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CURRENT TOPIC

Genes, Development and Evolution of the Placenta

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Through studies of transgenic and mutant mice, it is possible to describe molecular pathways that control the development of all major trophoblast cell subtypes and structures of the placenta. For example, the proliferation of trophoblast stem cells is dependent on FGF signalling and downstream transcription factors Cdx2, Eomes and Err2. Several bHLH transcription factors regulate the progression from trophoblast stem cells to spongiotrophoblast and to trophoblast giant cells (Id1/2, Mash2, Hand1, Stra13). Intercellular actions critical for maintaining stable precursor cell populations are dependent on the gap junction protein Cx31 and the growth factor Nodal. Differentiation towards syncytiotrophoblast as well as the initiation of chorioallantoic (villous) morphogenesis is regulated by the Gcm1 transcription factor, and subsequent labyrinth development is dependent on Wnt, HGF and FGF signalling. These insights suggest that most of the genes that evolved to regulate placental development are either identical to ones used in other organ systems (e.g., *FGF* and epithelial branching morphogenesis), were co-opted to take on new functions (e.g., *AP-2γ*, *Dlx3*, *Hand1*), or arose via gene duplication to take on a specialized placental function (e.g., *Gcm1*, *Mash2*). Many of the human orthologues of these critical genes show restricted expression patterns that are consistent with a conserved function. Such information is aiding the comparison of the human and mouse placenta. In addition, the prospect of a conserved function clearly suggests potential mechanisms for explaining complications of human placental development.
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INTRODUCTION

Comparing the placenta from one species to another can be a challenging task. On one hand, the gross anatomy of the placenta is at least superficially rather different among different species (Wooding and Flint, 1994). To complicate matters, even where analogous cell types exist, different names are often used to describe them in different species. The temptation is to either give up or assume that the placenta evolved independently into very different structures in different mammals. Although the differences are certainly interesting, a preoccupation with them obscures the conserved aspects of placental structure and function. At the most basic level, the placental trophoblast-derived structure fulfils two distinct functions in every mammalian species. First, it generates a large surface area for nutrient exchange, consisting of an epithelial barrier and underlying foetal blood vessels ('transport and barrier trophoblast') that comprise the chorioallantoic placenta. Second, trophoblast cells interact closely with the uterus (often after invading it) and produce growth factors, cytokines and hormones which target maternal physiological systems, resulting in provision of more blood flow and nutrient delivery to the feto-placental unit ('invasive and endocrine trophoblast'). Precisely how these structures and functions are orchestrated may vary among species, but the general principles are likely to remain the same. Because of the explosion of new information emerging from analysis of mutant and transgenic mice concerning the molecular control of placental development, it is an important time to deal with the issue of how to extrapolate this information to other species. Detailed descriptions and comparisons of the anatomy of the mouse and human placenta have been published elsewhere (Adamson et al., 2002; Georgiades et al., 2002). In this review, we present a framework for comparing the mouse and human placenta (Figure 1, Table 1) and provide molecular evidence in support (Table 2). Finally, we discuss how the identification of genetic pathways in mice has refined our thinking about the control of placental development and how it may have evolved.

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EVOLUTIONARY ORIGINS OF THE PLACENTA

The most primitive type of chorioallantoic placenta can be traced back to the egg-laying animals that, similar to eutherian

