The long terminal repeat is a determinant of cell tropism of maedi-visna virus

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Maedi-visna virus (MVV) is a lentivirus of sheep, mainly affecting the lungs and the central nervous system. Long terminal repeat (LTR) sequence variability is common in tissue culture-derived isolates of MVV as well as those of other lentiviruses. The role of this sequence variation in MVV replication has not been explored. PCR amplification of the LTRs of an MVV isolate revealed two product sizes, the larger containing a 53 bp duplication. PCR products containing the two size variants of the LTRs were cloned into an infectious molecular clone of MVV and the resulting chimeric viruses were tested for growth in various cell types. The chimeric virus containing only one copy of the 53 bp sequence was found to grow more slowly in sheep choroid plexus cells, sheep fibroblasts and sheep synovial cells than the virus with the 53 bp duplication. Both viruses grew equally well in macrophages. These results indicate that the LTRs determined the extended cell tropism of MVV.

Maedi-visna virus (MVV) is a member of the lentivirus subfamily of retroviruses, causing encephalitis (visna), pneumonia (maedi), mastitis and arthritis in sheep (Sigurdsson, 1954; reviewed in Georgsson, 1990). The primary target cells of MVV in vivo are considered to be of the monocyte lineage (Gendelman et al., 1986; Gorrell et al., 1992; Narayan et al., 1982), although viral RNA and DNA (Staskus et al., 1987; Stowring et al., 1985) as well as viral proteins (Georgsson et al., 1989) have been detected in other cell types, including neuroglia and endothelial cells. The molecular basis for the cell tropism of lentiviruses has not been fully elucidated. Human immunodeficiency virus cell tropism is determined largely by co-receptor usage (Deng et al., 1996; Feng et al., 1996), although the long terminal repeats (LTRs) have also been shown to vary in their efficiency of directing replication in various cell types (Canonne-Hergaux et al., 1995; Corboy et al., 1992). The LTRs have been shown to be important in determining cell tropism of equine infectious anaemia virus (EIAV) and feline immunodeficiency virus (Payne et al., 1999; Poeschla et al., 1998).

MVV was originally isolated from the brains of visna-affected sheep and was grown in sheep choroid plexus (SCP) cells, since visna lesions always occurred in the choroid plexus and virus was readily isolated from this tissue (Sigurdsson et al., 1960). However, not all field isolates of MVV grow well in SCP cells (Narayan et al., 1982; Querat et al., 1984) and it has been demonstrated that virus isolates from maedi-affected lungs grow more slowly in SCP cells than do the original visna strains (Andrésson et al., 1996; Thormar, 1965). In a previous study, the nucleotide sequence of the env gene and the LTR of a field strain, 1071, which was isolated from a maedi-affected lung, was determined (Andrésson et al., 1998). The sequenced clone contained only one copy of a 43 bp sequence in the LTR that is duplicated in the visna strains that have been sequenced (Andrésson et al., 1993; Braun et al., 1987; Sonigo et al., 1985; Staskus et al., 1991). However, half of the PCR products from the Hirt supernatant (Hirt, 1967) of virus-infected cells were larger, which would possibly indicate a duplication in the LTR.

In this study, we cloned the two size variants of LTR from the maedi strain in the infectious molecular clone of visna virus, KV1772kv72/67 (Andrésson et al., 1993) and tested the resulting chimeric viruses for replication in various cell types. The two LTR bands from maedi strain 1071 were isolated from an agarose gel, cloned in M13 and sequenced. The two sizes of LTR differed from each other only in that the larger one had a 53 bp duplication. The sequence that was duplicated overlapped the 43 bp repeat in the visna strains by 34 bp (Fig. 1). The duplication or lack of duplication is unlikely to be an artefact from the PCR, since we have never detected this kind of artefact in more than fifty PCR products from cloned LTRs that we have sequenced.

To examine whether the different LTRs might play a role in the cell tropism of MVV, chimeric viruses were constructed. The visna clone KV1772kv72/67 is contained in two plasmids (Skranb et al., 1999). The 3' region of clone KV1772kv72/67
from the XbaI site at position 7768 to a BamHI site flanking the 3′ U3 region (Andrésson et al., 1994) was cloned into a derivative of pAT153 plasmid containing an XbaI site (Twigg & Sherratt, 1980). The two variant LTRs were amplified by PCR with primers corresponding to base pairs 8460–8481 (forward) and 9177–9156 (reverse) and were cut with HindIII and SacI at positions 8643 and 9128. The equivalent HindIII–SacI band in the pAT153 3′ U3 region was then replaced by the HindIII–SacI bands from the two variants (Fig. 2). This clone was mixed in equimolar quantities with the 5′ U3 region of visna clone KV1772kv72/67 from a SphI site in the 5′ U3 region of the transfected DNA after one round of replication (Shimomoto & Temin, 1982). However, recombination of the two LTRs of the transfected DNA can occur (Bandyopadhyay et al., 1984). PCR and sequencing of the LTRs of the virus progeny from SCP cells revealed that 1071VA3 was heterogeneous due to recombination. The culture contained virus particles with the 43 bp duplication of the 5′ U3 from the KV1772kv72/67 backbone and, since the variant LTRs were 6% different in nucleotide sequence from KV1772kv72/67, the point of recombination could be located upstream of the duplication (data not shown).

