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Translation of Type C Viral RNAs in *Xenopus laevis* Oocytes: Evidence that the 120,000-Molecular-Weight Polyprotein Expressed in Abelson Leukemia Virus-Transformed Cells Is Virus Coded

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The genomic RNA of Abelson leukemia virus (AbLV) has been purified and translated in *Xenopus laevis* oocytes. The primary AbLV-specific protein synthesized is a polyprotein corresponding in molecular weight and immunological properties to a previously described p15 and p12 containing 110,000- to 130,000-molecular-weight polyprotein expressed in AbLV-transformed cells. In contrast, translation of woolly monkey sarcoma virus genomic RNA resulted in synthesis of a 55,000-molecular-weight polyprotein consisting of woolly helper virus p30, p15, and p12. These findings demonstrate the value of the *X. laevis* oocyte in vitro system for studies of translational products of replication-defective transforming viruses and establish the virus-coded nature of the nonstructural component of the 110,000- to 130,000-molecular-weight polyprotein expressed in AbLV-transformed cells.

Mammalian RNA type C replication-defective transforming viruses frequently cause morphological alteration of embryo fibroblasts in cell culture and induce tumors of a variety of histological types in vivo (1). Such viruses appear to represent recombinants between a portion of the leukemia helper virus genome and cellular genes coding for malignant transformation (15, 20). In view of the possibility that the cellularly acquired transforming sequences of these viruses may be etiologically involved in spontaneous tumors of their natural hosts, efforts have been undertaken to identify and develop immunological assays for their translational products. Cell lines nonproductively transformed by each of several such viruses, including one isolate of cat origin, feline sarcoma virus (6, 17, 18; A. S. Khan, D. N. Deobagkar, and J. R. Stephenson, J. Biol. Chem., in press), and two mouse-derived viruses, Abelson murine leukemia virus (AbLV) (9) and the T-8 MCF AKR-derived leukemia virus (12), express polyproteins of 100,000 to 130,000 molecular weight containing the two amino terminal *gag* gene proteins p15 and p12. In the feline system, the feline sarcoma virus-associated 130,000-molecular-weight polyprotein is efficiently immunoprecipitable by select feline leukemia virus-absorbed cat sera with high-titered antibody directed against the feline oncornavirus-associated cell membrane antigen (7, 18; Khan et al., in press), raising the possibility that this polyprotein may contain transformation (*src*)-specific sequences. In an effort to resolve whether the nonstructural components of such polyproteins are coded in entirety by the transforming virus genome, we have undertaken to translate AbLV genomic RNA in *Xenopus laevis* oocytes.

Viruses used were sucrose gradient-purified preparations obtained through the courtesy of R. V. Gilden (Frederick Cancer Research Center) and included Rauscher murine leukemia virus (R-MuLV) grown on JLS-V9 cells and the WB334 strain of woolly monkey virus (WSV) propagated on normal rat kidney (NRK) cells. This latter virus consists of approximately equal titers of replication-defective WSV and amphotropic mouse helper virus (16). Superinfection of the AbLV nonproductively transformed mouse cell line, ANN-1 (14), with wild-mouse amphotropic type C virus, 4070-A, generously provided by J. W. Hartley, National Institute of Allergy and Infectious Diseases (5), led to production of virus stocks containing approximately equal infectious titers of leukemia helper virus and pseudotype virions containing the AbLV genome in the amphotropic virus coat. Female *X. laevis* frogs were acquired from C. W. Fletcher, Hamp-

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Initial studies were undertaken to purify and characterize R-MuLV, WB334, and AbLV(4070-A) genomic RNA. The RNA profile obtained from sodium dodecyl sulfate (SDS)-Pronase treatment of density gradient-purified R-MuLV and subsequent sedimentation through a 15 to 35% sucrose gradient (Fig. 1A) shows a single prominent peak of absorbancy at 260 nm in the 70S region of the gradient. WB334 RNA similarly purified gave a major peak of absorbancy at around 70S in addition to a slower sedimenting peak of absorbancy in the 45 to 50S region of the gradient (Fig. 1B). This latter peak has been shown by Scolnick et al. (16) to contain genetic sequences corresponding to those of the WSV genomic RNA, whereas the 70S peak represents the amphotropic helper virus RNA. AbLV(4070-A) pseudotype virion RNA was also characterized by a peak of absorbancy in the 50S region of the gradient, and a less pronounced peak or shoulder at 70S (Fig. 1C).

