EVOLUTION OF PLACENTAL FUNCTION IN MAMMALS: THE MOLECULAR BASIS OF GAS AND NUTRIENT TRANSFER, HORMONE SECRETION, AND IMMUNE RESPONSES

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Carter AM. Evolution of Placental Function in Mammals: The Molecular Basis of Gas and Nutrient Transfer, Hormone Secretion, and Immune Responses. *Physiol Rev* 92: 1543–1576, 2012; doi:10.1152/physrev.00040.2011.—Placenta has a wide range of functions. Some are supported by novel genes that have evolved following gene duplication events while others require acquisition of gene expression by the trophoblast. Although not expressed in the placenta, high-affinity fetal hemoglobins play a key role in placental gas exchange. They evolved following duplications within the beta-globin gene family with convergent evolution occurring in ruminants and primates. In primates there was also an interesting rearrangement of a cassette of genes in relation to an upstream locus control region. Substrate transfer from mother to fetus is maintained by expression of classic sugar and amino acid transporters at the trophoblast microvillous and basal membranes. In contrast, placental peptide hormones have arisen largely by gene duplication, yielding for example chorionic gonadotropins from the luteinizing hormone gene and placental lactogens from the growth hormone and prolactin genes. There has been a remarkable degree of convergent evolution with placental lactogens emerging separately in the ruminant, rodent, and primate lineages and chorionic gonadotropins evolving separately in equids and higher primates. Finally, coevolution in the primate lineage of killer immunoglobulin-like receptors and human leukocyte antigens can be linked to the deep invasion of the uterus by trophoblast that is a characteristic feature of human placentation.

I. INTRODUCTION

A placenta is an organ for maternal-fetal exchange. There may have been a placenta in gnathostomes, the jawless fishes that are the common ancestors of cartilaginous and bony fishes (238). A majority of sharks are viviparous and have some form of placentation (142) as do many reptiles including skinks and snakes (27, 108). In the South American lizard *Mabuya*, for example, the placenta is every bit as complex as in any mammal (223, 385). It is the apposition of fetal membranes to the uterus that constitutes the basis for maternal-fetal exchange, and some definitions require the exchange to occur between fetal and maternal blood vessels (275).

In a choriovitelline placenta, the fetal vessels are supplied by the yolk sac vasculature. This type of placentation is common in sharks, reptiles, and marsupials (Metatheria). In addition, a choriovitelline placenta supports the early stages of development in many placental mammals (Eutheria), but with several exceptions, including higher primates.

A chorioallantoic placenta is supplied with blood by the allantoic vessels. It is the definitive type in all placental mammals and the structure from which they derive their name. It is, therefore, surprising how little we know about its function. It has long been clear that there is great variation in placental morphology (130). Evidence of this has continued to emerge with the availability of new techniques (86) and the study of a wider range of species (45). Yet the question posed in the 19th and early 20th century, Why is there variation in placental structure?, has not been addressed by physiologists. There is an impressive body of work on placental function in a small number of laboratory mammals; especially noteworthy is research on placental gas exchange in sheep (see Ref. 38) and amino acid transfer in rodents (170, 391). Nonetheless, comparative studies of placental function across a range of species are close to nonexistent.

There is now an opportunity to redress the balance at the molecular level. Considerable progress has been made in tracing the evolution of gene families such as those coding

for growth hormone and prolactin. These have been studied across a wide range of mammalian orders, and often the functional correlates of placentally expressed genes are known or can be implied. Similarly, there are broadly based studies on the evolution of the beta-globin genes that determine blood oxygen affinity and play a role in placental gas transfer. Elsewhere the molecular data are more limited. As an example, relatively few species have been canvassed for placental expression of amino acid transporter molecules; especially striking is the mismatch between our extensive knowledge of amino acid fluxes in the sheep and guinea pig and the dearth of molecular studies of amino acid transporters in the placenta of these species.

This review covers five topics: *1*) the role of fetal hemoglobins in maternal-fetal exchange of blood gases, *2*) strategies for the transfer of iron, *3*) transporter molecules for exchange of nutrients, *4*) placental hormone secretion, and *5*) placental immunology. Placental immunology is not fully within the remit of this review, but it was thought relevant to include recent work on the coevolution in primates of HLA-C (a major histocompatability class I molecule) and its cognate receptors. This is a persuasive example of the interplay between fetal and maternal factors that, intuitively, must have played a great role in the evolution of placental function.

A. Current Views on Mammalian Phylogeny

The classification of mammals has remained stable for many years (258), especially since Simpson's authoritative treatise on fossil and extant forms (329). Until recently it relied upon detailed analysis of the dentition and cranium supplemented with features of the postcranial skeleton and soft tissues. Developmental characters, including fetal membranes and placenta, were sometimes included (e.g., Ref. 328).

The focus has now shifted to phylogenetic analysis based on the nucleotide sequences of mitochondrial and nuclear genes (73, 74, 79, 210, 241, 263, 277, 278, 335, 336, 355). This approach has revealed new relationships among living Eutheria (Placentalia). The number of orders remains stable, but recent changes involve splitting of the insectivores and inclusion of whales and dolphins (Cetacea) with even-toed ungulates (Artiodactyla) in a new order Cetartiodactyla [now supported by fossil evidence (121)]. Importantly, molecular phylogenetics has shown that the placental mammals can be assigned to four superordinal clades (TABLE 1).

II. PLACENTATION

The fetal membranes include the amnion, chorion, yolk sac, and allantois. The manner in which they contribute to the placenta is described in detail elsewhere (45, 275, 378).

A. Choriovitelline Placentation

In many placental mammals, including the pig, horse, and ruminants, the embryo initially is supported by a yolk sac placenta. Later the yolk sac is displaced by the expanding exocelom and allantois and a chorioallantoic placenta is formed (87). Recent work involving somatic nuclear transfer in cattle (cloning) has shown yolk sac placentation to be a critical phase in development (267). Some mammals do not form a yolk sac placenta. In the human, for example, the yolk sac floats free in the exocelom; although it never forms a placenta, it has other important functions in early to midgestation (109, 186). In contrast, many mammals, including all rodents, retain a yolk sac placenta until term. There is then inversion of the germ layers with the yolk sac endoderm acting as a maternal-facing absorptive epithelium (294).

B. Chorioallantoic Placentation

1. Gross morphology

There is bewildering variation in placental structure, and only the salient features will be given here as there are several comprehensive reviews (45, 275, 378). In a diffuse placenta, such as that of the pig, the surface is covered with villi that interdigitate with crypts in the uterine epithelium. Alternatively, the villi may be gathered into tufts, forming microcotyledons, as they do in the horse. Most ruminants have cotyledonary placentas. Each cotyledon is a small disk and in number they vary from few in deer (oligocotyledonary) to many in bovids (polycotyledonary) (209). The zonary type of placenta is typical of carnivores, but is also found in manatees and elephants. The human placenta is a single disk; indeed, "placenta" means a flattened cake. Most rodents, including rats, mice, and guinea pigs, have a discoid placenta.

In addition to these structures, there are additional points of contact between fetal and maternal tissue. As an example, the lesser hedgehog tenrec (*Echinops telfairi*) has a discoid placenta, but the chorion extends well beyond the disk and is supplied by fetal capillaries, suggesting it participates in maternal-fetal exchange (44). In sheep, the membranes between the cotyledons are well vascularized and play an important role in exchange of water and solutes (32). As a third example, the human chorion laeve, which is not part of the placenta proper, produces prostaglandins that likely are involved in the initiation of labor (53).

2. Types of trophoblast

Trophoblast exhibits a range of phenotypes, and the terminology can try the patience of nonspecialists. Early in blastocyst development there is differentiation into an inner cell layer (cytotrophoblast) and an outer syncytial layer (syncytiotro-

Table 1. *The mammalian orders as currently recognized by molecular phylogenicists, their inclusion in four superordinal clades, and the distribution within orders of three types of placentation characterized by the tissues present in the interhemal barrier*

Superordinal Clade	Order	Examples	Placentation
Laurasiatheria	Carnivora	Most carnivores	Endotheliochorial
		Hyenas	Hemochorial
	Cetartiodactyla	Even-toed ungulates; whales and dolphins	Epitheliochorial
	Perissodactyla	Horses, tapirs	Epitheliochorial
	Pholidota	Pangolins	Epitheliochorial
	Chiroptera	Majority of bats	Hemochorial
		Six families, e.g., horseshoe bats	Endotheliochorial
	Erinaceomorpha	Hedgehogs	Hemochorial
	Soricomorpha	Shrews and moles	Endotheliochorial
Euarchontoglires	Lagomorpha	Rabbits, pikas	Hemochorial
	Rodentia	Most rodents	Hemochorial
		Kangaroo rats, spring hares	Endotheliochorial
	Scandentia	Tree shrews	Endotheliochorial
	Dermoptera	Colugos	Hemochorial
	Primates	Lemurs, lorises (strepsirrhines)	Epitheliochorial
		Tarsiers, monkeys, apes (haplorhines)	Hemochorial
Afrotheria	Afrosoricida	Otter shrews	Endotheliochorial
		Golden moles, tenrecs	Hemochorial
	Macroscelidea	Elephant shrews	Hemochorial
	Tubulidentata	Aardvark	Endotheliochorial
	Hyracoidea	Hyraxes	Hemochorial
	Sirenia	Manatees, dugong	Endotheliochorial
	Probiscidea	Elephants	Endotheliochorial
Xenarthra	Pilosa	Sloths	Endotheliochorial
		Anteaters	Hemochorial
	Cingulata	Armadillos	Hemochorial

phoblast). Where placentation is invasive, it usually is the syncytiotrophoblast that first penetrates the uterine epithelium.

Human placental villi have a maternal-facing layer of syncytiotrophoblast and an inner layer of cytotrophoblast. The syncytiotrophoblast is continually replenished from the cytotrophoblast, which can be regarded as a layer of proliferating stem cells (**FIGURE 1, *A*** and ***B***) (41, 179).

Even cellular trophoblast shows a remarkable diversity of forms. Some cells are multinucleated and/or show varying degrees of polyploidy (402). Usually they are giant cells, although the term *giant cell* in the placental literature includes cells that are not particularly large as well as cells that are maternal in origin (275). In the mouse, where substantive progress has been made in tracing cell lineages, trophoblast giant cells are found at various locations throughout the placenta (175).

Many types of trophoblast cells are able to invade maternal tissues. Best characterized is the extravillous trophoblast of human placenta (**FIGURE 1, *A*, *C***, and ***D***), which arises from proliferating cell columns and shows an intriguing succession of phenotypes as it penetrates deep into the endome-trium and the walls of the uterine vessels (201, 400). In the placenta of muroid rodents, invasive trophoblasts include the so-called glycogen cells (118). Even in placentas that are not regarded as invasive, because the uterine epithelium remains intact, trophoblast sometimes adopts an invasive phenotype. Ruminant placentas have a binucleated type of trophoblast that is able to form trinucleated cells or syncytial plaques by fusion with a maternal epithelial cell (377). Early in pregnancy in the horse, trophoblasts of the chorionic girdle invade deep into the uterine stroma and form the endometrial cups that secrete equine chorionic gonadotropin (92, 382).

Finally there is a type of trophoblast that will assume a central role in parts of the succeeding narrative. These are columnar epithelial cells that are highly polarized and equipped with the appropriate apparatus for pinocytosis and endocytosis.

3. The interhemal barrier

The number of tissue layers separating fetal and maternal blood varies across species and may even change in the course of development. This was the basis of a classification of placentas introduced by Grosser (130) and followed by
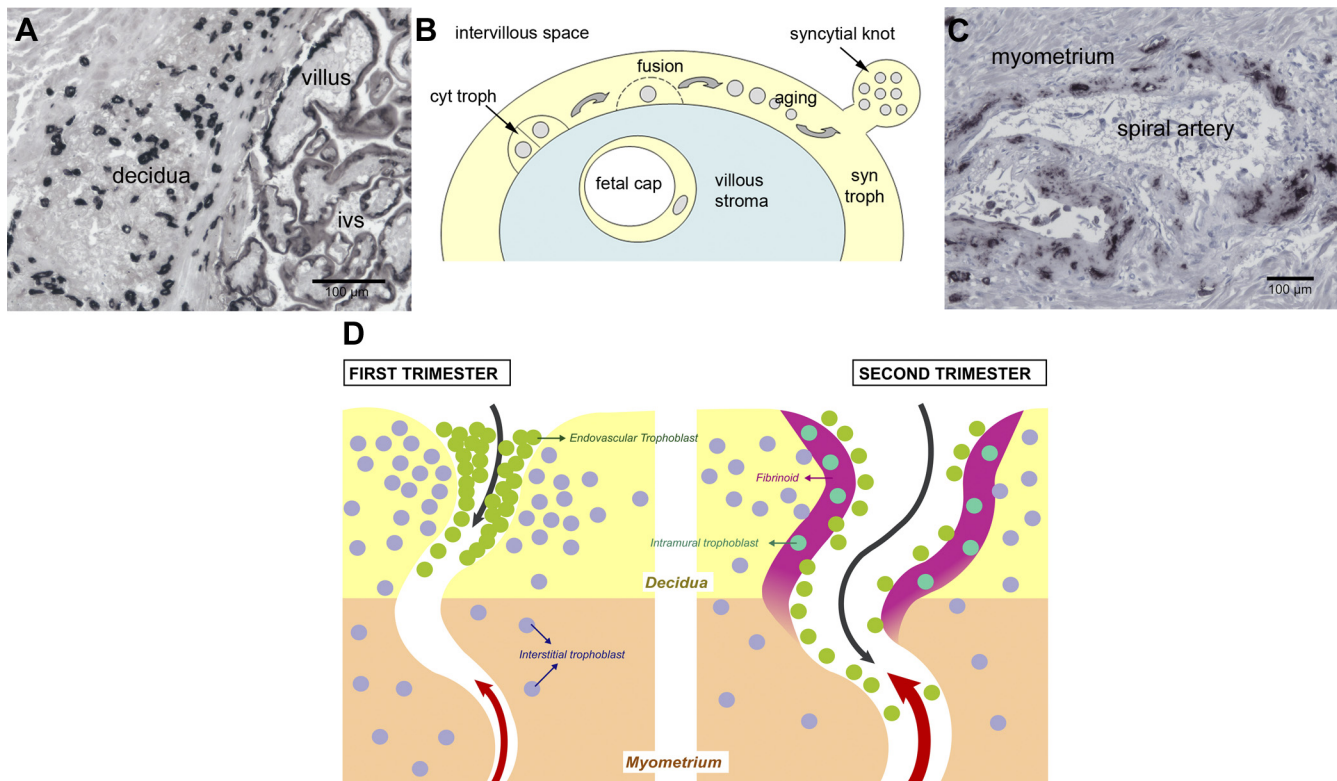
FIGURE 1. Villous and extravillous trophoblast in the placenta of human and great apes. *A*: gorilla placenta immunostained with the trophoblast marker cytokeratin 7/17 (black). At right, placental villi are seen with syncytiotrophoblast at the surface facing the intervillous space (ivs). At left, extravillous trophoblast is seen within the decidua. *B*: in human placenta, the villous syncytiotrophoblast (syn troph) is continually renewed by fusion with cytotrophoblasts (cyt troph). Aging of the syncytiotrophoblast leads to accumulation of apoptotic nuclei in syncytial knots. [From Carter (41).] *C*: chimpanzee placenta immunostained with cytokeratin 7/17 (black). In this section through a myometrial spiral artery, extravillous trophoblasts embedded in extracellular matrix are seen in the vessel wall. *D*: extravillous trophoblast invades the uterus by two routes and homes in on the spiral arteries. At left, the interstitial route is shown in blue and the endovascular route in green. By the second trimester at right, there is intramural trophoblast embedded in fibrinoid and lining the spiral artery. Note that trophoblast entering by the endovascular route (black arrow) moves against the direction of blood flow (red arrow). [From R. Pijnenborg R, Vercruysse L, and Hanssens M. *Placenta* 2006, 27: 939–958, 2006, with permission from Elsevier.]

most subsequent authors (10, 275). Based on ultrastructure (86), the Grosser classification usually is applied in a simplified form with three principal types: epitheliochorial, endotheliochorial, and hemochorial (45).

