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Nucleotide Sequence of the Jaagsiekte Retrovirus, an Exogenous and Endogenous Type D and B Retrovirus of Sheep and Goats

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The complete genome of the jaagsiekte sheep retrovirus (JSRV), the suspected etiological agent of ovine pulmonary carcinoma, has been cloned from viral particles secreted in lung exudates of affected animals and sequenced. The genome is 7,462 nucleotides long and exhibits a genetic organization characteristic of the type B and D oncoviruses. Comparison of the amino acid sequences of JSRV proteins with those of other retrovirus proteins and phylogenetic studies suggest that JSRV diverged from its type B and D lineage after the type B mouse mammary tumor virus but before the type D oncoviruses captured the *env* gene of a reticuloendotheliosislike virus. Southern blot studies show that closely related sequences are present in sheep and goat normal genomic DNA, indicating that JSRV could be endogenous in ovine and caprine species.

Jaagsiekte is the Afrikaans name of a naturally occurring contagious pulmonary bronchioalveolar adenocarcinoma primarily affecting sheep (7, 10, 45, 47) and, to a lesser extent, goats (40, 46). The primary lesion is the resultant transformation of type II epithelial alveolar cells (granular pneumocytes), with occasional metastasis to lymph nodes and extrathoracic tissues (10 to 50% of cases) (6). The disease is sporadic or endemic worldwide, except in Iceland, where it has been eradicated, and in Australia. Depending on country and breed of animal, the incidence varies from less than 1% to as high as 20% (48). Although a number of agents have been implicated in its etiology (45, 49), it is now generally accepted that the disease is caused by a retrovirus named the jaagsiekte sheep retrovirus (JSRV) (6, 48). Progress in research has been hampered because of the lack of an in vitro culture system. The source of virus was therefore limited to experimentally or naturally infected sheep lung washes, which were often coinfected with an ovine lentivirus (7, 31, 35, 37, 43). The two viruses can be separated by density gradient centrifugation and differentiated serologically (52). Interestingly, no detectable circulating antibodies against JSRV antigens have been detected in affected animals, a very unusual finding in retroviral infections. However, a p26 JSRV-associated protein is specifically recognized by antisera to the p27 capsid protein of Mason-Pfizer monkey virus (MPMV) and to mouse mammary tumor virus (MMTV) (41). Morphologically, JSRV, with its knoblike surface spikes, more closely resembles MMTV, the type B prototype, than MPMV, the type D prototype (32, 39). We have previously reported (52) that a JSRV-specific composite cDNA consisting of part of the *pol* gene and the 3′ end of the genome has homology to both type D and type B retroviruses. We now report the cloning and sequencing of the complete JSRV genome purified from ex vivo lung washes of diseased animals; we also report on the phylogenetic relationship of JSRV to the type D and B oncoviruses and present evidence that a closely related virus is endogenous in ovine and caprine species.

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MATERIALS AND METHODS

Virus production and purification. JSRV was purified from lung washings of experimentally infected sheep as previously described (47, 52).

cDNA and RT-PCR cloning. cDNA cloning was done essentially as previously described (52). Briefly, polyadenylated RNA was isolated from isopynic-gradient-purified virus, cDNA was synthesized by using an Amersham kit with oligo(dT) as primer, and after the addition of *Eco*RI linkers, the cDNAs were cloned either into λZAP II (Stratagene) or λgt 10 vectors. Recombinant clones were selected by using either labelled random primed cDNA from JSRV polyadenylated RNA or previously identified JSRV specific clones as probes. Clones were ordered relative to each other by (i) restriction mapping and (ii) nucleotide sequencing of the extremities and alignment of the predicted translational products against MPMV and MMTV protein sequences. cDNA of the 5′ end of JSRV genomic RNA was synthesized with 10 ng of polyadenylated RNA, 400 ng of the *gag* up primer (CAGGTCGACAATGGAGCGGTAGGACCA, positions 1117 to 1134 plus a *Sal*I restriction site tail), and Moloney murine leukemia virus (Mo-MuLV) reverse transcriptase (RT; BRL) in 20 μl of RT buffer. One-tenth of the reaction was polymerase chain reaction (PCR) amplified with 400 ng of *gag* up and U5 down (TATGTCGA CGTCCTGGTCGGATCCTCTCAACC, positions 18 to 37, with one mismatch to create a *Bam*HI site and a *Sal*I-*Aat*II tail) primers, and 2.5 U of *Taq* polymerase (Stratagene) in a volume of 50 μl. Thirty cycles were done, each consisting of 1 min at 94°C, 1 min at 60°C, and 4 min at 72°C. The amplified DNA was then purified on low-melting-point agarose and cloned into the *Sal*I site of a pBluescript II vector (Stratagene).

Shotgun cloning and sequencing. Relevant clones were sonicated, fractionated on low-melting-point agarose gels, and cloned into M13 vectors as described elsewhere (34).

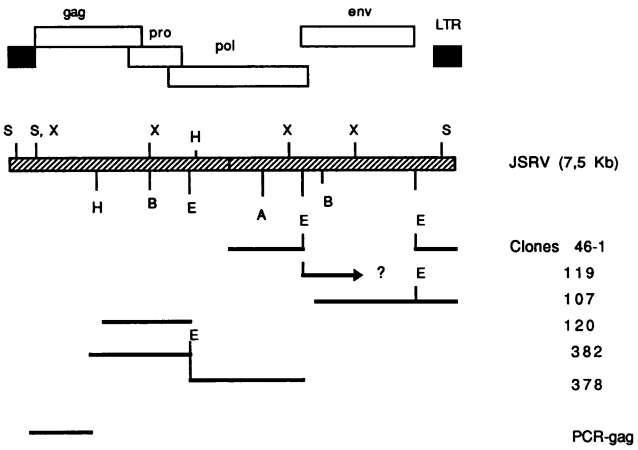

FIG. 1. Schematic representation showing the eight clones that were sequenced that cover the entire 7,462-nucleotide JSRV genome together with a simplified restriction map and genetic organization. Clone PCR-gag was amplified by using an RT-PCR reaction, and the other clones were from a library of oligo(dT)-primed cDNA. The ? indicates an undetermined 3′ end. Restriction endonucleases: E, *Eco*RI; B, *Bam*HI; S, *Sac*I; X, *Xho*I; A, *Aat*II; H, *Hin*dIII.

