Cell. Mol. Life Sci. 65 (2008) 3422 – 3432
1420-682X/08/213422-11
DOI 10.1007/s00018-008-8500-9

Cellular and Molecular Life Sciences

Coevolution of endogenous Betaretroviruses of sheep and their host

F. Arnaud[a,†], M. Varela[a,b,†], T. E. Spencer[c] and M. Palmarini[a,*]

[a] Institute of Comparative Medicine, University of Glasgow Veterinary School, 464 Bearsden Road, Glasgow, G61 1QH, Scotland (United Kingdom), Fax: +44 141 3302271, e-mail: m.palmarini@vet.gla.ac.uk
[b] Current address: Department of Veterinary Medicine, University of Cambridge, Madingley Road, CB3 0ES, Cambridge, England (United Kingdom)
[c] Laboratory for Uterine Biology and Pregnancy, Department of Animal Science, Texas A&M University, College Station, Texas (USA)

Online First 27 September 2008

Abstract. Sheep betaretroviruses offer a unique model system to study the complex interaction between retroviruses and their host. Jaagsiekte sheep retrovirus (JSRV) is a pathogenic exogenous retrovirus and the causative agent of ovine pulmonary adenocarcinoma. The sheep genome contains at least 27 copies of endogenous retroviruses (enJSRVs) highly related to JSRV. enJSRVs have played several roles in the evolution of the domestic sheep as they are able to block the JSRV replication cycle and play a critical role in sheep conceptus development and placental morphogenesis. Available data strongly suggest that some dominant negative enJSRV proviruses (i.e. able to block JSRV replication) have been positively selected during evolution. Interestingly, viruses escaping the transdominant enJSRV loci have recently emerged (less than 200 years ago). Thus, endogenization of these retroviruses may still be occurring today. Therefore, sheep provide an exciting and unique system to study retrovirus-host coevolution. (Part of a Multi-author Review)

Keywords. JSRV, enJSRVs, placenta, retrovirus, restriction factors, virus evolution, virus-host coevolution.

Introduction

Infection of a somatic cell by a retrovirus leads to stable integration of the DNA form of the viral genome (provirus) into the host cell genome [1]. Consequently, a provirus is intimately associated with an infected cell and will follow its fate whether it be mitosis or cell death like any other cellular gene. Retroviruses can also infect the germline of the host and in this case proviruses are transmitted vertically from generation to generation following Mendelian rules. Vertically transmitted retroviruses are called 'endogenous' retroviruses (ERVs) as opposed to 'exogenous' retroviruses which are instead transmitted horizontally from an infected to an uninfected host like any other virus [2, 3]. During evolution ERVs have colonized the genome of all eukaryotes where they account for a substantial portion of the genome (e.g. 8% of the human genome) [4].

The persistence of ERVs in the host genome is the result of a fine balance reached throughout evolution. ERVs are usually defective as a result of the accumulation of mutations, deletions, rearrangements and/or epigenetic modifications [2]. However, some ERVs have retained intact open reading frames (ORFs) for one or more of their genes after millions of years from the initial integration event, suggesting that they may be beneficial to their host. For example, some ERVs have the ability to interfere with the replication cycle of related pathogenic retroviruses [5–7]. In addition, accumulating evidence in humans, mice and sheep also support the idea that development of the placenta appears to require a functional envelope (Env)

† These authors contributed equally to this article.
* Corresponding author.

encoded by ERVs [8–14]. Thus, some ERVs may be viewed as having a symbiotic relationship with their host.

It is implicit that ERVs cannot have major deleterious effects for the host, otherwise they would be counter-selected during evolution. However, ERVs may persist if the deleterious effects they induce are transient or if they are counterbalanced by beneficial consequences. Most of the reported associations between disease and the expression of ERVs remain speculative [15]. However, ERV RNA can be copackaged with the genomes of exogenous retroviruses which can result in recombination and the appearance of novel pathogenic variants, as is the case for avian, murine and feline leukaemia viruses [2]. This phenomenon also generates concerns in the preparation of vectors for gene therapy since ERVs genomes present in packaging cell lines can contaminate therapeutic gene-delivery vehicles with replication-competent retroviruses.

ERVs can be classified into 'modern' and 'ancient' depending on whether original integration event of a determined provirus occurred after or before speciation [3]. Modern ERVs are closely related to infectious exogenous viruses, as they have not accumulated extensive deletions or mutations and in some cases they are still able to produce infectious viral particles. Some of these ERVs are often insertionally polymorphic, since they are not completely fixed in the population and are still undergoing endogenization, such as is the case for koalas and sheep [16, 17]. Ancient ERVs invaded the host genomes before speciation and thus are present in every individual of a particular species. Normally they have accumulated substantial mutations/deletions that render them replication-defective [3].

This review will focus on the endogenous betaretroviruses of sheep [18–20]. The domestic sheep (*Ovis aries*) provides a unique model system to study retrovirus-host coevolution in an outbred animal species. The pathogenic and oncogenic exogenous Jaagsiekte sheep retrovirus (JSRV) co-exists with highly related and biologically active ERVs, referred to as enJSRVs [18–21]. Several lines of evidence suggest that enJSRVs have become essential to sheep reproductive biology and have protected the host against incoming retroviral infections [13, 14, 21–23]. This article will review the current knowledge on these viruses and will focus in particular on the evidence suggesting that enJSRVs have been selected by the host, as they functioned as proper restriction factors against infection of related retroviruses.

Jaagsiekte sheep retrovirus

JSRV is the causative agent of ovine pulmonary adenocarcinoma (OPA), one of the major viral diseases of sheep and a unique large animal model for lung carcinogenesis [21, 24–26]. JSRV is a betaretrovirus phylogenetically related to simian and murine viruses such as Mason-Pfizer monkey virus and the mouse mammary tumour virus [21, 27]. OPA is characterized by the onset of a pulmonary adenocarcinoma originating from the differentiated epithelial cells of the distal lung (type II pneumocytes and Clara cells or a common precursor). *In vivo*, JSRV is preferentially expressed in tumour cells which originate from the differentiated epithelial cells of the lung, type II pneumocytes and Clara cells, although low levels of viral DNA and RNA are also found in lymphoid tissues [28–31] The viral long terminal repeats (LTRs), containing the promoter and enhancer regions, appear to be the main determinants of pulmonary tropism and interact *in vitro* with lung-specific transcription factors such as HNF-3β [32–35]. The genome of JSRV is approximately 7.5 Kb in length and harbours the typical retroviral genes gag, pro, pol and env (Fig. 1). The genes *gag*, *pro* and *pol* are in overlapping reading frames typical of betaretroviruses. The JSRV genome contains an additional ORF of unknown function referred to as *orf-x* and overlapping pol [21, 27, 36]. All the JSRV strains sequenced to date share an extremely high degree of similarity, suggesting that they are under minimal evolutionary pressure [27].

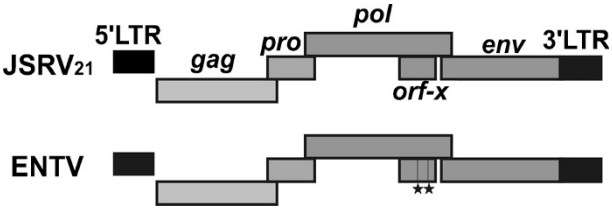

Figure 1. Genomic organization of the exogenous sheep betaretroviruses. JSRV (jaagsiekte sheep retrovirus) and ENTV (enzootic nasal tumor virus) show the typical retroviral genes *gag*, *pro*, *pol* and *env,* and two long terminal repeats (LTR). An open reading frame overlapping *pol* of unknown function termed *orf-x* is prematurely interrupted by two stop codons in ENTV (stop codons are shown by vertical bars and asterisks).

