

## Review

# Control of trophoblast cell differentiation: Lessons from the genetics of early pregnancy loss and trophoblast neoplasia

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Trophoblast cell differentiation is crucial to the morphogenesis of the placenta and thus the establishment of pregnancy and the growth and development of the embryo/fetus. In the present review, we discuss current evidence for the existence of regulatory genes crucial to trophoblast cell differentiation and placental morphogenesis. The elucidation of regulatory pathways controlling normal differentiation of trophoblast cells will facilitate the identification of sensitive junctures in the regulatory pathways leading to various developmental disorders, including those associated with the initiation of pregnancy, fetal growth retardation and gestational trophoblast disease.

**Key words:** differentiation, morphogenesis, placenta, placental morphogenesis, trophoblast regulatory genes.

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## Introduction

The placenta is an extraembryonic tissue that permits the embryo to develop within the female reproductive tract. Placental cells have an array of different functions vital to normal embryogenesis. These responsibilities of the placenta are assumed by cells of the trophoblast lineage. The appearance of trophoblast cells represents the initial differentiation event of embryogenesis (Gardner 1983). This progenitor population of trophoblast cells forming the outer layer of the blastocyst is referred to as trophoblast (Gardner 1983). Proliferative populations of trophoblast stem cells arise in proximity to the inner cell mass or its derivatives and are referred to as extraembryonic ectoderm and the ectoplacental cone (Rossant 1986). Trophoblast stem cells go on to differentiate along a multilineage pathway (Gardner & Bedington 1988; Soares *et al.* 1993).

In the rat and mouse, trophoblast differentiation is directed towards at least four recognizable phenotypes: (i) trophoblast giant cells; (ii) spongiotrophoblast cells; (iii) glycogen cells; and (iv) syncytial trophoblast cells

(Soares *et al.* 1993). Each of these cell types has unique functional and morphological attributes (Soares *et al.* 1993). Trophoblast giant cells and spongiotrophoblast cells represent endocrine cells of the rat placenta (Soares *et al.* 1991, 1993; Lu *et al.* 1994). Glycogen cells are transitory cell-types that accumulate glycogen and are surrounded by spongiotrophoblast cells (Davies & Glasser 1968). Syncytial cells arise by cell fusion and have a significant role in fetal–maternal exchange (Soares *et al.* 1993). The organization of these trophoblast cell types within the placenta is influenced by signals arising in both maternal and fetal compartments (Roby & Soares 1993). As the rat chorioallantoic placenta develops it becomes organized into two regions. Trophoblast giant cells, spongiotrophoblast cells, and glycogen cells situated at the decidua–placental interface comprise the junctional zone, while syncytial trophoblast cells and some trophoblast giant cells sandwiched between the junctional zone and the developing fetus constitute the labyrinth zone (Soares *et al.* 1993).

In this manuscript, we discuss current concepts regarding the control of trophoblast cell differentiation. Genetic evidence supporting the existence of trophoblast regulatory genes is reviewed and an *in vitro* model system derived from a rat choriocarcinoma is presented as a paradigm for the identification of trophoblast regulatory genes and regulatory networks.

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### Existence of specific trophoblast-regulatory genes

Pivotal genes responsible for the normal growth and differentiation of trophoblast cells are just beginning to be identified. Some of these genes may be required for the growth and differentiation of other specialized cell types, while some genes or combinations of genes must be unique to the trophoblast lineage. Disruption of the activities of these genes should compromise placental development and thus the well-being of the developing embryo/fetus. Identification of these important trophoblast-regulatory genes will provide a better understanding of trophoblast development and an avenue into the etiology of early pregnancy loss and various disorders and neoplasms of the placenta.

Considerable evidence exists for a genetic linkage to early pregnancy loss and spontaneous abortion (Simpson & Golbus 1992). The importance of genetic imprinting in the development of the trophoblast lineage is well appreciated (Surani *et al.* 1986; Solter 1988; Varmuza & Mann 1994). Consequences of autosomal aneuploidy on placental development have also been reported (Gearhart *et al.* 1986; Bersu *et al.* 1989).

Identification of specific regulatory genes responsible for trophoblast cell development is inherently difficult. On the one hand, mutations of these genes are likely prenatal lethals and thus pose significant problems in their identification and characterization. On the other hand, the regulation of trophoblast cells may be under redundant sets of controls. Thus, disruption of a single gene may not possess a marked phenotype.

Some limited progress in the identification of potential trophoblast regulatory genes has been presented (Fig. 1).