Single-stranded templates were sequenced by the dideoxy chain termination method with Klenow polymerase and gradient buffer gels. Sequence data were compiled by using an automatic shotgun sequencing program.

All computing was done via network link to the program package of BISANCE (8).

Phylogenetic analysis. Two programs were used to generate the multiple alignments: (i) the TREEALIGN program of Jotun Hein (13), which simultaneously solves the problem of multiple alignments and that of growing a parsimonous tree; and (ii) the CLUSTAL version V of Des Higgins (15). Trees were then grown by (i) the neighbor joining methods and bootstrap analysis included in CLUSTAL version V; (ii) PROTPARS, a parsimony program in the PHYLIP (Phylogeny Inference Package) version 3.4 package of J. Felsenstein (Department of Genetics, University of Washington, Seattle); and (iii) least-squares distance matrix programs with (KITSCH) or without (FITCH) the assumption of a molecular clock from the PHYLIP version 3.4 package of J. Felsenstein.

Cell lines and DNA analysis. The 15.4 cell line is an epithelial tumor cell line from a Jaagsiekte-affected animal (2). OFTR is a primoculture of ovine fetal tracheal cells of fibroblast type, CFSM are caprine fetal synovial membrane cells of fibroblast type, and SCP are sheep choroid plexus cells also of fibroblast type. Cell DNA (2 to 6 μg) was digested by restriction enzyme, separated on agarose gels, and transferred to nylon membranes. Blots were probed with a combination of cloned fragments representative of most of the genome. Hybridization conditions were 5× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate) 50% formamide, 0.5% Denhardt's solution, 0.2% sodium dodecyl sulfate, and 10 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); pH 7.0] at 42°C. Two stringent final washes (15 min each) were done in 0.1× SSC at 57°C as described previously (52).

Nucleotide sequence accession number. The nucleotide sequence of the complete genome of JSRV is deposited in GenBank under accession number M80216.

RESULTS

Using random primed cDNA of poly(A)$^+$ JSRV genomic RNA and previously described clone 46-1 (52), we "walked" the JSRV cDNA library. Seven clones were identified and sequenced. As clone 382 exhibited a mutation leading to a frameshift in the *pro* reading frame, the sequence was reascertained with clone 120. Four clones covering the 3′ end of the genome, including 107, 46-1, and two others (namely, 72 and 15, which are not shown in Fig. 1), gave divergent results for the 3′ end of the R region. For clones 46-1 and 72, there are 13 nucleotides between the poly(A) signal (positions 7444 to 7449) and the poly(A) tail (downstream of position 7462 or 13), but for clones 107 and 15, we found a much longer intervening sequence leading to polyadenylation at position 40. We hypothesized that the abnormal polyadenylation at position 40 represented a readthrough in the U5 region of the proviral long terminal repeat during 3′-end processing of the viral mRNA. This sequence was used to design an oligonucleotide primer specific for the 5′ end of the genomic RNA which, together with a primer in the sequenced *gag* region, was used to clone the 5′ end of the genome by RT-PCR (Fig. 1).

Viral sequence deletions resulting from clustered *Eco*RI sites at the extremities of cloned fragments are unlikely, since JSRV gene products in these regions are colinear with those of MPMV and MMTV. Therefore, we consider that this sequence is a faithful representation of the complete JSRV genome.

The genome is 7,462 nucleotides long, having a genetic organization typical of type B and D retroviruses, with overlapping *gag*, *pro*, and *pol* reading frames except for an extra open reading frame (ORF) named *X*, which overlaps *pol* (Fig. 2 and 3).

The long terminal repeat. Like all other retroviral long terminal repeats, those of JSRV are delineated by a polypurine tract (nucleotides 7158 to 7177) and a sequence complementary to the 3′ end of a tRNA, here $tRNA_{1,2}^{Lys}$ (primer-binding site [PBS], nucleotides 127 to 144), which is used for initiation of minus-strand synthesis. The long terminal repeat of the JSRV provirus is 397 bases in length and is bound by the inverted repeat sequences CTGC at positions 7178 to 7181 and GCAG at positions 122 to 125. We have found two alternative boundaries between R and U5, but we assume that the 13-nucleotide-long sequence which represents canonical spacing between the poly(A) signal and the poly(A)

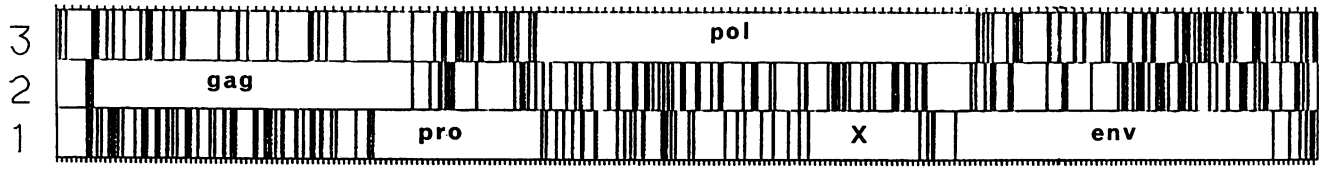

FIG. 2. Genetic organization of JSRV. Stop codons in the three reading frames are represented by vertical bars.