JSRV is unique among oncogenic retroviruses. Its envelope glycoprotein (Env) is a dominant oncoprotein as its expression is sufficient to induce cell transformation both *in vitro* [37–41] and *in vivo* [42, 43]. The retroviral Env is composed by a surface domain (SU) that interacts with the cellular receptor during viral entry and a transmembrane domain (TM)

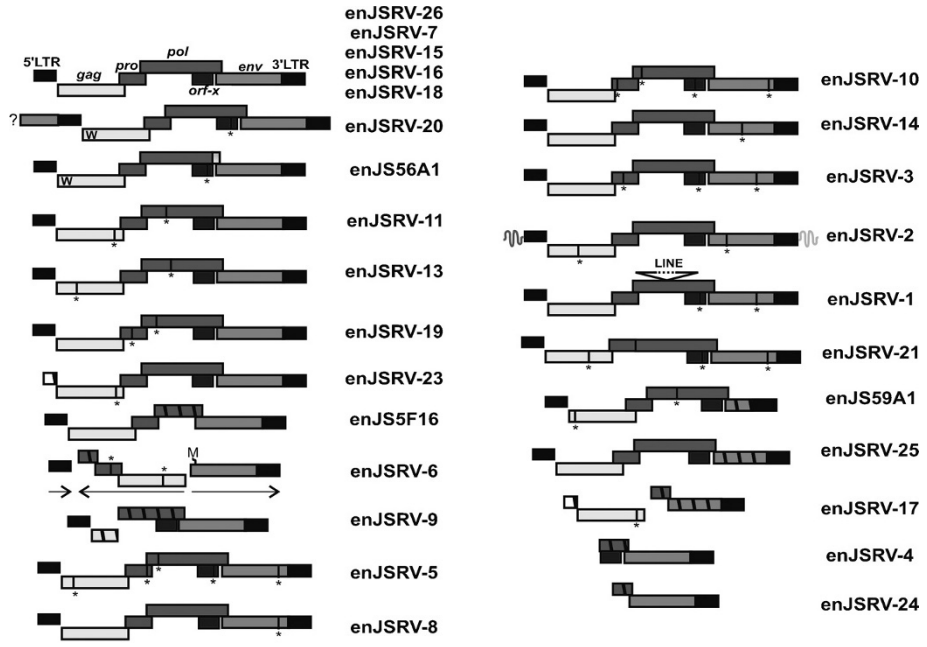

Figure 2. Genomic organization of the enJSRV proviruses present in the domestic sheep genome. Five enJSRVs display an intact genomic organization typical of replicative-competent proviruses (top). The 'W' in enJS56A1 and enJSRV-20 indicates the R21W substitution present in the Gag protein of these two transdominant proviruses. enJSRV-20 contains an *env* gene at its 5′ flanking region indicated by a box and a question mark (?). Stop codons are represented by vertical lines and an asterisk (*), whereas deletions are indicated by hatched boxes. enJSRV-6 harbours a recombined structure with internal sequence in the opposite direction compared to the rest of the provirus and the 5′ and 3′ LTRs. In enJSRV-6, the first methionine (indicated by the letter M) of the *env* gene is present after the usual start codon of the other enJSRV proviruses. enJSRV-2 does not possess a 6-bp duplication at the insertion sites of the provirus, likely suggesting a recombination event. The 3′ flanking regions of enJS56A1 and enJSRV-20 are essentially identical. enJSRV-1 contains a LINE element inserted within the *pol* gene. Figure reproduced from [16].

that fixes SU to the cell membrane. JSRV uses Hyaluronidase 2 as cellular receptor [39]. The main determinant of JSRV Env-induced cell transformation is present in the cytoplasmic tail (CT) of the TM domain. In particular, a Y-X-X-M motif in Env position 590–593 is critical for transformation of a variety of rodent and chicken cell lines [41, 44–46]. Although the mechanisms of transformation induced by JSRV Env are not completely understood, several pieces of evidence suggest that JSRV Env induces activation of the PI3 kinase-AKT (PI3K-AKT) and the Raf-MEK-MAPK signalling pathways [44, 46–49].

Enzootic nasal tumor virus (ENTV) is another exogenous betaretrovirus of sheep highly related to JSRV (Fig. 1). ENTV causes a nasal adenocarcinoma both in sheep and goats. ENTV shares many biological characteristics with JSRV. For example, both viruses use the same cellular receptor (Hyal-2) and both their Env proteins are dominant oncoproteins. Differences in the LTRs between JSRV and ENTV appear to be at the basis of the different tropism between these viruses [47, 50–53].

enJSRVs

The sheep genome contains at least 27 enJSRV proviruses (Fig. 2) [16, 19]. Most of the enJSRV loci possess defective genomes due to the presence of nonsense mutations, extensive deletions and recombinations; however, 5 of the 27 enJSRV proviruses (enJSRV-7, -15, -16, -18 and -26) display an intact genomic organization with uninterrupted ORF for all the retroviral genes. These proviruses are presumed to be recent integrations since 4 of them have identical 5′ and 3′ LTRs. In addition, enJSRV-16 and -18 are 100 % identical to each other at the nucleotide level. There is 85–89 % identity at the nucleotide level between the various enJSRVs and JSRV. The major differences lie in the U3 region within the LTR and in three regions along Gag and Env referred to as variable regions 1, 2 and 3 (VR1-2-3) [19]. VR1 and VR2 are 50 residues apart and reside in the matrix (MA) domain within *gag*, while VR3 comprises the last 67 amino acids of the Env glycoprotein. In particular, the CT of the TM domain of all enJSRVs Envs lack the Y-X-X-M motif that is present in the JSRV Env CT and is critical for virus-induced cell transformation. Indeed, none of the enJSRVs Env is able to induce transformation of rodent or chicken cell lines [16, 46].

Evolutionary history of enJSRVs

Retroviral integration into the genome of the host is essentially a random event. Thus, the evolutionary history of any particular ERV locus can be traced by determining its presence in different animals within the host species and within phylogenetically related species. The presence of a provirus in the same genomic location in animals of two different species indicates an integration event in the germline of a common ancestor before the split of those species. Thus, the 'age' of a provirus can be indirectly inferred by knowing the period during evolution in which two phylogenetically related species diverged [2].

Another way of estimating the age of a provirus is by determining the divergence of its 5′ and 3′ LTR and using this value as a molecular clock [54]. It can be assumed that the distal and proximal LTRs are identical at the time of proviral integration and divergence accumulated over time can be compared to the evolutionary rate of mammalian non-coding sequences. Thus, modern ERVs tend to have identical or nearly-identical 5′ and 3′ LTRs while ancient ERVs can show significant divergence, although homologous and heterologous recombination between proviruses can considerably alter these values [55–57].

Thus, the time of integration of enJSRVs can be estimated based on determining their presence in DNA samples from animals of different domestic sheep (*Ovis aries)* breeds and other related species within the Caprinae subfamily (Fig. 3). enJSRVs began to invade the sheep genome at least 5–7 million years ago, before the split between the genus *Ovis* (including domestic sheep and wild relatives) and the genus *Capra* (domestic goat and wild relatives) [58]. enJSRV proviruses have continued to invade the host genome throughout the evolution of the Caprinae and have continued to do so until very recently well after sheep domestication. Indeed, some enJSRV loci are restricted to *Ovis* spp. and present in all the species belonging to this genus (including the domestic sheep and species such as *Ovis nivicola, Ovis canadensis* and *Ovis dalli*) while others are present only in the domestic sheep (*Ovis aries*). Interestingly there are eight proviruses that are insertionally polymorphic within domestic sheep (e.g. these proviruses are present only in a proportion of domestic sheep). Sheep domestication began approximately 10000 years ago in South West Asia [59], and therefore it is possible to conclude that the insertionally polymorphic enJSRV loci have integrated less than 10000 years ago. Interestingly, there is evidence for at least one enJSRV provirus being less then 200 years old [16]. Thus, the invasion of the sheep genome by enJSRVs is likely still occurring today.

