TRANSLATION OF RAUSCHER MURINE LEUKEMIA VIRAL RNA

A MODEL FOR THE FUNCTION OF VIRUS-SPECIFIC MESSENGER

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SUMMARY

In cell-free systems 35S RNA from Rauscher murine leukemia virus is translated into group-specific antigen polypeptides, whereas envelope-related products are not detectable. Assuming that no internal initiation takes place, this means that the group-specific antigen gene is located at the 5' end and it may indicate that a sequence: group-specific antigen gene∿ polymerase gene∿envelope glycoprotein gene found in avian sarcoma virus also exists in the murine leukemia virus.

INTRODUCTION

C-type viruses contain as their principal RNA component a complex molecule with a molecular weight of approximately 6 x 10^6 daltons which sediments at 60-70S (1-3). When subjected to denaturing conditions this complex dissociates into two subunits with sedimentation coefficients of approximately 35S and a molecular weight of 3-4 x 10^6 (2-5). There is evidence that the two subunits contain the same genetic information (6-9). Recently a physical map of the viral genome has been constructed (10-12).

Non-defective avian sarcoma viruses have been shown to contain four genetic elements, namely the gag-gene, coding for the internal group-specific antigens, the pol-gene, for the reverse transcriptase, the env-gene, coding for the viral envelope glycoprotein, and a gene for the sarcomagenic cell transformation, the src- or onc-gene (9, 12-13). The location of these biological functions on the avian viral 35S RNA has been established (10-12).

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Abbreviations: gag, group-specific antigen; pol, reverse transcriptase; env, envelope; src, sarcomagenic; onc, oncogenic; R-MuLV, Rauscher murine leukemia virus

Polyribosomes from cells producing R-MuLV contain in addition to 35S RNA smaller virus-specific species of mRNA (14-18). Translation of these virus-specific RNA species in a reticulocyte cell-free system resulted in the synthesis of several different immunoprecipitable polypeptides, an indication of the presence of different virus messenger classes in cells producing R-MuLV (19).

Recently we have succeeded in translating the viral 35S RNA in different cell-free systems and in oocytes from *Xenopus laevis* (20-22). However, only a portion of the sequences of this virion 35S RNA was translated, as was determined by immunoprecipitation analysis of the products of translation performed with a polyvalent antiserum directed against R-MuLV. Therefore, no definite conclusion could be drawn concerning which types of polypeptides were synthesized. In this paper we provide evidence, by analysis with monospecific antisera directed against the gag- and env-products, that viral 35S RNA functions as messenger only for the synthesis of the gag-precursor, and, probably due to occasional readthrough, for a high molecular weight polypeptide which contains the gag and the adjacent gene, presumably the reverse transcriptase.

MATERIALS AND METHODS

R-MuLV was isolated from the plasma of leukemic mice (23), and treated with SDS and pronase. The 65S component of R-MuLV RNA was isolated by centrifugation in an isokinetic glycerol gradient (24) and denatured in 90% formamide at 50°C for 5 min. The resulting 35S RNA was purified by glycerol gradient centrifugation (24).

Native or denatured R-MuLV RNA was incubated for 60 min at 30°C in a reticulocyte cell-free system as described elsewhere (20-21), or injected into oocytes from *Xenopus laevis* (22). The R-MuLV-specific polypeptides synthesized in these systems were detected by indirect immunoprecipitation with polyvalent or monospecific antisera. The rabbit anti-R-MuLV serum was described previously (25). Rabbit anti-p30 and anti-p15(E),p12 sera are described elsewhere (26). Goat anti-gp69/71 and anti-p15 sera were described by Strand and August (27). The immunoprecipitates were analyzed on polyacrylamide slab gel gradients (7-18%) according to Berns *et al* (28). After staining and destaining the slab gels were treated with Me_2SO-PPO (29), dried under vacuum and placed in contact with a Kodak X-ray film for 2-3 days at -80°C.

RESULTS AND DISCUSSION

Vogt *et al* (30-31) have demonstrated that the major internal structural proteins of AMV are formed by cleavage of a common precursor polypeptide, and recently several other groups have reported the biosynthesis of gag- and

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Abbreviations: SDS, $NaDodSO_4$; p30, protein, 30,000 daltons; p15(E), envelope protein, 15,000 daltons; p12, protein, 12,000 daltons; gp69/71, glycoprotein, 69,000-71,000 daltons; p15, protein, 15,000 daltons; PPO, 2,5-diphenyloxazol; AMV, avian myeloblastosis virus; pr65, precursor, 65,000 daltons

