TRANSLATION OF AKR-MURINE LEUKEMIA VIRAL RNA IN AN E. COLI CELL-FREE SYSTEM

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SUMMARY: High molecular weight RNA isolated from the oncogenic type C murine leukemia virus, AKR-MuLV, stimulates the incorporation of radioactive amino acids into protein in an E. coli cell-free system. Analysis of the translational products by SDS polyacrylamide gel electrophoresis demonstrated the synthesis of at least three proteins corresponding in molecular weight to several authentic viral proteins. Positive immunoprecipitation tests also confirm the translational product as AKR-MuLV related. Although at least 18 proteins were found on analysis of disrupted murine leukemia virions, only three were synthesized in vitro in response to AKR-MuLV RNA in the E. coli cell-free system.

Experiments demonstrating type C viral RNA-directed protein synthesis in cell-free extracts of E. coli have been reported. RNA from avian myeloblastosis virus (AMV) has been shown to direct the synthesis of several proteins, one of which is identified by immunodiffusion as a group-specific antigen (1). Rauscher leukemia virus (RLV) RNA also has been reported to stimulate the incorporation of amino acids into protein in an E. coli cell-free system (2). However, immunological experiments identifying the in vitro synthesized product as viral related have not been reported. We report in vitro synthesis of proteins in an E. coli cell-free system in response to AKR-murine leukemia virus RNA and show that the product is immunologically reactive when tested with murine leukemia virus antisera. This type C virus was isolated from an AKR mouse spontaneously producing AKR virus and was propagated in NIH Swiss embryo cells (3).

MATERIALS AND METHODS

Murine leukemia virus, AKR-3T3, was grown and purified by double density gradient zonal centrifugation by Electro-Nucleonics Biological Laboratories and contained 10^{12} virus particles/ml. QB and f2 virus were purchased from Miles Laboratories. MuLV antiserum, goat anti-rat I_gG, and normal Fischer rat serum were prepared at Huntingdon Research Center. Viral RNA was isolated

from virions disrupted with SDS and extracted with chloroform-phenol (4,5). Viral 70S RNA was isolated by sucrose gradient sedimentation (6) and analyzed by polyacrylamide gel electrophoresis (7). The growth of an RNase I deficient strain of E. coli (MRE-600) and preparation of the S-30 extracts has been previously described (8).

Reaction mixtures (0.1 ml) contained 50 mM Tris-HCl, pH 7.8, 80 mM NH_4Cl, 10 mM DTT, 5 mM ATP, 0.5 mM GTP, 5 mM phosphoenol pyruvate, 5 µg pyruvate kinase, 25 µg E. coli tRNA, 0.1 mM each of the 19 non-radioactive amino acids and 20 µl of preincubated S-30. The amount of viral RNA, magnesium, and radioactive amino acid are given in each experiment. Incubations were carried out at 37°C for 30 minutes and reactions were stopped by the addition of 0.5 ml of 0.1 N KOH, incubated for another 15 minutes and precipitated with cold 10% Cl_3CCOOH. Samples for analysis by 7.5% polyacrylamide SDS gel electrophoresis (9) were prepared as previously described (10). Samples removed from reaction mixtures for immunoprecipitation studies were first treated with pancreatic RNase and dialyzed against cold phosphate buffered saline before use.

RESULTS AND DISCUSSION

Reaction mixtures were optimized for the incorporation of [^{3}H]-leucine into protein in response to homologous phage f2 messenger RNA. Maximum amino acid incorporation was obtained in reaction mixtures containing 8 mM magnesium; a 20% stimulation in [^{3}H]-leucine incorporation was seen when 10 mM dithiothreito was substituted for an equivalent concentration of 2-mercaptoethanol. A poor stimulation of [^{3}H]-leucine incorporation in response to total AKR-MuLV RNA was initially observed. Sucrose gradient centrifugation and polyacrylamide gel electrophoresis of these viral RNA preparations demonstrated the presence of large amounts of degraded low molecular weight RNA migrating in the 4S region. The inhibitory effect of low molecular weight (4S) viral RNA from AMV on amino acid incorporation in the E. coli system has previously been reported (11). Attempts to recover sufficient amounts of 70S RNA from these

