fied *SLG*- and *SRK*-related genes. Sequences encoded by this family of genes have been cloned from diverse plants and tissues, such as maize roots, carrot cell cultures, and *Arabidopsis* shoots and roots (6). The cell type–specific pattern of expression exhibited by the vegetatively expressed *SRK*-like genes of *Arabidopsis* (25) is consistent with the hypothesis that the *Brassica S*-locus genes are derived from ancestral genes having a very basic role in intercellular communication during plant development.

Characterization of these genes will determine how far the lessons learned from the study of SI can be applied to other plant systems. This question is of more than academic interest, for it will determine if the mechanism by which a plant controls the formation and growth of a pollen tube bears similarity to how it controls the growth of invading pathogens or the growth of its own cells in various phases of its development. Thus, further refinement of the mechanism of SI, including identification of activators and targets of the stigmatic proteins, will be important for understanding the fundamental nature of cell-cell signaling during the development of the plant body.

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Implantation and the Placenta: Key Pieces of the Development Puzzle

James C. Cross,* Zena Werb, Susan J. Fisher

The mammalian embryo cannot develop without the placenta. Its specialized cells (trophoblast, endoderm, and extraembryonic mesoderm) form early in development. They attach the embryo to the uterus (implantation) and form vascular connections necessary for nutrient transport. In addition, the placenta redirects maternal endocrine, immune, and metabolic functions to the embryo's advantage. These complex activities are sensitive to disruption, as shown by the high incidence of early embryonic mortality and pregnancy diseases in humans, as well as the numerous peri-implantation lethal mutations in mice. Integration of molecular and developmental approaches has recently produced insights into the molecules that control these processes.

In a remarkable series of events, implantation and placental development physically connect the mammalian embryo to its mother. Establishing this connection is the embryo's first priority, which is essential for its subsequent development. The importance of this simple fact is often overlooked, but is underscored by the temporal sequence in which the differentiated embryonic cell types appear. In mammals, the initial developmental decisions set aside three unique extraembryonic lineages that are the precursors of the placenta. The first differentiation event gives rise to trophoblasts, the specialized epithelial cells of the placenta that physically connect the embryo and the uterus. The remaining cells segregate at one pole of the embryo to form the inner cell mass (ICM). Endodermal and mesodermal components of the placenta are later derivatives of the ICM. In contrast, the differentiation of ICM cells that give rise to the embryo proper does not begin until the first placental structure has formed. The placenta also establishes functional connections that are critical for embryonic survival. For example, trophoblasts redirect the maternal endocrine system to create the hormonal milieu that directs changes in the uterus necessary for pregnancy to continue. Trophoblasts express paternal proteins and interact directly with maternal immune cells but somehow avoid rejection. Another critical step is the establishment of a hybrid vasculature in which the fetal trophoblasts, acting like endothelial cells, are in direct contact with maternal blood, where they transport nutrients and gases.

Failures in implantation and placental development are clinically important. About one-third of normal human pregnancies end in spontaneous abortion; 22% of such abortions occur before pregnancy is detected clinically (*1*). Similarly, failures in development during the peri-implantation period account for almost 80% of the embryonic loss that occurs in farm animal species (*2*, *3*). Even seemingly minor defects in placentation can have severe negative consequences. In humans, for example, abnormalities in the vascular connections result in preeclampsia, a disease of pregnancy with significant morbidity and mortality to both mother and fetus (*4*). Such disorders not only affect the health of the mother and fetus, but also represent significant societal costs. Currently, the approaches for diagnosis and treatment of diseases of pregnancy are limited mainly because of our inability to understand their causes.

Implantation and development of the placenta occur in a stepwise manner (Table 1). Recent analyses of naturally occurring mouse mutants and several mutants created by gene targeting experiments have highlighted these processes as major determinants of fetal growth and development (Table 2). The important conclusion from

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these observations is that most of the major roadblocks to development in utero occur during major transitions in the development of the placenta. These checkpoints primarily affect the placenta's ability to meet the cardiovascular demands of the embryo during its progressive growth. Conversely, even severe abnormalities in the development of the embryo, including the absence of major organ systems, often do not prevent the conceptus from being carried to term.

Preimplantation Development: Establishing the Trophoblast Lineage

After fertilization in the oviduct, a series of symmetrical cell divisions create a mass of totipotent cells, the morula, still enclosed within the zona pellucida. The first differentiation event occurs after compaction of the morula (approximately day 3.5 in the mouse) with formation of the blastocyst. Cells that lie on the outside of the morula become trophectoderm, leaving undifferentiated cells of the ICM surrounded by trophectoderm. Blastocyst formation occurs in vivo after the embryo has moved from the oviduct into the uterus, but there is little evidence that the maternal environment affects preimplantation development. Mouse embryos produced by in vitro fertilization can easily be cultured to the blastocyst stage in simple media. This does not, however, rule out the possibility that preimplantation embryos and maternal (oviductal or uterine) cells communicate—only that such communication is not essential for early development. The presence of the preimplantation embryo is clearly not required for priming the maternal environment, because cleavage-stage embryos and blastocysts can be successfully transferred to a nonpregnant, hormonally prepared uterus.

Allocation of cells to the trophoblast lineage is dictated by the position in which each cell finds itself at the morula stage and involves the development of epithelial-like characteristics. Although they are expressed in all cells in the morula, the cell adhesion molecule E-cadherin (uvomorulin) and the Na^+-K^+–adenosine triphosphatase (ATPase) become redistributed to the basolateral plasma membrane of the mural trophectoderm. The latter establishes a transcellular Na^+ gradient that drives the accumulation of fluid in the blastocoel (cavitation) (5), although pharmacological studies suggest that other transport mechanisms may also be used (6). Perturbing the function of E-cadherin with antisera prevents compaction of the mouse morula and subsequent blastocyst formation, including redistribution of Na^+-K^+–ATPase (5), similar to the effects of a mutation in the mouse E-cadherin gene (7). Trophoblasts that overlie the ICM (polar trophectoderm) continue to proliferate as long as they are proximal to the ICM. Cells away from the ICM that surround the blastocoel (mural trophectoderm) stop dividing and ultimately differentiate into primary trophoblast giant cells. The nature of the mitogenic signal emitted from the ICM is unknown, although placement of a second ICM into the blastocoel cavity induces a second zone of proliferative trophectoderm. In contrast, removal of the ICM results in terminal differentiation of the trophectoderm (8).

General epithelial cell markers, such as cytokeratin expression, are first detected at the compacted morula stage in those cells that will become trophoblasts (9). The receptor for colony-stimulating factor–1 (CSF-1), encoded by the c-*fms* gene, is first detected in cleavage-stage mouse embryos and becomes restricted to trophoblasts, in which a cell-specific promoter drives its expression after the blastocyst stage (10). Trophoblast interferon (IFN) τ is probably the earliest trophoblast-specific gene identified to date; in sheep and cows, expression begins at the blastocyst stage as soon as the trophectoderm forms (2).

How a cell's position in the morula ultimately results in changes in gene expression remains to be answered. The POU domain–containing transcription factor Oct-4 is expressed by all cells during cleavage-stage development, but its expression is downregulated with their differentiation into definitive cell lineages, including trophoblast (11). Although no definitive Oct-4 target genes have been reported, Oct-4 could transactivate the expression of genes critical for maintaining the undifferentiated state or it may repress other genes associated with differentiated activities. For example, ectopic expression of Oct-4 in trophoblasts represses the activity of promoters for IFN-τ (12) and the human chorionic gonadotropin (hCG) α subunit, another trophoblast-specific gene (13). Although Oct-4 may limit trophoblast development, there are no genes that are known to be essential for trophoblast commitment. The best candidate may be an unidentified gene encoded at the t^{12} locus, within the *t* complex (14). The t^{12} mutants do not form normal blas-

Table 1. Major milestones and checkpoints in mouse intrauterine development.

