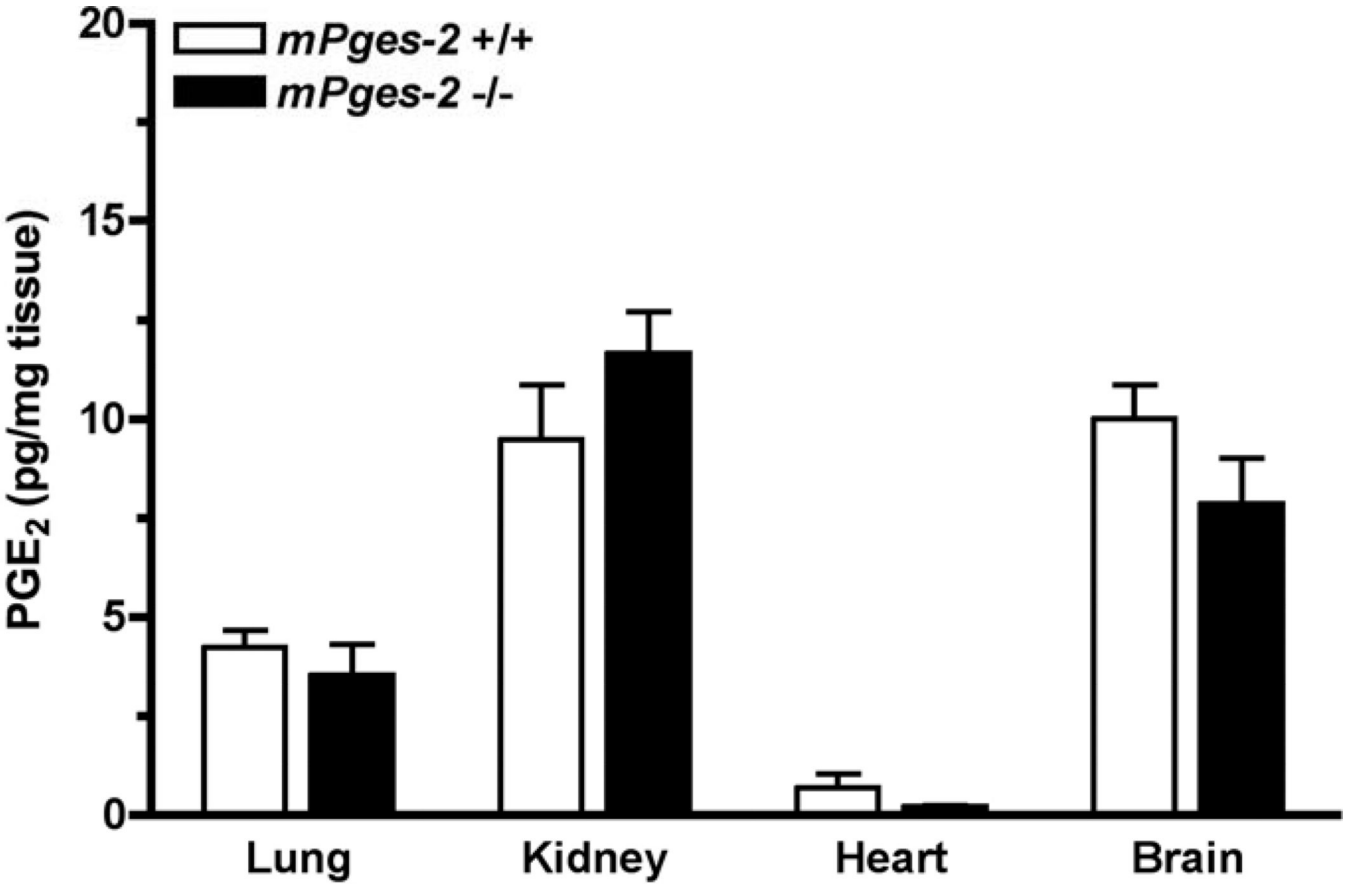

Fig. 3.

PGE_2 levels in *mPges-2* +/+ and −/− lung, kidney, heart, and brain. No significant difference in PGE_2 production by *mPges-2* −/− mice in the lung, kidney, heart and brain compared to wild-type controls. open bars, *mPges-2* +/+, closed bars, *mPges-2* −/−; (n=3–8 per group for all experiments). $p>0.05$ for all values.

