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# ENDOMETRIAL CELL SPECIFIC GENE ACTIVATION DURING IMPLANTATION AND EARLY PREGNANCY

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## TABLE OF CONTENTS



## 1. ABSTRACT

Human endometrium expresses numerous genes to achieve an optimal uterine environment for implantation and maintaining the pregnancy. In this review, we will summarize our previous observations on progestin regulated gene expression, estrogen metabolic enzymes, nitric oxide synthase, aromatase, IGF-I and II, IGFBP-1, prolactin and glycodelin. These genes are differentially activated in two types of endometrial cells during the menstrual cycle and early pregnancy. Multiple gene activation driven by progestin appears to be the major event responsible for the differentiation of endometrial cells. They play critical roles of endometrial cell function during implantation and pregnancy.

## 2. INTRODUCTION

Growth and differentiation of human endometrial glandular and stromal cells are cell-specific both temporally and spatially, although they are regulated by the same hormonal milieu produced by ovary. Endometrial

glandular epithelial cells proliferate in the follicular phase under the influence of estrogen. After ovulation, progesterone arrests the cell growth and subsequently promotes multiple-gene activations and cell differentiation. The endometrial stromal cells are quiescent with a moderate cell growth during the proliferative phase. In a fertile cycle, stromal cells undergo a second rapid growth accompanied by decidualization under the influence of higher levels of progesterone whereas glandular cells become attenuated in mid-late secretory phase. Differentiation of glandular cells is critical to prepare an optimal receptive stage for embryo implantation. Decidualization of stromal cells is essential for the attachment of the embryo. This attachment remains firm between decidua parietalis and the fetal membrane throughout gestation. The gestational endometrium is dominated by decidua parietalis containing well-differentiated decidual cells. The residual glands recede to the basalis and continue to secret high level of glycodelin (1) throughout the first trimester and they are viable in the decidual tissue at the term of gestation.

Progesterone induces numerous genes in endometrial glands and stromal cells during the secretory phase and gestation in a cell specific manner. The gene products, ranging from glycogen, growth regulating factors, hormones, steroids hormone metabolic enzymes, structural and matrix proteins and protease/protease inhibitors, are critical for the unique morphology of endometrium/decidua and play critical functional roles during implantation and gestation. Asynchronous development of glandular/stromal cells prevents embryo implantation and causes dedifferentiation of decidual cells, therefore disrupting the pregnancy. In this article, we summarize the progesterone regulation of a number of genes which are differentially expressed in endometrial glandular and/or stromal cells.

## 3. ENDOMETRIAL GLANDULAR CELLS GENE ACTIVATION

Numerous gene products have been identified in the human endometrial glandular cells during the menstrual cycle. We have made observations on the regulation of estrogen metabolic enzymes, nitric oxide system and glycodelin production. They are summarized below.

### 3.1. Induction of estrogen metabolic enzymes by progestin

17ß-hydroxy steroid dehydrogenase (17ß-HSD) type 2 and estrogen sulfotransferase (ESFT) are highly expressed in the endometrial glands in the secretory phase. 17ß-HSD catalyzes the interconversion of estradiol (E2) and estrone (E1) and ESFT converts the estrogen to estrogen sulfate which has little estrogenic activity.

The activity of 17ß-HSD increases >10 fold from proliferative to secretory endometrium (2). *In vitro*, we have shown that the progestin induced 17ß-HSD activity in a time- and dose-dependent fashion (3). Recently, Yang et al., have shown that the 17ßHSD promoter is activated by stromal factor induced by hPR (4) indicating that progesterone induced-protein synthesis in stromal cells is essential for the activation of 17ß-HSD in endometrium.

ESFT is present in the glandular epithelial cells of the secretory endometrium (5). The conversion to estrogen sulfate from free estrogen is not detectable in the proliferative phase and is active in the secretory phase (5). Progestin induced ESFT activity in glandular cells and in endometrial adenocarcinoma cell line, Ishikawa cells (6, 7). Estrogen sulfatase, catalyzing the conversion of estrogen sulfate to free estrogen, is present in both glandular epithelial and stromal cells (8) and it is induced during decidualization of the endometrial stromal cells (9).