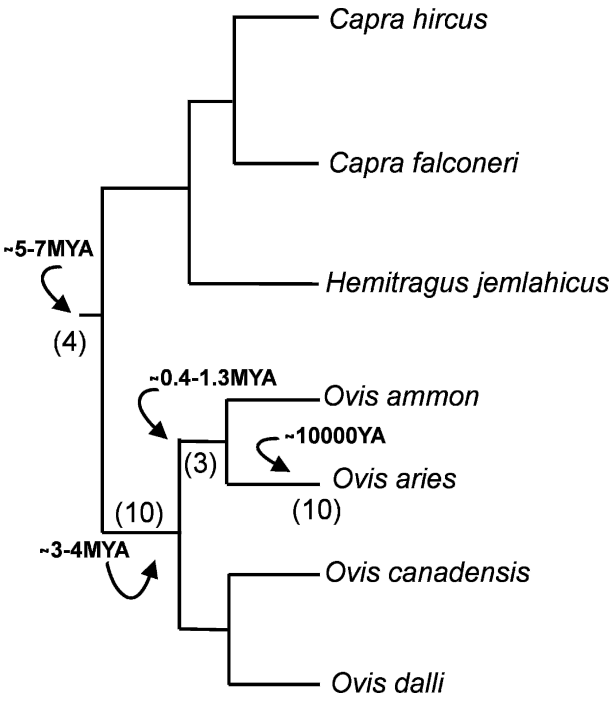

Figure 3. Insertion of enJSRV proviruses in the host genome. Simplified phylogenetic tree of the Caprinae, including the approximate time of divergence among major species. The approximate estimated dates of speciation between the *Ovis* and *Capra* genera as well as within the *Ovis* genus are indicated. The number of enJSRVs proviruses integrated at each time interval is indicated in parenthesis. Branch lengths are not shown to scale.

Role of enJSRVs in conceptus development and placenta morphogenesis

enJSRVs play a critical role in conceptus development and placental morphogenesis of sheep [13, 14, 23]. The earliest hints that enJSRVs could participate in some aspect of uteroplacental biology came from the observation that their RNA was particularly abundant in organs of the reproductive tract. However, enJSRV RNA could be detected by PCR-based assays in a variety of organs, including lungs, kidneys, thymus, bone marrow, spleen, mediastinal lymph nodes and leukocytes [19, 31, 60, 61]. The highest levels of enJSRV RNA are observed in the endometrial luminal and glandular epithelia of the uterus as well as in the epithelia of the oviducts and cervix [19, 31, 60, 61]. Lower levels of enJSRV RNA are also detected in the epithelia of the posterior and anterior regions of the vagina. *In situ* hybridization also detected enJSRV RNA in the lamina propria of the gut [19, 60, 61]. In the conceptus (embryo/foetus and associated extra-embryonic membranes), enJSRV RNA was observed in the mononuclear trophectoderm cells of the placenta, but was more abundant in the trophoblast giant binucleate cells (BNCs) and multinucleated syncytia, which are required for implantation and

form placentomes for nutrition of the conceptus [23]. Expression of enJSRV *env* in trophoblast cells starts at day 12 after mating, which is coincident with conceptus elongation and the production of interferon tau (IFNT), the pregnancy recognition signal [62]. Most interestingly, inhibition of enJSRV Env production by morpholino antisense oligonucleotides *in utero* retards blastocyst growth and elongation and inhibits trophoblast giant BNC differentiation, which culminates in loss of pregnancy [13]. These results, together with the fact that *HYAL2* mRNA, which functions as a cellular receptor for both JSRV and enJSRV [63], is detected in the trophoblast giant BNC and multinucleated syncytia of the conceptus [23], suggest that expression of enJSRV and HYAL2 is important for trophectoderm growth and differentiation in sheep [62]. Thus, sheep reproductive biology has become entirely dependent on enJSRV *env* expression. The abundant expression of ERVs in human and mouse placenta, in particular the presence of intact env genes in the syncytiotrophoblast, which have been preserved over millions of years, together with the observation that they elicit fusion of cells *in vitro*, has led to the speculation that independent ERVs were positively selected for a convergent biological role in placental morphogenesis during evolution [8–14].

In the endometrium, the level of enJSRV expression fluctuates during the oestrous cycle and early pregnancy. An increase in the abundance enJSRV RNA in the endometrial epithelium occurs between days 1 and 13, which correlates with an increase in the levels of progesterone and progesterone receptor [60, 64]. Moreover, the LTRs of some enJSRV loci respond moderately to progesterone in transient transfection assays [19]. Given that five of the enJSRV loci have intact ORF for all their genes, it is plausible that even enJSRV-derived viral particles are shed into the uterine lumen. Indeed, viral particles have been observed in the uterine epithelia and trophectoderm of the conceptus in sheep, although it is not known whether these particles have any biological function [65].

enJSRVs expression has also been investigated in sheep foetuses by *in situ* hybridization with an enJSRV env probe [63]. These experiments revealed a positive signal in the lymphoid cells of the lamina propria of the gut, in bronchial epithelial cells of the lungs and in the cortico-medullary junction of the thymus where it is thought that the final selection of T lymphocytes takes place. Expression of enJSRVs in these regions might tolerize sheep towards related exogenous betaretroviruses, and this could explain the lack of circulating antibodies against JSRV in OPA affected sheep [66, 67].

enJSRVs as restriction factors: early blockade

enJSRVs have been found to interfere with JSRV replication at early and late stages of the retroviral cycle. Expression of enJSRVs blocks viral entry probably by receptor interference, since JSRV is not able to enter a cell line derived from the ovine endometrial luminal epithelium (LE), which expresses high levels of enJSRVs. JSRV entry is not affected when the assay is performed using a cell line derived from the uterine stroma (ST), which does not express abundant enJSRVs [63]. The notion that enJSRVs block JSRV entry by receptor blockage is supported by the fact that enJSRVs and JSRV use the same cellular receptor for entry. This has been measured by the ability of murine leukemia virus (MLV)-based retroviral vectors pseudotyped with either enJSRVs or JSRV Env to transduce murine NIH-3T3 cells (normally resistant to JSRV entry) stably expressing the JSRV receptor [16, 63].

Thus, in addition to their vital function in placental development in sheep, enJSRVs Envs could also prevent JSRV (or enJSRV-like exogenous viruses) viral entry via receptor competition. The more recently integrated enJSRVs are insertionally polymorphic within the sheep population. Since the Env acts as an early restriction factor for JSRV, it can be speculated that the quantity and quality of enJSRVs present in the genome could influence the susceptibility or resistance of a particular individual or breed to JSRV infection.

enJSRVs as restriction factors: late blockade

enJSRVs can also block JSRV at late stages of the viral replication cycle. There are two proviruses whose expression has been proven to be transdominant towards JSRV: enJS56A1 and enJSRV-20. enJS56A1 and enJSRV-20 maintain intact ORFs for all the viral genes except for orf-x due to the presence of a premature stop codon. Moreover, enJS56A1 unlike enJSRV-20, contains a 2-bp deletion at the end of *pol*. Both enJS56A1 and enJSRV-20 can restrict JSRV viral exit via a mechanism known as JSRV late restriction (JLR) [68].

