Nucleotide Sequence of the Rauscher Murine Leukaemia Virus Long Terminal Repeat

By M. J. M. VAN DER FELTZ,* M. E. KRANENDONK-ODIJK, J. STARK AND N. J. DE BOTH

Department of Pathology, Faculty of Medicine, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

(Accepted 22 August 1986)

SUMMARY

The long terminal repeat (LTR) of Rauscher murine leukaemia virus (MuLV) has been sequenced. It differs in only three positions from the LTR of Rauscher spleen focus-forming virus (SFFV), and in four positions from the LTR of Rauscher mink cell focus-inducing virus (MCFV). It is unlikely that these differences account for differences in leukaemogenicity or tissue tropism of Rauscher MuLV, SFFV and MCFV. In contrast to the LTR of Friend MuLV, the Rauscher MuLV LTR contains only one copy of a tandem direct repeat. This repeat includes an enhancer core sequence.

Recently, research has been focused on the function of the retrovirus long terminal repeat (LTR). Several investigators have shown that the LTR of retroviruses determines tissue tropism (Chatis et al., 1984; DesGroseillers & Jolicoeur, 1984a; Rosen et al., 1985; Vogt et al., 1985). Others claim that it contributes to leukaemogenicity (DesGroseillers & Jolicoeur, 1984a, b; Oliff et al., 1984).

In the proviral form of retroviruses, the LTR is found on either side of the viral structural genes. It is formed during reverse transcription of the viral RNA genome and it includes important regulatory elements (Varmus, 1982). The LTR is subdivided into three regions: U3, R and U5 (Fig. 1). The U3 region contains enhancer and promoter sequences (‘CAT’ and ‘TATA’ boxes); a cap site is located on the junction of U3 and R, and a polyadenylation signal is found close to the junction between R and U5. U3 and U5 contain terminal inverted repeats which play a role in the integration of the provirus into the host DNA. For proviral transcription, the 5’ LTR is used as promoter.

The Rauscher murine leukaemia virus complex consists of several components including R-MuLV (murine leukaemia virus), R-MCFV (mink cell focus-inducing virus) and R-SFFV (spleen focus-forming virus). R-MuLV is an ecotropic replication-competent virus which predominantly causes chronic lymphoid leukaemia but sometimes causes myeloid leukaemia (de Both et al., 1985). R-MCFV is dualtropic, replication-competent and responsible for a slow erythroleukaemia (Van Griensven & Vogt, 1980). R-SFFV is a defective component which causes acute erythroleukaemia (Steeves, 1975).

We have cloned the LTR of R-MuLV, determined its nucleotide sequence and compared it to the sequence reported for the LTRs of R-SFFV, R-MCFV and of the related Friend virus. R-MuLV was cloned by endpoint dilution and infection of 3T3 cells (‘clone 9’). This clone produces only ecotropic, XC-positive, R-MuLV. Its 35S RNA is homogeneous as regards its oligonucleotide composition (Mol et al., 1982).

Mouse SC-1 cells (Hartley & Rowe, 1975) were infected with clone 9 virus; a Hirt extract was made from the cells (Hirt, 1967) 16 h after infection. The circular form of the viral DNA was isolated and molecularly cloned in β641. We obtained clones with one and two LTRs, which were subcloned in pBR328. From a pBR328 subclone containing viral DNA with one LTR we
isolated a *PstI* fragment containing this LTR (Fig. 2). Three fragments were subcloned in M13 mp8 and mp9 or mp19 (Fig. 2). Nucleotide sequencing was performed by the dideoxy-nucleotide termination method (Sanger *et al*., 1977) using the 15-base universal primer. For comparison, we have also subcloned and sequenced one corresponding fragment of the LTR from another isolate of R-MuLV (Habara *et al*., 1982). In Fig. 3 the sequences of both LTR isolates are shown and are compared with the sequence of the LTRs of other components of the Rauscher virus complex. A comparison with three components of the Friend virus complex is also made.

The R-MuLV clone 9 LTR contains 516 base pairs. The inverted repeats at the 5' and 3' termini are characteristic for several retroviruses. The various regulatory sequences are indicated. The overall homology between the R-MuLV LTR and the other LTRs of the Rauscher virus complex was greater than 98%, between R-MuLV and the Friend virus complex components (apart from the large repeat in F-MuLV) the homology was ≥ 94%. In an earlier report, the cDNA sequence of the R and U5 regions of uncloned Rauscher virus produced by JLS-V9 cells was described (Lovinger & Schochetman, 1979). This sequence is in agreement with our data.
Fig. 3. Nucleotide sequences of LTRs of R-MuLV clone 9, R-MuLV (RV-1; Habara et al., 1982), R-SFFV (Bestwick et al., 1984), R-MCF virus (Vogt et al., 1985), F-MuLV (Koch et al., 1984), F-SFFV, (Wolff et al., 1985) and R-MCFC virus (Koch et al., 1984). The sequence of the R-MuLV RV-1 LTR has only been partially determined. Gaps have been introduced to allow optimal alignment. Hyphens indicate those nucleotides which are homologous to R-MuLV clone 9. Dots indicate the number of base pairs in F-MuLV. Numbers on the right indicate the number of base pairs in R-MuLV clone 9. The promoter sequences ('CAT' and 'TATA' boxes) and the polyadenylation signal have been boxed. Putative enhancer sequences have been underlined as well as the 5' and 3' inverted repeats which play a role in the integration of the virus in the host genome.
The most obvious difference between R-MuLV and F-MuLV is that the LTR of the latter includes a direct tandem repeat which is not present in R-MuLV, R-MCFV and R-SFFV nor in F-MCFV and F-SFFV. In this tandem repeat, a stretch of nine nucleotides is found which is highly homologous to the so-called enhancer core sequence (Weiher et al., 1983). It is precisely in the enhancer sequence of the U3 region that tissue tropism and, in some cases, even leukaemogenic potential are supposed to be located (Chatis et al., 1984; DesGroseillers & Jolicoeur, 1984a, b; Oliff et al., 1984; Rosen et al., 1985; Vogt et al., 1985).

