Characterization of Viral RNA in Cells Transformed by Various Isolates of Moloney Murine Sarcoma Virus

By RICHARD HAMELIN, JEANNE DEVAUX, NICOLE HONORÉ, MARIE-ANNICK AUGER-BUENDIA AND ARMAND TAVITIAN*

INSERM, U-248, Unité de Génétique et Expression des Oncogènes, Faculté de Médecine Lariboisière–Saint-Louis, 10, avenue de Verdun, 75010 Paris, France

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SUMMARY

Intracellular polyadenylated viral RNA from cells infected by five different isolates of Moloney murine sarcoma virus (Mo-MuSV) was analysed by gel transfer hybridization. Genomic sizes of 4-6 kilobases (kb) for the m1-MuSV isolate, 5-2 kb for the m3- and 124-MuSV, 6-1 kb for the HT1-MuSV and 6-7 kb for the 78-A1-MuSV were determined. With the exception of the m1 strain, subgenomic RNA species were found in cells infected by the various isolates. However, no common subgenomic RNA containing \( v\)-mos sequences could be characterized. Each transformed cell line expressed a different set of viral RNA species in terms of size and structure.

Since the early isolation by Moloney of a murine sarcoma virus (Mo-MuSV) after injection of high doses of the replication-competent Moloney leukaemia virus (Mo-MuLV) in BALB/c mice (Moloney, 1966), several cloned Mo-MuSV have been characterized either from the original Mo-MuSV-MuLV complex or from Moloney sarcoma virus-induced tumours (Huebner et al., 1966; Bassinet al., 1971; Ball et al., 1973; Fischinger et al., 1974). All the Mo-MuSV isolates perform the same biological functions: induction of fibrosarcoma in newborn mice and transformation of mouse fibroblasts \textit{in vitro}; they share basic organizational features and have arisen by recombination between the Mo-MuLV virus and a set of sequences present in the host cellular genome (Scolnick et al., 1975; Frankel & Fischinger, 1976). As shown previously by comparative heteroduplex analysis (Donoghue et al., 1979a), genomic RNAs of a variety of Mo-MuSV isolates retain almost entirely the \textit{gag} gene but possess different deletions into the \textit{pol} gene. Most of the \textit{3'-located Mo-MuLV env} gene is deleted and substituted by the acquired cellular sequences. Transfection experiments using fragments of Mo-MuSV proviral DNA have shown that the transforming activity is located in the acquired cellular sequences (Anderson et al., 1979; Canaani et al., 1979; Oskarsson et al., 1980). Hence, the term \textit{mos}, derived from the virus name \textit{Moloney sarcoma}, was attributed to this gene (Coffin et al., 1981), which is called \textit{v-mos} and \textit{c-mos} according to its viral or cellular origin.

In their efforts to understand the mechanism of Mo-MuSV-induced transformation, several groups have attempted to identify the gene products encoded by \textit{v-mos}. By examining the coding potential of viral poly(A)\(^+\) RNA of different sizes in translation experiments \textit{in vitro}, they found a family of proteins supposedly encoded by the acquired cellular sequences (Papkoff et al., 1980; Lyons et al., 1980; Cremer et al., 1981). However, this search was hampered because of the lack of specific antisera against the Mo-MuSV tumours. Following the determination of the complete sequence of the \textit{v-mos} gene (Van Beveren et al., 1981) an antiserum was raised against a synthetic dodecapeptide corresponding to the C-terminus of the open reading frame (Papkoff et al., 1981). By using this antiserum a scarce protein doublet of approximately 37 kilodaltons (k dal) was specifically precipitated in Mo-MuSV-transformed cell lines (Papkoff et al., 1982).

Studies were also undertaken to characterize the intracellular RNA containing \textit{v-mos} sequences in murine cells transformed by 124-Mo-MuSV. The results appeared not to agree. By
electron microscopy studies of heteroduplexes, Donoghue et al. (1979a) characterized a subgenomic RNA 3.1 kilobases (kb) long, retaining part of the pol and env regions and containing the entire v-mos sequence. More recently, Dina (1982) found this species in the total cellular poly(A)+ RNA of 124-Mo-MuSV-transformed cells but detected only a 2.1 kb-long mRNA on the polyribosomes.