Days of gestation	Event
3.5	Blastocyst formation
4.25 to 4.5	Blastocyst activation
4.5 to 6	Implantation
6 to 8	Formation of the yolk sac and vitelline circulation
9 to 10	Development of the chorioallantoic placenta
8 to 18	Development of fetal vasculature
20 to 21	Birth

Table 2. Factors and genes critical for implantation and placental development.

Event	Factor	Reference
Blastocyst formation	t^{12}	(14)
Implantation	Estrogen	(18)
	LIF	(23)
Early postimplantation	t^{w73}	(104)
	β1 Integrin	(109)
	Egfr	(29)
	vav	(108)
	lethal yellow (A^y)	(105)
	l(5)-1	(107)
	Oligosyndactylism	(106)
	evx1	(110)
	Fgf4	(111)
	Blind	(113)
	Velvet coat	(113)
	t^0	(14)
	t^{w5}	(14)
	Fug1	(112)
	exed	(114)
Yolk sac development	Fibronectin	(115)
	α5 Integrin	(116)
Chorioallantois development	VCAM-1	(157)
	α4 Integrin	(156)
	Mash-2	(95)

tocysts, which implies that the t^{12} gene is essential, although its effect may not be specific for trophoblast commitment.

Implantation: Trophoblasts Connect the Embryo to the Uterus

In some mammals, including rodents and primates, implantation occurs soon after the blastocyst hatches from the zona pellucida—for example, at day 4.5 of development in mice (Fig. 1). This is not true in all mammalian species; in farm animals (horses, pigs, sheep, and cattle), the conceptus remains free within the uterus for several days before implantation (*15*). During implantation in mice, trophoblasts begin to attach to the receptive uterine epithelium and the uterus clamps around the blastocyst. Within a few hours of implantation, several remarkable events occur, including transformation of the uterine stroma (the decidual response), recruitment of inflammatory and endothelial cells, transepithelial invasion of trophoblasts into the endometrium, and apoptosis of the uterine epithelium (*16*).

The window for implantation. Whereas the preimplantation conceptus can develop without maternal cues, implantation requires an active dialog between the maternal cells and the blastocyst. The process demands exquisite synchrony in the development of the uterus and the blastocyst, a fact that probably accounts for the high rate of failure of embryo transfer in both humans and animals. The uterus undergoes dramatic developmental changes during the preimplantation period that are controlled by ovarian steroid hormones, estrogen (from follicles) and progesterone (from corpora lutea). The role of estrogens in controlling these and other reproductive functions suggests that fertility may be affected by environmental toxins that have potent estrogenic effects (*17*). Estrogen and progesterone prime the uterus for implantation, and in rodents, a secondary surge of estrogen secreted by ovarian follicles is the trigger that induces implantation. These hormonal signals and the uterine changes they elicit occur whether a viable conceptus is present or not. Ablating the surge of estrogen that occurs just before implantation (by ovariectomy or lactation) prevents the attachment reaction, and the blastocysts remain in diapause (delayed implantation). After a delay as long as 30 days, a single injection of estrogen induces implantation (*18*).

During delayed implantation, trophoblast-uterine interactions are actively inhibited by the uterus and blastocysts slow their metabolism (*16*, *18*). When such blastocysts are removed from the nonreceptive uterus and cultured, they proliferate, attach, and assume invasive behavior like their normal counterparts. Furthermore, the delayed blastocyst and uterus can be reactivated by injection of actinomycin D into the mother (*19*). Saccharides expressed on uterine cells may prevent blastocyst attachment. At implantation, MUC-1, a mucin-like integral membrane protein expressed on mouse uterine epithelium, is down-regulated, which suggests that it may be a barrier to blastocyst adhesion (*20*).

Estrogen triggers several events that allow implantation to begin. It acts on the uterine epithelium, inducing it to secrete cytokines, including members of the epidermal growth factor (EGF) family (*21*) and leukemia inhibitory factor (LIF) (*22*, *23*). Four members of the EGF family [EGF, transforming growth factor (TGF)–α, heparin-binding EGF (HB-EGF), and amphiregulin] are produced in the uterus during the peri-implantation period. TGF-α is expressed in large amounts in uterine tissue (*24*) but is not essential because implantation occurs normally in mice lacking a functional TGF-α gene (*25*). The expression pattern of HB-EGF is particularly striking. In normal pregnancy, the gene encoding this protein is expressed only in the luminal epithelium at the site of blastocyst apposition starting 7 hours before attachment (*21*). In delayed implantation, it is not expressed but is rapidly induced after estrogen injection. EGF receptors (EGF-R) are expressed on trophectoderm (*26*), and EGF promotes mouse trophoblast outgrowth (*21*) and blastocoel expansion (*27*) in culture, as well as human cytotrophoblast invasion (*28*). Despite these observations, mutation of the gene encoding EGF-R (*Egfr*) does not block blastocyst formation or the initial stages of implantation, although the embryos fail soon after (*29*). LIF, however, appears to have an essential role in triggering events required to initiate implantation. Normal embryos cannot implant in the uteri of mice with a null mutation in the LIF gene (*23*), but whether LIF acts on the blastocyst or has a paracrine effect on the uterus is unknown.

Implantation depends not only on maternal events that open the window for implantation, but also on secondary events that are triggered by the blastocyst itself. For example, interleukin-1β (IL-1β) is made by

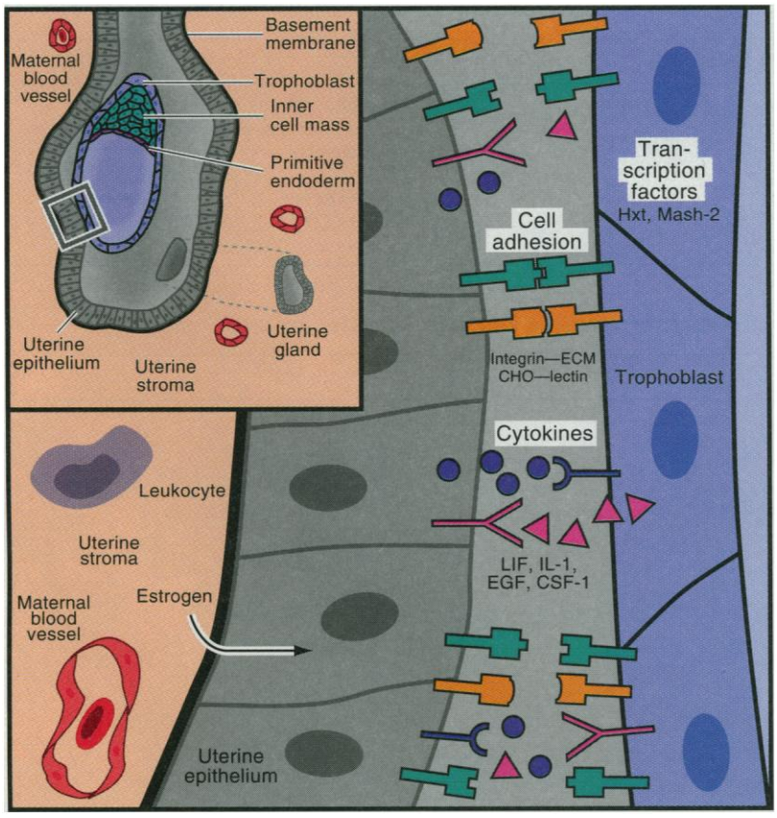

Fig. 1. Implanting blastocyst. At day 4.5 of mouse development, the blastocyst attaches to the uterine epithelium and the uterus clamps around the blastocyst. The uterus is nonreceptive to implantation until the "window of implantation" is opened by a surge of estrogen from the ovary. Estrogen makes the uterine epithelium permissive for blastocyst attachment and induces the release of cytokines such as LIF, IL-1, HB-EGF, and CSF-1. The adhesion mechanisms that regulate attachment are not understood, although it is likely that they include both carbohydrate (CHO)-lectin and integrin-integrin or integrin-ECM interactions.