#### Mouse mutations associated with abnormalities in trophoblast development

A few mutations in the mouse have been reported to affect trophoblast development (see McLaren 1976;

Copp 1995 for reviews). A brief description of the nature of the impact of each mutation on trophoblast cells follows. In each case, the specific genes responsible for trophoblast disruption have not been determined.

*agouti locus: A<sup>y</sup>* The *A<sup>y</sup>* mutation in the homozygous state is associated with peri-implantation lethality and a presumed defect in the development of trophoblast giant cells (Eaton & Green 1963; Pedersen & Spindle 1981; Papaioannou 1988). Cells of the inner cell mass may also be affected (Papaioannou 1988). The mutation represents a deletion of a large segment of DNA on chromosome 2 contiguous to the *agouti* gene locus resulting in constitutive expression of the *agouti* gene (Bultman *et al.* 1992). It is unclear whether the trophoblast defect is caused by the removal of a gene within the deleted segment or the overexpression of the *agouti* gene. The *agouti* gene encodes an antagonist for some types of melanocortin receptors (Lu *et al.* 1994). Whether melanocortin receptors are a component of signaling pathways regulating the development of trophoblast cells remains to be determined. The gene deleted in the *A<sup>y</sup>* mutation encodes for a novel RNA-binding protein termed *Raly*, which is constitutively expressed during early development (Michaud *et al.* 1993). Disrupting the *Raly* gene may result in a more generalized impairment of all embryonic and extra-embryonic cell types.

*albino locus: c<sup>6H</sup>* A deletion mutation at the *albino* locus on both alleles of mouse chromosome 7 results in embryonic lethality shortly after implantation (Lewis *et al.* 1976). The defect has been attributed to a failure in the growth of extraembryonic ectoderm and the ectoplacental cone. Trophoblast giant cell formation appears normal in the mutants, although trophoblast giant cells are reduced in number. Abnormalities are apparent by 7.5 days and death occurs by 8 days (Lewis *et al.* 1976). Magnuson and colleagues have

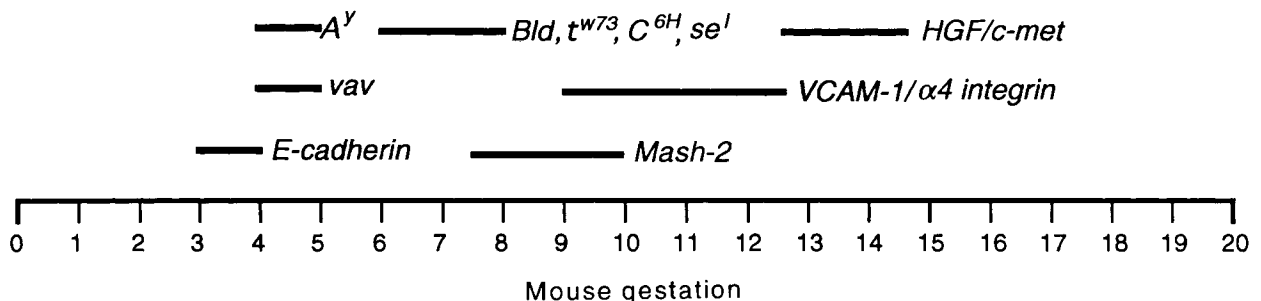


Fig. 1. Overview of putative trophoblast regulatory genes in the mouse. Genes were identified as spontaneous or induced mutations affecting trophoblast development and are presented relative to the onset of their actions during gestation. See text for further details. Abbreviations: Mash-2, mammalian member of the achaete scute family-2; HGF, hepatocyte growth factor; *c-met*, HGF receptor; VCAM-1, vascular cell adhesion molecule-1.

further defined this region of chromosome 7 narrowing the location of the gene responsible for the abnormalities in extraembryonic ectoderm development (Niswander *et al.* 1989; Sharan *et al.* 1991; Holdener-Kenny *et al.* 1992). The identity of this gene termed *exed* has not been reported.

**blind locus: Bld** The development of homozygous *Bld* embryos is retarded by 6.5 days and the embryos are dead by 7.5 days (Vankin & Caspari 1979). The defect is associated with an abnormality in trophoblast–uterine interactions attributed to aberrations in trophoblast development. Trophoblast giant cells fail to arise or arise late. Little additional information on the mutation is available beyond its localization to mouse chromosome 15 (Vankin & Caspari 1979).

**T/t locus: *t<sup>w73</sup>*** Homozygous *t<sup>w73</sup>* embryos exhibit disruptions in trophoblast development similar to those described for homozygous *Bld* embryos (Spiegelman *et al.* 1976; Vankin & Caspari 1979). The mutation maps to mouse chromosome 17, the site of an array of different genes known to affect growth and reproduction (Artzt 1984). The identity of the gene(s) affected by the *t<sup>w73</sup>* mutation has not been reported.