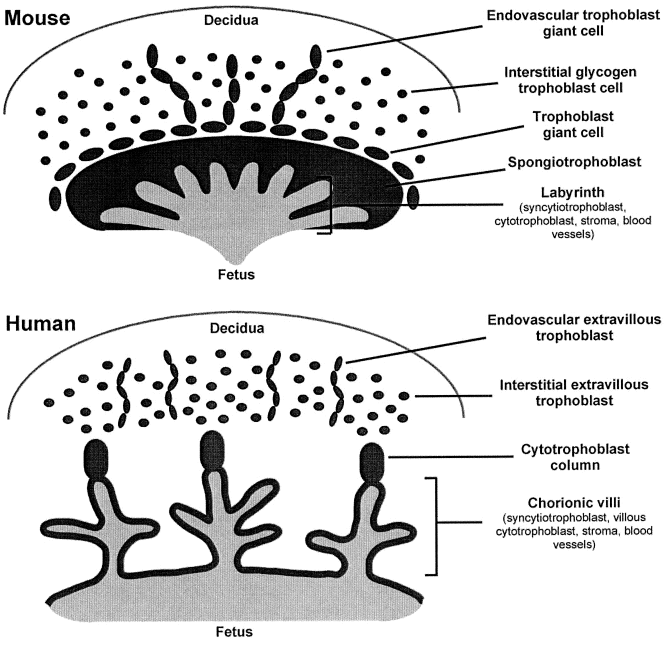

Figure 1. Comparative anatomy of the human and mouse placenta.

mammals, have two vascularized extraembryonic membranes (Wooding and Flint, 1994; Gilbert, 1997). The yolk sac derives nutrients from the yolk and is essential throughout development in reptiles and birds. Although it is often considered to be vestigial in eutherian mammals, the yolk sac is clearly essential for nutrition of the early embryo prior to the time that the placenta has formed (Cross et al., 1994). The chorioallantoic membrane that forms the placenta in higher mammals also exists in reptiles and birds albeit as a much simpler structure. In birds, the chorioallantoic membrane underlies the eggshell and is essential for gas exchange and for transporting calcium from the shell to the developing fetus. Clearly, the mammalian placenta has evolved to take on many additional roles dealing with intrauterine life, that are not undertaken by the chorioallantoic membrane in reptiles and birds. These include the production of hormones that alter systemic maternal functions and growth factors that alter the local uterine environment. In addition, trophoblast cells, in some species, either fuse with endometrial cells or invade into the uterus to promote a more intimate contact between conceptus and mother. It is not clear what to consider as the evolutionary precursor of the chorion in lower vertebrates and invertebrates and thus there is no way to identify candidate regulatory genes for the placenta by analogy to genetically tractable organisms like *Drosophila* and *C. elegans*.

COMPARATIVE PLACENTAL STRUCTURE: A GENERALIST'S VIEW

In most placental mammals, 'transport and barrier trophoblast' is organized into highly branched villous, tree-like folds (Figure 1). The organization of the villi is one basis for classifying placental structures (Wooding and Flint, 1994). The villi can be diffuse (pig, horse), or they can be tightly grouped into a band (cat, dog) or into round structures called cotyledons. Rodents have a single cotyledon (discoid placenta) whereas humans have multiple cotyledons, though they are consolidated into a cluster resembling a single disc. In ruminants (sheep, cow), multiple cotyledons are scattered across the placental surface. The structure of the trophoblast cell layer that covers the villi and the type of maternal cells that it directly contacts vary between species. These differences are another basis for placental classification (Wooding and Flint, 1994). In rodents and primates, the uterine epithelium (endometrium) is eroded such that maternal blood comes into direct contact with the trophoblast surface (haemochorial). Whereas rodents have three trophoblast cell layers (tri-chorial) including two syncytial layers and a single 'mononuclear' cell type of unknown function, primates have a single syncytial layer plus an underlying trophoblast stem cell layer. In ruminants and horses, the endometrium essentially remains intact and trophoblast cells lie in direct contact with uterine epithelium (epithelio-chorial).