Cells transfected with an expression plasmid for enJS56A1 do not release viral particles in the supernatant despite abundant Gag expression that can be detected in cell lysates [19]. This defect is transdominant over JSRV and has been mapped to residue 21 of enJS56A1 Gag [68] within the MA protein. JSRV displays an arginine (R) in position 21 which is highly conserved among members of the genus *Betaretrovirus*, while enJS56A1 harbours a trypto-

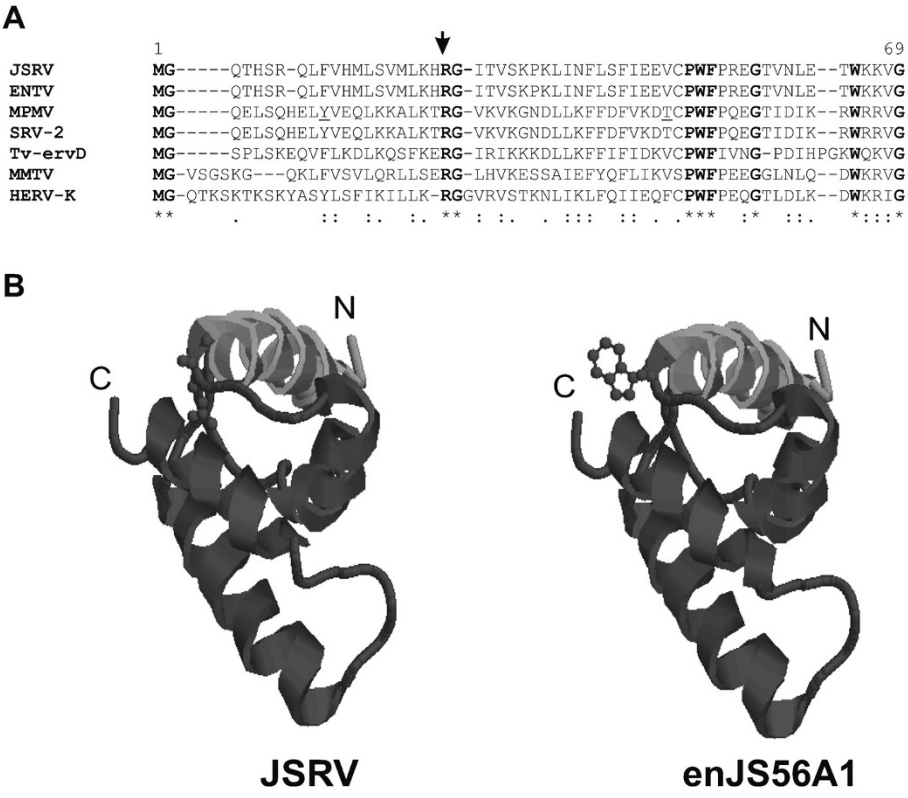

Figure 4. The residue W21 in enJS56A1 Gag is the major determinant of JSRV late restriction (JLR). (*A*) Alignment of the N-terminal amino acid sequence of the betaretroviruses Gag (JSRV = jaagsiekte sheep retrovirus; ENTV = enzootic nasal tumor virus; MPMV = Mason Pfizer monkey retrovirus; SRV-2 = simian retrovirus 2; Tv-ervD = brushtail possum type D endogenous retrovirus). Consensus symbols are shown as follows: '*' indicates identical residues, ':' indicates conserved substitutions, and '.' indicates semiconserved substitutions. Residue R21 in Gag is highly conserved in all betaretroviral sequences. (*B*) Modelling of the putative JSRV and enJS56A1 N-terminal matrix domains. The W21 and R21 residues are shown in a ball-and-stick format. The first α-helix is displayed in light grey. Both models were derived from the amino-terminal 92-amino acid residues of the JSRV/enJS56A1 Gag. The N and C terminals are indicated.

phan (W) (Fig. 4A). A single JSRV mutant where R21 is replaced with a tryptophan residue recapitulates the phenotype of enJS56A1 [68] (Fig. 4B).

JSRV and enJS56A1 Gag proteins co-localize very early after synthesis and co-immunoprecipitate when expressed in the same cell, suggesting that enJS56A1 acts in *trans* [22, 69]. Molecular complementation analysis indicates that enJS56A1 and JSRV likely co-assemble. Functional characterization of enJS56A1 and JSRV Gag proteins reveals that full-length enJS56A1 Gag proteins are necessary to restrict JSRV viral particle release. In particular, the deletion of the major homology region (MHR) of enJS56A1, a crucial domain of Gag necessary for viral assembly, impairs its ability to restrict JSRV, suggesting that proper assembly between JSRV and enJS56A1 is required for JLR to occur [69].

Betaretroviruses are believed to assemble in the pericentriolar region and to use the endosome trafficking machinery to be transported and released outside the cell [70, 71]. Interestingly, enJS56A1 and JSRV Gag proteins display different cellular localizations [69]. While JSRV Gag accumulates at the centrosome, enJSRV56A1 Gag mainly remains dispersed throughout the cytoplasm. Residue 21 in Gag is the main determinant of centrosomal targeting since the single mutant JSRV-R21W does not localize in the pericentriolar region, while the revertant mutant enJS56A1-W21R behaves as wild-type JSRV. As expected, centrosome targeting of JSRV is disrupted in cells co-expressing JSRV and enJS56A1 Gag, demonstrating that enJS56A1 Gag is transdominant over JSRV. Furthermore, the localization of JSRV Gag in the pericentriolar region is dependent on a functional microtubule network and dynein unlike enJS56A1 Gag localization [22]. In particular, JSRV hijacks the recycling endosome machinery since the block of the clathrin-dependent endocytosis or the selective knockdown by RNA interference of Rab11 proteins, involved in pericentriolar recycling endosome trafficking, dramatically reduces viral particle release [22, 72].

In order to further understand this unique viral interference mechanism, JSRV and enJS56A1 Gag intracellular localization and trafficking were studied in detail. By performing co-transfection experiments with enJS56A1 and a JSRV myristoylation mutant that accumulates at the centrosome, Arnaud and colleagues determined that JLR takes place before JSRV Gag reaches the pericentriolar region [16, 22, 73]. Unlike JSRV, enJS56A1 Gag proteins are found distant from the centrosome and do not co-localize with recycling endosomes. However, enJS56A1 Gag co-localizes with the aggresome marker GFP-250 [16, 22, 73]. This marker is a misfolded GFP protein that accumulates as aggresome precursors throughout the

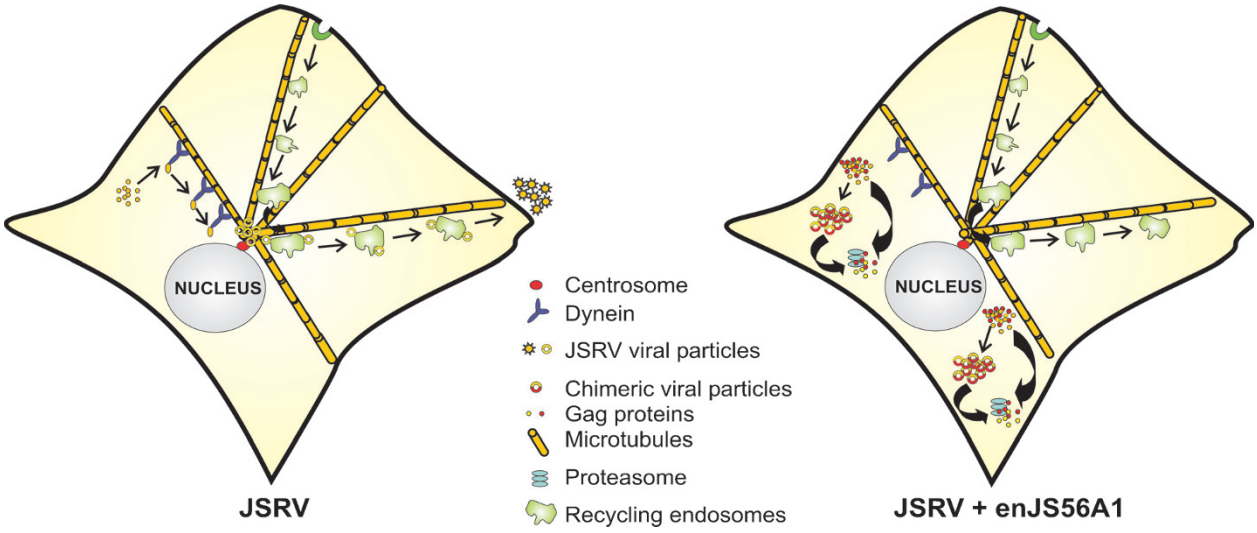

Figure 5. Model of JSRV Gag trafficking and JSRV late restriction. Figure reproduced from [22] with permission from the American Society for Microbiology.

