Endogenous retroviruses of sheep: a model system for understanding physiological adaptation to an evolving ruminant genome

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Endogenous retroviruses (ERVs) are present in the genome of all vertebrates and are remnants of ancient exogenous retroviral infections of the host germline transmitted vertically from generation to generation. Sheep betaretroviruses offer a unique model system to study the complex interaction between retroviruses and their host. The sheep genome contains 27 endogenous betaretroviruses (enJSRVs) related to the exogenous and pathogenic Jaagsiekte sheep retrovirus (JSRV), the causative agent of a transmissible lung cancer in sheep. The enJSRVs can protect their host against JSRV infection by blocking early and late steps of the JSRV replication cycle. In the female reproductive tract, enJSRVs are specifically expressed in the uterine luminal and glandular epithelia as well as in the conceptus (embryo and associated extraembryonic membranes) trophectoderm and *in utero* loss-of-function experiments found the enJSRVs envelope (env) to be essential for conceptus elongation and trophectoderm growth and development. Collectively, available evidence in sheep and other mammals indicate that ERVs coevolved with their hosts for millions of years and were positively selected for biological roles in genome plasticity and evolution, protection of the host against infection of related pathogenic and exogenous retroviruses, and placental development.

Introduction

Endogenous retroviruses (ERVs) are present in the genome of all vertebrates and are vertically transmitted as stable, inherited Mendelian genes (Boeke & Stoye 1997). ERVs are thought to arise from ancient infections of the germline of the host by exogenous retroviruses, and they have heavily colonized the genome of all animal species. The obligatory integration step of the retroviral replication cycle allowed, during evolution, the incorporation of the viral genome (provirus) into the host genome (**Fig. 1**). In fact, more that 40% of the human genome is comprised by transposable elements, and ERVs account for approximately 8% of the human

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genome (Kurth & Bannert 2010) (**Fig. 2**). Retrotransposition or re-infection of the germline can generate further insertions augmenting the number of ERVs loci in the genome (Gifford & Tristem 2003). A complete ERV "provirus", i.e. the retroviral genome integrated into the host cell genome, shares the same genomic structure of an exogenous retrovirus, which is four viral genes (*gag, pro, pol*, and *env*) flanked by two long terminal repeats (LTRs) (**Fig. 3**). The *gag* gene encodes for the major viral structural protein, while *pro* and *pol* encode for the viral enzymatic machinery necessary for the viral replication cycle. The *env* gene encodes for the envelope glycoprotein (Env) that is inserted in the lipid bilayer of the exterior membrane to form the viral envelope and mediates entry of the virus into susceptible cells via a receptor. The LTRs contain enhancer and promoter elements that direct expression of the viral genes.

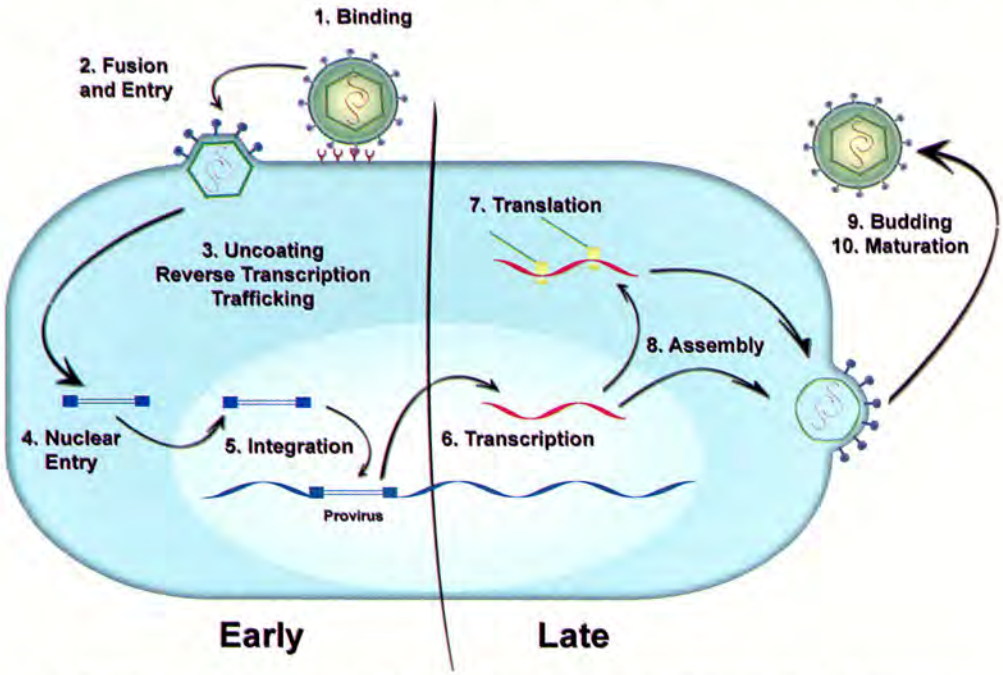

Fig. 1. Life cycle of retroviruses. The retroviral life cycle is arbitrarily divided into two phases, early and late. The stages in each phase are shown above. Interactions between viral and host cell restriction factors occur at every stage of the viral life cycle. The infecting virus attaches to a specific receptor on the cellular plasma membrane with the SU portion of the viral Env protein leading to fusion and entry. Reverse transcription then generates a double-stranded DNA copy of the RNA genome. The provirus is transported into the nucleus and integrated into chromosomal DNA. Transcription by the cellular machinery generates RNA copies that are then translated in the cytoplasm. Virion proteins and progeny RNA assemble at the cell boundary and the plasma membrane, and progeny virus is released into a mature viral particle.