17ß-HSD, ESFT and estrogen sulfatase regulate the intracellular concentration of E2. Among the three enzymes, 17ß-HSD is active during the entire menstrual cycle in both types of endometrial cells and its activity is intensified in glands of the secretory phase. 17ß-HSD, however, does not completely deplete the intracellular E2 since it catalyzes the interconversion between E1 and E2. The ESFT converts E2 to estrogen sulfate whereas estrogen sulfatase hydrolyzes the estrogen sulfates. Kinetic analysis showed that the $K_m$ for ESFT is ~2-20 nM, whereas the $K_m$ for estrogen sulfatase is 2 uM (8). The difference between the two $K_m$ values implies that intracellular conversion of E2 to estrogen sulfate is almost unidirectional at a lower E2 concentration, e.g., E2 levels in circulation or in endometrium. Indeed, a much lower level of E2 was detected in the secretory endometrium (8 and 1.8 pmol/gram of tissue in proliferative and secretory endometrium respectively; 10, 11). Therefore, induction of ESFT in the epithelial glands by progesterone intensifies the removal of E2 from endometrial glands. In addition, the lower $K_m$ of ESFT indicates that ESFT has high affinity to E2 and therefore it may compete with ER for the binding of E2. Since the levels of E2 are lower than the ER content in human endometrium (8 and 1.8 vs 25 and 7 pmol/ g of tissue in proliferative and secretory endometrium respectively, (10-12), the induction of the ESFT efficiently reduces the E2 levels and controls the extent of estrogenic actions in endometrial cells.

### 3.2. Nitric oxide synthase (NOS) and nitric oxide (NO) release in human endometrial glands

Nitric oxide (NO), a short-lived product of the enzymatic conversion of L-arginine to citrulin catalyzed by nitric oxide synthase (NOS), regulates multiple intracellular signaling pathways by interacting with redox systems, such as iron, thio groups, superoxide anion and cGMP. Human endometrial glandular epithelial cells exhibited NOS activity, i.e., NADPH-diaphorase histochemistry selectively labeled epithelial glands in the late secretory phase (13). Northern blot analysis revealed that glandular cells expressed a single size of endothelial NOS (eNOS) mRNA (4.5 kb). During the menstrual cycle, epithelial glands from early secretory endometrium showed a greater expression of eNOS mRNA. The highest eNOS expression was found in the glands of late secretory endometrium (Table 1) (13), which suggests that NO plays a role in the onset of menses. The cyclic variations of the mNRA levels suggest that eNOS gene expression is regulated by steroid hormones and locally produced peptide hormones. We found that cells incubated with relaxin (RLX) increased the eNOS mRNA content (13).

**Table 1.** Relative amounts of eNOS mRNA in the endometrial epithelial glands

| Tissue | No. specimens | eNOS mRNA |
|---|---|---|
| proliferative | 6 | 1 ± 0.6 (x±s.d.) |
| early secretory | 6 | 4 ± 3 |
| mid secretory | 5 | 0.6 ± 0.3 |
| late secretory | 6 | 8 ± 2 |

Adapted from reference 13

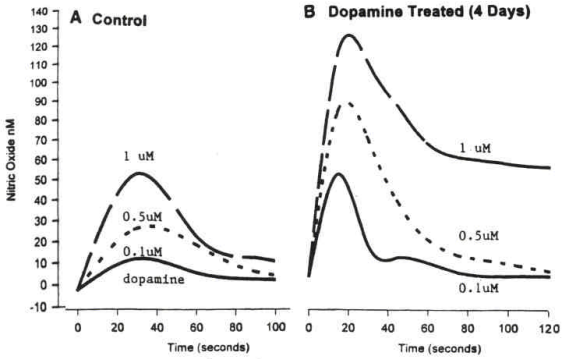


**Figure 1.** Effect of dopamine on NO release in human endometrial glandular epithelial cells. Glandular epithelial cells (proliferative) were cultured for 4 days in medium supplemented without (control) or with dopamine (1 μM). Cells from each culture condition were harvested and suspended in 0.5 ml serum free medium ($5x10^5$ cells) and equilibrated with NO-sensor for 30 min, to adjust the baseline. Various doses of dopamine were added to the cell suspension and NO release was recorded for one hour by an NO-selective microprobe (ISO-NO system; World Precision Instruments, World Precision Instruments [WPI], Sarasota, FL). (14).

