Interferon-\(\tau\) Induces Degradation of Prostaglandin H Synthase-2 Messenger RNA in Bovine Endometrial Cells Through a Transcription-Dependent Mechanism

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ABSTRACT

A series of experiments were undertaken to examine the effects of interferon (IFN)-\(\tau\) on regulation of prostaglandin H synthase (PGHS)-2 mRNA in bovine endometrial (BEND) cells as a means to elucidate the actions of IFN-\(\tau\) to maintain pregnancy. The objective was to determine if IFN-\(\tau\) mediates posttranscriptional regulation of PGHS-2 mRNA. Cells were treated with phorbol 12,13-dibutyrate (PdBu) for 3 h to induce PGHS-2 mRNA expression. Actinomycin D (0 or 1 \(\mu\)g/ml) or the p38 mitogen-activated protein kinase (MAPK) inhibitor, SB203580 (1 \(\mu\)M), were added at 3 h, followed by addition of IFN-\(\tau\) (0 or 50 ng/ml) at 3.5 h and extraction of RNA at 4.5 h. The concentrations of PGHS-2 mRNA were stable between 3 and 4.5 h regardless of actinomycin D. Simultaneous treatment of PdBu-treated cells with actinomycin D and SB203580 (1 \(\mu\)M) decreased PGHS-2 mRNA. Addition of IFN-\(\tau\) (50 ng/ml) reduced PGHS-2 mRNA, which was not observed when actinomycin D was present. Concurrent treatments of cells with SB203580 and IFN-\(\tau\) (5 ng/ml) decreased concentrations of PGHS-2 mRNA in an additive manner. Although IFN-\(\tau\) reduced PGHS-2 mRNA concentrations, phosphorylation of p38 MAPK was induced by IFN-\(\tau\), PdBu, and PdBu combined with IFN-\(\tau\) after 10 min of treatment. Both the p38 MAPK inhibitor and IFN-\(\tau\) decreased prostaglandin F\(_2\alpha\) secretion, and decreases were additive when the two were given together. In summary, activation of p38 MAPK by PdBu is required for continued presence of PGHS-2 mRNA and secretion of prostaglandin F\(_2\alpha\) in BEND cells. Interferon-\(\tau\) mediates a transcription-dependent mechanism, which induces degradation of PGHS-2 mRNA. However, the consequences of an IFN-\(\tau\)-induced activation of p38 MAPK warrant further investigation, because inhibition of p38 MAPK caused a degradation of PGHS-2 mRNA.

cytokines, female reproductive tract, kinases, signal transduction, uterus

INTRODUCTION

The prostaglandin H synthase (PGHS) enzyme catalyzes the conversion of arachidonic acid to prostaglandin (PG) H\(_2\) and is a rate-limiting enzyme for PG production. The two isoforms of PGHS are designated as PGHS-1, which is constitutively expressed, and PGHS-2, which is the inducible isoform [1]. Expression of PGHS-2 can be induced in many different cell types and tissues. Examples include cytokines acting on colonic epithelial cells [2] and endometrial cells [3], FSH and LH in ovarian follicles [4], endotoxins in macrophages [5], and tumor promoters acting on hepatocytes [6].

Normally, PGHS-2 is undetectable in most mammalian tissues, and expression occurs very rapidly. Thus, PGHS-2 is classified as an immediate early gene. In many cases, rapid induction of expression leads to rapid degradation of the mRNA and its protein [1]. Its sustained presence in tissues involves several regulatory mechanisms. In fibroblasts, for example, increased PGHS-2 mRNA results from a higher rate of PGHS-2 gene transcription [7]. Posttranscriptional regulation is also important. For example, serum withdrawal in mammary carcinoma cells increases the stability of PGHS-2 mRNA and, thus, its half-life [8]. Similarly, interleukin-1\(\alpha\) acts on a human cell line to increase the stability of PGHS-2 mRNA [9]. Thus, posttranscriptional mechanisms are required for the sustained presence of PGHS-2 mRNA in cells. Conversely, it is possible that acutely induced inhibition of PGHS-2 gene expression may involve stimulation of PGHS-2 mRNA degradation.

