TRANSLATION OF AVIAN MYELOBLASTOSIS VIRUS RNA IN A RETICULOCYTE CELL-FREE SYSTEM

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SUMMARY

AMV-RNA was translated into a precursor polypeptide of 76,000-80,000 daltons in a reticulocyte cell-free system. Besides this high molecular weight precursor, several smaller precursor polypeptides and the four major internal structural viral proteins were also synthesized. These virus-specific translation products were detectable after immunoprecipitation with antiserum against the gs-antigens of AMV.

INTRODUCTION

Recently Vogt and Eisenman (1) showed, by means of immunoprecipitation, that in AMV-infected primary chick fibroblasts some of the major structural proteins of AMV are derived by cleavage from a common precursor protein of 76,000 daltons. In further in vivo studies these authors identified five metabolically unstable AMV precursor polypeptides (2). The arrangement of the virion proteins in these in vivo precursor polypeptides was determined by means of inhibition of polypeptide chain initiation by pactamycin. Meanwhile, several papers appeared in the literature reporting the cell-free translation of RSV-RNA (3) and RLV-RNA (4, 5). Furthermore Ascione et al (6) described a cell-free mamma-

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Abbreviations:

AMV = avian myeloblastosis virus
gs = group-specific
RSV = Rous sarcoma virus
RLV = Rauscher leukemia virus
DTT = 1,4-dithiothreitol
SDS = sodium dodecyl sulfate
PPO = 2,5-diphenyloxazol
S30 = 30,000 x g supernatant fraction

lian system which upon addition of AMV-RNA was capable to stimulate the overall amino acid incorporation. However, since no product analysis was carried out by the latter authors, no conclusions could be drawn concerning the de novo synthesis of AMV-specific polypeptides. For this reason it seemed to be interesting to establish whether or not specific viral polypeptides were synthesized in a cell-free system programmed with AMV-RNA.

In the present paper we provide evidence for the in vitro synthesis of the same AMV-specific precursor polypeptides and structural proteins as have been found in vivo.

MATERIALS AND METHODS

Rabbit reticulocytes were prepared as described earlier (7) and lysed by addition of water. A 30,000 x g supernatant fraction of these lysed cells was used as cell-free system and incubations were performed at 30°C for 1 h.
The reaction mixture contained per ml: 0.6 ml of reticulocyte cell-free extract, 20 mM Tris-HCl, pH 7.4, 100 mM KCl, 2 mM $MgAc_2$, 0.2 mM spermidine, 2 mM DTT, 40 µg of AMV-RNA and 40 µCi [^{35}S]-methionine (specific activity ± 200 Ci/mmol). The ATP generating system and amino acid mixture were as described by Berns et al (8). The final volume of the incubation mixture was 25 µl.
AMV-RNA was obtained from purified virus which was derived from infected chicken plasma (9) and treated with SDS and pronase. The high molecular weight AMV-RNA was isolated by centrifugation in isokinetic glycerol gradients (10). The isolated 60S AMV-RNA was denatured by heating at 100°C for 3 min. For comparison 60S RLV-RNA was isolated and denatured in the same way as described for AMV. The AMV-specific polypeptides synthesized in the cell-free system were detected by indirect immunoprecipitation (11) with an antiserum prepared by hyperimmunization of a rabbit with Nonidet P.40 disrupted AMV in Freund's adjuvant (1). The RLV-specific polypeptides synthesized in a reticulocyte cell-free system programmed with RLV-RNA were immunoprecipitated with anti-RLV-serum (11). 10S Lens mRNA, kindly provided by Mr L. Cohen from our laboratory, was translated and treated with anti-AMV-serum to check for aspecific immunoprecipitation.
The products obtained upon immunoprecipitation were analyzed on SDS gel gradients (7-18%) according to Berns et al (8). After the electrophoretic run the slab gels were treated with Me_2SO-PPO and dried down on filter paper before scintillation autoradiography (12).

RESULTS AND DISCUSSION

The results of a typical translation experiment of AMV-RNA in a reticulocyte cell-free system are shown in Table I.

Due to the extremely high endogenous globin synthesis of the lysate system almost no stimulation of the amino acid incorporation was ob-

tained either after addition of lens 10S mRNA or of AMV-RNA. However, immunoprecipitation of the incubation mixtures with anti-AMV-serum revealed that only after programming the reticulocyte lysate with AMV-RNA a small amount of radioactivity was precipitable.

The specificity of the immunoprecipitation was checked by the following experiments:

1) Control incubations without mRNA or with 10S lens mRNA did not give rise to polypeptides which were precipitable with anti-AMV-serum.
2) After incubation of AMV-RNA in the reticulocyte system and addition of an antiserum directed against a non-related murine leukemia virus (anti-RLV-serum) no precipitation of polypeptides occurred. Likewise, no precipitable products could be detected upon incubation of RLV-RNA in the lysate followed by addition of an antiserum directed against the non-related avian myeloblastosis virus (anti-AMV-serum).