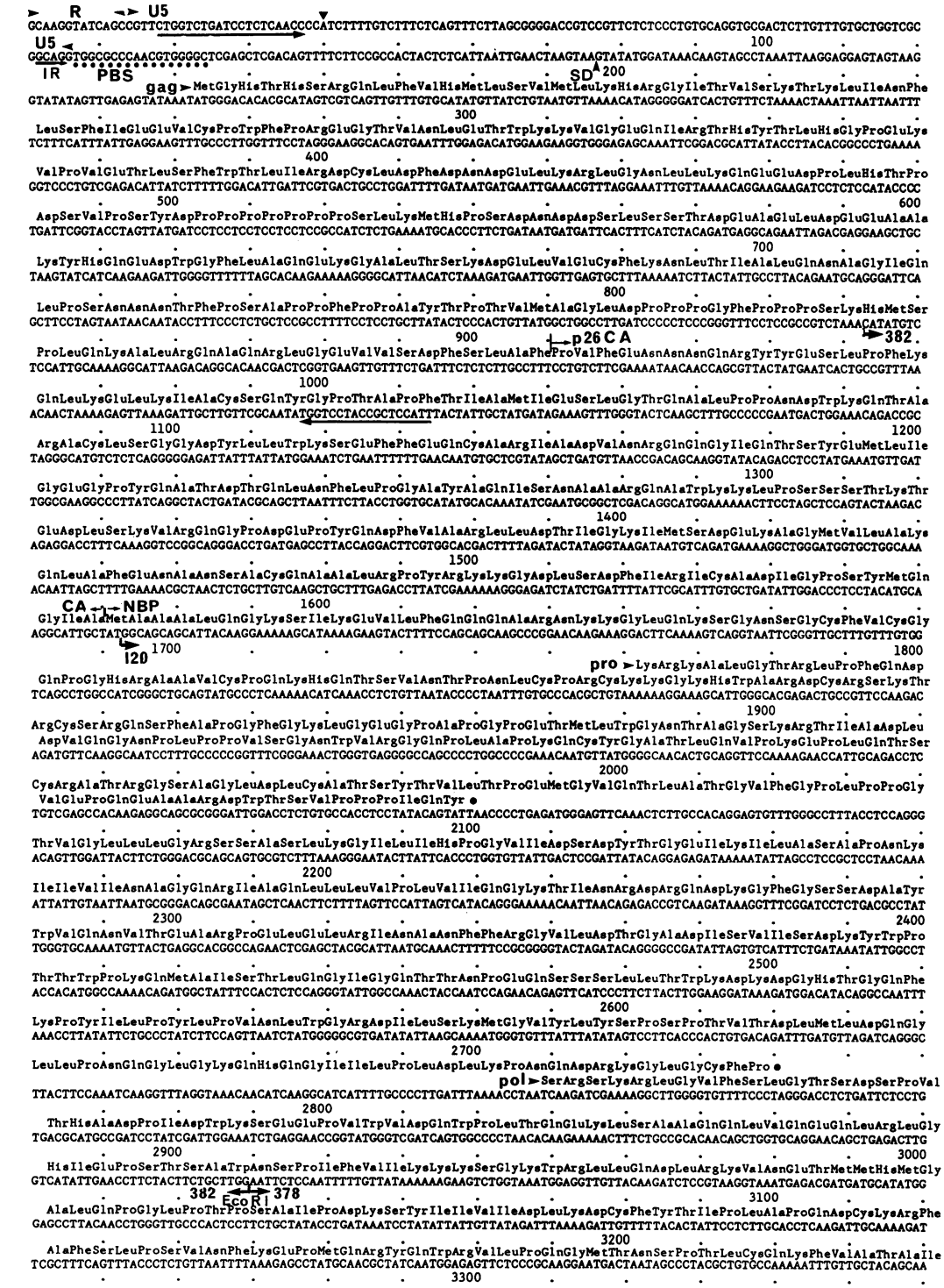

FIG. 3. Complete nucleotide sequence of the JSRV genome, from the cap site (position 1) to the polyadenylation site (position 7462), together with the deduced amino acid sequences of the relevant ORFs. The limits of U3, R, and U5 are indicated by arrows; the alternative polyadenylation site is capped with a closed triangle; the inverted repeats GCAG and CTGC, polypurine tract (PPT), PBS, TATA box, polyadenylation signal, enhancerlike sequences, relevant restriction sites, and extremities of the individual clones are also indicated. Oligonucleotide primers in U5 and *gag* which were used in the RT-PCR are underlined by arrows. ORFs were translated from the first ATG, except for *pro* and *pol*, which were translated from their beginnings, until the termination codon. Putative extremities of the matured *gag*-encoded capsid protein are indicated.