On further passage in SCP cells, there was selection for the variant with the duplication, and this was already an indication that the duplicated sequence enhanced growth in SCP cells. In the SCP cells infected with 1071VA4, the variant with the 53 bp duplication, there was no indication of recombination between the two LTRs. We then isolated VA3, without the duplication, by end-point dilution of the supernatant from the transfected FOS cells in blood-derived macrophages. PCR products from both LTRs were sequenced, with primers corresponding to base pairs 8460–8481 (forward) and 9177–9156 (reverse) to amplify the 3′ U3 region and 8865–8876 (forward) and 522–504 (reverse) to amplify the 5′ U3 region, with bp 8865–8876 as a sequencing primer. Nucleotide numbers are as published for KV1772kv72/67 (Andrésson et al., 1993). The sequence analysis revealed no heterogeneity in the LTRs.

As sheep blood-derived macrophages were permissible for all three viruses, these cells were used for preparation of virus stocks. Macrophage cultures were established as described previously (Torsteinsdóttir et al., 1997) and infected with
Maedi-visna virus LTRs

Fig. 3. Growth curves of the infectious molecular clone KV1772kv72/67 (a) and the two recombinant viruses 1071VA3 (b) and 1071VA4 (c) in various cell types: macrophages (a), FOS cells (b), SCP cells (c) and sheep fibroblasts (d). The cells were infected at an m.o.i. of 0.5. Virus replication was monitored by RT assays as described in the text.

Supernatants from the transfected FOS cells. Virus was harvested when CPE was clearly visible and equal amounts of virus (as determined by measuring RT activity, usually 1000–10000 c.p.m.) were used to infect fibroblasts from sheep longitudinal vertebral tendon, SCP cells and FOS cells as well as macrophage cultures. The m.o.i. was estimated by using a titrated KV1772kv72/67 stock as a standard and determining the correlation between TCID50 and RT activity. Growth was monitored by taking samples twice daily and measuring RT activity. Multiplicities of 0.5 and 5 were used. Fig. 3 shows growth curves when the cultures were infected with an m.o.i. of 0.5.

Although 1071VA3, the virus without the duplication, grew well in macrophages, it did not grow in fibroblasts or SCP cells and grew poorly in FOS cells. The FOS cells were consistently somewhat more permissive for 1071VA3 than were fibroblasts or SCP cells. At the end of the experiment, the identities of the virus strains were confirmed by PCR and sequencing. MVV strain 1071VA4, with the 53 bp duplication in the LTR, grew well in all cell types. The molecular clone KV1772kv72/67, with the 43 bp repeat, also grew well in all cell types tested. The same pattern was seen when an m.o.i. of 5 was used.

We have shown here that cellular tropism of MVV is determined by the LTR. The results do not allow us to conclude at what level of the replication cycle the LTR exerts its effect. However, the region in the LTR that needs to be duplicated for replication in SCP cells, FOS cells and sheep fibroblasts contains a number of known transcription factor-binding sites, including redundant AP-1 sites, a CAAAT sequence and a PEA-2 site, which has been implicated in the replication of EIAV in fibroblasts (Payne et al., 1999) and has also been suggested to be important in controlling MVV replication (Sutton et al., 1997) (Fig. 1). In the visna strain 1514, an AP-1 site proximal to the TATA box has been shown to be important for transcription in macrophages (Gabuzda et al., 1989; Hess et al., 1989). The receptor for MVV has not been identified, but there are indications that the receptor may be a relatively common molecule and that cell tropism is determined by cell-specific replication factors. This notion was supported by the findings of one study, that ovine lentivirus could enter a variety of cell types in vivo, but productive infection was restricted to macrophages (Brodie et al., 1995).

The importance in vivo of the broadening of cell tropism is unclear. The visna virus strains characterized in our laboratory were originally isolated from the choroid plexus and, although the macrophage is the main target cell of MVV, neurotropic strains may need to grow in other cell types in order to cross the blood–brain barrier. Although infrequent, productive infection has been demonstrated in endothelial cells (Georgsson et al., 1989). In this context, it is interesting to note that it has been shown that neuroinvasiveness of murine retroviruses is determined partly by the LTR (Portis & Lynch, 1998). The molecularly cloned visna virus used in the present
studies, KV1772kv72/67, is highly neurotropic and neurovirulent in vivo (Andrénsson et al., 1993). It is therefore well suited for studying neurotropism and neurovirulence. Studies on the role of the duplications in the LTR in neurotropism and pathogenicity are under way in our laboratory.

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References


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