PLACENTAL HORMONES

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HUMAN TROPHOBLASTS *IN VIVO*: THREE DIFFERENTIATION PATHWAYS

Trophoblasts are unique cells derived from the outer cell layer of the blastocyst which mediate implantation and placentation. Depending on their external environment, undifferentiated cytotrophoblasts can develop into **1) hormonally active villous syncytiotrophoblasts, 2) extravillous anchoring trophoblastic cell columns, or 3) invasive intermediate trophoblasts**[1] (Fig 1). Studies utilizing cultured cytotrophoblasts are beginning to elucidate the specific factors that mediate these pathways of trophoblast differentiation. This chapter will review the differentiation pathways of the cytotrophoblast, what is known about the factors that regulate trophoblast differentiation, the model systems used to study trophoblast biology, and the various hormones that have been shown to be made by these trophoblasts, both *in vitro* and *in vivo*.

Villous syncytiotrophoblast

The hormones secreted by the villous syncytiotrophoblast are critical for maintaining pregnancy[2,3]. Early in gestation, human chorionic gonadotropin (hCG) is essential to maintain corpus luteum progesterone production. Near the end of the first trimester, the mass of villous syncytiotrophoblast is large enough to make sufficient progesterone and estrogen to maintain the pregnancy. During the third trimester, large quantities of placental lactogen are produced, a hormone purported to have a role as a regulator of lipid and carbohydrate metabolism in the mother. Other syncytiotrophoblast products, to name a few, include pregnancy specific $ß_1$-glycoprotein[4], plasminogen activator inhibitor type 2[5], growth hormone[6], collagenases[7], thrombomodulin[8,9], and growth factor receptors[10,11,12]. The factors responsible for the regulated synthesis of these compounds has been the subject of a great deal of investigations, some of which will be reviewed below.

In vitro experiments have identified several compounds which are capable of differentiating cultured cytotrophoblasts towards an endocrine phenotype. These include cAMP[13,14,15], EGF[16] and hCG itself[17]. Cyclic AMP has been shown to upregulate hCG and progesterone secretion. In the case of hCG, the mechanism appears to be a direct upregulation hCG gene transcription via a cAMP regulatory region of the genome. For progesterone, increased synthesis appears to be due to a concerted upregulation of a number of enzymes responsible for progesterone biosynthesis, including the side chain cleavage enzyme and adrenodoxin complex—the first steps in the conversion of cholesterol to progesterone. Not only do these compounds upregulate hormone secretion, they also appear to down-regulate the synthesis of markers of the other pathways of trophoblast differentiation. For example, in the presence of 8-bromo-cAMP, cultured trophoblasts are induced to secrete large quantities of hCG[14]. At the same time, their synthesis and secretion of the trophoblast form of fibronectin, trophouteronectin[18]—a marker of junctional

trophoblasts (see Fig. 1)—is turned off[15]. This result suggests that mutually exclusive differentiation pathways result from stimulation by appropriate factors.

Trophoblasts seem to make more than one hormone at the same time—a difficult task for a cell. Once stimulated to become hormonally active, the trophoblast seems capable of producing at least two glycoproteins simultaneously[19], although electron microscopic immunochemistry has demonstrated that these products are located in different secretory vacuoles within the same cell[20]. This synchronous hormone production may help to explain why the syncytiotrophoblast is multinucleated: multiple copies of the genome may be necessary to allow this complex cell to make numerous products simultaneously while it continues to perform its other functions of absorption and waste excretion.