In the epitheliochorial type, the uterine epithelium remains intact. In principle, the interhemal barrier then has six layers of tissue. In practice, there are areas of extreme thinning, and connective tissue in particular may be pushed aside (**FIGURE 2A**). This type of placenta occurs in Cetartiodactyla, Perissodactyla, Pholidota, and Strepsirrhini (lower primates) **(TABLE 1)**.

In the endotheliochorial type of placenta, the uterine epithelium is lost, and there is contact between the trophoblast and the maternal capillary endothelium **(FIGURE 2B)**. This type is found in carnivores, elephants, manatees, and some bats **(TABLE 1)** (89).

In a hemochorial placenta, maternal blood comes directly in contact with the trophoblast **(FIGURE 2C)**. This placental type is typical of many rodents and primates including humans **(TABLE 1)**. According to the number of trophoblast layers, these placentas are referred to as hemomonochorial (e.g., human, guinea pig), hemodichorial (e.g., rabbit), or hemotrichorial (e.g., mouse) (86).

Currently there is much interest in how the interhemal barrier evolved. There is broad agreement that the epitheliochorial type evolved separately in the lower primates and the common ancestor of Cetartiodactyla, Perissodactyla, and Pholidota (353). Authors are equally divided, however, as to whether the common ancestor of placental mammals had an endotheliochorial placenta (249, 264) or a hemochorial one (84, 372).

C. Histotrophic Nutrition

Exchange of gases and nutrients between maternal and fetal blood is sometimes referred to as hemotrophic nutrition.
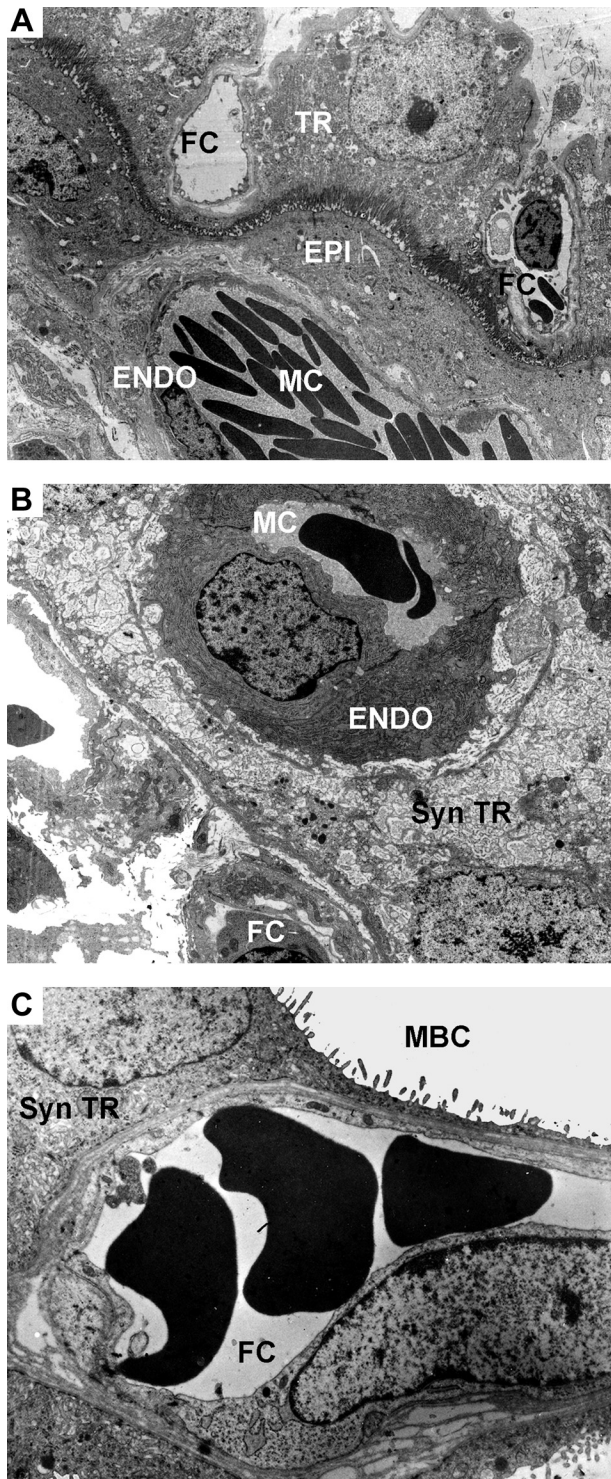
FIGURE 2. The three principal types of interhemal barrier. *A*: epitheliochorial placenta of a camelid. *B*: endotheliochorial placenta of the three-toed sloth (*Bradypus tridactylus*). *C*: hemochorial placenta of a hystricognath rodent, the degu (*Octodon degus*). ENDO, maternal endothelial cells; EPI, uterine epithelium; FC, fetal capillary including the vessel endothelium; MBC, maternal blood channel; MC, maternal capillary; Syn TR, syncytial trophoblast; TR, trophoblast cells. [From Mess and Carter (265), with permission from Elsevier. *Panel A* was kindly provided by Dr. Karl Klisch, University of Nottingham.]

However, there are alternate pathways involving absorption and phagocytosis by the trophoblast of secretions from uterine glands, tissue debris, and erythrocytes. This is called histotrophic nutrition (88).

A variety of accessory structures support histotrophic nutrition and invariably they involve columnar trophoblast. In the simplest form, known as an areola, the trophoblast forms an absorptive epithelium immediately above the opening of a uterine gland. The areola is commonly associated with epitheliochorial placentas such as those of swine and horses, but it also occurs in moles, which have endotheliochorial placentas. A similar but more elaborate structure is the chorionic vesicle found with epitheliochorial placentation in strepsirrhine primates. Another specialization for histotrophic nutrition is the uptake of maternal red blood cells as a source of iron. Hemophagous organs are discussed in a later section.

Evidence is emerging that histotrophic nutrition is important even in placentas that lack specialized structures. Early in human gestation uterine glands open into the intervillous space and their secretions, which include glycodelin A and the mucin MUC-1, are taken up by the villous trophoblast (36, 163, 193).

D. Reproductive Strategies

What is expected of a placenta depends to some extent on the reproductive strategy of the species. In altricial mammals, such as murine rodents, the newborn young are relatively immobile, have closed eyes, lack hair, and require close parental care (303). This pattern is associated with a large litter size and short gestation (248). In precocial mammals, such as ruminants, the newborns are relatively mature and mobile from the moment of birth. This strategy is associated with a smaller litter size and longer gestation (248, 303). The difference is important because the placenta is called upon to serve a new set of functions in mammals with long gestations. This is reflected in the very different endocrine functions subserved by the placenta in murine rodents and primates (40, 246).

III. PLACENTAL GAS EXCHANGE AND THE EVOLUTION OF BETA GLOBINS

The physiology of placental gas exchange is the subject of a classic treatise (266) that remains a valuable source of reference, despite more recent reviews (38, 42, 239, 373). The principal factors determining oxygen flux are the oxygen-diffusing capacity of the placenta, the rates of blood flow in the umbilical and uterine arteries, and the oxygen capacities and affinities of fetal and maternal blood (42). Fetal blood has a greater affinity for oxygen than maternal blood. This facilitates placental oxygen transfer and allows oxygen sat-

uration to be quite high at the low P_{O_2} levels encountered in umbilical venous blood (20, 266).

A. Globin Genes

Globins are found in virtually all kingdoms of organism. An ancestral hemoglobin gene was likely present before the divergence of plants and animals 1,500 million years ago. It has been speculated that the initial function of oxygen-binding hemoproteins was to protect cells from oxygen once this had appeared in the biosphere as a product of photosynthesis (149). Indeed, the function of embryonic hemoglobins, which appear early during mammalian development, very likely is to protect against free radicals derived from oxygen and nitric oxide such as superoxide and peroxynitrite. These hemoglobins have high affinity for oxygen (208) and appear well before there is an effective system of placental oxygen transfer. On this view of globin evolution, functions such as intracellular transport of oxygen and oxygen transport in blood were acquired later. The groundwork for this appears to have been laid by whole genome duplication in the vertebrate lineage (173).

Vertebrate hemoglobins are heterotetramers comprising two alpha chains, two beta chains, and a heme group. Multiple isoforms of each chain occur in mammals. They are determined by separate cassettes of genes situated on different chromosomes with separate regulatory elements (149). Publication of the genome of the platypus (*Ornithorhynchus anatinus*), a monotreme (365), has advanced our understanding of the evolution of these genes in marsupials and placental mammals (149, 172, 285, 291). There is a complex history encompassing many instances of gene duplication, inactivation, and deletion. **TABLE 2** lists the principal genes found in eutherian mammals by their current and traditional nomenclature (3).

Four alpha-globin genes are found in placental mammals (172). A species may have multiple copies as genes or pseu-

dogenes. In addition, a gene that is functionally active in one species may be a pseudogene in another. As an example, the human HBZ-T1 gene is a pseudogene in chimpanzee (172). Expression of alpha-globin genes is controlled by a highly conserved regulatory region upstream of the alpha-globin cassette (168). HBZ and HBA are developmentally regulated with expression of HBZ confined to the primitive erythroid cells of the yolk sac. The alpha-globin chain of fetal and adult hemoglobins, which concerns us here, is always coded by an HBA gene. There is a single beta-globin gene in lower vertebrates (285), but a number of duplication events in the eutherian lineage have yielded a cassette of five genes **(FIGURE 3)** (284).

B. Evolution of Beta Globins in Primates

The beta-globin genes are developmentally regulated. They are lined up on the same chromosome with a locus control region upstream (191). The closer a gene is to the control region, the earlier it is expressed. Thus the HBE gene is expressed during development of the early embryo. The next gene along is HBG. In strepsirrhine primates it is also expressed in the embryo (186, 304). In the lineage of catarrhine primates, however, duplication of the HBG gene resulted in two variants **(TABLE 2)**. The distance between the locus control region and HBG-T2 (γ^2-globin) increased sufficiently to allow its expression as the gamma chain of fetal hemoglobin. Meanwhile, recruitment of HBG-T2 for fetal expression was accompanied by a delay in expression of the HBB gene until near term of pregnancy (149).

A common device for increasing hemoglobin oxygen affinity is to decrease its sensitivity to allosteric cofactors like organic phosphates, protons, and chloride ions. This strategy is adopted by birds and mammals, such as deer mice, that survive at high altitude (338, 367). The beta globin expressed in the fetuses of higher primates has a substitution at one of the four residues binding 2,3-diphosphoglyc-

Table 2. *Alpha and beta globins of eutherian mammals and gene duplications relevant to the evolution of fetal hemoglobins in primates and ruminants*

| Globin Chain	Current Nomenclature	Previous Nomenclature	Catarrhine Primates Including Human	Ruminants
Alpha globins	HBZ	ζ-globin	Embryonic hemoglobin	Embryonic hemoglobin
	HBK	α^D-globin	Does not code for hemoglobin	Does not code for hemoglobin
	HBA	α^A-globin	Fetal and adult hemoglobin	Fetal and adult hemoglobin
	HBQ	θ-globin	Does not code for hemoglobin	Does not code for hemoglobin
Beta globins	HBE	ϵ-globin	Embryonic hemoglobin	Embryonic hemoglobin
	HBG	γ-globin	HBG-T1 in embryonic and HBG-T2 in fetal hemoglobin	Lost in Laurasiatheria including ruminants
	HBH	η-globin	Lost in Euarchontoglires including primates	Embryonic hemoglobin?
	HBD	δ-globin	Adult hemoglobin (about 3 % in human)	Pseudogene in ruminants
	HBB	β-globin	Adult hemoglobin (about 97 % in human)	Adult hemoglobin
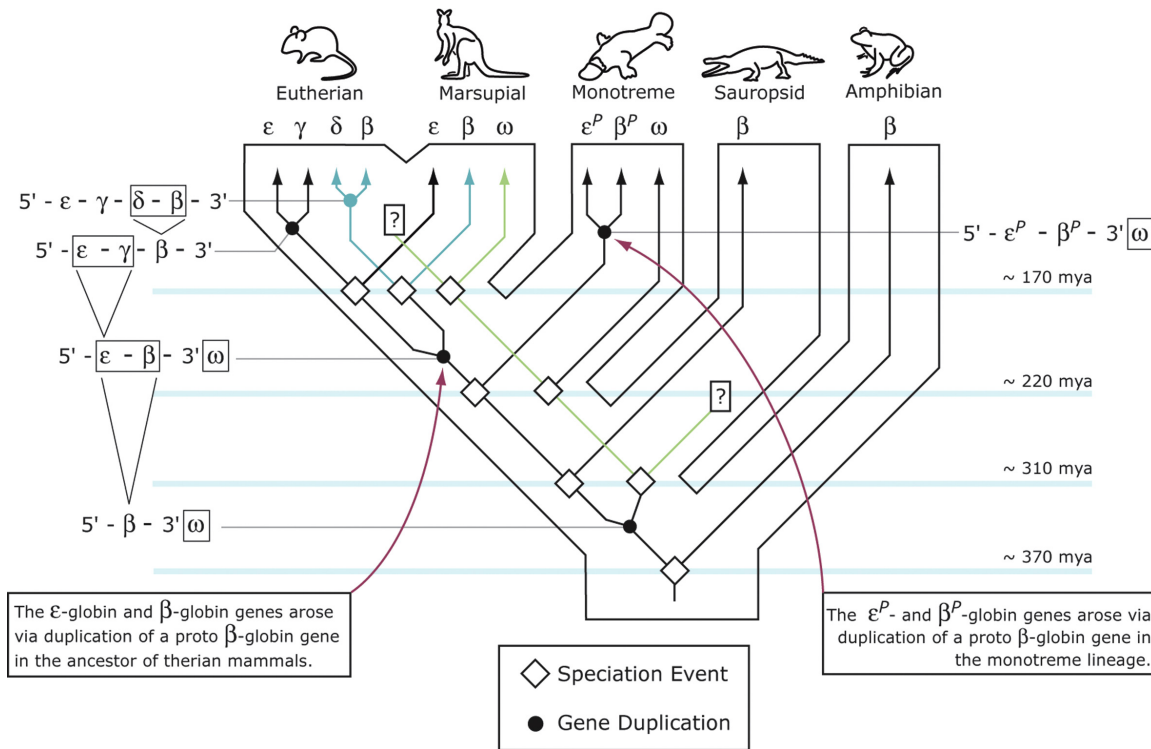
FIGURE 3. Hypothesis describing how the beta-globin genes evolved by successive duplication of an ancestral gene. In the eutherian lineage this gave rise to HBE, HBG, HBD, and HBB [ϵ, γ, δ, and β-globin] genes. [From Opazo et al. (285). Copyright 2008 National Academy of Sciences.]

erate (DPG). This results in a very low affinity for DPG and a high affinity for oxygen (22).

