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TRANSLATION OF AVIAN MYELOBLASTOSIS VIRUS GENOMIC RNA *IN VITRO*

A. MALÝ

Institute of Molecular Genetics, Academy of Sciences of Czech Republic, 166 37 Prague, Czech Republic

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Summary. - Defective viral particles (DVP) were isolated from the medium of chicken myeloblasts transformed by avian myeloblastosis virus (AMV) in the absence of helper viruses (non-producer myeloblasts). A 60S genomic RNA complex of AMV was isolated from DVP and translated *in vitro*. The predominant translation product was the *gag* polyprotein precursor $Pr76^{gag}$.

Key words: avian myeloblastosis virus; defective viral particles; 60S RNA; in vitro translation; gag polyprotein precursor

Introduction

AMV belongs to a group of defective avian leukaemia viruses (Hayman, 1983) and induces a relatively rapid acute myeloid leukaemia in infected chickens. The virus originated by recombination of myeloblastosis-associated virus type 1 (MAV-1) and the cellular gene c-*myb* (Perbal *et al.*, 1985). It is defective in its replication and depends for this function on MAV-2 as helper virus (Moscovici *et al.*, 1975) even though the plasma of sick chickens contains AMV and both MAV-1 and MAV-2.

Chicken myeloblasts transformed by AMV in the absence of MAVs (non-producer cells) have been found to release AMV as defective virus particles (Duesberg *et al.*, 1980). These particles contain a 60S RNA complex and the isolation of RNA from DVP is the way to obtain the 60S AMV genome for study.

Materials and Methods

BM2 cells (non-producers) were the kind gift of Dr. C. Moscovici and were grown as described previously (Malý and Krchnák, 1984).

Isolation of ^{3}H-labelled DVP. Most of the medium was withdrawn from the BM2 cell culture and stored at +4 °C. ^{3}H-uridine (0.9 - 1.1 TBq/mmol, Amersham) was added to the last 10 ml of medium to a concentration of 1.85 MBq/ml for 16 hr. Then both media were pooled (120 - 180 ml), centrifuged at 250 000 x g for 2 hr at +4 °C (MSE 65 rotor) and the pellet was resuspended in 4 ml TNE buffer (10 mmol/l Tris-HCl pH 7.4, 100 mmol/l NaCl, 1 mmol/l EDTA). The suspension was

loaded onto 1 ml 25 % sucrose in TNE buffer and the virus was pelleted at 45 000 rpm for 2 hr at +4 °C in an SW50.1 rotor (Beckman).

Isolation of RNA. The DVP pellet was dissolved in 1 ml of a lysis buffer (10 mmol/l Tris-HCl pH 7.4, 450 mmol/l NaCl, 45 mmol/l sodium citrate, 1 mmol/l EDTA, and 1 % sodium dodecyl sulphate (SDS)). 200 μg of Proteinase K (Boehringer) was added and the whole suspension lysed for 2 hr at 37 °C. The lysate was loaded on a linear sucrose density gradient (10–25 % sucrose in 10 mmol/l Tris-HCl pH 7.4, 50 mmol/l NaCl, 1 mmol/l EDTA, 0.1 % SDS) and the gradient was centrifuged at 38 000 rpm for 2 hr at 20 °C in an SW40 rotor (Beckman). After the centrifugation the gradient was fractionated dropwise into 40 fractions and 30 μl aliquots of each fraction were measured in a liquid scintillation counter. The 60S AMV RNA (peak of ^{3}H-uridine) was pooled and tRNA (10 μg) was added as a carrier. The NaCl concentration was adjusted to 200 mmol/l and the RNA was precipitated by ethanol.

Translation in vitro. RNA was precipitated, the pellet washed with 70 % ethanol, dried in vacuo, and the RNA dissolved in 12 μl of H_2O. 3 μl of RNA solution were translated in a 30 μl reaction mixture as described by Malý (1984).

SDS-polyacrylamide gel electrophoresis (SDS-PAGE). ^{35}S-methionine-labelled products of translation were fractionated according to size on SDS-polyacrylamide gel slabs (10 % acrylamide) using the discontinuous buffer system of Laemmli (1970). After electrophoresis the gel was processed for fluorography as described before (Malý, 1984).

Results

Total AMV RNA was isolated from DVP that had been liberated in a very low amount into the medium by non-producer myeloblasts. Therefore the procedure used for RNA isolation was as simple as possible to avoid any loss of material.

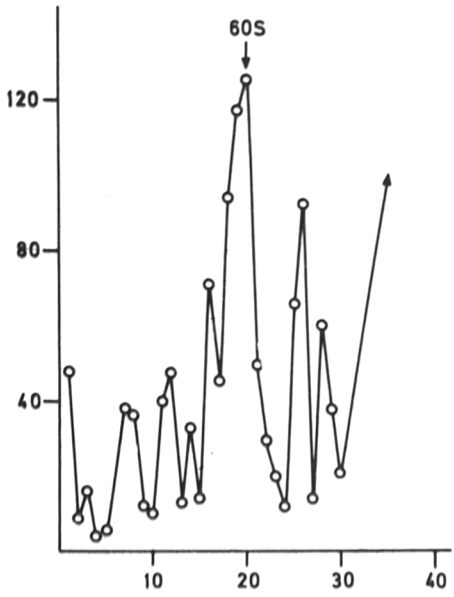

Fig. 1
Sucrose density gradient centrifugation of ^{3}H-uridine labelled AMV RNA
Isolated DVP were treated with protease in the presence of SDS and further fractionated on the sucrose gradient. The radioactivity of each fraction of the gradient was measured.
Abscissa: fraction number; ordinate: cpm.

