Characterization of a Large Genomic Size Moloney Murine Sarcoma Virus Produced by a Transformed Rat Cell Line

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SUMMARY

A rat cell line (78A1) transformed by the Moloney murine sarcoma virus–Moloney murine leukaemia virus (Mo-MuSV–MuLV) complex was found to produce a sarcoma virus different from the isolates previously described. Analysis of intracellular RNA of the 78A1 cell line by electrophoresis on agarose gel, and hybridization with DNA probes specific to M1-Mo-MuSV and v-mosMo sequences revealed a size of 6.7 kilo-bases (kb) for the RNA of this sarcoma virus. This genome is larger than those of m1, m3, HT1 and 124 isolates and slightly smaller than the myeloproliferative sarcoma virus genome (7.0 kb).

Moloney murine sarcoma virus (Mo-MuSv) is a replication-defective retrovirus arising from a recombination event between the genome of Moloney murine leukaemia virus (Mo-MuLV) and cellular sequences present within the normal mouse genome. These sequences, referred to as mosMo, are essential for the virus-transforming activity (Andersson et al., 1979).

After the isolation of the original Mo-MuSV–MuLV complex from a rhabdomyosarcoma in a Balb/c mouse by Moloney (1966), different cloned Mo-MuSV were characterized either in the original virus complex, or in various MuSV–MuLV-induced tumours (Fischinger et al., 1974; Ball et al., 1973; Huebner et al., 1966). Each of the MuSV isolates was shown to carry identical acquired cellular sequences [about 1.2 kilobase pairs (kbp)] which are inserted at different sites in the MuLV env gene (Donoghue et al., 1979). The cloned MuSV display a different set of deletions in the MuLV gag, pol and env genes, as observed by comparative electron microscopy of heteroduplex molecules (Donoghue et al., 1979). Furthermore, the complete nucleotide sequence of one of the isolates, 124-Mo-MuSV, was reported recently (Reddy et al., 1981; Van Beveren et al., 1981 b).

In the present report, we describe the intracellular retrovirus RNA in a transformed rat cell line, 78A1. This cell line was established by Bernard et al. (1967) by infecting Wistar-CF embryonic rat cells with an extract of Balb/c mouse tumours, obtained by inoculation of the original Mo-MuSV–MuLV complex into newborn animals. The biological properties of the transforming and helper viruses released by these cells were studied (Bernard et al., 1967, 1972; Devaux et al., 1980). 78A1 was shown to release a defective sarcoma virus together with the NB ecotropic Mo-MuLV and a xenotropic helper virus, probably amplified in the rat cells upon Moloney virus complex infection. We report that 78A1 cells produce an RNA species hybridizing specifically with v-mos sequences, which is larger than any of the previously described Mo-MuSV isolates.

Besides 78A1, several other cell lines were studied. 3T3-MLV is a fibroblastic murine cell line chronically infected by Mo-MuLV; FG10 cells are murine 3T3-FL cells, non-productively transformed by m1-MuSV (Fischinger et al., 1974); the hamster tumour HT1 cell line was isolated by Huebner et al. (1966); C3H cells infected with B88 virus (m3-MuSV strain) were a gift of Dr J. C. Chermann, Institut Pasteur, Paris, France.

Polyadenylated RNA extracted from monolayer cultured cells were purified by chromatography on oligo(dT)-cellulose columns and fractionated by agarose gel electrophoresis after denaturation in the presence of glyoxal (McMaster & Carmichael, 1977). The method of Thomas (1980) was used for transfer of RNA to nitrocellulose filters. Virus RNA was revealed by hybridization with nick-translated 32P-labelled DNA probes as described by Rigby et al. (1977).
Two different probes were used: first, proviral m1-MuSV DNA cloned in λgt WES λB by Vande Woude et al. (1979), which recognizes Mo-MuLV-derived as well as Mo-MuSV-specific sequences, and second, a fragment of the v-mos\textsuperscript{Mo} gene that we have subcloned in pBR322, as schematically represented in Fig. 1. A 4-1 kbp fragment containing the 3' end of the virus DNA was generated by \textit{Bgl}II digestion of a purified m1 \textit{EcoRI} insert. Partial \textit{PstI} digestion of the 2-0 kbp \textit{SmaI} fragment containing the entire v-mos gene allowed the construction of several recombinant plasmids, which have inserted various regions of the transforming gene. Plasmid pM6 carrying the B and C fragments was used as an MuSV-specific probe.

The patterns of intracellular virus RNA related to Mo-MuSV and MuLV are shown in Fig. 2(a). Four different major species were expressed in 78A1 rat cells (lane 1), and by comparison with the electrophoretic mobility of known virus species, Mo-MuLV genomic and subgenomic RNA present in 3T3-MLV cells (lane 3) and m1-MuSV RNA detected in FG10 cells (lane 5), we estimated their size to be 8-3, 7-0, 6-7 and 3-0 kb. The 8-3 kb and 3-0 kb species correspond most probably to the expression of NB ecotropic Mo-MuLV in 78A1 cells. The two additional RNAs revealed by the m1 probe appear as a doublet migrating slightly faster than the Mo-MuLV genomic RNA.