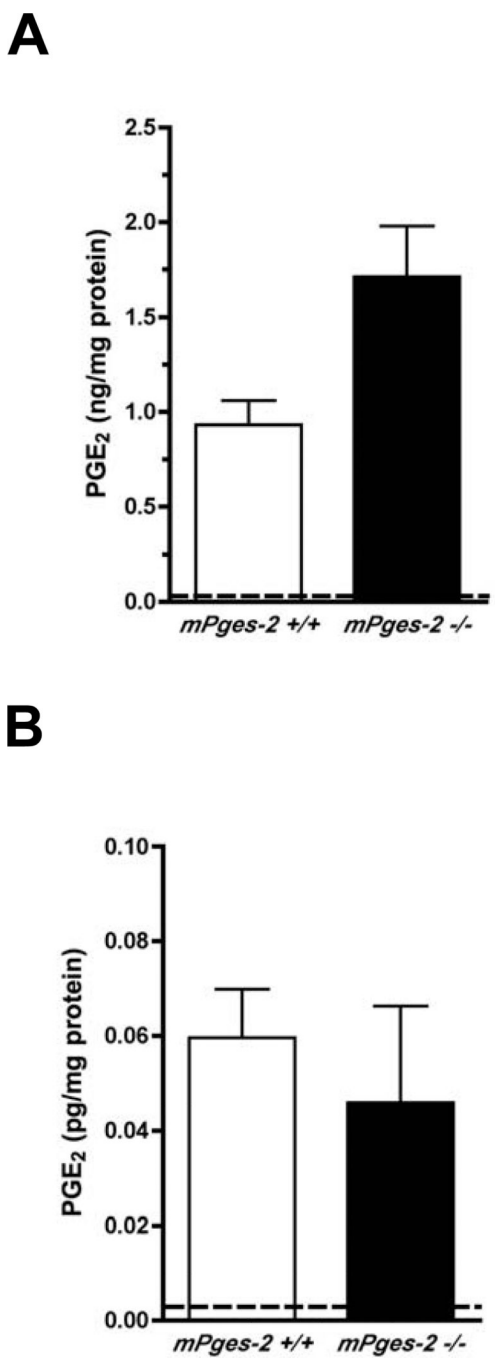

Fig. 4.

Liquid chromatography/mass spectrometry performed on duodenum and brain samples from *mPges-2* −/− mice and wild-type controls. No decrease in PGE_2 levels were detected in the samples from *mPges-2* −/− mice. PGE_2 levels were elevated in *mPges-2* −/− duodenum, although this difference was not statistically significant (A). No difference in PGE_2 levels were observed in *mPges-2* −/− brain tissue (B). Dotted line represents the lower detection limit for the assay (0.002 ng/mg protein), (n=3 for each group).

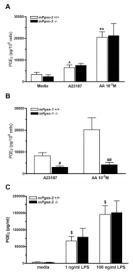

Fig. 5.

Acute and delayed PGE_2 production in *mPges-2* −/−, *mPges-1* −/− and respective wild-type control peritoneal macrophages. (A) Calcium ionophore (A23187) and arachidonic acid (AA) stimulated PGE_2 biosynthesis. PGE_2 was measured from supernatant pre- and post-stimulation. *mPges-2* −/− macrophages have no significant difference in PGE_2 release compared to *mPges-2* +/+ macrophages. *, p<0.05 (Mann-Whitney U-test), *mPges-2* +/+ media vs *mPges-2* +/+ A23187; **, p<0.001 (Mann-Whitney U-test), *mPges-2* +/+ media vs *mPges-2* +/+ AA. (B) *mPges-1* −/− macrophages have reduced levels of PGE_2. #, p<0.05, *mPges-1* +/+ vs *mPges-1* −/−, A23187 stimulation; ##, p<0.05, *mPges-1* +/+ vs *mPges-1* −/−, AA stimulation. (C) Macrophages were incubated in media containing 1 ng/ml or 100 ng/ml LPS for 16h. We observed a significant dose-dependent response increase in PGE_2 biosynthesis over PGE_2 levels measured in media alone. $, p<0.01; open bars indicate *mPges-2* +/+; closed bars, *mPges-2* −/−. (n=4–16 per group for each experiment).

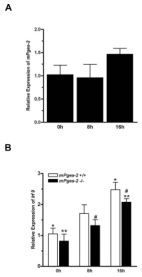

Fig. 6.

IFN-γ treated peritoneal macrophages. (A) Quantitative real-time PCR analysis of *mPges-2* mRNA in wild-type peritoneal macrophages treated with IFN-γ. No change in *mPges-2* expression was observed in IFN-γ stimulated macrophages. (B) Relative expression of *Irf9* expression in untreated peritoneal macrophages and macrophages stimulated with 100ng/ml IFN-γ for 8 hours and 16 hours. *Irf9* expression was induced in both wild-type and *mPges-2* −/− macrophages. *Irf9* expression was not significantly diminished in *mPges-2* −/− peritoneal macrophages upon stimulation with IFN-γ compared to wild-type controls. Open bars, *mPges-2* +/+; closed bars, *mPges-2* −/−. *, $p<0.01$, unstimulated wild-type vs 16h IFN-γ treated wild-type peritoneal macrophages; **, $p<0.001$, unstimulated *mPges-2* −/− vs 16h IFN-γ treated *mPges-2* −/− peritoneal macrophages; # $p<0.05$, 8h IFN-γ treated *mPges-2* −/− vs 16h IFN-γ treated *mPges-2* −/− peritoneal macrophages (n=4–9 per group).