ERVs are widespread throughout vertebrate genomes (Herniou *et al.* 1998). Some ERVs are highly related to exogenous retroviruses, including Jaagsiekte sheep retrovirus (JSRV), mouse mammary tumor virus (MMTV), feline leukemia virus (FeLV), and avian leukemia virus (ALV), which are currently active and infect sheep, mice, cats and chickens, respectively (Boeke & Stoye 1997). These ERVs are generally referred to as "modern" ERVs, because they integrated

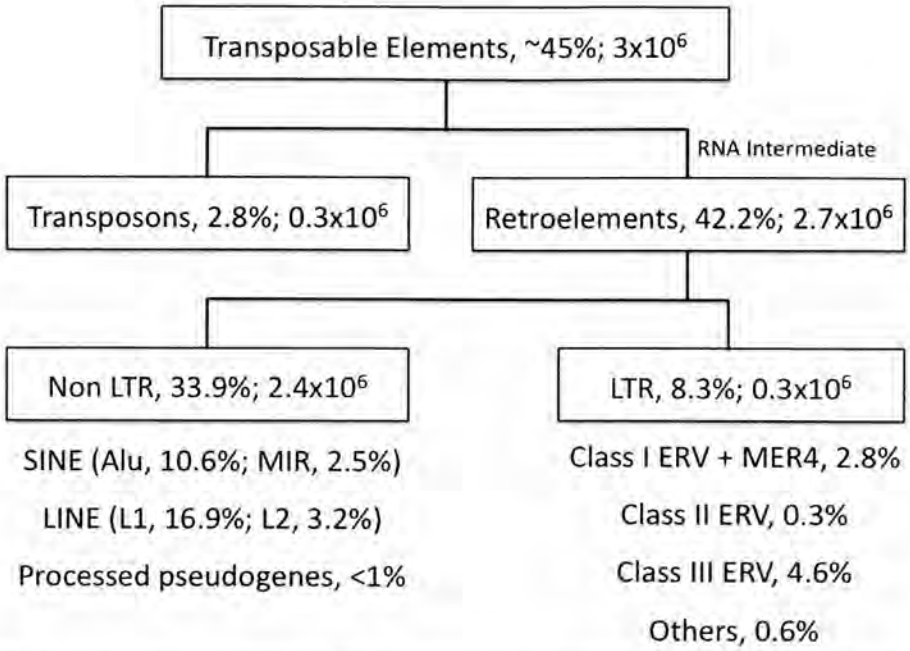

Fig. 2. Endogenous retroviruses are transposable elements. Transposable elements are stretches of DNA that cut and splice themselves out of the genome into another region, contributing to genetic diversity in a variety of organisms (Bannert & Kurth 2004, Biemont & Vieira 2006). Approximately 45% of the human genome is composed of transposable elements. Transposable elements can be divided into DNA transposons, which act *via* a DNA intermediate, and retrotransposons that use a RNA intermediate. DNA transposons comprise 2.8% of the transposable elements of the human genome, while the remaining 42.2% are retrotransposons. Retrotransposons can be further divided into non LTR elements (33.9%), comprising the long and short interspersed elements (LINEs and SINEs respectively), and LTR elements (8.3%) that are endogenous retroviruses (ERVs). Figure adapted from Bannert & Kurth (Bannert & Kurth 2004).

into the host genome after speciation and are closely related to exogenous viruses that are still infectious. Indeed, most ERVs do not have an exogenous counterpart. Some modern ERVs are still able to produce infectious virus due to the lack of inactivating mutations. Modern ERVs can also have insertionally polymorphic loci, since they are not completely fixed in a particular population and are still undergoing endogenization. In contrast, "ancient" ERVs invaded the genomes before speciation and, consequently, are present in every individual at the same genomic location of phylogenetically related species (Coffin 2004). Over time, ERV accumulate mutations, insertions, and recombinations, particularly if their expression brings deleterious consequences for the host (Boeke & Stoye 1997). Consequently, the majority of integrated retroviral sequences are non-functional remnants ("fossils") of exogenous related sequences.

The biological significance of ERVs has been debated for several decades and in the past they were generally thought to be "junk DNA" (Bock & Stoye 2000), but mounting evidence reveals that selected ERVs have a variety of beneficial roles to their host (Jern & Coffin 2008, Varela *et al.* 2009, Kurth & Bannert 2010). The presence of transcriptionally active ERVs with intact open reading frames conserved million of years after integration support the idea that some

ERVs were selected and co-opted by the host for specific biological roles until they became true essential genes (de Parseval & Heidmann 2005). In this review, we will focus on the biological roles of sheep JSRV-related endogenous betaretroviruses (enJSRVs) in genome plasticity and evolution, protection from exogenous and pathogenic JSRV, and placental development.