cytoplasm. GFP-250 overexpression leads to the formation of an aggresome that accumulate at the centrosome where intermediate filaments of vimentin reorganize to form a cage. enJS56A1 Gag co-localizes with aggresome precursors dispersed in the cytoplasm but not with GFP-250 accumulated at the centrosome. Consistent with this result, proteasomal inhibitors trigger the formation of enJS56A1 Gag-containing aggresomes in a microtubule-dependent manner [22]. Thus, enJS56A1 Gag levels in the cell are maintained by continuous synthesis and rapid proteasome-mediated degradation which results in the formation of pre-aggresomal cytoplasmic bodies, while JSRV Gag proteins are not affected by proteasome inhibitors. As illustrated in Figure 5, available results support a model where the JSRV Gag, in absence of enJS56A1 Gag, reaches the pericentrosomal area where it engages the cellular trafficking machinery to reach the cell membrane. enJS56A1 Gag instead behaves like an unfolded or misfolded protein, probably due to the presence of a tryptophan residue in position 21 that potentially affects the general conformation of the protein. enJS56A1 Gag associates with JSRV Gag early after its synthesis, resulting in the formation of multimers and/or chimeric viral particles that are unable to traffic to the cell membrane and are ultimately sequestrated in pre-aggresome structures and degraded by the proteasomal machinery of the cell.

Sequential counteradaptations between endogenous and exogenous sheep betaretroviruses

The interplay between endogenous and exogenous retroviruses and their host has been difficult to study in outbred animal species. For example, the concept that ERVs can protect the host against incoming pathogenic retroviruses has been mainly developed experimentally in *in vitro* studies and in laboratory mice strains [1]. Thus, sheep have provided a unique model to study ERV-host co-evolution given the co-existence in this animal species of exogenous retroviruses and biologically active endogenous retroviruses that are still infecting the germline of the host [16].

As mentioned above both the transdominant enJS56A1 and enJSRV-20 possess a tryptophan residue in Gag position 21 (in place of the 'wild-type' arginine residue) which is the main determinant of JLR. These two proviruses entered the sheep genome within the last 3 million years (before and during speciation of the *Ovis* genus). However, most likely the exogenous virus from which these transdominant proviruses derived must have possessed an arginine residue in position 21 of Gag (and not the transdominant tryptophan) in order to replicate and successfully infect the germline of the host.

Thus, sheep can be used as a model system to address the question of whether transdominant interfering proviruses have indeed played a role in protecting the host against related exogenous viruses. Strong clues on whether the transdominant forms of these two proviruses have been subject of purifying selection have been obtained by tracking the presence/absence

of enJS56A1 and enJSRV-20 (with R21 or W21 in Gag) in *Ovis* spp. Available data suggest that indeed both proviruses have been positively selected in the domestic sheep [16]. enJS56A1 and enJSRV-20 possess W21 in 31/31 samples from *Ovis aries* (domestic sheep) of various breeds tested (Fig. 6). The argali (*Ovis ammon*, a species closely related to the domestic sheep) possess both enJS56A1 and enJSRV-20 bearing either an arginine or a tryptophan in position 21. However, the same proviruses harbour only the arginine residue in the North American wild sheep species *Ovis nivicola, Ovis canadensis* and *Ovis dalli,* which are more distantly related to the domestic sheep than the argali [16]. Thus, available data suggest that (i) enJS56A1 and enJSRV-20 acquired defective and transdominant Gag proteins in two temporally distinct events during evolution; (ii) these two transdominant proviruses were positively selected before or during sheep domestication around 10 000 years ago; and (iii) became fixed in the sheep genome. Indeed, enJS56A1 and enJSRV-20 Gag proteins displaying an interfering phenotype would have provided protection against an exogenous pathogenic betaretrovirus like JSRV. However, the exogenous JSRV currently circulating is preferentially expressed in the respiratory tract, unlike enJSRVs, which are mostly expressed in the genital tract. Thus, it is possible that the ancestor of the currently circulating JSRV may have had a tropism for the reproductive tract. enJSRVs expression may have provided a selective pressure on the exogenous virus to switch tropism from the genital tract towards the respiratory tract where there is low or no enJSRV expression.

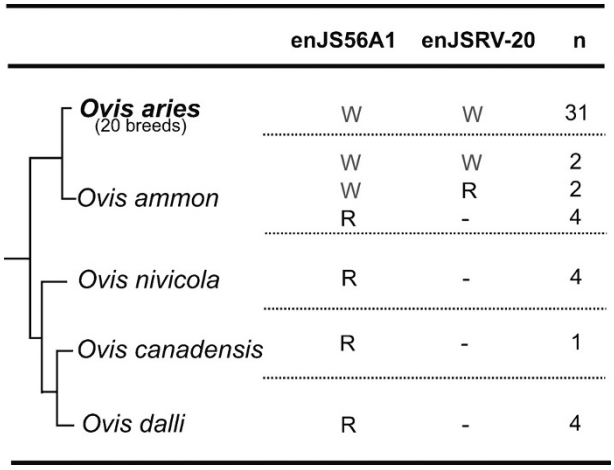

Figure 6. Fixation of the Gag R21W substitution in the transdominant proviruses enJS56A1 and enJSRV-20. Schematic representation of the genotypes of enJS56A1 and/or enJSRV-20 in *Ovis* species. The phylogenetic tree indicates only relationships and is not proportional to time. Figure reproduced from [16].

As mentioned before, five enJSRV proviruses (enJSRV-7, -15, -16, -18 and -26) have an intact genomic structure, are able to release viral particles when expressed *in vitro* and are insertionally polymorphic in the sheep population, suggesting that their original integration occurred $< 10\,000$ years ago [16]. enJSRV-26 in particular is extremely rare and was found only in a Texel ram (whose DNA was used to construct the BAC library screened for enJSRV proviruses) [16]. None of the 330 sheep DNA samples tested from different breeds, including Texel sheep from different geographical locations and some relatives of the enJSRV-26-positive ram, harboured enJSRV-26. Thus, given the history of the selection of the Texel breed, it appears that enJSRV-26 integrated within the last 200 years and may be a unique integration event in a single animal [16].

Interestingly, enJSRV-26, which is the most recent enJSRV provirus detected so far, is able to escape JLR induced by enJS56A1 and enJSRV-20. On the other hand, the enJSRVs that are able to express viral particles are fully susceptible to JLR analogously to the exogenous JSRV. Therefore, proviruses escaping JLR emerged and infected the germline less than 200 years ago. The selection of escape mutants for JLR indicates that the transdominant enJS56A1/enJSRV-20 have indeed been used as true restriction factors against related exogenous retroviruses (Fig. 7).