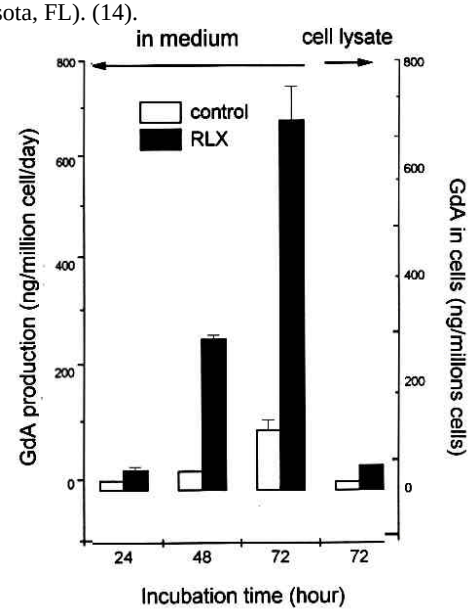


**Figure 2.** Effect of RLX on the production rate of GdA in human endometrial glandular cells. Glandular cells were isolated from an early secretory endometrium and incubated with 100 ng/ml of RLX. Culture media were collected after 24, 48 and 72 h of incubation. Cells lysates were collected at the end of 72 h of incubation (23).

Of the substances that trigger the NO release, we found that dopamine and morphine stimulate a transient surge of NO release whereas estrogen, progesterone or RLX had no acute effect of NO release (14). NO release induced by dopamine or morphine was dose-dependent (14). Cells treated with dopamine for 4 days enhanced the dopamine-induced NO release, 4- to 6- fold, with the peak of the NO surge shifting from 35 to 15 sec (Figure 1). Although RLX did not acutely trigger the NO release, RLX enhanced the capacity of NO release (14). Dopamine and RLX are locally produced in the fetal maternal compartment (15, 16), thus, NO release may occur *in vivo*. It is likely that NO release has a great impact on the development of the vascular system in endometrium, since NO promotes endothelial cell proliferation, migration and neo-vascular angiogenesis. However, excessive NO release causes abnormal bleeding. The regulation of NO release should be further investigated to ascertain its physiological role in endometrium.

### 3.3. Glycodelin secretion in endometrial glands

Human endometrium and decidua secrete glycodelin-A (GdA), previously known as placental protein-14, pp14. In normal ovulatory cycle, GdA secretion begins 4-5 days after ovulation. The production rate of GdA continues to increase in decidua during early gestation (17-19). Temporal expression of GdA is critical for fertility regulation since GdA inhibits the binding of sperm to the zona pellucida (20) and its immunosuppressive activity enhances implantation (21). To facilitate reproduction, an optimal expression of GdA requires a minimal production rate at the time of fertilization followed by an immediate increase during the process of implantation as it occurs in a natural reproductive cycle. However, infertility associated with fertilization or implantation occurs readily which may be, in part, caused by irregular GdA production. Thus, it is important to identify factors that regulate the endometrial cells GdA gene activation.

GdA gene activation has been associated with progesterone since only the progesterone-sensitized endometrium in the luteal phase induces the GdA secretion (17). Serum GdA levels, however, did not correlate to the serum progesterone levels (22). Thus, other factors must be involved for induction of GdA in glandular cells (23). We have shown that RLX stimulates GdA production in human glandular epithelial cells. *In vitro,* cells incubated with RLX consistently increased the GdA production rate (2-6 fold, Figure 2) and the GdA mRNA content (2-11 fold). In contrast, a lesser effect (~40% increase) was seen when cells were incubated with progestin. *In vivo,* RLX given to women with normal menstrual cycle increased their circulating GdA concentrations (24). These data indicate that RLX stimulates GdA production *in vivo* and *in vitro*. Hausermann *et. al*., (26) reported that estrogen and progesterone stimulated uterine GdA production when baboons were pretreated with hCG indicating multiple factors regulate the GdA expression.

For the transcriptional regulation, we found that Sp1 transcription factor activates the basal promoter

activity. In addition, ligand activated hPR modulated the glycodelin promoter activity through the sp1 sites in human endometrial adenocarcinoma cells (25). These observations indicate that the effect of progesterone on GdA expression involves multi-factors rather than through a simple pathway.