preparations for translation were unsuccessful. A positive correlation, however, between viral preparations which demonstrated high endogenous reverse transcriptase activity (12) and high levels of undegraded native 70S RNA was made. RNA extracted from these preparations gave a significant stimulation of amino acid incorporation as shown in Table 1. A 13-fold stimulation in [^{3}H]-

RNA DIRECTED PROTEIN SYNTHESIS
IN *E. coli* CELL FREE SYSTEM

Template	μg RNA	Mg^{++} Conc. (mM)	[^{3}H] Leucine cpm Incorporated
None	-	8	630
f_2	10	8	140,000
QB	10	8	109,000
AKR-MuLV			
Exp. 1	10	8	8,400
Exp. 2	10	8	10,000

Table 1. Reaction mixtures, as described in Materials and Methods, contained in 0.1 ml total volume 10 μg each of either phage f2, QB, or AKR-MuLV RNA, 10 μC_i of [^{3}H]-leucine (specific activity 25 C_i/mmol) and 8 mM magnesium. Reactions were removed anddincubated in 0.1 N KOH for 15' at 37°C and the radioactivity in acid insoluble precipitates was determined.

leucine incorporation above the background value is seen when 10 μg of AKR-MuLV is added to reaction mixtures containing 8 mM magnesium. This increase in [^{3}H]-leucine incorporation is linear for 30 minutes and dependent on the amount of AKR-MuLV RNA added. In contrast, the addition of homologous f2 or QB RNA results in [^{3}H]-leucine incorporation an order of magnitude higher. Proteins synthesized in vitro in response to total AKR-MuLV RNA were analyzed by SDS acrylamide gel electrophoresis and compared to patterns given by disrupted AKR-MuLV virions. As shown by the densitometer profile in Fig. 1, at least 18 distinct polypeptides are seen on analysis of disrupted AKR-MuLV virus particles. As shown in Fig. 1 (middle curve), the major in vitro synthesized protein migrates in the region of 30,000 daltons; two minor polypeptides corresponding in molecular weight to 12,000 and 9,000 daltons respectively are also seen (Fig. 1). These

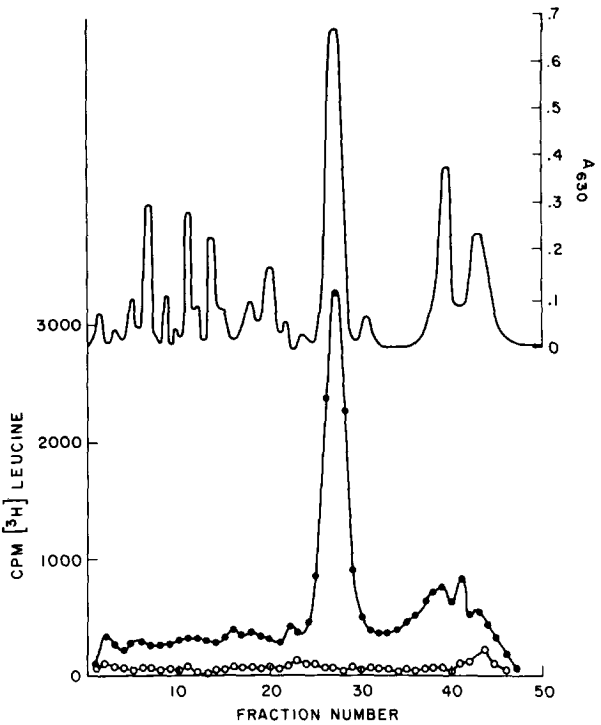

Fig. 1. SDS-polyacrylamide (7.5%) gel analysis of AKR-MuLV proteins: (a) upper curve (——) pattern of proteins from disrupted virions; (b) middle curve (•——•) radioactive pattern of proteins synthesized in vitro in response to AKR-MuLV RNA; and (c) lower curve (o——o) without the addition of RNA. Gels (15 cm) were run at 2.5 mA/gel for 4 hrs at 22°C. Gels loaded with marker proteins and viral proteins were stained with Coomassie Blue and scanned at 630 nm. Radioactivity in gel slices was determined as described (14).