The ^{3}H-uridine labelled DVP lysate thus obtained was loaded on a sucrose gradient and the radioactivity profile of the fractionated gradient was estimated. The overall radioactivity was very low and there were many peaks (Fig. 1), but in the 60S RNA position of the gradient (fraction number 20) the peak was distinct. The total RNA isolated from chicken leukaemic (AMV) plasma virus (Malý, 1984) was run in a similar and parallel gradient and 60S RNA was also detected in fraction number 20 (results not shown).

The ^{3}H-uridine labelled 60S AMV RNA (Fig. 1, fractions 16–23) was pooled, prepared for translation in a rabbit reticulocyte lysate system, translated, and ^{35}S-methionine labelled polypeptides were analyzed by SDS-PAGE (Fig. 2). 60S RNA isolated from the virus of leukaemic plasma (Malý, 1984) and likewise translated was used as a control of the *in vitro* translation of the retroviral genome complex.

The sole product of translation of AMV 60S RNA (Fig. 2, lane c) that was absent in the endogenous reaction mixture (Fig. 2, lane a) was Pr76^{gag}. This was

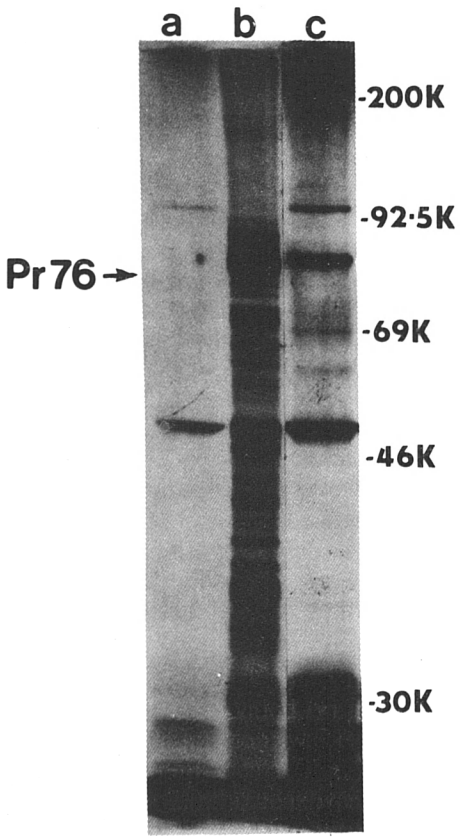

Fig. 2
Fluorograph of the SDS-PAGE of *in vitro* translation products
Lane a: 10 μl of reaction mixture incubated without any viral RNA (endogenous reaction, control). Lane b: 4 μl of reaction mixture containing 1 μg of 60S RNA isolated from AMV from leukaemic plasma. Lane c: 10 μl of reaction mixture containing 60S RNA isolated from DVP. The M_r values of size markers are indicated on the right side. The position of the *gag* polyprotein precursor (Pr76) is indicated on the left side.

in contrast to many polypeptides translated from the 60S RNA isolated from the virus of leukaemic plasma (Fig. 2, lane b); of these, however, only the 76 K polypeptide was immunoprecipitable by anti-*gag* serum (Malý, 1984). The Pr76$^{gag\ (AMV)}$ (Fig. 2, lane c) also seemed to be immunoprecipitable by this antiserum but the signal, probably owing to the small amount of template AMV RNA, was very weak (results not shown).

Discussion

Analysis of intracellular virus-specific proteins in AMV-transformed non-producer chicken myeloblasts had shown unprocessed Pr76^{gag} and the common *gag-pol* precursor (Pr180$^{gag\text{-}pol}$) as the main virus specific proteins (Duesberg *et al.*, 1980). These cells release DVP in a quantity of 5–10 % of the virion production by myeloblasts transformed by AMV in the presence of helper(s) (Duesberg *et al.*, 1980; Schulz *et al.*, 1981). DVP contain, among other nonviral proteins, the *gag* proteins p27, p19, p15, and p12 of infectious avian tumour viruses. However, the particles lack detectable polymerase protein and activity (Duesberg *et al.*, 1980). The AMV genome in DVP is made of 7.5 kb RNA and, based on oligonucleotide analysis (Duesberg *et al.*, 1980), heteroduplex mapping (Souza *et al.*, 1980), and sequencing (Rushlow *et al.*, 1982; Klempnauer *et al.*, 1982), the structure of the AMV genome has been estimated to be 5'-*gag-pol-myb*-3'.

In the present investigation we have translated *in vitro* the AMV RNA genome complex isolated from DVP and the predominant polypeptide product was Pr76^{gag}. Similar results have been obtained when AMV genome RNA separated from helper RNA by electrophoresis was translated *in vitro,* comparison with background polypeptides was not made, but the products of the translation seemed to be Pr76^{gag} and Pr180$^{gag\text{-}pol}$ (Schulz *et al.*, 1981). Pr76^{gag} was likewise detected by immunoprecipitation of an *in vitro* reaction mixture where the template RNA isolated from virus released into the supernatant of BM2 cells superinfected by MAV-2 (Klempnauer *et al.*, 1983) was translated. This polyprotein, however, had probably been mostly synthesized on the genome RNA of the helper.

Because Pr76^{gag} synthesized in non-producer cells seems to stay unprocessed intracellularly (Duesberg *et al.*, 1980), it would be interesting to find out if the *in vitro* synthesized polypeptide is capable of being processed correctly.

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