The 'invasive and endocrine trophoblast' subpopulation is divergent across species in the extent of uterine invasion and the types of maternal-acting hormones that it can produce. Ruminant bi-nucleate cells migrate essentially only the distance of one cell diameter to enter the endometrium (Wooding and Flint, 1994). In contrast, rodent trophoblast giant cells and glycogen trophoblast cells migrate several hundred microns into the uterus (Adamson et al., 2002), though still a relatively modest distance compared to the analogous cells in humans, the extravillous cytotrophoblast cells (Figure 1). The cell adhesion molecules and matrix-degrading proteinases that mediate cell invasion in humans are expressed by trophoblast giant cells in mice (Cross et al., 1994). The characteristic feature of rodent trophoblast giant cells is that they are polyploid (Zybina and Zybina, 1996), as a result of repeated rounds of DNA replication in the absence of intervening mitoses, an unusual type of cell cycle term called endoreduplication (MacAuley et al., 1998). Human extravillous cytotrophoblast (Berezowsky et al., 1995) and bovine binucleate cell nuclei (Klisch et al., 1999) are polyploid, though ploidy tends to be 4–8 N compared with up to 1024 N in rodents (Zybina and Zybina, 1996). This is different than syncytiotrophoblast cells, that form as the result of trophoblast cells exiting the cell cycle and fusing together to form a multi-nucleated syncytium with each nucleus remaining diploid (Figure 2, Table 1).

Two general types of invasion are observed in the human and rodent placenta (Pijnenborg et al., 1981) (Figure 1). Trophoblast giant cells in rodents show invasion that is strictly associated with maternal spiral arteries that bring blood to the implantation site (Adamson et al., 2002). Glycogen trophoblast cells, by contrast, invade into the interstitium of the maternal decidua and show no close association with the spiral arteries (Adamson et al., 2002). Differences in marker expression by these two cell types suggest that the endovascular and

Table 1. Comparison of structures in the human and mouse placenta

	Human	Mouse
A. Invasive and endocrine trophoblast		
Formal name	Extravillous cytotrophoblast	Trophoblast giant cell
Invasive	Yes	Yes
Proliferative	No	No
DNA content	Mononuclear polyploid (4 N–16 N)	Mononuclear polyploid (up to 1000 N)
B. Transport and barrier trophoblast		
Formal name	Chorionic villi	Labyrinth
Haemochorial	Yes	Yes
Syncytiotrophoblast surface	Yes	Yes
Formed by cell fusion	Yes	Yes
Nuclear DNA content	Diploid	Diploid

Table 2. Comparative gene expression in the human and mouse trophoblast cell lineage

Gene	Human	Mouse
Gcm1	Syncytiotrophoblast and precursor	Syncytiotrophoblast and precursor
Hand1	Trophectoderm	Trophectoderm, trophoblast giant cells
Id2	Villous cytotrophoblast	Chorionic trophoblast
Mash2	Column cytotrophoblast	Spongiotrophoblast
Met	Villous cytotrophoblast	Chorionic trophoblast
Mmp9	Invasive cytotrophoblast	Trophoblast giant cells
Pecam1	Endovascular cytotrophoblasts	Endovascular trophoblast giant cells
Tef5	Syncytiotrophoblast	Syncytiotrophoblast
uPA	Invasive cytotrophoblast	Trophoblast giant cells

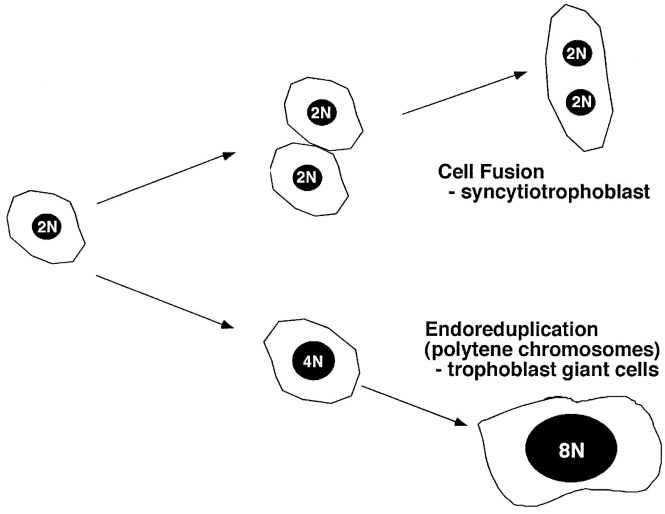

Figure 2. Formation of multi-nucleated syncytiotrophoblast and mononuclear, polyploid trophoblast giant cells.

interstitial cells represent distinct cell types (Adamson et al., 2002). Distinct endovascular and interstitial routes of trophoblast invasion are observed in the placental bed of humans (Pijnenborg et al., 1981) but, unlike in rodents, molecular markers have not been identified that can distinguish them.