Interferon (IFN)-\(\tau\) is classified as a type I IFN and is secreted by trophoblastic cells of the growing embryo in ruminants [10–12]. It binds to type I IFN receptors on endometrial cells [13] and suppresses oxytocin-induced secretion of PGF\(_2\alpha\) in vivo [14] and from primary endometrial epithelial cells in culture [15, 16]. Suppression of PGF\(_2\alpha\) secretion leads to maintenance of the corpus luteum (CL) to sustain a pregnancy [17]. Studies with a bovine endometrial (BEND) cell line [18, 19] and primary epithelial cells [16] demonstrated that IFN-\(\tau\) decreases concentrations of PGHS-2 mRNA and protein and subsequent PGF\(_2\alpha\) secretion. It was suggested that IFN-\(\tau\) may inhibit transcription of the PGHS-2 gene, because the promoter for the PGHS-2 gene has IFN stimulatory response elements (ISREs) [19, 20]. Type I IFNs also induce transcription of IFN regulatory factor (IRF)-1 and IRF-2 [21], and IRF-1 directly increases expression of IRF-2 [22]. In BEND cells, IFN-\(\tau\) stimulates IRF-1 expression [23]. However, IRF-2 negatively regulates IRF-1 [21]. The IRF-2 is a potent transcriptional inhibitor of IFN-stimulated genes and is expressed in the luminal epithelium of the ovine uterus [24]. Thus, IRF-2 might affect PGHS-2 mRNA expression negatively in BEND cells. An alternative negative regulatory mechanism could be an indirect effect mediated through IFN-\(\tau\)-induced gene transcription of other endometrial proteins that alter transcription rate, turnover of PGHS-2 mRNA, and/or functionality of PGHS-2 protein.

The p38 mitogen-activated protein kinase (MAPK) enzyme regulates the stability of PGHS-2 mRNA. Inhibition of p38 MAPK in different cell types, induced by a variety
of agents, resulted in reduced expression and/or increased turnover of PGHS-2 mRNA [8, 25, 26]. In human monocytes [27] and human alveolar macrophages [5], endotoxin-induced expression of PGHS-2 mRNA was reduced by p38 MAPK inhibitors, suggesting that p38 MAPK activity is required for stabilization of PGHS-2 mRNA. Agents that stimulate expression of PGHS-2 mRNA also induce p38 activity [27]. Use of ceramide in fibroblasts induced activity of extracellular signal-regulated kinase (ERK)/MAPK and p38 MAPK [28]. Similarly, use of phorbol 12,13-dibutyrate (PdBu), a tumor-promoting agent, induced activation of protein kinase C (PKC) and, subsequently, ERK/MAPK and p38 MAPK in human astrocytoma cells [29]. Use of PdBu in BEND cells also activates PKC [30] and leads to activation of ERK/MAPK [19], expression of PGHS-2 mRNA, and secretion of PGF_2α [18, 19].

Treatment of primary bovine endometrial epithelial cells and BEND cells [18] with IFN-γ resulted in a decrease in PGHS-2 mRNA expression. The stability of PGHS-2 mRNA may be regulated by p38 MAPK, and it needs to be characterized within endometrial cells relative to IFN-γ regulation of PGHS-2 mRNA. Our hypothesis is that IFN-γ decreases the concentration of PGHS-2 mRNA via regulation of mRNA degradation because of an IFN-γ-induced decrease in p38 MAPK activity within endometrial cells.

The objectives of the present study were to determine if p38 MAPK is required for sustained presence of PGHS-2 mRNA induced by PdBu and if IFN-γ increases the degradation of PGHS-2 mRNA in BEND cells.

