

# Placental endogenous retrovirus (ERV): structural, functional, and evolutionary significance

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## Summary

That endogenous retrovirus (ERV) is present within the placenta of humans and other mammals has been known for the past 25 years, but the significance of this observation is still not fully understood. Much molecular biological data have emerged in recent years to support the earlier electron microscopic data on the presence of placental ERV. The evidence for ERV in animal and human placental tissue is presented, then integrated with data on the the presence of ERV in a range of other tissues, in particular teratocarcinoma cells. Placental invasiveness and maternal immunosuppression are then discussed in relation to metalloproteinase secretion, the immunosuppressive potential of retroviruses, and placental growth factors, while the evidence for a functional link between placental proto-oncogenes and trophoblast malignancy is reviewed. Finally, placental development, structure, and life span are discussed within an evolutionary context. The hypothesis that one or more ancient trophoblastic ERVs could have played a role in the evolution and divergence of all placental mammals is evaluated. *BioEssays* **20**:307-316, 1998. © 1998 John Wiley & Sons, Inc.

## INTRODUCTION:

### ENDOGENOUS RETROVIRUS EVERYWHERE

The available literature on both exogenous (infective) retroviruses and endogenous retroviruses (ERV), which are germ-line-integrated into host DNA,<sup>1-3</sup> continues to expand. This article places emphasis on the expression of ERV in animal

and human placenta (see also Lyden and Johnson<sup>4</sup> for a recent survey), with the aim of restating, expanding, and updating our earlier concepts on ERV involvement in placental structure and function, and in the evolution of placental mammals.<sup>5,6</sup>

The widespread detection and cellular expression of animal and human endogenous retrovirus (HERV) components raises the question of their biological/physiological, pathological, and evolutionary relevance. Early data involved detection of endogenously expressed retrovirus-like particles (RLPs) by electron microscopy (EM), followed by isolation of RLPs, measurement of reverse transcriptase (RT) activity, and tissue labeling with antibodies to ERV proteins. Subsequent molecular methods (i.e., polymerase chain reaction [PCR], gene in situ hybridization and gene sequencing), however, have led to the detection of integrated retroviral (proviral) sequences within the DNA of animal and human cells. The discovery of the existence and expression of such sequences (complete and defective) within specific tissues and cultured cell lines has posed major questions as to the possible physiological and pathological roles of ERVs in both

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Abbreviations: EM, electron microscopy; EGF epidermal growth factor; ERV, endogenous retrovirus; EVT, extravillar trophoblast; HERV, human endogenous retrovirus; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; HTDV, human teratocarcinoma-derived virus; IGF, insulin-like growth factor; IL, interleukin; MMLV, mouse/murine mammary leukemia virus; MNGC, multinucleate giant cell; MuLV, murine leukemia virus; PDGF, platelet-derived growth factor; PTN, pleiotrophin; *Ras*-GAP, *Ras* GTPase activating protein; Rb, retinoblastoma; RLP, retrovirus-like particle; RT, reverse transcriptase; SLE, systemic lupus erythromatosus; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinase

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animals and humans. The original provirus concept advanced by Temin,<sup>7</sup> supported by Baltimore<sup>8</sup> and others, proposes that retrovirus-related genes are normal components of the genome, with amplification potential (apparently, up to one-third of the human genome may be degenerate retroviral transcripts), and that the infective exogenous retroviruses are derived from such sequences. This does not exclude the possibility of later reinfection of the germline genome with a mutated exogenous retrovirus, since multiple variant copies of endogenous proviruses are distributed through the genome.<sup>9</sup> Recent evidence indicates that, once integrated into the germline genome, ERV sequences evolve much more slowly than do their exogenous proviral counterparts (with integration, viral production and cellular release, following infection of a non-germline cell), and that they can therefore be considered as permanent symbiotic, normal components of the genome<sup>2,4</sup> or as parasitic DNA sequences held in check.

#### DETECTION AND ISOLATION OF ANIMAL AND HUMAN PLACENTAL ERV

Throughout the 1970s, C-type retrovirus(es) were detected by EM in the normal placenta of baboons and a number of other primates, cats, mice, and guinea pigs<sup>6</sup> (see Daniel and Chilton<sup>10</sup> for review), as well as in humans.<sup>11,12</sup> These data were supported by the isolation of retroviral particles from the baboon and rhesus monkey.<sup>13,14</sup> The use of naturally occurring human antibodies in cord sera and monoclonal antibodies against human T-cell leukemia virus and a partial HERV protein sequence enabled immunolocalization of the purported human placental ERV to syncytiotrophoblast and choriocarcinoma cells,<sup>15,16</sup> together with immunoblotting of placental peptides. Correlation of placental extracts and retroviral isolates with the presence of RT was important, as was the placental expression of proviral mRNA.<sup>17</sup> Antigenic interaction was also established between placenta and antibodies present in the serum of patients with autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis, and leukemia, but the possibility of cross-reactivity and even artefacts cannot be ruled out.