Conclusions

Sheep betaretroviruses and their hosts offer a unique model system to study the biology and the biological relevance of endogenous retroviruses. It is clear that enJSRVs have played several roles in the evolution of this animal species by interfering with the replication of exogenous retroviruses and by facilitating early sheep embryo development. We speculate that the protection of the host against pathogenic retroviruses was the primary reason for the selection of enJSRVs in the host, while their role in reproduction biology can be seen as an 'acquired' secondary role.

enJSRVs are preferentially expressed in the epithelium of the genital tract and especially in the uterus. Given that the tropism of enJSRVs appears to be driven mainly by their LTRs (which are remarkably similar within all proviruses), it is also probable that the exogenous 'ancestor' that originated these viruses possessed a tropism for the genital tract and the uterus. Any viral infection in the uterus would have most likely had catastrophic consequences for reproduction. Thus, it is conceivable that an epidemic of such virus would favour selection of those animals with enJSRV proviruses with transdominant properties

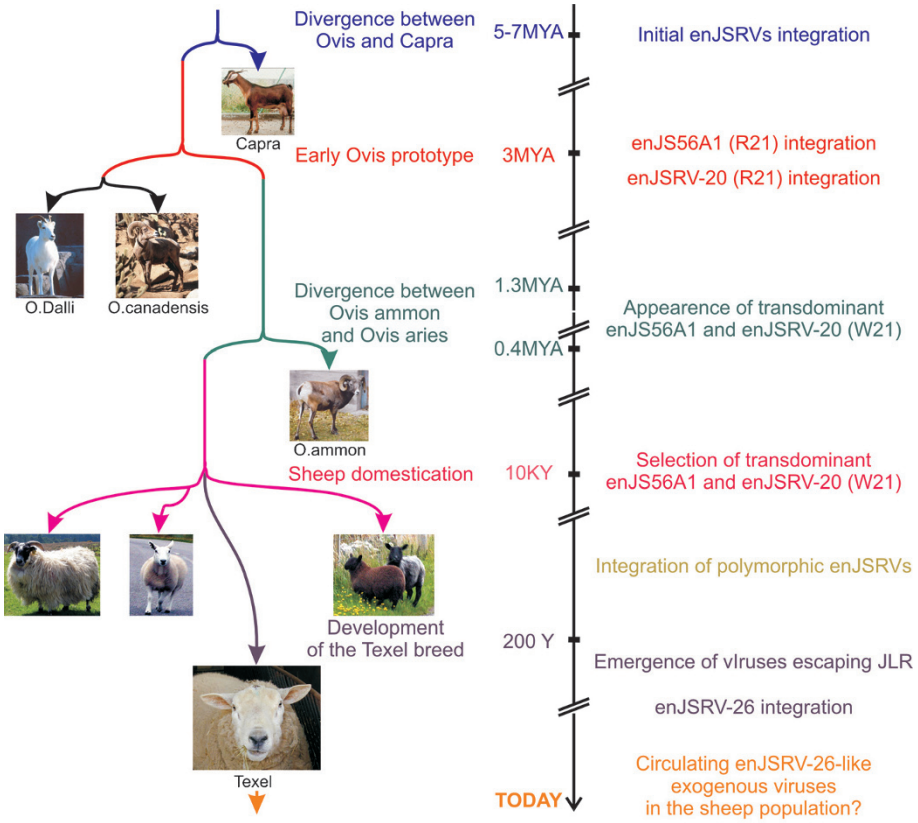

Figure 7. Evolutionary history of enJSRVs and their host.

over their exogenous counterpart. In turn, enJSRVs may have favoured the selection of the exogenous JSRV circulating today with a tropism for the respiratory tract (conferred by its LTRs).

Available data suggest that 'endogenization' of enJSRVs in the sheep genome is still ongoing and has not reached equilibrium. enJSRV-26, the most recent enJSRV provirus, is able to escape JLR conferred by enJS56A1 and enJSRV-20 and likely represents another exogenous sheep betaretrovirus that is currently circulating undetected in the sheep population. The recent selection of viruses escaping JLR is the strongest evidence to date that ERVs have been used by the host as one of their innate mechanisms to combat retroviral infections.

It is unlikely that the initial selection of enJSRVs in the host was due to their role in conceptus development and placental morphogenesis. These are fundamental processes that from the evolutionary point of view are older than enJSRVs and must have been possessed by the host before the colonization by these viruses. Instead, it is more likely that enJSRVs 'accidentally' favoured conceptus development and increased reproductive fitness of the host. Subsequently, during evolution the host may have lost its own mechanisms governing these reproductive processes as they became redundant. The placenta is one of the most diverse organs among major mammalian taxa. It is possible that different ERVs have played a convergent role in the development of the placenta in different animal species.

In conclusion, the three-way interaction between enJSRVs, their exogenous counterparts and domestic sheep provides a unique look into the complex interplay between retroviruses and their hosts during evolution.

Acknowledgments. We are grateful to the members of the Laboratory of Viral Pathogenesis of the University of Glasgow Faculty of Veterinary Medicine for stimulating discussions. Work in the laboratory of the authors is supported by a programme grant of the Wellcome Trust, by a Strategic Research Developmental Grant by the Scottish Funding Council and by NIH grant HD052745. M.P. is a Wolfson-Royal Society Research Merit Awardee.

1 Coffin, J. M., Hughes, S. H. and Varmus, H. E. (1997) pp. 843 Colld Spring Harbor Laboratory Press, New York.
2 Boeke, J. D. and Stoye, J. P. (1997) in: Retroviruses, pp. 343–436 (Coffin, J. M., Hughes, S. H. and Varmus, H. E. (eds.), Cold Spring Harbor Laboratory Press, Plainview, NY.
3 Coffin, J. M. (2004) Evolution of retroviruses: fossils in our DNA. Proc. Am. Philos. Soc. 148, 264–280.
4 Gifford, R. and Tristem, M. (2003) The evolution, distribution and diversity of endogenous retroviruses. Virus Genes 26, 291–315.
5 Gardner, M. B., Kozak, C. A. and O'Brien, S. J. (1991) The Lake Casitas wild mouse: evolving genetic resistance to retroviral disease. Trends Genet. 7, 22–27.

6 Kozak, C. A., Gromet, N. J., Ikeda, H. and Buckler, C. E. (1984) A unique sequence related to the ecotropic murine leukemia virus is associated with the Fv-4 resistance gene. Proc. Natl. Acad. Sci. USA 81, 834–837.

7 Payne, L. N. and Pani, P. K. (1971) A dominant epistatic gene which inhibits cellular susceptibility to RSV (RAV-0). J. Gen. Virol. 13, 455–462.

8 Blaise, S., de Parseval, N. and Heidmann, T. (2005) Functional characterization of two newly identified Human Endogenous Retrovirus coding envelope genes. Retrovirology 2, 19.

9 Blond, J. L., Beseme, F., Duret, L., Bouton, O., Bedin, F., Perron, H., Mandrand, B. and Mallet, F. (1999) Molecular characterization and placental expression of HERV-W, a new human endogenous retrovirus family [In Process Citation]. J. Virol. 73, 1175–1185.

10 Dupressoir, A., Marceau, G., Vernochet, C., Benit, L., Kanellopoulos, C., Sapin, V. and 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. USA 102, 725–730.

11 Mallet, F. , Bouton, O., Prudhomme, S., Cheynet, V., Oriol, G., Bonnaud, B., Lucotte, G., Duret, L., Mandrand, B. (2004) The endogenous retroviral locus ERVWE1 is a bona fide gene involved in hominoid placental physiology. Proc. Natl. Acad. Sci. USA 101, 1731–1736.

12 Mi, S., Lee, X., Li, X., Veldman, G. M., Finnerty, H., Racie, L., LaVallie, E., Tang, X. Y., Edouard, P., Howes, S. et al. (2000) Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis Nature 403, 785–789.