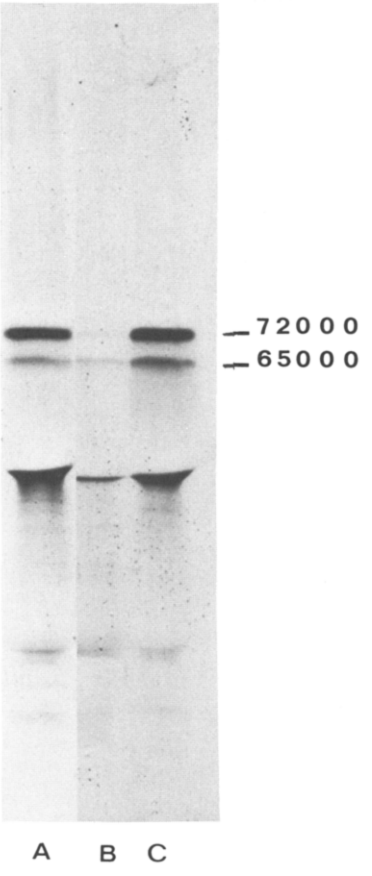

fig. 1. Autoradiography of an SDS gel electrophoresis pattern of [^{35}S] -methionine-labeled and immunoprecipitated polypeptides which were synthesized in reticulocyte cell-free incubations programmed with 35S R-MuLV RNA.
(A) immunoprecipitation with a polyvalent antiserum directed against R-MuLV;
(B) immunoprecipitation with a polyvalent antiserum directed against R-MuLV after addition of an excess of unlabeled p30 to the incubation mixture;
(C) immunoprecipitation with a monospecific antiserum directed against purified p30.

env-precursor polypeptides in C-type virus producing cells (25-26, 32-34). The precursor for the env-glycoprotein was found in the 82,000-90,000 molecular weight region on SDS-gels, whereas the gag-precursor was localized at 65,000-75,000 daltons.

Addition of oncogenic viral RNA to cell-free systems resulted in the synthesis of several virus-specific polypeptides. These polypeptides can be detected after immunoprecipitation with polyvalent antisera directed against the viral structural proteins (25), followed by analysis with SDS-gel elec-

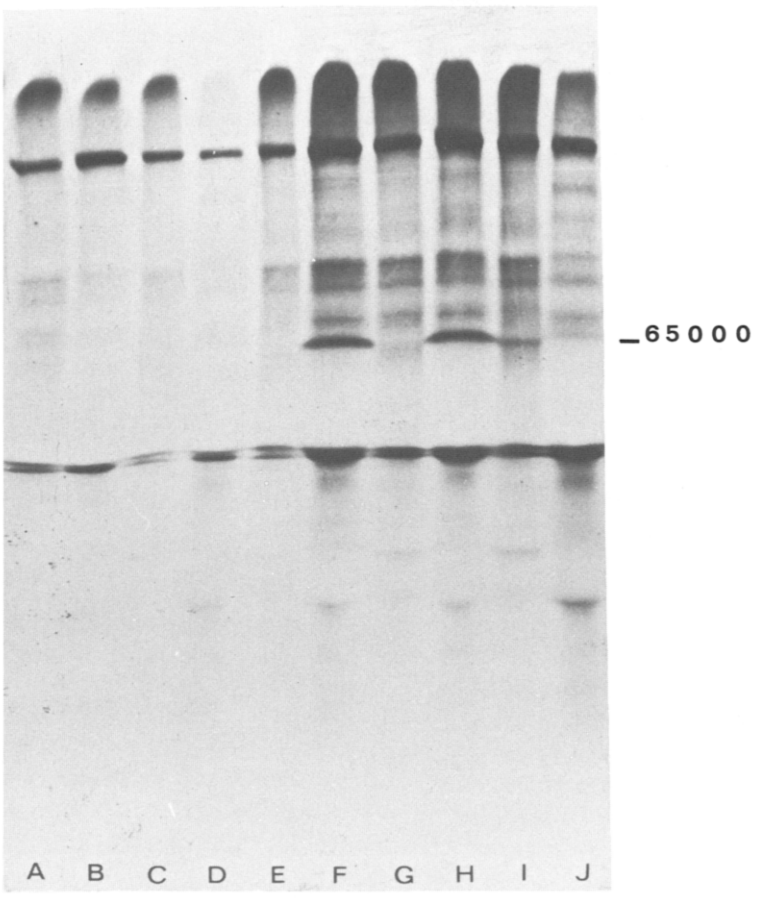

fig. 2. Autoradiography of an SDS gel electrophoresis pattern of ^{35}S-methionine-labeled and immunoprecipitated polypeptides which were synthesized in oocytes from Xenopus laevis programmed with 35S RNA.
Control (no RNA injected). Immunoprecipitations with (A) polyvalent antiserum directed against R-MuLV; (B) anti-gp69/71 serum; (C) anti-p30 serum; (D) anti-p15 serum; (E) anti-p15(E),p12 serum.
35S R-MuLV RNA injected oocytes immunoprecipitated with (F) polyvalent antiserum directed against R-MuLV; (G) anti-gp69/71 serum; (H) anti-p30 serum; (I) anti-p15 serum; (J) anti-p15(E),p12 serum.