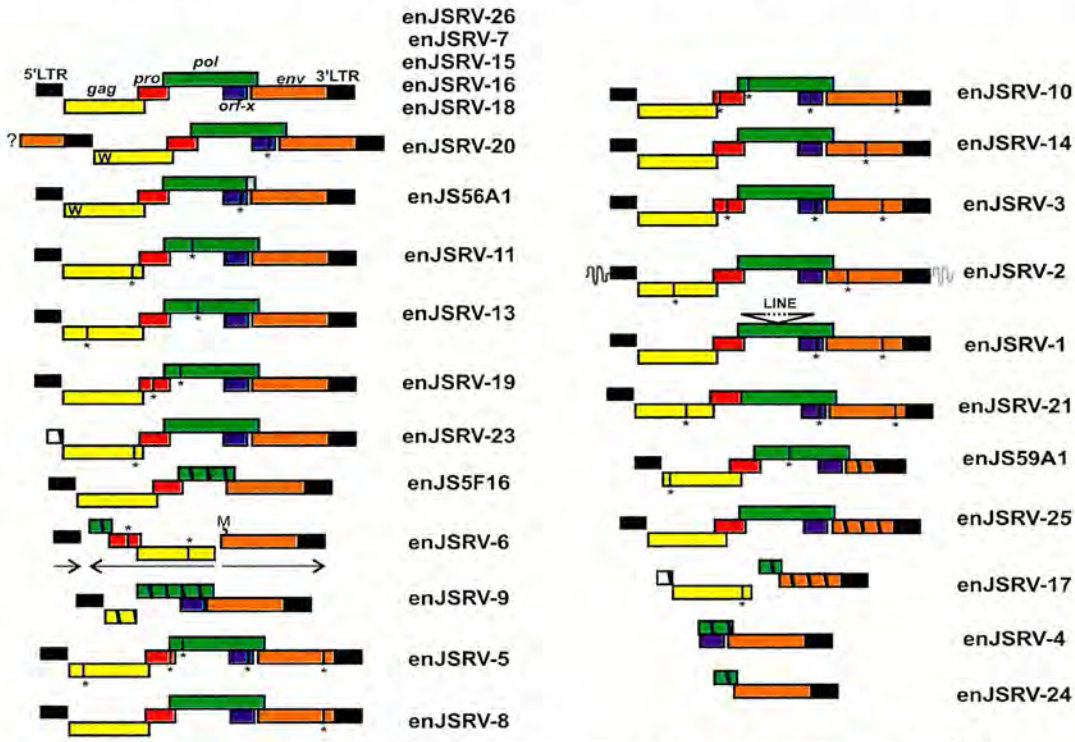

Fig. 3. Representative enJSRVs proviruses present within the sheep genome. Five enJSRVs display an intact genomic organization typical of replication competent proviruses (top). The "W" present in the Gag protein of enJS56A1 and enJSRV-20 indicates the R21W substitution that confers transdominant properties to these two proviruses. The 5′ flanking region of enJSRV-20 contains an *env* gene indicated by a box and a question mark (?). Vertical lines with an asterisk (*) represent stop codons, while hatched boxes indicate deletions. enJSRV-6 harbours a recombined structure with internal sequence in the opposite direction compared to the 5′ and 3′ LTRs of the provirus. The first methionine (indicated by the letter M) of the *env* gene of enJSRV-6 is present after the usual start codon. Figure reproduced from Arnaud and coworkers (Arnaud *et al.* 2007a).

Endogenous and exogenous betaretroviruses of domestic sheep: enJSRVs/JSRV

Domestic sheep harbor at least 27 copies of ERVs in their genome, termed enJSRVs (**Fig. 3**), because they are highly related to the exogenous and pathogenic Jaagsiekte sheep retrovirus (JSRV) (Arnaud *et al.* 2007a). JSRV is the causative agent of ovine pulmonary adenocarcinoma, a transmissible lung cancer of sheep (Palmarini *et al.* 1999). A unique feature of JSRV among oncogenic retroviruses is that its Env glycoprotein is the main determinant of cell transformation both *in vitro* and *in vivo* (Maeda *et al.* 2001, Wootton *et al.* 2005, Caporale *et al.* 2006). The JSRV Env is able to induce lung adenocarcinomas in immunocompetent sheep when expressed by a JSRV-based vector under the control of the JSRV LTR (Caporale *et al.* 2006). Although the mitogen activated protein kinase (Ras-MEK-MAPK), Rac1, and phosphoinositide 3-kinase (PI3K-AKT-mTOR) pathways are implicated in JSRV-induced cell transformation, it

still remains to be determined how the viral Env engages the cell signaling network to activate these pathways (Palmarini *et al.* 2001b, Maeda *et al.* 2005, De Las Heras *et al.* 2006, Varela *et al.* 2006, Maeda & Fan 2008).

The majority of the 27 enJSRV proviruses in sheep are defective as a result of deletions, nonsense mutations, and recombinations; however, five enJSRV proviruses contain intact genomes with uninterrupted open reading frames for all retroviral genes (**Fig. 3**) (Arnaud *et al.* 2007a). These enJSRV loci are insertionally polymorphic in the domestic sheep population. JSRV and enJSRVs have a high degree of similarity (~85-89% identity at the nucleotide level). enJSRVs are present in sheep and goats, but not cattle or other species. Integration of enJSRVs began before the split between the genus Ovis and the genus Capra, approximately 5 to 7 million years ago, and continued after sheep domestication (~ 10,000 years ago) (Arnaud *et al.* 2007a, Chessa *et al.* 2009) (**Fig. 4**). Interestingly, a particular enJSRV provirus, enJSRV-26, is thought to have integrated in the host less than 200 years ago and may be a unique integration event occurred in a single animal (Arnaud *et al.* 2007a). Thus, the enJSRVs are most likely still invading the sheep genome.