TABLE I

$[^{35}S]$-methionine incorporation and immunoprecipitation of a reticulocyte cell-free system programmed with mRNA.

RNA added	$[^{35}S]$-methionine incorporated in CCl_3COOH-precipitable cpm	anti-AMV-serum precipitable cpm
none	10.8×10^6	30
10S lens mRNA	11.2×10^6	56
denatured AMV-RNA (35S)	10.1×10^6	1053
native AMV-RNA (60S)	11.1×10^6	3115

3) Incubation mixtures programmed with viral or lens mRNA, supplemented with BSA, and immunoprecipitated with anti-BSA-serum did not contain any radioactive component. The analysis of the [^{35}S]-methionine labeled and immunoprecipitable products synthesized under direction of native or denatured AMV-RNA are depicted in Fig. 1.

60S AMV-RNA (Fig. 1: 1) gives rise to the synthesis of virus-specific polypeptides with molecular weights of 80, 76, 66, 50, 32, 25, 18, 14,

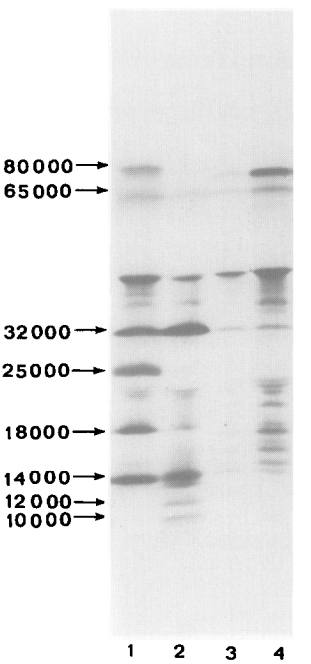

FIGURE: 1

Autoradiography of the SDS gel analysis of immunoprecipitable polypeptides synthesized in vitro in a reticulocyte cell-free system which has been programmed with oncogenic viral RNA.
Incubations and immunoprecipitations were performed as described in the Materials and Methods section.
The X-ray film was exposed in contact with the dried gel for 3 days.

Incubation with:
1) native AMV-RNA
2) denatured AMV-RNA
3) native RLV-RNA
4) denatured RLV-RNA

12, and 10 x 10^3. Denatured AMV-RNA (Fig. 1: 2) leads to the synthesis of the polypeptides with molecular weights of 66, 32, 18, and 14 x 10^3. In the latter case a much smaller quantity of the high molecular weight polypeptides was synthesized.

Native and denatured RLV-RNA give rise to the synthesis of polypeptides with molecular weights of 72, 65, and 15 x 10^3 beside some smaller polypeptides (Fig. 1: 3 and 4) (5). Control immunoprecipitations of incubations programmed with 10S lens mRNA or without RNA did not reveal any bands.

Our in vitro translations of AMV-RNA are in good agreement with the in vivo studies of Vogt et al (2). The latter authors found five unstable AMV-specific precursor polypeptides, namely pr76, pr66, pr60, pr32, and pr12 in addition to the viral internal structural proteins p28 and p14 (where pr stands for precursor polypeptide, p for polypeptide present in the virion, whereas the number indicates the molecular weight in daltons x 10^{-3}).

By incubating isolated AMV-RNA in a cell-free system we were able to demonstrate the synthesis of the same precursor polypeptides (pr76-80, pr66, pr32 and pr12) and some additional polypeptides, probably cleavage products of these precursors. Furthermore we found the internal structural proteins p28 (however, 25,000 daltons in our system), p10 and p14 (in our systems 12,000 and 10,000 daltons, respectively (13)). It is striking that, whereas the in vivo and in vitro translation of AMV-RNA and processing of the precursor polypeptides in the corresponding products is very similar, the situation with RLV is greatly different (5). In the latter case a number of polypeptides synthesized in vivo could not be detected in the in vitro experiments. This may be explained by one or both of the following reasons:

1) The precursor for the glycoproteins found in vivo in the RLV system (11) is not detectable in vitro (5), whereas such a precursor has

not been observed in the in vivo and in vitro system for AMV because the anti-AMV-serum is directed only against the gs-antigens.

2) The enzymic cleavage of the precursors in the AMV system is different from that in the Rauscher system. Since we used an S30 fraction, in which membranes have been removed, it is possible that a number of membrane-bound proteolytic enzymes, responsible for the processing of the RLV precursor, are lacking.

In any case it may be concluded from our results that the enzymes responsible for the processing of the AMV precursor polypeptides are present in the reticulocyte S30 lysate since the same viral precursors and structural polypeptides which are present in vivo are also synthesized in the in vitro system. Furthermore upon prolonged incubation of AMV-RNA in the S30 lysate (up to 18 h) the high molecular weight precursors (pr76-80) disappear, whereas the amount of structural proteins increases. This means that, whereas initiation and elongation continue only for 3-4 h in our lysate system, enzymic cleavage continues upon prolonged incubation. Nevertheless, our results do not support the idea that for the translation of oncorna virus RNA in vitro specific messenger recognition factors are required.

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