EVIDENCE FOR A NON-HEMIN REGULATED TRANSLATIONAL REPRESSOR IN FRIEND LEUKEMIA VIRUS TRANSFORMED MURINE PROERYTHROBLASTS

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SUMMARY

Lysates prepared from Friend leukemia virus transformed murine proerythroblasts, unlike those prepared from rabbit reticulocytes, are not significantly stimulated by hemin over a wide concentration range. Mixing of rabbit reticulocyte and Friend leukemia cell lysates, in the absence or presence of added hemin, results in the inhibition of synthesis of reticulocyte proteins. A translational repressor has been partially purified from these leukemic cells.

Globin synthesis in both rabbit reticulocytes (1,2) and their cell-free lysates (3,4) is controlled by the availability of hemin. The cessation of protein synthesis observed in the absence of added hemin appears to be due to a block in initiation of new globin chains (3-7). In addition, it has been shown that hemin enhances the synthesis of all proteins in reticulocyte lysates, including those programmed by exogenous mRNAs (8,9). Hemin also appears to stimulate protein synthesis in intact Krebs II ascites tumor cells and their cell-free extracts (10). It has been proposed that hemin either prevents the formation of an inhibitor of initiation (7,11) or that it interacts with an initiation factor thus preventing its inactivation (12).

Friend leukemia cells (FL cells) are murine proerythroblasts that have been transformed by Friend leukemia virus. They can be stimulated to further differentiation and to synthesize hemoglobin by dimethylsulfoxide (13,14). Although the onset of induced hemoglobin synthesis in these cells appears to be controlled at the transciptional level (15), evidence has been presented for elements of posttranscriptional control (16,17).

We wish to report the apparent lack of stimulation by hemin of protein synthesis in lysates prepared from unstimulated FL cells and the partial

purification of what appears to be a non-hemin regulated translational repressor related to this phenomenum.

MATERIALS AND METHODS

Preparation of Lysates.

The procedure used for the preparation of rabbit reticulocyte lysates was that described by Adamson et al. (3).

Murine spleen cells transformed by Friend leukemia virus (FSD-1/clone 4) were the kind gift of Dr. W. Ostertag (Max-Planck Institut fur Experimentelle Medizin, Gottingen, Germany). These cells, designated here as FLC, were grown in suspension culture as previously described (18). Lysates of cells harvested in the logarithmic phase of growth were prepared as described for rabbit reticulocytes (3) except that total lysis of the cells was accomplished by additional mechanical disruption in a Dounce homogenizer.

Preparation of FLC Translational Repressor.

The FLC translational repressor was partially purified from the 105,000 x g supernatant of a FLC lysate by chromatography on Sephadex G-200 and microgranular DE-52 cellulose (Whatman Biochemicals Ltd., Kent, England). The inhibitory activity, which is eluted from Sephadex G-200 slightly behind the void volume upon development of the column with a solution containing 10 mM Tris·HCl (pH 7.5) and 100 mM KCl, was applied to a column packed with DE-52 equilibrated with the same buffer solution. The inhibitory fraction was then recovered by elution with a solution containing 10 mM Tris·HCl (pH 7.5) and 250 mM KCl, dialyzed against 10 mM Tris·HCl (pH 7.5)-50% glycerol (v/v), and stored at -80° until used.

Standard Assay System.

Protein synthesis was measured in incubation mixtures containing the following in a final volume of 100 µl: 10 mM Tris·HCl (pH 7.5), 90 mM KCl, 1.5 mM $MgCl_2$, 5 mM dithioerythritol, 0.5 mM ATP, 0.2 mM GTP, 15 mM creatine phosphate, 26 units/ml creatine phosphokinase, 0.1 mM [^{14}C] leucine (40 Ci/mol), 0.1 mM all other [^{12}C] amino acids, and 20 µl of rabbit reticulocyte

and/or FLC lysate. Where indicated, hemin (bovine, Type I) was added to give the desired concentration. Incubation was carried out at 34° for 30 min unless otherwise indicated. Reactions were stopped by diluting 25 µl aliquots with 0.40 ml of a cold solution containing 1 mM [^{12}C] leucine and 0.50 N NaOH. After incubation for 10 min at 37°, the samples were made 5% in trichloroacetic acid and allowed to stand for 5 min at room temperature. The precipitate formed was collected on nitrocellulose filters (0.45 µm pore size, type HAWG, Millipore Corp., Bedford, Mass.), washed with three 5 ml portions of 5% trichloroacetic acid and counted by liquid scintillation.