**short ear locus: *se<sup>l</sup>*** The short ear lethal (*se<sup>l</sup>*) mutant is a postimplantation lethal when homozygous (Dunn 1972). The phenotype of the mutation is characterized by an absence of mesoderm and an excessive proliferation of extraembryonic ectoderm resulting in an increased number of trophoblast giant cells (Dunn 1972). Trophoblast abnormalities are apparent between 6.5 and 7.5 days of gestation. The mutation may indirectly affect trophoblast development via its effects on mesoderm development. Nonetheless, the *se<sup>l</sup>* mutant represents the discovery of an important restraint on trophoblast growth. The mutation consists of a large deletion located between the dilute and short ear loci on mouse chromosome No. 9 (Kingsley *et al.* 1992). The identity of the regulatory gene(s) has not been determined.

#### *Putative trophoblast regulatory genes identified by gene targeting*

By chance, a few putative trophoblast regulatory genes have been identified via homologous recombination and gene targeting experiments (see Copp 1995 for review).

**E-cadherin** E-cadherin is a cell–cell adhesion molecule critical for the formation of epithelia. E-cadherin

negative embryos show disruptions in the formation of the blastocyst and die around the time of implantation (Larue *et al.* 1994). Affected embryos fail to form a trophoctodermal epithelium and a blastocoelic cavity (Larue *et al.* 1994). However, *in vitro* trophoblast giant cell outgrowth by E-cadherin null blastocysts was not disrupted, providing evidence for the dissociation of components of trophoblast cell development (Larue *et al.* 1994).

**Mash-2** The existence of another trophoblast-regulatory gene comes from research on a mammalian member of the achaete-scute family (Mash) of basic helix-loop-helix (bHLH) transcription factors. The *Mash-2* gene has recently been shown to play a role in the development of the mouse placenta (Guillemot *et al.* 1994). *Mash-2* expression is restricted to trophoblast cells (Guillemot *et al.* 1994). *Mash-2* null embryos show an aberration in spongiotrophoblast and glycogen cell development and die at midgestation (Guillemot *et al.* 1994). Trophoblast giant cell and labyrinthine syncytial trophoblast cell lineages do not appear to be directly affected. The *Mash-2* gene is genetically imprinted and has been localized to chromosome 7 contiguous to a number of genes also known to be genetically imprinted (Guillemot *et al.* 1995). Both paternal and maternal *Mash-2* alleles are expressed in trophoblast cells early in development; however, as gestation proceeds only the maternal *Mash-2* allele is expressed (Guillemot *et al.* 1995). Trophoblast cell target genes for *Mash-2* action have yet to be determined. *Mash-2* interacts with consensus nucleotide sequences, termed E-Boxes, known to bind other bHLH transcription factors (Johnson *et al.* 1992). The 5' flanking regulatory DNA of a spongiotrophoblast-specific gene, prolactin-like protein-A, contains putative E-Boxes (Vuille *et al.* 1993) and may be a target for *Mash-2*.

**vav** *Vav* is a component of tyrosine kinase signaling pathways and when overexpressed can transform cells (Bustelo *et al.* 1994). A number of functional domains relevant to signaling pathways are present within the *vav* protein; however, a specific function for *vav* remains to be elucidated (Coppola *et al.* 1991; Bustelo *et al.* 1994). Development of *vav* null mutant embryos arrests around the time of implantation (Zmuidzinas *et al.* 1995). Trophoblast cells are prominent sites for *vav* expression early during embryogenesis and trophoblast differentiation is markedly disrupted in *vav* null mutant embryos (Zmuidzinas *et al.* 1995). At this juncture, we can only speculate that *vav* participates in a tyrosine kinase signaling pathway regulating trophoblast cell growth and/or differentiation.

*Hepatocyte growth factor/scatter factor (HGF/SF)/c-met* HGF/SF is a potent mitogen and morphogen for a variety of cell types and hypothesized to mediate mesenchymal directed actions on epithelial growth and differentiation (Michalopoulos & Zarnegar 1992). Within the developing placenta, HGF/SF and its receptor, the transmembrane tyrosine kinase, *c-met* are expressed in the allantoic mesenchyme and labyrinthine trophoblast cells, respectively (Uehara *et al.* 1995). Targeted disruption of HGF/SF or *c-met* results in prenatal lethality between embryonic days 13 and 15 (Schmidt *et al.* 1995; Uehara *et al.* 1995). The prenatal lethality appears, at least in part, to result from a failure of the labyrinth zone of the chorioallantoic placenta to properly develop (Schmidt *et al.* 1995; Uehara *et al.* 1995). HGF/SF has also been shown to specifically stimulate the proliferation of labyrinthine trophoblast progenitors (Uehara *et al.* 1995).