mouse trophoblasts starting at the blastocyst stage (*30*), and the type I IL-1 receptor is expressed on trophoblasts, uterine epithelium, and endometrial stroma (*30*). Treatment of mice with IL-1 receptor antagonist (IL-1ra) prevents implantation. Blastocysts in these mice that neither attach nor induce the decidual response remain free in the uterus, similar to the appearance of blastocysts during delayed implantation. However, IL-1ra has no effect on blastocyst attachment and trophoblast outgrowth in culture (*30*). It is not clear if trophoblast-derived IL-1β acts on the uterus, although autocrine effects are possible. IL-1β is also produced by human trophoblasts (*31*) in which it can induce several markers of trophoblast differentiation, including aromatase activity (*32*), release of corticotropin-releasing factor, adrenocorticotropin hormone, hCG (*33*), and gelatinase B (*34*).

Trophoblast-uterine adhesion. At implantation, the previously nonadhesive apical surface of the trophectoderm becomes adhesive. The molecules that mediate binding of trophoblasts to the uterine epithelium are not well defined, although blastocysts can interact with a wide variety of substrates, including tissue culture plastic (*35–38*). By analogy with lymphocyte extravasation (*39*), carbohydrate-lectin interactions might mediate initial blastocyst adhesion, which is then stabilized by binding of integrins to their extracellular matrix (ECM) ligands. Mouse blastocysts express several carbohydrate structures, including a selectin ligand, sialylated Lewis^x (*40*). They also synthesize proteoglycans such as perlecan, the basement membrane form of heparan sulfate proteoglycan (*41*). Inhibition of heparan sulfate blocks embryonic outgrowth on laminin, fibronectin, or isolated mouse uterine epithelial cells (*37*). This interaction may be relevant in vivo because both mouse and human uterine epithelial cells express heparan sulfate–binding proteins (*42*) that could interact with the trophoblast proteoglycans. Uterine epithelial cells also express an H-type-I carbohydrate around the time of implantation (*43*), and the abembryonic trophectoderm acquires the ability to specifically bind H-type-I structures at the late blastocyst stage (*38*, *44*). Saccharides that carry this epitope inhibit embryo attachment to endometrial monolayers in vitro (*38*, *44*). Uterine glycosaminoglycans, such as chondroitin sulfate and hyaluronic acid (HA), may also participate in adhesion because blastocysts attach and spread on HA in culture (*45*). During the process of forming a decidua, HA is rapidly cleared from the ECM of the uterine wall opposite the implantation site (*46*). Thus, HA could facilitate embryonic invasion and migration, and its absence could restrict attachment.

At least in culture, integrins are also critical for trophoblast-ECM interactions; a broad spectrum antiserum against integrins blocks mouse trophoblast outgrowth on ECM ligands (*36*). However, their specific roles during implantation in vivo are uncertain. The preimplantation mouse blastocyst produces a broad repertoire of integrins and ECM ligands, but so far only integrins recognized by an antiserum against $\alpha V_\beta 3$ integrin have been detected on the apical surface of the trophoblasts. αV integrins bind several ECM components, including perlecan, although probably not intact laminin or type IV collagen. As mouse blastocysts mature and acquire the ability to attach to ECM, the α7β1 integrin, a laminin receptor, is up-regulated (*47*). Coincidentally, uterine stromal cells up-regulate their expression of laminin and collagen (*48*). In addition, uterine epithelial integrins are modulated during the peri-implantation period. In humans, $\alpha V_\beta 3$ integrin is expressed by uterine epithelium only between days 19 to 24 of the menstrual cycle, the period of optimum uterine receptivity (*49*). The relevance of this observation is supported by the fact that this integrin staining does not appear in the epithelium of infertile women with luteal phase abnormalities.

Transformation of the uterus. The tissue-specific response of the uterus to an implanting embryo (the decidual response) that occurs in rodents and primates is remarkable. The initial stages share many features with the acute inflammatory response (*50*, *51*). Vascular changes occur, such as increased permeability of uterine blood vessels (as demonstrated by injection of dyes such as pontamine blue). In addition, inflammatory cells are rapidly recruited to the implantation site and several proinflammatory cytokines are produced in the uterus (*52*). In rodents, uterine angiogenesis actually anticipates implantation (*51*, *53*), which suggests that blastocysts are

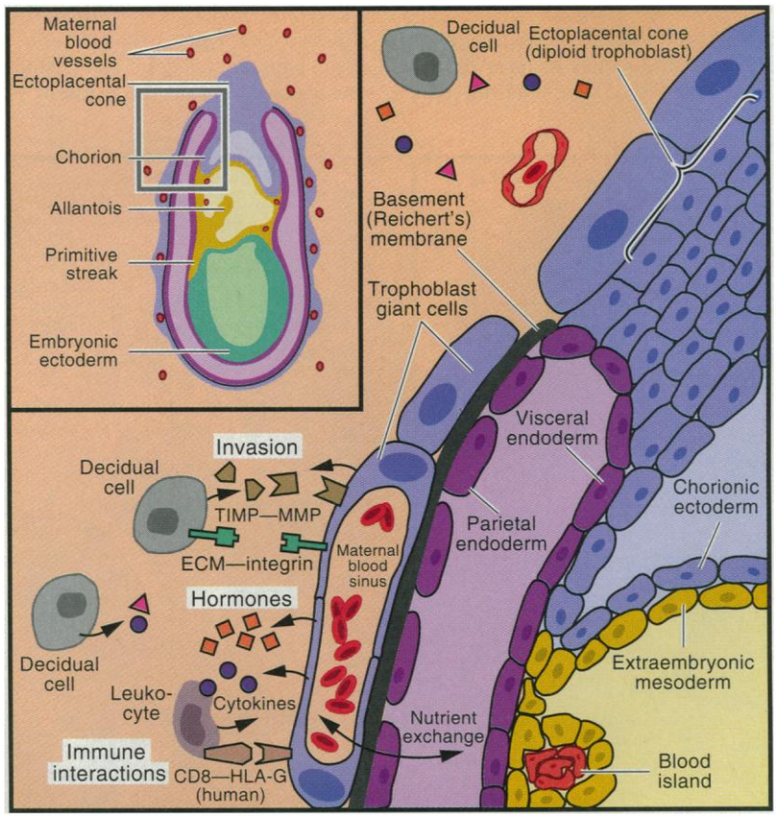

Fig. 2. Early postimplantation stage placenta (yolk sac placenta). By day 7.5 of mouse development, the decidual response has occurred and is characterized by apoptosis of the uterine epithelium, transformation and growth of decidual stromal cells, and recruitment of inflammatory and immune cells. Trophoblast giant cells surround the conceptus and invade the decidua, a process that is mediated by matrix metalloproteinases (MMP) and changes in the expression of adhesion molecules such as integrins. In humans, trophoblasts within the decidual tissue express the nonclassical MHC class I molecule HLA-G, which can interact with maternal $CD8^+$ T lymphocytes. Trophoblasts also secrete growth factors, cytokines, and hormones that alter maternal endocrine, immune, and cardiovascular functions. At this stage, the parietal yolk sac (trophoblast giant cells and the underlying parietal endoderm cells) forms the principal transport organ. Gastrulation has begun and extraembryonic mesodermal cells form blood islands that are the precursors of fetal endothelial and blood cells. These cells also form the allantois that will ultimately fuse with the chorion to form the chorioallantoic placenta.

