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CHAPTER FOUR

The Phylogeny of Placental Evolution Through Dynamic Integrations of Retrotransposons

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Abstract

Trophoblasts, a major constituent of the placenta, are known to express genes derived from various endogenous retroviruses (ERVs) as well as LTR retrotransposons. However, the evolutionary significance of *ERV*-derived genes involved in placental development has not been well characterized. In this review, we catalog the diverse morphology of placental structure among mammalian species with note of counterintuitive developments. We then detail the history of ancient placenta development with paternally expressed gene 10 (*Peg10/Sirh1*), *Peg11/Sirh2*, and *Sirh7/Ldoc1* as LTR retrotransposons, followed by independent captures of ERV-*env*-related genes such as *Syncytin-1*, *-2*, *-A*, *-B*, *-Rum1*, and *Fematrin-1* responsible for trophoblast cell fusion, resulting in multinucleate syncytiotrophoblast formation, and possibly morphological diversification of placentas. Because the endogenization of retroviral infections has occurred multiple times independently in different mammalian lineages, and some use the same molecules in their transcriptional activation, we speculate that *ERV* gene variants integrated into mammalian genomes in a locus-specific manner have replaced the genes previously responsible for cell fusion. Moreover, ERVs also work as transcriptional regulators of various genes such as interferon (*IFN*)-stimulated genes. The "baton pass" hypothesis suggests that

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evolutionary events caused by multiple successive retrotransposon integrations, possibly resulting in effective fusogenic activity, downstream gene transcription in a temporal and spatial manner, and/or increased diversity of placental structures.

1. PLACENTA: STRUCTURAL DIVERSITY

Following fertilization, the early embryonic cells begin to differentiate for the first time into an inner cell mass (ICM) and an outer trophectoderm, forming the blastocyst. The ICM differentiates into the embryo as well as the amnion, yolk sac, and allantois, whereas the trophectoderm develops into chorionic membrane, later becoming a major part of conceptus side of placenta. In most mammals, the allantois displaces the yolk sac from the trophoblast, resulting in chorioallantoic placentation.[1] However, in some other mammalian species such as rodents and lagomorphs, the yolk sac forms a maternal-facing absorptive epithelium, known as an inverted yolk sac placenta, and it persists until term.[2] This may reflect predominance of a yolk sac or inverted yolk sac placenta form of placentation in early mammals, after which chorioallantoic placentation developed and provided reproductive advantages.

1.1 Classification of Placenta

The placenta is a transient organ that provides an interface for metabolic exchange between the fetus and the mother. The placenta is composed of both the fetal chorion and the maternal uterine endometrium. Placentas are classified according to the distribution of chorionic villi: diffuse (pigs and horses), cotyledonary (ruminants), zonary (dogs and cats), and discoids (murines and primates) (Fig. 1); constituent cell types as well as anatomical structures vary considerably among mammalian species. Placentas are also classified by the number of placental cell layers that separate the fetal blood from the maternal blood: epitheliochorial, endotheliochorial, and hemochorial (Fig. 2). In an epitheliochorial placenta, the various cell types that form a layer between the maternal and fetal blood are: (1) the endothelium of the maternal capillary, (2) uterine endometrium (stroma and/or decidua), (3) the epithelial layer of the uterine endometrium, (4) the layer or layers of trophoblasts that make up the chorionic epithelium, (5) fetal connective tissues, and (6) the endothelium of the fetal capillary. Regardless of the number of cell types in between the mother and fetus, maternal nutrients and gases must traverse all intervening cell/tissue layers to reach fetal

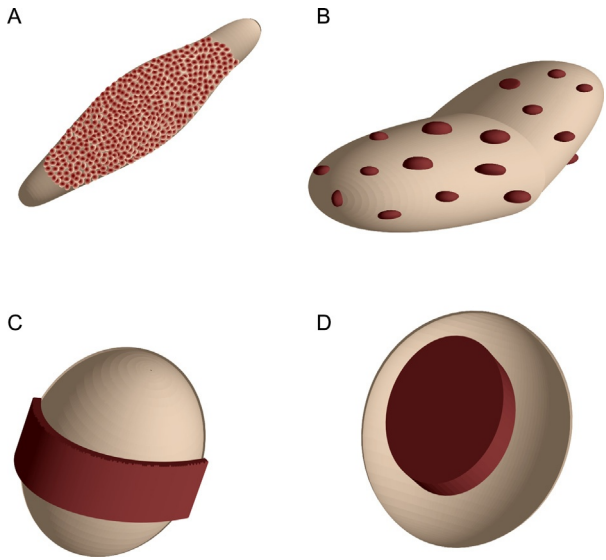

Fig. 1 Placental classification by distribution of chorionic villi. Gross anatomy showing diffuse (pig), cotyledonary (ruminants), zonary (dogs and cats), and discoid (rodents and primates). (A) Diffuse placentas have uniform distribution of chorionic villi that cover the surface of the chorion. (B) Cotyledonary placentas have numerous, discrete button-like structures called cotyledons. (C) Zonary placentas have a band-like zone of chorionic villi. (D) Discoid placentas form a regionalized disc-like structure.

circulation from which waste materials must then be expelled back to the maternal circulatory system.