Fractions from the 70S region of the R-MuLV RNA gradient were alcohol precipitated, redissolved in water at a concentration of 1 mg/ml, and injected into *X. laevis* oocytes. After incubation of the oocytes in [^{3}H]leucine-labeling media, extracts were subjected to immunoprecipitation by various sera to separate the virus-specific proteins from endogenous translation products. Immunoprecipitates were chromatographed on SDS-polyacrylamide slab gels, and the protein bands were visualized by fluorography. As shown in Fig. 2, the major product of R-MuLV translation was a 65,000-molecular-weight protein which was immunoprecipitated by antisera directed against disrupted R-MuLV, R-MuLV p30, p15, p12, and p10 but not by either normal goat serum or anti-R-MuLV gp70 (Fig. 2 A to G), and therefore appears to represent the previously described 65,000-molecular-weight R-MuLV *gag* gene-coded precursor polyprotein (10, 13). The appearance of protein bands at molecular weights of 30,000, 15,000 and 12,000 which were efficiently immunoprecipitable by antisera directed against R-MuLV p30, p15, and p12, respectively (Fig. 2 C to E), demonstrates posttranslational cleavage of a portion of Pr65 into its component structural proteins and defines the specificities of the antisera used. For instance, although p30 is the major protein precipitated by anti-R-MuLV p30, a smaller amount of p12 (Fig. 2C) was also observed. Anti-R-MuLV p15 was the least specific of the sera used in that it precipitated not only p15, but also p30 and p12 (Fig. 2D). In contrast, anti-R-MuLV p12 only recognized p12 (Fig. 2E), whereas anti-R-MuLV p10 did not precipitate p30, p15, or p12 to significant extents (Fig. 2F). Although this

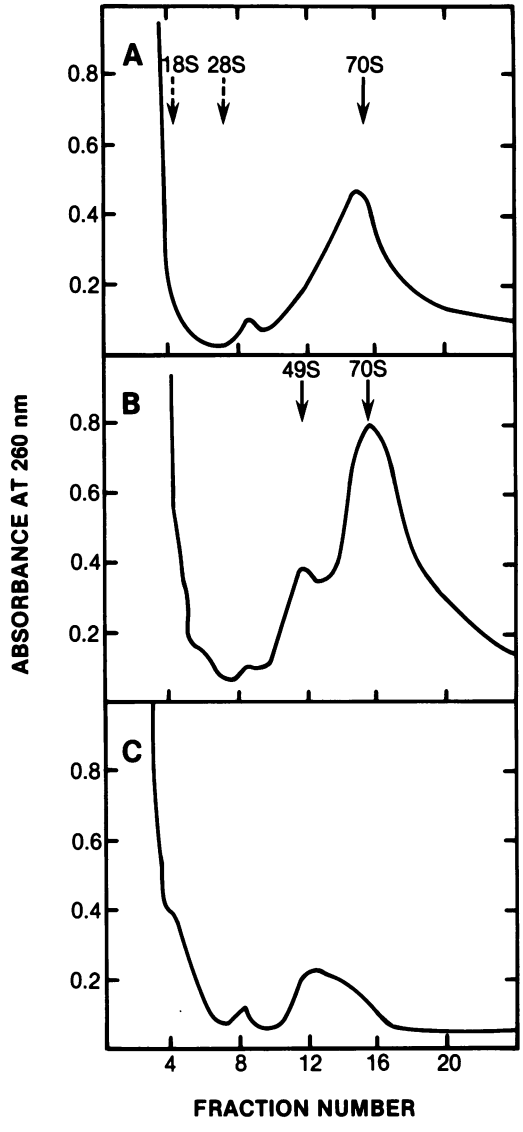

FIG. 1. *Sedimentation profiles of RNA extracted from (A) R-MuLV, (B) WB334, and (C) AbLV(4070-A) density gradient-purified viruses. Viral genomic RNA was extracted by resuspension of 5 to 10 mg of pelleted virus in TNE buffer (0.01 M Tris-hydrochloride [pH 8.0]-1% NaCl-1 mM EDTA) containing 1% SDS, 0.5 mg of Pronase (Calbiochem, La Jolla, Calif.), and 1% β-mercaptoethanol. The sample was incubated at 37°C for 30 min before sedimentation through a 15 to 35% (wt/wt) sucrose gradient in TNE at 40,000 rpm for 5 h at 4°C in an SW 41 rotor. Gradients were fractionated with a density gradient fractionator, model 640 (Instrument Specialties Co., Lincoln, Neb.), and absorbance at 260 nm was recorded by an Instrument Specialties Co., model UA-5 absorbance monitor. Fractions used for translation in the oocytes were (A) 13 to 17; (B) 11 to 13 and 14 to 17; and (C) 12 to 15.*