perceived by the uterus before they attach. The embryo may not elicit these responses with a specific stimulus because many of the vascular and stromal changes are reproduced by introducing a foreign agent (such as oil) into the uterine lumen (*54*). In addition to the vascular changes, several cellular changes occur. For example, the uterine epithelium is lost and poorly characterized stromal (decidual) cells undergo an epithelioid transition and proliferate, producing a massively thickened uterine wall. The decidua also contains large numbers of macrophages and lymphocytes with unusual properties. In humans, these cells include large numbers of $CD16^-$ and $CD56^+$ (N-CAM) natural killer–like cells, as well as lymphocytes with γ, δ-type T cell receptors (*55*, *56*). The latter cells show unusual immunologic properties, such as reduced alloreactivity and responsiveness to stimulation by CD3 antibody (*56*).

The Early Placenta

After implantation in the mouse, the polar trophectoderm proliferates to form the ectoplacental cone (Fig. 2) and later the spongiotrophoblast layer (Fig. 3). Trophoblast stem cells in the ectoplacental cone are also precursors of the chorionic ectoderm. The outermost trophoblasts of the ectoplacental cone differentiate into secondary trophoblast giant cells. Trophoblast giant cells lie on the outside of the placenta, forming the interface with maternal cells in the decidua. These unusual cells stop proliferating even though DNA replication continues (endoreplication) (*8*, *57*). This produces polyploid cells that have specialized functions. Several genes that are involved in the acquisition of trophoblast-specific functions are regulated during trophoblast differentiation. For example, the expression of gelatinase B (*58*), α1 integrin (*47*), placental lactogen (PL) (*59*), and pregnancy-specific glycoprotein (PSG) (*60*) increase during trophoblast giant cell formation.

Parietal endoderm cells migrate from the enlarging ICM (now called the egg cylinder) onto the basal surface of the trophoblast layer and deposit an extensive basement membrane (Reichert's membrane) between the two cell types (*61*) (Fig. 2). At this stage, parietal endoderm and trophoblast giant cells comprise the earliest placental structure (parietal yolk sac). This structure is critical for the absorption of nutrients from maternal blood that appears in sinuses surrounding the conceptus. After gastrulation, extraembryonic mesoderm lines the inner surface of the visceral endoderm, producing the visceral yolk sac. These cells differentiate into hemangioblasts (blood islands) (day 7.5) (Fig. 2), the first vascular (endothelial and hemopoietic) cells in the conceptus that ultimately give rise to the primitive circulatory system (vitelline circulation).

Trophoblast transport of nutrients. Nutrients are transported across the parietal yolk sac and absorbed by the visceral yolk sac (Fig. 2). The placenta is not merely a sieve but has highly specialized transport properties. In many respects, trophoblasts function as an endothelium; in contrast to most polarized cells, the trophoblast apical domain is in direct contact with maternal blood. This presents an interesting problem with regard to how these cells target membrane proteins that transfer substances to and from the fetus. For example, in most epithelial cells, the transferrin receptor, which is involved in iron transport, is mainly localized to the basolateral surface. Sorting is accomplished by direct transport from the trans Golgi network and by efficient recycling after endocytosis (*62*). However, in BeWo cells, a human trophoblast cell line, about a third of the transferrin receptors are found on the apical cell surface as a result of bidirectional transcytosis (*63*). Thus, it is likely that trophoblasts use novel mechanisms to target receptors whose function is critical to fetal development. In addition to transporting nutrients, trophoblasts transfer other molecules such as growth factors. For example, embryos with a null mutation in their TGF-β1 gene are able to develop normally in utero because of placental transport of TGF-β1 from the mother (*64*).

Trophoblast and placental development among different species. Early steps in embryonic development, including establishment of cell lineages that make up the placenta, proceed similarly among all vertebrates. However, the form that the placenta takes is extremely variable (*15*). In primates and rodents, trophoblasts are invasive, breaching uterine vessels; as a result, maternal blood is in direct contact with trophoblasts (hemochorial placenta). In the pig, however, no invasion occurs and trophoblasts are apposed to uterine epithelium throughout the course of pregnancy (epitheliochorial placenta). In ruminants, most trophoblasts are noninvasive, but specialized zones of trophoblast (cotyledons) invade into and fuse with uterine epithelium, forming binucleate trophoblasts (synepitheliochorial placenta) that secrete proteins of the PL family. In horses, trophoblasts within the chorionic girdle invade the uterus and differentiate

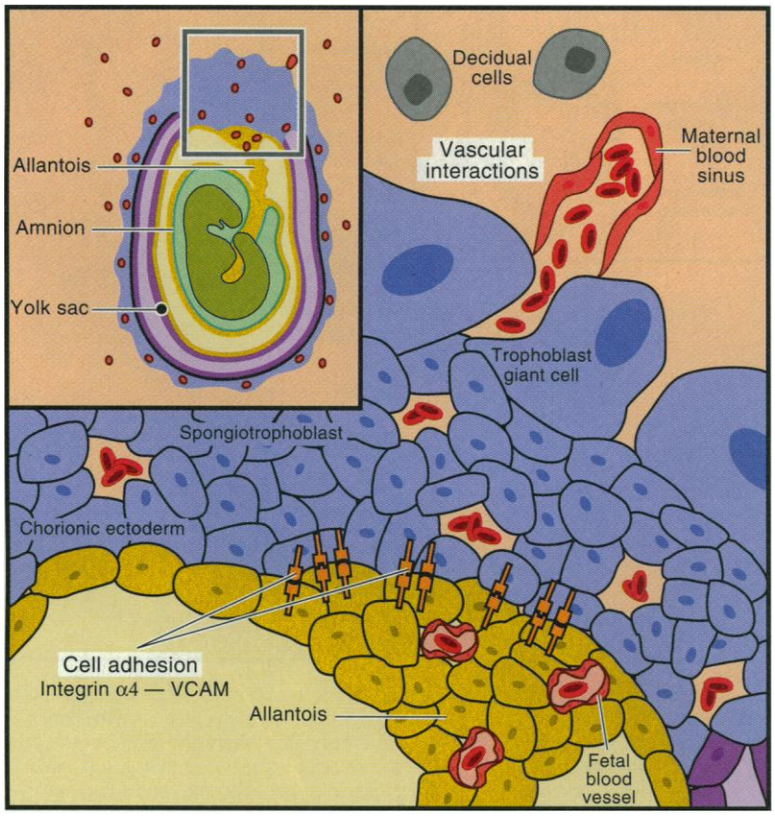

Fig. 3. Late postimplantation stage placenta (chorioallantoic placenta). By day 9.5 of mouse development, the allantois has fused with the chorion, a process mediated by interactions between VCAM-1 and α4 integrin. Thereafter, the labyrinthine layer develops in which there is extensive intermingling of maternal blood and fetal blood vessels in the chorioallantois (umbilical cord). Subsequent growth of the fetus depends on formation of this exchange organ. The decidua has largely regressed, and placental development is essentially complete.

into hormone-secreting cells. In humans, two differentiated trophoblast populations form. Proliferative cytotrophoblast stem cells are anchored to basement membranes surrounding the stromal cores of two types of chorionic villi. In floating villi, cytotrophoblasts fuse to form an overlying syncytium that is in direct contact with maternal blood, mediating nutrient and gas exchange. In anchoring villi, cytotrophoblasts also differentiate into a syncytium that covers most of their surface, but at discrete sites subpopulations of cytotrophoblasts leave the basement membrane and form columns of cells. These columns give rise to the invasive subpopulation of cytotrophoblasts that attaches to and invades the uterus and its arterial system. Both cytotrophoblasts and syncytiotrophoblasts secrete hormones (hCG and human PL).