torphoresis (20-22). Predominantly two virus-specific polypeptides with molecular weights of 65,000 and 72,000, respectively, were identified (fig. 1A). We wondered whether the 65,000 daltons polypeptide was identical to the gag-precursor found in infected cells (25-26). Furthermore it had to be elucidated whether the 72,000 daltons polypeptide was related to the gag-pr65 or to the env-precursor, since the molecular weight of the non-glycosylated protein moiety of the env-precursor might well be 72,000 (34-35).
In order to discriminate between these alternatives, we determined the com-

petition between the putative labeled gag-related polypeptides, synthesized in a cell-free system and immunoprecipitable with a polyvalent antiserum, and p30, by adding an excess of unlabeled p30 (the major internal gag-protein) (26)(fig. 1B). It appeared that p30 competed both with the 65,000 and 72,000 molecular weight polypeptides. Thus both polypeptides must contain gag-determinants. Likewise, immunoprecipitation with a monospecific antiserum directed against p30 precipitates equally well the polypeptides of 65,000 and 72,000 daltons, respectively (fig. 1C). Immunoprecipitation with monospecific antisera directed against gp69/71 or p15(E),p12, the proteins that are derived from a common env-precursor (26), did not reveal any virus-specific bands (not shown). Therefore, we conclude that upon programming cell-free systems with viral RNA only polypeptides that are gag-related are synthesized.

Since no glycosylation takes place *in vitro*, we analyzed the virus-specific polypeptides synthesized in *Xenopus laevis* oocytes, a system which is able to glycosylate polypeptides. Injection of viral 35S RNA and immunoprecipitation with polyvalent or monospecific antisera directed against p30 or p15 reveals the synthesis of the 65,000 molecular weight gag-precursor (fig. 2F, H and I), whereas monospecific antisera directed against gp69/71 or p15(E),p12 did not precipitate an env-precursor or the viral gp69/71 or p15(E),p12 (fig. 2G and J). It has recently been shown that in R-MuLV infected cells this anti-gp69/71 or anti-p15(E),p12 sera precipitate both the common env-precursor and the processed viral proteins (26). We conclude that injection of 35S RNA into *Xenopus laevis* oocytes leads, as in cell-free systems, exclusively to the biosynthesis of gag-related products. In this connexion it has to be mentioned that Mueller-Lantzsch *et al* (36) identified 35S RNA as the only virus-specific messenger in polyribosomes isolated from virus-infected cells. These polyribosomes were immunoprecipitated with an antiserum directed against p30.

The fact that we were unable to detect very large precursor polypeptides corresponding to the total viral information does not necessarily mean that such a polyprotein does not exist. If its concentration is extremely low, e.g. due to rapid processing, it would escape detection. Therefore, we investigated the polypeptides synthesized in a reticulocyte cell-free system programmed with R-MuLV RNA, in the presence of a high concentration (3 mM) of canavanine, an arginine analog which prevents cleavage of polypeptides by trypsin-like enzymes (fig. 3). Instead of a very high molecular weight polypeptide, two polypeptides with molecular weights of 72,000 and

