ANALYSIS OF MURINE ONCORNAVIRUS 4S RNA FOR THE PRESENCE OF 'SPOT 1 RNA'

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1. Introduction

A 4S RNA species, known as Spot 1 RNA, has been identified in RSV (Rous Sarcoma Virus) by bidimensional polyacrylamide gel electrophoresis [1]. It represents the most plentiful form of 4S RNA, and also the form which is most strongly associated with the 70S RNA genome of RSV [1,2].

This RNA has been identified as tRNAtrp by aminoacylation and by determination of its primary structure [3,4]. Experiments involving tagging with α-^{32}P-dATP [2,5], heat denaturation of the RNA genome [2,5], reassociation of Spot 1 RNA to the 30–40S RNA subunits [6,7] and more recently inhibition by aminoacylation with tryptophan of the loss of priming activity after periodic acid oxydation [8] have provided evidence for its role as a primer of the in vitro transcription of the genome of two avian oncornaviruses, RSV and AMV (Avian Myeloblastosis Virus).

In order to determine whether Spot I RNA may act as a primer [9] in the transcription of the murine

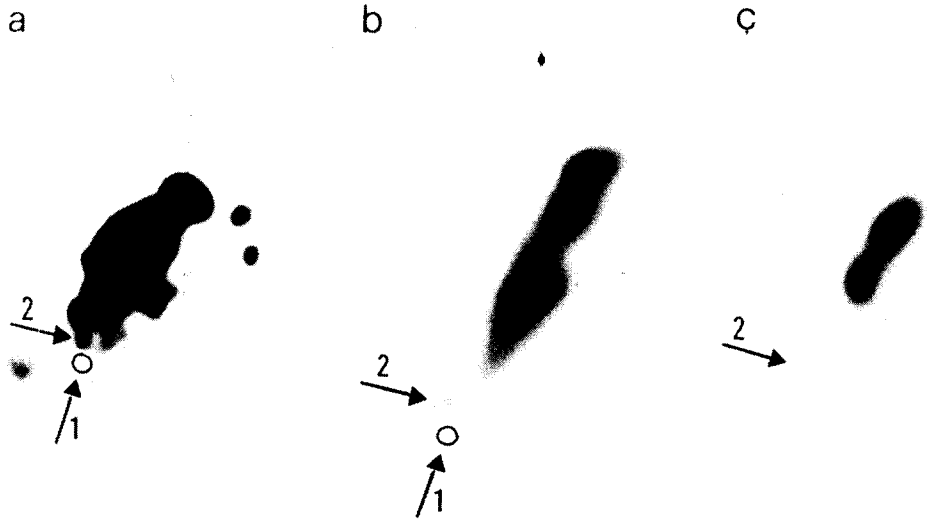

Fig.1. Two-dimensional polyacrylamide gel electrophoresis pattern of 4S RNAs obtained as described in methods by sucrose gradient centrifugation: (a) cellular 4S RNAs from 78A$_1$ cells; (b) free viral 4S RNAs from M-MSV(MLV); (c) associated viral 4S RNA from M-MSV (MLV) released by heating to 60°C. The patterns obtained by heating to 60°C or 80°C were identical. The first dimension (10% polyacrylamide gel) is from right to left and the second dimension (20% polyacrylamide gel) is from top to bottom. Only the 4S region of the first dimension was transferred on top of the 20% gel. 1 and 2 indicate spots of which the fingerprints are shown respectively in fig.2 and fig.4.

oncornavirus genome, it has been sought in M-MSV (MLV) (Moloney–murine sarcoma–leukemia virus complex) and in GLV (Gross leukemia virus).

2. Methods

78A$_1$ cells [10] producing M-MSV(MLV), and ERTh cells [11] producing GLV were grown as monolayers in Eagle's minimum essential medium (MEM) supplemented with 10% foetal calf serum. The cells were labelled for a 24-h period with 25 μCi of carrier-free ^{32}P orthophosphate per ml of phosphate-free MEM supplemented with 10% dialyzed foetal calf serum. Viral and cellular RNA was extracted with phenol as previously described [12,13].