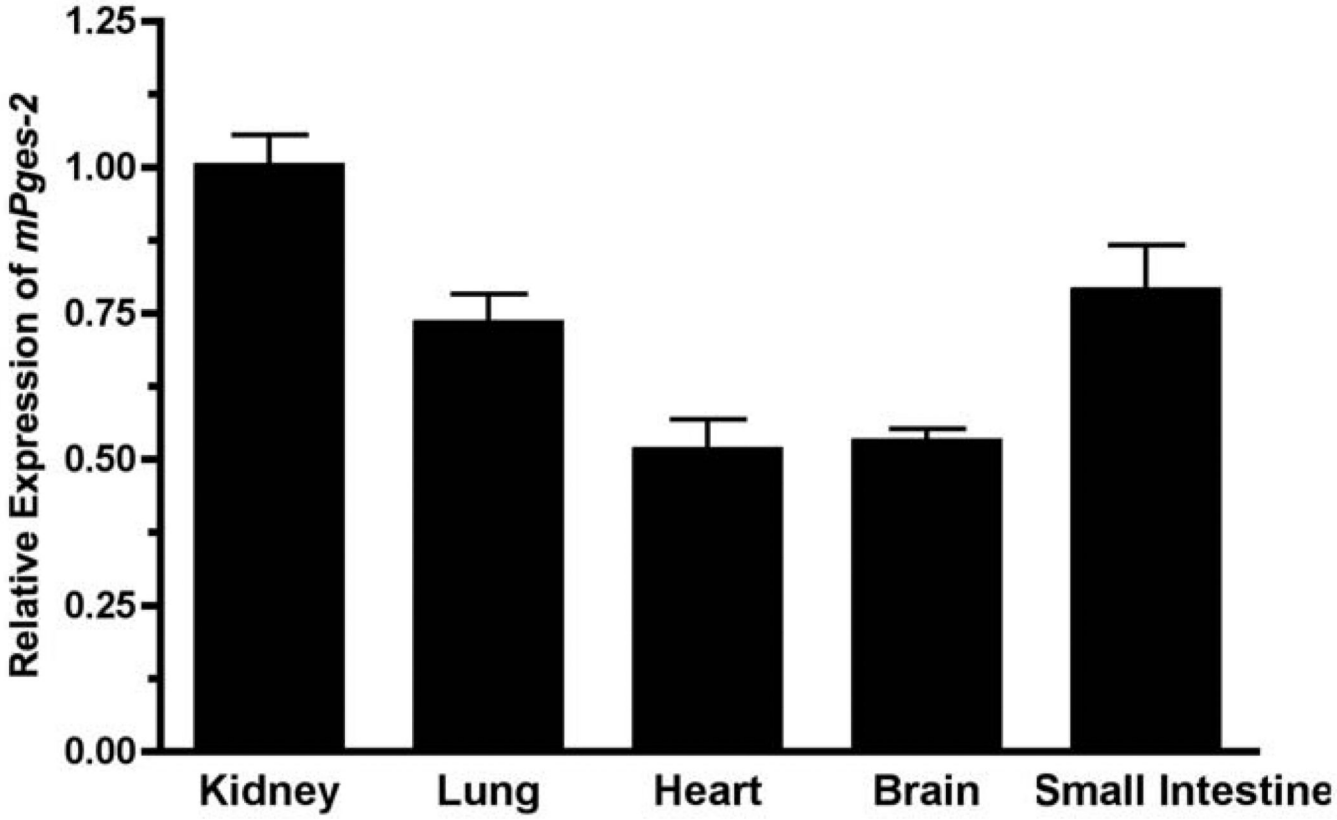

Fig. 7.

Relative expression of *mPges-2* in various tissues. Quantitative real-time PCR was used to assess *mPges-2* expression in multiple organs. Expression levels were normalized to *Gapdh* and are represented as fold change relative to kidney. *mPges-2* is ubiquitously expressed in the mouse, with higher expression in the kidney, lung, heart, brain and small intestine (n=3 per group).

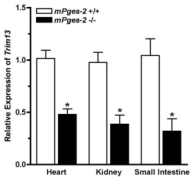

Fig. 8.

Trim13 expression in *mPges-2* −/− mice. Expression of *Trim13* was assessed by quantitative real-time PCR using *Gapdh* as an internal control. *Trim13* levels are significantly decreased in *mPges-2* −/− kidney, small intestine and heart. Open bars, *mPges-2* +/+; closed bars, *mPges-2* −/−. *, $p<0.005$ relative to wild-type expression levels. (n=5–6 per group).