The 1990s have shown renewed interest in human placental ERV, particularly spurred by the work of Johnson and colleagues,<sup>18</sup> who, using immunological methods, investigated the expression and isolation of human placental ERV. This group of workers isolated a human placental C-type retrovirus (p1.17) possessing RT activity and performed ultrastructural characterization,<sup>4,19</sup> followed by the generation of a murine monoclonal antibody to the retroviral particle.<sup>20</sup> This monoclonal antibody was immunohistochemically reactive to placental syncytiotrophoblast and immunogold EM labelling localized the antigen primarily to the basal submembranous region (i.e., within the fetal extracellular matrix/interstitial space, directly beneath the syncy-

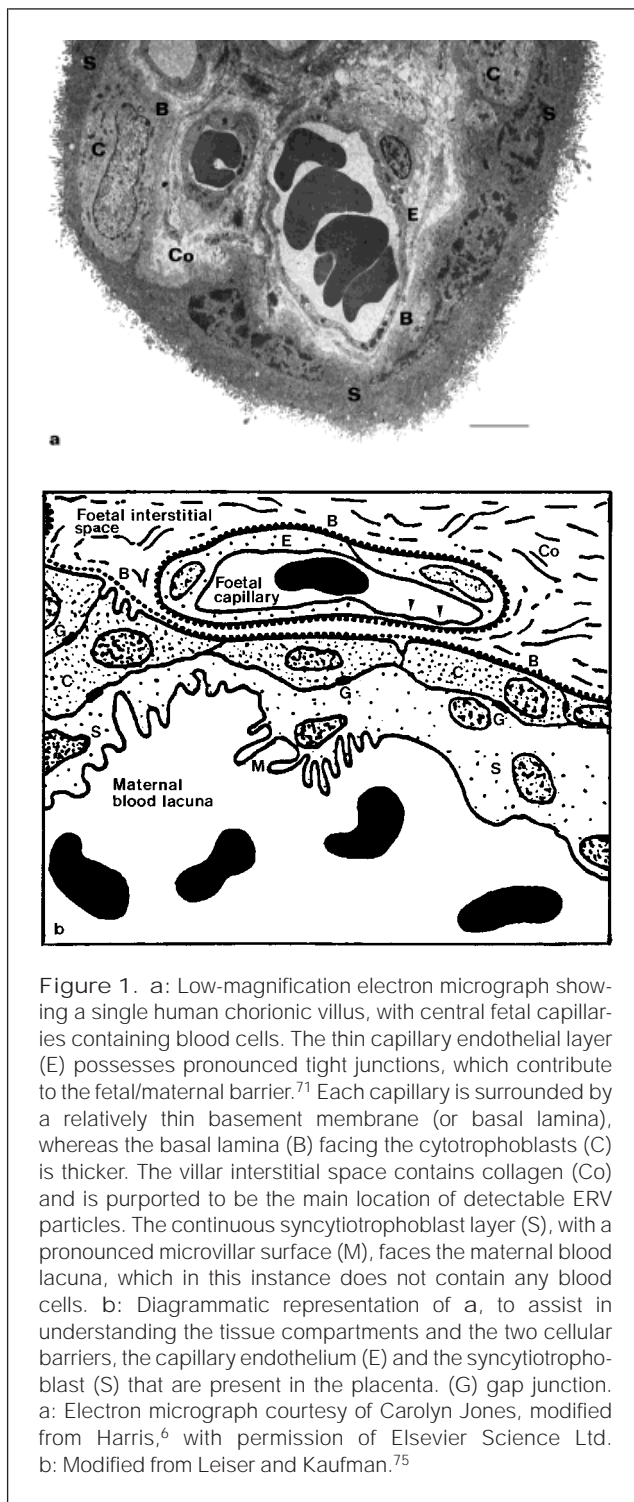
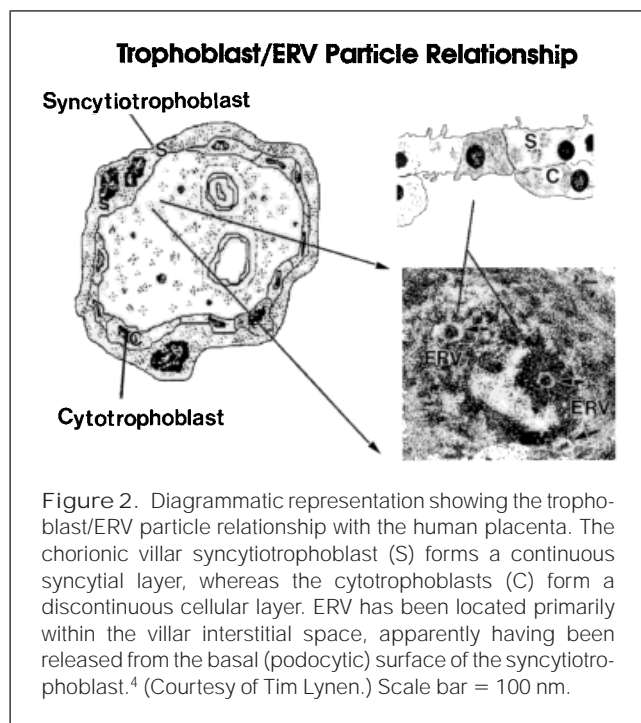