In the present report, we characterize the intracellular genomic and subgenomic viral RNA in five cell lines, each transformed by a different isolate of Mo-MuSV. We confirm the sizes previously determined in heteroduplex studies for the various genomic RNAs (Donoghue et al., 1979a). We observe a considerable variability in the intracellular subgenomic RNAs expressed in cells transformed by the various Mo-MuSV isolates.

The following cell lines were used: (i) the G-8-124 mouse cell line (Ball et al., 1973) producing an excess of 124-Mo-MuSV over the helper Mo-MuLV (gift of Dr J. K. Ball, University of Western Ontario); (ii) the FG 10 mouse 3T3-FL cell line, non-productively infected with ml-Mo-MuSV (Fischinger et al., 1974); (iii) the S+L+ cell clone C243 carrying m3-Mo-MuSV (Bassin et al., 1971); (iv) the HT1-Mo-MuSV-transformed non-producer hamster cells (clone P25) isolated from a hamster tumour (Huebner et al., 1966); (v) the 78 A1 rat cell line, established by Bernard et al. (1967) by infecting Wistar CF embryonic rat cells with the original Mo-MuSV-MuLV complex; a sixth cell line, NIH-3T3 murine fibroblasts infected by Mo-MuLV, was used as a helper-positive–MuSV-negative control. All cell lines were grown in MacCoy’s medium containing 10% heat-inactivated foetal calf serum.

Total cellular RNA was extracted as previously described (Hamelin et al., 1973). Polyadenylated RNA was selected twice on oligo(dT)—cellulose columns (P-L Biochemicals) (Aviv & Leder, 1972), and denatured by heating for 20 min at 50 °C in 50% dimethyl sulphoxide, 1 M-glyoxal and 10 mM-sodium phosphate buffer pH 7 (McMaster & Carmichael, 1977). They were then subjected to electrophoresis in 1.25% agarose gels at 80 V for 5 h and blotted overnight onto nitrocellulose filters as described by Thomas (1980). Ribosomal 28S and 18S RNAs were run on a parallel lane as markers and were revealed by acridine orange staining (McMaster & Carmichael, 1977). Prehybridization, hybridization, and washing procedures were as described by Wahl et al. (1979).

Several DNA fragments derived from λml, a proviral ml-Mo-MuSV DNA cloned in λgt WES, λB by Vande Woude et al. (1979a) were used as hybridization probes (Fig. 1). The EcoRI–BglII 5' and 3' parts of ml-MuSV were subcloned in the EcoRI and BamHI sites of pBR322. A v-mos-specific fragment was subcloned from the 3' end recombinant into pBR322 by partial hydrolysis with PstI (Devaux et al., 1982). Other fragments were obtained by sucrose gradient purification after hydrolysis with the indicated restriction enzymes. They correspond to U3- and gag-specific sequences and to a DNA fragment containing both the U5 sequence and the sequence 5' to the consensus donor splice. The location of the m1°Mo-MuSV genes was deduced by comparing the 2ml restriction map (Vande Woude et al., 1979a) with the complete nucleotide sequences of 124-Mo-MuSV (Van Beveren et al., 1981; Reddy et al., 1981) and Mo-MuLV (Shinnick et al., 1981).

The different DNA fragments were 32P-labelled by nick-translation (Rigby et al., 1977) to specific activities of approximately 108 ct/min/μg using [α-32P]dCTP from New England Nuclear (3000 Ci/mmol). Approximately 105 ct/min of 32P-labelled DNA was used per cm2 of nitrocellulose sheet. After an overnight hybridization at 42 °C in sealed plastic bags, the filters were washed, dried and exposed to X-ray film (Kodak X-Omat S) with a DuPont Cronex Lightning intensifying screen at −80 °C.

Before re-using the nitrocellulose filters, the hybridized probe was removed by two washes at 100 °C with 1 x SSC (0.15 M-NaCl, 0.015 M-sodium citrate) for 20 min. The effectiveness of washing was checked by autoradiography and subsequent prehybridization and hybridization with a new probe were performed after the complete removal of radioactive label on the filter.