MOLECULAR PATHWAYS REGULATING PLACENTAL DEVELOPMENT

Insights from gene knockout mice

The mouse is an extremely powerful system for understanding the control of development because of the ability to alter and ablate the expression of genes through transgenic and knockout mouse technologies. These experiments have produced an amazingly long list of genes that are essential for placental development [reviewed in (Cross et al., 1994; Rinkenberger et al., 1997; Cross, 2000; Hemberger and Cross, 2001; Rossant and Cross, 2001)]. The detailed analysis of these genetically altered mice, combined with the similarity of some of the mutant phenotypes, has allowed us to describe these genes as part of genetic pathways, and to gain precise understanding of the cellular and molecular functions of these pathways in tissue development (Cross, 2000; Hemberger and Cross, 2001; Rossant and Cross, 2001). Two

general lessons have emerged from this work. First, development of the two major placental substructures—'transport and barrier trophoblast' and 'invasive and endocrine trophoblast'—is regulated by distinct genetic pathways (Cross, 2000). Second, intercellular signalling events regulate many different aspects of trophoblast cell development (Rossant and Cross, 2001).

Trophoblast stem cells. Trophoblast stem cells are defined as those cells that have the potential to give rise to all differentiated trophoblast cell subtypes. They can be isolated from the blastocyst and early post-implantation stage mouse conceptuses by culturing them in the presence of fibroblast growth factor-4 (FGF4) and feeder cells (Tanaka et al., 1998). Removal of either factor results in cell proliferation arrest and differentiation to trophoblast giant cells. The transcription factors Cdx2 and Eomes are regulated by FGF signalling and are in turn also essential for the maintenance of trophoblast stem cells (Chawengsaksophak et al., 1997; Tanaka et al., 1998; Russ et al., 2000). Another transcription factor, AP-2γ, is also essential for trophoblast stem cell maintenance (Auman et al., 2002; Werling and Schorle, 2002), but whether it is regulated by FGF is unclear. FGF4 is expressed by the inner cell mass at the blastocyst stage and subsequently in the epiblast (embryonic ectoderm) whereas the FGF receptor, FGFR2, is expressed in trophoblast cells immediately adjacent to the epiblast (chorion or extraembryonic ectoderm) (Rossant and Cross, 2001). Because FGFs are matrix associated, the effect of FGF4 is restricted to this close paracrine effect. After gastrulation, when the epiblast cells lose their immediate contact with the chorionic trophoblast, it is unclear whether the FGF-dependent trophoblast stem cell population continues to persist. It is certainly possible that another FGF is produced to sustain the cells. FGF-dependent TS cell lines have not been established from mouse conceptuses after E7.5, however, implying that trophoblast stem cells may not persist (Rossant and Cross, 2001).

Two other signalling systems appear to regulate the maintenance of trophoblast stem cells and suppress differentiation, at least at later times during development. The nuclear receptor Err2/Errβ (encoded by the *Esrrb* gene) is expressed in the extraembryonic ectoderm after E7.5 and *Esrrb*-deficient embryos show premature loss of the extraembryonic ectoderm and ectoplacental cone, with an associated increase in trophoblast giant cells (Luo et al., 1997). Err2/Errβ appears to be a constitutively active nuclear receptor, but upon binding to the estrogen analogue diethylstilbestrol (DES), transcriptional activity is repressed (Tremblay et al., 2001). Consistent with this, DES treatment of a cultured TS cell line promotes the loss of stem cell character and increases the formation of trophoblast giant cells (Tremblay et al., 2001). A TGFβ family member, Nodal, also has a role in regulating trophoblast development in that trophoblast giant cell differentiation is enhanced in Nodal-deficient embryos (Ma et al., 2001). Curiously, trophoblast giant cells themselves express Nodal (Ma et al., 2001). Although there is no direct evidence to suggest how Nodal production from giant cells might negatively regulate giant cell formation, it implies that different trophoblast subtypes can interact with each other.