Trophoblast invasion. Trophoblast invasion anchors the placenta to the uterine wall. Human trophoblasts are extremely invasive; they traverse the uterine epithelium and invade the decidua, the inner third of the myometrium and the maternal arteries. Rodent trophoblasts are less overtly invasive in vivo, although they are highly invasive in vitro. Trophoblasts produce proteinases that degrade the ECM, including gelatinase B (also called matrix metalloproteinase–9 or MMP-9), a matrix metalloproteinase made by both rodent and human trophoblasts. Gelatinase B is required for both mouse (*58*) and human (*65*) trophoblast invasiveness in vitro. Furthermore, changes in its synthesis correlate with gestation-related changes in trophoblast behavior. In humans, cytotrophoblast production and activation of gelatinase B peak during the first trimester, coinciding with maximal invasive behavior in vivo (*28, 65, 66*). In mice, both trophoblast invasion and gelatinase B expression by trophoblast giant cells peak at day 7.5 (*58, 67*).

Another class of proteinase, urokinase-type plasminogen activator (uPA), is made by both human cytotrophoblasts (*68*) and mouse trophoblast giant cells (*69*). However, inhibition of uPA activity does not limit trophoblast invasion in vitro (*58, 65*). Moreover, embryos that lack either a functional uPA gene (*70*) or the low density lipoprotein receptor–related protein (*71*), which is required for uPA receptor internalization, implant normally. This does not completely rule out a function for uPA, because it may have a role in the activation of metalloproteinases or in regulating fibrin deposition in areas where maternal blood vessels are breached. Such a role would be consistent with observed effects on fibrinolysis (but not ECM lysis or fertility) in transgenic animals that overexpress uPA (*72*).

In addition to producing proteinases that degrade the ECM, trophoblasts also change their adhesive properties during invasion. This occurs in vivo as the cells leave their basement membrane. In humans and mice, the anti-adhesive protein, tenascin, is produced at sites where trophoblasts start to invade (*73, 74*). Subsequently, both human (*73*) and mouse (*47*) trophoblasts undergo three major transitions in their expression of integrins and ECM components. Human cytotrophoblast stem cells within the villi express $\alpha6\beta4$ integrin, a receptor for epithelial laminin. As they leave the basement membrane, they down-regulate the $\alpha6\beta4$ integrin and begin to express the $\alpha5\beta1$ integrin, a fibronectin receptor, along with a fibronectin-rich pericellular ECM. Within the uterine wall, they produce $\alpha1\beta1$ integrin, a receptor for laminin and type IV collagen. The integrin switching that occurs in vivo is recapitulated when villous stem cells are cultured. Under these conditions, antibodies to laminin, type IV collagen, or $\alpha1\beta1$ integrin inhibit cytotrophoblast invasiveness, which suggests that integrin interactions with these ligands promote uterine invasion. Conversely, antibodies to the $\alpha5\beta1$ integrin strongly enhance invasion, and addition of its ligand, fibronectin, inhibits invasion. These results suggest that the interaction between $\alpha5\beta1$ integrin and fibronectin primarily restrains cytotrophoblast invasiveness (*75*).

Mouse embryos transplanted to an ectopic site invade uncontrollably (*76*), which suggests that decidual factors might control trophoblast invasion. TGF-β promotes the deposition of ECM and inhibits the production of matrix metalloproteinases in other cell types (*77*). TGF-β1 is expressed by both human decidua (*78*) and cytotrophoblasts (*79*), although it does not inhibit human cytotrophoblast invasiveness in vitro (*34*). Broad spectrum proteinase inhibitors (for example, α_2-macroglobulin), as well as more specialized inhibitors [for example, tissue inhibitors of metalloproteinases (TIMPs)], of trophoblast and decidual origin are likely important for limiting trophoblast invasion. α_2-Macroglobulin (*80*), and TIMP-1, TIMP-2, and TIMP-3 are expressed in rodent decidua (*67, 81*). TIMPs abolish trophoblast invasiveness in culture (*58, 65*), but mice with a null mutation in the gene encoding TIMP-1 have normal fertility (*82*), which suggests that it is not a critical inhibitor. Trophoblasts may also limit their own invasion because human cytotrophoblasts up-regulate TIMP-3 in parallel with gelatinase B in vitro (*83*) and TIMP-3 is expressed by the labyrinthine trophoblast in the mouse placenta at day 14 (*84*).

Disruption of human trophoblast invasion in preeclampsia. Preeclampsia, which occurs only in humans, is a clinically important example of dysfunctional trophoblast invasion. It is most common in primagravidas and affects 7 to 10% of all pregnancies (*4*). Many of the maternal signs that develop in the late second or third trimester (increased maternal blood pressure, renal dysfunction, and edema) can be explained by the altered function of maternal blood vessels (*85*). There is also a profound impact on the fetus, frequently resulting in intrauterine growth retardation and perinatal mortality. Although the cause of preeclampsia is unknown, the evidence strongly implicates the action of placental trophoblasts as the underlying cause (*86*). In preeclampsia, cytotrophoblast invasion is shallow and uterine arteriole invasion is nearly absent, resulting in poor blood perfusion of the placenta (*87*). Moreover, the characteristic pattern of integrin switching that takes place during normal trophoblast differentiation does not occur in preeclampsia (*87*). Invading cytotrophoblasts in preeclamptic placentas up-regulate expression of the fibronectin receptor ($\alpha5\beta1$ integrin) and an extensive repertoire of ECM components, as they do during normal pregnancy, but they fail to up-regulate expression of $\alpha1\beta1$ integrin. This adhesion defect cannot be corrected by removing the cells from the maternal environment, because cytotrophoblasts isolated from preeclamptic placentas do not undergo integrin switching in vitro and express almost no gelatinase B (*88*). Together, these results suggest that the adhesive and degradative phenotype of cytotrophoblasts in preeclampsia restricts their invasion.

Determining why cytotrophoblast differentiation is abnormal in preeclampsia could help unravel the etiology of this syndrome. In preeclampsia, the placental bed is relatively hypoxic because of the lack of endovascular invasion by cytotrophoblasts. When normal first trimester human cytotrophoblasts are cultured under hypoxic conditions, they express an integrin pattern characteristic of cytotrophoblasts in preeclampsia and reduce their production of gelatinase B, as well as their invasive capacity (*89*). These results suggest that maternal perfusion of the placenta not only supplies blood to the fetus, but also creates an optimal environment for trophoblast differentiation along the invasive pathway.