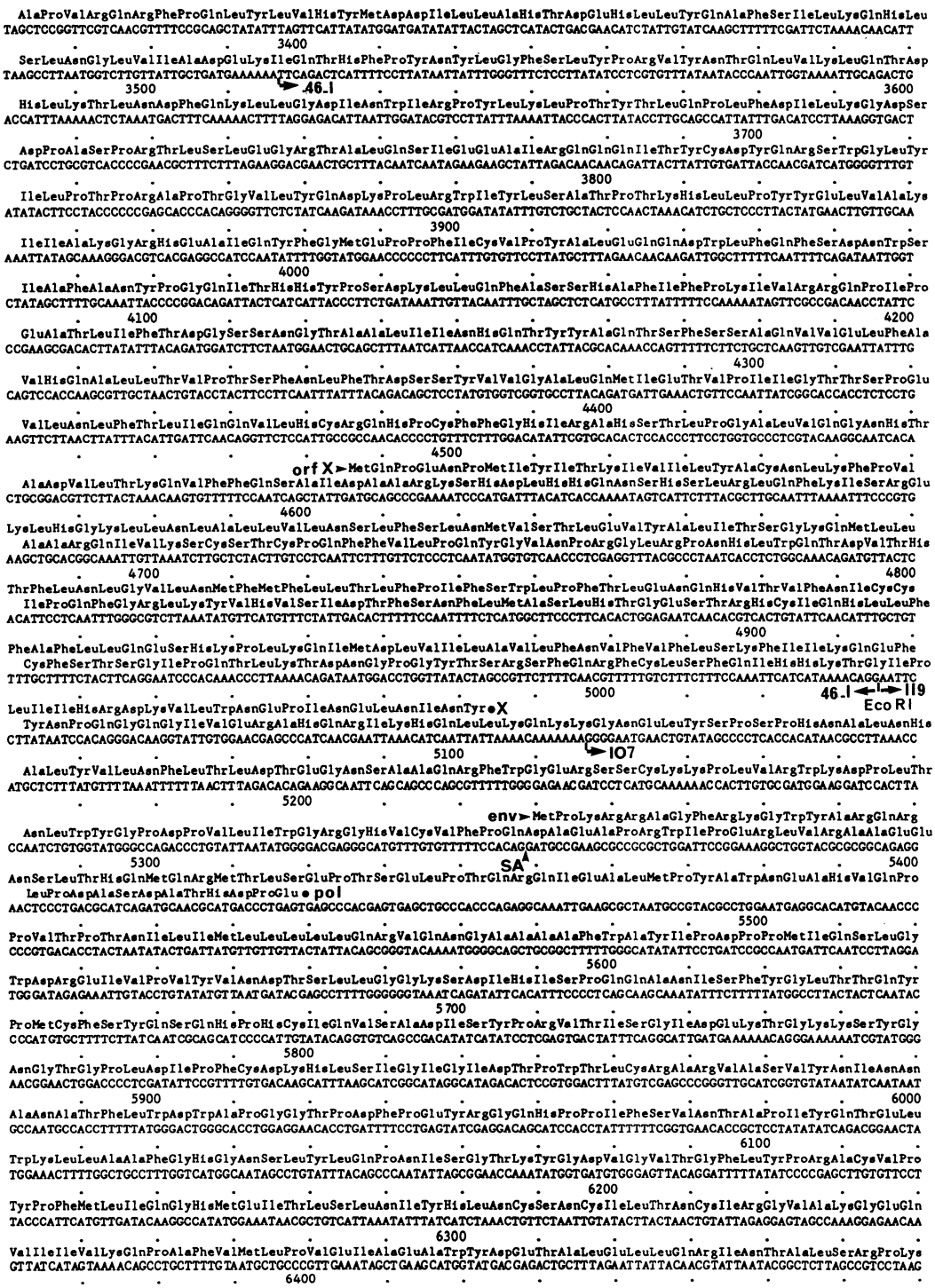

FIG. 3—*Continued.*

tail is the true R region. Hence, U3 is 271 bp, R is 13 bp, and U5 is 113 bp long. The U3 region usually contains regulatory signals for viral transcription. In addition to the TATA box, we have highlighted two 19-bp tandemly repeated sequences (positions 7347 to 7365 and 7366 to 7384), a sequence [TGGC$(A)_4$GCCA, nucleotides 7221 to 7232] resembling an NF-1 binding site, and a core C/EBP putative binding site at positions 7387 to 7396.

Viral proteins. (i) **Gag.** The Gag precursor polyprotein (nucleotides 263 to 2098, Fig. 3) is a 612-amino-acid polypeptide with a calculated molecular weight of 68 kDa. The precursor is probably myristilated, since its amino terminus Met-Gly is identical to those of many other myristilated retroviral Gag proteins. Alignment of the predicted JSRV Gag polyprotein with those of MPMV, squirrel monkey retrovirus (SMRV), and MMTV shows that there is a strong

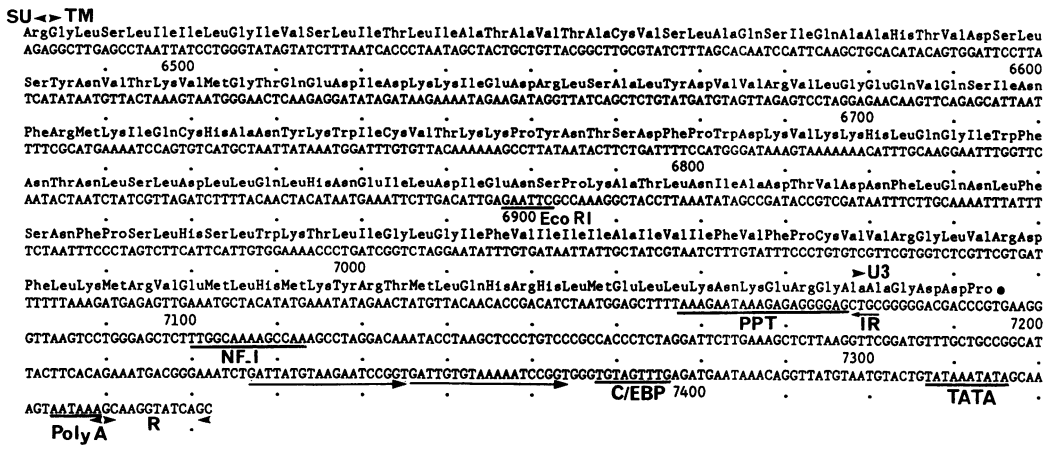

FIG. 3—*Continued.*

sequence conservation corresponding to the boundaries of the capsid proteins (CA) of these three viruses (14, 16). We therefore tentatively assigned the N terminus of the JSRV CA to positions 1031 to 1033 and its C terminus to positions 1694 to 1696, implying a protein with a calculated molecular weight of 24.6 kDa, which is in close agreement with the observed JSRV *gag*-related p26 recognized by anti-MPMV p27 serum. The COOH extremity of the nucleocapsid protein (NC) is less certainly assigned, although the MPMV cleavage site Tyr/Gly-Ala is found in the same relative position in JSRV Gag. This would make an NC of 9.6 kDa, very similar to that of MPMV, which, according to its basic content, migrates as p14. Separated by 13 residues are two Cys-X_2-Cys-X_4-His-X_4-Cys sequences thought to mediate the binding of NC to the genomic RNA.