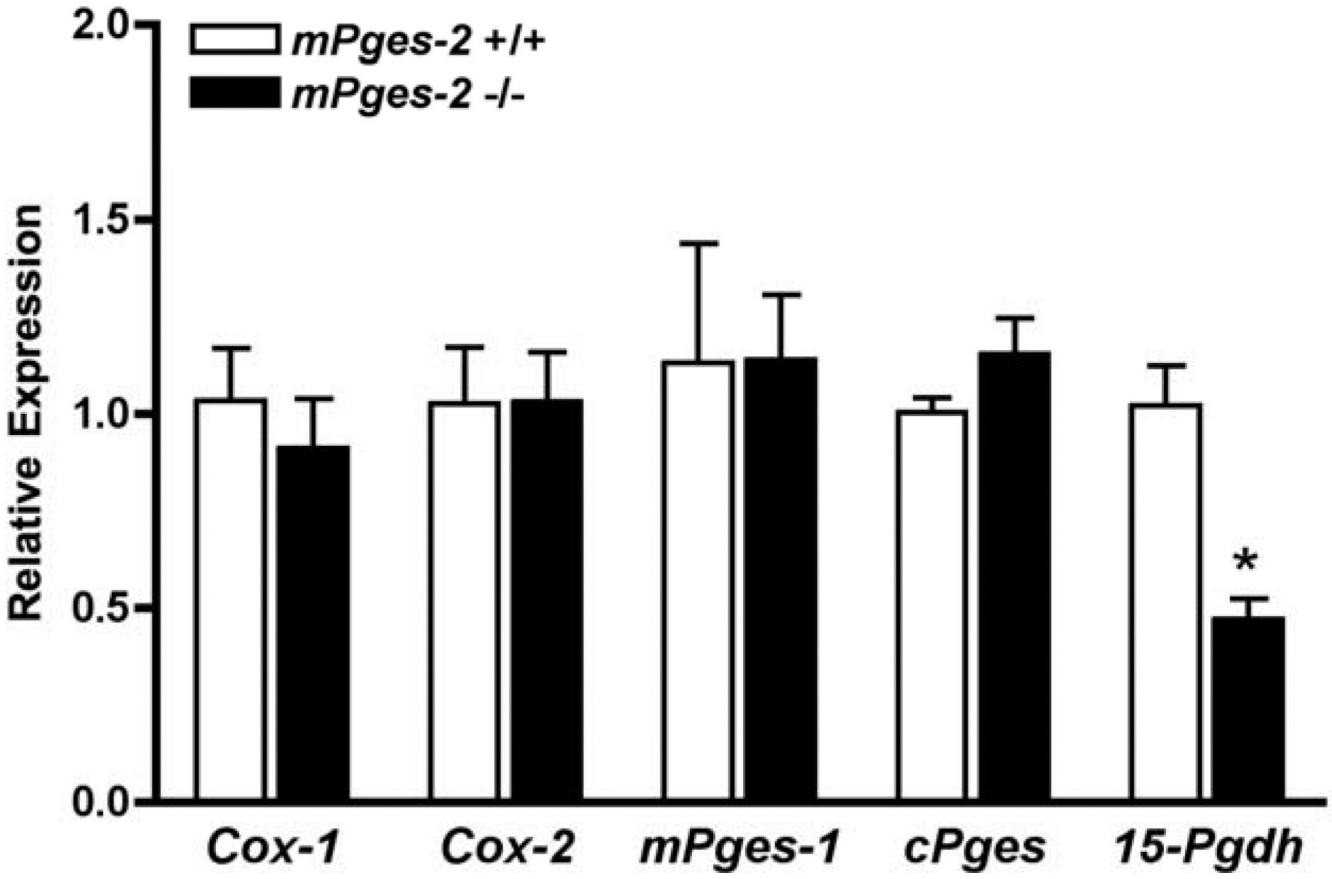

Fig. 9.

Quantitative real-time PCR analysis of the enzymes responsible for the synthesis and metabolism of PGE_2. Expression levels of the genes of interest were normalized to *Gapdh* and are represented as a fold change relative to wild-type. Expression of *Cox-1, Cox-2, mPges-1, cPges* and *15-Pgdh* were assessed in the small intestine. Open bars, *mPges-2* +/+; closed bars, *mPges-2* −/−.

Table 1

Candidate genes from microarray analysis

Gene Name	Accession #	Fold Change in mPges-2 −/− Heart	Validated by qPCR
Plekhh1	AK122464	+2.8	no
Trim13	NM_023233	−2.5	yes
Fasn	NM_007988	−1.9	no
Myh7	NM_080728	−1.8	no
Sf3b4	NM_153053-1.6	−1.6	no
Tnxip	AK004653	−1.5	no