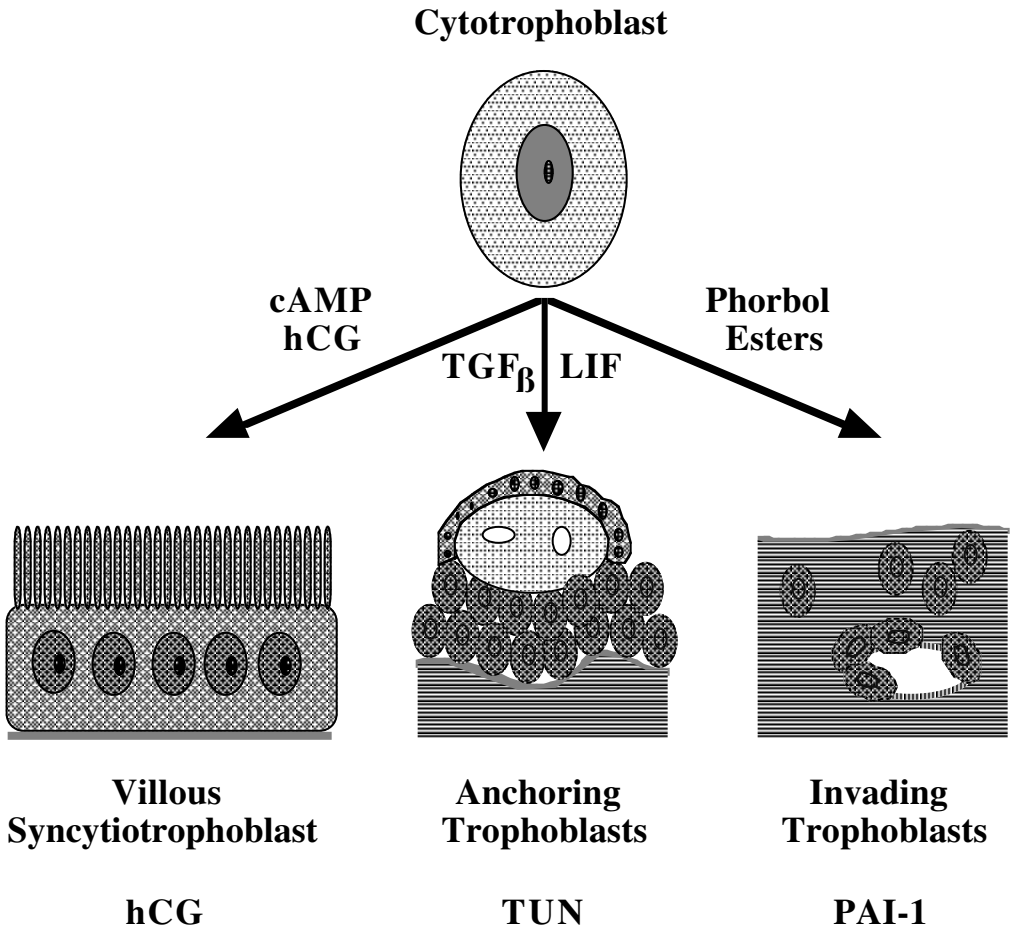

Fig. 1. Pathways of trophoblast differentiation . Just as the basal layer of the skin gives rise to keratinocytes, the cytotrophoblast—the stem cell of the placenta—gives rise to the differentiated forms of trophoblasts. **Left)** Within the chorionic villi, cytotrophoblasts fuse to form the overlying syncytiotrophoblast. The villous syncytiotrophoblast makes the majority of the placental hormones, the most studied being hCG. cAMP, EGF, and even hCG itself have been implicated as stimulators of this differentiation pathway. In addition to upregulating hCG secretion, cAMP has also been shown to down-regulate trophouteronectin (TUN) synthesis. **Center)** At the point where chorionic villi make contact with external extracellular matrix (decidual stromal ECM in the case of intrauterine pregnancies), a population of trophoblasts proliferates from the cytotrophoblast layer to form the second type of trophoblast—the junctional trophoblast. These cells form the anchoring cell columns that can be seen at the junction of the placenta and endometrium throughout gestation. Similar trophoblasts can be seen at the junction of the chorion layer of the external membranes and the decidua. The junctional trophoblasts make a unique fibronectin—trophouteronectin—that appears to mediate the attachment of the placenta to the uterus. TGFß and LIF have been shown to induce cultured trophoblasts to secrete increased levels of trophouteronectin, while down-regulating hCG secretion. **Right)** Finally, a third type of trophoblast differentiates towards an invasive phenotype and leaves the placenta entirely—the invasive intermediate trophoblast. In addition to making human placental lactogen, these cells also make urokinase and plasminogen activator inhibitor-1 (PAI-1). Phorbol esters have been shown to increase trophoblast invasiveness in *in vitro* model systems and to upregulate PAI-1 in cultured trophoblasts. The general theme that comes from these observations is that specific factors are capable of shifting the differentiation pathway of the cytotrophoblast towards

one of the above directions, while turning off differentiation towards the other pathways. See text for details.

Anchoring trophoblasts

It has been generally accepted that some form of cell-extracellular matrix interaction takes place at the attachment interface between the anchoring trophoblasts and the uterus. Recently, a specific type of fibronectin—*trophouteronectin (TUN)*—has been implicated as the protein responsible for the attachment of anchoring, extravillous trophoblasts to the uterus throughout gestation[18,21]. This specialized form of fibronectin appears to be made wherever trophoblasts contact extracellular matrix proteins. The factors that may be responsible for activating trophoblast TUN production include TGFß[22] and leukemia inhibitory factor (LIF)[23]. TGFß has been identified in the region of the utero-placental junction, possibly made by both decidual cells in that area and by the trophoblasts themselves[24]. LIF has been identified in human endometrium[25], but has not been shown to be made by trophoblasts. Interestingly, both TGFß and LIF have been shown to upregulate TUN secretion from cultured trophoblasts while down-regulating hCG secretion[22, 23](Fig. 1).

Invading trophoblasts

As human gestation progresses, invasive populations of extravillous trophoblasts attach to and interdigitate through the extracellular spaces of the endo- and myometrium. The endpoint for this invasive behavior is penetration of maternal spiral arteries within the uterus[26]. Histologically, trophoblast invasion of maternal blood vessels results in disruption of extracellular matrix components and development of dilated capacitance vessels within the uteroplacental vasculature. Biologically, trophoblast-mediated vascular remodeling within the placental bed allows for marked distensibility of the uteroplacental vessels, thus accommodating the increased blood flow needed during gestation. Abnormalities in this invasive process have been correlated with early and mid-trimester pregnancy loss, preeclampsia and eclampsia, and intrauterine growth retardation[27] .