Ectoplacental cone and spongiotrophoblast. At early post-implantation stages of development, trophoblast cells in the ectoplacental cone lie between the chorion and the outer layer of trophoblast giant cells. It is thought that ectoplacental cone cells later become the spongiotrophoblast, largely based on gene expression patterns. For example, the genes *Tpbp/4311* (Lescisin et al., 1988) and *Flt1* (He et al., 1999) are expressed in the ectoplacental cone and spongiotrophoblast, and not in trophoblast giant cells, extraembryonic ectoderm or labyrinth. Importantly though, expression of *Tpbp/4311* and *Flt1* is first detectable only in a few cells at the apex of the ectoplacental cone at E7.5 and 8.5. In contrast, *Tpbp/4311* and *Flt1* are expressed throughout the spongiotrophoblast layer at later times in development, albeit not at uniform levels in all cells. This shows that the ectoplacental cone is not uniform and is composed of more than one cell type, though the functions of the two populations is not clear.

The basic helix-loop-helix (bHLH) transcription factor Mash2 is expressed in the chorion, ectoplacental cone and later the spongiotrophoblast (Guillemot et al., 1994; Nakayama et al., 1996). *Mash2*-deficient embryos show an absence of spongiotrophoblast by E10.5 as well as increased number of giant cells and a secondary failure of the labyrinth to form (Guillemot et al., 1994; Tanaka et al., 1997). The mutant phenotype is interesting because, despite early expression, *Mash2* mutants have a normal ectoplacental cone and chorion up to at least E8.5, implying that *Mash2* gene is only required for the later maintenance of the ectoplacental cone-derived cells and not for their initial formation. While *Mash2* mRNA is expressed in both the outer part of the ectoplacental cone, coincident with *Tpbp/4311* and *Flt1*, it is also expressed in the inner part (Guillemot et al., 1994; Nakayama et al., 1996). Other members of the bHLH transcription factor family that are obligate DNA binding partners for Mash2, Alf1/HEB and Itf2, are only expressed in the inner part of the ectoplacental cone (Scott et al., 2000). This pattern is informative because it implies that Mash2 function is actually limited to the inner cells and, therefore, that these cells are somehow linked to the maintenance of the spongiotrophoblast. This is likely due to Mash2 promoting trophoblast proliferation (N.D. and J.C.C., unpublished observations). It is notable that in addition to Mash2, its potential partners Alf1/HEB and Itf2 are also expressed also in the chorion (Scott et al., 2000). Whether they are able to dimerize with Mash2 is unclear, however, since cells of the chorion also express Id1 and Id2 (Jen et al., 1997). The Id proteins are dominant–negative HLH factors that can dimerize with Alf1/HEB, Itf2 as well as the related E12/47 proteins (Norton, 2000).