Fig. 2 shows the pattern of the Mo-MuSV-related poly(A)-containing RNA extracted from cells infected by five different Mo-MuSV isolates and from the 3T3-MuLV cell line containing only the Mo-MuLV component. Each blot was successively hybridized with the v-mos (lanes 1), U5 (lanes 2), gag (lanes 3) and U3 (lanes 4) probes. Two RNA species, 8.3 kb and 3.0 kb long,
Fig. 1. Characterization of the different probes used in this work. (a) Location of the viral genes on the genomic RNA of the m1 strain of Mo-MuSV. (b) Partial restriction endonuclease map (according to Vande Woude et al., 1979b) of two BgII–EcoRI DNA fragments of m1 subcloned in pBR322 (Devaux et al., 1982). M, Mink; ---, pBR322 sequences; LTR, long terminal repeated sequences; B, BglII; bg, BgI; E, EcoRI; H, HinfI; K, KpnI; P, PstI; S, SacI; Sm, Sinai; X, XbaI; Xh, XhoI. (c) The location of viral genes within the restriction fragments deduced from the published sequence data on 124-Mo-MuSV (Reddy et al., 1981; Van Beveren et al., 1981) and Mo-MuLV (Shinnick et al., 1981).

Fig. 2. Size determination of Mo-MuSV-specific RNA in transformed cells. Approximately 5 μg of polyadenylated RNAs from several cell lines were denatured by glyoxal treatment, electrophoresed in agarose gel and transferred to nitrocellulose. They were then hybridized sequentially with various 32P-labelled nick-translated DNA probes. Poly(A)+ RNA was extracted from: (a) 3T3-MLV cells; (b) FG 10 cells; (c) C243 cells; (d) G-8-124 cells; (e) HT1 cells; (f) 78 A1 cells. The probes were respectively v-mos-specific (lanes 1), U3-specific (lanes 2), gag-specific (lanes 3) and U5-specific (lanes 4). The positions of 28S and 18S ribosomal RNA electrophoresed on a parallel lane are indicated as size markers.

were revealed with the U5 and U3 probes in 3T3-MuLV cells (lanes a2 and a4). These RNA species correspond respectively to the genomic and the spliced mRNA for Mo-MuLV env gene (Rothenberg et al., 1978). The v-mos-specific probe did not hybridize with the Mo-MuLV species (lane a1) while the gag-specific probe revealed only the genomic RNA (lane a3). These results demonstrated the specificity of each probe. Among the different cell lines transformed by Mo-MuSV, G-8-124 (lanes d) and 78 A1 (lanes f) are productively transformed cells and contain both the 8·3 kb and 3·6 kb Mo-MuLV-related RNA species.
The patterns of intracellular RNA related to Mo-MuSV, as revealed by hybridization to the v-mos-specific probe, are shown in the first lane of each blot. The genomic size of each Mo-MuSV isolate, determined by agarose gel mobility, was respectively 4.6 kb for the m1 isolate (lane b1), 5.2 kb for the m3- and 124-Mo-MuSV (lanes c1 and d1), 6.1 kb for the HT1-MuSV (lane e1) and 6.7 kb for the Mo-MuSV produced by the 78 A1 cells (lane f1). These values are in agreement with those obtained by Donoghue et al. (1979a) using electron microscopic visualization of heteroduplexes between the DNA of 124-Mo-MuSV and the RNAs of various Mo-MuSV isolates.

There was also a considerable variability in the subgenomic v-mos-related RNA. No detectable hybridization to subgenomic RNA was observed with the various probes in the non-producer FG 10 cells transformed by the ml-Mo-MuSV isolate (lanes b).

In cells transformed by the m3- and 124-Mo-MuSV strains which displayed similar genomic length and structure (Donoghue et al., 1979a), the v-mos-specific probe did not reveal any subgenomic RNA species although it hybridized intensely with the 5.2 kb genomic RNA (lanes c and d). A discrete subgenomic RNA, 3.5 kb long, was clearly observed, however, when the 5' and 3' end-specific and the gag-specific probes were used (compare lanes 2, 3 and 4 with lanes 1 in blots c and d in Fig. 2). In HT1 cells, two Mo-MuSV subgenomic RNA species, about 3.0 kb and 1.4 kb in size, were detected. Both bands hybridized to the v-mos-specific probe (lane e1) but only the smaller one was revealed by the U5 probe (lane e2) and none by the gag probe (lane e3) even after longer exposures. The pattern obtained with the 78 A1 cell line has been already described (Devaux et al., 1982): besides the 6.7 kb genomic RNA, a 3.0 kb-long species was revealed by the v-mos probe (lane f1). This 3.0 kb band could not be separated from the 3.0 kb Mo-MuLV env mRNA; since the latter species hybridizes to both the U5- and U3-specific probes, it was not possible to determine whether the former band also contained U5 and U3 sequences (lanes f2 and f4).