In the epitheliochorial placenta, the uterine luminal epithelium is in direct contact with the chorionic trophoblast. This type of placentation is found in several orders including even-toed ungulates, whales, dolphins, and lower primates (Strepsirhini). In an endotheliochorial placenta, a loss of uterine epithelium and stromal thinning results in the endothelium of the maternal capillaries being located close to the trophoblast. This type of placenta is seen in carnivores, but it is also found in elephants (Proboscidea).[3] In a hemochorial placentation, maternal blood is directly in contact with the trophoblast, functioning without the capillary endothelium. This type of placentation is seen in many rodents and in higher primates including humans.

It was once thought that placental evolution had proceeded from the least invasive, epitheliochorial, to most invasive, hemochorial placentation. Using phylogenetic and statistical analyses of genomic and morphological data, Wildman *et al.* proposed that the ancestral eutherian placenta had a

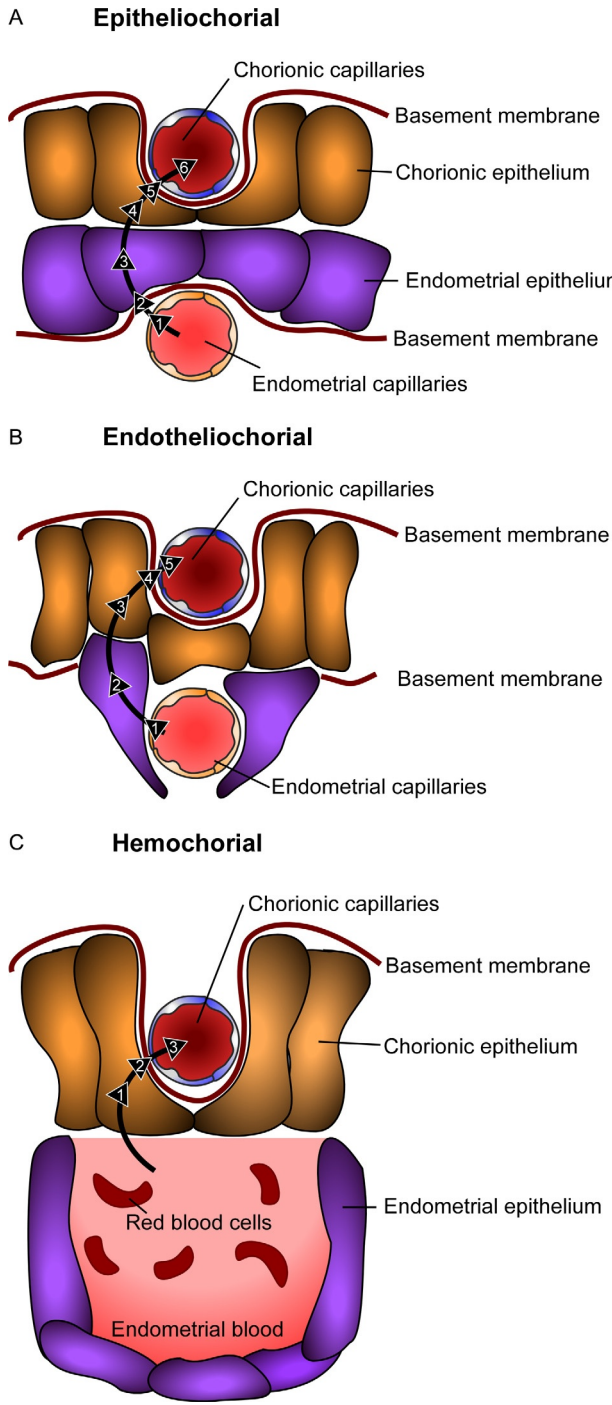

Fig. 2 See legend on opposite page.

hemochorial placental interface with discoid shape and labyrinthine maternofetal interdigitation.[4] However, recent phylogenetic analyses of molecular data sets do not fully support the notion that the highly invasive trophoblast is the most recently evolved or least refined form of placentation. For example, hyenas belong to the order carnivore, but unlike dogs and cats, the hyena undergoes hemochorial placentation.[5] These placental variations and similarities across separate phylogenic trees provide evidence for convergent evolution in mammalian placentation[6] (Table 1 and Fig. 3).

1.2 Evaluation of Evolution From Syncytiotrophoblast

Syncytiotrophoblast cells, components of the chorionic membrane, are generated from trophectodermal fusion and are located next to the maternal cell components. These cells manage efficient nutrient/gas exchange, enable the production of placental lactogen, and chorionic gonadotropin, and are also involved in immunotolerance of the conceptus by the maternal immune system.[11,12] In hemochorial placentation, syncytiotrophoblasts can be divided into three types depending on the number of trophoblast layers. The placenta of some murine species is characterized as monochorial placenta with a single layer of syncytiotrophoblast. Although the human placenta does not have a labyrinth zone like the murine species, still it can be classified as a monochorial placenta. In contrast, murines including Muridae and Cricetidae have three trophoblast layers and are therefore classified as trichorial placentation.[13] The first layer closest to the maternal stroma/decidua consists of mononucleate cytotrophoblast cells, and the second and third trophoblast layers are composed of syncytiotrophoblast cells. Beavers, rabbits, and bats have dichorial placentas, with the first layer of syncytiotrophoblast cells and the second of cytotrophoblast cells. Carnivora including Felidae and Canidae have two trophoblast layers, with the first