*Vascular cell adhesion molecule-1 (VCAM-1)/ $\alpha$ 4 integrin* VCAM-1 is a transmembrane glycoprotein mediating cell–cell interactions among a number of different cell types via the cell surface receptor,  $\alpha$ 4 integrin (Gurtner *et al.* 1995; Kwee *et al.* 1995; Yang *et al.* 1995). The expression patterns of VCAM-1 and  $\alpha$ 4 integrin within the developing placenta resemble those for HGF/SF and *c-met*. VCAM-1 is expressed at the tip of the invading allantoic mesenchyme, whereas  $\alpha$ 4 integrin is expressed on the surface of the trophoblast cells interacting with the allantoic mesenchyme (Gurtner *et al.* 1995; Kwee *et al.* 1995; Yang *et al.* 1995). Disruption of either gene results in failure of fusion and vascularization of the chorioallantoic placenta and death at midgestation (Gurtner *et al.* 1995; Kwee *et al.* 1995; Yang *et al.* 1995). Interactions mediated by VCAM-1 and  $\alpha$ 4 integrin are responsible for determining the surface area available for maternal–fetal exchange. Collectively, HGF/SF/*c-met* and VCAM-1/ $\alpha$ 4 integrin represent two important signaling systems used in the establishment of the labyrinthine syncytial trophoblast.

### Paradigms for identifying trophoblast-regulatory genes

The above information supports the existence of genes regulating trophoblast cell differentiation but does not provide a framework for identifying other pivotal trophoblast-regulatory genes or regulatory networks.

A method utilized in the characterization of many differentiation processes has been the dissection of cell differentiation through *in vitro* analyses. Using *in vitro* methods, tissue-specific regulatory genes and signaling pathways have been identified in muscle, neuronal, bone, skin, adipose, hematopoietic, and pit-

uitary cell differentiation systems (Watt 1989; Karin *et al.* 1990; Orkin 1992; Olson 1993; Stein & Lian 1993; Smyth *et al.* 1993).

Most trophoblast-related research has involved the use of primary human term placental cell cultures, rodent primary placental cell cultures, and a few human choriocarcinoma cell lines (Ringler & Strauss 1990; Soares *et al.* 1993). The purity of the primary cultures and their spontaneous differentiation has been a problem and the available human choriocarcinoma cell lines suffer from their limited abilities to express a differentiated phenotype. Nonetheless, through the use of these cell culture systems evidence has emerged supporting the involvement of cAMP/protein kinase A and tyrosine kinase signaling pathways, and the GATA family of transcription factors in regulating expression of at least some aspects of the differentiated trophoblast cell phenotype (Strauss *et al.* 1992; Rebut-Bonneton *et al.* 1993; Steger *et al.* 1994).

A major obstacle hindering our understanding of trophoblast cell differentiation in any species is the absence of culture systems that permit dissection of events in trophoblast cell differentiation throughout the transition from the proliferative trophoblast phenotype to the terminally differentiated trophoblast phenotype.

### Rcho-1 trophoblast cell line

A very useful *in vitro* culture model for studying trophoblast cell differentiation has been established from a rat choriocarcinoma. The rat choriocarcinoma was experimentally generated by Dr Shinichi Teshima and his colleagues at the National Cancer Institute (Tokyo, Japan), found to be transplantable, and to have significant endocrine effects on its hosts (Teshima *et al.* 1983). Cell lines with trophoblast characteristics were independently established from the choriocarcinoma by Dr Michel Vandeputte's laboratory at the University of Leuven, Belgium (Verstuyf *et al.* 1990) and by our laboratory (Faria & Soares 1991). The trophoblast cell line derived in our laboratory has been termed *Rcho-1*. The cell line consists of a homogenous population of trophoblast stem cells that proliferate or can be induced to differentiate depending upon the conditions of their culture (Faria & Soares 1991; Hamlin *et al.* 1994). *Rcho-1* trophoblast cell differentiation is incremental, recapitulating the normal ontogeny of giant cell differentiation-specific gene expression (Hamlin *et al.* 1994; Yamamoto *et al.* 1994). This feature permits the dissection of regulatory mechanisms necessary for progression along the trophoblast giant cell differentiation pathway (Fig. 2). Differentiation appears restricted to the trophoblast giant cell lineage.