## 4. ENDOMETRIAL STROMAL CELLS GENE EXPRESSION DURING DECIDULALIZATION

Endometrial stromal/decidual cells grow continuously throughout the gestation to accommodate the growing embryo/fetus during pregnancy. Thus, decidualization of endometrial stromal cells involves both proliferation and differentiation, spanning from mid secretory phase of the menstrual cycle to the term pregnancy. To analyze the process of decidualization, we have developed a culture system to decidualize the stromal cells in the absence of embryo and glandular epithelial cells. Stromal cells are morphologically transformed into decidual cells after 20 to 30 days' incubation with progestin and RLX (27). Transmission and scanning electron microscopy revealed that *in vitro* decidualized stromal cells were abundant with parallel channels of rough-surfaced endoplasmic reticulum and gap junctions. The intercellular space was enlarged and abundant in secretory granules which were attached to the projected endoplasmic reticular firbrils (27). The overall structures are almost identical to 6-10 week gestational decidual cells (28). The endometrial stromal/decidual cell culture provides a model to dissect the process of decidualization caused by multiple gene expression.

To illustrate the biochemical changes in this system, we classified decidualization as the growth phase and the differentiation phase. In the growth phase, stromal cells proliferate and transform into predecidual cells (5 to 10 days progestin exposure). In the differentiation phase, terminally differentiated decidual cells are formed (after 10-15 days progestin and RLX exposure). During the growth phase, progestin transiently induces DNA synthesis, aromatase, IGF-I, fibronectin (FN) and a moderate amount of prolactin (PRL) and insulin-growth factor binding protein-1 (IGFBP-1, pp12). In the differentiation phase, progestin reduces DNA synthesis and constitutively induces IGF-II, RLX, FN and large amounts of PRL and IGFBP-1. The induction patterns of these genes and their products regulated by progestin and other factors are summarized below.

### 4.1. IGF-I and II mRNA expression regulated by progestin

The effect of progestin and anti-progestin was studied during decidualization of endometrial stromal cells in long-term culture (29). During the first 10 days of culture, medroxy progesterone acetate (MPA) stimulated the expression of IGF-I mRNA by >4 fold in pre-decidualized cells. IGF-I mRNA decreased to the basal level in prolonged culture when cells were decidualized. In contrast, MPA suppressed the IGF-II mRNA level by 60% in predecidual cells. Anti-progestin RU486 reduced IGF-I mRNA by 50-90% and transiently increased IGF-II mRNA in progestin-pretreated cells. IGF-II was highly expressed after 20 days incubation with MPA (~5 fold increase from Day 5-10 to Day 20 of culture). These observations indicate that progestin and anti-progestin differentially regulate the IGF-I and IGF-II mRNA levels in human endometrial stromal/decidual cells.

### 4.2. DNA synthesis regulated by progestin, IGF-I and II, IGFBP-1

Endometrial stromal cells proliferate under the influence of progestin and IGF-I and II (30). Endometrial stromal cells treated with IGF-I or IGF-II or progestin increased their rate of DNA synthesis 3-6 fold. In a time study IGF-I or IGF-II stimulated DNA synthesis 3 fold in 24 hours. An equivalent increase by MPA required 48 hours. IGF-I stimulated DNA synthesis was inhibited completely by IGFBP-1. Non-phosphorylated and phosphorylated forms of IGFBP-1 were equipotent inhibitors of IGF-I stimulated DNA synthesis. IGFBP-1 also inhibited progestin-stimulated DNA synthesis. Phosphorylated IGFBP-1 was a 3-4 fold more potent inhibitor of progestin stimulated DNA synthesis than the non-phosphorylated form. In long term culture of the endometrial stromal cells, progestin exerts a biphasic effect on DNA synthesis, i.e., stimulation at an early stage of cell culture (less than 10 days in culture) and inhibition in advanced stages (beyond 10 days). Progestin induced DNA synthesis was inhibited completely by an equimolar concentration of RU486. Relaxin also inhibited progestin-enhanced DNA synthesis after 7 and 14 days of incubation. The inhibitory effect of RLX is likely caused by enhanced production of IGFBP-1. These observations indicate that progestin regulate an autocrine growth control loop in the endometrium that involves IGFs, IGF receptors and IGFBP-1.

### 4.3. Aromatase activity in endometrial stromal cells

Progestin induces the aromatase activity in endometrial stromal cells. The aromatase activity stimulated by progestin reached a maximum between 2-5 day of incubation with a subsequent decline in prolonged culture. The stimulation was enhanced by estrogen, RLX or the cAMP stimulating agent, forskolin and inhibited by RU486 (31-33). An optimal condition to stimulate aromatase in culture was at the concentration of progestin (0.01 –0.1 uM) and estrogen (0.04 – 40 nM) similar to the plasma concentrations of these steroids after conception and in the early gestation (34) suggesting aromatase *in situ* increases the E2 levels in stromal cells, therefore, the estrogenic effects. Aromatase was also found in stromal cells of adenomyosis and endometriosis (35, 36) suggesting that E2 synthesized *in situ* may have an adverse effect on adenomyosis and endometriosis.