C. Developmental Regulation of Blood Oxygen Affinity in Rodents and Lagomorphs

Rodents and lagomorphs do not have a fetal hemoglobin (187). The difference in oxygen affinity between fetal and maternal blood is due to the low DPG content of fetal red blood cells (187, 295). Two enzymes concerned with DPG metabolism are developmentally regulated in the laboratory rat (188). The first is 2,3-biphosphoglycerate mutase, which converts 1,3-DPG to 2,3-DPG. The corresponding gene (*bpgm*) is developmentally regulated, and no enzyme activity can be detected in fetal red blood cells. In contrast, the activity of pyruvate kinase is 10-fold higher in fetal than in adult red blood cells. This enzyme catalyzes the rate-limiting step in glycolysis and, since the breakdown product of DPG is 3-phosphoglycerate, it determines the rate at which DPG is metabolized. In the fetal rabbit, the high pyruvate kinase activity may depend on a discrete fetal isozyme (106), although this supposition needs to be revisited in the light of recent genomic information (389).

D. Evolution of Beta Globins in Ruminants

Convergent evolution of a fetal hemoglobin with high oxygen affinity has occurred in the ruminant lineage following

tandem duplication of the HBB gene (236). HBB-T3 **(TABLE 2)** is expressed in the fetus, where it codes for a beta chain with high innate affinity for oxygen.

In other members of Cetartiodactyla, including the domestic pig (*Sus scrofa*) and Weddell seal (*Leptonychotes weddellii*) (306), the higher oxygen affinity of fetal blood is due to a low intraerythrocytic concentration of DPG, much as in rodents and lagomorphs (23).

IV. PLACENTAL TRANSFER OF IRON

Iron-containing heme is an important component of the globins and of the cytochromes and cytochrome oxidase of the electron transport chain. Other significant proteins contain iron-sulfur clusters (12). Iron is useful because of its ability to act as an electron acceptor. However, if iron is present in excess, this same property renders it toxic, because it can generate free radicals. That is largely solved by storing iron as ferritin.

Although a limited number of species have been studied, we know of three different strategies for supplying the fetus with iron. In the first of these, trophoblast binds and internalizes circulating transferrin, releasing the iron to the fetal side. This requires the expression in trophoblast of an appropriate set of existing genes and is restricted to hemochorial placentas where the trophoblast has access to circulating, transferrin-bound iron. In a strategy adopted by

some species with epitheliochorial placentas, iron is secreted from uterine glands as uteroferrin and taken up by columnar trophoblast. Finally, maternal blood may be ingested by trophoblast and the iron extracted from hemoglobin. The most striking aspect here is the development of special structures for the purpose, but the release of iron from heme again requires expression of appropriate genes within columnar trophoblast cells.

Copper is a component of many enzymes; examples are cytochrome *c* oxidase, multicopper ferroxidase, and lysyl oxidase (116). The placental transfer of iron and copper is closely linked (255).

A. Placental Uptake of Transferrin in Primates and Rodents

In hemochorial placentas, the trophoblast has direct access to the maternal circulation and can take up iron directly from the blood, where it circulates bound to transferrin. Transferrin receptors are located in clathrin-coated pits at the apical surface of the syncytiotrophoblast (FIGURE 4A). Transferrin binds to the receptor, and the entire complex is internalized by receptor-mediated endocytosis. The vesicles are uncoated and protons are pumped into them, lowering the pH to 5.5, at which point ferric iron (Fe^{3+}) dissociates from apotransferrin. It is converted to the more soluble ferrous iron (Fe^{2+}) by an endosomal reductase and transported out of the vesicle by the divalent metal transporter DMT1 (*SLC11A2*) (119, 337). Apotransferrin and the receptor are returned to the cell surface and recycled.

Iron leaves the syncytiotrophoblast at the basolateral membrane via ferroportin 1 or IREG1 (*SLC40A1*) (33). Ferrous iron must then be oxidized to ferric iron by a multicopper ferroxidase (67). In the absence of this enzyme, ferrous iron would stay bound to ferroportin, which would be ubiquitinated, internalized, and degraded (12, 69). Although the
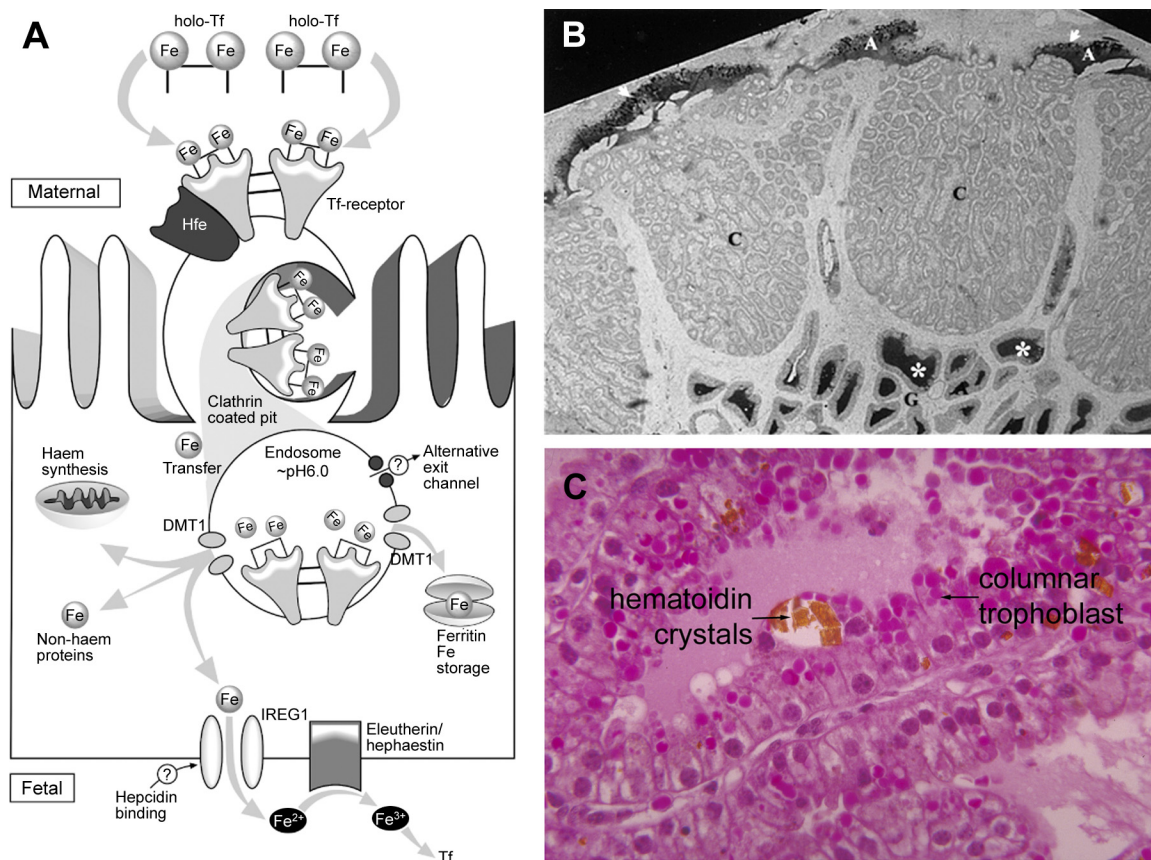

FIGURE 4. Three strategies for fetal acquisition of iron. *A*: in human placenta, transferrin (holo-Tf) is captured by receptors (Tf-receptor) on the maternal-facing membrane of the syncytiotrophoblast. After internalization, the pH of the endosome is lowered to release the iron, which then is pumped out by the divalent metal transporter (DMT1). At the fetal side, iron leaves the trophoblast via ferroportin (IREG1) and is oxidized by a multicopper ferroxidase (here shown as eleutherin/hephaestin). [From McArdle et al. (255), with permission from John Wiley and Sons.] *B*: in equine placenta, iron in the form of uteroferrin is secreted by the uterine glands (G) to the areolas (A) whence it is taken up by columnar trophoblast (arrows). Immunostaining for uteroferrin (black) is restricted to these structures and is not seen in the microcotyledons (C). [From Wooding et al. (381), with permission from Elsevier.] *C*: in tenrec placenta, columnar trophoblast takes up maternal red cells by phagocytosis. Iron is released by the action of heme oxygenase, and the breakdown products are stored as crystals of hematoidin. [From Carter et al. (44), with permission from Elsevier.]

placental enzyme resembles ceruloplasmin and hephaestin, it recently was shown to be encoded by a distinct gene and given the name zyklopen (57). DMT1 is also expressed in the basolateral membrane where it could participate in the export of iron (33).

Whatever the mechanism, iron has still to cross the villous stroma and capillary endothelium before reaching the fetal blood. All we know with certainty is that, once there, iron is bound by fetal apotransferrin. Similar mechanisms likely operate in other simian primates, since binding of transferrin to the microvillous membrane of trophoblast has been demonstrated in the baboon placenta (98, 112).

Endocytosis of transferrin-bound iron has been documented in rats (256) and guinea pigs (351, 352). Transferrin receptor, DMT1, and ferroportin are expressed in rat placenta (11, 115). Zyklopen, the newly characterized multi-copper ferroxidase, is expressed in mouse placenta (57). Curiously, knockout of the *Slc11a2* gene in mice did not prevent accumulation of iron by the fetus, indicating that DMT1 is not essential for placental iron transport in this species (136). Perhaps it can be substituted by one of the zinc transporters ZIP8 (*Slc39A8*) or ZIP14 (*Slc39A14*) (189); the former is known to be expressed by murine placenta (364). Notwithstanding, rodents are useful models for studying perturbations in placental iron transfer (114).

B. Uptake of Uteroferrin in Pigs and Horses

In pigs and horses, iron is supplied to the fetus as uteroferrin, an iron-rich glycoprotein secreted from the uterine glands to specialized structures called areolas. In midpregnancy, the porcine endometrium secretes uteroferrin at an estimated rate of 1–2 g/day (242). Uteroferrin is taken up by the polarized trophoblast of the areolas (311). It reaches the blood and is cleared by the fetal liver where the iron is extracted and utilized for hemoglobin synthesis. Some uteroferrin is excreted with the fetal urine and reaches the allantoic sac; iron exchange between uteroferrin and transferrin may occur in this location (282).

The human ortholog of the uteroferrin gene is tartrate-resistant acid phosphatase type 5 (*ACP5*) (349). It is a useful marker of osteoclasts, where it is expressed on the ruffled membrane and has been assigned a role in regulating lacunar pH and thus in bone remodeling (199). The gene is present in a wide variety of mammalian genomes (287), but clearly has acquired a new function in species that rely on histotrophic nutrition. This has been achieved without gene duplication (349). Expression of uteroferrin mRNA and protein in porcine endometrial glands is progesterone dependent (327). Estrogen stimulates secretion but inhibits transcription. The transcriptional regulation of the porcine gene begs to be reexamined as some earlier experiments were subject to cloning artifacts (349).

The placenta of the horse is rather more complex than that of the pig, although both are epitheliochorial. Uteroferrin is secreted by the endometrial glands and taken up by the areolar trophoblast through a pinocytotic or endocytotic process **(FIGURE 4B)** (83, 381). As in the pig, secretion of uteroferrin by equine endometrial glands is progesterone dependent (257).

Uteroferrin may play a subsidiary role in iron supply to the ruminant fetus. Immunostaining for uteroferrin has been demonstrated in the uterine glands and some trophoblast cells of the water buffalo (*Bubalus bubalis*) (292). The uteroferrin gene is highly conserved and is expressed in the endometrium of all mammals including monotremes (287). It may not contribute to iron transfer in all species, but the placenta of the cat exhibits strong acid phosphatase activity (376) and certainly deserves renewed examination.

C. Hemophagous Regions

In many placentas, especially those of the endotheliochorial type, there is controlled seepage of maternal blood and the erythrocytes are taken up by columnar trophoblast. Indeed, it has been known since 1889 that the primary source of iron in carnivores is heme derived from maternal red blood cells (231). The trophoblast is often arranged in elaborate structures known as hemophagous regions (35, 88). In shrews this function is performed by the yolk sac (46, 207).

These processes have yet to be characterized at the molecular level, although it does seem likely that the columnar trophoblast in hemophagous regions would express genes similar to those expressed in enterocytes. These include heme oxygenase, which opens up the ring structure, releasing ferric iron and creating biliverdin, which is rapidly reduced to bilirubin. Bilirubin may form crystals of hematoidin, and these are often found in the trophoblast of hemophagous regions (44), whence they are conveniently discarded with the placenta at birth **(FIGURE 4C)**.

D. Placental Uptake of Copper in Primates and Rodents

High-affinity copper transporters constitute a highly conserved gene family. Thus there are resemblances between the *Ctr1* gene of yeast (*Saccharomyces cerevisiae*) and the human *CTR1* gene that include conserved methionine residues (305). Human *CTR1* is expressed by syncytiotrophoblast and may be responsible for uptake of copper from maternal blood (255). However, the protein has yet to be localized to the microvillous membrane and might instead facilitate transport away from the fetus across the basal membrane (151). Since the concentration gradient is from mother to fetus, this could be an important protective mechanism to maintain fetal copper homeostasis (151).

Two copper ATPases are expressed in human placenta. ATP7A (also known as Menkes disease-associated protein, MNK) is located at the basolateral membrane (150, 152) and may assist in transfer of copper to the fetus (153). ATP7B (Wilson disease protein, WND) is found in the perinuclear compartment whence it can translocate to the microvillous membrane (150, 152). A model has been proposed where ATP7B assists in clearing copper from the fetal circulation. It is supported by experiments on cultured Jeg-3 cells (153) but cannot fully be reconciled with the observations in ATP7B mutant rats (see Ref. 255).