Genetic Control of Trophoblast Differentiation

Transcriptional regulation of trophoblast differentiation. When trophoblasts are removed from the placenta and cultured, they rapidly differentiate, but the factors that promote or limit this process in vivo are poorly understood. Several transcription factors are expressed in trophoblasts, including the zinc finger–containing factor Rex-1 (*90*),

the homeodomain-containing factor Pem (*91*), and a member of the GATA family, GATA-3 (*92*). However, these factors are also expressed in other cell types, and their roles in trophoblast commitment and differentiation have not been tested. Members of the basic helix-loop-helix (bHLH) family of transcription factors are important cell lineage determinants throughout evolution in many cell types (*93*). Recently, two bHLH factors were identified that give the first insights into the control of trophoblast differentiation. *Mash-2*, initially identified in a preneuronal cell line (*94*), was recently shown to be expressed in large amounts in trophoblasts (*95*). A screen for bHLH factors expressed in blastocysts resulted in the identification of another bHLH factor, *Hxt*, that is expressed in trophoblasts in mice and sheep (*96*). *Hxt* induces the commitment of cells to differentiate into trophoblasts, as shown by injection of *Hxt* into uncommitted blastomeres of cleavage-stage mouse embryos. Whether *Hxt* is an essential trophoblast determinant awaits genetic analysis.

In rodents, the balance between proliferation of trophoblasts and differentiation into nonproliferative trophoblast giant cells is regulated by these trophoblast-specific bHLH transcription factors, as well as by the negative HLH factors (*Id*). The expression of *Id-1* (*96, 97*) and *Id-2* (*96, 98*) is high in proliferative cells and is down-regulated during differentiation. Ectopic expression of *Id-1* reduces the ability of rat trophoblasts to differentiate in vitro (*96*), an effect similar to the activity of this gene in regulating the differentiation of other cell lineages (*99*). *Mash-2* is also an important regulator of trophoblast proliferation; its expression diminishes as mouse trophoblasts differentiate into giant cells (*95*), and mutation of the *Mash-2* gene results in an increase in the number of giant cells at the expense of the proliferative cell population, resulting in a diminished spongiotrophoblast layer (*95*). In contrast to *Mash-2* and *Id-1*, *Hxt* expression persists and even increases in mouse trophoblasts as they form giant cells. Overexpression of *Hxt* in rat trophoblast stem cells reduces their proliferation and promotes differentiation (*96*), consistent with the hypothesis that *Hxt* regulates trophoblast giant cell formation. The mechanisms that control giant cell formation and endoreduplication are mysterious, although they may be analogous to the formation of differentiated multinucleated myotubes that is promoted by myogenic bHLH transcription factors such as MyoD (*93*). The ability of MyoD to induce myogenic conversion of fibroblasts depends on the interaction of the retinoblastoma protein (Rb) with the bHLH domain of MyoD (*100*). Presumably this interaction couples the process of differentiation with exit from the normal cell cycle.

Trophoblast development is controlled by imprinted genes. Both the maternal and paternal genomes are required for mammalian embryogenesis. This is demonstrated by the fact that isoparental embryos fail in development soon after implantation (*101*). Androgenotes, which possess two copies of the paternal genome, develop abnormally with little embryonic but extensive trophoblast development. Conversely, parthogenotes, which possess two copies of the maternal genome, usually die early. Occasionally they develop further and have reasonably normal embryonic structures but poorly developed placentas (*102*). These observations suggest that trophoblast development depends on imprinted genes that are expressed only from the paternally derived allele. Normal trophoblast proliferation is maintained by contact with the ICM (*8*). ICMs from parthogenotes, like those from normal blastocysts, placed inside normal trophoblast vesicles induce trophoblast proliferation. In contrast, trophoblasts from parthogenotes do not proliferate in response to normal ICMs (*101*), which indicates that imprinting affects the expression of genes necessary for reception of the mitogenic signal. Although several genes are now known to be imprinted (*103*), the nature of the ICM-derived signal is unknown.

Genetic Control of Early Post-Implantation Development

Although no embryonically expressed genes are known to be essential for initiating implantation, several naturally occurring mouse mutants fail in development soon after the onset of implantation (Table 2). Embryos with mutations in the *t* complex, t^{w73} (*104*), and at the *lethal yellow* (*105*), *oligosyndactylism* (*106*), and *l(5)-1* (*107*) loci begin to implant but fail to develop. The phenotype of these defects is similar to that of mouse embryos that are homozygous for mutations generated by homologous recombination in the *vav*, β1-integrin, and *Egfr* genes. The *vav* proto-oncogene is expressed by trophoblasts in blastocysts, and mutation of the gene results in embryonic mortality between days 4.5 and 7.5 (*108*). Although the precise timing of the failure is unclear, *vav* mutant blastocysts are unable to hatch from the zona pellucida in vitro, which suggests an early defect. Mutants in the β1 integrin gene develop normally to the blastocyst stage, begin to implant and induce decidualization, but die shortly thereafter (*109*). Similarly, a null allele at the EGF-R locus (*Egfr*) (*29*) is a peri-implantation lethal mutation; EGF-R–deficient blastocysts begin to implant, and although the ICMs die soon after, they appear to have altered adhesive properties suggestive of trophoblast abnormalities.

Embryonic lethality occurs slightly later after implantation for mouse embryos with mutations in the even-skipped (*evx1*) (*110*), fibroblast growth factor (*Fgf*)–4 (*111*), and *Fug1* genes (*112*), and for naturally occurring mutants at the blind, velvet coat (*113*), t^0, and t^{w5} (*14*) loci. Although these mutants die early, the primary defect appears to be in the embryo proper, rather than in the extraembryonic lineages. Conversely, *exed* mutants also die somewhat later but have abnormalities in the development and survival of trophoblasts (*114*). Several gene mutations result in lethal abnormalities in the fetal vasculature. Homozygous mutant embryos in which genes for either fibronectin (*115*) or its receptor (α5 integrin) (*116*) are inactivated have deformed embryonic vessels; the visceral yolk sac, vitelline vasculature, and amnion are also defective, resulting in hemorrhages. Mutations in *Mgat-1* (*117*), *Notch-1* (*118*), and c-*myc* (*119*) also result in abnormal yolk sac development, and the vitelline circulation appears to lack blood cells. Embryos with these mutations survive until around day 10, dying when the vitelline circulation and chorioallantoic placenta become critical. This indicates that if the parietal yolk sac has normal absorptive functions, the vitelline vasculature is not critical for normal development until the demands of fetal growth increase.

Placental Control of Maternal Functions

Trophoblast control of maternal endocrine functions. Progesterone is required throughout pregnancy to maintain a proper uterine environment. In some species, the placenta itself secretes progesterone in the latter half of gestation (*120, 121*). However, early in gestation conceptuses of all species induce endocrine changes in the mother that ensure continued secretion of progesterone from corpora lutea in the ovary. This event is one of the earliest maternal physiological responses to pregnancy that differentiates a normal ovarian cycle from pregnancy and is called maternal recognition of pregnancy (*122*). Although hormone production by trophoblasts is critical for initiating this process, the molecular components differ among species (*120*). In rodents, prolactin-like hormones are critical for the control of maternal physiology throughout gestation. Their functions include inducing development of the mammary gland and sustaining production of progesterone from the corpora lutea in the ovary (luteotrophic effect). The act of mating induces pulsatile prolactin release from the posterior pituitary gland that sustains production of progesterone be-

yond the nonmated cycle length of 4 days to about 9 days. Therefore, even mating to a nonfertile male induces pseudopregnancy. Prolactin-like hormones produced by trophoblasts become the primary luteotrophins in the latter half of pregnancy. In rodents, trophoblast giant cells secrete several prolactin-like hormones in a highly ordered sequence (59); PL I production peaks first, followed by peaks in secretion of PL II and proliferin. Prolactin-like hormones are also produced by cells in the decidua (*123*).