Figure 1. a: Low-magnification electron micrograph showing a single human chorionic villus, with central fetal capillaries containing blood cells. The thin capillary endothelial layer (E) possesses pronounced tight junctions, which contribute to the fetal/maternal barrier.<sup>71</sup> Each capillary is surrounded by a relatively thin basement membrane (or basal lamina), whereas the basal lamina (B) facing the cytotrophoblasts (C) is thicker. The villar interstitial space contains collagen (Co) and is purported to be the main location of detectable ERV particles. The continuous syncytiotrophoblast layer (S), with a pronounced microvillar surface (M), faces the maternal blood lacuna, which in this instance does not contain any blood cells. b: Diagrammatic representation of a, to assist in understanding the tissue compartments and the two cellular barriers, the capillary endothelium (E) and the syncytiotrophoblast (S) that are present in the placenta. (G) gap junction. a: Electron micrograph courtesy of Carolyn Jones, modified from Harris,<sup>6</sup> with permission of Elsevier Science Ltd. b: Modified from Leiser and Kaufman.<sup>75</sup>

tiotrophoblast and cytotrophoblast layer). The cellular organization of the human chorionic villus is shown in Figure 1a, with a diagrammatic interpretation of the tissue structure in Figure 1b. The relationship between the in situ localization of



ERV particles and the cellular structure of the placenta (as defined by Lynen) is shown in Figure 2. Detection of ERV within the fetal interstitial compartment of the placenta need not necessarily preclude release of such material from the opposite, microvillar maternal-facing surface of the syncytiotrophoblast, where passage of ERV through the blood lacuna would lead to direct entry to the maternal circulation and contact with the myometrium, and interaction with the maternal immune system. It is believed that, at implantation and placental formation, cytotrophoblasts or multinucleate giant cells (MNGCs), or both, do invade the uterine myometrial wall (as extravillar trophoblast; EVT), but throughout most of pregnancy the villus trophoblast is actually in contact with the maternal blood lacunae, rather than the myometrium. The invasive capacity and mobility of the decidual extravillar trophoblastic MNGCs remains unknown, since the formation of MNGCs within the myometrium, and other peripheral tissue sites such as the lung, could occur through cytotrophoblast fusion at these sites, as is believed to be the mechanism for formation of the chorionic villar syncytiotrophoblast.

It should be emphasized that the EM search and positive identification of human placental ERV in situ is by no means a simple task, even for the most experienced placental pathologist or retrovirologist. Detection of RLPs budding at the cell surface (for C- and D-type virions) has usually been taken as a clear indication of retrovirus formation, but such data have proved hard to obtain from human placental tissue. Thus, it would appear that, in the human placenta, the number of forming RLPs within the cytotrophoblasts and syncytiotropho-

blast, and extracellular ERV released from these cells, is rather low. Nevertheless, the expression of some retroviral proteins from defective ERV sequences, without RLP formation, remains a possibility.

By immunoblotting, first-trimester placental extracts were shown to contain a reactive 17- to 22-kDa protein.<sup>20</sup> Other groups have provided supportive data for the existence and expression of endogenous retroviral (ERV3) gene products in the human placenta.<sup>21–25</sup> Significantly, no ERV3 expression could be detected in choriocarcinoma cell lines<sup>25</sup> and cytotrophoblasts<sup>21</sup> but was seen in syncytiotrophoblast within placenta and hydatidiform moles.<sup>22</sup> Although HERVs do not carry oncogenes, choriocarcinoma ERV3 RNA transcripts encode a characteristic zinc-finger cellular protein.<sup>25</sup> Cross-reactivity of antibodies to HIV-1 proteins with proteins present in normal human placenta,<sup>26</sup> specifically to the extravillous trophoblast cells,<sup>27</sup> presents the intriguing possibility that an inherent antigenic similarity to the endogenous *env* gene product to HIV-1 gp120/40 underlies the immunosuppressive capability of the invasive trophoblast (see below for further discussion).

#### ENDOGENOUS RETROVIRUS (ERV) IN OTHER TISSUES

Evidence for endogenous C-type murine leukemia related proviruses (MuLVs) and B-type mouse mammary tumor virus (MMTV)-related proviruses has been established in the literature for a considerable number of years. Endogenous intracisternal A-type RLPs are another proviral element dispersed throughout the mouse genome, which have recently been shown to be present in established mouse plasmacytomas, with expression related to proviral hypomethylation.<sup>28</sup> Of evolutionary interest is the observation by Springer et al.<sup>29</sup> that the sea urchin *Tripneustes gratilla* contains an ancient retrovirus-like element expressing long terminal repeats and an open reading frame for the *gag* and *pol* genes. Thus, sea urchins, and indeed other invertebrates, have probably been inserting mobile retroelements into their genomes through active replication and DNA insertion cycles for the past 200 million years.