Cellular 4S RNA was obtained by centrifugation of total cellular RNA on a 5–30% sucrose gradient (26 h at 24 000 rev/min in a SW-25-1 Spinco rotor). Viral RNA was separated by sedimentation in a 5–30% sucrose gradient (90 min at 40 000 rev/min in a SW-41 Spinco rotor) into 70S RNA, and a light fraction containing the free viral 4S RNA. Following denaturation (3 min at 60°C or 80°C) the 70S RNA was re-fractionated in a 5–30% sucrose gradient (4 h at 40 000 rev/min in a SW-41 Spinco rotor) in 30–40S RNA representing the subunits of the viral genome and a light fraction containing the viral associated 4S RNA. All sucrose gradients were prepared in 0.01 M Na acetate, 0.1 M NaCl buffer pH 5.

Bidimensional polyacrylamide gel electrophoresis was carried out according to the technique of Ikemura and Dahlberg [14]. RNAs eluted from the gel were fingerprinted after T$_1$ RNase hydrolysis by electrophoresis on cellulose acetate at pH 3.5, followed either by electrophoresis on DEAE paper in 7% formic acid [15] or by homochromatography on thin-layer DEAE cellulose plate [16]. Pseudouridylic acid and ribothymidylic acid were analysed by thin-layer chromatography on cellulose plate [17].

3. Results

Figure 1 shows the autoradiograph of a 20% polyacrylamide gel, which represents the second dimension of a bidimensional electrophoretic separation (10%, 20%) of cellular (78A$_1$) and viral (M-MSV

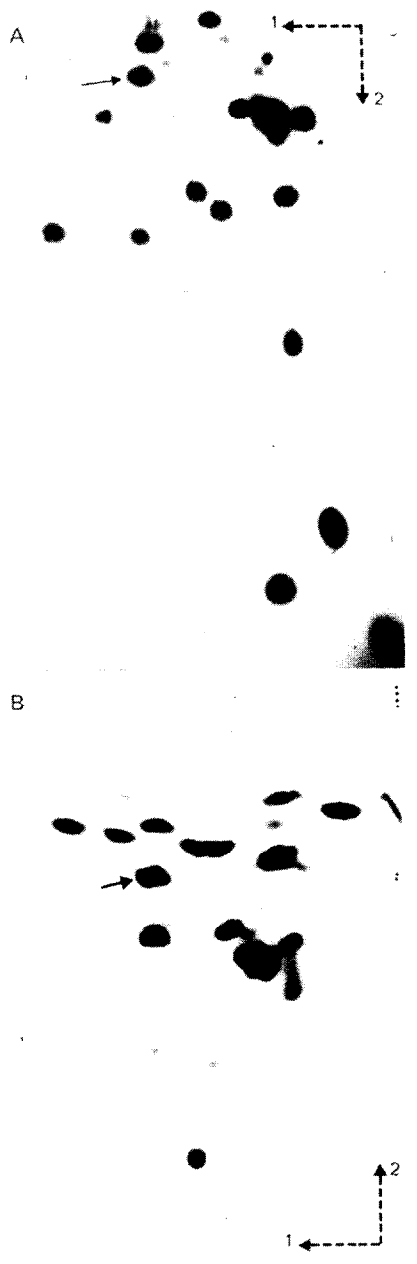

Fig.2. T$_1$ fingerprints of spot 1 RNA isolated from cellular 4S RNAs (fig.1a). First dimension in A and B is electrophoresis at pH 3.5 on cellulose acetate. The second dimension is (in A) electrophoresis in 7% formic acid on DEAE paper and (in B) homochromatography on thin layer DEAE cellulose plate. The homomixture is a 3% concentrated solution of yeast RNA (from B.D.H.) hydrolyzed with KOH for 30 min and dialyzed against 7 M urea.