Rodents are useful models of maternal-fetal copper transfer. Knockout of *Ctr1* in mice is embryonic lethal (225). Copper deficiency in pregnant rats causes structural abnormalities in the neonates, some of which can be linked to the copper-containing enzyme lysyl oxidase, which is responsible for forming lysine-derived cross links in collagen and elastin (116). The interplay between copper and iron is nicely illustrated by the downregulation in copper-deficient pregnant rats of placental expression of *TfR1* and one of the transcripts of *Slc11a2* (11).

V. SUBSTRATE TRANSFER

Substrate transfer is an essential function of the placenta, yet in this area molecular biology has contributed little compared with what already is known from integrative studies of fetal and placental physiology (21). It is a relatively easy task to transport substrates from maternal to fetal blood when there is a single layer of trophoblast. In the human placenta, this is accomplished by expression of the appropriate genes for glucose and amino acid transporters and incorporation of the proteins in the microvillous and basal membranes of the trophoblast layer. However, even the mouse and rat present a challenge (180), because there are three layers of trophoblast and this adds a further level of anatomical complexity. In endotheliochorial and epitheliochorial placentas, the trophoblast no longer has access to the maternal blood. Nevertheless, carbohydrates and amino acids [although few lipids (21)] do cross the many cell layers of the sheep placenta.

A. Sugar Transporters

1. Human placenta

Sugar transporters have a deep evolutionary history (375). In mammals there is a family of facilitative transporters usually referred to as GLUT1–14; the corresponding genes are *SLC2A1–14* (395). Those known to be expressed in human placenta **(TABLE 3)** include the glucose transporters GLUT1, GLUT3, and GLUT4. In addition, GLUT9 is expressed as two splice variants (25). Both GLUT10 and a splice variant of GLUT11 are expressed in human placenta (395), but their function here is unknown (25). The same is true of GLUT12 which was found in the syncytiotrophoblast of first trimester placentas but not at term (133). Given the great antiquity of this gene family, it is perhaps not unexpected that no placenta-specific genes have been evolved. GLUT11 is absent from the mouse and rat genome (319), but this represents a loss in murid rodents rather than a gain in primates. The sodium-dependent glucose transporters SGLT1–6 (*SLC5A1–6*) seem not to be expressed in human placenta (395).

GLUT1 is expressed in both the microvillous and basal membranes of the syncytiotrophoblast **(FIGURE 5)** (185) and is thought to be mainly responsible for glucose transfer (180). The distribution of the transporter is asymmetric, however, with a threefold greater density at the maternal-facing microvillous membrane (184), suggesting that transport across

Table 3. *Facilitated hexose transporter genes and gene products and evidence for their expression in human placenta*

| Gene	Protein	Substrates	Expression in Human Placenta	Location
SLC2A1	GLUT1	Glucose, galactose, mannose, glucosamine	mRNA and protein	Microvillous and basal membranes
SLC2A2	GLUT2		Not determined	Not determined
SLC2A3	GLUT3	Glucose, galactose, mannose, maltose, xylose, dehydroascorbic acid	mRNA and protein	Microvillous membrane and fetal capillary endothelium
SLC2A4	GLUT4	Glucose, dehydroascorbic acid, glucosamine	mRNA and protein	Villous stroma
SLC2A5-8	GLUT5-8		Not determined	Not determined
SLC2A9	GLUT9	Glucose, fructose	mRNA and protein	Microvillous (GLUT9b) and basal (GLUT9a) membranes
SLC2A10	GLUT10	Glucose, galactose	mRNA	
SLC2A11	GLUT11	Glucose, fructose	mRNA	
SLC2A12	GLUT12	Glucose	mRNA and protein	Villous stroma
SLC2A13-14	GLUT13-14		Not determined	Not determined
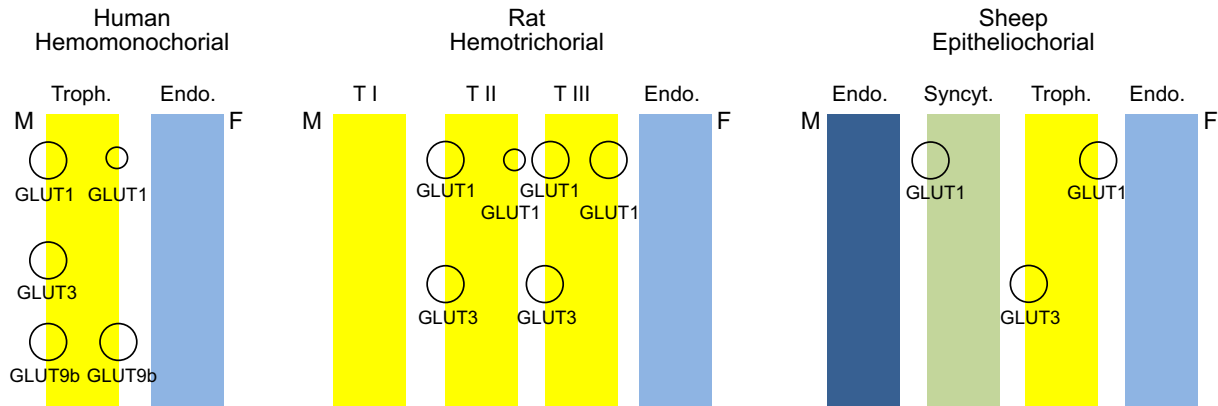
FIGURE 5. Sugar transporters. In human placenta, transporters (GLUT) are located to the microvillous (maternal-facing) and basal membranes of the syncytiotrophoblast (Troph.) GLUT3 is expressed in the fetal capillary endothelium (Endo.), but its exact location is not known. In rat placenta, the cytotrophoblast layer (TI) does not express transporters, but they are found as shown in the maternal-facing and basal membranes of the two syncytiotrophoblast layers (TII and TIII). In sheep placenta, GLUT1 is found at the basal membranes of maternal syncytium (Syncyt.) and trophoblast and GLUT3 in the microvillous membrane of the trophoblast. Transporters are not found in either maternal or fetal endothelium. Spaces between layers are for illustrative purposes; in rat placenta, for example, gap junctions connect TII and TIII and may provide a route for glucose transfer. M, maternal side; F, fetal side.

the basal membrane may be rate limiting. The isoforms of GLUT9 localize asymmetrically with 9a predominating in the basal and 9b in the microvillous membrane (25). This has focused attention on GLUT9a as a possible alternative to GLUT1 for glucose transfer across the basal membrane. In addition, GLUT9 may transport fructose across the syncytiotrophoblast (25). GLUT3 is expressed at the microvillous membrane (34) and in the fetal capillary endothelium (156). Recent work suggests it to be of greater importance for glucose transfer in the first trimester than at term (34). The insulin-responsive GLUT4 isoform is associated with perinuclear membranes of first trimester syncytiotrophoblast (94) and at term is expressed in the villous stroma (388). Its role in glucose transfer remains uncertain.

2. Rodents and lagomorphs

GLUT1 and GLUT3 are expressed in rat placenta (399). The precise location of the proteins **(FIGURE 5)** has been determined by immunofluorescence and at the ultrastructural level by immunogold labeling. Neither transporter is expressed by the cytotrophoblast, but this layer (TI) is discontinuous and unlikely to present a barrier to glucose (342). GLUT1 is found in both layers of syncytiotrophoblast (TII and TIII) at both the maternal-facing and basal membranes (326, 342). However, there are only small amounts of GLUT1 in the basal membrane of TII. Moreover, GLUT3 is present only on the maternal-facing membranes of TII and TIII (326) and cannot account for glucose transfer between the two layers. Therefore, gap junctions have been proposed as an additional route for transfer of glucose from TII to TIII (326, 342).

Comparing the placentas of littermates in mice, it was shown that *Slc2a1* (coding for GLUT1) was upregulated in small placentas (61). In line with this, placental size was smaller but *Slc2a1* expression higher in undernourished mice (62).

The rabbit has a hemodichorial placenta, i.e., with two trophoblast layers. GLUT1 has been localized to the maternal-facing surface of the outer layer of syncytiotrophoblast. GLUT3 was expressed in the inner layer of cytotrophoblast, but the precise localization of the protein is difficult to assess (204).

3. Ruminants

The sheep is an ideal model to explore the flux of glucose between mother, placenta, and fetus (157, 158). Both GLUT1 and GLUT3 are expressed in the ovine placenta (66, 68, 82), but the exact location of the proteins was not known until quite recently. Where the barrier is thinnest, glucose must cross consecutively through maternal capillary endothelium, a maternal syncytial layer, trophoblast, and the fetal capillary endothelium. There was no appreciable expression of transporters on blood vessels, so the focus was on the trophoblast and maternal syncytium. GLUT1 was localized innermost and outermost, i.e., to the basal membranes of both layers **(FIGURE 5)**. The apical surfaces were characterized by interdigitating microvilli and here GLUT3 was expressed, although only on the trophoblast (379). Similar patterns of expression were seen in goat, sheep, and red deer (*Cervus elephas*) (379).

B. Amino Acid Transporters

The amino acid supply to the fetus has long been perceived as a determinant of growth, and there is an extensive literature, much of it predating molecular studies. Thus transport sys-

tems have been characterized in terms of their activities, adopting the somewhat esoteric terminology used in other studies of transport epithelia (274). These systems are defined by which substrates are transported and which excluded, whether or not they are Na$^+$ dependent, and if they can be blocked by specific inhibitors (392). An example is System A, which transports neutral amino acids, including exogenous methyl aminobutyric acid (MeAIB), excludes cationic and anionic amino acids, and is inhibited by methyl alanine (392).

In recent years, the genes responsible for transport activity in human epithelia have been characterized, and this has led to a welcome improvement in classification and terminology (59, 214). Thus, as shown in **TABLE 4**, three genes code for transporters with the properties of System A and a member of the same family codes for the System N transporter. With few exceptions, orthologous genes can be found in the genomes of other mammals, but their placental expression has been described in only a few, rat and mouse being those best explored.

1. Human placenta

The literature on transporter activities in human placenta is extensive (see Refs. 274, 392). Recent work has focused on

Table 4. *Amino acid transport systems with associated genes and gene products and evidence for expression in human placenta*

Transport System	Gene	Protein	Substrates	Expression in Human Placenta
Sodium-coupled neutral amino acid transporters				
A	*SLC38A1*	SNAT1	Neutral amino acids	mRNA and protein
A	*SLC38A2*	SNAT2	Neutral amino acids	mRNA and protein
A	*SLC38A4*	SNAT4	Neutral amino acids	mRNA and protein
N	*SLC38A3*	SNAT3	Gln, His	Absent
High-affinity glutamate and neutral amino acid transporters				
X$^-_{AG}$	*SLC1A1*	EAAT3	Anionic amino acids	Protein
X$^-_{AG}$	*SLC1A2*	EAAT2	Anionic amino acids	Protein
X$^-_{AG}$	*SLC1A3*	EAAT1	Anionic amino acids	Protein
ASC	*SLC1A4*	ASCT1	Ala, Ser, Cys, anionic amino acids	mRNA
B^O	*SLC1A5*	ASCT2	Neutral amino acids	mRNA; protein expressed only by cytotrophoblasts
Sodium- and chloride-dependent transporters				
β	*SLC6A6*	TAUT	β Ala, Tau	mRNA
B$^{O,+}$	*SLC6A14*	ATB$^{O,+}$	Neutral and cationic amino acids	No data
Gly	*SLC6A9*	GLYT1	Gly	No data
Cationic amino acid transporters				
y$^+$	*SLC7A1*	CAT1	Cationic amino acids	mRNA
y$^+$	*SLC7A2*	CAT2B	Cationic amino acids	mRNA
y$^+$	*SLC7A4*	CAT4	Cationic amino acids	mRNA
Glycoprotein-associated amino acid transporters				
asc	*SLC7A10* (light chain)	Asc1	Small neutral amino acids	mRNA
	SLC3A2 (heavy chain)	4F2hc		
asc	*SLC7A12* (light chain)	Asc2	Small neutral amino acids	mRNA for light chain
	Unknown heavy chain			
b$^{O,+}$	*SLC7A9* (light chain)	b$^{O,+}$	Neutral and cationic amino acids, Cys	mRNA for light chain; heavy chain protein not found
	SLC3A1 (heavy chain)	rBAT		
L	*SLC7A5* (light chain)	LAT1	Large neutral amino acids	mRNA and protein
	SLC3A2 (heavy chain)	4F2hc		
L	*SLC7A8* (light chain)	LAT2	Neutral amino acids	mRNA
	SLC3A2 (heavy chain)	4F2hc		
y$^+$L	*SLC7A7* (light chain)	y$^+$ LAT1	Neutral and cationic amino acids	mRNA
	SLC3A2 (heavy chain)	4F2hc		
y$^+$L	*SLC7A6* (light chain)	y$^+$ LAT2	Neutral and cationic amino acids	mRNA
	SLC3A2 (heavy chain)	4F2hc		
Aromatic amino acid transporter (monocarboxylate transporter family)				
T	*SLC16A10*	TAT1	Aromatic amino acids	mRNA

studying gene expression in the syncytiotrophoblast layer as well as the membrane localization of the gene products. This knowledge can be related to new or existing data on transporter activities. It must be said from the outset that none of these transporter genes is exclusive to the placenta and there is no evidence to date of gene duplication to serve placenta-specific functions. There is expression in human placenta of five types of amino acid transporter; **TABLE 4** follows the nomenclature for solute carriers established by the HUGO Gene Nomenclature Committee (http://www.genenames.org/).

A) SODIUM-COUPLED NEUTRAL AMINO ACID TRANSPORTERS. System A is important for the supply of neutral amino acids. It has been localized to the maternal-facing microvillous membrane of the syncytiotrophoblast in human placenta (190). Its activity there is decreased in placentas from pregnancies that end with babies born small-for-gestational-age (SGA) (243). The transporter is also found at the basal membrane of the syncytiotrophoblast (171). All three genes responsible for System A activity are expressed in placenta. Their products (SNAT1, -2, and -4) are present in syncytiotrophoblast (76, 77), and SNAT4 is functional in microvillous membrane vesicles, especially in the first trimester. In contrast, SNAT1 may play a greater role closer to term (75). A fourth gene coding for SNAT3 and responsible for System N activity is not expressed in human placenta (37).

B) HIGH-AFFINITY GLUTAMATE AND NEUTRAL AMINO ACID TRANSPORTERS. There is some evidence for expression of these transporters in placenta (14, 200). Expression of ASC2 (System B^0) has attracted some interest (213) as it acts as the receptor for syncytin, the endogenous retrovirus-W envelope gene that is ascribed a role in the syncytialization of trophoblast. It appears, however, that ASC2 expression is restricted to the cytotrophoblast layer (159). It is unlikely, therefore, to be involved in placental amino acid transport.

C) SODIUM- AND CHLORIDE-DEPENDENT TRANSPORTERS. Included here is System β responsible for uptake of taurine. Expression of the transporter has been demonstrated in placenta-derived cell lines (307).