MATERIALS AND METHODS

Materials

Recombinant bovine IFN-γ (1.08 × 10^7 U antiviral activity/mg) was a generous gift from Dr. R. Michael Roberts (University of Missouri, Columbia, MO). TRizol reagent was purchased from Invitrogen Corporation (Carlsbad, CA). Polyethylene terephthalate Costar six-well plates and culture dishes (100 mm × 20 mm) were from Corning Glass Works (Corning, NY). Polyethylene terephthalate culture flasks (185 cm²) were from Sargest Inc. (Newton, NC). Acrylamide, N,N'-methylenebisacrylamide, sodium dodecyl sulfate, and Nonidet-P 40 were purchased from BDH Laboratory Supplies (Poole, U.K.). Coomassie brilliant blue, bromophenol blue, β-mercaptoethanol, NaOH, Tris, Tris-HCl, TEMED, ammonium persulfate, formaldehyde, acetic acid, Tween 20, NaCl, EDTA, NaF, glycerol, glycine, and methanol were from Fisher Scientific (Pittsburgh, PA). The PdBu, Hanks balanced salt (HBSS) Eagle minimum essential medium, antibiotic-antimycotic solution (AbAm), insulin, d-valine, horse serum, aprotinin, leupeptin, pepstatin, NaPO_4, EGTA, Na_2VO_3, PMSF, actinomycin D, and BSA were from Sigma Chemical Co. (St. Louis, MO). TRIzol reagent was purchased from Invitrogen Corporation (Carlsbad, CA). Polyethylene terephthalate Costar six-well plates and culture dishes (100 mm × 20 mm) were from Corning Glass Works (Corning, NY). Polyethylene terephthalate culture flasks (185 cm²) were from Sargest Inc. (Newton, NC). Acrylamide, N,N'-methylenebisacrylamide, sodium dodecyl sulfate, and Nonidet-P 40 were purchased from BDH Laboratory Supplies (Poole, U.K.). Coomassie brilliant blue, bromophenol blue, β-mercaptoethanol, NaOH, Tris, Tris-HCl, TEMED, ammonium persulfate, formaldehyde, acetic acid, Tween 20, NaCl, EDTA, NaF, glycerol, glycine, and methanol were from Fisher Scientific (Pittsburgh, PA). The PdBu, Hanks balanced salt (HBSS) Eagle minimum essential medium, antibiotic-antimycotic solution (AbAm), insulin, d-valine, horse serum, aprotinin, leupeptin, pepstatin, NaPO_4, EGTA, Na_2VO_3, PMSF, actinomycin D, and BSA were from Sigma Chemical Co. (St. Louis, MO). Fetal bovine serum was from Hyclone Laboratories, Inc. (Logan, UT). The P32 Labeled PGHS-2 cDNA was used for Northern blot analyses of the mRNA. Total RNA was isolated from confluent BEND cells with TRIzol according to the manufacturer’s specifications. Twenty micrograms of cellular RNA was fractionated in a 1.5% agarose-formaldehyde gel, stained with ethidium bromide, blotted to BioTrans nylon membrane, and hybridized. The PGHS-2 [33] cDNA was a gift from Dr. J. Siros (University of Montreal, St. Hyacinthe, QC, Canada). The P32-labeled PGHS-2 cDNA was used for Northern blot analyses of the mRNA.

Preparation of Cell Extracts

At the end of the culture periods, plates were transported to a cold room (4°C). The culture medium was discarded, and the cells were rinsed twice in ice-cold PBS. Cells were scraped from plates in the presence of 1 ml of whole-cell extract buffer (50 mM Tris [pH 8.0], 300 mM NaCl, 20 mM NaF, 1 mM Na_2VO_3, 1 mM NaPO_4_2, 1 mM EDTA, 1 mM EGTA, 0.5 mM PMSF, 10% [v/v] glycerol, 0.5% [v/v] NP-40, and 10 μg/mL each of aprotinin, leupeptin, and pepstatin) and then placed on a rotor (Labconco, La Jolla, CA). The extracts were then centrifuged at 10,000 × g for 10 min and the supernatant was collected. Protein concentrations were determined in supernatants according to the method described by Bradford [34].

Western Blot Analysis of Phosphorylated p38 MAPK

Fifty micrograms of protein was loaded onto 10% acrylamide denaturing gel, submitted to SDS-PAGE, and electrophoretically transferred to nitrocellulose membranes. Membranes were blocked for 2 h at room temperature in 1% (w/v) BSA in Tris-buffered saline (TBS; 10 mM Tris-base, pH 7.5) and probed with antibodies against phosphorylated p38 MAPK diluted (1:1000) in 0.1% BSA in TBS with 0.1% Tween 20 (TBS) for 2 h at room temperature. Blots were then incubated with the secondary antibody (anti-rabbit immunoglobulin G; 1:5000 dilution in 0.1% BSA in TBS) for 1 h. Proteins were detected by chemiluminescence and analyzed by densitometry (AlphaImager 2000;Alpha Innotech Corporation, San Leandro, CA).
RESULTS

