Characterization of the Poly (A)$^+$ and the Poly (A)$^-$ RNAs in the Native and Denatured Genomes of Oncornaviruses

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SUMMARY

The native and denatured 60 to 70S RNAs of two murine oncornaviruses and one simian oncornavirus were examined for their poly (A) content. In the native genome, a significant proportion of poly (A)$^-$ RNA was found and intact 60 to 70S RNA complex was identified in this fraction. The amount of poly (A)$^-$ RNA in the native genome was related to cellular growth and seemed to be independent of virus maturation. The subunits obtained after thermal denaturation consisted of approx. 2/3 poly (A)$^+$ and 1/3 poly (A)$^-$ RNA.

The poly (A)$^-$ subunits were mainly composed of 20 to 28S RNA and the poly (A)$^+$ subunits of 30 to 35S RNA. The results of competitive molecular hybridization of these two fractions with virus cDNA suggested that the two species of subunits possessed similar nucleotide sequences.

Polyriboadenylic acid [poly (A)] sequences have been found in the genomic RNA of retroviruses (Gillespie, Marshall & Gallo, 1972; Green & Cartas, 1972; Lai & Duesberg, 1972). Several hypotheses have been elaborated concerning the function of poly (A) present in virus RNA but this function is not yet understood.

Some authors claimed that the intact 60 to 70S RNA always contained poly (A) (King & Wells, 1976). The existence of poly (A)-rich and poly (A)-lacking 35S RNA subunits was reported by several investigators (Ihle, Lee & Kenney, 1974; Wang & Duesberg, 1974) and it was suggested that this heterogeneity might have a biological meaning.

In the present report we characterize the virus RNA populations that differ in their poly (A) content and demonstrate the existence of 60 to 70S RNA lacking poly (A) sequences. We first analysed the 60 to 70S complex of the native virus genome and then the subunits obtained by thermal denaturation of this RNA complex.

Three cell lines producing C-type virus particles were used for preparing labelled viruses: (1) The 78A$_1$ rat cell line chronically infected by a mouse sarcoma virus (Moloney strain) M-MSV(MLV), isolated by Bernard, Boiron & Lasneret (1967). (2) The ERTh rat cell line chronically infected by the Gross leukaemia virus (GLV) isolated by Ioachim (1969). (3) The NRK rat cell line chronically infected by the simian sarcoma virus (SSV-1) isolated by Theilen et al. (1971).

Cells were grown as monolayers in Eagle’s minimum essential medium supplemented with 10 % calf serum. For labelling experiments, the cells were incubated in the same medium without phosphate and with 25 µCi $^{32}$P/ml. The supernatant was usually harvested after 24 h of labelling. The procedures used for virus purification have been described previously (Emanoil-Ravicovitch et al. 1973). Nucleic acids were extracted from the purified virus by the cold phenol-SDS procedure as already described (Galibert et al. 1966) and native RNA genome was separated from the low mol. wt. RNAs.

Poly (A)$^+$ and poly (A)$^-$ RNAs were separated on unesterified cellulose columns, as
described previously (Larsen et al. 1973). As a control for the cellulose chromatography method we have done parallel experiments on Millipore membranes as described by Lee, Mendecki & Brawerman (1971). Both methods gave similar results. Poly (A)− and poly (A)+ were analysed by electrophoresis in polyacrylamide-agarose gels (Tiollais et al. 1972) or by centrifugation in sucrose gradients. The cDNA used for hybridization was prepared as described by Tavitian et al. (1974).

The experiments were performed to purify the intact 60 to 70S RNA complex based on its poly (A) content. The percentage of the total native RNA input separated by chromatography on a cellulose column for three retroviruses was calculated. In a first experimental set, a significant percentage (20 to 30 %) of native virus RNA was found to be poly (A)− for the three studied viruses [M-MSV(MLV), GLV and SSV-1].

Several hypotheses could explain this high percentage of poly (A)− population: (a) this fraction might represent degraded 60 to 70S RNA; (b) the secondary structure of the 60 to 70S RNA complex is such that the poly (A) segments are situated in a supercoiled region and therefore could not bind to the column [presence of cryptic poly (A)]; (c) the virus population is heterogeneous in its genome with respect to the poly (A) content; it may be that some viruses possess genomes devoid of poly (A). We attempted to answer this question by further characterization of poly (A)+ and poly (A)− nucleic acid populations, isolated by cellulose column chromatography.

Nucleic acid materials from poly (A)− and poly (A)+ fractions were separated, pooled and analysed by sucrose gradient centrifugation for each kind of virus. The centrifugation profiles of the M-MSV(MLV) genome are presented in Fig. 1. As shown in this figure, all the native genome before cellulose column chromatography consisted of high mol. wt. RNA (Fig. 1a). The poly (A)− and poly (A)+ fractions (Fig. 1b and c respectively) were also represented by high mol. wt. RNA. A few small RNAs (around 4S RNA) were also present in both fractions. This is probably due to lability of the 60 to 70S RNA after passing through the cellulose column. These results show, however, the presence of an apparently intact 60 to 70S RNA complex lacking poly (A).

To confirm the hypothesis of the presence of cryptic poly (A) in the secondary structure of 70S RNA complex, thermal denaturation experiments were performed on the poly (A)− population. These studies show that the poly (A)− fraction was devoid of poly (A) sequences since, after heating, more than 90 % of this fraction did not bind to the cellulose column. These results suggest the existence not only of viruses with poly (A)+ native genome RNA but also of viruses with poly (A)− native genome RNA. We analysed different properties capable of giving a more homogeneous virus population with regard to the presence of poly (A) sequences.