D) CATIONIC AMINO ACID TRANSPORTERS. Three genes code for System y^+ which transports cationic amino acids (there is also a pseudogene). Their products (CAT1, CAT2B, and CAT4) are expressed in human placenta (194). On the basis of functional studies in *Xenopus laevis* oocytes, it has been suggested that CAT2B is most likely to act at the microvillous membrane and CAT1 at the basal membrane of syncytiotrophoblast (111).

E) GLYCOPROTEIN-ASSOCIATED AMINO ACID TRANSPORTERS. Transporters in this category are dimers comprising light and heavy chains coded by separate genes (37). In most cases the heavy chain is 4F2hc. System L transports large neutral amino acids. Two genes code for the light chains of the transporter, and their products (LAT1 and LAT2) are expressed by both BeWo cells and syncytiotrophoblast (314). It has been proposed that transport across the microvillous membrane depends on LAT1, whereas the basal membrane uses LAT2 (212). In addition, System y^+L is expressed in human placenta (211), and y^+LAT1 acts at the basal membrane (15, 212).

System asc transports small neutral amino acids. Two genes code for the light chains, and expression at the mRNA level has been detected in placental tissues (52, 110). There are no functional studies to date.

Finally, System $b^{0,+}$ transports neutral and cationic amino acids. Although message for the light chain has been detected in placental tissue (99), the product of the heavy chain gene (rBAT) was not found in term placentas (15) nor could any evidence be found for System $b^{0,+}$ activity at the microvillous or basal membranes of the syncytiotrophoblast (15).

F) THE AROMATIC AMINO ACID TRANSPORTER. The aromatic amino acid transporter (coded by *SLC16A10*), which belongs to the monocarboxylate transporter family, has not been studied in human placenta despite a report of expression at the mRNA level (290).

2. Rodents

The techniques developed for isolating membrane vesicles from human placenta have to a limited extent been applied to the rat and mouse. Thus System A can be demonstrated in membranes isolated from syncytiotrophoblast layer TII (122, 216). Functional studies show that the activity of this system is upregulated in mouse models of intrauterine growth restriction (62, 64), although not in a rat model (123).

Several transporter genes have been studied in rat placenta: *1*) sodium-coupled neutral amino acid transporters [*Slc38a1*, -2, and -4 (183, 280)], 2) high-affinity glutamate and neutral amino acid transporters [*Slc1a1* and -6 (245, 254)], 3) a cationic amino acid transporter [*Slc7a1* (244, 245)], and 4) glycoprotein-associated amino acid transporters [*Slc3a1* and -2 (95, 281)]. Increasingly this work is being extended to mouse placenta, i.e., *Slc38a1*, -2, and -3 (61); *Slc1a1*, -2, -3, and -6 (253); and *Slc7a1* (253). In general, localization has been by immunohistochemistry with nowhere near the level of resolution achieved for glucose transporters. An exception is one of the sodium-dependent transporters, which has been shown to be expressed at the protein level in membranes isolated from layer TII of mouse placenta [*Slc6a6* (216)].

In the guinea pig there is a mismatch between functional and molecular studies on the placental transfer of amino acids. The work of Yudilevich on unidirectional flux of amino acids across the placenta (80, 369, 392) is considered

important because of its theoretical underpinning (31). This research relied on a technique for perfusion of the guinea pig placenta in situ that had been developed earlier (28, 313). It has not been followed up at the molecular level except for a single study that measured the expression (but not the localization) of the system A transporter *Slc38a2* (321). It would be useful further to explore these transporters at the molecular level in the guinea pig, a rodent with a hemomonochorial placenta that is in many respects a better model for human pregnancy than either rat or mouse (40).

3. Ruminants

The gravid ewe is an excellent model for integrative studies of fetal and placental physiology. It has been used to great effect to elucidate the transfer of diverse amino acids including calculation of the bidirectional fluxes between mother, placenta, and fetus (21, 309). Unfortunately, this has yet to be followed up by studies at the molecular level of the expression and location of amino acid transporters.

VI. PLACENTA AS AN ENDOCRINE ORGAN

Placenta has a high rate of oxygen consumption that for much of gestation exceeds that of the fetus (24). The energy requirement is in large part due to the synthesis of structural proteins and peptide hormones (39). The rate of secretion of a single hormone from human placenta, human placental lactogen, is about 1 g/day towards term, which exceeds that of any other peptide hormone (197). The focus of this section is on the evolution of peptide hormones that influence growth and metabolism in mother and fetus.

There are several fascinating examples of convergent evolution. Arguably the most interesting involves the luteotrophic factors that prevent regression of the corpus luteum (CL); in different orders they have been evolved from prolactin, luteinizing hormone, and interferons.

Pregnancy maintenance is often dependent on the placenta. It may secrete peptide hormones to maintain the CL, as in muroid rodents, or be directly responsible for synthesis of progesterone and other gestagens, as in ruminants and primates. This section will focus on the evolution of genes coding for placental expression of peptide hormones.

A. Growth Hormone and Prolactin

Growth hormone and prolactin are pituitary hormones thought to share an ancestral gene. Growth hormone (GH) promotes postnatal growth and has a range of metabolic activities. Prolactin (PRL) has a wider range of effects that can be grouped into six categories (30, 107). One function in mammals is to promote growth of the mammary gland and milk secretion. In several orders of mammals there have been episodes of rapid evolution of one or both of these genes (103). This has often been accompanied or followed by gene duplication. The new genes are expressed in the placenta as placental growth hormones or placental lactogens (PL). In different orders PLs have arisen by duplication of the GH gene or the PRL gene **(FIGURE 6)**. The nomenclature used here will be conservative, although for the >30 prolactin genes and pseudogenes identified in mouse and rat genomes, a much improved annotation is available (332).

B. GH-like Hormones

1. Human and great apes

A gene cluster found on chromosome 17 codes for five GH-like proteins. Four of them are expressed in placenta (56), specifically in the syncytiotrophoblast clothing the placental villi (232, 320). The fifth gene, hGH-N, is expressed in the anterior pituitary (56), and its product is secreted in pulsatile fashion. Tissue-specific expression of the genes is dependent on a locus control region situated 14.5–32 kb 5' to the promotor region (192, 340). There is an interesting parallel here to the β-globin gene cluster discussed in a previous section. Of particular note is hGH-V, which is secreted from the placenta to the maternal circulation from around 15 wk rising to a plateau that is maintained from 20 wk until term (49, 105, 269). It supplants pituitary GH, which ceases to be secreted from ~21–25 wk (50). In contrast to the pulsatile secretion of pituitary GH, there is continuous secretion of the placental hormone (96), which may alter maternal metabolism during pregnancy. Because GH promotes gluconeogenesis, lipolysis, and anabolism, it has been suggested that the placental variant increases nutrient availability to the fetus and placenta (219). In addition, placental GH is a key regulator of insulin-like growth factor I (IGF-I) (49, 50, 269). The protein is not secreted to the fetal circulation and therefore cannot control fetal growth directly (105).

Two GH-like genes (hCS-A and hCS-B) code for hPL, previously known as chorionic somatomammotropin. A fourth gene (hCS-L), which is expressed in placenta at much lower levels, differs significantly in encoded sequence (56). hPL is first found in maternal plasma at ~6 wk and rises linearly to reach a plateau by ~30 wk of gestation (196). hPL plays a poorly defined role in mammary gland development (146, 196). Its main action, on maternal metabolism, is designed to ensure glucose and amino acid availability to the fetus (146). In contrast to hGH-V, some hPL enters the fetal circulation and can bind to receptors in fetal liver (169).

The GH locus of the chimpanzee (*Pan troglodytes*) includes genes for GH-N, GH-V, and three PL-like genes (293). One PL-like gene, predicted to be functional, is orthologous to a
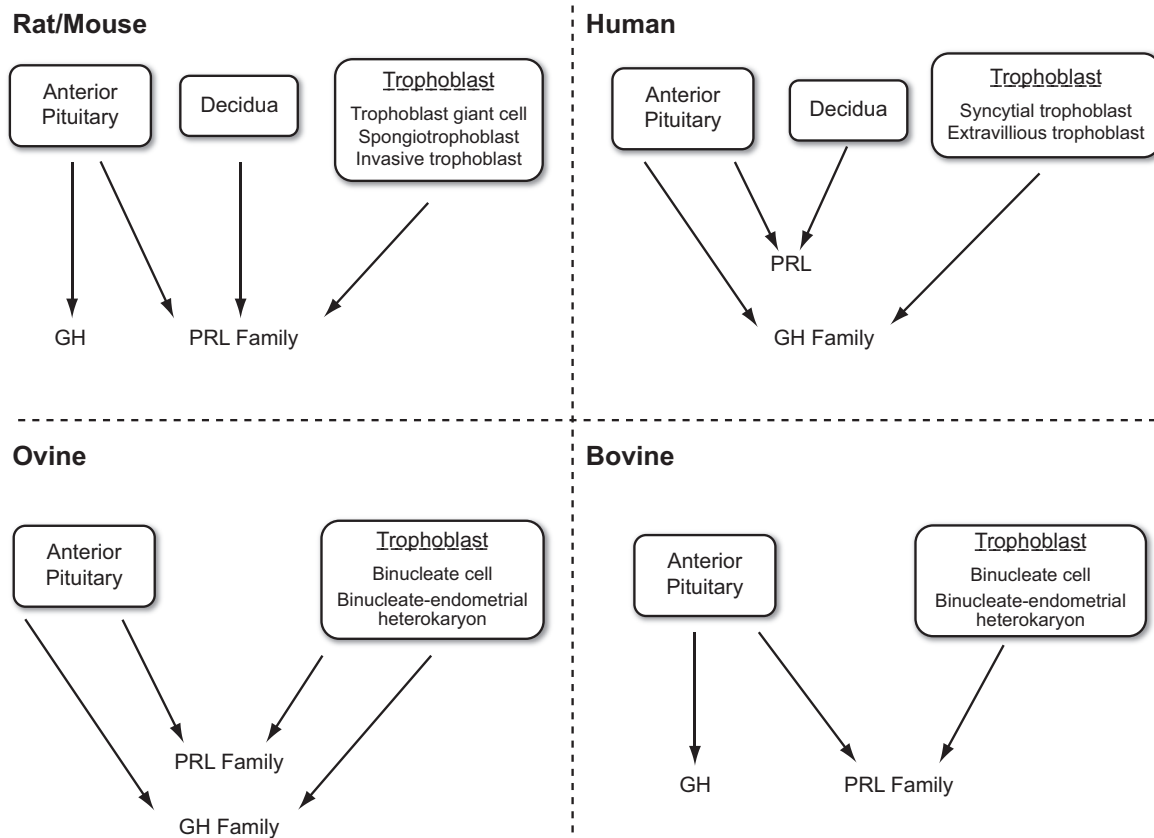
FIGURE 6. In different orders placental lactogens have arisen by duplication of the growth hormone (GH) gene or the prolactin (PRL) gene normally expressed in the anterior pituitary. Thus placental lactogens were derived from prolactin in rodents and from growth hormone in primates. There is some variation among ruminants with bovine trophoblast expressing a number of prolactin-like genes and sheep trophoblast expressing in addition a duplicate growth hormone gene. [From Soares (331). Copyright 2004, licensee BioMed Central Ltd.]

human pseudogene (293, 312). The genome of lowland gorilla (*Gorilla gorilla*) has two GH and four PL-like genes, but the duplication within the PL-like cluster is different from that in the chimpanzee (318).

2. Other primates

Prosimians such as the slow loris (*Nycticebus pygmaeus*) and bush baby (*Otolemur crassicaudatus*) have a single gene coding for pituitary GH (2, 363). In this they resemble mammals from most nonprimate orders (357).

In contrast, as many as 40 GH-like genes and pseudogenes have been identified in a genomic library from the white-fronted capuchin (*Cebus albifrons*) (362) and 14 sequences of GH-like genes obtained from three other New World monkeys (127, 230). The gene duplication from which this cluster originated was independent of that occurring on the lineage of Old World monkeys and apes (361). A cluster of eight genes and pseudogenes has been sequenced in the marmoset (*Callithrix jacchus*) (361, 362), and six in Geoffroy's spider monkey (*Ateles geoffroyi*) (312), but the organization of the gene locus has not been completely determined for other New

World monkeys (127). At least three GH-like genes are expressed in the placenta of the black-headed spider monkey (*A. fusciceps*), and they have been named *GHB*, *GHC,* and *GHD* (288).

The rhesus monkey (*Macaca mulatta*) has a cluster of six GH-like genes (127). Four of them have been sequenced from cDNAs, confirming they are expressed in the placenta (126). The status of the other two is unresolved, but they do seem to be functional genes coding for PLs (126).

The last common ancestor of catarrhine and platyrrhine primates likely had a single GH gene. It has been suggested, however, that it already had acquired placental expression (288). If so, placental expression was maintained in both lineages with separation of function occurring after two independent series of gene duplications in the catarrhine and platyrrhine lineages, respectively (288). This would be in line with a model proposed by Forsyth and Wallis (103) to explain the rapid evolution of GH-like and PRL-like genes by function switching until the functions were separated by gene duplication **(FIGURE 7A)**.
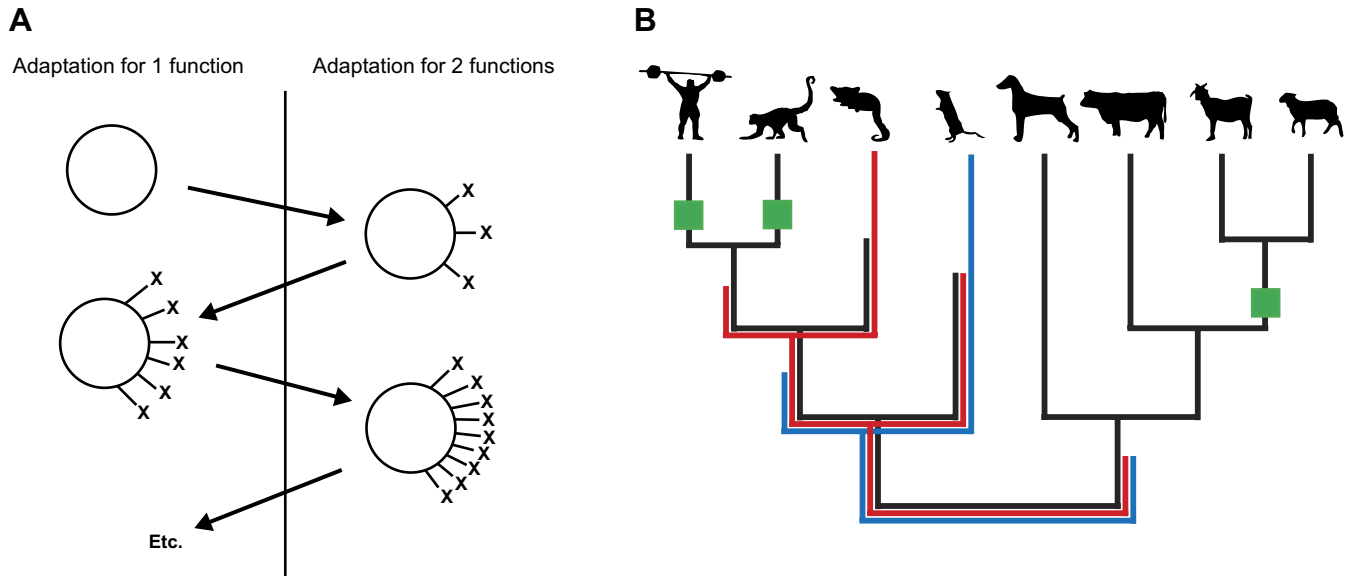
FIGURE 7. Evolution of GH-like and PRL-like genes. *A*: rapid evolution of the *GH* and *PRL* genes can be explained by structural adaptation to serve a second function. If the second function ceases to be important for a while, there will be readaptation better to serve the original function. Repeated cycles of switching between functions may continue until gene duplication allows a second protein to take over the new function. [From Forsyth and Wallis (103), with permission from Springer Science + Business Media.] *B*: putative *GH* gene tree for representative species of Boroeutheria; from left, human, spider monkey, galago (strepsirrhine primate), rat (rodent), dog (carnivore), goat, and sheep (ruminants). The model assumes three GH genes (depicted in black, red, and blue) were present in the last common ancestor of Boroeutheria. Truncated lines represent gene loss. Additional gene duplications and losses occurred in some lineages but are not shown. The green boxes indicate placental expression of GH-like genes. [From Papper et al. (288). Copyright 2009 National Academy of Sciences.]