Effect of IFN-τ and p38 MAPK on Degradation of PGHS-2 mRNA

At 4.5 h, cells exposed to PdBu and to PdBu with actinomycin D had the same PGHS-2 mRNA concentration as cells harvested at 3 h after PdBu treatment (Fig. 1). Addition of IFN-τ for a 1-h period beginning at 3.5 h reduced the abundance of PGHS-2 mRNA ($P < 0.01$) (Fig. 1), and the IFN-τ-induced reduction in PGHS-2 mRNA was blocked by addition of actinomycin D ($P < 0.01$) (Fig. 1). The IFN-τ caused a reduction in existing PGHS-2 mRNA concentration, because expression of PGHS-2 mRNA at 4.5 h of PdBu stimulation was comparable to that at 3 h of PdBu stimulation. Inhibition of p38 MAPK caused by the addition of SB203580 along with PdBu and actinomycin D led to a reduction in PGHS-2 mRNA compared to cells treated with PdBu and actinomycin D together ($P < 0.01$) (Fig. 1). Thus, p38 MAPK appeared to be essential to maintaining stability of the PGHS-2 mRNA. The p38 MAPK inhibitor-induced increase in degradation of PGHS-2 mRNA was comparable to that induced by IFN-τ. Collectively, experimental results indicate that IFN-τ increased the degradation of PGHS-2 mRNA through a mechanism that requires gene transcription. In contrast, p38 MAPK appears to directly sustain the concentration of PGHS-2 mRNA in a manner that is not transcriptionally dependent.

Complementary Effects of IFN-τ and the p38 MAPK Inhibitor to Reduce PGHS-2 mRNA

At the end of the starvation period (0 h), cells were stimulated with PdBu (100 ng/ml). At 3.5 h, IFN-τ (0 or 5 ng/ml) was added to the cells. At 3.5 h, IFN-τ (0 or 5 ng/ml) was added. Cells were collected at 4.5 h for RNA extraction. Autoradiographic bands were scanned for densitometric values. Data were analyzed using values for glyceraldehyde phosphate dehydrogenase as covariate and expressed as the least-squares mean ± SEM. The PdBu-induced, steady-state concentrations of PGHS-2 mRNA were reduced by both SB203580 and IFN-τ ($P < 0.01$). Main effects of SB203580 ($P < 0.01$) and IFN-τ ($P < 0.01$) were detected. Means with different letters are significantly different ($P < 0.01$).

PdBu-Induced Phosphorylation of p38 MAPK

Treatment of cells with either PdBu or IFN-τ for 10 min induced phosphorylation of p38 MAPK ($P < 0.01$) (Fig. 3). The antibody recognizes p38 MAPK that is phosphorylated on threonine-180 and tyrosine-182 residues. Concurrent addition of PdBu and IFN-τ stimulated phosphorylation of p38 MAPK, but no more than when given separately.

Involvement of p38 MAPK in PdBu-Induced PGF2α Secretion from BEND Cells

After starvation, cells were pretreated with SB203580 (0 or 1 µM) for 1 h (i.e., −1 h). At 0 h, PdBu (100 ng/ml) was added concurrently with IFN-τ (0 or 50 ng/ml). Medium was sampled at 6 h for determination of PGF2α con-
The p38 MAPK inhibitor (1 μM) decreased PdBu-induced PGF$_{2\alpha}$ secretion from BEND cells at 6 h ($P < 0.01$) (Fig. 4). The IFN-γ also reduced significantly the PdBu-induced secretion at 6 h ($P < 0.01$) (Fig. 4). Treatment with both the inhibitor and IFN-γ caused a further reduction in the secretion of PGF$_{2\alpha}$, ($P < 0.05$) (Fig. 4). Main effects of p38 MAPK inhibitor and IFN-γ were detected ($P < 0.01$). Responses indicated an additive inhibitory effect of the p38 MAPK inhibitor and IFN-γ on secretion of PGF$_{2\alpha}$.