As would be anticipated when considering invasive cells, these trophoblasts produce a variety of proteases[28],[29],[30] and protease inhibitors[5] which are utilized to regulate the invasive process. In addition to the protease systems, invasive trophoblasts also make protein hormones, most notably human placental lactogen[31].

IN VITRO MODEL SYSTEMS TO STUDY TROPHOBLAST DIFFERENTIATION

The most commonly used approaches for examining the regulation of hormone production by trophoblasts have come from *in vitro* studies. Model systems developed to study placental and trophoblast function have included placental organ and explant culture, trophoblast culture, chorion laeve culture, choriocarcinoma cell line culture, and placental perfusion studies[1]. Recently, most investigators have turned to trophoblast cell culture since it eliminates the complications of more heterogeneous cell systems. Since the cytotrophoblast is the precursor of all other trophoblasts, a variety of methods have been proposed to purify this cell type from the human placenta[4,32,33,34,35,36,,37,38,39,40,41].

We have demonstrated by time-lapse cinematography that when these mononuclear cytotrophoblasts are placed in Dulbecco's Modified Eagles' Medium (DMEM) containing 20% (v/v) heat-inactivated fetal calf serum (FCS), they flatten onto the culture surface within 3-12 h, migrate towards each other to form aggregates within the first 24 h, and over the next 24 h of culture, form syncytiotrophoblasts[4]. Concomitant with these morphologic changes, these trophoblasts synthesize and secrete a number of cell products, including protein hormones, peptide hormones, steroid hormones, growth factors, and cytokines. We and others have used these cells to elucidate the products of trophoblast differentiation and to explore the mechanisms by which their synthesis and secretion is regulated.

TROPHOBLASTS AS ENDOCRINE CELLS

Trophoblasts synthesize and secrete a vast array of endocrine products (for reviews see references [2,3,42,43,44,45,46]). Collectively, these hormones function to regulate trophoblast growth and differentiation, affect fetal growth and homeostasis, modulate maternal immunologic, cardiovascular and nutritional status, protect the fetus from infection, and prepare the uterus and mother for parturition.

PROTEIN HORMONES

Chorionic gonadotropin

The most widely studied trophoblast hormone product is chorionic gonadotropin. This glycoprotein is critical to pregnancy since it rescues the corpus luteum from involution, thus maintaining progesterone secretion by the ovarian granulosa cells. Its usefulness as a diagnostic marker of pregnancy stems from the fact that it may be one of the earliest secreted products of the conceptus. Ohlsson et al[47] have demonstrated by *in situ* hybridization that ß-hCG transcripts

are present in human blastocyst trophoblasts prior to implantation. Placental production of hCG peaks during the eighth to the tenth week of gestation, and tends to plateau at a lower level for the remainder of pregnancy. This difference in the rate of hCG secretion may be mimicked to some extent by trophoblasts cultured from first versus third trimester placentae. Kato and Braunstein[48] have demonstrated that trophoblasts from first trimester placentae secrete greater amounts of hCG than trophoblasts purified from term placentae, suggesting that cultured trophoblasts may retain the regulatory effects of their *in situ* milieu even after several days of culture.

What regulates hCG synthesis and secretion in the trophoblast? Workers have attempted to discover what regulates hCG synthesis and secretion by examining likely factors *in vitro*. Table 1 summarizes our current knowledge of the regulatory factors that appear to modulate hCG secretion in trophoblasts.

Table 1

Regulation of trophoblast hCG secretion

Factor	Trophoblasts (Trimester)	Effect on hCG Secretion	References
cAMP	Term	Stimulates	14
hCG	Term	Stimulate	17
GnRH	Term	Stimulates	49, 50
GnRH	First, Term	Not clear	51
ß-adrenergic agonists	First	Stimulates	52
Dexamethasone	Term	Stimulates	53
Inhibin	Term	Inhibits	54, 55, 56
Activin	Term	Potentiates GnRH simulation of hCG secretion	56
Activin	First	Stimulates	57
EGF	First, Term	Stimulates	16
Thyroid hormone	First, Term	Stimulates	58

Thyroid Stimulating Hormone	Term	Inhibits	[59]
Interleukin-1.	First	Stimulates	[60]
Interleukin-6	First	Stimulates	[61]
Basement Membrane	First	Stimulates	[62]
Decidual Protein	Term	Inhibits	[63]
Prolactin	Term	Inhibits	[64]

Novel effects of hCG

In addition to the commonly accepted functions of hCG as the rescuer of corpus luteum function and the stimulator of fetal Leydig cells[46], hCG may have other roles to play in gestation. Shi et al[17] have shown that hCG can promote the differentiation of cytotrophoblasts into syncytiotrophoblasts, suggesting that this hormone may function in an autocrine fashion to commit villous cytotrophoblasts to become villous syncytiotrophoblasts. Thus, in the middle of the placenta where hCG concentrations would be expected to be high, cytotrophoblast stem cells would tend to differentiate and fuse with the overlying syncytium to further the growth of the placental mass. At the same time, the tendency towards anchoring or invasive phenotypes would be suppressed. The cytotrophoblasts near the placental-uterine junction might be exposed to lower local concentrations of hCG and be more able to be shifted to the other pathways of trophoblast differentiation. Milwidsky et al[29] demonstrated that hCG markedly suppressed trophoblast secreted serine protease and urokinase activities. Again, hCG would tend to inhibit the trophoblast from functioning in a phenotype other than the hormonally active villous syncytiotrophoblast. Both of these studies suggest that a high hCG environment tends to maintain villous syncytiotrophoblast differentiation (Fig. 1).