3. Ruminants

An analysis of GH genes in Cetartiodactyla indicates an episode of rapid evolution that is restricted to the ruminant lineage (247). Thus, although two nucleotide sequences are found in the giraffe (*Giraffa camelopardalis*) and hippopotamus (*Hippopotamus amphibius*), these do not translate into different protein sequences (247). A similar situation exists in the most basal ruminant, the chevrotain (*Tragulus javanicus*), although with a single amino acid difference between the protein sequences (360). It is unclear whether these findings reflect gene duplication or the existence of two alleles of a single gene.

A single GH gene is found in cattle, and it is not expressed in the placenta (128). Duplication of the GH gene did, however, occur in sheep and goat (359). An intriguing feature of GH gene duplication in these ruminants is that the duplicate genes exist in an allelic polymorphism with the single form (359). GH secretion by the placenta of sheep involves expression both of the original GH gene and the new one. At the tip of the fetal villi, GH-like protein is located to syncytiotrophoblast, whereas at the base of the villi it is expressed in mono- and binucleated trophoblast cells (218). Placentally expressed ovine GH (oGH) is involved in secretion of "uterine milk" from the endometrial glands (279). Moreover, in sheep, in contrast to primates, GH is secreted from the placenta to the fetal circulation (217).

4. Evolution of GH-like genes in Boroeutheria

Papper et al. (288) have constructed a putative GH gene tree for Boroeutheria, a higher level clade that includes primates, rodents, carnivores, and ruminants (FIGURE 7*B*). They postulate that the last common ancestor of Boroeutheria (97% of extant placental mammals) had three GH genes but that gene losses occurred in each of the descendant lineages. Subsequent gene duplications were associated with the expression of GH-like genes in anthropoid primates, sheep, and goats (green boxes in FIGURE 7*B*).

C. PRL-like Genes

1. Human and other primates

Humans and other higher primates have a single PRL gene that is expressed primarily by the anterior pituitary. The same is true of most orders of mammals with the notable exception of rodents and cetartiodactyls (103). However, although PRL is not expressed in the placenta, it is strongly induced in the decidualized endometrium during early pregnancy (60, 251). In primates, including human and black-headed spider monkey (*Ateles fusciceps*), endometrial transcription of PRL is initiated from the long transposable element MER39, which is unique to the mammalian superorder Euarchontoglires (85).

2. Rodents

Multiple PRL-like genes cluster with the PRL gene on chromosome 13 in mouse (371) and 17 in rat (286). The two major hormones in muroid rodents are PL-I and PL-II. Thus, in mouse and rat, trophoblast giant cells produce PL-I (*Prl3d1*) in mid-gestation and PL-II (*Prl3b1*) during the latter half of gestation. In rat, a variant form of PL-1 (*Prl3d4*) is synthesized by spongiotrophoblast cells in the latter part of pregnancy (70). Promoter regions well upstream of the transcriptional start site direct placenta-specific expression of PL-II in mouse (233, 325) and rat (286, 322). In addition, mouse and rat placenta express other PRL-like proteins. In mouse they include proliferin (*Prl2c2*) produced in giant cells and proliferin-related protein (*Prl7d1*) from cytotrophoblasts in the junctional zone. A subset of PRL-like proteins is expressed by the trophoblast that invades maternal arteries (4, 370).

In muroid rodents such as mouse, rat, and hamster (334), PRL has acquired a luteotrophic function. Newly formed corpora lutea of rat secrete progesterone for only 2 days unless rescued by PRL secretion from the pituitary. The critical event initiating twice daily surges of PRL is stimulation of the uterine cervix during coitus (135). As pregnancy advances, PRL is supplemented by PLs (113). PRL, PL-I, and PL-II silence expression of 20α-hydroxysteroid dehydrogenase (20α-HSD), which otherwise would catabolize progesterone (396). PL-I is a glycosylated protein peaking at midgestation, whereas PL-II is nonglycosylated and predominates in late pregnancy (113). By midpregnancy rising concentrations of PL-I have resulted in suppression of pituitary PRL by activating dopaminergic neurons in the arcuate nucleus (227). In addition to maintaining ovarian progesterone production, PLs are important to mammary gland development.

Proliferin and proliferin-related protein are positive and negative regulators of angiogenesis (226). Proliferin acts through the mannose-6-phosphate/insulin-like growth factor II receptor, attaching to its mannose-6-phosphate binding site to stimulate angiogenesis (226, 354). Although proliferin is a circulating hormone, it may also act locally to stimulate neovascularization of the placenta. The timing of proliferin expression coincides with the development of the placental labyrinth (182). A mid-gestation switch results in decreasing expression of proliferin and activation of proliferin-related protein, thus restricting further vascularization (182).

Several PRL-like proteins (PLP-A, -B, and -C) are expressed in the trophoblasts that invade the mesometrial triangle (also known as the metrial gland). The timing of the invasion is precise. It occurs in the last week of pregnancy and coincides with the disappearance from the mesometrial triangle of uterine natural killer (uNK) cells (5). PLP-A binds to uNK cells (276), and this trophoblast-uNK signaling

pathway apparently results in suppression of interferon-γ secretion (5).

3. Ruminants

At least eight PRL-like genes are expressed in the placenta of cattle (220). They are found as a cluster on chromosome 23 (78). One of the translated proteins is heavily glycosylated and has been well characterized as bovine PL. An equivalent but nonglycosylated placental hormone is found in sheep as ovine PL (oPL) (63) as well as in the goat (317). The remaining PRL-like genes are conventionally referred to as prolactin-related proteins (PRPs). In cattle only one PRP is known to be translated.

There is considerable sequence divergence between ovine and bovine PLs (356). Ruminant PLs and PRPs have been said to affect CL function, uterine gland development, intermediary metabolism, and mammary gland development. These postulates are not always backed by empirical evidence (331). An important function of oPL is to stimulate secretion by the endometrial glands of "uterine milk" that is taken up by the trophoblast. A careful set of studies has established that secretion of histotrophe requires sequential action on the glandular epithelium of steroids, interferon-τ (IFN-τ) (134), oPL and placentally expressed oGH (279). IFN-τ is secreted by the blastocyst prior to attachment. Implantation and placentation is not completed until 50–60 days of gestation. In the meantime, intercaruncular endometrial glands undergo hyperplasia that is largely dependent on secretion of oPL (279). The hormone is secreted by binucleated trophoblast cells (198, 380, 384). Subsequently oGH of trophoblastic origin causes glandular hypertrophy and increased production of histotrophe (279).

Ruminant PLs are secreted into both fetal and maternal blood (124). The mean concentration in maternal blood rose between 80–100 and 121–130 days, whereas the concentration in fetal blood fell in the same interval (124). There is evidence that oPL regulates growth of the fetus, presumably by supporting its nutrient supply (13).

The concentration of oPL in maternal plasma starts to decline 10–15 days before birth and at delivery is ~50% of that measured at mid-gestation (346). Thorburn (348) has suggested a role for oPL in pregnancy maintenance involving inhibition of prostaglandin synthesis. Thus the decline in oPL towards term could contribute to the increased production of prostaglandins leading up to parturition (348).

Of the other PRL-like genes, bPRP-I is expressed as RNA and protein in binucleated cells (268) and found at high concentration in the uterine fluid (202). The bPRPs are regarded as orphan ligands since they lack most of the residues identified as determinants for binding to PRL and GH receptors (125) (see below).

4. Elephant

The prolactin gene of the African elephant (*Loxodonta africana*) has undergone rapid evolution, although without gene duplication (358). Therefore, it is of interest that strong expression of prolactin by elephant trophoblast has been postulated based on immunostaining with an antibody raised against human PRL (390). Gestation in elephants is maintained by large accessory corpora lutea. The source of the luteotrophic factor seems likely to be the trophoblast (390). Expression of PRL in the pregnant uterus was found in an independent study, but here the source was said to be the endometrium (85), where immunostaining for PRL is weak or absent (390).

D. GH and PRL Receptors

The GH and PRL receptors (GHR and PRLR) are members of the cytokine receptor superfamily. Receptor dimerization is an absolute requirement for initiating signal transduction by either type of receptor (125). Each hormone has two receptor binding sites. The hormone initially binds to a receptor via site 1. It can then bind through site 2 to a second receptor to form the active 1:2 complex (125).

The receptor genes have undergone accelerated evolution in lineages where there has been duplication of genes for their ligands (229). There has not, however, been duplication of the receptors themselves. In the human, hPL has evolved fidelity for PRLR and hGH-V for GHR (138). Because levels of hPRL and hPL both increase in late gestation, Haig (138) mooted the intriguing possibility that hPL acts as an antagonist at the PRLR. This has yet to be tested.

Ruminant PLs can bind to both types of receptor. They are agonists at PRLR but antagonists at GHR since they bind to site 1 but cannot achieve homodimerization (120, 165). Ovine PL is, however, able to form heterodimers by binding to GHR through site 1 and PRLR through site 2 (26, 164). There is evidence from transfection experiments that the heterodimer can activate signaling pathways enabling ovine PL to mimic some of the effects of ovine GH (26).

E. Insulin-like Growth Factors

Insulin-like growth factors (IGFs) act in an endocrine or paracrine fashion to promote cell proliferation, differentiation, and migration. Placental growth in mice is lessened in the absence of *Igf2* gene, but not in the absence of *Igf1* (16, 71, 237). The importance of IGF-II in placental growth and development is further underlined by experiments involving placenta-specific knockout of *Igf2* in mice (64, 215). Other than in general terms, there is little to be said about evolution of the insulin-like growth factors in the placenta. However, some of the earliest studies that used null mutations

and transgene expression to explore fetal and placental development involved this system (81). Additionally, the first demonstration of parental imprinting concerned the genes for *Igf2* and its receptor *M6p/Igf-2r* (18, 72).

The two peptides, IGF-I and IGF-II, have at least two receptors, and there are six high-affinity binding proteins (IGFBPs). The expression of the IGF system in placenta of human (144), mouse (47, 81), and other mammals (145) has been reviewed extensively as has their putative role in control of fetal growth (104).

The effects of IGF-I, and many actions of IGF-II, are mediated through the IGF-1 receptor (IGF-1R) which has a similar structure to the insulin receptor. In mice, null mutation of the *Igf1r* gene slows prenatal growth (237). The IGF-2 receptor (M6P/IGF-2R) binds IGF-II with high affinity, and its principal function is clearance of IGF-II. Consequently, null mutation of the *M6p/Igf-2r* gene results in large birth weights (222). Additional receptors may be involved in the growth-promoting effects of IGF-II (16), including the insulin receptor (240).

1. The kinship theory of genomic imprinting

Genomic imprinting is an epigenetic form of gene regulation resulting in silencing of one of the parental alleles. The phenotype of the conceptus is determined by the paternal allele of *Igf2* (72, 101) and the maternal allele of *Igf2r* (18). This reciprocal imprinting led to formulation of a genetic conflict model (139) predicting that expression of the paternal genome would tend to increase offspring size (*Igf2* promotes fetal growth), whereas expression of the maternal genome would tend to restrict it (*M6p/Igf-2r* restricts fetal growth by sequestering the growth factor).

In more general terms, the kinship theory of genomic imprinting (374) builds on the assumption that allocation of resources to the developing fetus will increase the fitness of the offspring but decrease the mother's fitness for future reproduction (139). Although commonly known as the conflict theory, it was early pointed out (273) that paternal and maternal genomes must cooperate to produce viable offspring. Therefore, it is likely that the mother retains overall control of resource allocation with parental imprinting "tinkering at the margins" of this control (374). The reciprocal imprinting of *Igf2* and *Igf2r* remains the most convincing example in support of the kinship theory. The theory may also go some way towards explaining the rapid evolution of placental lactogens (138, 288). The theoretical framework of kinship theory has been carefully developed by its proponents (374), yet it is invoked indiscriminately by others to explain all manner of variation in placental structure and function. Kinship theory arose initially from a consideration of reproduction in seed plants (140). Therefore, it is sobering to note that imprinted *maternally ex-*

pressed gene 1 (*Meg1*) in maize acts to promote rather than restrict allocation of nutrients to the offspring (65).

Interestingly, while imprinting of *IGF2* is ubiquitous among mammals, imprinting of *M6P/IGF-2R* is not. The protein first acquired an IGF binding site in marsupials and is already imprinted in the opossum (*Didelphis virginiana*) (205). *M6P/IGF-2R* is imprinted in Cetartiodactyla (sheep, cattle, pigs) as well as in rodents (206). However, imprinting appears to have been lost in Euarchonta, the clade that includes primates. Biallelic expression of *M6P/IGF-2R* has been shown in the Phillipine colugo (*Cynocephalus volans*), common tree shrew (*Tupaia glis*), the ring-tailed lemur (*Lemur catta*), and in a large sample of human tissues (206). This suggests lineage-specific loss of *M6P/IGF-2R* imprinting in Euarchonta with retention of imprinting in the sister group Glires, comprising rodents and lagomorphs (206). These comparative data are interesting in relation to the kinship theory as it pertains to the placenta, since *M6P/IGF-2R* is imprinted in marsupials, which develop mainly in the pouch, but not in higher primates, which have a highly invasive form of placentation.

F. Gonadotropins

Luteinizing hormone (LH) belongs to a family of glycoprotein hormones expressed in the anterior pituitary. They have a common α-subunit and a hormone-specific β-subunit. In some mammals, LH is expressed in the placenta and is then referred to as chorionic gonadotropin (CG).