13 Dunlap, K. A., Palmarini, M., Varela, M., Burghardt, R. C., Hayashy, K., Farmer, J. L. and Spencer, T. E. (2006) Endogenous retroviruses regulate peri-implantation conceptus growth and differentiation. Proc. Natl. Acad. Sci. USA 103, 14390–14395.

14 Dunlap, K. A., Palmarini, M. and Spencer, T. E. (2006) Ovine endogenous betaretroviruses (enJSRVs) and placental morphogenesis. Placenta Suppl. A, S135–S140.

15 Moyes, D., Griffiths, D. J. and Venables, P. J. (2007) Insertional polymorphisms: a new lease of life for endogenous retroviruses in human disease. Trends Genet. 23, 326–333.

16 Arnaud, F., Caporale, M., Varela, M., Biek, R., Chessa, B., Alberti, A., Golder, M., Mura, M., Zhang, Y. P., Yu, L. et al. (2007) A paradigm for virus-host coevolution: sequential counter-adaptations between endogenous and exogenous retroviruses. PLoS Pathogens 3, e170.

17 Tarlinton, R. E., Meers, J. and Young, P. R. (2006) Retroviral invasion of the koala genome. Nature 442, 79–81.

18 DeMartini, J. C., Carlson, J. O., Leroux, C., Spencer, T. and Palmarini, M. (2003) Endogenous retroviruses related to jaagsiekte sheep retrovirus. Curr. Top. Microbiol. Immunol. 275, 117–137.

19 Palmarini, M., Hallwirth, C., York, D., Murgia, C., de Oliveira, T., Spencer, T. and Fan, H. (2000) Molecular cloning and functional analysis of three type D endogenous retroviruses of sheep reveal a different cell tropism from that of the highly related exogenous jaagsiekte sheep retrovirus. J. Virol. 74, 8065–8076.

20 Palmarini, M., Mura, M. and Spencer, T. E. (2004) Endogenous betaretroviruses of sheep: teaching new lessons in retroviral interference and adaptation. J. Gen. Virol. 85, 1–13.

21 Palmarini, M., Sharp, J. M., De las Heras, M. and Fan, H. (1999) Jaagsiekte sheep retrovirus is necessary and sufficient to induce a contagious lung cancer in sheep. J. Virol. 73, 6964–6972.

22 Arnaud, F., Murcia, P. R. and Palmarini, M. (2007) Mechanisms of late restriction induced by an endogenous retrovirus. J. Virol. 81, 1762–1772.

23 Dunlap, K. A., Palmarini, M., Adelson, D. L. and Spencer, T. E. (2005) Sheep endogenous betaretroviruses (enJSRVs) and the hyaluronidase 2 (HYAL2) receptor in the ovine uterus and conceptus. Biol. Reprod. 73, 271–279.

24 Fan, H. (2003) Jaagsiekte Sheep Retrovirus and Lung Cancer, Springer-Verlag, Berlin.

25 Palmarini, M. and Fan, H. (2001) Retrovirus-induced ovine pulmonary adenocarcinoma, an animal model for lung cancer. J. Natl. Cancer Inst. 93, 1603–1614.

26 Palmarini, M., Fan, H. and Sharp, J. M. (1997) Sheep pulmonary adenomatosis: a unique model of retrovirus-associated lung cancer. Trends Microbiol. 5, 478–483.

27 Palmarini, M. and Fan, H. (2003) Molecular biology of jaagsiekte sheep retrovirus. Curr. Top. Microbiol. Immunol. 275, 81–115.

28 Platt, J. A., Kraipowich, N., Villafane, F. and DeMartini, J. C. (2002) Alveolar type II cells expressing jaagsiekte sheep retrovirus capsid protein and surfactant proteins are the predominant neoplastic cell type in ovine pulmonary adenocarcinoma. Vet. Pathol. 39, 341–352.

29 Palmarini, M., Cousens, C., Dalziel, R. G., Bai, J., Stedman, K., DeMartini, J. C. and Sharp, J. M. (1996) The exogenous form of Jaagsiekte retrovirus is specifically associated with a contagious lung cancer of sheep. J. Virol. 70, 1618–1623.

30 Palmarini, M., Dewar, P., De las Heras, M., Inglis, N. F., Dalziel, R. G. and Sharp, J. M. (1995) Epithelial tumour cells in the lungs of sheep with pulmonary adenomatosis are major sites of replication for Jaagsiekte retrovirus. J. Gen. Virol. 76, 2731–2737.

31 Palmarini, M., Holland, M. J., Cousens, C., Dalziel, R. G. and Sharp, J. M. (1996) Jaagsiekte retrovirus establishes a disseminated infection of the lymphoid tissues of sheep affected by pulmonary adenomatosis. J. Gen. Virol. 77, 2991–298.

32 McGee-Estrada, K. and Fan, H. (2006) In vivo and in vitro analysis of factor binding sites in Jaagsiekte sheep retrovirus long terminal repeat enhancer sequences: roles of HNF-3, NF-I, and C/EBP for activity in lung epithelial cells. J. Virol. 80, 332–341.

33 McGee-Estrada, K., Palmarini, M. and Fan, H. (2002) HNF-3ß is a critical factor for the expression of the Jaagsiekte sheep retrovirus (JSRV) long terminal repeat in type II pneumocytes but not in clara cells. Virology 292, 87–97.

34 McGee-Estrada, K., Palmarini, M., Hallwirth, C. and Fan, H. (2005) A Moloney murine leukemia virus driven by the Jaagsiekte sheep retrovirus enhancers shows enhanced specificity for infectivity in lung epithelial cells. Virus Genes 31, 257–263.

35 Palmarini, M., Datta, S., Omid, R., Murgia, C. and Fan, H. (2000) The long terminal repeats of Jaagsiekte sheep retrovirus (JSRV) are preferentially active in type II pneumocytes. J. Virol. 74, 5776–5787.

36 Rosati, S., Pittau, M., Alberti, A., Pozzi, S., York, D. F., Sharp, J. M. and Palmarini, M. (2000) An accessory open reading frame (orf-x) of jaagsiekte sheep retrovirus is conserved between different virus isolates. Virus Res. 66, 109–116.

37 Allen, T. E., Sherrill, K. J., Crispell, S. M., Perrott, M. R., Carlson, J. O. and DeMartini, J. C. (2002) The jaagsiekte sheep retrovirus envelope gene induces transformation of the avian fibroblast cell line DF-1 but does not require a conserved SH2 binding domain. J. Gen. Virol. 83, 2733–2742.

38 Maeda, N., Palmarini, M., Murgia, C. and Fan, H. (2001) Direct transformation of rodent fibroblasts by jaagsiekte sheep retrovirus DNA. Proc. Natl. Acad. Sci. USA 98, 4449–4454.

39 Rai, S. K., Duh, F. M., Vigdorovich, V., Danilkovitch-Miagkova, A., Lerman, M. I. and Miller, A. D. (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. USA 98, 4443–4448.

40 Liu, S. L. and Miller, A. D. (2005) Transformation of madin-darby canine kidney epithelial cells by sheep retrovirus envelope proteins. J. Virol. 79, 927–933.

41 Zavala, G., Pretto, C., Chow, Y. H., Jones, L., Alberti, A., Grego, E., De las Heras, M. and Palmarini, M. (2003) Relevance of Akt phosphorylation in cell transformation induced by Jaagsiekte sheep retrovirus. Virology 312, 95–105.

42 Caporale, M., Cousens, C., Centorame, P., Pinoni, C., De las Heras, M. and Palmarini, M. (2006) Expression of the Jaagsiekte sheep retrovirus envelope glycoproteins is sufficient to induce lung tumor in sheep. J. Virol. 80, 8030–8037.