In addition to intrinsic factors, it is clear that trophoblast development is regulated by cell extrinsic factors. Cell–cell interactions are important as embryos that are deficient for the gap junction protein Cx31 (expressed in the spongiotropho-

blast) show a transient alteration in the spongiotrophoblast layer (Plum et al., 2001). The proliferation of human trophoblast cells in vitro is highly regulated by oxygen tension. Primary cells that are cultured in low oxygen proliferate, as they would be expected to do in vivo, whereas cells cultured in an atmosphere with 20 per cent oxygen do not proliferate and instead differentiate into the invasive trophoblast subtype (Genbacev et al., 1996; Genbacev et al., 1997). There are two ways to interpret these data. On one hand one could conclude that low oxygen suppresses differentiation. Alternatively, one could argue that exposure of early trophoblast cells to a high oxygen level might be toxic and that the response is to arrest cell proliferation and to differentiate. A precedent for trophoblast cells responding to a toxic insult by differentiating is that proliferating trophoblast cells exposed to radiation either die or differentiate (MacAuley et al., 1998; Heyer et al., 2000). No matter the effect by which trophoblast cells respond to different oxygen levels, the mechanism of low oxygen action is likely mediated by the transcriptional complex HIF1α/Arnt. *Arnt*-deficient embryos show a placental phenotype in which the labyrinth and spongiotrophoblast layers are reduced in size, and giant cell numbers are increased (Adelman et al., 2000). Culture of TS cells in low oxygen promotes their differentiation into spongiotrophoblast-type cells and suppresses giant cell formation (Adelman et al., 2000).

Trophoblast giant cells. Trophoblast giant cells form from the mural trophectoderm immediately after implantation (primary giant cells) and then more giant cells emerge from the differentiation of cells at the outer edge of the ectoplacental cone (secondary giant cells) (Cross et al., 1994; Hemberger and Cross, 2001; Rossant and Cross, 2001). The differentiation of both primary and secondary giant cells is dependent on the bHLH factor Hand1 (Riley et al., 1998; Scott et al., 2000). Hand1 and Mash2 have opposing functions and indeed in vitro they show mutually antagonistic activities (Scott et al., 2000). In *Hand1* mutants, *Mash2* mRNA expression fails to be shut down (Riley et al., 1998) which may be functionally important because ectopic expression of *Mash2* in vitro is sufficient to block giant cell differentiation (Cross et al., 1995; Kraut et al., 1998; Scott et al., 2000). Suppression of Mash2 DNA-binding activity is also likely the mode of action of the I-mfa protein (Kraut et al., 1998). I-mfa binds to Mash2 in vitro and embryos lacking I-mfa show fewer giant cells than normal (Kraut et al., 1998). This phenotype is not observed in some genetic backgrounds, however, indicating that this function is modulatory but not essential.

The differentiation of trophoblast giant cells is thought to be a 'default' pathway in the sense that if primary trophoblast cells are cultured (in the absence of FGF) they spontaneously differentiate into giant cells (Rossant, 1986). Despite this, it is clear that some extrinsic cues can regulate giant cell differentiation. Whereas low oxygen levels, at least in vitro, can block giant cell formation (Adelman et al., 2000), treatment with retinoic acid stimulates giant cell differentiation both in vitro and in vivo (Yan et al., 2001). Retinoid signalling is likely to be important for normal trophoblast development because mutations in retinoic acid receptors have placental phenotypes (Wendling et al., 1999; Sapin et al., 2000). In addition, the retinoic acid-regulated gene *Stra13* is expressed in trophoblast giant cells in vivo as a part of normal development (Boudjelal et al., 1997), and *Stra13* expression increases during the differentiation of TS cells in vitro (M. Hughes and J.C.C., unpublished observations).

Glycogen trophoblast cells. The cell lineage origins of glycogen trophoblast cells are not exactly clear. However, they first appear within the spongiotrophoblast layer before they begin to migrate into the decidual tissue of the mother and they express the *Tpbp/4311* gene, a gene otherwise exclusively expressed in spongiotrophoblast (Adamson et al., 2002). Therefore, it is very likely that they are a specialized subtype of spongiotrophoblast. The only molecular clues that we have about the control of their development is that fewer glycogen trophoblast cells are present *Igf2* in mutants (Lopez et al., 1996), and that more glycogen cells is a common finding in several single gene mutants (Li and Behringer, 1998; Takahashi et al., 2000; Frank et al., 2002).