hCG As A Marker Of Gestational Health

The measurement of hCG levels during gestation has recently become of great interest to obstetricians, sparked largely as a result of the observation of Bogart et al that maternal second trimester hCG levels with trisomy 21 fetuses are two-fold greater than in gestations with normal fetuses[65]. Since then an abundance of literature has appeared linking higher than normal hCG levels (1.8 to 10 multiplies of the mean) with Down, Turner and Kleinfelter syndrome fetuses, trisomy 13, and trisomy 20, and lower than normal hCG levels with Trisomy 18 fetuses[66,67,68]. In addition to genetic abnormalities, abnormally low levels of hCG have been shown to be associated with early embryonic failure[69].

Degradation Pathways of hCG

HCG is made in high concentrations during the first trimester of pregnancy. What prevents this hCG from entering the fetal circulation and deranging the developing fetal endocrine system? While intact (non-nicked) hCG is biologically active, nicked hCG and degraded ß-core fragment (ß-core) are inactive. Once nicked, hCG splits into free-subunit and nicked free-subunit which are degraded further or rapidly cleared from the circulation[70]. A granulocyte/macrophage elastase nicks hCG at 44-45 and 47-48 *in vitro*[71]. Immunohistochemistry of first, second and third trimester placentas utilizing antibodies specific for intact, nicked, and ß-core fragment revealed degraded hCG species in the villous core macrophages (Hofbauer cells) adjacent to active hCG-producing trophoblast tissue[72]. These results suggest that villous core macrophages may protect the fetus from exposure to high levels of hCG by degrading excessive hCG that diffuses towards the fetal circulation (Fig. 2). Once degraded, these inactive forms may then diffuse out of the villi and into the maternal circulation or into the fetal circulation where they are filtered into the fetal urine and eventually urinated into the amniotic cavity by the fetus.

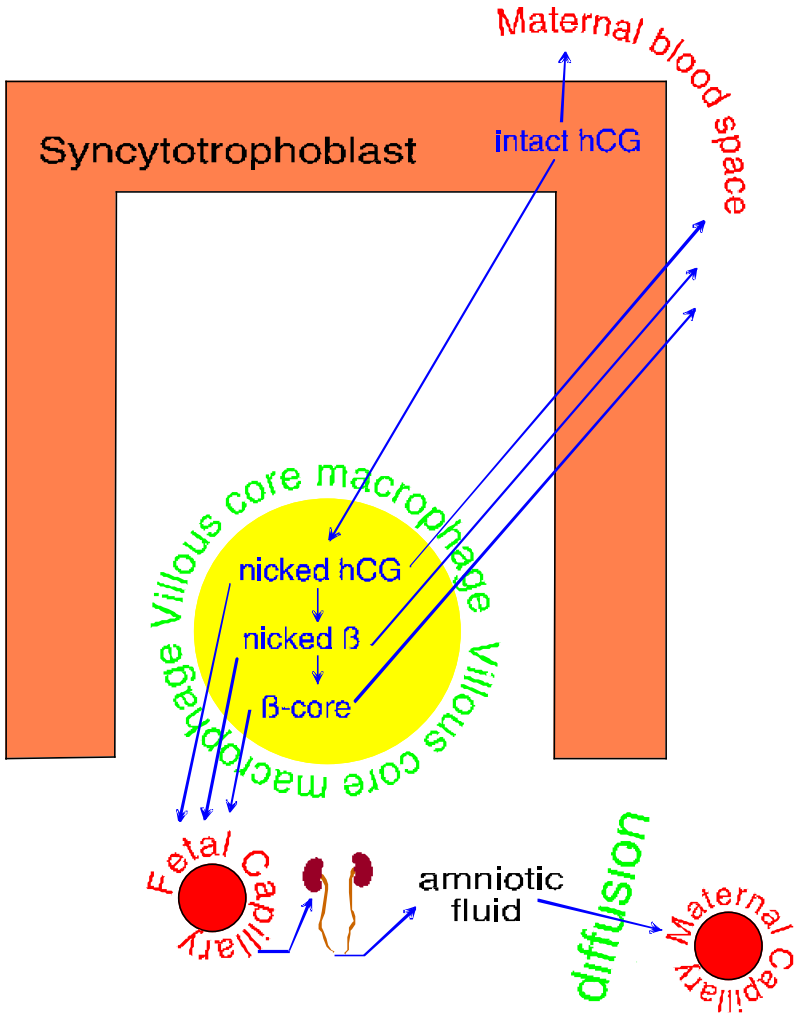

Fig. 2. HCG degradation pathway in the placenta. Most of the hCG synthesized by the syncytiotrophoblast layer of the chorionic villi is secreted into the intervillous space, whereupon it is carried to the maternal systemic circulation. Because of the extremely high concentrations of hCG within these cells, some of the hCG diffuses into the villous core. The villous core macrophages may take up and breakdown the hCG as a way to protect the fetus from high levels of gonadotropin. The hCG breakdown products diffuse both into the maternal and fetal circulations, and via the fetal circulation and urinary system, enters the amniotic fluid. (Figure drawn by Laurence Cole).