Fig. 2 Placental diversity based on cell/tissue layers between fetus and the mother. (A) In the epitheliochorial placenta (pig and horse), both the endometrial epithelium and the epithelium of chorionic villi are intact, and therefore, there are six-cell layers for maternal nutrients and gasses to reach the fetal blood: (1) endometrial cell layer of the maternal capillaries, (2) endometrial interstitium, (3) endometrial epithelial layer, (4) chorionic epithelium, (5) chorionic interstitium, and (6) endothelial cell layer of chorionic capillaries. (B) In the endotheliochorial placenta (cats and dogs), both the endometrial epithelium and underlying interstitium are eroded and maternal capillaries are directly exposed to epithelial cells of the chorion. (C) In the hemochorial placenta (rodents and primates), the chorionic villi are in direct apposition to maternal pools of blood. This results in direct exchanges of nutrients and gases, which move through only three tissue layers to reach the fetal blood.

Table 1 Placental Diversity and Animals That Could Be Assigned to That Category

		Epitheliochorial			Syndesmochorial	Endotheliochorial		Hemomonochorial			
Diffuse		Mono nucleate	Mono/bi nucleate	Multinucleate							
		Pig, Whale, Dolphin	Horse	Bush baby							
Cotyledonary					Mono/bi/ multinucleate						
					Cow, sheep						
Zonary						Mononucleate	Multinucleate	Mononucleate			Multinucleate
						Elephant, kangaroo rat	Dog, cat, earless seal	Hyrax			Hyena
Discoid	Bi					Multinucleate		Multinucleate			
						Treeshrew		Rhesus macaque, Japanese macaque			
	Mono					Star nosed mole		Mono (mononucleate)	Mono (multinucleate)	Bi (mono–multinucleate)	Bi (mono-multi–multinucleate)
								Tenrec, jerboa	Guinea pig, human (Primates)	Beaver, rabbit, Vespertilio sinensis	Mouse, rat, hamster

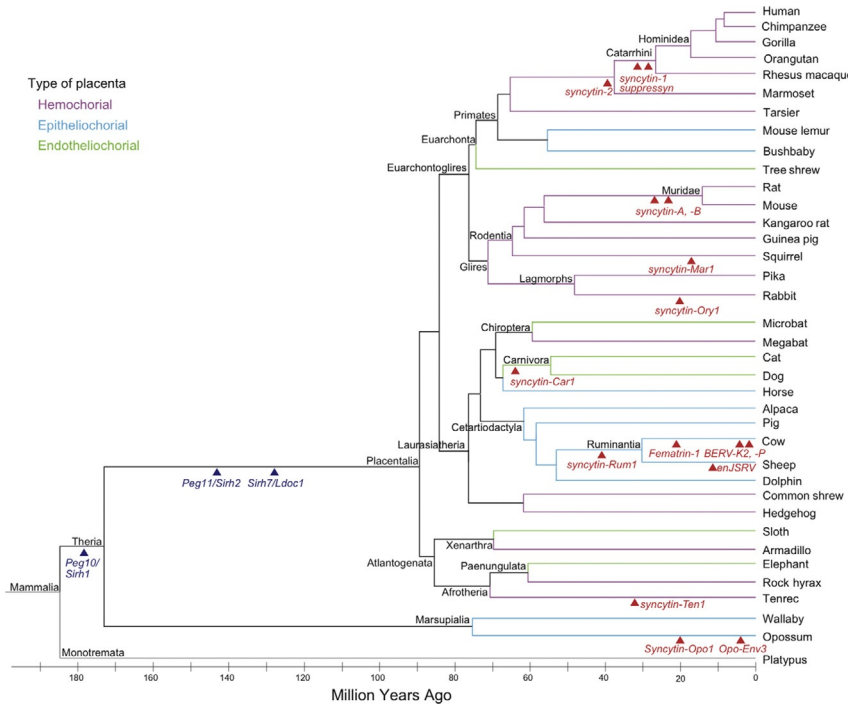

Fig. 3 Evolution and diversity of placenta in mammalian species and integration of genes derived from ERVs and LTR retrotransposons. This phylogenetic tree of mammalian species was based on the data shown in dos Reis *et al.*[7] and Imakawa *et al.*[8] An integrated gene known to be related to placental development that is derived from LTR retrotransposons or ERVs is shown in *blue* or *red,* respectively. The branch color designates the type of placenta[3,9,10] as shown in the *upper left panel.*

layer being cytotrophoblast and the second syncytiotrophoblast.[14] In the Bovidae family, syncytiotrophoblast cells are not formed; rather, through acytokinetic mitosis, the trophectoderm forms binucleate cells (BNCs), which migrate and fuse with epithelial cells, resulting in the formation of trinucleate cells (TNCs) localized in the uterine stroma.[15] These TNCs may serve functions similar to syncytiotrophoblasts in these species.[16] In animals with epitheliochorial placentation such as horses, camels, pigs, hippopotami, and cetaceans, syncytiotrophoblast layer is not formed; however, BNCs can be found in the horse placenta and *Galago* (bushbabies) possess syncytiotrophoblasts.[16] Although Procaviidae (Hyrax), *Tenrec ecaudatus*, and Dipodidae possess hemochorial placentation, syncytiotrophoblast cells are not formed in their placentas.[17–19] These observations indicate that although the placentas serve analogous functions of nutrition and gas

exchange, their morphologies are far more diversified, and that their morphology can vary beyond the history of their specific section of the mammalian evolutionary tree (Table 1 and Fig. 3).