**DISCUSSION**

Treatment of BEND cells with PdBu induces ERK/MAPK activity, and the MEK1 inhibitor, PD98059, completely blocked PdBu-stimulated PGHS-2 mRNA transcription and PG secretion [19]. The PdBu-induced activation of PKC in BEND cells [30] causes a subsequent activation of the Raf1/MEK1/ERK1/2 signaling pathway with transcription of c-jun and c-fos transcription factors leading to the transcription of PGHS-2 mRNA [19]. The dependency on PKC activation is further supported by the observation that inhibition of PKC activity by a specific inhibitor (e.g., G109203X) blocks the PdBu stimulation of PGF$_{2\alpha}$ secretion in BEND cells. Indeed, PKCoB appears to be the activated isotype of PKC family in BEND cells [30].

In the present series of experiments, the importance of p38 MAPK activity for PdBu-induced secretion of PGF$_{2\alpha}$ was evident in BEND cells. Treatment of cells with PdBu induced a rapid phosphorylation of p38 MAPK at 10 min. Furthermore, preincubation of cells for 1 h with a specific p38 MAPK inhibitor, 1 μM SB203580, decreased PGF$_{2\alpha}$ secretion measured at 6 h after PdBu stimulation. The SB203580 is a highly specific, cell-permeable inhibitor of p38 kinase and does not inhibit significantly other members of the MAPK family, such as ERKs and c-jun N-terminal kinase (JNK), even at 100 μM concentrations (Calbiochem). Dean et al. [27] concluded that the effects of SB203580, at concentrations up to 2 μM, are highly specific for the inhibition of p38 MAPK. A pivotal regulatory role of p38 MAPK in response to PdBu has been emphasized by other studies in a variety of cell types for reducing turnover of mRNAs [29, 37, 38]. Indeed, in the present experiment, inhibition of p38 MAPK with SB203580 increased degradation of PGHS-2 mRNA.

The PGHS-2 is one of the critical enzymes for production of PGF$_{2\alpha}$ by BEND cells. It is an immediate response gene, the expression of which is evident by 30 min after PdBu stimulation [19]. However, PGHS-2 mRNA is degraded rapidly or is highly unstable, with a half-life of 1 h in human lung and kidney cells [39]. Ristimaki et al. [9, 39] suggested that posttranscriptional regulation is important to sustain a PGHS-2 presence and proposed that antiinflammatory responses induced by glucocorticoids increase turnover of PGHS-2 mRNA. Indeed, a role for dexamethasone was indicated for the destabilization of PGHS-2 mRNA via an inhibition of p38 MAPK [40]. Several other studies [8, 25, 27] have demonstrated the importance of p38 MAPK activity to maintain PGHS-2 mRNA stability. In the present experiment, actinomycin D was added to BEND cells after 3 h of PdBu treatment, and the PGHS-2 mRNA levels were measured 90 min after addition of actinomycin D. In cells that were treated with or without actinomycin D, PGHS-2 mRNA levels were constant and not different. Simultaneous addition of the p38 MAPK inhibitor and actinomycin D caused a major decrease in PGHS-2 mRNA levels by 90 min. This response indicates that transcription was not required for stabilization of PGHS-2 mRNA by p38 MAPK and that the SB203580-induced degradation of PGHS-2 mRNA was caused by direct inhibition of p38 MAPK. Thus, p38 MAPK appears to sustain mRNA concentrations of PGHS-2.

The present results as well as those of Pru et al. [19] suggest that PdBu induces both ERK/MAPK and p38 MAPK pathways (i.e., PdBu stimulated phosphorylation of p38 MAPK) to increase PGHS-2 mRNA in BEND cells. It may be that the ERK/MAPK pathway is required for PdBu induction of PGHS-2 mRNA expression and p38 MAPK is activated to sustain the stability of PGHS-2 mRNA. These differential effects of ERK/MAPK and p38 MAPK support the conclusions that p38 MAPK activation alone is not enough to induce PGHS-2 expression [5] and that p38 MAPK is involved in PGHS-2 mRNA stability [26].