(ii) Pro. The protease is probably expressed as a fused Gag-Pro polypeptide by a ribosomal frameshifting as for MMTV (17). This ORF (326 residues; calculated molecular weight, 35 kDa), like those of other type D and B oncoviruses, comprises two parts: a pseudoprotease or protease-like (PrL) domain of about 180 residues (3, 22, 33) and an active protease (PR) which exhibits the core amino acid sequence of cellular aspartyl protease Leu-Asp-Thr-Gly (residues 198 to 201, nucleotide start at 2473). A PrL domain is also found in some lentiviruses but located in the *pol* gene between the RNase H and the integrase domain (22). Alignment of the JSRV PrL and PR domains against those of these lentiviruses shows that the PrL polypeptide is even more conserved than the protease, with respective percentages of amino acid identities of 36 and 24 against feline immunodeficiency virus, 32 and 28 against caprine arthritis-encephalitis virus, and 30 and 25 against the South African strain of visna virus. It has been shown by computer analysis that the PrL domains of retroviruses were related to dUTPases (24) and, more recently, that the feline immunodeficiency PrL protein indeed exhibits a dUTPase activity. A similar activity was also present in the type D (MPMV and SRV1) viral particles (11). It is thus likely that JSRV PrL also encodes a dUTPase activity which could be packaged into the virions.

(iii) Pol. Pol is likely to be translated as a fused Gag-Pro-Pol polypeptide. The boundaries of RT and integrase domains (Fig. 3) have been assigned by homology to other retroviral Pol proteins. The putative RT active site Tyr-Met-Asp-Asp (3) starts at nucleotide 3405.

(iv) X ORF. An additional ORF (Orf X) is found at positions 4465 to 5103. The first ATG is well into the ORF at position 4616, implying a predicted polypeptide of 166 amino acids. Orf X is unusual in its location, is very hydrophobic, and yields no meaningful homologies when aligned with sequences in the National Biomedical Research Foundation data bank. As we have not been able to detect any viral transcripts in the cultured tumor cell lines 15.4 and JS7 (data not shown), we do not know if there is a specific transcript associated with this ORF. We computed the codon usage along four 500-bp fragments of the *pol* gene, the last one being overlapped by the X ORF, looking for an X ORF-induced alteration of the codon usage in the fourth fragment. No such alteration was observed (Table 1), suggesting that the hypothetical X ORF coding potential exerts few if any constraints on the codon usage of the *pol* gene. Moreover, the codon usage of X ORF was found to be very different from that of *pol*, weakening the prospect of X ORF being a coding gene.

(v) Env. Overlapping the end of *pol*, the *env* ORF encodes the precursor of the viral envelope glycoprotein. There are two ATG codons 7 residues apart at the beginning of this ORF (positions 5329 to 5331 and 5350 to 5352), but since a

TABLE 1. Codon usage of the *pol* gene and X ORF[a]

Amino acid	Codon	% Usage at:				
		pol nucleotides				X ORF nucleotides
		3102–3602	3603–4103	4104–4604	4605–5105	4606–5106
F	TTT	66.7	85.7	76.9	81.8	43.8
F	TTC	33.3	14.3	23.1	18.2	56.3
Y	TAT	90	75	50	100	25
Y	TAC	10	25	50	0	75
H	CAT	100	66.7	60	64.33	0
H	CAC	0	33.3	40	5.7	100
Q	CAA	76.9	83.3	78.6	93.3	16.7
Q	CAG	23.1	16.7	21.4	6.7	83.3
R	CGT	50	25	25	41.7	0
R	CGC	16.7	0	50	16.7	0
R	CGA	0	62.5	25	33.3	0
R	AGG	0	0	0	0	100

[a] Codon usages along four 500-bp fragments of the *pol* gene and the X ORF are indicated for phenylalanine (F), tyrosine (Y), histidine (H), glutamine (Q), and four of the six codons for arginine (R).

TABLE 2. Amino acid identities of JSRV proteins and those of type B and D retroviruses

Virus (type)[a]	% Identical amino acids			
JSRV (?)	Gag[b]	Pro	Pol	Env
MPMV (D)	53.1	51.9	56	
SRV-1 (D)	53.5	51.9	55.4	
SMRV-H (D)	36.7	48	52.5	
MMTV (B)	37.7	44.4	49.5	27.1
HERV-K (B)	34.8	41.8	45.6	23.9

[a] SRV-1, simian retrovirus 1; SMRV-H, SMRV from a human B-cell line.
[b] From the putative NH2 extremity of core p26 of JSRV to the COOH terminus of Gag.

splice acceptor consensus sequence is found 1 base upstream of the second, we assume that Env is initiated at methionine 5350, making a precursor of 617 amino acids. A potential cleavage site in the sequence RPKR/GLS (position 6484) defines the C terminus of the outer membrane or surface protein (SU) and the NH2 extremity of the transmembrane protein (TM). Three hydrophobic segments can be readily identified in the Env precursor. The first one, 13 amino acids long, beginning 61 residues downstream of the start site, corresponds to the signal peptide (positions 5530 to 5577). The second, 25 residues long, situated just downstream of the SU-TM cleavage site, probably corresponds to the fusiogenic domain, and the third, 24 residues long (positions 6997 to 7071) is the anchor, membrane-spanning domain of TM which is followed by the cytoplasmic tail. There are 12 cysteines and nine potential N-linked glycosylation sites, three of which are found in TM.

The calculated molecular weight of the unglycosylated Env precursor is 69 kDa. Assuming an average molecular weight of 2.1 kDa for each carbohydrate moiety (21) and a cleavage site of the signal peptide at residue 73, the calculated molecular weight of mature SU protein is 47 kDa and that of TM is 33 kDa. As the viral particles produced in the lung surfactant have never been purified to sufficient homogeneity, in the absence of specific antisera, there are no observed counterparts for the predicted gp47 and gp33.