Expression of HERV sequences by peripheral blood mononuclear cells of healthy individuals<sup>30</sup> correlates with the EM observation of RLPs budding from both normal and HIV-1 infected lymphoid cells, although no evidence for RT or antigenic cross-reactivity with known retroviral proteins was presented.<sup>31</sup> The search for ERV associated with human mammary carcinoma has been surrounded by difficulties for at least the past 20 years. Convincing evidence, however, has built up in recent years, with the discovery of RLPs possessing reverse transcriptase activity in human mammary carcinoma cell lines.<sup>32,33</sup> In addition, firm evidence from PCR has shown high homology between the MMTV *env* sequence and a sequence present in a significant percent-

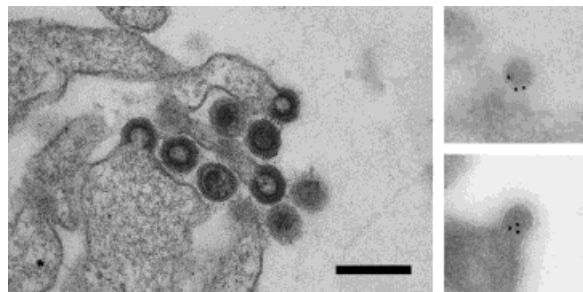


Figure 3. HTDV-K particles budding from a human teratocarcinoma cell line (lhs) and two HTDV-K particles (rhs) immunolabelled with colloidal gold particles, using serum from a seminoma patient. (Electron micrographs courtesy of Klaus Boller.) Scale bar = 200 nm.

age of human breast carcinomas. The presence of the relatively commonly occurring monocyte/macrophage-derived multinucleate giant cells in mammary carcinoma has not yet been correlated with the presence of RLPs and RT activity.<sup>34</sup>

Perhaps the strongest evidence for extra-placental HERV has come from studies at the Paul Ehrlich Institute in Langen, Germany, with the full structural and molecular biological characterization of human teratocarcinoma-derived retrovirus (HTDV), also termed HERV-K.<sup>35–38</sup> Figure 3 shows a group of HERV-K particles budding from the surface of a cultured teratocarcinoma cell, together with the immunogold labeling of HERV-K particles, using serum from a seminoma patient. HERV-K has also been associated with leukocytes from patients with essential thrombocythemia, polycythemia vera, and chronic myeloid leukemia.<sup>39</sup> Very recently, HERV-K related sequences, Gag protein and RLP particles have been detected in the placenta<sup>40</sup> and also in trophoblastic and other germ cell (e.g., ovarian and testicular) tumors.<sup>41</sup>

The presence of ERV in human oocytes and spermatogenic cells confirms the strong germ cell-related expression of these HERVs,<sup>10</sup> which correlates with their expression in the normal placental syncytiotrophoblast, fetal tissues, and germline tumors (Table 1). It is clear, however, that distinction of the HERV-K related family from the HERV-R (ERV3) family occurs, the former being expressed primarily in the teratocarcinomas and the latter in the placenta and trophoblast-derived tumors.

#### PLACENTAL INVASIVENESS AND IMMUNOSUPPRESSION

In common with other cellular systems where cell fusion occurs, such as myotube formation from myoblasts, osteoclast formation from granulocyte–macrophage precursor cells and sperm–oocyte fusion (and possibly also spermatogonia fusion in senescent seminiferous epithelium), cytotro-

TABLE 1. Main Human Endogenous Retrovirus (HERV) Families\*

HERV family name	Principal cell/ tissue location
HERV-E (HERV4.1, HML) <sup>a</sup>	Monocytes, placenta
HERV-H (RTLH-H)	Placenta, teratocarcinoma, lung cancer, seminoma, lymphoid cells, bladder cancer
HERV-K <sup>b</sup> (HTDV)	Teratocarcinoma lines, myeloid leukemia, seminoma, lymphoid cells
HERV-K(C4)	No data on gene expression
HERV-L <sup>c</sup>	Placenta
HERV-P (HuERS, HuRRS-P)	No data on gene expression
HERV-R (ERV3; SY1–4) <sup>d</sup>	Placenta, hydatid mole, sebaceous gland, U-937 monocyte line, fetal tissue
ERV9 (XA34–38)	Glioma cell line, placenta, teratocarcinoma
RR HERV-I	Ovarian teratocarcinoma
No name <sup>e</sup>	Human mammary carcinoma T47D and MCF-7 lines

\*An increasing number of HERV family members (often with defective sequences or incomplete expression, for both) are being detected. HERV terminology in the literature remains somewhat confusing; further categorization is required.

<sup>a</sup>Homology to MuLV.

<sup>b</sup>HERV-K10 homology to MMTV.

<sup>c</sup>Homology to human foamy retrovirus, present in mouse and primates.

<sup>d</sup>At least one family member (SY-2) has homology to primate and mouse ERV, chorionic gonadotropin responsive.

<sup>e</sup>Homology to MMTV and the HERV-K family; steroid responsive.