Apparently, the presence of more than one copy of this sequence is not required for pathogenicity, since MCFV and SFFV are leukaemogenic and contain only one copy of the enhancer core sequence. Proviruses containing LTRs with two enhancer repeats may be transcribed more efficiently than those with only one enhancer and may therefore be more pathogenic. In experiments carried out with recombinant retroviruses, Oliff et al. (1984) have shown that recombinants of the non-leukaemogenic Ampho 4070 with the F-MCFV envelope (env) gene and F-MuLV LTR (containing two copies of the repeat element) cause leukaemia in 38% of the inoculated mice. However, recombinants of Ampho 4070 with F-MCFV env and F-MCFV LTR (containing one copy of the repeat element) induce leukaemia in only 14% of the cases. Another example in which tandem repeats of the enhancer may influence the degree of leukaemogenicity of a virus is described for radiation leukaemia virus by Rassart et al. (1986). In the case of the Rauscher virus complex, there is no correlation between increased pathogenicity and the number of copies of the tandem repeat. Both R-MuLV and R-MCFV which cause long latency chronic leukaemia have one copy of the repeat as does R-SFFV which causes acute leukaemia.

It is also suggested that the deletion of one copy of the repeat is required to generate more pathogenic viruses, e.g. F-MCFV and F-SFFV from F-MuLV (Wolff et al., 1985). This seems plausible especially for F-SFFV. So far, all isolates of F-SFFV investigated, from both the anaemia and the polycythaemia strains, have only one copy of the repeat. In a more recent paper, however, the same authors (Wolff & Ruscetti, 1986) demonstrate that recombinants of F-SFFV with LTRs containing one or two copies of the repeat are equally pathogenic.

The R-SFFV LTR differs in no more than three positions from the LTR of R-MuLV clone 9, both having one copy of the repeat. Therefore, the argument that deletion of one copy of the repeat is required to generate more pathogenic viruses does not pertain in the case of Rauscher virus either.

Apart from the LTR, other regions of the viral genome influence the degree of leukaemogenicity (DesGroseillers & Jolicoeur, 1984b; Oliff et al., 1985). The env region plays an important role in the generation of MCF viruses and in the induction of erythroleukaemia (Evans & Cloyd, 1985; Linemeyer et al., 1982; Oliff et al., 1984; Van Griensven & Vogt, 1980). Some authors speculate that SFFV was formed from an MCF virus which had undergone a specific deletion in the env gene. This is borne out by sequence data of Rauscher and Friend MCFV and SFFV (Bestwick et al., 1984; Koch et al., 1984; Vogt et al., 1985).

Apart from leukaemogenicity, the LTR can also confer tissue tropism. The experiments carried out with recombinant viruses, e.g. Moloney MuLV (M-MuLV) with an F-MuLV LTR (Chatis et al., 1984) show that the F-MuLV LTR can change the target cell for leukaemia from a lymphoid cell to an erythroid cell. The reciprocal recombinant induces almost exclusively lymphomas. A recombinant of R-MCFV with an M-MuLV LTR gives rise to lymphomas as well (Vogt et al., 1985), but the reciprocal recombinant of this combination has not been tested.

Koch et al. (1984) have found that there are certain nucleotides in the LTR which are consistently different between lymphoid and erythroid leukaemia-inducing viruses. They occur in positions 20, 21, 29, 30, 32, 128, 226, 289 and 334 (Fig. 3). In R-SFFV and in R-MuLV clone 9, those nucleotides considered specific for erythroleukaemia-inducing viruses have been conserved. In contrast to the Friend virus complex, where both SFFV and MuLV components can induce rapid erythroleukaemia (Troxler & Scolnick, 1978), the Rauscher virus components have a different tissue tropism. R-SFFV always causes acute erythroid disease, whereas R-MuLV generally causes lymphoid and myeloid leukaemia (de Both et al., 1985). This difference in target cell specificity cannot be deduced from differences in the LTR.
The difference in tissue tropism between R-MuLV and R-SFFV is probably the result of two different mechanisms for the induction of the leukaemia as is suggested by Vogt et al. (1985). In the acute erythroid disease, the R-SFFV env product may act as a mitogen for the erythroid target cell, and the enhancer in the LTR may then increase this mitogenic stimulation. The mechanism of leukaemia induction by R-MuLV is probably less related to the env gene, although some authors suggest that for other viruses MCFV formation is also required for the induction of lymphatic leukaemia (Cloyd, 1983). For chronic leukaemia viruses, several examples have been described of 'insertional activation' by retrovirus promoter or enhancer insertion, either close to a known oncogene (Hayward et al., 1981; Corcoran et al., 1984; Steffen, 1984) or in a specific chromosomal region (Nusse & Varmus, 1982; Tsichlis et al., 1983; Cuypers et al., 1984; Silver & Kozak, 1986). Both ecotropic MuLVs and MCFVs are capable of activating oncogenes in T cell lymphomas (Selten et al., 1984).

Our results favour the conclusion that the biological difference between R-SFFV, R-MCFV and R-MuLV cannot be correlated to differences in the nucleotide sequence of their respective LTRs.

We thank Dr A. Habara for sending us R-MuLV clone RV-1.

REFERENCES


(Received 23 June 1986)