enJSRVs viral interference

enJSRVs can block JSRV replication at both early and late stages of the retroviral cycle (**Fig. 1**). Both JSRV and enJSRVs use the same cellular receptor for entry called HYAL2 (hyaluronidase 2), a glycosylphosphatidylinositol (GPI)-anchored protein (Rai *et al.* 2001, Arnaud *et al.* 2007a). As described for other viruses, enJSRVs Env can prevent JSRV entry by a classic mechanism of receptor interference (Spencer *et al.* 2003). In addition, two enJSRV loci (enJS56A1 and enJSRV-20) can block JSRV replication at a late stage of the retroviral replication cycle by a block referred to as JSRV late restriction (Mura *et al.* 2004, Arnaud *et al.* 2007a, Murcia *et al.* 2007). These two transdominant proviruses entered the host genome 3 million years ago before and during speciation within the genus *Ovis* (Arnaud *et al.* 2007a) (**Fig. 4**). They subsequently acquired in two temporally distinct events a defective Gag polyprotein *via* a substitution of an arginine at the position 21 (typical of a replication competent virus) to a tryptophan residue. JSRV late restriction likely occurs *via* the production of defective Gag protein by the transdominant proviruses that form viral particles and/or multimers with the functional Gag proteins, which then accumulate in the cytoplasm as pre-aggresome structures that are subsequently degraded by the proteasome. Therefore, the transdominant proviruses prevent Gag proteins of the competent virus to interact with the trafficking cellular machinery and ultimately the release of viral particles (Arnaud *et al.* 2007b, Murcia *et al.* 2007).

Available evidence strongly supports the hypothesis that selection of transdominant enJSRV loci protected sheep against infection with related exogenous retroviruses, including JSRV and perhaps enzootic nasal tumor virus or ENTV. Both proviruses with transdominant (protective) genotype/phenotypes became fixed in the host genome of the domestic sheep (*Ovis aries*), supporting the idea of their positive selection during or immediately before sheep domestication (9,000 years ago) (**Fig. 4**). These data support the hypothesis that ERVs could help the host to fight retroviral infections (Palmarini *et al.* 2004).

ERVs and placental development

ERVs have been speculated to play a physiological role in placenta morphogenesis for almost three decades considering that retroviral particles have been frequently observed in the reproductive tract (Kalter *et al.* 1975, Smith & Moore 1988, Harris 1991). In fact, ERVs are

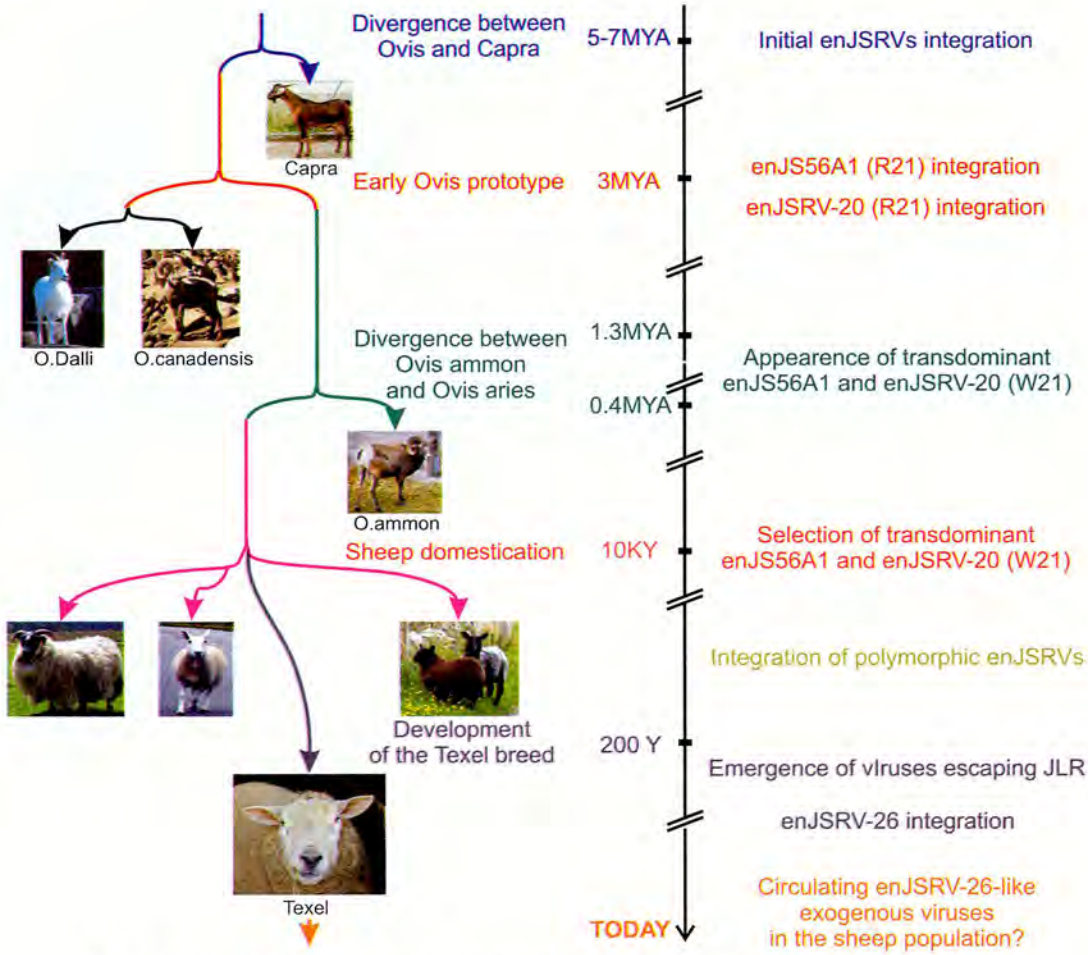

Fig. 4. Dates and events associating the evolutionary history of enJSRVs and their host. Schematic diagram illustrating key events of the evolutionary history of enJSRVs with estimated dates during the evolution of the domestic sheep and the Caprinae. Figure reproduced from Arnaud and coworkers (Arnaud *et al.* 2008).