43 Wootton, S. K., Halbert, C. L. and Miller, A. D. (2005) Sheep retrovirus structural protein induces lung tumours. Nature 434, 904–907.

44 Liu, S. L., Lerman, M. I. and Miller, A. D. (2003) Putative phosphatidylinositol 3-kinase (PI3K) binding motifs in ovine betaretrovirus Env proteins are not essential for rodent fibroblast transformation and PI3K/Akt activation. J. Virol. 77, 7924–7935.

45 Cousens, C., Maeda, N., Murgia, C., Dagleish, M. P., Palmarini, M. and Fan, H. (2007) In vivo tumorigenesis by Jaagsiekte sheep retrovirus (JSRV) requires Y590 in Env TM, but not full-length orfX open reading frame. Virology 367, 413–421.

46 Palmarini, M., Maeda, N., Murgia, C., De-Fraja, C., Hofacre, A. and Fan, H. (2001) A phosphatidylinositol 3-kinase docking site in the cytoplasmic tail of the Jaagsiekte sheep retrovirus transmembrane protein is essential for envelope-induced transformation of NIH 3T3 cells. J. Virol. 75, 11002–11009.

47 Liu, S. L., Duh, F. M., Lerman, M. I. and Miller, A. D. (2003) Role of virus receptor Hyal2 in oncogenic transformation of rodent fibroblasts by sheep betaretrovirus env proteins. J. Virol. 77, 2850–2858.

48 De Las Heras, M., Ortin, A., Benito, A., Summers, C., Ferrer, L. M. and Sharp, J. M. (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.

49 Maeda, N., Fu, W., Ortin, A., de las Heras, M. and 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.

50 Alberti, A., Murgia, C., Liu, S. L., Mura, M., Cousens, C., Sharp, M., Miller, A. D. and Palmarini, M. (2002) Envelope-induced cell transformation by ovine betaretroviruses. J. Virol. 76, 5387–5394.

51 Ortin, A., Cousens, C., Minguijon, E., Pascual, Z., Villarreal, M. P., Sharp, J. M. and Heras Mde, L. (2003) Characterization of enzootic nasal tumour virus of goats: complete sequence and tissue distribution. J. Gen. Virol. 84, 2245–2252.

52 Cousens, C., Minguijon, E., Dalziel, R. G., Ortin, A., Garcia, M., Park, J., Gonzalez, L., Sharp, J. M. and de las Heras, M. (1999) Complete sequence of enzootic nasal tumor virus, a retrovirus associated with transmissible intranasal tumors of sheep. J. Virol. 73, 3986–3993.

53 Cousens, C., Minguijon, E., Garcia, M., Ferrer, L. M., Dalziel, R. G., Palmarini, M., De las Heras, M. and Sharp, J. M. (1996) PCR-based detection and partial characterization of a retrovirus associated with contagious intranasal tumors of sheep and goats. J. Virol. 70, 7580–7583.

54 Johnson, W. E. and Coffin, J. M. (1999) Constructing primate phylogenies from ancient retrovirus sequences. Proc. Natl. Acad. Sci. USA 96, 10254–10260.

55 Hughes, J. F. and Coffin, J. M. (2001) Evidence for genomic rearrangements mediated by human endogenous retroviruses during primate evolution. Nat. Genet. 29, 487–489.

56 Hughes, J. F. and Coffin, J. M. (2004) Human endogenous retrovirus K solo-LTR formation and insertional polymorphisms: implications for human and viral evolution. Proc. Natl. Acad. Sci. USA 101, 1668–1672.

57 Hughes, J. F. and Coffin, J. M. (2005) Human endogenous retroviral elements as indicators of ectopic recombination events in the primate genome. Genetics 171, 1183–1194.

58 Hernandez Fernandez, M. and Vrba, E. S. (2005) A complete estimate of the phylogenetic relationships in Ruminantia: a dated species-level supertree of the extant ruminants. Biol. Rev. Camb. Philos. Soc. 80, 269–302.

59 Ryder, M. L. (1983) Sheep and Man. Gerald Duckworth, London.

60 Palmarini, M., Gray, C. A., Carpenter, K., Fan, H., Bazer, F. W. and Spencer, T. E. (2001) Expression of endogenous betaretroviruses in the ovine uterus: effects of neonatal age, estrous cycle, pregnancy, and progesterone. J. Virol. 75, 11319–11327.

61 Spencer, T. E., Stagg, A. G., Joyce, M. M., Jenster, G., Wood, C. G., Bazer, F. W., Wiley, A. A. and Bartol, F. F. (1999) Discovery and characterization of endometrial epithelial messenger ribonucleic acids using the ovine uterine gland knockout model. Endocrinology 140, 4070–4080.

62 Spencer, T. E., Johnson, G. A., Bazer, F. W., Burghardt, R. C. and Palmarini, M. (2007) Pregnancy recognition and conceptus implantation in domestic ruminants: roles of progesterone, interferons and endogenous retroviruses. Reprod. Fertil. Dev. 19, 65–78.

63 Spencer, T. E., Mura, M., Gray, C. A., Griebel, P. J. and 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.

64 Spencer, T. E. and Bazer, F. W. (1995) Temporal and spatial alterations in uterine estrogen receptor and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. Biol. Reprod. 53, 1527–1543.

65 Kalter, S. S., Heberling, R. L., Helmke, R. J., Panigel, M., Smith, G. C., Kraemer, D. C., Hellman, A., Fowler, A. K. and Strickland, J. E. (1975) A comparative study on the presence of C-type viral particles in placentas from primates and other animals. Bibl Haematol 40, 391–401.

66 Ortin, A., Minguijón, E., Dewar, P., García, M., Ferrer, L. M., Palmarini, M., Gonzalez, L., Sharp, J. M. and De las Heras, M. (1998) Lack of a specific immune response against a recombinant capsid protein of Jaagsiekte sheep retrovirus in sheep and goats naturally affected by enzootic nasal tumour or sheep pulmonary adenomatosis. Vet. Immunol. Immunopathol. 61, 229–237.

67 Sharp, J. M. and Herring, A. J. (1983) Sheep pulmonary adenomatosis: demonstration of a protein which cross- reacts with the major core proteins of Mason-Pfizer monkey virus and mouse mammary tumour virus. J. Gen. Virol. 64, 2323–2327.

68 Mura, M., Murcia, P., Caporale, M., Spencer, T. E., Nagashima, K., Rein, A. and Palmarini, M. (2004) Late viral interference induced by transdominant Gag of an endogenous retrovirus. Proc. Natl. Acad. Sci. USA 101, 11117–11122.

69 Murcia, P. R., Arnaud, F. and Palmarini, M. (2007) The transdominant endogenous retrovirus enJS56A1 associates with and blocks intracellular trafficking of the JSRV Gag. J. Virol. 81, 1762–1772.

70 Sfakianos, J. N. and Hunter, E. (2003) M-PMV capsid transport is mediated by Env/Gag interactions at the pericentriolar recycling endosome. Traffic 4, 671–680.

71 Sfakianos, J. N., LaCasse, R. A. and Hunter, E. (2003) The M-PMV cytoplasmic targeting-retention signal directs nascent Gag polypeptides to a pericentriolar region of the cell. Traffic 4, 660–670.

72 Ullrich, O., Reinsch, S., Urbe, S., Zerial, M. and Parton, R. G. (1996) Rab11 regulates recycling through the pericentriolar recycling endosome. J. Cell Biol. 135, 913–924.

73 Garcia-Mata, R., Bebok, Z., Sorscher, E. J. and Sztul, E. S. (1999) Characterization and dynamics of aggresome formation by a cytosolic GFP-chimera. J. Cell Biol. 146, 1239–1254.