Syncytiotrophoblast and morphogenesis of the labyrinth. The labyrinth layer of the placenta forms starting at E8.5 when allantoic mesoderm attaches to the basal surface of the chorion layer (Cross, 2000). While the chorion continues to have proliferating cells within it, some of trophoblast cells begin to differentiate early on E9 and form primary branchpoints for the villous structure. Syncytiotrophoblast cell differentiation also begins early on E9 and the *Gcm1* gene is necessary for both the initiation of morphogenesis and syncytiotrophoblast differentiation (Anson-Cartwright et al., 2000). *Gcm1* encodes a transcription factor that is homologous to a factor that controls an important cell fate decision in the nervous system in Drosophila (Wegner and Riethmacher, 2001). While *Gcm1* expression in mice appears to be essentially restricted to the placenta, it probably has an analogous cellular function in that it regulates a cell fate decision between chorion trophoblast and the progenitors of villi and syncytiotrophoblast. *Gcm1* is the only gene identified to date that has been shown to be critical for syncytiotrophoblast differentiation.

Several other transcription factors are critical for development of the labyrinth layer after initiation of morphogenesis, such as Dlx3 (Morasso et al., 1999), Pparγ (Barak et al., 1999) and Esx1 (Li and Behringer, 1998). The cellular function of these factors, and how they are regulated, is not entirely clear. In contrast, genes encoding several components of known biochemical pathways show similar mutant phenotypes (Hemberger and Cross, 2001; Rossant and Cross, 2001). Because of the similarity of their phenotypes, and localized sites of expression or function, it is possible to describe signalling pathways among different cell types at the chorioallantoic interface (Hemberger and Cross, 2001; Rossant and Cross, 2001). One example is the Wnt signalling pathway. Deficiency for *Wnt7b* (Parr et al., 2001) or genes encoding the

Wnt-activated transcription factors, *Tcf/Lef1* (Galceran et al., 1999), is associated with failures in chorioallantoic attachment. This may reflect a requirement for Wnt signalling in either polarity of the trophoblast cells in the chorion or induction of expression of the cell adhesion molecule α4 integrin in the mesothelial cells underlying the basal surface of the chorion (Parr et al., 2001). Wnt signalling is also required later in labyrinth development, as shown by mutant phenotypes for the *Wnt2* (Monkley et al., 1996) and *Fzd5* (encoding a Wnt receptor) (Ishikawa et al., 2001) genes. The *Wnt2* gene is expressed by the allantois and *Fzd5* appears to be expressed in the trophoblast component of the labyrinth. Growth factor signalling through receptor tyrosine kinases is also required for chorioallantoic morphogenesis (Rossant and Cross, 2001). Whereas a null mutation in *Fgfr2* is associated with early blastocyst-stage phenotype, consistent with a role in trophoblast stem cell proliferation (Arman et al., 1998), a hypomorphic mutation results in a chorioallantoic branching defect (Xu et al., 1998). A similar phenotype is observed in mutants for the *Hgf* (Schmidt et al., 1995; Uehara et al., 1995) and *c-Met* (encoding the HGF receptor) (Bladt et al., 1995) genes. Likewise mutation of genes encoding signalling adaptor proteins (Grb2, Sos1, Mek1, p38) and transcription factors (Fra1, JunB) known to be downstream of receptor tyrosine kinases all produce similar phenotypes (Rossant and Cross, 2001). Similar to Wnt2/Fzd5, the major downstream responses of the receptor tyrosine kinases are in the trophoblast component of the labyrinth (Rossant and Cross, 2001). The major impact of these findings is that it focuses attention on signalling interactions between allantoic mesoderm and trophoblast as important factors in labyrinth development.