See references 1–3 for further details on subfamilies and references.

phoblast fusion to generate the single-cell-thick syncytiotrophoblast appears to involve the release of metalloproteinase. In the case of the invasive syncytiotrophoblast these metalloproteinases (e.g., type IV collagenases) are believed to be actively involved in collagen degradation at the implantation site, with collagenase presence clearly defined in both the syncytiotrophoblast and the underlying cytotrophoblasts.<sup>42,43</sup> The extent of cytotrophoblast/syncytiotrophoblast invasion and extracellular collagen degradation increases under conditions of acute fasting<sup>44</sup> and when oxygen deprivation is induced in rhesus monkeys (by aortic stricture).<sup>45</sup> These studies indicate a physiological response from the fetus and placenta, leading to progressive endometrial invasion. That fetal extravillar trophoblast (EVT) cells do enter the maternal circulation under normal circumstances is not surprising,<sup>46,47</sup> and noninvasive fetal prenatal genetic diagnosis of such cells is likely to be routinely performed in the future. The old concept of placental *pseudotumor* and *physiological metastasis* of placental trophoblast during pregnancy (particularly to the breasts) refer to this invasive situation. Isolated

subpopulations of cultured human placental trophoblast cells<sup>48</sup> all secrete type IV collagenases and urokinase plasminogen activator, exhibit fusogenic capacity and thereby create MNGCs. Transplantation of rat trophoblast within the brain of adult rats (to escape all physiological anti-invasive response) was found to readily establish vasculature, cellular differentiation, and proliferation in a manner essentially equivalent to the normal placenta.<sup>49</sup> In particular, the ability to degrade brain extracellular matrix and destroy blood vessels to create vascular lacunae can be considered indicative of a useful physiological *in vivo* model for extravillous trophoblastic growth.

The immunology of the human placental trophoblast has been addressed in some detail by Johnson,<sup>50</sup> with emphasis upon the various ways the forming embryo can protect itself from the mother's immunological response. Factors of greatest importance appear to be the absence of trophoblastic major histocompatibility molecules, the presence of unique nonpolymorphic HLA molecules and the expression of complement regulatory proteins. In addition, the release of immunosuppressive factor(s) by the placenta has been investigated in recent years. A trophoblast-derived human choriocarcinoma cell line has been found to secrete a 5- to 6-kDa protein possessing the ability to suppress the proliferative response of both human and murine lymphocytes.<sup>51</sup> Within the present context, however, the ability of many infective retroviruses to elicit an immunosuppressive response<sup>52,53</sup> is of greatest importance. A 17-amino acid peptide of HIV-1 gp41 shows immunosuppression and possesses sequence homology with the equivalent evolutionarily conserved peptide in both C- and D-type retroviruses.<sup>53</sup> This was also indicated by the data of Cianciolo et al.<sup>54</sup> and correlated to a peptide sequence in the env protein of ERV9<sup>55</sup> and ERV3 (HERV-R).<sup>24</sup>

A role for growth factors/cytokines and/or their receptors in the regulation of trophoblast invasion remains unclear. The immunosuppressive cytokine interleukin-10 (IL-10), produced by placental cells, could contribute to maternal tolerance<sup>56</sup> and a similar claim has been advanced for the role of the interferons,<sup>57</sup> but any relationship to suppression of either endogenous or exogenous retroviral expression in this tissue can only be speculative. Transforming growth factor- $\beta$  (TGF- $\beta$ ) possesses a marked antiproliferative action on normal trophoblast cells, apparently due to upregulation and cellular production of tissue inhibitor of metalloproteinases 1 (TIMP-1).<sup>58</sup> Of interest is the additional observation that the choriocarcinoma cell lines JAR and JEG-3 failed to produce TIMP-1.<sup>58</sup> Platelet-derived growth factor (PDGF) has been indicated as a growth factor for cytotrophoblasts,<sup>59</sup> with an autostimulatory loop regulated by the number of cytotrophoblasts carrying cell surface PDGF  $\alpha$ -receptors. A similar role has been advanced for the insulin-like growth factors 1 and 2 (IGF-1, IGF-2).<sup>60</sup>

Further studies on the myometrial extracellular matrix and trophoblast invasion are important, as is the detailed understanding of the role of glycan synthesis and deposition, and the involvement of cellular adhesion molecules, such as fibronectin, laminin, and the integrins, needs to be established.<sup>61</sup>

#### PLACENTAL PROTO-ONCOGENES AND TROPHOBLASTIC MALIGNANCY

The complex role of proto-oncogenes in placental development and regulation may be closely connected with both the action of growth factors and the induction of malignancy.<sup>62</sup> In particular, the *vav* proto-oncogene has been implicated in normal trophoblast development and implantation.<sup>63,64</sup> A potential involvement of the oncosuppressor gene products p53 and retinoblastoma susceptibility (Rb) in the control of trophoblastic proliferation and of *c-myc* in the control of both proliferation and differentiation has also been suggested.<sup>65</sup> Reduction of placental p53 from early gestation to term has been shown,<sup>66</sup> in parallel with an increase in the proto-oncogene BLC-2. This finding was interpreted as indicative of maintenance of resistance to apoptosis in terminally differentiated syncytiotrophoblast, with low expression of BLC-2 by choriocarcinoma cells rendering them more susceptible to apoptosis. Reciprocal expression of the proto-oncogene *c-erbB2* and epidermal growth factor receptor (EGF-R) by varying trophoblast cellular populations has provided evidence that the former may be important for trophoblast invasion and differentiation whereas the EGF-R may be implicated in trophoblast proliferation.<sup>67</sup> The claim has been advanced<sup>68</sup> that there is an inverse correlation between villous trophoblastic expression of the *Ras* GTPase activating protein (*Ras*-GAP) and the invasive potential of trophoblastic tumors; normal placentas and noninvasive hydatidiform moles express *Ras*-GAP, whereas the extravillous cytotrophoblasts of invasive moles and choriocarcinomas do not. Detection of a defective HERV genome within the open reading frame of the human growth factor pleiotrophin (PTN) led Schulte et al.<sup>69</sup> to the conclusion that tissue-specific expression of PTN, as a result of this insertion, might be related to the aggressive growth of human choriocarcinoma and possibly of the human trophoblast as well.