### 4.4. Up-Regulation of progesterone receptor by progestin

Down-regulation of the progesterone receptor (hPR) by its ligand has been demonstrated in breast cancer cell lines and in rat uterus. However, in the endometrial stromal cells, reduction of the PR level is not apparent in the luteal phase of the menstrual cycle. We have determined the effect of progestin on hPR and PR mRNA

in human endometrial stromal cells (37). Western blot analysis showed that progesterone or MPA increased the two isoforms, hPR-A and hPR-B, in stromal cells but reduced them in glandular epithelial cells (37). Progestin increased the hPR-A and hPR-B mRNA by 2- to >10-fold in the stromal cells of 12 specimens. A time study showed that the increase in hPRmRNA required at least 2-3 days' incubation with progestin and that the high mRNA levels, especially hPR-A, were maintained beyond 10 days of progestin incubation. The stimulatory effect of progestin was inhibited by RU486 and by cycloheximide, suggesting that the up-regulation requires a ligand binding and *de novo* protein synthesis. Progestin also increased the stability of PRmRNA in endometrial stromal cells. These results demonstrated that progestin exerts an up-regulation of hPR by increasing the steady-state level of PR mRNA specifically in human endometrial stromal cells.

In addition, we found that hPRs auto-regulates the promoter activity of the hPR gene. The ligand-activated hPR induced the basal promoter activity mediated through Sp1 site in the promoter region between -55 to +31 bp. Our study showed that hPR enhanced the binding of dephosphorylated Sp1 to the Sp1 site (MCE, in press).

**4.5. Fibronectin gene activation in human endometrial stromal cells**

Fibronectin (FN) is one of the major components of the matrix proteins, which forms the decidual basement membrane. We found that progestin induced the synthesis of FN and FN mRNA content in human endometrial stromal cells (38). Immunofluorescence staining showed that fibronectin was uniformly distributed in the intracellular and extracellular regions in stromal cells treated with MPA for 14 days. MPA or progesterone increased the synthesis and secretion of FN dose dependently. MPA was more effective than progesterone and the stimulation was inhibited by the anti-progestin, RU486. The maximal stimulation occurred after 6 days treatment with MPA. Prolonged culture beyond 16 days reduced the rate of synthesis and secretion to 40% of the maximum. Fibronectin mRNA content in stromal cells was also increased by MPA with the maximal level maintained between 6-15 days of incubation and reduced to less than 40% afterward. The profile of the induction in mRNA content coincides with the FN production rate in human endometrial stromal cells.

**4.6. Endometrial stromal/decidual cell PRL and IGFBP-1: secretion patterns, mRNA levels and rate of transcription**

Secretions of PRL and IGFBP-1 from human endometrium is a continuous process spanning from the mid-secretory phase to the entire gestation. The secretion patterns of PRL and IGFBP-1 and mRNA content induced by progestin and RLX have been investigated in a long-term culture of endometrial stromal cells (19, 39, 40). Secretions of PRL and IGFBP-1 were undetectable in un-stimulated stromal cells and a moderate increase in predecidual cells. After sequential administration of MPA and RLX for a month, daily production PRL and IGFBP-1 reached 1 and 50-100 ug /$10^6$ cells respectively (19, 39). In these cultures, cells exhibited morphological changes consistent with pre-decidual and decidual cell formations (1 to 8 days and 10 to 30 days incubation respectively) indicating that endometrial cells are producing growth regulating factors, such as TGFß and RLX to enhance the morphological changes as well as the gene expression of PRL and IGFBP-1.

Both PRL and IGFBP-1 undergo post translational glycosylation. In addition, IGFBP-1 synthesized in endometrial stromal/decidual cells is highly phosphorylated at multiple serine sites (41). Phosphorylation stabilizes the IGFBP1 and its ability to inhibit the progestin enhanced DNA synthesis (30). PRL and IGFBP-1 mRNA contents were increased by MPA and RLX to different extent in long term culture (~100 and ~1000 fold, Fig.3) even though they are regulated by the same set of hormones. Different degree of induction is likely controlled by the promoter context of PRL and IGFBP-1.