1.3 Significance of Syncytiotrophoblast Formation

Trophoblast cells, components of the outermost membrane, are located next to the maternal endometrium and must exhibit invasive properties to form an effective maternal–fetal vascular relationship. However, excessive invasiveness of trophoblast cells may cause too much stress to the uterus, resulting in uterine bleeding as well as inciting an immune response against the embryo. Although the uterine endometrium has means to control trophoblast invasiveness, trophoblasts themselves may have evolved to limit their own invasiveness into the uterine endometrium. One of these mechanisms appears to be slowing transit through their own cell cycles. Inhibition of cell cycles causes endoreduplication, forming trophoblast giant cells. In humans and murine species, syncytiotrophoblasts are formed through cytotrophoblast cell fusion, resulting in the cessation of cell cycles[20] and the regulation of trophoblast invasion of the maternal endometrium. Syncytialization may not be the only mechanisms through which trophoblasts control their invasiveness. Hyrax is known to have hemochorial placentation, but its trophoblasts do not form a syncytium. This independent appearance of similar functions in different clades is a prime example of convergent evolution.

2. PLACENTA ERVs

Transposable elements make up at least 45% and 40% of human and mouse genomes, respectively,[21,22] of which *ERV*s and long terminal repeat (LTR) retrotransposons account for a respective 8% and 10% of these genomes. Nucleotide structures of *ERV*s and LTR retrotransposons are quite similar to each other; both contain 5′- and 3′-LTRs in each terminus as regulatory elements and *gag*, *pro*, and *pol* protein-coding genes, but an *env*-coding gene that corresponds to a spike protein of viral envelope is included only in ERVs. In both retrotransposons, nucleotide structures largely consist of mutations including insertions and deletions. However, a number of protein-coding genes of ERVs and LTR retrotransposons are still actively transcribed in certain situations.

It has been estimated that a primitive placenta emerged in a mammalian ancestor 150–166 million years ago (MYA).[23] In considering LTR

retrotransposon integrations into the mammalian genomes, there appeared to be occasional *ERV* endogenization events throughout mammalian evolution; it is hypothesized that earlier ones were necessary for structural formation of the placenta, while the latter facilitated morphological diversity of the placentas during mammalian evolution.

2.1 Sirh Family

A group of genes derived from the sushi-ichi LTR retrotransposon is called sushi-ichi-related retrotransposon homolog (*SIRH*) family, comprising 12 genes (*Sirh*1–12) in mammals. In this review, three *Sirh* family genes that are directly involved in placental structure and functioning will be discussed.

2.1.1 Peg10/Sirh1

Paternally expressed 10 (*Peg10/Sirh1*), a maternally imprinted gene, is expressed in trophoblasts and placentas.[24] *Peg10* contains *gag* and *pol* regions of retroviral genomes. A gene ablation study demonstrated that *Peg10* knockout mice suffered early embryonic death, resulting from severe placental defects with loss of spongiotrophoblasts and the labyrinth layer of the placenta. The labyrinth layer is essential for the exchange of nutrients and gases between maternal and fetal compartments. Integration of the gene is believed to have occurred 148 MYA, after the split with monotremes,[25] suggesting that this has contributed to the emergence of primitive placentation.

2.1.2 Peg11/Sirh2

Paternally expressed 11/retrotransposon-like 1 (*Peg11/Rtl1*) is a paternally imprinted gene with *gag* and *pol* regions of retroviral genomes.[26] The gene is believed to function in nutrient and gas exchanges at the allantochorion membranes; ablation of this gene causes early postnatal death resulting from malnutrition-like symptoms during gestation.[27] As this gene is conserved among placental mammals, this gene likely became integrated into the genome soon after marsupials diverged from eutherian mammals.

2.1.3 Sirh7/Ldoc1

Another LTR retrotransposon-derived *Sirh7/Ldoc* [sushi-ichi retrotransposon homolog 7/leucine zipper, downregulated in cancer 1, also called mammalian retrotransposon-derived 7 (*Mart7*)] has recently been characterized.[28] Ablation of this gene is associated with abnormal placental cell differentiation/maturation, leading to an overproduction of placental

progesterone and placental lactogen (PL1) from trophoblast giant cells. Based on these observations, genes of the *Sirh* family likely contributed to the emergence of a primitive placenta. It should be emphasized, however, that the functions of *Sirh* family genes inferred to date are derived from gene ablation studies in present-day murines. Many years must have been required for these *Sirh* family genes to gain the functions we now recognize. Moreover, the observations that *Sirh* family genes are integrated into similar loci across mammalian species strongly indicate that these genes are highly conserved throughout mammalian evolution.