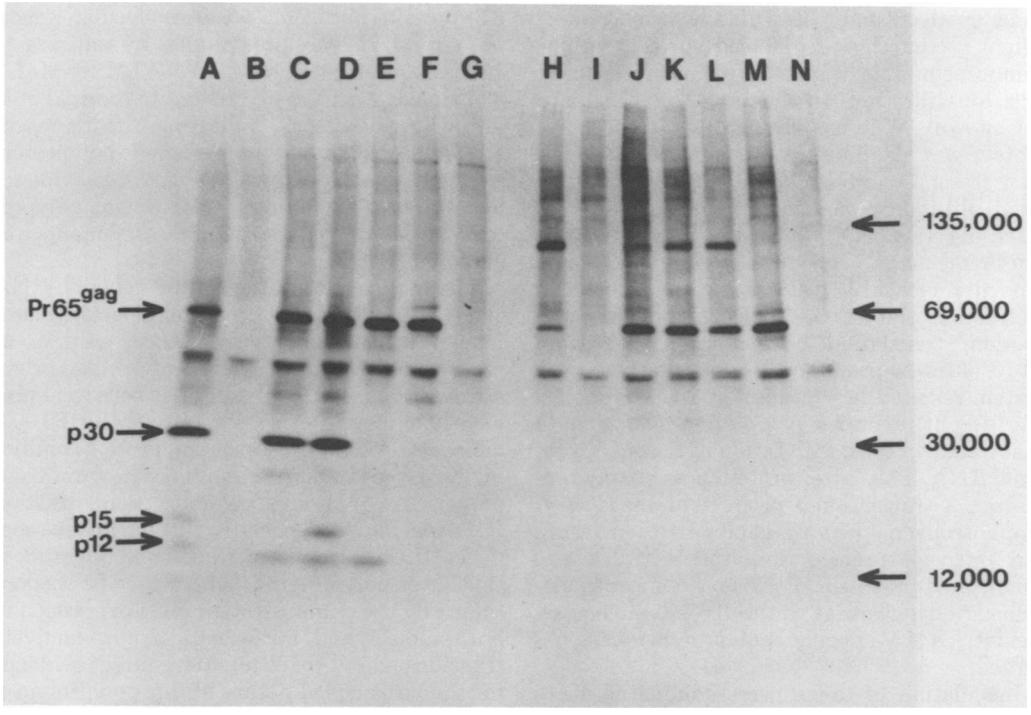

FIG. 2. *Immunoprecipitation and SDS-polyacrylamide gel electrophoretic analysis of [^{3}H]leucine-labeled viral proteins translated in X. laevis oocytes from R-MuLV (A to G) and AbLV(4070-A) pseudotype (H to N) genomic RNA. After removal from the frog, oocytes were maintained in Barth media (4), and injections were performed with an MN 358 Special Micropositioner (Bunton Instruments, Rockville, Md.) and a 10-μl syringe attached to a micrometer screw. Viral RNA sucrose gradient fractions pooled as indicated in the legend to Fig. 1 were precipitated with 2 volumes of ethanol and 0.1 volume of 2.0 M sodium acetate, pH 5.0, at −20°C. RNA was pelleted from the alcohol by centrifugation at 8,000 rpm at −20°C in a Sorvall SS-34 rotor. After removal of the alcohol, the pellet was dried briefly in a dessicator under vacuum before resuspension of the RNA in water to a concentration of 1 mg/ml. Oocytes were injected with 40 nl of the RNA solution and were incubated for 20 h at 20°C in Barth media containing 1 mCi of [^{3}H]leucine (New England Nuclear, Boston, Mass.) (>110 Ci/mmol) per ml, washed three times with unlabeled Barth media, and dounced in PBSTDS (10 mM $NaPO_4$ [pH 7.2], 1.0% Triton X-100, 0.9% NaCl, 0.5% deoxycholate, and 0.1% SDS) buffer containing 0.1% leucine. After clarification of the extracts to remove lipid and cell debris, 2 × 10^6 trichloroacetic acid-precipitable counts were incubated with 5 μl of antiserum to immunoprecipitate virus-specific translational products. After 12 to 16 h, 50 μl of a 20% suspension of protein A-Sepharose CL-4B (Pharmacia, Piscataway, N. J.) in PBSTDS was added to adsorb the immune complexes. Sepharose-bound protein A immune complexes were washed three times in PBSTDS buffer, resuspended in 20 μl of sample buffer (0.0625 M Tris-hydrochloride [pH 6.7], 1% SDS, 10% glycerol, 2.5% β-mercaptoethanol, and 0.1% bromophenol blue), heated for 2 min at 90°C, and subjected to electrophoresis through a 5% acrylamide–0.133% bisacrylamide stacking gel and a 9-cm separation gel with a 5 to 20% polyacrylamide gradient (30:08, acrylamide:bisacrylamide) in a Tris–glycine–SDS buffer system described by Laemmli (7) at 30 to 35V. Radioactivity was visualized by scintillation autoradiography (3). Sera included goat anti-R-MuLV (A, H); anti-R-MuLV gp70 (B, I); anti-R-MuLV p30 (C, J); anti-R-MuLV p15 (D, K); anti-R-MuLV p12 (E, L); anti-R-MuLV p10 (F, M); and normal goat sera (G, N). ^{125}I-labeled molecular weight standards included β-galactosidase (135,000), bovine serum albumin (69,000), and R-MuLV p30 (30,000).*