The second objective of the present study was to investigate if IFN-γ exerted a role in posttranscriptional regulation of PGHS-2 mRNA. Studies in BEND cells showed that IFN-γ decreased PdBu-stimulated expression of PGHS-2 mRNA by 1 h [19] and that of PGHS-2 protein by 3 h [18]. The IFN-γ may be regulating both transcriptional and non-transcriptional mechanisms to exert inhibitory effects on...
PGHS-2 gene and protein expression as well as secretion of PGF$_{2\alpha}$. Pru et al. [19] failed to demonstrate any inhibitory effect of IFN-$\tau$ on any step of the Raf1/MEK1/ERK1/2 pathway. Because the effect of IFN-$\tau$ on PGHS-2 mRNA is very rapid, examination of the effect of IFN-$\tau$ on p38 MAPK activation is important as an alternative mechanism of nontranscriptional regulation of PGHS-2 mRNA. Contrary to our hypothesis, Western blots for phosphorylated p38 MAPK revealed that IFN-$\tau$ also induced phosphorylation of p38 MAPK. Similarly, IFN-$\tau$ induced phosphorylation and activation of p38 MAPK in bovine primary myometrial cell cultures [41] but also induced PGHS-2 mRNA expression. The IFN-$\tau$ could have differential effects depending on cell types and the amount of IFN-$\tau$ used. For example, Parent et al. [42] demonstrated distinct responses to IFN-$\tau$ (same isoform as that used in the present experiment) depending on the concentration used in primary bovine epithelial cell cultures. At concentrations less than 1 $\mu$g/ml, IFN-$\tau$ suppressed PG secretion, whereas at 20 $\mu$g/ml, PG secretion and expression of PGHS-2 were increased. Moreover, Xiao et al. [31] demonstrated in primary cultures that IFN-$\tau$ decreased PGHS-2 mRNA expression in epithelial cells but increased expression in stromal cells. The IFN-$\tau$ stimulation of p38 MAPK phosphorylation in BEND cells may be related to some other functional roles of IFN-$\tau$. Type I IFNs stimulate p38 MAPK activation in a variety of cell types [43–45]. Uddin et al. [44] demonstrated that the p38 MAPK pathway was required for IFN-$\alpha$-dependent transcriptional activation. Moreover, Mayer et al. [43] and Verma et al. [45] indicated a role for p38 MAPK in mediating the growth-inhibitory effects of IFN-$\alpha$. Li et al. [46] reported that p38 MAPK activation is required for type I IFN-dependent transcription to mediate IFN responses. It was demonstrated that the p38 MAPK and its downstream effectors are essential for transcription of type I IFN-regulated genes that have ISRE and gamma-activated sequence (GAS) elements in their promoters. Perhaps, IFN-$\tau$ activation of p38 MAPK leads to expression of a protein, not yet identified, that may cause an increased degradation of PGHS-2 mRNA.

One-hour treatment of cells with IFN-$\tau$ after previous 3-h stimulation with PdBu was enough to decrease the concentrations of PGHS-2 mRNA. Interestingly, when transcription was blocked by the addition of actinomycin D for 30 min before the addition of IFN-$\tau$ at 3 h after PdBu stimulation, the inhibitory effect of IFN-$\tau$ on PGHS-2 mRNA was blocked. Thus, the decrease in PGHS-2 mRNA caused by IFN-$\tau$ is dependent on transcription. The magnitude of the decrease in the message induced by IFN-$\tau$ (50 ng/ml) was similar to that caused by addition of p38 MAPK inhibitor. As mentioned previously, PdBu-treated cells with or without actinomycin D had similar PGHS-2 mRNA concentrations. Thus, the IFN-$\tau$ effect to decrease PGHS-2 was through an increased degradation of the PGHS-2 mRNA and was transcription dependent. This effect was independent of that induced by the inhibition of p38 MAPK, because the p38 MAPK inhibitor acted in the presence of actinomycin D (SB203580 acts in a similar fashion in the absence of actinomycin D; data not shown). The SB203580-induced decrease in stability of PGHS-2 mRNA is attributed to an inhibition in p38 MAPK activity. In contrast, IFN-$\tau$ induction of an increase in degradation of PGHS-2 mRNA required transcriptional products that are yet to be identified. In the BEND cell model, the transcriptional effect of IFN-$\tau$ and the nontranscriptional effect of the p38 MAPK inhibitor on degradation of PGHS-2 mRNA ultimately caused additive and independent inhibitory effects on PGF$_{2\alpha}$ secretion.