Human placental lactogen (hPL)

This potent glycoprotein is made throughout gestation, increasing progressively until the 36th week, where it can be found in the maternal serum at a concentration of 5-15 μg/ml, the highest concentration of any known protein hormone. The major source of hPL appears to be the villous syncytiotrophoblasts, where it is made at a constant level throughout gestation[73] In addition to the villous syncytiotrophoblast, hPL has been identified in invasive intermediate trophoblasts during the first trimester[31,74], as well as the third trimester[75]. In addition to identifying hPL within trophoblasts *in situ*, experiments have shown that cultured first trimester trophoblasts secrete hPL *in vitro*[40]. Sakbun et al[73] have also identified hPL mRNAs in cultured trophoblasts. Hoshina et al[76], working with choriocarcinoma cell lines, have proposed that hPL gene expression occurs after α-hCG and ß-hCG gene expression, suggesting that hPL is a product of a more differentiated trophoblast. Kliman et al have also shown that intracytoplasmic α-hCG appears prior to intracytoplasmic hPL in cultured term trophoblasts[19].

The factors that regulate hPL synthesis and secretion are not as well studied as for hCG. Kato and Braunstein[77] have demonstrated that the secretion of hCG and hPL are discordant during the first 5 days of term trophoblast culture, suggesting different regulatory pathways for these hormones. Dodeur et al[40] demonstrated that dibutyryl cAMP stimulated hPL secretion from cultured first trimester trophoblasts. Maruo et al[16] have shown that EGF, in addition to increasing hCG secretion by cultured human trophoblasts, also augments hPL secretion by these cells. Handwerger et al[78] showed that high density lipoproteins (HDL) stimulate the release of hPL from human placental explants, while Wu and Handwerger showed that HDL stimulates hPL release from cultured trophoblasts via a protein kinase-C-dependent pathway[79]. Finally, Petit et al[80] have demonstrated that angiotensin II stimulates hPL release by cultured trophoblasts, while opioids stimulate hPL release via a calcium influx mechanism[81].

Chorionic adrenocorticotropin (cACTH)

An ACTH-like protein, lipotropin, and ß-endorphin have all been identified in placental extracts[82], presumably all derived from the common precursor pro-opiomelanocortin[83]. Liotta et al[84] demonstrated that cACTH is synthesized by cultured placental cells, and Al and Fox[85] have demonstrated cACTH within villous syncytiotrophoblasts by immunohistochemistry. Mulder et al[86] demonstrated that isoproterenol stimulated cACTH secretion by placental explant cultures, while Waddel and Burton demonstrated cACTH release by perfused human placenta. The physiological role of placental cACTH is unclear. As with other placental hormones, it may represent a shift from maternal to placental control (see Table 2).

Parathyroid hormone-related protein (PTH-rP)

Calcium transport across that trophoblast layer from maternal to fetal circulations is controlled, at least in part, by a calcium responsive membrane protein found on the cytotrophoblast plasma membrane[87]. This protein appears to be the same one found in parathyroid cells, suggesting that calcium levels around the trophoblasts can regulate the secretion of the trophoblast equivalent of PTH: PTH-rP. Using specific anti-PTHrP monoclonal antibodies, Hellman et al[88] were able to show that cytotrophoblasts, and to a lesser extent, syncytiotrophoblasts, contained large quantities of PTH-rP. Given the parallel calcium sensitivity between purified cytotrophoblasts and parathyroid cells and the content of PTH-rP hormone within the same cells, it appears that trophoblasts again have been shown to contain all the cellular machinery necessary to regulate their own physiology, independent of maternal intervention.

Growth hormone (chorionic somatomammotropin)

Growth hormone can be measured in high levels in the cord blood of a normal term fetus. The fetal pituitary does not seem to be the source of this hormone since experimental decapitation in animal systems does not affect fetal growth significantly[89] and anencephalic fetuses—which can have little pituitary tissue—are normal in weight. The source of growth hormone appears be the placenta. Syncytiotrophoblasts contain the message for the placental form of growth hormone—growth hormone variant (GH-V)[90], and cultured human trophoblasts secrete GH-V[91]. The origin of the difference between adult GH and placental GH-V appears to be due to alternate splicing in the placental form[92].