Thus far, through the use of the *Rcho-1* trophoblast

cell line, tyrosine kinase signaling systems have been implicated in the control of trophoblast giant cell differentiation (Hamlin & Soares 1995) and regulatory mechanisms controlling trophoblast giant cell differentiation-specific gene activation, including the involvement of the GATA family of transcription factors have been demonstrated (Shida *et al.* 1993; Vuille *et al.* 1993; Ng *et al.* 1994; Yamamoto *et al.* 1994).

The value of this *in vitro* system is in the efficiency of generating mechanistic information about trophoblast cell differentiation that can be formulated into hypotheses and tested *in vivo*.

**Control of trophoblast cell differentiation**

In the following paragraphs, we attempt to synthesize data obtained from several sources relating to the development of the trophoblast lineage (Fig. 3).

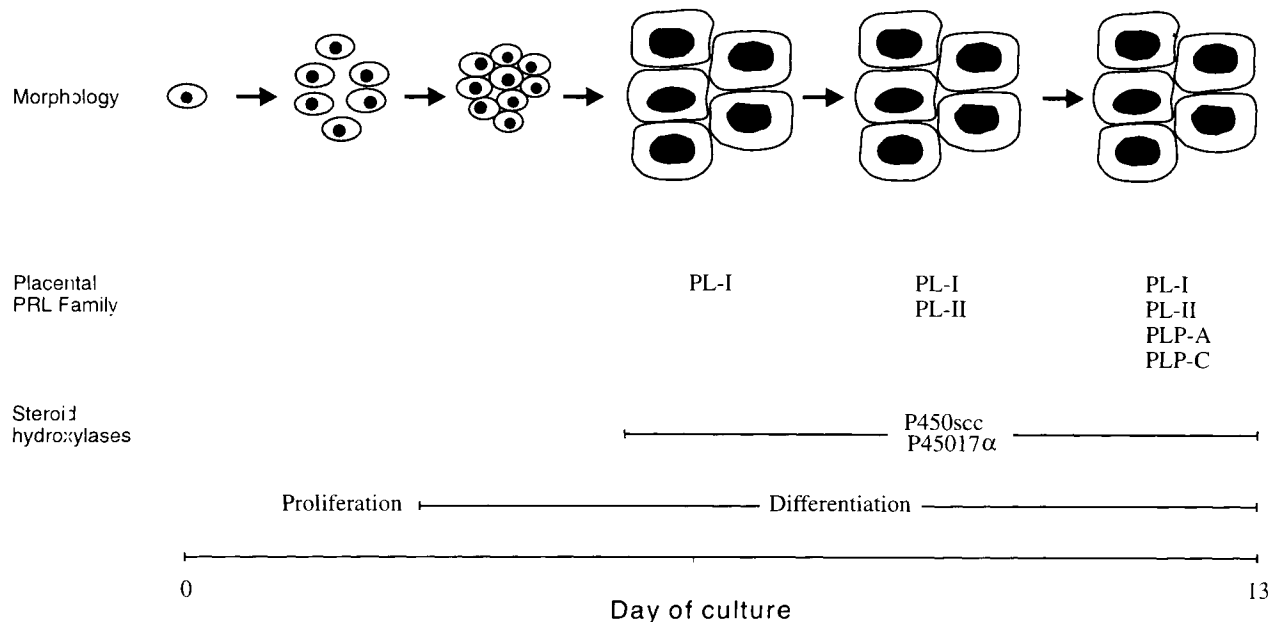
*Trophoblast cell determination*

A bHLH transcription factor, termed *Hxt*, has recently been implicated in the commitment of cells to differentiate along the trophoblast lineage (Cross *et al.* 1994). Such information is similar to regulatory models previously developed for muscle cell differentiation (Olson 1993). Only preliminary observations concerning *Hxt* have been presented (Cross *et al.* 1994). Consequently, the overall significance of the bHLH transcription factor is difficult to assess.

*Trophoblast cell proliferation*

The identification of trophoblast cell growth modulatory factors has been elusive. The placenta is a rich source of a variety of different growth factors and receptors for growth factors (Adamson *et al.* 1987; Ohlsson *et al.* 1993; Kanai-Azuma *et al.* 1994), many of which have been proposed to regulate trophoblast cell proliferation. However, the existence of convincing evidence for the identification of a growth factor(s) promoting trophoblast cell proliferation is meager. There are a number of technical problems associated with studying the proliferation of trophoblast cells. First of all, trophoblast cell proliferation *in vitro* is an aberration. Upon removal from their *in situ* location, trophoblast cells spontaneously differentiate (Rossant 1986; Soares *et al.* 1993; Ringler & Strauss 1990). The factors necessary to prevent spontaneous differentiation have not been elucidated. Second, homogenous cultures of primary trophoblast cells are difficult to obtain from any species. Some reports of *in vitro* growth promotion of placental cells are potentially confounded by non-trophoblast elements of the placenta (Ringler & Strauss 1990; Ohlsson *et al.* 1993). Finally, as DNA synthesis can be associated with trophoblast cell differentiation, the only reliable measure of trophoblast cell proliferation is an increase in trophoblast cell number. These apparent difficulties have made identification of factors that promote trophoblast cell proliferation an arduous task.