A panel of antibodies to distinguish immunologically between members of the differentiating trophoblast cellular population throughout pregnancy and also in the trophoblastic malignancies is not yet available. The use of a monoclonal antibody against an epithelial surface antigen on human trophoblastic cells (Ber-EP4)<sup>70</sup>, however, indicates that it is possible to define early gestational cytotrophoblast and choriocarcinoma cells selectively. Although trophoblastic tumors are relatively rare, they are particularly aggressive in some instances (as mentioned above), probably assisted by the escape of metastasizing tumor cells into the maternal

circulation via the maternal placental lacunae. Thus, molecular and immunological assays are required to assist the pathologist to produce a rapid diagnosis of trophoblastic and other germline tumors.

#### PLACENTAL DEVELOPMENT, STRUCTURE, AND LIFE SPAN

After fertilization, early mammalian development is initially characterized by the proliferation of the extraembryonic trophoectoderm, rather than the embryo itself. The subsequent formation of the placenta, from the more rapidly dividing polar trophoectoderm at the endometrial attachment site, leads to the creation of the definitive cellular structure (the placenta), which is not a part of the fetus, but presents the site of first-line interaction with the mother for purposes of fetal nutrition, removal of waste metabolites, and the exchange of respiratory gasses to and from the fetal circulation. The extensive chorionic villation combined with the pronounced microvillar surface of the syncytiotrophoblast contribute to the maximization of the membrane surface for maternal–fetal exchange. It should, however, be noted that the syncytiotrophoblast microvillated surface facing the maternal blood lacunae performs extremely active endocytosis; coated pits are readily detectable along and at the base of the microvilli, together with internalized coated vesicles. The intense endocytotic activity of the syncytiotrophoblast, indicated by this structural evidence, is required for nutrition of the rapidly growing fetus. The fetal-facing surface of the syncytiotrophoblast/cytotrophoblast layer (adjacent to the basal lamina; see Fig. 1) has far fewer microvilli and is sometimes termed the podocytic surface, because of some superficial structural similarity to the renal glomerular podocyte–basement membrane interface.

Physiological metastasis of extravillar cytotrophoblasts within the maternal circulation together with the progressive myometrial invasion indicate that an exceptional immunological situation (but with immunosuppression localized and directed toward the fetal placenta alone) must be occurring within the mother to prevent rejection of placental tissue. Indeed, the placenta itself has unique properties that it, and the organized trophoblast, with its cellular cytotrophoblasts and fused syncytiotrophoblast layer (Fig. 1), contain structural features unlike any other cell type.<sup>71</sup> Furthermore, the special role of the placenta is limited to the weeks or months of pregnancy, after which it must be totally ejected by the maternal body.

The role of apoptosis, if any, in the ultimate degeneration of placental tissue remains to be firmly established. Ultrastructural indications from the formation of clustered nuclei containing condensed chromatin (nuclear/syncytial knots) in human placental syncytiotrophoblast at term<sup>6,72</sup> (even more dramatically under conditions of rhesus incompatibility), is characteristic of apoptosis. The claim that apoptosis can be identified

in human trophoblastic cells by in situ nick end labeling of fragmented DNA<sup>73</sup> provides evidence that controlled cell death does play a role in the terminal differentiation of the placenta. Discontinuities in the syncytiotrophoblast layer, associated with fibrin deposition, are believed to trigger apoptosis of the adjacent syncytiotrophoblast,<sup>74</sup> but apoptosis had not been previously linked to terminal placental differentiation or structural changes associated with full term.

It is well known that the placenta in different mammalian species exhibits considerable structural diversity.<sup>75</sup> What is less well known is that even the dasyurid marsupials create a transient invasive placental structure with a syncytiotrophoblast,<sup>76,77</sup> which possesses considerable cellular similarity to that of the placental mammals. The creation of the syncytiotrophoblast layer, initially in immediate contact with the maternal endometrial tissue, extracellular matrix, and then body fluids in the maternal blood lacunae (Figs. 1, 2) is a fundamental within almost all placenta (there are a few exceptions, such as the pig and horse, in which a cellular trophoblast is maintained). In all mammals with a syncytiotrophoblast it is now accepted that cell fusion events occur, whereby the outermost layer of placental cytotrophoblasts, with well-organized desmosomal/gap junctions between them, undergo cell–cell fusion to create the single-cell-thick syncytiotrophoblast barrier between the maternal and fetal circulations.<sup>6,72,78,79</sup> Gap junctions remain between the syncytiotrophoblast and adjacent unfused cytotrophoblasts, and gap junction remnants can be detected within the syncytiotrophoblast.<sup>6,72</sup> Indeed, the expression pattern of connexins within the gap junctions of the trophoblast has been related to placental differentiation and control of myometrial invasion.<sup>80</sup> Interestingly, the trophoblast/syncytiotrophoblast alone has been considered to represent an incomplete barrier to transplacental exchange,<sup>71</sup> since the existence of well-defined cadherin arrays of the zonula adherens and discontinuous zonula occludens/tight junctions between the endothelial cells of the adjacent fetal capillaries may also contribute to the restriction of the transplacental exchange of macromolecules.