1. Human

Duplication of the LH gene has given rise to six copies of the β-subunit gene for CG found on chromosome 19 along with a single copy of the LH β-subunit gene (301, 344). Only two of the copies are known to be expressed (343).

Secretion of hCG is initially from cytotrophoblast and later by syncytiotrophoblast (250). The plasma concentration rises rapidly in the 4 wk following implantation and peaks at 8–10 wk of pregnancy (54). Binding of hCG to LH receptors on the CL prevents its regression and ensures continued progesterone production for the maintenance of pregnancy (148).

2. Other primates

The evolution of the LH β-subunit gene is an excellent example of how genes with new functions arise by duplication of existing genes (252). A single gene is expressed in the pituitary of strepsirhine primates and tarsiers (252). The initial duplication event occurred after divergence of anthropoid primates from tarsiers. At about the same time there was a frame shift that resulted in the incorporation of a 3′-untranslated region (344). The COOH-terminal pep-

tide conveys a longer half-life on primate CG that enables it to stimulate progesterone secretion by the CL. In addition, the COOH-terminal peptide contains *O*-glycosylation sites that ensure apical targeting of the protein rather than secretion from the basolateral side as in the pituitary (181, 252). This is important because the gene is expressed in the maternal-facing syncytiotrophoblast of the villi such that apically secreted products are delivered directly to the intervillous space.

The CG β-subunit gene has continued to evolve in the primate lineage. New World monkeys have several copies of the gene, although only one is functional (252). In Old World monkeys, there are four copies in the rhesus macaque but six in the dusky leaf monkey (*Trachypithecus obscurus*) (252). Orangutan (252) and chimpanzee (141) each has five gene copies compared with six in the human genome.

3. Equids

Placental secretion of CG has arisen by convergent evolution in equids (members of Perissodactyla). In this instance, a single gene codes for the β-subunit of pituitary LH and CG (58, 324). Interestingly, in equids, the β-subunit gene acquired a COOH-terminal extension, which might be important for placental expression (324). The α-subunit also acquired characteristics that enabled it to be expressed in placenta (100).

Equine CG (eCG; once known as pregnant mare serum gonadotropin) is secreted by trophoblasts located in the endometrial cups. Equids have epitheliochorial placentation, a type that normally is noninvasive. Early in pregnancy, however, a subset of trophoblast cells migrates from a thickening in the chorion called the allantochorionic girdle, penetrates the maternal epithelium, and migrates deep into the endometrium to form the endometrial cups (8, 9, 92). It is here that the equine chorionic gonadotropin is made, as can be demonstrated by immunostaining and by immunogold staining for electron microscopy (90, 382). The life of the endometrial cup cells is limited due to an intense maternal immunological response. They begin to degenerate after day 70, and serum levels of eCG peak between 50 and 75 days of gestation (6).

Secondary CL develop in the ovaries under the influence of eCG and pituitary FSH. They maintain pregnancy until around 100 days of gestation, when the placenta assumes this role (reviewed in Ref. 7).

4. Guinea pig

Muroid rodents have a single LH β-subunit gene expressed in the pituitary. However, in the guinea pig, a hystricognath rodent, several lines of evidence point to secretion of a CG-

like protein from the placenta peaking in the third week of gestation (177). A protein has been isolated with suitable physicochemical, biological, and immunological properties (17) and localized by immunohistochemistry to the syncytiotrophoblast lining the labyrinth (17). As in equids, there has been no duplication of the β-subunit gene (323), but unlike in equids and primates, there is no COOH-terminal peptide domain. Recently β-subunit RNA was isolated from placental RNA, confirming that the gene is transcriptionally active in the placenta, although the product could not be demonstrated by Northern blotting (323).

If the peptide is involved in maintenance of the CL, it is interesting that secretion falls after the fourth week coincident with a decline in ovarian progesterone and rise in placental progesterone secretion (161). There is an interesting parallel to human pregnancy in that the ovaries are not required in the latter part of gestation (160) when pregnancy probably is maintained by placental steroids (393).

G. Pregnancy-Specific Glycoproteins

Human placenta secretes a large amount of pregnancy-specific β_1-glycoprotein, and in mice and monkeys, the corresponding protein is essential for the successful completion of pregnancy (29, 155). Pregnancy-specific glycoproteins (PSG) comprise one subfamily of the carcinoembryonic antigen (CEA) family, the other being the CEA cell adhesion molecule (CEACAM) subfamily (195). There are 23 CEA genes and pseudogenes in the human genome clustered on chromosome 19 (19, 195, 339). In mice, 31 genes are found in a cluster on chromosome 7 (195, 394). Human PSGs are composed of an immunoglobulin variable-like domain and a variable number of constant-like domains.

A recent phylogenetic analysis of CEA genes, comprising all major clades of mammal, concluded that PSG genes had evolved in only a few orders and were closely associated with invasive placentation (195). There were, for example, no PSG genes in dogs or cattle. Expansion of PSG genes likely occurred independently in primates and rodents (260, 394), since they are found neither in lower primates (bush baby and gray mouse lemur) nor in lagomorphs (pika and rabbit), the sister group to rodents (195).

1. Human and non-human primates

PSG is detectable in maternal blood in the third week of gestation (132). It reaches a concentration of 200–400 μg/ml at term (234) and is then the most abundant placental protein in maternal blood. PSG is selectively secreted to the maternal circulation (131). There are 10 human *PSG* genes (195), but they share greater than 90% nucleotide and 80% amino acid identity (261). The protein has been localized to the syncytiotrophoblast of human placenta (55, 345). The biological role of PSGs has not been resolved (261). The

view that they may act to modulate maternal immune responses is supported by the ability of recombinant human PSGs to induce secretion by monocytes of several anti-inflammatory cytokines (137, 330).

Fifteen PSG-related genes are found in the baboon, and one of these is highly expressed in term placenta (397). In addition, PSG-related genes occur in the genomes of great apes (chimpanzee and orangutan) as well as in rhesus macaque and African green monkey or grivet (*Chlorocebus aethiops*) (195, 398).

2. Rodents

The *Psg* gene locus of the mouse comprises 17 members (259). Transcripts of eight of these genes can be detected in mouse placenta with low or absent expression in other tissues (394). Gene expression occurs in primary and secondary giant cells and the spongiotrophoblast layer of the mouse placenta (55, 387). A similar expression pattern has been described in rat placenta (308). In addition to *Psg* genes, another member of the CEA family (*Cea5*) is expressed in trophoblast giant cells during early placental development (102).

Evidence that murine PSGs interact with the maternal immune system includes upregulation of interleukin-10 in macrophages by PSG-18 (368) and binding of PSG-17 to the CD-7 receptor of macrophages (366), inducing secretion of several cytokines (137).

3. Equids

At least five equine CEACAM-related genes appear to encode secreted proteins (195). Moreover, two corresponding EST sequences have been identified in horse trophoblast cDNA libraries, suggesting these are true PSG genes (195). The horse has an epitheliochorial placenta, but as earlier explained, a subset of invasive trophoblast cells forms endometrial cups. Kammerer and Zimmerman (195) speculate that PSG secreted by these cells could cause local suppression of the maternal immune response.

H. Interferons

The luteotrophic factor in ruminants is IFN-τ secreted by the trophectoderm (162). Secretion begins at the blastocyst stage and peaks during conceptus elongation (134). The *IFNT* gene arose through duplication of the *IFNW* (IFN-ω) gene in the lineage of pecoran ruminants, and further duplications gave rise to multiple genes and pseudogenes (315); *IFNT* is not found in closely related species such as pig, hippopotamus, or llama (224). In the process of its evolution, the promotor region of *IFNT* lost its viral control elements and acquired two regions responsible for trophectoderm-specific expression (97, 147). One of these contains a binding sequence for the transcription factor Ets-2 which

plays a key role in the transient expression of *IFNT* (97). Receptors for IFN-τ are located in the endometrium (143), where it acts to suppress secretion of the luteolytic factor PGF$_{2\alpha}$ (162). Since attachment of the trophectoderm to uterine epithelium silences IFN-τ gene expression (134), it is not strictly a placental hormone.

FIGURE 8 summarizes the various forms of luteotrophic factor found in mammals and highlights those, such as IFNT, that have evolved following gene duplication events.

I. Pregnancy-Associated Glycoproteins

Pregnancy-associated glycoproteins (PAGs) are the products of a family of genes found in artiodactyls, where they have undergone rapid evolution (176). Most mammals have but a single *PAG*-like gene (341), but the cow, for example, has 18 *PAG* genes and 14 pseudogenes (347). PAGs belong to a wider family of aspartic peptidases and have undergone two rounds of duplication. The products of the first round, called "ancient PAGs," retain the active site. In pig and cow, they are expressed at the microvillous junction between the uterine epithelium and trophoblast (386). Here they may function as linking molecules (386) and play a role in fetal-maternal anchorage (228). The second round of duplication was restricted to the ruminant lineage, and many of the resultant gene products lack the active site of the enzyme. They are expressed predominantly on the surface of binucleate cells (386). Gene duplication is often associated with the assumption of new functions, and it has been speculated that the PAGs expressed on binucleate cells
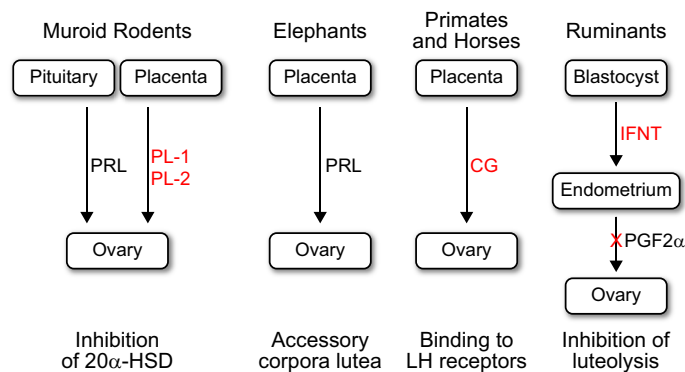

FIGURE 8. Molecular evolution of luteotrophic factors. Signals arising by gene duplication are shown in red. In muroid rodents (e.g., mouse, rat), pituitary prolactin (PRL) released in response to coitus acts by inhibiting 20α-hydroxysteroid dehydrogenase (20α-HSD); subsequently, this function is assumed by placental lactogens (PL-1 and PL-2). In elephants, placental expression of the *PRL* gene is responsible for pregnancy maintenance by accessory corpora lutea. In primates and horses, chorionic gonadotropins (CG) derived from duplication of the luteinizing hormone (LH) gene are expressed by trophoblast and maintain corpus luteum function in the first months of gestation. In ruminants, interferon-τ (IFNT) derived from duplication of the *IFNW* gene is secreted by the blastocyst and acts on the endometrium to inhibit the luteolytic signal prostaglandin F$_{2\alpha}$ (PGF2α).

engage in immunological camouflage and facilitate maternal tolerance of this invasive type of trophoblast (386).

VII. PLACENTAL IMMUNOLOGY

A. Expression of Major Histocompatibility Molecules by Trophoblast

Much has been made of the fetus as an allograft (262), and this has led to study of the mechanisms that convey immune tolerance of the trophoblast. They may well differ with the type of placentation (271). This section will be limited to primates and deal with recent advances concerning the major histocompatibility (MHC) class I antigens and their receptors, many of which belong to the killer immunoglobulin-like receptor (KIR) family. Human MHC molecules are referred to as human leukocyte antigens (HLA). Both HLA and KIR exhibit an extraordinary degree of allelic polymorphism. The interaction between these two families is of interest due to a putative connection to differences within catarrhine primates in the depth and extent of trophoblast invasion (43).

Extravillous trophoblasts express HLA-C, -E, and -G at the cell surface, but not HLA-A and HLA-B, which are highly polymorphic and convey resistance to infection (167). HLA-E is an ancient gene and is not polymorphic; nor is HLA-G, a novel form found in primates and expressed only on the surface of extravillous trophoblast (178). HLA-C is the only one of these antigens that is both polymorphic and expressed by invading trophoblast cells (167). There are two main epitopes of HLA-C, C1 and C2, that act as the principal ligands for NK cells through the KIR.

B. Evolution of MHC-C Ligands and Their Receptors in Primates

The leukocyte population of the uterus is unusual in that ~70% are uterine natural killer (uNK) cells (221). These differ phenotypically from the natural killer cells found in the circulation. They play a role in the remodeling of the uterine spiral arteries as well as in regulating the depth of trophoblast invasion (154). These cells express KIRs of which there are inhibitory and excitatory variants regulated by a cassette of genes on chromosome 19 (289). Importantly, there are two variants: haplotype A codes mainly inhibitory, whereas haplotype B codes both inhibitory and additional excitatory KIRs (**FIGURE 9A**) (270). This appears to be of importance in relation to reproductive success. There is increased risk of recurrent abortion, intrauterine growth restriction, or preeclampsia when the mother is homozygous for haplotype A and carries a fetus where the trophoblast expresses HLA-C2 (**FIGURE 9B**) (166, 167).