Thus far, the data indicate that trophoblast prolifer-



**Fig. 2.** Schematic overview of the *Rcho-1* trophoblast giant cell differentiation model. Timing of the morphological and functional parameters of differentiation are based on earlier reports (Faria & Soares 1991; Hamlin *et al.* 1994). Abbreviations: PRL, prolactin; PL-I, placental lactogen-I; PL-II, placental lactogen-II; PLP-A, prolactin-like protein-A; PLP-C, prolactin-like protein-C; P450scc, cytochrome P450 side chain cleavage enzyme; P45017α, cytochrome P450 17α hydroxylase.

ation is dependent upon extrinsic signals that utilize tyrosine kinase signaling pathways and  $TGF\beta$  is a negative modulator of trophoblast cell proliferation (Roby *et al.* 1994; Hamlin & Soares 1995).

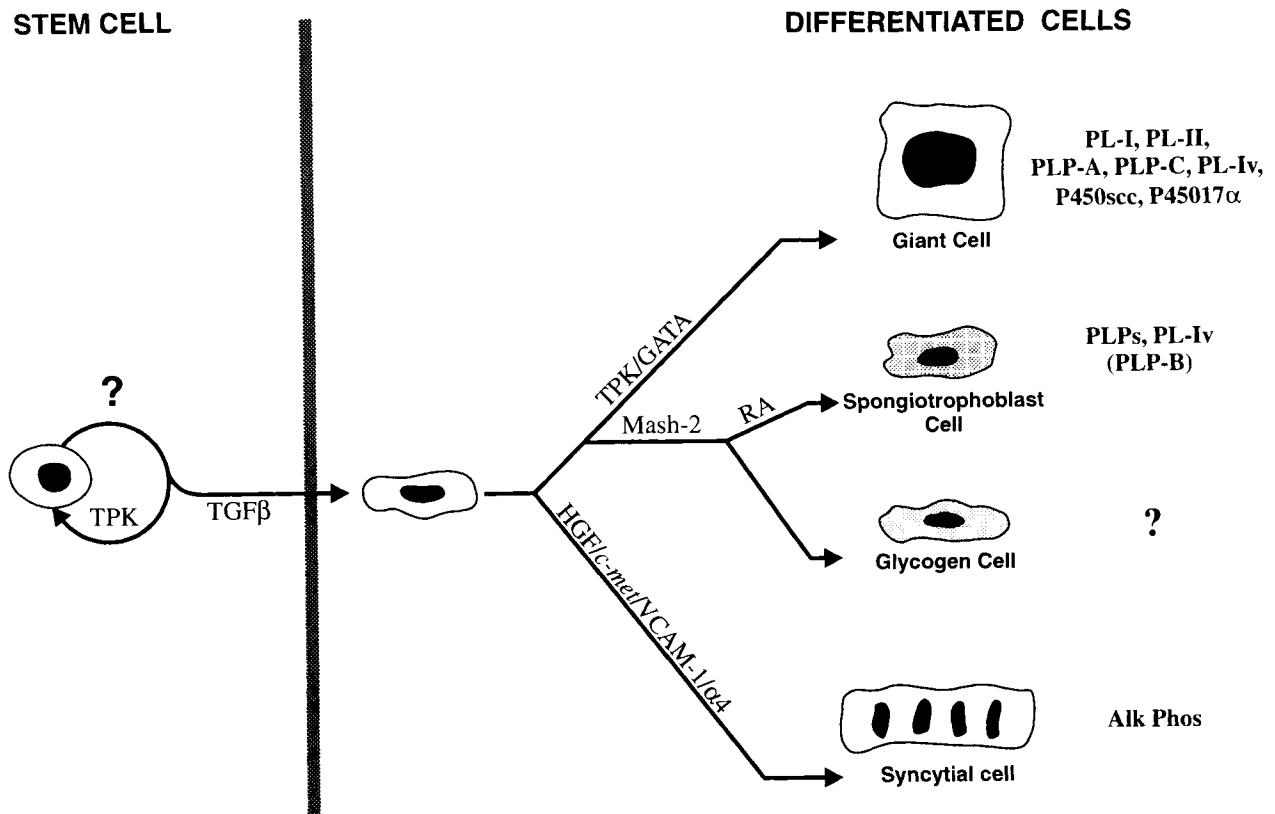
Regulators of trophoblast proliferation may differ depending upon the specific branch of the trophoblast cell lineage. For example, HGF/SF appears to specifically expand the labyrinthine trophoblast cell population (Uehara *et al.* 1995). Progenitors for the trophoblast giant cell, spongiotrophoblast and glycogen cell populations may be expanded by different sets of signals. Some of these modulators may be influenced by the mutations affecting trophoblast development described above.