Homologies with other retroviruses and phylogeny. JSRV protein sequences were aligned to those of the type B and D retroviruses and scored for the percentage of amino acid identity. As there is no reliable alignment between the N terminus of Gag polyproteins until the beginning of the CA protein, with most of these viruses, this part was omitted. There is a clear genetic dichotomy between the most JSRV-related viruses, with *gag*, *pro*, and *pol* on one hand and *env* on the other (Table 2). Although there is more than 50% amino acid identity between JSRV, MPMV, and simian retrovirus type 1 Gag, Pro, and Pol proteins, there is no reliable alignment of their envelope precursor proteins. Conversely, Env proteins of JSRV, MMTV, and human endogenous retrovirus with lysine PBS (HERV-K) are homologous, although there is less than 38% amino acid identity between their Gag proteins (30.4% for the whole length of Gag). Homology between JSRV and type B Env proteins is not very high in terms of amino acid identities, but the relationship is evident in terms of overall structural organization (Fig. 4), particularly for the second half of the molecules, where numerous cysteines and glycosylation sites are shared. In contrast, the skeletons of MPMV and SMRV Env proteins show weak similarities to those of JSRV and the type B retroviruses but are highly homologous to that of the type C reticuloendotheliosis virus (REV-A) (44).

This study was extended by phylogenetic analysis of Pol and Env subdomains of these and other viruses. Numerous trees were grown, either by using parsimony or by distance matrix methods. The overall topology was conserved in most of the trees. The deviations from the neighbor joining tree presented in Fig. 5A are (i) in the group of lentiviruses, the South African strain of visna virus most often diverged first; (ii) in the KITSCH tree, SMRV and JSRV exchanged positions; and (iii) in parsimony analysis, a clade of MMTV and HERV-K diverging between hamster intracisternal-type A particles and JSRV is equally parsimonious to the successive divergences presented in the figure. A bootstrap analysis of a neighbor joining tree for the RT subdomain shows a clear clade in which the type A (hamster intracisternal type A particles), B, and D retroviruses successively evolved (Fig. 5A) in agreement with previous reports (1, 23, 51). JSRV diverged between the B prototype MMTV and the D prototype MPMV. Even though this tree is unrooted, KITSCH analysis and previous works (9, 51) pointed out a root between the Mo-MuLV-baboon endogenous virus clade and the other retroviruses. In the absence of a published RT sequence for REV-A, the position of this type C virus is not indicated on this tree. However, it has been shown elsewhere (23, 44) that the avian REV-A branched from the Mo-MuLV lineage before baboon endogenous virus diverged. Concerning the extracellular domain of the Env transmembrane proteins, a similar bootstrapping analysis which is also strongly supported by all other tree-growing programs yielded a very different relationship. JSRV still grouped with the type B clade, but the type D retrovirus TMs evolved from a REV-A-like ancestor (23, 44). Because recombinational events in the branches of the tree preclude the possibility of using a molecular clock in the tree-growing program and because there is no known outgroup envelope sequence, there is no way of internally placing a reliable root for the transmembrane tree. Assuming that the root is the same as that of the RT tree, then a single recombinational event involving the prototype D *env* gene after the divergence of JSRV in the A-B-D clade could explain the anomalous positions of the type D envelope proteins. The position of the baboon endogenous virus envelope in the tree would be best explained by a second recombination between this retrovirus and the *env* gene of a MPMV progenitor (23).

Endogenous nature of JSRV. The absence of detectable circulating antibodies in affected animals made us question whether the pathogenic exogenous JSRV could have an endogenous counterpart in the same way as its close relative MMTV. To investigate this, we analyzed genomic digests of (i) the 15.4 epithelial tumor cell line (2), two fetal primary cells of fibroblast type from sheep and goats (OFTR and CFSM), and a primary culture of choroid plexus fibroblasts. All were found to contain JSRV-like sequences as integrated proviral DNA without any detectable linear or circular unintegrated proviruses (Fig. 6). *Sac*I cut the exogenous JSRV three times, twice in the long terminal repeats (position 7217) and once upstream of the PBS (position 151). Therefore, the observed 7-kb *Sac*I fragment represents a major part of the provirus (Fig. 6B). The 7.5-kb fragment in OFTR DNA (Fig. 6B, lane 3) corresponds to incomplete digestion of the PBS site in this particular experiment. However, the restriction pattern for *Eco*RI and other enzymes (data not shown) is identical to those of 15.4 and French-ovine choroid plexus sheep DNA. The inferred restriction map of the endogenous sheep JSRV-like virus is