In order to localize on this pattern the RNA species carrying v-mos-transforming sequences, RNA from 78A1 cells was separated under similar conditions and hybridized with the DNA of plasmid pM6. This probe does not allow the detection of Mo-MuLV RNA (Fig. 2a, lane 4), whereas it reveals the presence of the m1-MuSV 4-6 kb RNA (lane 6). As shown in Fig. 2(a), lane 2, a major RNA species of 6-7 kb and two minor species of 5-2 and 3-0 kb were revealed in the rat cell line. The two larger species were also found in the virions produced by 78A1 cells (results not shown) and are therefore presumed to be genomic RNAs, whereas the 3-0 kb species would be an Mo-MuSV subgenomic RNA. Furthermore, when the RNA separated in Fig. 2(a), lane 2, was subsequently hybridized with the m1 probe only one band was visualized in the 3-0 kb region (lane 1), indicating that the sizes of Mo-MuSV subgenomic RNA from 78A1 cells and Mo-MuLV \textit{env} mRNA are identical. The 7-0 kb RNA species which does not hybridize with the v-mos-specific probe, may represent either a deleted helper virus genome, or a recombined species of Mo-MuLV with other unknown sequences.

Fig. 2(b) compares the size of the Mo-MuSV RNA expressed in 78A1 cells (lane 1), and of the RNA from previously characterized Mo-MuSV strains, HT1 (lane 2), m1 (lane 3) and m3 (lane 4). These results clearly confirm the estimated size of 78A1 virus RNA obtained from Fig. 2(a): the 6-7 kb RNA migrated slower than the genomic RNA of HT1-MuSV (6-1 kb), m3-MuSV (5-2 kb) and m1-MuSV (4-6 kb). The 5-2 kb RNA species from 78A1 co-migrated with the m3-MuSV genomic RNA. This weak RNA band was present in variable amounts in most preparations of 78A1 virus and cytoplasmic RNA. It could either represent an MuSV genomic RNA, or result from a partial and selective degradation of the major 6-7 kb MuSV species. The 3-0 kb RNA
Fig. 2. Electrophoretic pattern of Mo-MuSV-MuLV intracellular RNA in agarose gels. Polyadenylated RNA from various cell lines were denatured by glyoxal treatment and subjected to electrophoresis (3-6 V/cm for 5 h) in a 1.25% agarose gel. The RNA was transferred to a nitrocellulose filter and assayed for virus species with the indicated nick-translated 32P-labelled DNA (10^5 ct/min/cm^2). Autoradiography was performed for 24 h. (a) RNA from 78A1 cells, 3T3-MLV cells and FG10 cells were hybridized with the 2ml probe (lanes 1, 3 and 5 respectively) and with the 'mos'-specific pM6 probe (lanes 2, 4 and 6 respectively). (b) RNA from 78A1 cells (lane 1), HT1 hamster cells (lane 2), FG10 cells (lane 3), and C3H-B88 cells (lane 4) were hybridized with the Mo-MuSV-specific pM6 probe.

species co-migrated with the subgenomic RNA from HT1 and seemed slightly larger than the subgenomic RNA from m3-MuSV-infected C3H cells.

Hybridization analysis of the virus RNA synthesized in 78A1 cells with a 'v-mos'-specific DNA probe has shown the occurrence of an MuSV genomic RNA which is larger, on agarose gel mobility criteria, than the genome of any known Mo-MuSV variant. This RNA species was also found to hybridize with Mo-MuLV probes specific for the long terminal repeats (LTR) and 'gag' gene sequences (J. Devaux et al., unpublished results). This Mo-MuSV isolate might have been selected in our laboratory from the original Mo-MuSV–MuLV complex or generated later during successive passages in vivo in Balb/c mice and in vitro in embryonic rat cells. It has been shown to transform fibroblasts in vitro and induce sarcoma in newborn mice (C. Bernard, unpublished results).

It is worth noticing that the myeloproliferative sarcoma virus (MPSV), which is derived from Mo-MuSV–MuLV and exclusively contains sequences of these two viruses, has also a large genomic size (7-0 kb) (Pragnell et al., 1981). Preliminary studies on the biological activity of the 78A1 virus complex indicate that there is no induction of myeloproliferative disease when injected into adult mice. It would be interesting to compare further the genomic organization and the biological activity of MPSV with our Mo-MuSV isolate. Restriction enzyme mapping and microscopic examination of heteroduplex molecules formed by the 6-7 kb genome with known molecularly cloned proviral DNA (such as Mo-MuLV, m1 and HT1-MuSV) are under current investigation. Molecular cloning of this Mo-MuSV isolate would permit studies providing additional information on its genetic structure and organization.
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REFERENCES


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