In primates and horses, trophoblasts produce chorionic gonadotropins that have luteotrophic effects analogous to those of the prolactins (*120, 124*). In some farm animal species (pigs, sheep, and cows), the production of progesterone by corpora lutea is maintained not by luteotrophic compounds, but rather by paracrine factors (antiluteolysins) that prevent uterine production of prostaglandin-$F_2\alpha$, the factor that triggers regression of the corpora lutea in a nonpregnant animal (*120*). In pigs, the antiluteolytic agent produced by trophoblasts is estrogen (*125*), whereas in ruminants the active factor is a member of the type I IFN family, IFN-τ (*2*). IFN, which has many effects on the immune system, is expressed in the placental bed in several mammalian species (*2*). This finding prompted the suggestion that IFN is universally important in pregnancy, particularly for modulating the maternal immune system (*126*). Although IFN may be universally expressed in the placenta, the nature and amount of IFN produced differ widely among species (*2*). Indeed, IFN-τ genes are restricted to ruminants (artiodactyls) (*127*). This may reflect the fact that although all species produce some form of IFN as a consequence of placental development, only the ruminant species use an IFN to initiate maternal recognition of pregnancy.

Trophoblast interactions with the maternal immune system. A paradox about pregnancy is that the placenta, a semi-allograft of fetal tissue, avoids maternal immune rejection. Fetal trophoblasts, which lie in direct contact with maternal immune cells, use several mechanisms to subvert normal maternal immune responses. These include secretion of factors that may suppress local immune responsiveness, selective expression of immune antigens critical to alloreactivity, and impaired responses to immune-activating cytokines present in the placental bed. Intriguingly, some viruses use these same general mechanisms to escape detection by the host immune system. Although these activities are of considerable biological interest, whether immune-mediated mechanisms lead to significant embryonic losses clinically remains controversial.

Placental factors that inhibit immune functions in vitro include hormones (progesterone, human PL, prolactin, and estrogens), pregnancy-associated α2-glycoprotein, pregnancy-associated plasma protein-A, and α-fetoprotein (*128*). Human and mouse placentas also produce several cytokines, including CSF-1 (*129*), IL-1β (*34*), IL-6 (*130*), TGF-β (*131*), activin (*132*), inhibin (*132, 133*), and IL-10 (*134*). IL-10 is of considerable interest because it reduces the proliferation of cytotoxic T helper (T_H1) cells (*135*). The Epstein-Barr virus produces a homologous protein, BCRFI, that may allow virus-infected cells to avoid immune surveillance (*136*).

Trophoblasts selectively express antigens that are recognized by effector immune cells. They do not synthesize major histocompatiblity complex (MHC) class II antigens, but specific subpopulations express unusual MHC class I molecules. This is of interest because these antigens are major determinants used by immune cells to distinguish self from nonself (*137*). Invasive human cytotrophoblasts express a nonclassical class Ib molecule, HLA-G, which is trophoblast cell–specific (*138, 139*). Conversely, they do not express classical MHC class Ia molecules (for example, HLA-A, B, and C) that are characteristically produced in other cell types. HLA-G functions as a typical MHC class I α chain in that it associates with β_2-microglobulin (*138*) and binds to CD8 (*140*). Expression of HLA-G protects against killing by natural killer cells (*141*). Unlike the genes encoding HLA-A, B, and C, the gene encoding HLA-G exhibits limited polymorphism (*138*). Thus, protein encoded by paternally derived HLA-G genes is not recognized as foreign by the maternal immune system. In addition, the relative lack of polymorphism of the gene encoding HLA-G may restrict the repertoire of peptide antigens that fetal trophoblasts present to maternal T cells, although the nature of these peptides and their importance in communication between trophoblast and maternal immune cells are unknown. MHC class I molecules are also expressed by trophoblasts in other species. In rats, trophoblasts express a nonclassical class I MHC antigen that is genetically imprinted (*142*). In horses, only invasive trophoblasts express class I antigens, although the identity of the genes has not been determined (*143*).

Why human trophoblasts do not express the classical MHC class Ia molecules is incompletely understood. Their genes are constitutively expressed at low levels by most adult cells and their transcription is stimulated by cytokines, including IFN, that activate specific enhancer elements (*144*). However, the IFN-responsive enhancer is not functional in trophoblasts, because trophoblasts are selectively resistant to IFN. In mice, IFN-γ does not upregulate MHC expression in the placenta (*145*). Likewise, neither IFN-α nor IFN-γ induce the transcription of MHC class I genes in the JEG-3 human trophoblast line, because of the uncoupling of specific IFN signaling pathways. In trophoblasts, activation of the IFN-α–responsive transcription factor ISGF2 occurs normally, whereas activation of ISGF3 does not (*146*). Similar defects in IFN signaling occur in cells that are infected by adenovirus and hepatitis B virus (*147*).

Although the maternal immune system has been viewed as antagonistic to placental function, there is evidence that cytokines produced by epithelial or immune cells of the uterus may promote trophoblast development (*52*). In vivo treatment with proinflammatory cytokines reduces the rate of spontaneous abortion in CBA × DBA/2 mice (*148*). CSF-1 is expressed by the uterine epithelium (*149*) and may interact with its receptors that are present on trophoblasts beginning at the blastocyst stage (*10*). CSF-1 enhances the rate of blastocyst development in culture (*150*). Although this cytokine is not absolutely required for reproduction, CSF-1–deficient *op/op* mice have reduced fertility (*52*). Trophoblast-lymphocyte interactions are not critical for pregnancy, however. For example, mice that are deficient in β_2-microglobulin (*151*) or class I heavy chain peptide transporters (*152*), and that do not express MHC class I antigens, have normal fertility. Similarly, fertility is not compromised in SCID (severe combined immunodeficiency) mice, which lack lymphocytes, or *beige* mice, which lack natural killer cells including the natural killer–like cells that are resident in the uterus (*153*).

The Mature Placenta

Organogenesis in the mouse embryo is largely complete by midgestation. From this point until birth, the major transformation in the embryo proper is its substantial growth. In contrast, there is a dramatic transition in the rodent placenta around day 10; the chorioallantois replaces the yolk sac as the primary means of nutrient, gas, and waste exchange. Failure in chorioallantois formation is incompatible with continued development (Table 2). The allantois, which begins to form at day 7 in the mouse, spans the exocoelom and fuses with the chorion by day 9 (Fig. 3). Fetal vessels intermingle closely with maternal blood sinuses, creating by day 10 the loose network of vascular cells that comprises the mature labyrinthine layer (*61*).