molecular weights are estimated by comparison with known molecular weight standard proteins examined in the same system. When 70S AKR-MuLV RNA was used in place of total AKR-MuLV RNA to direct [^{3}H]-leucine incorporation a similar pattern was seen. The lower profile shows the radioactivity pattern of proteins synthesized in reaction mixtures incubated for 30 minutes to which no RNA was added and represents endogenous E. coli RNA-directed protein synthesis.

It is interesting to note that the major in vitro synthesized protein migrates on gels in the position of the major protein species found in disrupted AKR-MuLV virions. This protein corresponds in molecular weight to 30,000 daltons and has been previously identified as gs antigen (13). Since the products synthesized in vitro in response to AKR-MuLV RNA correspond in molecular weight to several authentic viral proteins, a fidelity of translation is suggested. Additional evidence based on the antigenic properties of these proteins was obtained using antisera prepared against total MuLV proteins. As shown in Table 2, the products synthesized in vitro in response to two different viral

IMMUNOPRECIPITATION OF PROTEINS SYNTHESIZED *IN VITRO* IN RESPONSE TO AKR-MuLV RNA

	Input[a] cpm [^{3}H] Leucine-Labelled Protein	cpm Precipitated		Percent Precipitation
		(Control Sera)	(MuLV Antisera)	
Exp. 1	3479	380	1876	43
Exp. 2	3284	442	1690	38
–	363	157	161	1.1

Table 2. Samples were prepared for immunoprecipitation as described in Materials and Methods. In vitro synthesized labelled protein was incubated with 10 µl of MuLV antiserum for 45 minutes at 30°C after which 25 µl of goat antiserum directed against rat gamma globulin was then added and incubation continued overnight at 5°C. The resulting precipitates were washed three times with 0.5 ml of cold PBS and radioactivity was determined. Specific precipitation is determined by subtracting the value obtained in precipitates using normal Fischer rat serum from total cpm precipitated with MuLV antisera. Percent precipitation represents cpm specific precipitation divided by cpm input value.

RNA preparations gave 43% and 38% precipitation respectively when reacted with MuLV antisera. Values for non-specific precipitation were determined by reacting the in vitro synthesized product with serum prepared from normal Fischer rats. Proteins synthesized in reaction mixtures to which no viral RNA was added did not react with MuLV antisera and values were usually less than one percent.

When one considers (a) that the major protein synthesized in vitro in

response to AKR-MuLV RNA is in the molecular weight range of gs antigen, 30,000 daltons, and (b) the immunological data which indicates that about 40% of the in vitro synthesized product is reactive with antisera containing antibodies against MuLV gs antigen (3+ complement fixation at 1:80 dilution), it seems likely that the viral RNA contains the information for gs antigen and that this information can be translated in the E. coli cell-free system. This, to our knowledge, is the third report on translation of type C viral RNA in a bacterial system. Translation of type C viral RNA in mammalian systems has proven difficult Although the universality of the genetic code has been established, various restrictive and modulating factors are surely involved in the efficiency of translation in heterologous systems. For example, in this study one must note the low level of amino acid incorporation in response to AKR-MuLV RNA as compared to values achieved when homologous f2 messenger RNA was used. Also possibly related is the observation that only relatively low molecular weight proteins are synthesized in response to AKR-MuLV RNA in this system whereas the mature virus particle contains at least 18 proteins, many of high molecular weight.

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