A high rate of reproductive failure in humans, as well as the occurrence of preeclampsia, is often seen as the price paid for
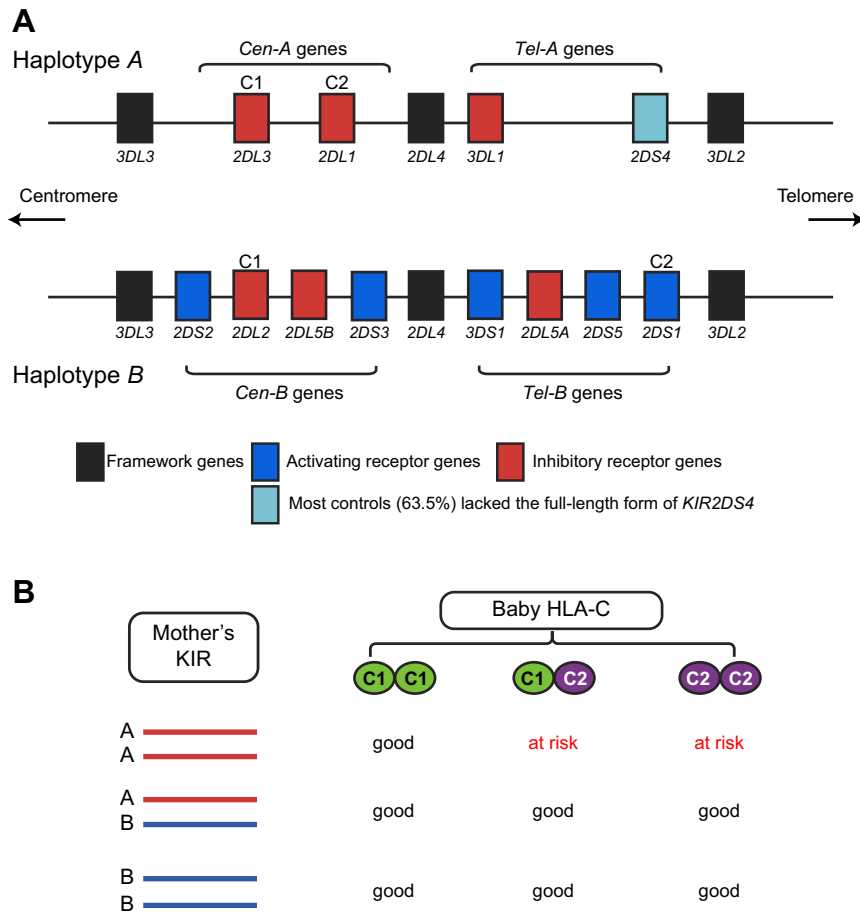
FIGURE 9. HLA-C and its cognate receptors. *A*: receptors for HLA-C occur as two KIR haplotypes as in this representative example. Haplotype A has inhibitory receptors for HLA-C1 (*KIR2DL3*) and HLA-C2 (*KIR2DL1*). Haplotype B has the inhibitory receptor for HLA-C1 but an activating receptor for HLA-C2 (*KIR2DS1*). [From Hiby et al. (166), with permission from American Society for Clinical Investigation.] *B*: pregnancy outcome is affected when a mother with haplotype A carries a baby with one or two HLA-C2 alleles. [From Moffett and Hiby (270), with permission from Elsevier.]

deeper trophoblast invasion (for critical reviews, see Refs. 249, 300). Old World monkeys and humans differ in respect of trophoblast invasion of the uterus and its blood vessels. In both humans and monkeys, trophoblast invades by the endovascular route travelling down the arteries against the direction of blood flow (91). In humans, however, extravillous trophoblast also invades directly from the basal plate into the decidualized endometrium (FIGURE 1D); this does not happen in monkeys (48). A more important difference may be the depth reached by the trophoblast and the depth to which the consequent widening of the spiral arteries extends. In Old World monkeys it is only the arterial segments within the endometrium that are affected. In humans, trophoblast invasion and spiral artery remodeling extend beyond the relatively shallow endometrium and into the inner third of the myometrium (296). This enables a higher rate of blood flow and an increase in oxygen supply to the fetus that is seen as critical to the development of a large brain (249).

Recently we were able to show that the gibbons or lower apes resemble Old World monkeys (299), whereas the routes and depth of trophoblast invasion in chimpanzee (297) and gorilla (298) resemble those of humans. There is an interesting parallel in the evolution of MHC-C, which is absent in monkeys and gibbons, but arises as an invariant form in orangutans. There it bears the C1 epitope that also occurs in some MHC-B haplotypes. The C2 epitope first appears in gorilla, bonobo, chimpanzee, and human (283) (TABLE 5). The concept has emerged that the connection is a causal one, with MHC-C evolving to allow deeper trophoblast invasion in great apes and parallel evolution of KIRs designed perhaps to control the invasion by more aggressive extravillous trophoblast. This would be consonant with the earlier hypothesis linking trophoblast invasion in humans to preeclampsia and intrauterine growth restriction, since both are characterized by much shallower trophoblast invasion than in a healthy pregnancy. The revised hypothesis would be that when trophoblasts expressing HLA-C2 encounter uNK cells expressing only inhibitory KIRs (genotype AA), the result is shallower invasion and reduced transformation of the spiral arteries affecting especially the myometrial segments (43).

Table 5. *Route and depth of trophoblast invasion in primates in comparison to the evolution of the MHC class I antigen HLA-C*

Primate Species	Route and Depth of Trophoblast Invasion	Presence or Absence of MHC-C
Rhesus macaque (*Macaca mulatta*)	Endovascular; restricted to spiral arteries of decidua	Absent
Gibbons (data from several species)	Endovascular; restricted to spiral arteries of decidua	Absent
Sumatran orangutan (*Pongo abelii*)	Not known	MHC-C (single locus)
Western gorilla (*Gorilla gorilla*)	Interstitial and endovascular; reaches inner third of myometrium	MHC-C1 and MHC-C2
Bonobo (*Pan paniscus*)	Not known	MHC-C1 and MHC-C2
Common chimpanzee (*Pan troglodytes*)	Interstitial and endovascular; reaches inner third of myometrium	MHC-C1 and MHC-C2
Human (*Homo sapiens*)	Interstitial and endovascular; reaches inner third of myometrium	HLA-C1 and HLA-C2

This in turn would increase the risk of pregnancy failure (166).

If this is the case, should not chimpanzees and gorillas experience similar patterns of reproductive failure? Although there are case reports of eclampsia in great apes, the evidence suggests this is a rare event (43). One explanation might be that the KIR genes have evolved along separate paths in great apes and humans (203) and that there is nothing in the chimpanzee corresponding to the A haplotype in humans. Lest it seem farfetched that chimpanzees have a more balanced system than humans, it should be recalled that the concomitant function of MHC class I antigens and KIRs is to convey resistance to infection. On the basis of human population studies, it has been shown that immune functions and reproductive functions exert opposite effects on the polymorphic HLA and KIR systems (117). Therefore, it is conceivable that selection pressure driven by disease resulted in loss of an activating receptor in the A haplotype, whereas in chimpanzees this imbalance did not occur.

Finally, it may be remarked that Neanderthals are thought to have given birth to large-brained babies and may have suffered similar reproductive challenges (302). There was gene flow between modern humans and archaic humans such as Neanderthals (129) and Denisovans (310). Some of the HLA allotypes thus acquired have been subject to positive selection in modern humans, particularly HLA-B which is concerned more with T-cell responses (1). It remains to be determined whether the genes passed from archaic to modern humans were beneficial in terms of reproduction.

The co-evolution of MHC-C and KIR2D in primates raises an interesting question. In theory, this could have happened in two steps. That would, however, have necessitated a step where MHC-C2 occurred without a receptor or a C2 receptor without a ligand. A more probable scenario is a four-step process (with three mutations) and maintenance of function at each step **(FIGURE 10)** (283).

It is appropriate to conclude this review with the HLA-KIR story, since it offers a glimpse of the selection pressures operating on one aspect of placental function. It alerts us to the possibility that evolution driven, for example, by epidemic infection may impact secondarily on placental function. In most other areas of placental physiology, we are faced with the end points of evolution but can only guess at the forces that shaped them.

VIII. PERSPECTIVES

Comparative genomics is yielding new insights into the evolution of physiological function. Whole genome duplication, though comparatively rare, did play a critical role in the evolution of vertebrates (350). It may have had a part in the differentiation of globins into molecules designed for oxygen transport rather than simple oxygen sequestration (173). Tandem duplications of genes or groups of genes is more common and in the mammalian lineage led to the further evolution of beta globins with the emergence of embryonic, fetal, and adult hemoglobins (285). The key element here is that a duplicated gene can acquire a new function that may be reflected in expression at a given time point or in a specific tissue such as trophoblast. An example of the latter is the evolution in primates of hCG, which is secreted by the trophoblast but signals by binding to the LH receptor.

A. Convergent Evolution

Molecular phylogenetics has given us a better understanding of the relation between mammals at the level of order (278) and family (263). One of the most striking findings is that eutherian mammals fall into four distinct clades **(TABLE 1)**. When this is taken into account, it is apparent that there has been much convergent evolution of placental structure and function. A feature of placental structure that has long figured in classifications of placentation is the interhemal barrier (130). Even if this complex character is reduced to three states
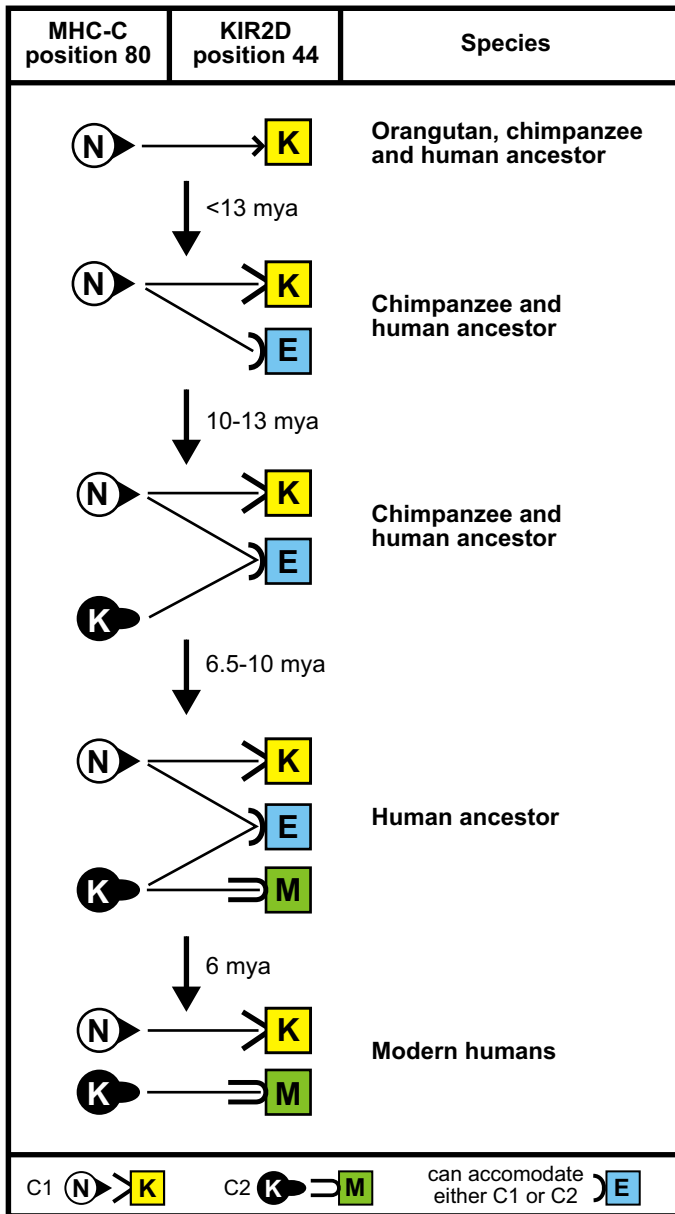
MHC-C position 80	KIR2D position 44	Species

FIGURE 10. Model proposed to explain how coevolution of MHC-C2 and KIR2D in primates could have occurred with maintenance of function at each stage. Circles depict MHC-C with N for C1 and K for C2. Squares depict KIR2D with K for lysine, E for glutamate, and M for methionine at position 44. The starting point is C1 and the C1 receptor as still found in gibbons. In step one, mutation of K to E gave a receptor capable of binding C1 or C2 as found in orangutans. MHC-C2 appeared in step two. A C2 specific receptor appeared in step three with mutation of K to M. In the final step, the more broadly reactive C1+C2 receptor was eliminated to achieve the situation found in modern humans. Estimates of the evolutionary time scale are given as million years ago (mya). [From Older Aguilar et al. (283). Copyright 2010, The American Association of Immunologists, Inc.]

(epitheliochorial, endotheliochorial, and hemochorial), it is difficult to be sure which represents the ancestral condition (84, 265). It can be concluded with some confidence that there have been multiple conversions between the two invasive types. Thus even this basic character shows signs of convergent evolution (249) **(TABLE 1)**.

Certainly there has been convergent evolution within many of the systems discussed here. The evolution of beta globins in primates and ruminants to yield high-affinity fetal hemoglobins is one example. Even more striking is the separate derivation in rodents, ruminants, and primates of GH-like and PRL-like placental hormones and the convergent evolution in equids and primates of chorionic gonadotropins.

B. Linking Molecules to Placental Function

Embryology was at the cutting edge of science in the late 19th and early 20th centuries. There was then a clear focus on comparative studies; close attention was paid to insectivores as they were regarded to be primitive mammals (46). In this era, developmental research relied heavily on studies of structure. In contrast to other organ systems, however, there was little follow through at the level of function. Development of the sheep model for study of the unstressed fetus was an important exception as it enabled measurements of placental blood flows, maternal-fetal gas exchange, and substrate transfer (21, 24).

Molecular biology offers a fresh opportunity to bridge the gap between structure and function. Already it has greatly increased our understanding of placental development (316). As this review has shown, molecular tools are valuable in examining gene expression and the localization of translated proteins at the cellular level and across the full time course of placental development. Occasionally this is sufficient to explain function, an example being expression of uteroferrin by the uterine glands of certain species to supply iron to the fetus. In many cases, however, the molecular information begs to be complemented by more classical physiological studies aimed to explain the function of expressed genes. As an example, many of the proteins secreted by trophoblast are discharged preferentially into the maternal bloodstream to act on maternal tissues, yet extraordinarily little is known about their effects on maternal physiology. They tend to be described in the vaguest of terms. When statements are underpinned by fact, they do not necessarily point to appropriate animal models. As has been shown, the placental lactogens of rodents, ruminants, and primates have evolved convergently by duplication of two separate genes and very likely do not have functional equivalence.

The placenta is first and foremost devoted to maternal-fetal exchange. One area where integrative physiology has made important advances is placental handling of nutrient transfer. Especially for the amino acids, there is substantial information available from work in guinea pigs and sheep (21, 170, 392). Some of this can be linked directly to the crucial question of how amino acids are supplied to the human fetus in normal pregnancies and in case of fetal growth restriction (51). Remarkably, there are virtually no complementary studies in sheep and guinea pig at the molecular level.

Instead, the tendency has been to miniaturize physiological techniques for application in pregnant mice (401). As an

example, it is now possible to measure System A activity as transfer of MeAIB across the mouse placenta in situ and apply the method in a model of fetal growth restriction based on reduced placental expression of IGF-I (64, 215). Laudable though these efforts may be, the mouse is not an appropriate model for human placentation (40). The chorioallantoic placenta supplies the fetus for little more than half of a 3-wk gestation. A yolk sac placenta supports early embryonic development and functions through term; no equivalent structure exists in higher primates. The mouse has a labyrinthine placenta with three trophoblast layers in the interhemal membrane (40). As already discussed, mouse and human placental hormones are quite different (see also Ref. 246) and the immunology of placentation may differ as well (271).

Because of its greater size, the rat is more amenable to experimental manipulation than the mouse, and its physiology is better known (333). From the point of view of the placenta, however, it shares the same drawbacks as the mouse. Comparative studies have value in themselves, but from the viewpoint of understanding human pregnancy, primates are the best models.

The most recent findings from comparative genomics show there still is much to be learned about evolution at the molecular level. Around 5.5% of the mammalian genome has undergone purifying selection with most of the constrained elements residing in intronic and intergenic regions rather than protein-coding exons (235). A recent comparative study additionally detected 3,700 candidate exons that hitherto had escaped annotation. They tended to be more tissue specific than known exons (235), and it will be exciting to learn how many are expressed by trophoblast. In conclusion, molecular biology has greatly increased our understanding of placental evolution, and there is much more to come. It is important that the new findings are followed through at the functional level and that today's physiologists do not repeat the error of a previous generation who failed to capitalize on the advances in embryology made at the start of the last century.

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DISCLOSURES

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