Product Analysis.

Analysis of the products synthesized in these cell-free systems was done on sodium dodecyl sulfate-polyacrylamide gels under the conditions described by Weber and Osborn (19). After staining with Coomassie brilliant blue, the gels were destained electrophoretically, cut into 2 mm slices and counted by liquid scintillation in 10 ml of 10% Biosolve (Beckman Instruments, Inc., Palo Alto, Ca.) in toluene counting fluid containing 5.0 g of 2-5 diphenyloxazole per liter of toluene. Counting efficiency was 14%.

RESULTS AND DISCUSSION

Unlike rabbit reticulocyte lysates, FLC lysates are not appreciably

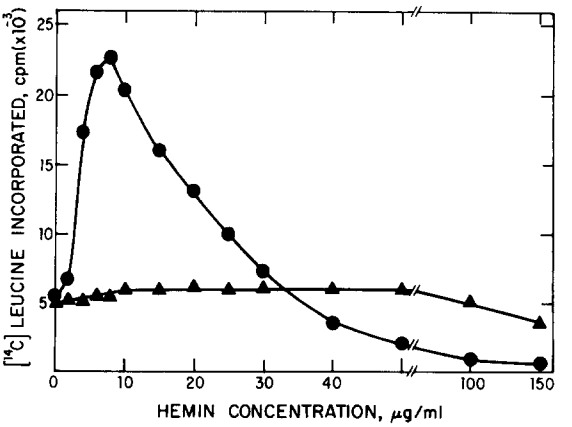


Figure 1. The effect of hemin on protein synthesis in rabbit reticulocyte and FLC lysates. Protein synthesis was measured as described under Methods at the indicated hemin concentrations. ●——● Reticulocyte lysate; ▲——▲ FLC lysate.

TABLE I

Effect of Mixing Rabbit Reticulocyte and FLC Lysates on Total Protein Synthesis

Volume Added, μl		[^{14}C] Leu Incorporated
Reticulocyte	FLC	cpm
20	0	3,364
40	0	7,539
0	20	6,500
0	40	11,200
20	20	7,520

Incubations were carried out as described under Methods, except that the indicated volumes of lysate were used.

stimulated by hemin over a wide concentration range. This is shown in Figure 1. While reticulocyte lysates may be stimulated 4 to 5 fold at the optimum hemin concentration under the conditions used, the maximal stimulation observed in FLC lysates is approximately 20% above the incorporation obtained in the absence of added hemin. This low level of stimulation with the FLC lysate is similar in magnitude to that observed with lysates from Krebs II ascites tumor cells (10). It may reflect small amounts of a hemin-controlled repressor or an effect on other enzyme systems in these cells.

The observed difference in the response to hemin in these two systems led us to investigate the effect that mixing of the lysates would have on protein synthesis. As shown in Table I, doubling the volume of reticulocyte or FLC lysate added to the incubation mixture results in a concomitant increase in protein synthesis. However, mixing of equal volumes of reticulo-

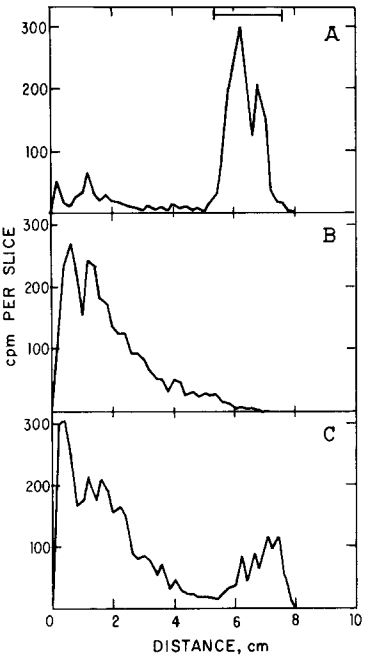
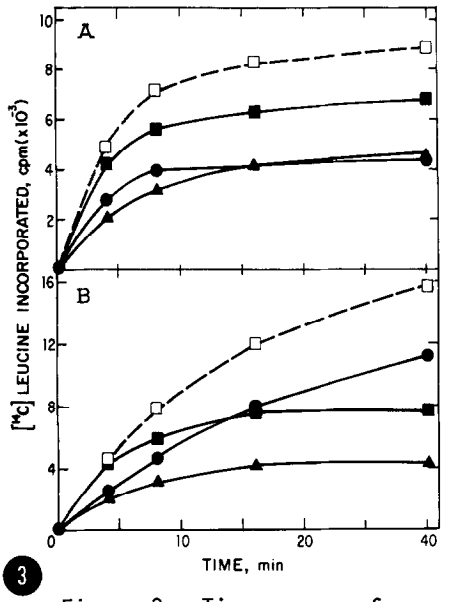