The morphogenesis of the chorioallantoic placenta appears to result from two different mesenchymal-

epithelial interactions. Signals emanating from the inner cell mass govern early peri-implantation trophoblast growth (Gardner 1983). Postimplantation trophoblast cell expansion is modulated by positive modulators arising from fetal mesenchyme, an inner cell mass derivative (HGF/SF; Uehara *et al.* 1995) and negative modulators arising from maternal mesenchyme, decidua ( $TGF\beta$ ; Tamada *et al.* 1990; Hamlin & Soares 1995). Other positive and negative trophoblast cell proliferation modulators arising from maternal and fetal mesenchymal or possibly trophoblast sources are likely to be identified in the future.

#### *Trophoblast giant cell pathway*

Trophoblast giant cell differentiation is initiated through the development of cell-cell interactions and is facili-



**Fig. 3.** Schematic diagram of the rat trophoblast cell lineage. A putative trophoblast stem cell gives rise to four distinct pathways of differentiation: (i) trophoblast giant cells; (ii) spongiotrophoblast cells; (iii) glycogen cells; and (iv) syncytial cells. Trophoblast giant cells are the sole placental sources of placental lactogen-I (PL-I), PL-II, cytochrome P450 side chain cleavage (P450scc or CYP11A), and cytochrome P450 17 $\alpha$ -hydroxylase (P45017 $\alpha$  or CYP17), and minor contributors to prolactin-like protein-A (PLP-A), PLP-C, and PL-I variant (PL-Iv) production. Spongiotrophoblast cells are the major sources of each of the PLPs and PL-Iv and the only trophoblastic source of PLP-B. Syncytial cells express a unique form of alkaline phosphatase. Question marks denote uncertainties regarding the control of trophoblast cell differentiation and markers for glycogen cells. Putative regulatory genes implicated in directing trophoblast differentiation are indicated. Abbreviations: TPK, tyrosine protein kinase;  $TGF\beta$ , transforming growth factor- $\beta$ ; GATA, family of transcription factors binding GATA *cis*-elements; *Mash-2*, mammalian member of the achaete scute family-2; RA, retinoic acid; HGF, hepatocyte growth factor; *c-met*, HGF receptor; VCAM-1, vascular cell adhesion molecule-1;  $\alpha 4$ ,  $\alpha 4$  integrin.

tated by the removal of mitogenic stimuli (Faria & Soares 1991; Hamlin *et al.* 1994; Hamlin & Soares 1995). Trophoblast giant cell differentiation is characterized by endoreduplication (continued DNA synthesis in the absence of cell division) and the stage specific expression of members of the prolactin (PRL) gene family and two steroid hydroxylases (Faria & Soares 1991; Hamlin *et al.* 1994; Yamamoto *et al.* 1994, 1995a,b). Progression along the trophoblast giant cell differentiation pathway and maintenance of the differentiated trophoblast giant cell phenotype are under intrinsic control mechanisms, involving, at least in part, tyrosine kinase signaling pathways (Hamlin & Soares 1995; Chapman & Soares unpubl. data). *Src* family tyrosine kinases are the most prominent tyrosine kinases activated in proliferating and differentiating trophoblast cells (Hamlin & Soares 1995; Hamlin & Soares, unpubl. data). Activation of one of the *Src* family tyrosine kinases, *lyn*, is directly coupled to trophoblast giant cell differentiation (Hamlin & Soares, unpubl. data). Such observations are consistent with the role of *lyn* in other cell types where it is known to arrest the cell cycle and promote differentiation (Kharbanda *et al.* 1994a,b; Scheuermann *et al.* 1994). Additionally, *lyn* kinase can be activated via treatment with agents known to cause endoreduplication in normal cell types (Kharbanda *et al.* 1994a,b). Thus, we propose that *lyn* participates in the initiation, progression and/or maintenance of trophoblast giant cell differentiation.