Conservation of molecular pathways between mice and humans

Because many of the genes that are essential for trophoblast development in mice show patterns of expression and functions that are restricted to one specific trophoblast subtype (Table 2), a demonstration of where the human orthologues are expressed helps to make explicit functional comparisons between the human and mouse placenta. Although this has only been done for a limited number of genes, the information is quite revealing (Table 2). Mouse chorionic trophoblast and human villous cytotrophoblast cells both act as stem cell populations and are identified by expression of *Id2/ID2* (Jen et al., 1997; Janatpour et al., 2000). *Mash2* is expressed in the 'intermediate' type of trophoblasts in the EPC/spongiotrophoblast in mice (Guillemot et al., 1994), and its human orthologue (*MASH2* or *HASH2*) is detected in cytotrophoblast cell columns (Alders et al., 1997; Janatpour et al., 1999). In both organisms, this gene is down regulated during trophoblast differentiation (Guillemot et al., 1994; Janatpour et al., 1999; Scott et al., 2000). Invasive, extravillous cytotrophoblast cells in humans and trophoblast giant cells in rodents share expression of the matrix-degrading enzyme MMP9 (Librach et al., 1991; Alexander et al., 1996), the cell adhesion molecule $\alpha_1\beta_1$ integrin (Sutherland et al., 1993; Damsky et al., 1994) and the transcription factor Stra13 (Boudjelal et al., 1997; Janatpour et al., 1999). HAND1 expression in human trophoblast has been detected in trophectoderm of blastocysts (Knofler et al., 2002), similar to mouse (Cross et al., 1995), and in choriocarcinoma cells (Knofler et al., 1998) but not as yet in the mature placenta. *Gcm1/GCM1* is expressed in a defined subset of cells within the labyrinth in mice (Anson-Cartwright et al., 2000) and the chorionic villi in humans (Janatpour et al., 1999; Nait-Oumesmar et al., 2000; D.B., J.C.C. and J.C.P.K., manuscript submitted). The syncytiotrophoblast layers of the murine labyrinth in mice and the floating chorionic villi in humans are characterized by *Tef5/TEF5* expression (Jacquemin et al., 1998).

Given the considerable conservation in trophoblast subtype-specific expression between mice and humans, it seems likely that the molecular functions are conserved as well. While human systems are not amenable to genetic manipulation in the same way as the mouse, a limited amount of data is available that supports the hypothesis of conserved trophoblast cell subtype-specific functions. GCM1 and TEF5 have been shown to transactivate the syncytiotrophoblast-expressed genes encoding aromatase (CYP19) (Yamada et al., 1999) and chorionic somatomammotropin (Jiang et al., 1999), respectively. Mis-expression of ID2 in cultured human trophoblast reduces their invasion and supports the maintenance of markers typical of stem cells (Janatpour et al., 2000). The more complete functional data in mice provided by knockout mice for a broader range of genes should clearly help to suggest molecular mechanisms underlying the developmental defects that are associated with human diseases such as spontaneous abortions, preeclampsia and intrauterine growth restriction.

CONCLUSIONS: MOLECULAR EVOLUTION OF THE PLACENTA

When molecular biology investigations began into the control of placental development and regulation of placental-specific genes, the conventional wisdom was to go looking for genes that were uniquely expressed there. The logic was that the most important developmental regulators would be trophoblast-specific factors. Today though, it is clear that most of the genes that have been shown to be essential for placental development are also involved in the development of other organs and that expression of only a very limited number of genes is restricted to the placenta. This implies that evolution of the placenta among vertebrates did not involve invention of an entirely new set of genes. Rather, nature has re-used existing pathways for functions common to other systems (e.g., FGF signalling and branching morphogenesis in the lung as well as the placenta), re-cycled old genes to take on new functions (e.g., *Hand1* functions in placenta, heart and neural crest), and duplicated and diverged existing multi-gene families to provide a pool of 'genetic talent' dedicated to placental

development (e.g., *Gcm1, Mash2*). The variations in placental structures that we see among eutherian mammals may simply reflect slightly more or less elaborate use of the 're-use, re-cycle and duplicate' plan.

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