abundant in the genital tract and placenta of various animal species (Harris 1991, Harris 1998). Indeed, a number of intact ERV *env* genes have been identified in primates (syncytin-1 and -2) (Venables *et al.* 1995, Blond *et al.* 2000, de Parseval *et al.* 2003), Muridae (syncytin-A and -B in mouse, rat, gerbil, vole, and hamster) (Dupressoir *et al.* 2005), and rabbits (syncytin-Ory1) (Heidmann *et al.* 2009). In each case, the protein products of the nonorthologous ERV *env* genes, termed syncytins, are highly fusogenic in transfection assays and preferentially expressed in the syncytiotrophoblast. The syncytiotrophoblast is a multinucleated cell that lines the outer surface of the placenta, derived by intercellular fusion of mononuclear cytotrophoblast cells, and responsible for the transport of oxygen, nutrients and waste products, production of hormones, and immune tolerance (Watson & Cross 2005). In both humans and mice, one of the two syncytins (human syncytin-2 and mouse syncytin-B) is immunosuppressive and, rather unexpectedly, the other (human syncytin 1 and mouse syncytin-A) is not, although both are

able to induce cell-cell fusion (Mangeney *et al.* 2007). Syncytin-A plays an important biological role in syncytiotrophoblast development, because *syncytin-A* null mice die *in utero* due to failure of trophoblast cells to fuse and form one of the two syncytiotrophoblast layers present in the mouse placenta (Dupressoir *et al.* 2009). Given that some syncytins are immunosuppressive, they may play a role in fetomaternal tolerance, although this concept has not been mechanistically tested *in vivo* (Mangeney *et al.* 2007). The presence of intact env genes that are expressed in the multinucleated syncytiotrophoblasts of the placenta and preserved over thousands of years, together with the observation that they elicit fusion of cells in vitro, led to the speculation that ERVs play an essential role in placental development and were positively selected for a convergent fundamental role in the evolution of placental mammals and development of viviparity (Villarreal 1997, Mi *et al.* 2000, Dupressoir *et al.* 2005, Heidmann *et al.* 2009).

In sheep, enJSRVs are abundantly expressed in the epithelia lining the different tissues of the female reproductive tract (vagina, cervix, uterus and oviduct) (Palmarini *et al.* 2000, Dunlap *et al.* 2005). In the uterus, both enJSRVs RNA and protein are detected specifically in the endometrial luminal epithelium (LE) and in the glandular epithelia (GE) (Spencer *et al.* 1999, Palmarini *et al.* 2000, Palmarini *et al.* 2001a). In addition, the enJSRVs *env* are expressed in the trophectoderm cells of the placenta in a temporal fashion that is coincident with key events in conceptus elongation and onset of trophoblast giant binucleate cell (BNC) differentiation (Dunlap *et al.* 2005). Within the placenta, enJSRVs are most abundant in the trophoblast giant BNC and multinucleated synytial plaques within the placentomes throughout pregnancy. The RNA of enJSRVs is first detected in the conceptus on day 12 (Dunlap *et al.* 2005). Interestingly, hyaluronoglucosaminidase 2 (HYAL2), a cellular receptor for both JSRV and enJSRVs Env (Rai *et al.* 2001, Arnaud *et al.* 2007a), is detected exclusively in the BNC and the multinucleated syncytial plaques of the placenta (Dunlap *et al.* 2005). These observations led to the hypothesis that enJSRVs and HYAL2 are important for placental growth and differentiation in sheep (Spencer *et al.* 2007). Indeed, injection of morpholinos that inhibit enJSRV Env production into the uteri of pregnant sheep on day 8 of pregnancy compromised conceptus elongation, resulting in reduced mononuclear trophoblast cell outgrowth and loss of trophoblast giant BNC differentiation (Dunlap *et al.* 2006). The biological role of HYAL2 in sheep conceptus development and differentiation has not been determined. **Figure 5** presents a current hypothesis on the biological roles of enJSRVs Env and HYAL2 in trophoblast development and differentiation in the sheep conceptus during early pregnancy.

Interestingly, the enJSRVs Env have a high degree of similarity with the oncogenic exogenous JSRV Env; thus, it is tempting to speculate that both endogenous and exogenous JSRV Env share similar mechanisms to induce trophoblast proliferation/differentiation and cell transformation, respectively, since placental morphogenesis has features similar to tumorigenesis and metastasis (Soundararajan & Rao 2004, Ferretti *et al.* 2007). Indeed, the Ras-MEK-MAPK, Rac1, and PI3K-AKT-mTOR signalling pathways involved in JSRV-induced cell transformation are important regulators of trophoblast growth and differentiation in human and rodent placentae (Pollheimer & Knofler 2005).