Binelli et al. [23] demonstrated that IFN-$\tau$ induced tyrosine phosphorylation, homo- and heterodimer formation, nuclear translocation, and DNA binding of signal transducer and activator of transcription (STAT) proteins 1, 2, and 3 in BEND cells. Expression of IRF-1 protein was evident at 1 h after IFN-$\tau$ treatment in BEND cells [23]. The IFN-$\tau$ induced gene transcription in bovine and ovine uteri [47–51]. One product of IFN-$\tau$-induced transcription is ISG17 [52]. The ISG17 gene encodes a protein, ISG17, which is a 17-kDa ubiquitin homologue. This protein was originally named ubiquitin cross-reactive protein, because it shared amino acid sequence homology with ubiquitin [48]. ISG17 undergoes conjugation with cytosolic uterine proteins [53]. However, to our knowledge, these cytosolic proteins have not yet been identified. Such conjugations may occur with some proteins, which regulate stability of specific mRNAs. Stability of mRNA is regulated by AU-rich sequences within the 3'-untranslated region (UTR) [54], and the PGHS-2 transcript possesses these sequences in the 3'-UTR [33]. Specific proteins, such as HuD, HuR, and AUFI, bind to the 3'-UTR region of mRNAs and regulate their stability either positively or negatively [54–56]. Perhaps an IFN-$\tau$-induced protein, such as ISG17, could alter the availability of such proteins, leading to a destabilization or degradation of PGHS-2 mRNA even though p38 MAPK is activated by IFN-$\tau$.

Earlier studies indicate that low concentrations of IFN-$\tau$ inhibit secretion of PGF$_{2\alpha}$ and expression of PGHS-2 that is reversed by high concentrations of IFN-$\tau$ [42, 57]. In vivo production of IFN-$\tau$ is correlated positively with embryo size. Well-developed embryos produce a sufficient amount of IFN-$\tau$ to inhibit pulsatile secretion of PGF$_{2\alpha}$ and luteolysis through a block in expression of the oxytocin receptor (OTR) [58, 59]. In maintained pregnancies, expression of PGHS-2 is increased in the bovine endometrium [60, 61]. In pregnant mammals, PGHS-2 is needed for pregnancy-associated events, such as regulation of localized immune function, angiogenesis and regulation of blood flow, and development of implantation sites [62–65]. The increased expression of PGHS-2 is reflected by an increase in basal secretion of PGF$_{2\alpha}$ in the bovine [66, 67]. The potential to secrete luteolytic pulses, which would antagonize pregnancy in cattle, is abolished by an absence of OTRs because of IFN-$\tau$ [58, 59]. Xiao et al. [16] documented that even nanogram concentrations of IFN-$\tau$ could lower oxytocin-binding capability of cultured bovine epithelial cells. Thus, in embryos with low IFN-$\tau$ secretory potential that are destined to die (i.e., reduced embryo size at Day 17), pulsatile secretion of PGF$_{2\alpha}$ would be decreased, leading to a temporal delay in luteolysis. Van Cleef et al. [68] reported significant increases in the proportion of heifers with extended cycles that were inseminated but subsequently not diagnosed as pregnant (e.g., experienced embryo mortality) compared to noninseminated cyclic heifers. Consequently, low concentrations of IFN-$\tau$ would inhibit the pulsatile secretion of PGF$_{2\alpha}$ via impairment in oxytocin signaling as well as an increase in the degradation of PGHS-2 mRNA. Pregnancy will not be maintained because of an absence of PGHS-2 for implementation of PG-dependent pregnancy processes, but the temporary absence of pulsatile secretion of PGF$_{2\alpha}$ contributes to a delay in CL regression that is finally overridden by induction of OTR as a default in the absence of IFN-$\tau$.

In conclusion, the negative effects of IFN-$\tau$ on PdBu-
induced expression of PGHS-2 mRNA and secretion of PGF2α in BEND cells seems to be transcription dependent. This effect possibly was exerted partially by increased degradation of the PGHS-2 mRNA in BEND cells. Also, PdBu acts to activate the p38 MAPK pathway along with the ERK/MAPK pathway to stimulate rapid activation of the PG cascade.

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