The acquired cellular sequences of Mo-MuSV were shown by transfection assays to be responsible for the transforming activity of the virus (Anderson et al., 1979; Canaani et al., 1979). These sequences are located at the 3' end of the viral genome. The v-mos transforming protein could be expressed via three different mechanisms: (i) as a polyprotein translated from the entire MuSV genome, (ii) as a v-mos-specific protein translated from a subgenomic spliced mRNA or (iii) as a v-mos-specific protein translated from a complete MuSV genome at an internal initiation site. A fusion polyprotein was never characterized in Mo-MuSV-transformed cells, except in the case of cells infected with ts 110, a thermosensitive mutant of Mo-MuSV 349 (Peltier-Horn et al., 1980, 1981). In contrast, an overlapping set of v-mos-coded proteins has been identified by translation experiments in vitro (Papoff et al., 1980; Lyons et al., 1980; Cremer et al., 1981). In this work, we examined the intracellular viral RNA in cells infected by five different isolates of Mo-MuSV. Poly(A)+ RNA blots were hybridized with various nick-translated DNA fragments of the cloned ml-Mo-MuSV. Under our experimental conditions, we could not detect any v-mos spliced mRNA in m1-, m3- and 124-transformed cells. A 3.5 kb RNA species was detected in m3- and 124-Mo-MuSV-transformed cells, but was not v-mos-specific. The 78 A1 and the HT1 cell lines contain a mos-specific RNA 3.0 kb long. In the 78 A1 cell line, this RNA species is not the direct transcript of a deleted Mo-MuSV provirus since Southern blot analysis of genomic DNA shows the presence of only one copy of a complete provirus (M. Quibriac, unpublished results). However, this 3.0 kb mos-specific RNA co-migrates with the spliced mRNA for the env gene of Mo-MuLV, so that we cannot confirm the presence of U5 and U3 sequences and determine whether this RNA is spliced. In HT1 cells, the 3.0 kb RNA does not hybridize with U5 and U3 probes. In contrast, the same cells contain a 1.4 kb RNA species hybridizing with U5, v-mos and U3 sequences, but not with a gag-specific probe. This RNA species is too short to contain the entire v-mos gene, but other reports suggest that the 5' end of v-mos is not required for the transforming activity of the gene (Dina, 1982; Rechav et al., 1982).

The subgenomic RNA species observed in the cells transformed by the various Mo-MuSV isolates are quite disparate. Some of them contain mos sequences and others do not. It is noticeable that there exists a considerable variability in the sizes and structures of the various
genomic RNAs, and acceptor and donor splice sequences could have been either deleted or created in the course of the generation of the various Mo-MuSV isolates by recombination events. Donoghue et al. (1979b) described a 3-1 kb RNA species in Mo-MuSV-infected G-8-124 cells, but Dina (1982) did not find this species in the poly(A)+ fraction of polysomal RNA. We found a similar mos-related species in 78 A1 and HT1 cells, but the 3-5 kb RNA species we detected in m3- and 124-Mo-MuSV-infected cells did not contain mos sequences.

Among the set of viral subgenomic RNA species described in this paper, none has really proved to be the messenger for the transforming protein. The problems raised by the detection of such an mRNA can account for the results obtained by Papkoff et al. (1982): by using an antiserum raised against a synthetic dodecapeptide, they immunoprecipitated a very low level of the 37 kdal v-mos-specific protein in the cytoplasm of 124-Mo-MuSV-infected cells. Finally, no expression of c-mos sequences has ever been detected in normal cells (Gattoni et al., 1982) even during embryonic development (Müller et al., 1982), the only exception being a 1-2 kb mos-specific RNA species found in a mouse myeloma (Rechavi et al., 1982).

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