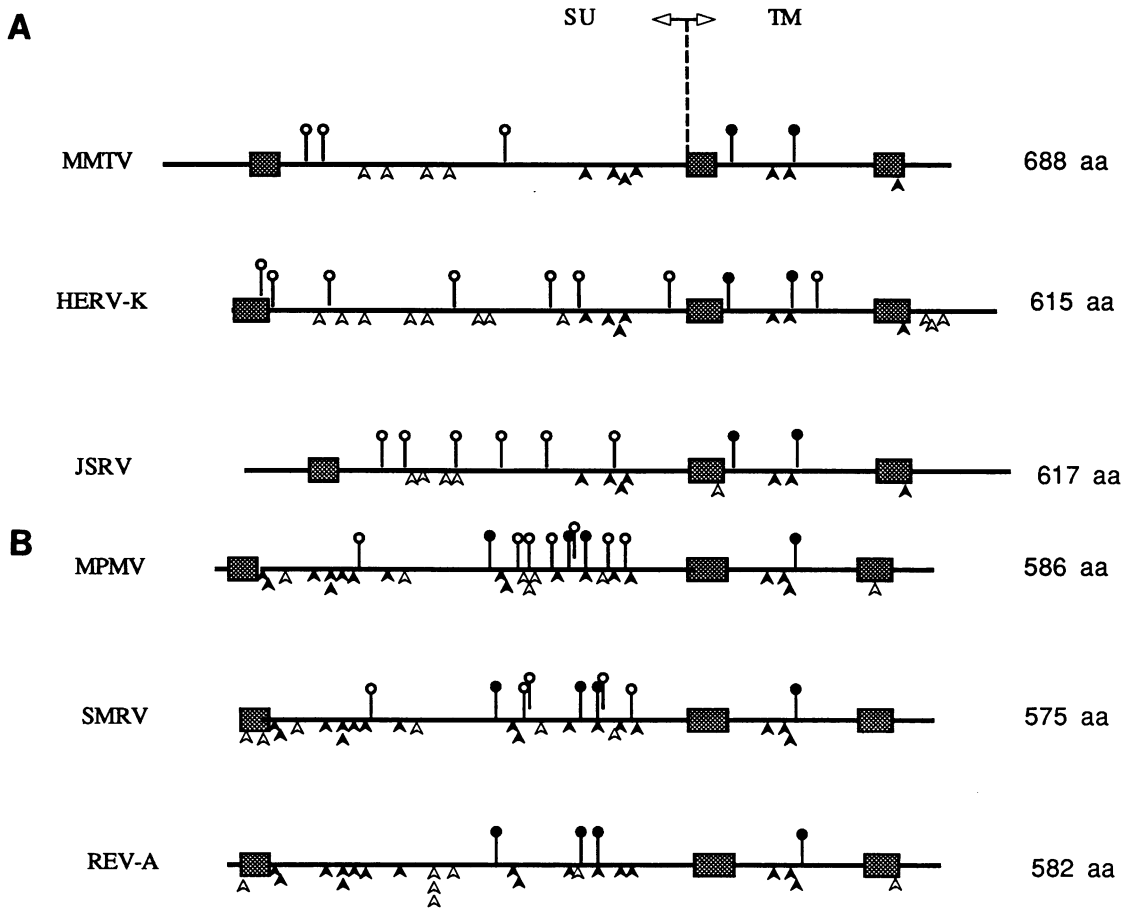

FIG. 4. Structural features of Env precursors of MMTV, JSRV, and HERV-K (A) and of MPMV, SMRV human isolate (SMRV-H), and REV-A (B). Cysteines and glycosylation sites shared by the three viruses in each group are indicated by closed symbols. Symbols: ▩, hydrophobic residues; ♀, unique glycosylation sites; ♀, shared glycosylation sites; ▲, unique cysteines; ▲, shared cysteines. aa, amino acids.

mostly but not completely identical to that of the exogenous JSRV, indicating a close relationship between the two viruses. Stringent hybridizations of sheep DNA with *gag*-specific or *env*-specific probes (not shown) yield essentially the same conclusions; i.e., the JSRV-related sequences in normal sheep DNA are homologous to JSRV along the entire genome. However, the presence of additional faint bands indicates that the endogenous sequences are heterogenous, as is usually observed for other endogenous retroviruses. The somewhat different restriction pattern of the goat DNA implies that the goat endogenous provirus is more distantly related to the exogenous sheep JSRV than to the endogenous sheep provirus (Fig. 6B and C, lanes 4, and data not shown). Dot blot analysis using the cloned PCR-*gag* fragment as internal standard and Southern blots probed with JS 378 *pol* fragment indicate that JSRV-like sequences are present at tens of copies in ovine and caprine species but absent in human and mouse DNAs (data not shown).

DISCUSSION

In agreement with biochemical, serological, and morphological reports, sequence information confirmed that JSRV is related to the type B and D oncoviruses. They share the same characteristic genetic organization, exhibiting a separate *pro* reading frame. Although JSRV Gag, Pro, and Pol show a higher relatedness with the type D than the type B retroviruses, JSRV Env is related only to type B but not to type D Env. Phylogenetic analysis suggest that JSRV evolved from the A-B-D lineage and diverged from it after HERV-K and MMTV type B but before MPMV and SMRV type D diverged. After the JSRV diverged, a recombinational event leading to the capture of a REV-A-like *env* gene by the progenitor of the type D viruses could have taken place. This could explain why the Env proteins of MPMV and SMRV are not in line with those of JSRV and the type B viruses, although they are still highly related for Gag, Pro, and Pol. In conclusion, because the Env "skeletons" essentially determine the morphology of the spikes on the viral envelope, which was a characteristic used in the classification of types B and D, JSRV is more type B than type D.

The finding that probes of cloned JSRV cDNA hybridized to endogenous sequences in sheep and goat DNA was not unexpected. For many years, we had speculated on this possibility because of the failure to detect circulating antibodies in the sera of experimentally or naturally infected sheep. It is unlikely that this failure results from the poor quality of the antigens in the viral pellets, because sera directed against the heterologous MPMV and MMTV *gag* proteins cross-reacted with the JSRV p26 in the same