Prolactin

Human prolactin, which is 67% homologous to hPL, is found in high levels in maternal serum and amnionic fluid during pregnancy[93]. It's major function appears to be related to lactation. Paradoxically, prolactin levels drop after delivery, even when breast feeding occurs. This observation can be partially explained by studies that have shown prolactin expression in the placenta. Al and Fox[85] and Sakbun et al[110] demonstrated by immunohistochemistry that villous syncytiotrophoblasts contain prolactin. More recently, Wu et al[94], utilizing both immunohistochemistry and *in situ* hybridization for prolactin, demonstrated that only decidual cells contain the message for prolactin, while the trophoblasts contain only the prolactin protein—suggesting an active uptake of prolactin by trophoblasts. The function of absorbed trophoblast prolactin is not known.

Hypothalamic hormones: production and regulation

The placenta appears to produce a number of hypothalamic hormones, including gonadotropin-releasing hormone (GnRH), corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH) and growth hormone-releasing hormone (GHRH) (for recent reviews, see [95] and [46]). GnRH was first identified within villous cytotrophoblasts by immunochemical staining of intact placentae[96]. More recently, Petraglia et al[97] have demonstrated GnRH secretion by cultured trophoblasts and have shown that estrogen augments cAMP induction of trophoblast GnRH secretion.

CRH is found in maternal serum at low levels during the first and second trimesters of uncomplicated pregnancies, but rises dramatically in the third trimester of normal gestations[46] or earlier if there are pregnancy complications resulting from such factors as prematurity, diabetes, or hypertension[98]. This CRH appears to be secreted by placenta, amnion and decidua. Riley et al[98] found high levels of CRH within the syncytiotrophoblasts and intermediate trophoblasts of term placentas, but not within the cytotrophoblasts. Okamoto et al[99] found CRH message in third trimester placenta, but not first or second trimester. CRH is also made and secreted by cultured trophoblasts[100]. Robinson et al[101] have demonstrated that glucocorticoids stimulate CRH release by cultured trophoblasts. Adding a further level of complexity to the regulatory signals impinging on the placenta, Petraglia et al[102] have shown that neurotransmitters and peptides modulate the release of immunoreactive CRH, and that interleukin-1-ß increases both CRH and ACTH release from cultured human trophoblasts[103]. The precise role of placental CRH in pregnancy is not known[104,105]. However, Riley and Challis[106] have speculated that CRH may serve to initiate labor, since it is found in abnormally high levels in premature labor patients. It is possible, on the other hand, that factors that induce labor may secondarily stimulate trophoblasts to physiologically upregulate CRH production, which in turn increases fetal cortisol levels, which may serve to mature the fetus in preparation for extrauterine life.

TRH has been shown to be made by the placenta, although its posttranslational processing appears to be different from that found in the hypothalamus[107]. The biological role of this releasing hormone in pregnancy is not known. Similarly, GHRH has also been identified in the human placenta[108], but its cellular localization and function are unknown.

Relaxin

Relaxin, a small insulin-like protein hormone, is found in maternal serum throughout gestation[109]. Although the only sites of relaxin synthesis had been considered to be the corpus

luteum and decidua, Sakbun et al[110], using anti-peptide antibodies, demonstrated immunoreactivity for the C-peptide and/or prorelaxin in villous cytotrophoblasts. More recently, Sakbun et al[111] have demonstrated relaxin secretion by cultured trophoblasts. Trophoblast derived relaxin may, therefore, play an important role in maternal ECM modification as parturition approaches. This hypothesis is supported by the clinical observation that relaxin deficiency of the placenta can be a cause of cervical dystocia[112].

Cytokine Growth Factors

A number of growth factors, including transforming growth factors α and ß (TGFα, TGFß), and epidermal growth factor (EGF) have been identified in trophoblasts, both *in vitro* and *in vivo*. TGFß has been identified by immunohistochemistry in first and third trimester human placenta[113], especially in the syncytial trophoblasts and the cell columns of first trimester anchoring villi. This finding supports the hypothesis that trophoblast derived TGFß—as well as decidual derived TGFß[24]—at the utero-placental junction may stimulate the anchoring trophoblasts to make TUN[22], the placental fibronectin found in this location[18] (Fig. 1).

EGF and the EGF receptor have been localized to the syncytiotrophoblast in intrauterine and ectopic pregnancies[114], suggesting a potential autocrine role for EGF in placental growth. TGFα, an EGF-like hormone, has also been identified in the placenta throughout gestation, but in the cytotrophoblasts of the chorionic villi[115]. Both EGF and TGFα were able to stimulate cultured cytotrophoblasts to increase their mitotic rate[115].

Activin and Inhibin

Activin and inhibin are closely related dimeric glycoprotein hormones. Inhibin is a heterodimer of α and ß subunits (which exist as two distinct peptides: ßA or ßB), while activin is a homodimer of two inhibin ß-subunits. The placenta produces all three subunits: α, ßA and ßB[116,117]. In the non-pregnant state inhibin is made in the human testis and granulosa cells of the ovary and functions to inhibit FSH release from the pituitary. During pregnancy, the major source of inhibin appears to be the placenta[118]. Immunohistochemistry has revealed inhibin to be localized within both cyto and syncytiotrophoblasts, while *in situ* hybridization for α and ßA subunits revealed message only in the cytotrophoblasts, suggesting synthesis occurs in the cytotrophoblast layer followed by transport of finished product to the overlying syncytium[118]. In addition to these observations made *in situ*, inhibin has been shown to be secreted by cultured trophoblasts *in vitro*[119], the secretion of which can be increased by EGF[120] and prostaglandins[121].