latter serum did precipitate p10, the band was not sufficiently intense to be seen in the gel shown in Fig. 2.

In both mink and mouse cells nonproductively transformed by AbLV, we have previously reported the expression of a 110,000- to 130,000-molecular-weight polyprotein containing MuLV p15 and p12 as well as a possible transformation-specific nonstructural component (9). Translation of AbLV genomic RNA in the present study resulted in synthesis of a 120,000-molecular-weight polyprotein which was efficiently immunoprecipitated by antisera to detergent-disrupted R-MuLV, anti-R-MuLV p15, and anti-R-MuLV p12 but not by anti-R-MuLV p10 or gp70 (Fig. 2H to N). This latter protein migrated

under co-electrophoresis with a high-molecular-weight [^{35}S]methionine-labeled polyprotein immunoprecipitable from AbLV-transformed mink cells by either anti-R-MuLV p12 or p15 (data not shown). Whereas the AbLV-specific polyprotein was also immunoprecipitable, although to a lesser extent by anti-R-MuLV p30, this latter serum also contained significant anti-p12 reactivity (Fig. 2C). Thus, the question as to whether the AbLV-specific polyprotein contains a portion of MuLV p30 remains unresolved. In addition to the 120,000-molecular-weight polyprotein, translation of genomic RNA from AbLV(4070-A) pseudotype virions in the oocyte system resulted in synthesis of a 65,000-molecular-weight precursor polyprotein analogous to that obtained from translation of R-MuLV genomic RNA. This latter protein presumably represents a translational product of the 4070-A amphotropic helper virus genome. In contrast to the extensive processing of the R-MuLV *gag* gene-coded precursor, Pr65, no significant post-translational cleavage of the 120,000-molecular-weight, AbLV-specific polyprotein was observed.

Inoculation of the slower sedimentng 45 to 50S WB334 RNA described in Fig. 1B into *X. laevis* oocytes resulted in synthesis of a major protein at a molecular weight of around 55,000 which was immunoprecipitable by antisera to detergent-disrupted R-MuLV, woolly monkey leukemia virus (WLV), R-MuLV p30, and to a limited extent by high-titered anti-R-MuLV p10 (Fig. 3, F–I). Translation of the more rapidly sedimenting (70S) viral RNA resulted in synthesis not only of the 55,000-molecular-weight polyprotein, but also a 65,000-molecular-weight protein which was precipitable by antisera to detergent-disrupted R-MuLV, WLV, R-MuLV p30, and R-MuLV p10, but not by normal goat serum (Fig. 3A to E), indicating it to represent an amphotropic *gag* gene-coded polyprotein analogous to that shown in Fig. 2. No evidence for a high (>55,000)-molecular-weight polyprotein containing WLV structural components was obtained (Fig. 3).