Conclusions

ERVs are present in the genomes of all vertebrates (Gifford & Tristem 2003) and can be used as DNA fossils to unravel virus-host coevolution over millions of years (Coffin 2004, Chessa *et al.* 2009). The domestic sheep constitutes a powerful model to study the biological significance

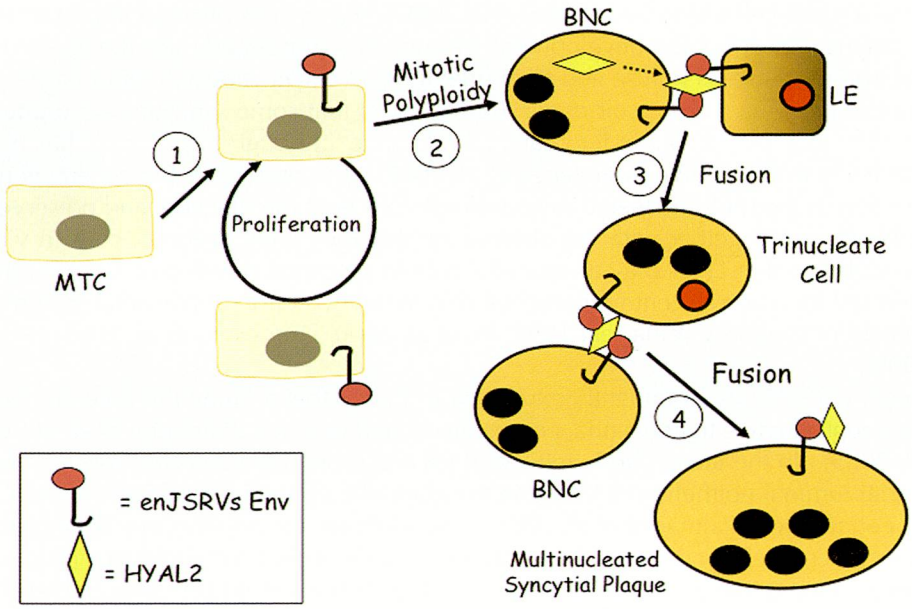

Fig. 5. Hypothesis on the biological role of enJSRVs Env and HYAL2 in trophoblast differentiation in sheep. During pregnancy, trophoblast giant binucleate cells (BNC) begin to differentiate from mononuclear trophoblast cells (MTC) on day 14. First, MTC begin to express enJSRVs envelope (Env) in the conceptus on day 12 (Step 1). Second, results from microscopy studies support the idea that binucleated trophectoderm cells or trophoblast giant BNC are derived from karyokinesis without cytokinesis (endoreduplication) or mitotic polyploidy (Step 2). Next, the newly formed BNC that are co-expressing enJSRVs *env* and *HYAL2* initially fuse with enJSRVs *env*-expressing endometrial luminal epithelial (LE) cells, forming a trinucleated fetomaternal hybrid cell (Step 3). During this period, the BNC and LE cells express enJSRV *env* RNA, whereas only the BNC express *HYAL2*. In fact, *HYAL2* mRNA is not detectable in uterine cells. By days 20 to 25, virtually all of the endometrial LE cells are fused with the BNC. Fourth, other newly formed BNC fuse with trinucleate cells to form a multinucleated syncytial plaque (Step 4). During most of gestation, the BNC continue to differentiate from the MTC and then fuse with each other and existing multinucleated syncytia to form multinucleated syncytial plaques with 20-25 nuclei. The multinucleated syncytial plaques and BNC form the basis of the cotyledons of the placenta that interdigitate with caruncles of the endometrium to develop and form placentomes.

of ERVs given the contemporary presence in this animal species of a pathogenic exogenous retrovirus (JSRV) and the biologically active enJSRVs (Arnaud *et al.* 2007a, Arnaud *et al.* 2008). During evolution, the first driving force that positively selected and fixed enJSRVs in sheep population was their ability to protect their host against infection by related pathogenic retroviruses (Palmarini *et al.* 2004, Arnaud *et al.* 2008). However, the enJSRVs Env are also essential for placental development in sheep (Dunlap *et al.* 2006). Collective evidence from studies of primates, rodents, rabbits and sheep supports the idea that independently acquired, nonorthologous ERVs were positively selected for a convergent physiological role in evolution and development of the placenta. Studies with enJSRVs and JSRV as well as other ERVs expressed in the placenta help us understand how ERVs evolved from infectious elements to essential genes (de Parseval & Heidmann 2005).

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References

Arnaud F, Caporale M, Varela M, Biek R, Chessa B, Alberti A, Golder M, Mura M, Zhang YP, Yu L, Pereira F, Demartini JC, Leymaster K, Spencer TE & Palmarini M 2007a A paradigm for virus-host coevolution: sequential counter-adaptations between endogenous and exogenous retroviruses. *PLoS Pathog* **3** e170.

Arnaud F, Murcia PR & Palmarini M 2007b Mechanisms of late restriction induced by an endogenous retrovirus. *J Virol* **81** 11441-11451.

Arnaud F, Varela M, Spencer TE & Palmarini M 2008 Coevolution of endogenous betaretroviruses of sheep and their host. *Cell Mol Life Sci* **65** 3422-3432.

Bannert N & Kurth R 2004 Retroelements and the human genome: new perspectives on an old relation. *Proc Natl Acad Sci U S A* **101 Suppl 2** 14572-14579.

Biemont C & Vieira C 2006 Genetics: junk DNA as an evolutionary force. *Nature* **443** 521-524.

Blond JL, Lavillette D, Cheynet V, Bouton O, Oriol G, Chapel-Fernandes S, Mandrand B, Mallet F & Cosset FL 2000 An envelope glycoprotein of the human endogenous retrovirus HERV-W is expressed in the human placenta and fuses cells expressing the type D mammalian retrovirus receptor. *J Virol* **74** 3321-3329.

