Adenine-rich RNA in the Mycovirus of *Allomyces arbuscula*

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**SUMMARY**

An adenine-rich fraction has been extracted from the mycovirus infecting the Phycomycete *Allomyces arbuscula*. This fraction which accounts for approximately 8% of the total RNA is heterogeneous and contains tracts of 25 to 45 nucleotides in length. The majority of the poly(A) tracts have a mol. wt. of \(1.2 \times 10^4\) and a sedimentation value of 2 to 3S.

The mycovirus infecting the Phycomycete *Allomyces arbuscula* contains a three segmented double-stranded RNA genome (Khandjian, Turian & Eisen, 1977). About 8% of the total \(^{32}\)P-labelled RNA is resistant to pancreatic RNase A in conditions where double-stranded RNA is digested, namely heat denaturation before RNase treatment, while no acid precipitable radioactivity is recovered after alkaline hydrolysis. Furthermore, resistance of this material to RNase A and T₁ provides evidence for a polyadenylic acid [poly(A)] sequence.

Mycelium was grown in the presence of \(^{32}\)PO₄ and the virus was purified by high-speed centrifugation followed by sucrose and CsCl density gradients (Khandjian et al. 1977). \(^{32}\)P-labelled virus RNA was obtained from purified particles by two different procedures: (a) particles were dialysed against 0.048 M-Na-phosphate buffer, pH 6.8, and lysed by addition of an equal volume of 8 M-urea containing 2% (w/v) SDS. The lysate was applied to a column of hydroxylapatite and washed with several volumes of 0.048 M-Na-phosphate buffer. The \(^{32}\)P-labelled RNA was eluted with 0.48 M of the same buffer (Khandjian et al. 1977). (b) Extraction at 60 °C with phenol equilibrated with 10 mM-tris-HCl, pH 8.5, containing 10 mM-EDTA. Both batches of RNA were dialysed against 10 mM-tris-HCl, pH 7.5, containing 0.5 M-KCl before being fractionated by affinity chromatography on a polyuridylic acid [poly(U)] agarose column, type 6 (P-L Biochemicals, Inc.). Elution of the column with buffer containing 0.5 M-KCl yielded an RNA fraction I. Bound poly(A) was then eluted with buffer containing no KCl to give fraction II (approx. 8% of total).

RNA I which was not bound to the poly(U) column was identified after acrylamide gel electrophoresis as the three segmented double-stranded RNA which has previously been studied. In contrast, the poly(A) fraction II migrated ahead of tRNA and was found to be heterogeneous in size. The poly(A) tracts were estimated to be composed of 25 to 45 nucleotides with a mean value of 37, corresponding to a mol. wt. of \(1.2 \times 10^4\) and a mean sedimentation value of 2 to 3S (Fig. 1). Nucleotides were extracted from the gel after alkali digestion of the corresponding slices at 37 °C for 18 h. The base composition was determined after thin-layer chromatography on PEI-cellulose (Merck No. 5579) by the method of East (1968). The RNA had a base composition of 84% AMP, 10% UMP and 3% CMP + GMP.

The poly(A) fraction is an integral part of the virion since it was recovered from the particles after the two different centrifugation procedures used. It is unlikely that the virus preparation was contaminated with poly(A) sequences derived from degraded host cell messenger RNAs.
Reovirus, the best studied prototype of double-stranded RNA viruses, contains an adenine-rich RNA molecule (Bellamy & Joklik, 1967; Shatkin & Sipe, 1968). This adenine-rich molecule is not linked to pieces of the double-stranded RNA genome and unlike most other RNA viruses, reovirus messengers contain no detectable adenylic acid polymers at their 3'-termini (Stoltzfus, Shatkin & Banerjee, 1973; see also review by Silverstein, Christman & Acs, 1976). On the other hand, poly(A) tracts have been demonstrated associated with the double-stranded RNA from *Saccharomyces cerevisiae* strains carrying the killer character (Vodkin, Katterman & Fink, 1974; Shalitin & Fischer, 1975). The relation between the killer character and the presence of virus-like particles is well established (Herring & Bevan, 1975). In the case of the mycovirus infecting *A. arbuscula*, the adenine-rich RNA has been identified as a free molecule by the two different extraction procedures used. This reduces but does not exclude the possibility of an artifact of preparation which might have caused the breakage of single-stranded poly(A) tails associated with one or all of the three different double-stranded RNA species.

Free poly(A) sequences have been proposed as a possible inhibitor of endonuclease activity in eukaryotic cells, and in so doing can prevent the degradation of messenger RNA devoid of poly(A) tails (Levy *et al*. 1975). Since all viruses must perform messenger RNA synthesis (Baltimore, 1971), one might expect that the adenine-rich molecule would play a role in the protection of this messenger.

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**Fig. 1.** Polyacrylamide gel electropherogram of 32P-labelled poly(A) fraction obtained by poly(U) agarose chromatography. Samples were subjected to electrophoresis for 120 min at 5 mA per tube through a 12 cm 3.5 % acrylamide-0.5 % agarose-composite gel (Peacock & Dingman, 1968). Gels were calibrated with 4 and 5S RNA from *E. coli* according to Gould, Pinder & Matthews (1969) and sliced into 2 mm sections for radioactivity determination.
REFERENCES


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