How does *lyn* communicate to the nucleus in order to activate or sustain the expression of trophoblast giant cell differentiation-specific genes? *Lyn* may directly activate differentiation-dependent transcription factors via tyrosine phosphorylation or may indirectly activate other signaling pathways culminating in the activation of differentiation-dependent transcription factors. A likely indirect mechanism for signaling to the nucleus for members of the *Src* family involves activation of the *ras* pathways (Courtneidge *et al.* 1993; Pickett & Gutierrez-Hartmann 1994).

Two trophoblast giant cell genes transcriptionally activated during differentiation (placental lactogen-I, PL-I and cytochrome P450 side chain cleavage enzyme, P450scc) are potentially downstream events coupled to tyrosine kinase pathways. 5' Flanking regulatory DNA of both genes contain consensus sequences consistent with the involvement of tyrosine kinase signaling pathways and the GATA family of transcription factors (Shida *et al.* 1993; Ng *et al.* 1994; Yamamoto *et al.* 1995a). Although GATA factors modulate PL-I gene expression (Ng *et al.* 1994), it is not clear how GATA factors are activated during trophoblast giant cell differentiation. Mitogen-activated protein kinase, a downstream component of the *ras* pathway, has recently

been shown to phosphorylate and modulate GATA-2 activation in hematopoietic stem cells (Towatari *et al.* 1995). It is important to appreciate that the control of trophoblast giant cell specific gene expression is likely multi-factorial, involving the coordinated activities of multiple *cis*-acting elements and multiple *trans*-acting factors. Identification of *cis*-acting elements and *trans*-acting factors regulating P450scc promoter activation in differentiating trophoblast giant cells will provide insight into regulatory circuits from the cell surface to the nucleus controlling trophoblast giant cell differentiation.

#### *Spongiotrophoblast and glycogen cell pathways*

Spongiotrophoblast and glycogen cells arise during the morphogenesis of the chorioallantoic placenta (Soares *et al.* 1993). As indicated above, *Mash-2* is pivotal to the development of these components of the chorioallantoic placenta; however, its targets remain to be elucidated (Guillemot *et al.* 1994). Further insight into the development of these cell types has been hindered by the lack of suitable *in vitro* models. Some progress has recently been made with the establishment of a primary culture system for rat spongiotrophoblast cells (Lu *et al.* 1994). This culture system is compatible with spongiotrophoblast cell differentiation, expresses robust levels of members of the PRL gene family, and is susceptible to manipulation. Retinoic acid has a profound modulatory effect on the phenotype of these cultured spongiotrophoblast cells affecting their pattern of expression of members of the PRL gene family (Lu *et al.* 1994). Glycogen cells show a more complex appearance and disappearance during gestation (Davies & Glasser 1968). The absence of markers and *in vitro* systems for studying glycogen cells has limited progress in understanding the pattern of their differentiation.

#### *Syncytial trophoblast cell pathway*

The development of the labyrinth zone is dependent upon signals arising from the embryo/fetus (Roby & Soares 1993). Syncytial trophoblast cells are the epithelial component of a classic epithelial-mesenchymal relationship within the labyrinth zone of the chorioallantoic placenta. The targeted disruption of HGF/SF, *c-met*, VCAM and  $\alpha 4$  integrin has led to the identification of mesenchymal-epithelial signaling pathways involved in the morphogenesis of the labyrinth zone of the chorioallantoic placenta (Gurtner *et al.* 1995; Kwee *et al.* 1995; Schmidt *et al.* 1995; Uehara *et al.* 1995; Yang *et al.* 1995). Presumably, these signaling events are critical for initiating events leading to the fusion of trophoblast stem cells and their subsequent differentiation into syncytial trophoblast cells. Further pro-

ress will be facilitated by the development of syncytial trophoblast markers and *in vitro* culture systems. Syncytial trophoblast cells comprise an epithelium involved in nutrient and waste transport. Thus, specific transporter proteins and functions may prove useful for monitoring syncytial trophoblast cell differentiation. Rat syncytial trophoblast cells express a unique alkaline phosphatase protein that has been used to monitor labyrinthine trophoblast cell development (Campbell *et al.* 1991; Roby *et al.* 1993).

## Conclusions

Our understanding of trophoblast cell differentiation has benefited from several important technical advancements and a fair amount of serendipity. The expanding use of gene targeting strategies will undoubtedly lead to the fortuitous identification of additional trophoblast regulatory genes. However, deciphering their role in the scheme of trophoblast cell differentiation and placental morphogenesis will require the development of other approaches including *in vitro* strategies for culturing various trophoblast cell lineages and precise means of monitoring the differentiation state of the cells. Placentation is essential for the propagation of viviparous species. It is anticipated that the fundamental regulatory processes governing the differentiation of the parenchymal cells of the placenta, trophoblast cells, will be conserved across species.

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