2.2 ERV-*env*

The effects of *Sirh* family gene integration into mammalian genomes are not sufficient to explain placental function and morphological diversity among mammalian placentas, because they are similarly integrated and fixed in the mammalian genomes. Integration of the *ERV* family genes occurred multiple times independently among mammalian species, which make these genes prime candidates for the emergence of efficient nutrient and gas exchange as well as for structural diversification of mammalian placentas. *Env* proteins of mammalian ERVs have been extensively studied, because *Env* proteins are required for viral infection to the host cells through specific receptors, and those of mammalian ERVs can also induce cell–cell fusion in host cells.[6,29] In humans, 18 ERV-*env* nucleotide structures have been identified, among which 16 genes have full coding *env* genes and are transcribed in several healthy tissues; however, only three of these 18 ERV-*env*s possess fusogenic activity.[30,31]

2.2.1 Syncytin-1 and -2

Syncytin-1 belongs to the HERV-W family, and *syncytin-2* belongs to the HERV-FRD family, both of which express env proteins and possess fusogenic activity.[32] In 2000, syncytin-1 was found in human syncytiotrophoblasts, and its fusogenic activity was demonstrated in cytotrophoblasts.[33,34] In the in vitro assay, *syncytin-1* mRNA is upregulated through an increase in cAMP levels when human choriocarcinoma BeWo cells are treated with forskolin, resulting in the fusogenic activity of this cell type. Cell fusion was also demonstrated in African green monkey COS-7, human rhabdomyosarcoma TE671, and human embryonic kidney-derived 293 T cells when these cells were transfected with *syncytin-1* expressing plasmids. In addition, the fusogenic activity of syncytin-1 was found in insect Sf9 cells. In syncytin-2-treated cells, fusogenic activity was found in feline

G355-5 and human 293 T cells.[32] Both syncytin-1 and -2, comprising 538 amino acids, contain surface (SU) and transmembrane (TM) subunits, which are cleaved by a furin protease, and their interaction is required for their fusogenic activity.[35,36]

A transcription factor, glial cell missing factor homolog 1 (*GCM1*), is required for the transcriptional regulation of *syncytin-1* and *-2* genes. The *GCM* family genes are present even in drosophila and are regarded as master regulators.[37] Previously, a multispanning transmembrane protein, CD9, was found as a regulator for the *GCM1* gene.[38] This protein is involved in the invasive behavior of cancer cells and in cell fusions between sperm and egg as well as myoblasts in muscle development.[39,40] *CD9* mRNA and protein are increased in BeWo cells when the cells are treated with forskolin. In addition, CD9 was downregulated by a protein kinase A (PKA) inhibitor, implicating regulation of GCM1 expression through the cAMP/PKA intracellular signaling system. It is possible that as *syncytin-2* is regulated by GCM1, *syncytin-2* is also regulated through the CD9 and cAMP/PKA signaling system. Furthermore, the transcription of *syncytin-2* gene is silenced epigenetically by CpG methylation in human 293 cells.[41] These findings suggest that *syncytin-1* and *-2* gene regulation has not been fully characterized and it awaits further investigation.

The expression of *syncytin-1* and *-2* transcripts differs in their cellular locations. In in situ hybridization studies, *syncytin-1* mRNA is found in syncytiotrophoblasts, whereas *syncytin-2* transcripts are found only in cytotrophoblasts. In addition, the abundance of *syncytin-1* transcripts is maintained throughout the entire gestational period; however, the level of *syncytin-2* decreases during the latter part of pregnancy.[42] Syncytin-1 receptor, RD114/mammalian type D retrovirus receptor (*ASCT2*), is expressed in both syncytiotrophoblast and cytotrophoblasts.[43] Syncytin-2 receptor, major facilitator superfamily domain containing 2 (*MFSD2*), is found only in cytotrophoblasts where syncytin-2 is expressed.[44] Upon forskolin treatment in BeWo cells, *syncytin-1* mRNA increases; however, its receptor *ASCT2* mRNA level decreases.[45] Based on these findings, it is possible that syncytin-1 may work not only on its primary syncytiotrophoblast target but also the secondary target of cytotrophoblasts. In contrast, MFSD2 expression is found only in cytotrophoblasts, and syncytin-2 may work only on cytotrophoblasts.[46] Recombinant protein produced from the *syncytin-2* gene possesses immunotolerizing activity, but the syncytin-1 protein does not, strongly suggesting that syncytin-1 and -2 may function differently in humans.

In the phylogenetic analysis, *syncytin-1* gene is found only in Catarrhini, but not in Platyrrhini, indicating that among primates, *syncytin-1* entered the primate lineages after Catarrhini separation from Platyrrhini lineages approximately 40 MYA.[47] In Catarrhini, the premature termination codon and/or frameshift mutations on *syncytin-1* genes provide evidence that functional syncytin has existed in primates after the separation from Old World monkeys approximately 25 MYA. It cannot be determined whether anthropoids were able to use inactivated *syncytin-1* gene or whether Old World monkeys were unable to use the activated *syncytin-1* gene. In any case, the function of syncytin-1 in the placental formation differs even among the Catarrhini lineages. On the contrary, syncytin-2 is conserved from Platyrrhini to humans (>87.9%) and regardless of lineage, it possesses fusogenic activity in a similar manner, suggesting that this gene has endured for more than 40 million years.[32]