The in vitro translation of the genomic RNA of two representative replication-defective mammalian transforming viruses, one (AbLV) of mouse, and the second (WSV) of primate origin are described. AbLV-transformed cells were previously shown to express a 110,000- to 130,000-molecular-weight precursor polyprotein containing MuLV p15 and p12 and nonstructural component(s) containing possible transformation (*src*)-specific sequences (9, 12). Suggestive evidence that the nonstructural component(s) of this latter polyprotein is AbLV-specific was obtained by the demonstration of its expression in both mouse and mink cells nonproductively transformed by AbLV (9). More direct evidence for the virus-coded nature of this polyprotein is provided in the present study by its in vitro translation after inoculation of AbLV genomic RNA into *X. laevis* oocytes. An alternative, although less likely, possibility is that the AbLV polyprotein represents a translational product of a spliced mRNA species containing genetic sequences corresponding to the 5′ terminus of the AbLV *gag* gene covalently linked to cellular sequences located adjacent to the integrated viral genome. Precedent for spliced type C viral

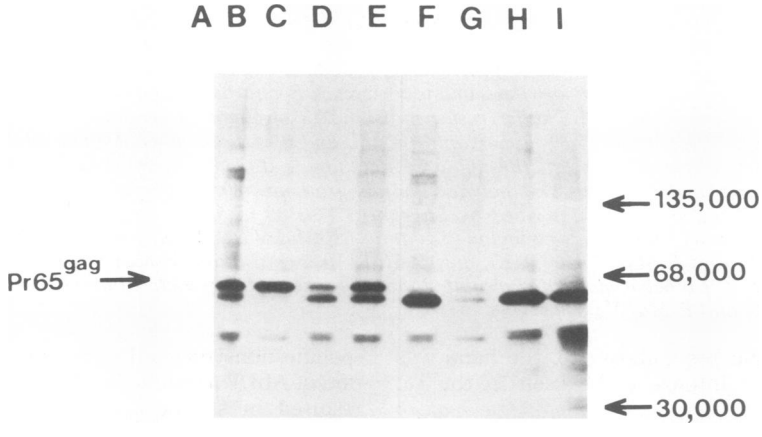

FIG. 3. *Immunoprecipitation and SDS-polyacrylamide gel electrophoretic analysis of [^{3}H]leucine-labeled viral proteins translated in X. laevis oocytes from WB334 70S RNA (A to E) and WB334 45 to 50S RNA (F to I). Sera included normal goat (A); anti-R-MuLV (B, F); anti-R-MuLV p10 (C, G); anti-R-MuLV p30 (D, H); and anti-WLV (E, J). ^{125}I-labeled molecular weight standards include β-galactosidase (135,000), bovine serum albumin (69,000), and R-MuLV p30 (30,000).*

mRNA species containing noncontiguous RNAs has been reported (8, 11). If the latter were the case, however, it would be necessary to postulate either the incorporation of this spliced mRNA species into pseudotype virions with equal efficiency as the amphotropic helper virus genomic RNA or, alternatively, less efficient incorporation but more efficient translation of the spliced mRNA.

In contrast to AbLV, however, the WSV genome codes both in vivo (2) and in vitro (Fig. 3) for a protein of a molecular weight of around 55,000 containing WLV p15, p30 and p12. In this respect, WSV closely resembles the S^+L^- strain of Moloney murine sarcoma virus (2). The inability to demonstrate a precursor polyprotein either in WSV-transformed cells or by in vitro translation of the WSV genome may indicate either that such a protein does not exist, or alternatively, that it is subject to rapid post-translational processing and that its nonstructural component, presumably containing transformation-specific sequences, is not recognized by presently available antisera. If the latter model were correct, the translation of a nonstructural WSV-coded protein in *X. laevis* oocytes, at levels coordinate with those of the WSV *gag* gene proteins, would be predicted. Such a protein should be detectable in a fully cleaved form in the event that appropriate sera for its recognition become available. The possibility, however, that the WSV-transforming protein is encoded by a spliced mRNA species, analogous to that coding for type C viral envelope glycoprotein (8, 11) which does not become incorporated into mature virions, cannot be excluded.

The results of the present study thus provide direct evidence favoring the possibility that the nonstructural portion of the 110,000- to 130,000-molecular-weight polyprotein expressed in AbLV-transformed cells is virus coded. The ability both in the present study, and in previous studies, to identify transforming virus-coded precursor polyproteins containing structural and nonstructural components has been limited to those transforming viruses which code only for p15 and p12. The in vitro translation system described should facilitate further analysis of such virus-coded polyproteins and may provide a means of testing sera from various sources for antibody directed against their nonstructural components.

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ADDENDUM IN PROOF

Witte et al. (Proc. Natl. Acad. Sci. U.S.A. **75**:2488–2492, 1978) have independently described an AbLV-coded polyprotein analogous to that reported in the present study.

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