Genes controlling chorioallantoic placenta formation. A number of mouse mutants, produced by homologous recombination, die at the critical juncture when the cho-

rioallantoic circulation becomes primary. A null allele in the *Brachyury* gene produces abnormalities in axial development, but it also prevents the allantois from making contact with the chorion (*154*), similar to the effects of a mutation in the DNA methyltransferase gene (*155*). The underlying cause of these defects is unclear, but they probably reflect generalized mesodermal abnormalities. Other mutations appear to disrupt cell adhesion events specifically. Vascular cell adhesion molecule–1 (VCAM-1) is expressed on vascular endothelium during inflammation and mediates leukocyte migration from blood into tissues. The α4 integrins mediate ECM and cell-cell adhesions by interacting with fibronectin and VCAM-1, respectively. The α4 integrin is expressed in the chorion, whereas VCAM-1 is expressed at the tip of the allantois. In up to half of homozygous embryos with targeted mutations in genes encoding either α4 integrin (*156*) or VCAM-1 (*157*), the allantois does not fuse with the chorion (Fig. 3). Embryonic death occurs 1 to 3 days later, presumably a result of lack of maternal nutrients. Fibronectin, another potential ligand for α4 integrin, is expressed in both the allantois and the chorion, although chorioallantoic fusion is not affected by mutation of the fibronectin gene (*115*). VCAM-1 also plays a role in placental formation after chorioallantoic fusion. In the subset of VCAM-1–deficient embryos in which chorioallantoic fusion occurs, allantoic mesoderm is abnormally distributed over the chorionic surface (*157*).

As discussed earlier, *Mash-2* regulates trophoblast development, although homozygous mutant embryos survive until around day 10. The placentas of these embryos show an abundance of trophoblast giant cells and a relative lack of the spongiotrophoblast layer, presumably because of premature trophoblast differentiation. The labyrinthine layer is also poorly developed in *Mash-2* mutants; although chorioallantoic fusion occurs, this layer lacks the network of fetal vessels and maternal blood sinuses characteristic of a normal placenta. The timing of embryonic loss suggests that the primary abnormality in the placenta is in the development of the chorioallantois and not in the development of the avascular spongiotrophoblast layer. Guillemot and co-workers used a clever trick to show that the defect is exclusively placental and not embryonic (*95*). When tetraploid and diploid blastomeres are aggregated, the tetraploid cells contribute to placental structures, although poorly, if at all, to the embryo proper (*158*). Aggregation of tetraploid cells to *Mash-2* mutant embryos completely rescued development of homozygous mutant embryos through gestation and resulted in live born, healthy, and fertile homozygote mutants (*95*).

Mouse mutants that die late in embryonic development. Once a functional placenta has formed, even mouse embryos with massive malformations in major organ systems survive until birth. The exceptions are embryos with defects in their cardiovascular or hematopoietic systems. For example, embryos homozygous for the *Mov13* insertion mutation lack type I collagen and die because of ruptured blood vessels (*159*). Mice with mutations in N-*myc* (*160*) and RXRα (*161*) have heart abnormalities. Embryos with mutations in their α4 integrin or VCAM-1 genes that survive without defects in chorioallantois formation die later in development as a result of defects in cardiac development (*156*, *157*). Finally, some embryonic mutants, such as lethal alleles of the receptor c-*kit* and its ligand (*W* and *Sl* loci, respectively), are severely anemic because of defects in hematopoietic stem cells (*162*).

Birth: Death of the Placenta

The placenta dies when the fetus is born. The concept that the placenta may have a finite lifespan and ages, however, is controversial. It has been suggested that age-related changes in the morphology of chorionic villi reflect maturation rather than senescence (*163*). However, as pregnancy proceeds, cytotrophoblast stem cells rapidly lose the ability to differentiate. Production of gelatinase B decreases (*65*), and integrin switching is impaired. For example, whereas first trimester human cytotrophoblasts upregulate α1β1 integrin during culture, full-term cytotrophoblasts do not (*75*). Because production of both gelatinase B and α1β1 integrin is required for invasion, it is not surprising that term cells show only a small fraction of the invasive capacity of cells isolated from early gestation placentas (*28*, *65*). Whether these changes in gene expression are indicative of trophoblast aging remains to be determined.

Summary

Implantation and placental development offer fascinating insights into how the earliest developmental decisions are made in mammals. There is clearly active participation of the cells of both conceptus and mother to control whether implantation occurs. Thereafter, the placenta redirects maternal endocrine and immune systems, as well as establishes the vascular connections between mother and embryo that are critical for sustaining fetal growth. Abnormalities in these placental functions or in the development of the fetal cardiovascular system appear to account for the bulk of embryonic mortality during postimplantation intrauterine life.

The first genes that regulate implantation and development of the placenta have now been identified. Methods that are becoming standard for identifying genes that control differentiation of other cell types have yielded candidate genes for regulating development of the trophoblast lineage, implantation, and formation of the mature placenta. In addition, placental biologists have benefitted from genetic experiments designed to test the function of molecules originally not known to contribute to placental development. For the primary investigator, who is most often a cell or molecular biologist, characterizing the exact defect of mutant mice with abnormal placental phenotypes can be difficult. Compared to organs that are critical for extrauterine survival, the placenta has been neglected by developmental biologists. As a result, there is relatively little available information concerning its function. Another problem, less readily solved, is that placental mutations are often lethal during the peri-implantation period, a major roadblock to determining the effects of genes on subsequent development of the embryo proper. Clearly, rescue of mutations that affect extraembryonic lineages by aggregating mutant embryos with tetraploid embryos (*95*) is an important means of circumventing this problem. Continued advances in our understanding of implantation and placental development will undoubtedly lend insights into clinically important causes of embryonic mortality.

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Fertility Decline in East Asia

Griffith Feeney

With the fall of fertility in China to near or below replacement levels in the early 1990s, the whole of East Asia may now be said to have completed a demographic transition. Its experience lies between that of the West and the many developing countries in which demographic transition is now under way. The main features and possible underlying causes of the fertility declines in Japan, Taiwan, South Korea, and China during this century are discussed. Fertility decline in East Asia is interesting both in its own right, as a chapter in the history of human reproduction, and for the light it may shed on fertility decline in the rest of the world.

In the not too distant past, most children reaching adulthood in the world would have seen roughly half of their brothers and sisters die. With declining mortality in the 1900's, the childbearing habits of earlier times would lead to historically unprecedented numbers of surviving children. Declining fertility has tended to correct the balance, reducing family size to more or (usually) less than past levels, with associated improvements in quality of life.

The most profound consequences initially have been in the lives of women, for whom the bearing and rearing of children may now occupy a smaller fraction of adult life and energies. In the long run, however, so great a change in the lives of women must induce comparable alterations in society at large. The fertility decreases have been too recent for us to know their eventual effects, even in the developed countries in which these changes first occurred.

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Demographic Transition

The defining features of demographic transition are the levels of fertility, mortality, and family limitation before and after the transition occurs (*1*). Before transition, less than half of all children in East Asia survived to adulthood. For families (*2*) to reproduce themselves, the mother had to bear a large number of children to compensate for mortality loss. After the transition, nearly all children survived to adulthood. If couples did not act to limit their fertility, they might have twice or three times as many surviving children as they would have had in the past. Nearly all couples adopt some form of family limitation, however, limiting fertility to an average of about two children per woman (*3*).

Pretransition levels of mortality vary widely, but risks of death are generally an order of magnitude or more higher than modern levels. Fertility levels vary widely (*4*), but the average level of fertility is always much lower after the transition (*5*).

Family limitation is necessarily widespread after the transition, but the pretransition situation is variable. Primitive methods of family limitation can be very effective, and were systematically practiced in some pretransition populations. There is evidence of family limitation in premodern China (*6*, *7*) and in Tokugawa, Japan (*8*, *9*).

Fertility, Mortality, and Surviving Children

A woman's fertility may be described numerically by plotting the number of children she has borne at any given time and age. Surviving children are likewise described by a graph of number of children versus time and age. Averages for groups of women may be computed by averaging numbers of children born and surviving for each age of woman.

When mortality risks are low, as in the currently developed countries, the average curve describing surviving children does not differ appreciably from the corresponding curve of children ever born until well after most children have left home. For most of human history, however, mortality