Activin appears to stimulate trophoblast hCG secretion[55,57], while inhibin can suppress hCG secretion in term placental explants[122]. Interestingly inhibin does not appear to inhibit hCG secretion in first trimester explants, suggesting that inhibin-activin regulation of hCG may explain the long perplexing observation that hCG secretion peaks in the first trimester and decreases thereafter in spite of the fact that trophoblast mass continues to rise throughout pregnancy.

Renin

The placenta often functions as if it also had a systemic pressure regulating system. The renin and angiotensinogen system is critical for systemic fluid and pressure homeostasis. In the case of the kidney, a decrease in renal perfusion leads to an increase in renin production which triggers a cascade of events that leads to an increase in perfusion of the kidney. Preeclampsia presents clinically as a systemic increase in maternal blood pressure during pregnancy. The trigger for this increase appears to be a decrease in uteroplacental blood flow to the placenta via the maternal spiral arteries. The signal that the placenta utilizes to induce this change is not known, but the finding of renin within the placenta[123] suggests that this hormone may function in the placenta much as it does in the kidney.

Calcitonin

Since the placenta synthesizes a PTH related protein and appears to regulate PTH-rP via extracellular calcium levels, it is not unexpected that trophoblasts also secrete calcitonin[124], the counterpart to PTH in calcium homeostasis. As with hCG secretion, the addition of cAMP to placental cultures increased calcitonin secretion.

PRODUCTION AND REGULATION OF STEROID HORMONES

Progesterone

The significance of placental elaboration of progesterone was revealed by Diczfalusy and Troen[125], who showed that bilateral oophorectomy between 7 and 10 weeks of gestation had little impact on the conceptus or urinary pregnanediol levels.

More recently, we have been able to demonstrate progesterone secretion by cultured term trophoblasts[4]. In addition we have identified various components of the steroidogenic machinery

necessary for progesterone biosynthesis within cultured trophoblasts[126]. Like hCG, progesterone synthesis and secretion seems to be upregulated by cAMP agonists[14,127]. Treatment of cultured trophoblasts with 8-bromo-cAMP induces a marked upregulation of the cholesterol side-chain cleavage enzyme (P-450$_{scc}$). This enzyme is the rate limiting step responsible for the conversion of cholesterol to pregnenolone. Consistent with these studies is the work of Moore et al[128] who have identified a cyclic adenosine 3',5'-monophosphate response element in the human gene for P-450$_{scc}$. Additional insight into the regulation of progesterone synthesis in the trophoblast has come from the work of Chaudhary et al[129]. They showed that while cAMP was able to upregulate progesterone secretion in cultured trophoblasts, the addition of anti-hCG antibodies blocked the effect. They also could show that anti-hCG antibodies prevented the normal upregulation of P-450$_{scc}$ in the presence of the nucleotide. Shi et al[130] also showed this anti-hCG antibody effect on trophoblast progesterone secretion, and in addition demonstrated that GnRH also upregulates trophoblast progesterone secretion. These results suggest that progesterone synthesis and secretion may be regulated in an autocrine fashion by trophoblast hCG and GnRH.

Estrogen

The placenta does not have all the necessary enzymes to make estrogens from cholesterol, or even progesterone. Human trophoblasts lack 17α-hydroxylase and therefore can not convert C_{21}-steroids to C_{19}-steroids, the immediate precursors of estrogen. To bypass this deficit, dehydroisandrosterone sulfate (DHA) from the fetal adrenal is converted to estradiol-17ß by trophoblasts[131]. Not surprisingly, trophoblasts contain the necessary enzymes to make this conversion[2], namely sulphatase, 3ß-hydroxysteroid dehydrogenase/ $^{5\rightarrow4}$-isomerase (3ßHSD), and aromatase. Lobo and Bellino[132] have demonstrated that cultured trophoblasts synthesize aromatase, and that cAMP appears to stimulate aromatase production by these cells. Nestler demonstrated that insulin-like growth factor II[133], and more recently, insulin itself[134], inhibits aromatase in cultured human trophoblasts, possibly explaining why diabetic women who are treated with high levels of insulin may have lowered estrogen levels.

MARCHING TO THE BEAT OF A DIFFERENT DRUMMER

One of the common themes in placental biology is that trophoblasts make many proteins that are found in other parts of the body, but with minor—yet presumably important—differences. We see this most clearly with hCG and luteinizing hormone (LH), which share identical α-

subunits and have ß-subunits that are 80% homologous (with hCG having an additional 24-amino acid extension at the carboxy-terminus). Other parallel proteins are shown in Table 2.