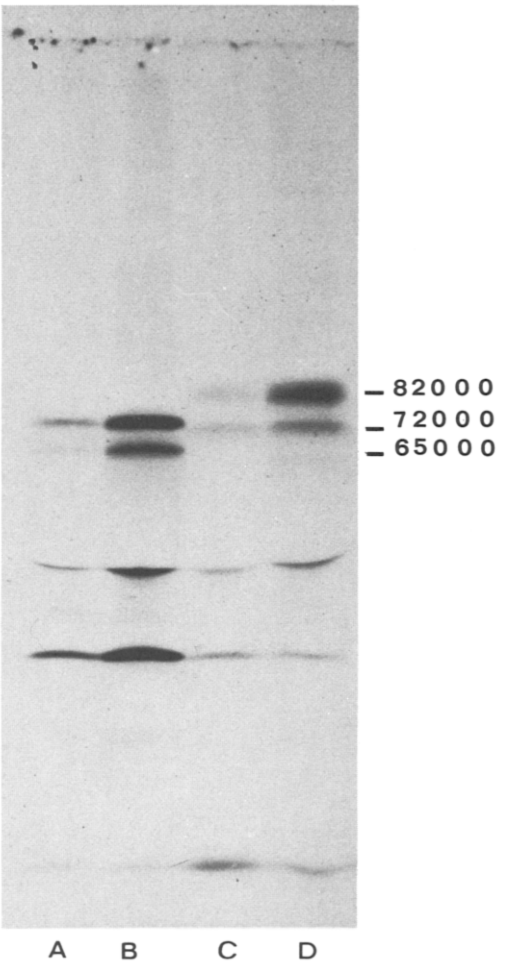

fig. 3. Autoradiography of an SDS gel electrophoresis pattern of ^{35}S -methionine-labeled and immunoprecipitated polypeptides which were synthesized in reticulocyte cell-free incubations programmed with native or denatured R-MuLV RNA.
Virus-specific polypeptides synthesized in the absence of canavanine after addition of (A) native, or (B) denatured viral RNA.
Virus-specific polypeptides synthesized in the presence of canavanine after addition of (C) native, or (D) denatured viral RNA.

82,000 are synthesized under these conditions, whereas no 65,000 daltons polypeptide was present (cf. fig. 3B and 3D). Both polypeptides again were precipitable with an anti-p30 or anti-p15 serum and are, therefore, related to the gag-precursor. The observed change in molecular weight of the gag-polypeptides in the presence of canavanine may be due to prevention of the tryptic cleavage or to an altered conformation of the polypeptides. Also addition to cell-free systems of a number of known protease inhibitors

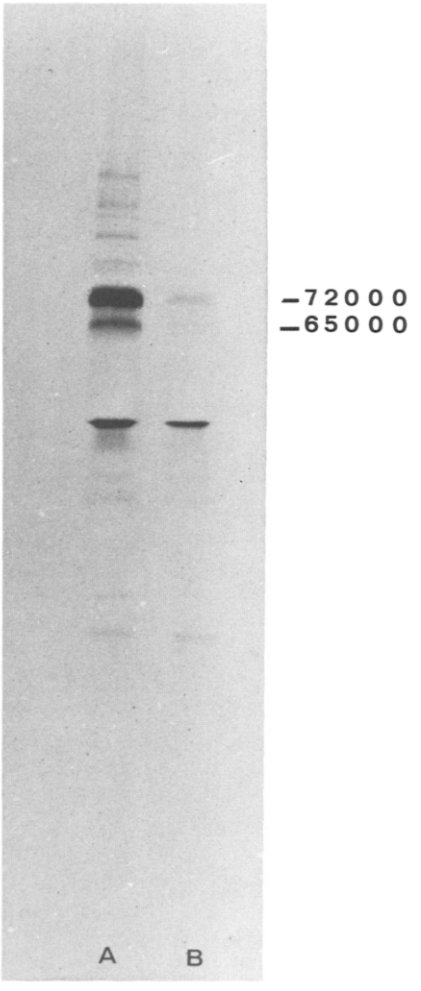

fig. 4. Autoradiography of an SDS gel electrophoresis pattern of ^{35}S-methionine-labeled polypeptides synthesized in a reticulocyte cell-free system and immunoprecipitable with a polyvalent antiserum directed against R-MuLV. Immunoprecipitated products synthesized after addition of (A) 35S, or (B) 65S R-MuLV RNA.

such as TPCK, TLCK or trasylol did not result in the appearence of high molecular weight precursors corresponding to the total viral information. However, upon programming the reticulocyte lysate with 35S viral RNA a small amount of discrete polypeptides are synthesized with molecular weights up to 150,000 (fig. 4). These polypeptides are immunoprecipitable

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Abbreviations: TPCK, tosylphenylalanine chloromethylketone; TLCK, tosyllysine chloromethylketone

with anti-p30 serum but not with anti-gp69/71 serum. Taking into account the recently proposed gene order gag-pol-env (10-12, 37), our results suggest that these high molecular weight polypeptides contain the gag-precursor linked to the reverse transcriptase. The synthesis of this high molecular weight polypeptide may be due to occasional readthrough at the termination site of the gag-gene (cf. ref. 34).

Our findings can be explained by the assumption that, whereas 35S viral RNA directs mainly the synthesis of the gag-precursor, a readthrough mechanism occurring at low frequency is responsible for the production of a small amount of a gag-pol precursor. This would also explain the relative low concentration of reverse transcriptase synthesized as compared with the gag-products. Since, obviously, 35S RNA does not direct the synthesis of env-related products, it has to be established whether virus-specific mRNAs with lower sedimentation values (which have been found on polyribosomes from virus-infected cells (14-18)) are responsible for the formation of env-polypeptides.

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