Although decidual and pituitary secrete the same PRL protein, the transcriptional regulation is fundamentally different in the two types of cells. The decidual PRL transcription initiation site is located 6 kb upstream of the pituitary specific cap site (42). Thus, it is expected there are different sets of regulatory elements in the two systems. Gellersen et al, (43-45) have shown that protein kinase A is essential for the activation of decidual PRL gene. Other active sites are still under investigation.

**4.7. Differential Effect of RU486: super induction of IGFBP-1 and PRL**

As described in the previous sections, progestin stimulates DNA synthesis, IGF-I/IGF-II, aromatase and hPR, IGFBP-1 and PRL. Thus, anti-progestin, such as RU486, is likely to inhibit the gene activation. We found that RU486 alone had no effect on these genes. However, in progestin-primed endometrial stromal cells, RU 486 caused a super induction of PRL and IGFBP-1 (3 to 30 fold after one day incubation of RU486) (40, 46). On the other hand, DNA synthesis, IGF-I/IGF-II and aromatase were immediately inhibited by RU486 (29, 30, 33) . Induction of PRL and IGBP1 by RU486 may be mediated through the membrane progesterone receptor (mPR), although conventional nuclear hPR is also essential for the gene expression. The endometrial mPR, however, has not been identified and requires further investigation.

**4.8. Regulation of IGFBP-1 promoter activity in human endometrial stromal/decidual cells**

IGFBP-1 is the most active gene expressed during the progressive decidualization. It is constitutively expressed in decidual cells in the hormonal milieu of pregnancy. Thus, IGFBP-1 gene promoter provides a system to identify functional transcription factors specifically active in endometrial stromal/decidual cells.

The transcription cap site of the IGFBP-1 gene is identical in the decidua and the liver (40). We found that

**Table 2.** Regulation of hIGFBP-1 promoter by the Nuclear Proteins In Hepg2 And Stromal/Decidual Cells

| Cis-element | Location | Trans-activators | |
|---|---|---|---|
| | | **HepG2** | **Stroma/decidua** |
| CCAAT | -72 bp | HINF1(activation) | NF-Y(repression) |
| PRE1/PRE2 | -186/-95 bp | GR(activation) | PRA/PRB(activation) |
| IRE | -103 to >200bp | IREBP (repression) | (IREBP?) (repression) |
| Sp1 | -2.8,-2.7,-2.6kb | (unknown) | Sp1/Sp3 (activation) |

the two systems share a few common active sites, CCAAT, PREs/GREs and CRE in the proximal promoter region (Table 2). However, decidual cells contain transcription factors that are able to activate the distal promoter region, which is silent in HepG2 cell. The interaction between cis- and trans-activators regulated by nuclear proteins in stromal/decidual cells are summarized below.

The promoter activity derived from p3.6CAT (3600 bp IGFBP-1 promoter 5' to the CAT reporter gene) was minimal in un-stimulated cells. A time study over a 13 day period of culture showed that the promoter activity increased exponentially to $>10^4$ fold in cells treated with MPA and RLX, which parallels the IGFBP-1 secretion pattern and mRNA accumulation (Figure 3). Deletion analysis showed that two regions in the IGFBP-1 gene promoter are responsible for the activation. (47-49). The basal promoter region between -1 to -300 bp contains multiple sections of regulatory sequence including a cis-element CCAAT (-72 bp), PRE1 / PRE2 and CRE sites. In HepG2 cells, CCAAT was activated by HINF-1, a hepatic nuclear protein that is not expressed in endometrial cells. We found that in stromal/decidual cells NF-Y down regulated the promoter activity (50). The endogenous NF-Y mRNA was reduced by progestin (50). Thus, different sets of transcription factors regulate the CCAAT function in two types of organs, i.e., activation by HINF-1 in HepG 2 cells and repression by NF-Y in stromal cells. Progestin reverses the repression by reducing the endogenous NF-Y (50).

The PRE1/PRE2 (GRE1/GRE2) sites were active in HepG2 cells co-transfected with glucocorticoid receptor and dexamethesone (51). The same PRE sites (Table 2) were activated by endogenous hPR or when co-transfected with hPR expression vector in the progestin-treated stromal cells (52).

Insulin and IGF-I inhibit the transcription of hIGFBP-1 gene and was found to be mediated through the IRE site (insulin response element) (51). The nuclear protein (IRE-BP) that interacts with IRE is still under investigation.