Some might consider that one measure of biological competency in mammalian evolution is the length of pregnancy (i.e., the time of physiologically efficient placental function/survival) and the resulting extent of fetal maturity at birth. Thus the placenta of whales and the elephant, and other mammals producing at birth highly mature mobile offspring, may merit more detailed study than they have been given thus far. Nevertheless, the diversity of placental survival from only a few days for the primitive placenta of the dasyurid marsupials through to the 22 months of the elephant, indicates that some well-controlled physiological mechanisms are operational in all cases. These may relate to growth factor, growth factor receptor and proto-oncogene

**TABLE 2.** Placental ERV Expression: Possible Biological and Evolutionary Roles

1. Placental ERV expression could influence, or be influenced by, growth factor/cytokine and proto-oncogene interactions, and be related to the release of metalloproteinases and subsequent myometrial invasion by the trophoblast.
2. The expression of ERV env gene product within the surface membranes of placental cytotrophoblasts could be of significance for membrane-membrane interaction and cell fusion to form the syncytiotrophoblast. (Metalloproteinases may also play a part in the potentiation of cell-cell fusion.)
3. Placental ERV env proteins that conserve the retroviral peptide sequence defined as being immunosuppressive could play a role in a localized maternal immunosuppression, permitting the initial trophoblastic attachment, invasion, and overall placental survival.
4. ERV expression within the placental trophoblast may account at least in part for the pseudotumor-like and metastatic properties of this extraembryonic tissue.
5. Placental ERV expression could be part of a control mechanism for the control of placental survival and for terminal apoptotic degeneration.
6. An amplification or increase in expression of trophoectoderm ERV within some premammalian species (some 100+ million years ago) could ultimately have resulted in the evolution and diversity of all placental mammals.

Modified from Johnson et al.<sup>18</sup>

expression, and placental hormonal factors such as chorionic gonadotropin,<sup>21,81</sup> perhaps in addition to the presence of (and interaction with) endogenous ERV gene products.

A fundamental property of many exogenous retroviruses is their ability to create cell-cell fusion, either by direct *fusion from without* or by *fusion from within*. After cellular integration and proviral expression, fusion of infected with uninfected (or infected) cells can occur.<sup>6,22</sup> The correlation of these properties with the action of endogenous retroviral provirus products in cytotrophoblastic cells, in particular that of the *env* gene, which expresses the equivalent to the exogenous retroviral envelope glycoprotein (gp120/40) (in HIV-1 this glycoprotein is believed to be involved in viral attachment and cell-cell fusion in vitro and possibly also in vivo), underlies the proposal that a member of the placental HERV-R family could be involved in the creation of the syncytiotrophoblast. Inhibition of fusion of cultured cytotrophoblasts by antibodies against ERV proteins and the use of transgenic constructs could possibly provide useful assay systems to provide firm evidence for the role of placental ERV. A summary indicating the possible involvement of HERV in placental structure and function, is given in Table 2.

## PLACENTAL BIOLOGY AND EVOLUTION

The adaptive physiological role provided by the placenta in the development of all eutherian and metatheurian mammals is obvious. Yet, to pose the leading question, "Could an ancient ERV, selectively expressed within the extraembryonic trophoectoderm, be responsible for the creation of the placenta and the tumor-like invasive properties of the trophoblast?" is reasonable. To provide a conclusive answer, however, is not currently possible. An answer may eventually emerge from phylogenetic analysis of the evolutionary lineages of the various animal<sup>82</sup> and human ERV families and cell fusion studies performed using cultures of well-defined trophoblast subpopulations. Further studies on primitive mammals such as the marsupials, with transient placentae, could also contribute significantly. Gene and protein sequence homology of ERV between both close and distant mammalian species has already provided suggestive evidence that several proviruses were inserted into the germline many millions of years ago.<sup>83,84</sup> Hence, the possibility that retroviruses have coexisted with eukaryotic cells (within vertebrate and invertebrate cells) throughout their evolution, and have in some instances given rise to stable germline ERVs (as well as exogenous infective retroviruses) must be considered.