Retroviruses are released by budding at the cellular membrane in an apparently unstable configuration (immature viruses) and several hours later they are converted into a more stable form (mature viruses). Experiments were carried out to determine whether the changes occurring during the virus maturation process involve variation in the poly (A) content of native genome. We analysed the poly (A) content of native genome in the virus harvested at 1/2 h intervals (young virus). No differences were noted in the percentage of poly (A)− and poly (A)+ fractions of the native genome between the young virus and the mature virus (24 h harvest).

In contrast, when we changed the cellular growth conditions, we obtained, less than 10 % of poly (A)− 60 to 70S RNA. In this second series of experiments, we used a new set of foetal calf serum in the culture medium which accelerated the cellular multiplication. All the other culture conditions being the same, the cells in such cultures reached a subconfluent
density after 24 h at 37 °C while in the previous experimental set they grew more slowly and reached the same confluency at 48 h. These results suggest a relationship between stimulation of cell growth and amount of poly (A) in the virus 60 to 70S RNA.

As the 60 to 70S RNA genome of oncornaviruses can be converted by heating into subunits and low mol. wt. (LMW)-associated RNA (Bader & Steck, 1969), it was of interest to investigate the poly (A) content of all the RNAs that composed the 60 to 70S native complex. As a first step, thermal denatured total 70S complex containing 30 % of poly (A)- RNA was analysed by sucrose gradient sedimentation as shown in Fig. 2(a). It consisted of three main fractions as previously described in murine retroviruses (East et al. 1973; Emanoil-Ravicovitch et al. 1975): (1) 30 to 35S RNA; (2) 20 to 26S RNA; (3) 4 to 10S RNA. When the 60 to 70S RNA was denatured and thereafter fractionated by chromatography on a cellulose column, the sedimentation profiles of poly (A)- and poly (A)+ fractions presented in Fig. 2(b) were obtained. It is of interest to note that the poly (A)+ fraction is mainly composed of 30 to 35S RNA subunits, while the poly (A)- fraction contains essentially 20 to 28S RNA subunits and LMW-associated RNAs. In a second step we characterized the subunits of poly (A)+ and poly (A)- 60 to 70S RNA populations. After denaturation, the 60 to 70S RNA lacking poly (A) is mainly composed of 20 to 28S RNA species while 60 to 70S RNA containing poly (A) has the same composition as the total denatured genomic complex, as shown in Fig. 2(a).

These results raise the question of whether poly (A) containing and poly (A) non-containing RNA represent different forms of the genomic virus RNA. For this purpose comparative and competitive molecular hybridizations of poly (A)- and poly (A)+ subunits to virus re-cycled cDNA were performed. We could not detect significant differences in the nucleotide sequences of the two types of viral subunits by this method. These data are in

Fig. 1. Sucrose density gradient sedimentation of the native virus genome of M-MSV(MLV). The 32P-labelled virus RNA was centrifuged through a 5 to 30 % sucrose gradient at 4 °C in a Spinco SW41 rotor at 40000 rev/min for 4 h. (a) Native virus genome before cellulose column chromatography. (b) Poly (A)- nucleic acid population of the native virus genome obtained by cellulose column chromatography. (c) Poly (A)+ nucleic acid population of the native virus genome obtained by cellulose column chromatography. The positions of the ribosomal 28S and 18S RNA are shown in the figures.
agreement with those obtained by fingerprints of avian retrovirus genome subunits (Wang & Duesberg, 1974; Coffin & Billeter, 1976).

Differences in sedimentation coefficients of the poly (A)$^+$ and the poly (A)$^-$ populations could be due to the fragility of the fraction lacking poly (A). One argument in favour of such an assumption is the fact that poly (A) acts as a general inhibitor of endonuclease activity and in so doing prevents the degradation of mRNAs (Levy et al. 1975).

The presence of the 20 to 28S RNA species could also be due to degradation during RNA extraction (King & Wells, 1976). However, if this was the case, one has to explain the presence of 60 to 70S RNA in the poly (A)$^-$ fraction. Moreover, after thermal denaturation the presence of a large amount of the poly (A)$^-$ RNA indicates that our RNA preparations are not massively degraded. We cannot discard the possibility of a specific active nuclease that may remove exposed poly (A) tails of 60 to 70S native genomes. However, the possibility of this kind of specific cleavage occurring only on 30% of the poly (A)$^+$ molecules is difficult to envisage and very unlikely.

We conclude that the percentage of poly (A)$^-$ RNA decreases greatly when the growth of producing cells is stimulated. A relationship between cell growth and virus RNA was also previously reported by Paskind, Weinberg & Baltimore (1975). It should be pointed out that the presence of poly (A)$^-$ messenger RNA has been described in eukaryotic cells (Milearek, Price & Penman, 1974) and furthermore retrovirus murine RNA lacking poly (A) was found in infected cells (C. J. Larsen, personal communication). These facts, taken with the present work, suggest that an intracellular event could be involved in the virus genome poly (A) content: it may well be that when optimal cellular growth conditions are not reached some virus subunits lack poly (A) but are nevertheless assembled into virus particles forming a 60 to 70S RNA complex.
Short communications

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REFERENCES


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