Bock M & Stoye JP 2000 Endogenous retroviruses and the human germline. *Curr Opin Genet Dev* **10** 651-655.

Boeke JD & Stoye JP 1997 Retrotransposons, endogenous retroviruses and the evolution of retroelements. In *Retroviruses*, pp. 343-436.

Caporale M, Cousens C, Centorame P, Pinoni C, De Las Heras M & Palmarini M 2006 Expression of the jaagsiekte sheep retrovirus envelope glycoprotein is sufficient to induce lung tumors in sheep. *J Virol* **80** 8030-8037.

Chessa B, Pereira F, Arnaud F, Amorim A, Goyache F, Mainland I, Kao RR, Pemberton JM, Beraldi D, Stear MJ, Alberti A, Pittau M, Iannuzzi L, Banabazi MH, Kazwala RR, Zhang YP, Arranz JJ, Ali BA, Wang Z, Uzun M, Dione MM, Olsaker I, Holm LE, Saarma U, Ahmad S, Marzanov N, Eythorsdottir E, Holland MJ, Ajmone-Marsan P, Bruford MW, Kantanen J, Spencer TE & Palmarini M 2009 Revealing the history of sheep domestication using retrovirus integrations. *Science* **324** 532-536.

Coffin JM 2004 Evolution of retroviruses: fossils in our DNA. *Proc Am Philos Soc* **148** 264-280.

De Las Heras M, Ortin A, Benito A, Summers C, Ferrer LM & Sharp JM 2006 In-situ demonstration of mitogen-activated protein kinase Erk 1/2 signalling pathway in contagious respiratory tumours of sheep and goats. *J Comp Pathol* **135** 1-10.

de Parseval N & Heidmann T 2005 Human endogenous retroviruses: from infectious elements to human genes. *Cytogenet Genome Res* **110** 318-332.

de Parseval N, Lazar V, Casella JF, Benit L & Heidmann T 2003 Survey of human genes of retroviral origin: identification and transcriptome of the genes with coding capacity for complete envelope proteins. *J Virol* **77** 10414-10422.

Dunlap KA, Palmarini M, Adelson DL & Spencer TE 2005 Sheep endogenous betaretroviruses (enJSRVs) and the hyaluronidase 2 (HYAL2) receptor in the ovine uterus and conceptus. *Biol Reprod* **73** 271-279.

Dunlap KA, Palmarini M, Varela M, Burghardt RC, Hayashi K, Farmer JL & Spencer TE 2006 Endogenous retroviruses regulate periimplantation placental growth and differentiation. *Proc Natl Acad Sci U S A* **103** 14390-14395.

Dupressoir A, Marceau G, Vernochet C, Benit L, Kanellopoulos C, Sapin V & Heidmann T 2005 Syncytin-A and syncytin-B, two fusogenic placenta-specific murine envelope genes of retroviral origin conserved in Muridae. *Proc Natl Acad Sci U S A* **102** 725-730.

Dupressoir A, Vernochet C, Bawa O, Harper F, Pierron G, Opolon P & Heidmann T 2009 Syncytin-A knockout mice demonstrate the critical role in placentation of a fusogenic, endogenous retrovirus-derived, envelope gene. *Proc Natl Acad Sci U S A* **106** 12127-12132.

Ferretti C, Bruni L, Dangles-Marie V, Pecking AP & Bellet D 2007 Molecular circuits shared by placental and cancer cells, and their implications in the proliferative, invasive and migratory capacities of trophoblasts. *Hum Reprod Update* **13** 121-141.

Gifford R & Tristem M 2003 The evolution, distribution and diversity of endogenous retroviruses. *Virus Genes* **26** 291-315.

Harris JR 1991 The evolution of placental mammals. *FEBS Lett* **295** 3-4.

Harris JR 1998 Placental endogenous retrovirus (ERV): structural, functional, and evolutionary significance. *Bioessays* **20** 307-316.

Heidmann O, Vernochet C, Dupressoir A & Heidmann T 2009 Identification of an endogenous retroviral envelope gene with fusogenic activity and placenta-specific expression in the rabbit: a new "syncytin" in a third order of mammals. *Retrovirology* **6** 107.

Herniou E, Martin J, Miller K, Cook J, Wilkinson M &

Tristem M 1998 Retroviral diversity and distribution in vertebrates. *J Virol* **72** 5955-5966.

Jern P & Coffin JM 2008 Effects of retroviruses on host genome function. *Annu Rev Genet* **42** 709-732.

Kalter SS, Heberling RL, Helmke RJ, Panigel M, Smith GC, Kraemer DC, Hellman A, Fowler AK & Strickland JE 1975 A comparative study on the presence of C-type viral particles in placentas from primates and other animals. *Bibl Haematol* **40** 391-401.

Kurth R & Bannert N 2010 Beneficial and detrimental effects of human endogenous retroviruses. *Int J Cancer* **126** 306-314.

Maeda N & Fan H 2008 Signal transduction pathways utilized by enzootic nasal tumor virus (ENTV-1) envelope protein in transformation of rat epithelial cells resemble those used by jaagsiekte sheep retrovirus. *Virus Genes* **36** 147-155.

Maeda N, Fu W, Ortin A, de las Heras M & Fan H 2005 Roles of the Ras-MEK-mitogen-activated protein kinase and phosphatidylinositol 3-kinase-Akt-mTOR pathways in Jaagsiekte sheep retrovirus-induced transformation of rodent fibroblast and epithelial cell lines. *J Virol* **79** 4440-4450.