Based on these observations, it is postulated that (a) *syncytin-2* entered the anthropoid lineages and acquired immunotolerance as well as fusogenic activity in cytotrophoblasts, generating primitive syncytiotrophoblasts and (b) *syncytin-1* then entered the Catarrhini lineages. In primates, syncytin-1 enables fusion between syncytiotrophoblasts and cytotrophoblasts. In humans, the fusion between cytotrophoblasts is initiated on days 7–11 days of gestation, and the fusion between syncytiotrophoblasts and cytotrophoblasts continues until the end of pregnancy.[48] Because syncytiotrophoblasts do not possess the ability to proliferate, it is possible that the syncytiotrophoblasts may maintain cellular activity through their fusion with cytotrophoblasts. If this is the case, syncytin-1 could be required to extend placental activity, resulting in a longer gestational period. A relatively short gestation period is seen in Platyrrhini and Old World monkeys; *syncytin-1* is not found in Platyrrhini, whereas the open reading frame of this gene is truncated and not functional in Old World monkeys.

2.2.2 Syncytin-A and -B

From the results of murine genome analysis, two *env* genes with their fusogenic activity in vitro were found and named *syncytin-A* and *-B*.[49] Mouse placenta consists of three trophoblast layers, and syncytin-A is found in the second layer, known as syncytiotrophoblast layer-I (ST-I), whereas syncytin-B is localized in the third or syncytiotrophoblast layer-II (ST-II).[50] Ablation of *syncytin-A* results in a lack of ST-I formation and these embryos die between days 11.5 and 13.5 of pregnancy.[51] Because syncytin-A exhibits fusogenic activity in Green monkey Vero and human 293 T cells,

syncytin-A is involved in trophoblast cell fusion. Similar to human syncytins, GCM1 works as a transcription factor targeting the upstream region of the *syncytin-A* gene, regulating in the initiation of its transcription.[52] This is in agreement with the observation that *GCM1* gene ablation blocks the development of labyrinth zone in mouse placentas.[53] On the other hand, *syncytin-B* gene ablation does not result in embryonic death, although ST-II layer formation is insufficient and the number of pups born is smaller than that for control mice with functioning *syncytin-B*. In in vitro assay, syncytin-B exhibits fusogenic activity only in canine MDBK cells; however, it possesses strong immunosuppressive activity.[46] Findings for syncytin-B in murine trophoblasts closely resemble those for syncytin-2 in human cytotrophoblasts.

In a phylogenetic analysis, both *syncytin-A* and *-B* entered murine lineages approximately 20 MYA (Fig. 3). In Rodentia, *syncytin-A* and *-B* are found in Muroidea, but not in the rest of Myomorpha.[49] It should be noted that Muroidea possess a trichorial placenta and Myomorpha have monochorial placenta. It is thought that (a) an unknown gene X entered Rodentia, which was involved in the formation of monochorial placenta; (b) after Muridae's divergence from Myomorpha, Muridae incorporated *syncytin-A* and *-B*, which had fusogenic and immunosuppressive activity, respectively, and produced two layers of syncytiotrophoblasts; and (c) Muroidea with *syncytin-A* and *-B* lost the unknown gene X, resulting in the formation of the three trophoblast layers as we know them today. With regard to the function of each syncytin, the *syncytin-A* and *-B* genes are likely homologous to the human *syncytin-1* and *-2*.

2.2.3 ERVs in Ruminants

Unlike mammalian species discussed earlier, syncytiotrophoblasts do not exist in ruminant placentas. Instead, ruminant trophoblasts form cytotrophoblast BNCs, which fuse with maternal uterine epithelia, resulting in TNCs in the bovine or syncytial plaques in sheep and goats. It should be noted that these represent the only heterologous cell fusions between fetal and maternal cells in mammalian species. Using BLAST search on the bovine genome, two ORF regions derived from ERV-*env* genes were found and named as bovine endogenous retrovirus K1 (*BERV-K1*) and *BERV-K2*.[54] In addition to these ERVs, several other ERVs were recently found: *Syncytin-Rum1*,[55] *BERV-P*,[56] and endogenous Jaagsiekte sheep retroviruses (*enJSRV*).[57] Regions corresponding to *gag* and *pol* are lost from the nucleotide structures of *BERV-K1*, but its *env* region is well conserved.

On the other hand, *gag*, *pol*, and *env* regions are conserved in *BERV-K2*.[54] The expression of BERV-K1 mRNA and protein is much greater than those of BERV-K2.[58] In the comparison between nucleotide structures of *BERV-K1* and *BERV-K2*, integration of *BERV-K1* occurred much earlier than for *BERV-K2*. Nucleotide structure differences could be considered as reflecting relative degrees of endogenization of these genes. Recently, BERV-K1 was demonstrated as having strong fusogenic activity and identified as a main factor involved in TNC formation, and was therefore named as *Fematrin-1*.[58] It was also reported that *syncytin-Rum1* was inserted into ruminant genomes, including cattle and sheep, and was possibly involved in fetomaternal cell-to-cell fusion in both species.[55] However, *Fematrin-1* is integrated into the bovine genome, but not in the sheep genome, and it is now believed that *syncytin-Rum1* was integrated into ruminant genomes 20 MYA, while *Fematrin-1* was integrated into the bovine genome 11 MYA.[59]

3. HYPOTHESIS OF GENE EVOLUTION THROUGH BATON PASS

Genes originated from ERVs emerge mainly through viral infections and/or retrotranspositions; they do not typically form clusters in the genome. For example, *Fematrin-1* was integrated into the intron 18 of FAT tumor suppressor homolog 2 (*FAT2*), and no *ERV*s were found around the region.[54,58] Moreover, the integration of an exogenous virus is always associated with various components of genes (i.e., *gag*, *pol*, and *env*) as well as transcription regulatory sites (LTRs), and therefore their evolutionary pathways of *ERV* acquisition must operate in a functionality- and locus-specific manner. Indeed, a phylogenetic analysis of *env* genes derived from ERVs (ERV-*env*s), many of which are called "syncytins," clearly shows that those origins are phylogenetically distinct (Fig. 4).