The major difference of IGFBP-1 gene activation in the gestational endometrium and in the hepatic system lies in the regulatory sequence in the distal promoter region. The region between -2.6 to -3.4 kb mediates >95% of the total promoter activity derived from -3.3 kb to +68bp (19,21). The distal promoter region appears to be specifically activated in decidual cells and little activity was found in HepG2 cells (47). This region contains multiple Sp1 sites. Binding analysis revealed that that Sp1/Sp3 bound to this region. Further investigation is needed to evaluate the enormous power of activation in this region that occurs only in decidual cells.

## 5. DIVERISIFIED FUNCTIONS OF NUCLEAR hPR IN HUMAN ENDOMETRIAL CELLS

Progesterone is the key steroid hormone that is responsible for the activation of those genes in endometrial glandular/stromal cells described in the previous sections. Research has been focused on the ligand binding to the nuclear receptor hPR-A and hPR-B. In endometrial stromal/decidual cells, the ligand activated hPR stimulates the IGFBP-1 transcriptional activity through its binding to PRE1/PRE2 sites (Table 2) (53). We have shown that hPR-A isoform is a stronger trans-activator than hPR-B for the activation of the IGFBP-1 gene. The promoter activity increased by the hPR-A was significantly higher than hPR-B (52), in contrast to the observations in several endocrine sensitive breast cancer cell lines in which hPR-B is a stronger transactivator and hPR-A acts as a repressor (54). These observations indicate that hPR-A and hPR-B can have dissimilar effects on different promoters in different cell types. Activation by hPR-A is physiologically relevant since the hPR-A is the dominant isoform in decidualized stromal cells (37).

hPR function is not limited to the interaction with the PRE site. We found that ligand activated hPR modulates the glycodelin (25), hPR (MCE, in press) and fibronectin (unpublished observations), all of which were mediated through the Sp1 sites. Our study indicates that dephosphorylated Sp1 interacts with hPR and subsequently enhanced the binding to the DNA, thus increasing the promoter activity (MCE, in press). Sp1 enhanced activity is just one of the many examples to illustrate multifunctional roles of hPR. In endometrial cells, we and others have shown that progesterone stimulates numerous genes essential for the transformation of stromal cells into decidual cells in vitro (27) and in vivo However, PRE containing genes are limited. Thus, interaction of PR with other transcription factors provides diversified routes to mediate the progestational functions.

Steroid hormone receptor interaction with other transcription factors becomes seemingly important as it expands the functions of the nuclear receptor to numerous target genes lacking hormone responsive elements (25, 55-57). Such interaction, however,

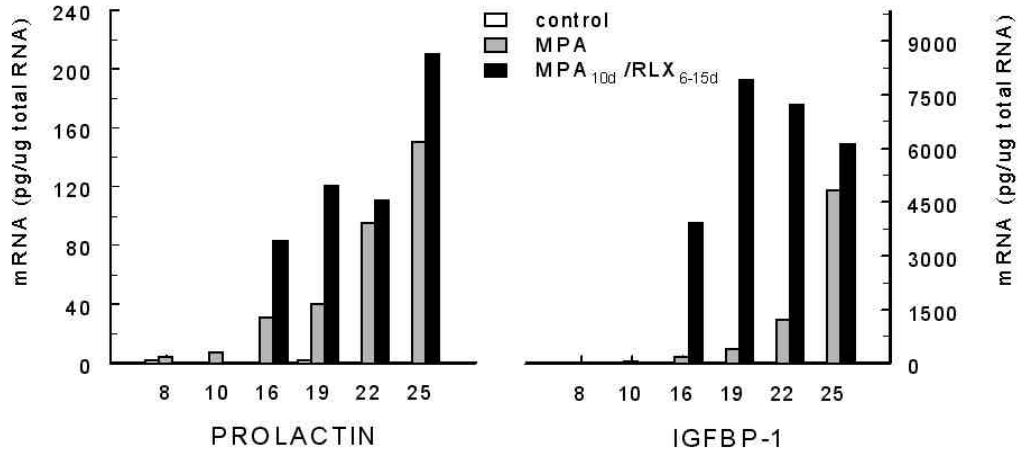


**Figure 3.** Stromal cells isolated from a secretory endometrium were incubated with or without hormone. RNA was subjected to Northern blot analysis. The amounts of mRNA were estimated from a standard curve constructed from PRL and IGFBP-1 sense RNA. Adapted from reference (7).

requires individual investigation since they involve diversified mechanisms.

## 6. ACKNOWLEDGMENTS

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