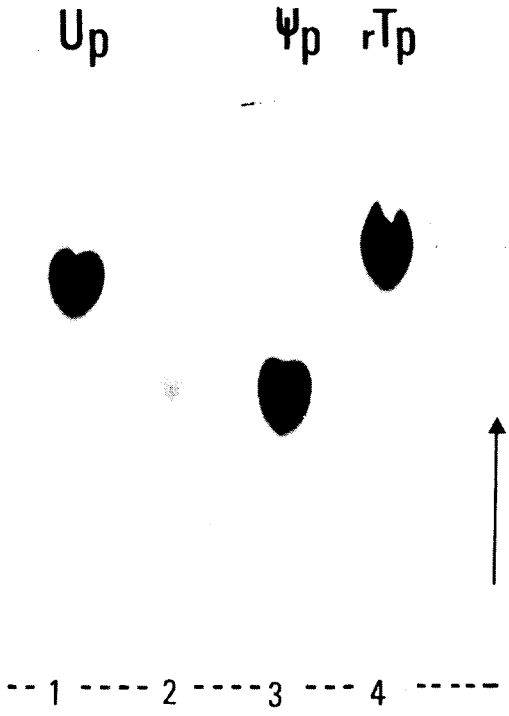

Fig.3. The spot indicated by an arrow in fig.2, has a mobility identical to (U_2, C_1) Gp oligonucleotide. It was eluted, digested with KOH and fractionated by electrophoresis on Whatman 3 MM paper at pH 3.5. The radioactive material migrating like Up was recovered and chromatographed on a cellulose thin-layer plate (20 × 20 cm) with isopropanol, water and concentrated HCl (70:15:15) as solvent (row 2 in this figure). Up, Ψp and rTp used as markers were prepared from a mixture of tRNA, rows 1,3 and 4 in this figure. The interrupted line indicates the origin of migration.

Fig.4. T_1 fingerprint of Spot 2 RNA isolated by 2D gel electrophoresis from cellular 4S RNAs (fig.1a). The fingerprinting system used is that described under (fig.2b).

(MLV)) free and associated 4S RNA, All the spots which, on the basis of their mobility, could have represented spot 1 RNA [1] were recovered from the gel, hydrolysed by T_1 RNase, and fingerprinted. One of these spots which was separated from the cellular 4S RNA (fig.1a) gave a T_1 fingerprint (fig.2) which was identical to that of spot 1 RNA [1]. Furthermore this RNA possessed (as does Spot 1 RNA) the sequence ΨΨCGp (fig.3) instead of the sequence rTΨCGp, which is common to most sequenced transfer RNAs, providing further proof of its identity with Spot 1 RNA.

Spot 1 RNA was also demonstrated in free (M-MSV (MLV)) viral 4S RNA (fig.1b) and characterized by its T_1 fingerprint and by the presence of the sequence ΨΨCGp.

As can be seen in figs.1a and 1b, spot 1 RNA migrated close to spot 2. This latter spot, which is easily identified by its fingerprint (fig.4) was found among the viral associated RNA (fig.1c). However despite the presence of this migration marker, Spot 1 RNA, was not detected in this viral associated fraction.

Identical results were obtained in experiments using RNA from ERTh cells and GLV.

4. Discussion

Bidimensional polyacrylamide gel electrophoretic separation of cellular (78A_1 and ERTh) 4S RNA, and free viral (M-MSV(MLV)) and (GLV) 4S RNA, allows the ready demonstration of Spot 1 RNA in these two fractions. The quantity of radioactivity present in Spot 1 RNA from free viral 4S RNA, represented approximately 1% of the total radioactivity in this fraction. This percentage is comparable to that of

Spot 1 RNA found in cellular 4S RNA, showing that it is not selectively concentrated in virions. The analysis of M-MSV(MLV) and GLV associated 4S RNA for the presence of Spot 1 yielded a negative result, despite the presence of a migration marker (Spot 2), suggesting that Spot 1 RNA is either absent from this fraction, or else exists at a level too low to be detected. This result may be contrasted with the situation obtaining in avian oncornaviruses where Spot 1 RNA, which primes the in vitro transcription of these viruses is present in relatively large amounts in the free 4S RNA fraction, and represents the major form of associated 4S RNA [1].

This finding suggests that the reverse transcriptase associated with murine oncornaviruses uses an alternative RNA primer for the in vitro transcription of the genome. This result is in keeping with Waters' report that the most strongly aminoacylated species of RNA in the 4S RNA fraction liberated by heat treatment above 60°C of the AKR virus genome corresponds to proline tRNA [18].

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