In human *syncytin-1* and *-2* genes, the transcription factor GCM1 is the control element, regulating their expression.[37,41] CD9 is another upstream factor controlling GCM1 and *syncytin* genes.[38] Just as in humans, GCM1 also controls mouse *syncytin-A* and *-B* gene expression. During evolution, integration of one *ERV* could also be followed by another integration of *ERV*; newly acquired *ERV*s could function in trophoblast cell fusion and possibly placental morphogenesis with greater efficacy than the preexisting gene. Fematrin-1, for example, possessed much better fusogenicity than the earlier

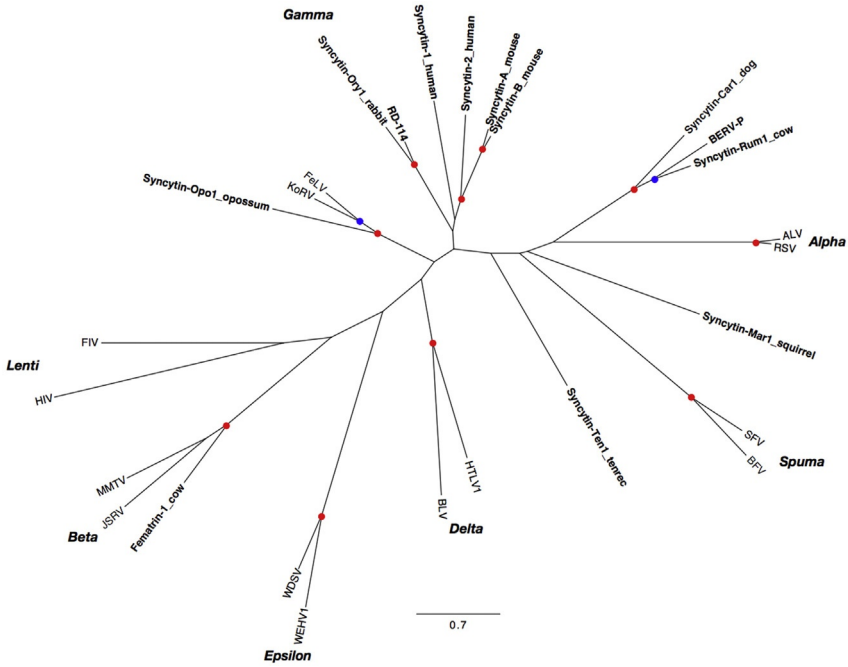

Fig. 4 Maximum likelihood (ML) tree of "syncytins" and Env sequences of various retroviruses. The ML tree was constructed as follows: (1) amino acid sequences of 12 Env sequences derived from endogenous retroviruses including *Syncytins* and 15 Env sequences of exogenous retroviruses that are representative in each genus of retroviruses—*Alpharetrovirus*, *Betaretrovirus*, *Gammaretrovirus*, *Deltaretrovirus*, *Epsilonretrovirus*, *Lentivirus*, and *Spumavirus*—were downloaded from NCBI database, (2) transmembrane regions were aligned using MAFFT L-INS-i[60] and gapped sites were removed using trimAl,[61] (3) LG amino acid replacement model[62] plus gamma distribution and invariant site (G + I) was selected using ProtTest3,[63] and (4) the phylogeny was inferred using RAxML program with 1000 rapid bootstrapping tests.[64] Twelve amino acid sequences of *Env* genes derived from ERVs used in this study were shown as follows: Syncytin-1 and -2 of humans, NP_055405.3 and NP_997465.1, respectively; Syncytin-A and -B of mice, NP_001013773.1 and NP_775596.1, respectively; Syncytin-Ory1 of rabbits, ACZ58381.1[65]; Syncytin-Car1 of dogs, AEX32761.1[66]; Fematrin-1,[58] Syncytin-Rum1,[55] and BERV-P[56] Env of cows, BAJ72717.1, NP_001292383.1, and BAN14723.1, respectively; Syncytin-Mar1 of squirrels, AHZ59674.1[67]; Syncytin-Ten1 of tenrecs, NP_001292515.1[68]; Syncytin-Opo1 of opossums, NP_001295306.1.[69] Fifteen Env amino acid sequences of exogenous retroviruses are shown as follows: ALV (Avian leukosis virus, *Alpharetrovirus*), AAU06813.1; RSV (Rous sarcoma virus, *Alpharetrovirus*), NP_056885.1; JSRV (Jaagsiekte sheep retrovirus, *Betaretrovirus*), AAD45228.2; MMTV (mouse mammary tumor virus, *Betaretrovirus*), BAA03768.1; FeLV (feline leukemia virus, *Gammaretrovirus*), AAA93093.1; KoRV (koala retrovirus, *Gammaretrovirus*), AAF15099.1; RD114 (RD114 retrovirus, *Gammaretrovirus*), YP_001497149.1; BLV (bovine leukemia virus,