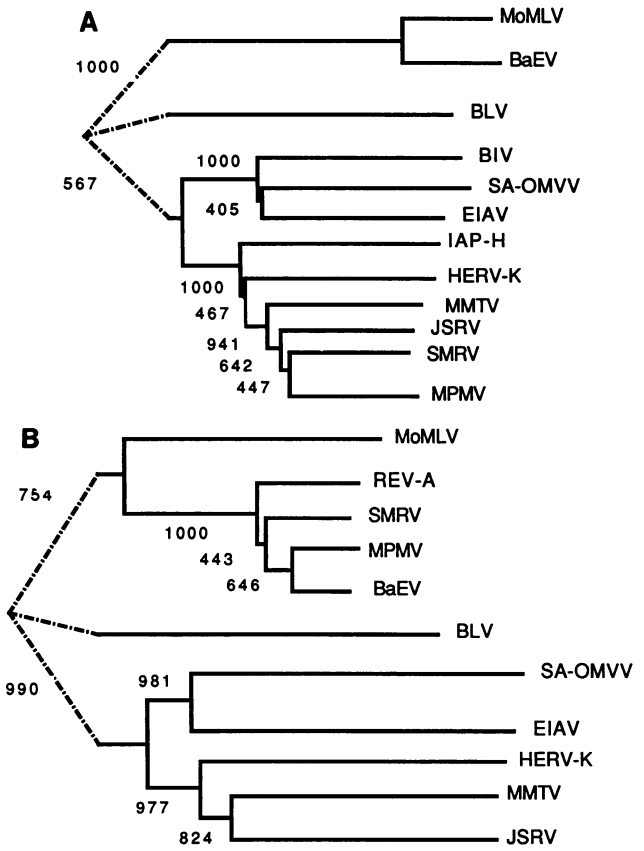

FIG. 5. Phylogenetic trees of RT (A) and transmembrane (B) domains of baboon endogenous virus (BaEV) (18), Mo-MuLV (42), bovine leukemia virus (BLV) (38), South African ovine maedi visna virus (SA-OMVV) (34), bovine immunodeficiency virus (BIV) (12), equine infectious anemia virus (EIAV) (19), hamster intracisternal type A particles (IAP) (28), HERV-K (29), MMTV (25), JSRV (this work), MPMV (44), REV-A (50), and SMRV human isolate (SMRV-H) (27). For RT, the datum subset is a 220-site-long domain aligned to SA-OMVV nucleotides 2220 to 2858 or Mo-MuLV nucleotides 2778 to 3416. For the transmembrane, the datum subset is a 152 (for REV-A)- to 195 (EIAV)-amino-acid-long domain aligned to JSRV nucleotides 6472 to 7044, corresponding mostly to the extracytoplasmic transmembrane, from the beginning of the RPKR consensus sequence of the SU-TM cleavage site to a residue in the hydrophobic membrane-spanning domain of TM. Sequences were aligned by using the multiple alignment program CLUSTAL version V, and trees were grown by the neighbor joining method. Numbers indicate the numbers of occurrences of this particular grouping in 1,000 trials of bootstrap resampling; horizontal branch lengths are proportional to evolutionary distances measured as percentages of divergence.

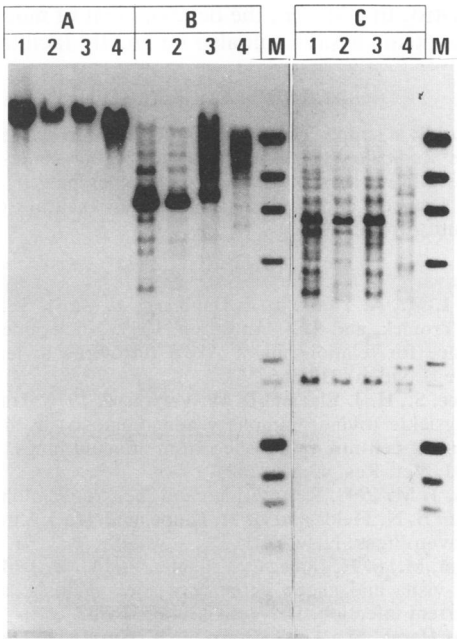

FIG. 6. JSRV-like sequences in normal sheep and goat DNAs, either undigested (A) or digested by *Sac*I (B) or *Eco*RI (C). Genomic DNAs were analyzed by Southern blotting and probed with a combination of cloned fragments representative of the whole JSRV genome under stringent washing conditions (T_m − 12°C). Lanes: 1, 15.4 sheep tumor cell line; 2, French-ovine choroid plexus sheep DNA; 3, sheep OFTR DNA; 4, goat CFSM DNA; and M, *Hin*dIII-digested λ DNA and *Hae*III-digested φX174 DNA.

preparation (39, 48). A possible explanation could be that the very closely related endogenous JSRV antigens are expressed in the neonatal period during ontogeny, leading to the depletion of JSRV-specific T and/or B lymphocytes and thereby establishing a state of tolerance for the endogenous and eventually the exogenous virus.

There have been reports of a low incidence of natural jaagsiekte in goats (36). The disease has also been experimentally transmitted by the sheep isolate to newborn goats, although with a reduced efficiency (40, 46). Recently, a JSRV- and MPMV-related virus was identified in nasal fluids and tumors from goats with nasal adenocarcinomas (5). To date, it is not known whether the natural goat virus is more closely related to the endogenous virus of goats or the exogenous sheep virus.

Ovine lentiviruses and JSRV often coexist in the same diseased animals (30, 31, 37, 43), and to our knowledge, there is no country devoid of one virus-induced pathology which is not also devoid of the other. This suggests that the lentivirus and JSRV could be synergistic in their transmission and/or their pathogenicity, even though they are individually pathogenic in sheep (20, 39). In fact, it has been reported that the lentivirus infection and its transmission to in-contact animals occurred to a greater extent in JSRV-infected sheep (4). The lentivirus has been shown to induce in experimentally infected sheep a mild immunosuppression (26) which may affect other viral or bacterial infections.

In field cases, the incubation period of ovine pulmonary carcinoma ranges from months to years, but serial passages in newborn lambs of a JSRV isolate contaminated with a lentivirus were associated with a decrease in the incubation period to as little as 3 weeks (39). Such an acute transformation suggests that the oncogenic potential of the virus is encoded by a viral gene. However, neither *env* nor ORF X shares homology with known oncogenes, and because we did not detect any evidence of JSRV transcription in the cultured tumor cell lines, it is still not known whether ORF X is actually a gene or an unusually long ORF which arose by chance, as suggested by its atypical codon usage. A study of JSRV transcription in tumor biopsy samples will help address this issue.

Having the JSRV cloned and sequenced will now enable us to express the viral proteins in vitro and thereby overcome the problems associated with the inability to grow the

virus in vitro. In addition, the development of much-needed JSRV diagnostic assays will also be greatly facilitated.

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