That ancient prokaryotic genomes were introduced and integrated in an endosymbiotic capacity into the primitive eukaryotic cell, generating both mitochondria and chloroplasts, is now widely accepted. It is similarly possible that we may in the future consider at least some of the ERVs to be ancient genomic symbionts/controlled parasitic DNAs; because of the longevity of their integration such ERVs must be considered as normal permanent genetic elements, whereas others may have been amplified from existing progenes or germline integrated from exogenous retroviruses at more recent evolutionary dates.<sup>85</sup> The life cycles of the various retroelements and the reverse flow of genetic information are summarized diagrammatically in Figure 4. Further molecular evidence that endogenous retroviruses (and other retroelements) play a role in host evolution and, specifically, in normal and oncogenic cellular differentiation in mammalian development is likely to be forthcoming.

## CONCLUSIONS

The presence of ERV in animal and human placenta leads to the interpretation that such gene products may play a role in placental formation (cell fusion), placental function (fetal/maternal barrier formation; initial endometrial/myometrial invasion), and also placental survival throughout pregnancy (role in immunosuppression and possibly apoptosis). To extend these considerations within an evolutionary context to account for the actual existence of the extraembryonic trophoblast and a role in the divergence and success of the placental mammals, while speculative, is not beyond the

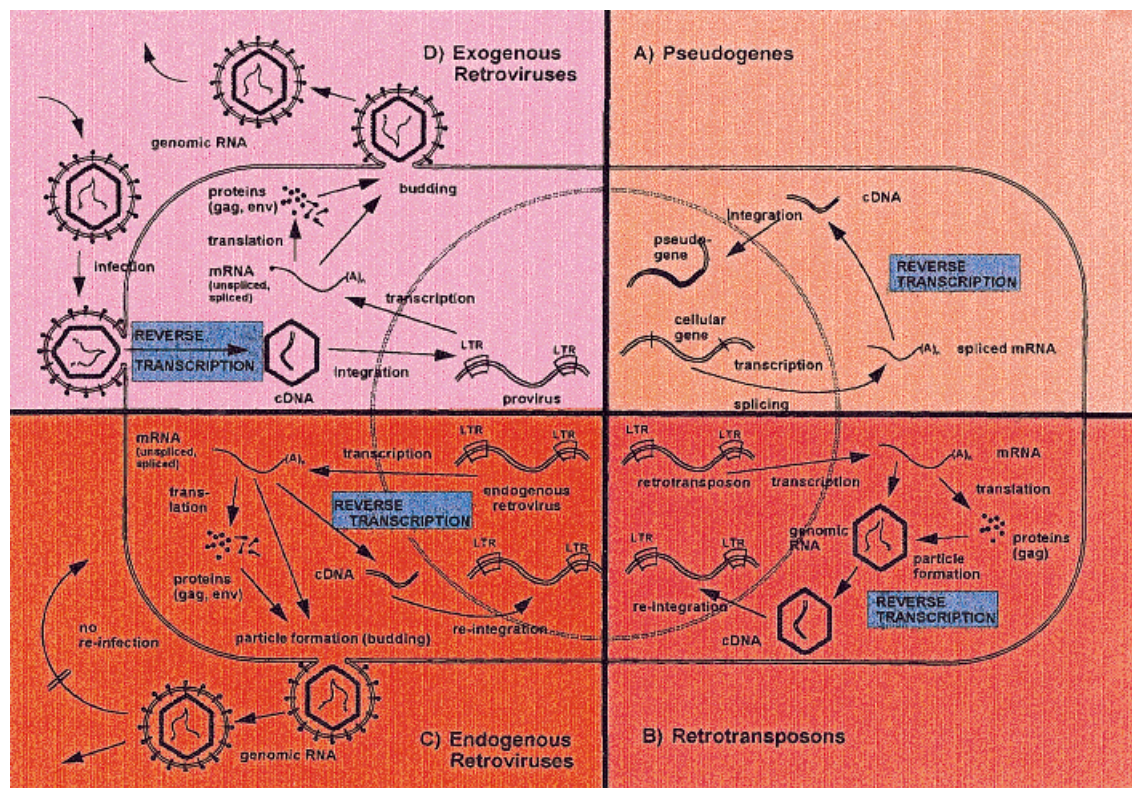


Figure 4. Life cycles of retroelements. A: Generation of pseudogenes. B: Transposition of retrotransposons. C: Expression and amplification of ERVs. D: Replication cycle of exogenous retroviruses. (Diagram courtesy of Roswitha Löwer, from Löwer et al.,<sup>2</sup> with permission of the National Academy of Sciences, USA.)

bounds of reasonable possibility. It is clear, however, that further immunological and molecular biological studies on the expression and location of ERVs within fish, reptiles, and birds<sup>82</sup> are vitally necessary to establish whether there are homologies between these retroelements and mammalian ERVs. Likewise, further comparative studies on the primitive placental tissue of marsupial mammals may generate useful indications as to the evolutionary significance of the ERV3 family of retroviruses.

#### ACKNOWLEDGMENTS

The author thanks Klaus Boller, Tim Lynen, Paul Agutter, Tracie Bunton and Milan Nermet, for reading and critically commenting on the manuscript. Previously published and unpublished material was readily made available for use in this article by Carolyn Jones, Klaus Boller, Roswitha Löwer, and Tim Lynen, along with much encouragement.

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