(Continued)

Syncytin-Rum1 under physiological conditions.[55,58,70] These successive *ERV* acquisitions, therefore, are called a "baton pass": a new *ERV* replaced the preexisting *ERV* gene and acquired the role that that gene had played,[8,59,71] while the previous gene may either be lost or coopted for another function such as immunosuppressive ability.[6,72] In genes incorporated into reproductive processes through the baton pass, the integration of *ERV*s must be locus specific because they could be transcribed through their own LTRs or instead be transcribed along with placenta-specific genes.[58,73]

In our hypothesis of the baton pass, successive *ERV*s had replaced the preexisting genes and carried out their functions more effectively than their predecessors. The baton pass with *ERV*s in the placenta could also be seen in biological systems other than the process of placentation, such as innate immunity involving interferons (IFNs). IFNs are proinflammatory signaling molecules released upon infection, which promote transcription of IFN-stimulated genes (*ISG*s). When ISGs are activated, *cis*-regulatory elements of these genes are bound by the transcription factors IRF (interferon regulatory factor) and STAT signal transducer and activator of transcription.[74] Quite recently, Chuong *et al.* reported that *ERV*s could have shaped a transcriptional network of the IFN response, in which lineage-specific ERVs had dispersed numerous IFN-inducible enhancers in mammalian genomes. One *Gammaretrovirus*, *MER41*, was endogenized in the genome of an anthropoid primate ancestor 45–60 MYA, and six subfamilies (*MER41A*, *B*, *C*, *D*, *E*, and *G*) are now fixed in the human genome.[75] Deletion of these ERV elements in the human genome impaired expression of adjacent interferon gamma (IFNG)-induced genes, including activation of the gene *Absent in Melanoma* (*AIM2*) inflammasome. These authors also found ancestral sequences of *MER41*-like LTRs in lemuriformes, vesper bats, carnivores, and artiodactyls. Also, reconstructed *MER41*-like LTR in dogs and cows can drive robust IFNG-inducible reporter activity in HeLa cells,

Fig. 4—Cont'd *Deltaretrovirus*), AAO21338.2; HTLV1 (human T-cell lymphotropic virus type 1, *Deltaretrovirus*), NP_057865.1; WDSV (Walleye dermal sarcoma virus, *Epsilonretrovirus*), NP_045939.1; WEHV1 (Walleye epidermal hyperplasia virus types 1, *Epsilonretrovirus*), AAD30049.1; FIV (feline immunodeficiency virus, *Lentivirus*), NP_040976.1; HIV1 (human immunodeficiency virus 1, *Lentivirus*), AAC97548.1; BFV (bovine foamy virus, *Spumavirus*), AAN08117.1; SFV (Simian foamy virus, *Spumavirus*), AAA19979.1. *Env* genes derived from ERVs are shown in *bold letters*. *Red* and *blue circles* indicate that the clade was supported by $\geq$95% or $\geq$80% bootstrap values, respectively.

suggesting that *ERV*s may have independently expanded the IRF regulatory network in multiple mammalian lineages.

This hypothesis could also be applied to explain the variety of placental structures occurring among mammals. Although placental structures exhibit an abundance of diversity, trophoblast functions and their fusogenic activity exhibit more similarity than differences regardless of their invasive versus noninvasive trophoblast nature. Because of its distribution among mammalian genomes, *Peg10* may be a common gene initially required for the evolution of placental mammals.[25,26] While maintaining the same functions, placental structures have been diversified due to successive acquisition of new genes such as ERV-*env*s. When new *ERV*s that possess and provide reproductive advantages are integrated into the host genome, those new *ERV*s may then be retained in the population, eventually becoming common to the species.

4. CONCLUDING REMARKS

In this review, the expression of newly acquired and placenta-specific *ERV*s has been discussed pertaining to placental diversity and mammalian evolution. The main idea presented is that although *Peg10/Sirh1*, *Peg11/Sirh2*, and *Sirh7/Ldoc1* were integrated into primitive placentas, ERV-*env*s with fusogenic activity were independently and successively integrated into mammalian genomes. It should be noted that ERV-*env*s are not orthologous viral genes, but exhibit similar fusogenic functions. These independent integrations of ERV-*env*s must have caused structural diversification in a relatively short time. In the baton pass hypothesis, newly acquired ERV-*env*s replaced the preexisting genes and performed their functions much better than the predecessors.

What happened to old or preexisting genes once their function was taken over by the acquisition of new *ERV*s? Those genes tend to lose their function through mutations and/or deletions within a species. However, it is also possible that while those genes may have lost their original functions, they could have acquired alternative functions in other cell types or tissues. If this is the case, placental variations may still be proceeding through intermediate stages and their evolutionary history may still be far from over. Further experimentation should be carried out, through which other examples of the baton pass, function, and/or regulatory mechanisms of functional gene transcription, can be identified.

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