Table 2
Placental Hormones and their Systemic Counterparts

Placental Hormone	Non-placental Counterpart	Counterpart Source
hCG	LH	Pituitary
hPL	GH	Pituitary
	hPRL	Pituitary
ACTH-like protein	ACTH	Pituitary
PTH-related protein	PTH	Pituitary
Hypothalmic-like-releasing hormones	GnRH, TRH, CRH, somatostatin	Hypothalamus

Why does the placenta make unique proteins, different from the forms seen in the rest of the body? Could it be that the placenta contains primitive versions of the genes for the hormones seen in other locations? Or do the placental versions of these proteins have unique characteristics that give them specific, needed, functions in gestation? There is some evidence for the latter explanation. For example, hCG has a far greater half-life than its counterpart hormone LH, due largely to hCG's carboxy-terminus 24 amino acid extension[135],[136]. This longevity may help hCG achieve the specific and needed functions of this gonadotrope. The advantages of the other placental hormone variants are not as clear.

BEHIND EVERY HEALTHY BABY IS A HEALTHY PLACENTA

The second major theme that is apparent from this review of the placental hormones and their regulatory pathways is that the placenta achieves independence from its host, the mother. Unlike the rest of the endocrine organs of the body that are interrelated at many levels through the hypothalmic-pituitary-end-organ model, the placenta takes all these levels and compresses them into one cell type—the trophoblast (Fig. 3). Much like the shifting of the control of the space shuttle from Cape Kennedy to the Johnson Space Center in Houston once lift-off has been achieved, the placenta takes over many regulatory functions of the mother to insure optimal

control of the gestation. These include indirect effects on the endometrium through maintenance of ovarian progesterone during the initial phases of pregnancy, direct effects on the endometrium at the time of implantation, modification of the maternal immune response, regulation of energy metabolism in the mother, modification of maternal blood supply to the placenta and control of systemic circulatory pressures, regulation of corticosteroid synthesis during stress, regulation of calcium transport, and control of local growth of the placenta and fetus. This concerted, complex regulatory machinery of the trophoblast has but one goal—the birth of a healthy child.

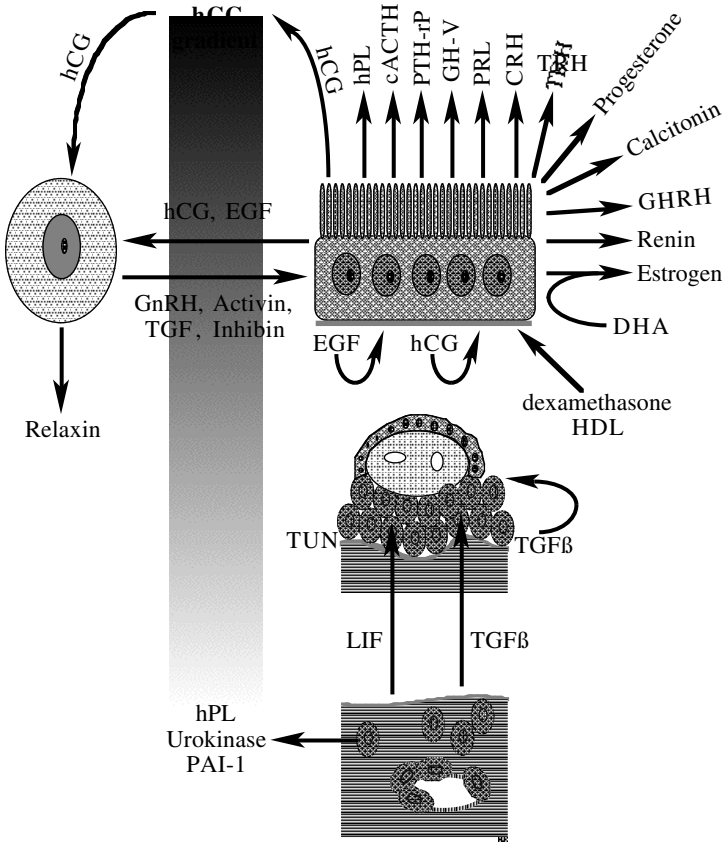

Fig. 3. Summary of placental hormones and regulatory interrelationships. The villous syncytiotrophoblast is the major source of placental hormones. Hormones from the cytotrophoblasts (paracrine), from the syncytiotrophoblast itself (autocrine) and from the maternal circulation (endocrine) regulate syncytiotrophoblast function. In turn, hormones from the syncytiotrophoblast regulate cytotrophoblast function, modulate maternal physiology and promote fetal growth. An hCG gradient is created by the villous syncytiotrophoblasts which maintains villous differentiation. As the hCG levels drop, cytotrophoblasts can differentiate towards an anchoring or invasive phenotype. Anchoring trophoblasts receive both autocrine and paracrine signals to make TUN. Within the endo- and myometrium, invasive trophoblasts make hPL and markers of migrating cells. See text for details and abbreviations.

SYNOPSIS

Behind every healthy baby is a healthy placenta. The placenta creates this healthy environment for the fetus by producing a wide variety of hormones that shifts the control of many regulatory functions away from the mother to the fetus to insure optimal control of the

gestation. The cells which mediates this process are the trophoblasts—unique cells derived from the outer cell layer of the blastocyst which mediate implantation and placentation.

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