Maeda N, Palmarini M, Murgia C & Fan H 2001 Direct transformation of rodent fibroblasts by jaagsiekte sheep retrovirus DNA. *Proc Natl Acad Sci U S A* **98** 4449-4454.

Mangeney M, Renard M, Schlecht-Louf G, Bouallaga I, Heidmann O, Letzelter C, Richaud A, Ducos B & Heidmann T 2007 Placental syncytins: Genetic disjunction between the fusogenic and immunosuppressive activity of retroviral envelope proteins. *Proc Natl Acad Sci U S A* **104** 20534-20539.

Mi S, Lee X, Li X, Veldman GM, Finnerty H, Racie L, LaVallie E, Tang XY, Edouard P, Howes S, Keith JC, Jr. & McCoy JM 2000 Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature* **403** 785-789.

Mura M, Murcia P, Caporale M, Spencer TE, Nagashima K, Rein A & Palmarini M 2004 Late viral interference induced by transdominant Gag of an endogenous retrovirus. *Proc Natl Acad Sci U S A* **101** 11117-11122.

Murcia PR, Arnaud F & Palmarini M 2007 The transdominant endogenous retrovirus enJS56A1 associates with and blocks intracellular trafficking of Jaagsiekte sheep retrovirus Gag. *J Virol* **81** 1762-1772.

Palmarini M, Gray CA, Carpenter K, Fan H, Bazer FW & Spencer T 2001a Expression of endogenous betaretroviruses in the ovine uterus: effects of neonatal age, estrous cycle, pregnancy and progesterone. *J Virol* **75** 11319-11327.

Palmarini M, Hallwirth C, York D, Murgia C, de Oliveira T, Spencer T & Fan H 2000 Molecular cloning and functional analysis of three type D endogenous retroviruses of sheep reveals a different cell tropism from that of the highly related exogenous jaagsiekte sheep retrovirus. *Journal of Virology* **74** 8065-8076.

Palmarini M, Maeda N, Murgia C, De-Fraja C, Hofacre A & Fan H 2001b A phosphatidylinositol-3-kinase (PI-3K) docking site in the cytoplasmic tail of the Jaagsiekte sheep retrovirus transmembrane protein is essential for envelope-induced transformation of NIH3T3 cells. *Journal of Virology* **75** 11002-11009.

Palmarini M, Mura M & Spencer TE 2004 Endogenous betaretroviruses of sheep: teaching new lessons in retroviral interference and adaptation. *J Gen Virol* **85** 1-13.

Palmarini M, Sharp JM, De las Heras M & Fan H 1999 Jaagsiekte sheep retrovirus is necessary and sufficient to induce a contagious lung cancer in sheep. *Journal of Virology* **73** 6964-6972.

Pollheimer J & Knofler M 2005 Signalling pathways regulating the invasive differentiation of human trophoblasts: a review. *Placenta* **26 Suppl A** S21-30.

Rai SK, Duh FM, Vigdorovich V, Danilkovitch-Miagkova A, Lerman MI & Miller AD 2001 Candidate tumor suppressor HYAL2 is a glycosylphosphatidylinositol (GPI)-anchored cell-surface receptor for jaagsiekte sheep retrovirus, the envelope protein of which mediates oncogenic transformation. *Proc Natl Acad Sci U S A* **98** 4443-4448.

Smith CA & Moore HD 1988 Expression of C-type viral particles at implantation in the marmoset monkey. *Hum Reprod* **3** 395-398.

Soundararajan R & Rao AJ 2004 Trophoblast 'pseudotumorigenesis': significance and contributory factors. *Reprod Biol Endocrinol* **2** 15.

Spencer TE, Johnson GA, Bazer FW, Burghardt RC & Palmarini M 2007 Pregnancy recognition and conceptus implantation in domestic ruminants: roles of progesterone, interferons and endogenous retroviruses. *Reprod Fertil Dev* **19** 65-78.

Spencer TE, Mura M, Gray CA, Griebel PJ & Palmarini M 2003 Receptor usage and fetal expression of ovine endogenous betaretroviruses: implications for coevolution of endogenous and exogenous retroviruses. *J Virol* **77** 749-753.

Spencer TE, Stagg AG, Joyce MM, Jenster G, Wood CG, Bazer FW, Wiley AA & Bartol FF 1999 Discovery and characterization of endometrial epithelial messenger ribonucleic acids using the ovine uterine gland knockout model. *Endocrinology* **140** 4070-4080.

Varela M, Chow YH, Sturkie C, Murcia P & Palmarini M 2006 Association of RON tyrosine kinase with the Jaagsiekte sheep retrovirus envelope glycoprotein. *Virology* **350** 347-357.

Varela M, Spencer TE, Palmarini M & Arnaud F 2009 Friendly viruses: the special relationship between endogenous retroviruses and their host. *Ann N Y Acad Sci* **1178** 157-172.

Venables PJ, Brookes SM, Griffiths D, Weiss RA & Boyd MT 1995 Abundance of an endogenous retroviral envelope protein in placental trophoblasts suggests a biological function. *Virology* **211** 589-592.

Villarreal LP 1997 On viruses, sex, and motherhood. *J Virol* **71** 859-865.

Watson ED & Cross JC 2005 Development of structures and transport functions in the mouse placenta. *Physiology (Bethesda)* **20** 180-193.

Wootton SK, Halbert CL & Miller AD 2005 Sheep retrovirus structural protein induces lung tumours. *Nature* **434** 904-907.