Figure 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the products synthesized in rabbit reticulocyte, FLC and mixed lysate systems. Incubation conditions were as described under Methods except that [^{14}C] leucine (320 Ci/mol) was used. Aliquots of 10 µl were analyzed. Electrophoresis was carried out as described under Methods. The limit bars indicate the position of authentic rabbit globin. A, reticulocyte lysate; B, FLC lysate; C, mixed lysate system.

cyte and FLC lysates does not result in the additive incorporation of [^{14}C] leucine into protein. The products formed with the reticulocyte, FLC and mixed lysates were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, with the results shown in Figure 2. Synthesis of rabbit globin is reduced by 60% in the mixed lysate system, as indicated by summating the counts in the portion of the gels within the limit bars shown in Figure 2A. Authentic rabbit globin migrates into this area of the gels under the conditions used. There appears to be no decrease in the synthesis of FLC protein in the reaction mixture containing both types of lysates.

Time courses for [^{14}C] leucine incorporation in reticulocyte, FLC and mixed lysate systems in the absence and presence of added hemin are shown in Figures 3A and 3B respectively. In both cases the extent of protein synthesis



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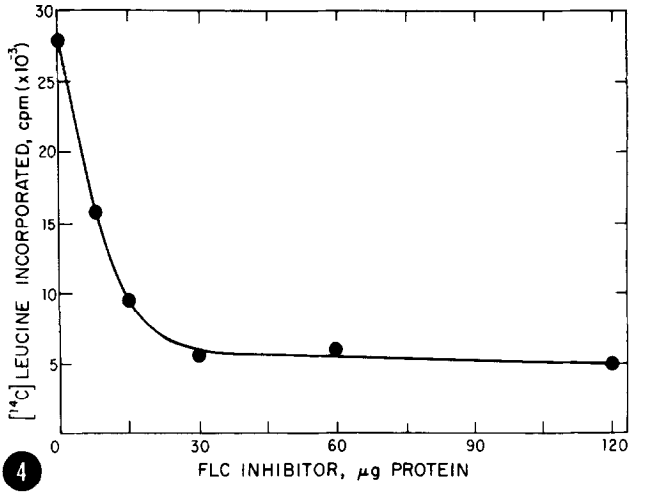

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Figure 3. Time courses for protein synthesis in rabbit reticulocyte, FLC and mixed lysate systems. [^{14}C] leucine incorporation was determined at the indicated times. A, in the absence of added hemin; B, in the presence of 8 μg/ml hemin. ●—● Reticulocyte lysate; ▲—▲ FLC lysate; ■—■ mixed lysate system; □—□ theoretical curve assuming additive incorporation for the mixed lysate system.

Figure 4. The effect of FLC inhibitor on [^{14}C] leucine incorporation in a reticulocyte lysate. Protein synthesis was measured in the presence of 8 μg/ml hemin and the indicated amounts of partially purified FLC inhibitor. Incorporation in the absence of added hemin was 6,500 cpm.

observed in the mixed lysate system is lower than the sum of incorporation for separate incubation of the individual lysates. However, the initial rate with or without added hemin in the mixed lysate system is approximately that expected for a simple additive effect of the individual lysates. This result suggests that inhibition of synthesis may take place at peptide initiation. The inhibition of protein synthesis observed in the mixed lysate system in the presence of added hemin does not appear to be a consequence of a shift in the hemin requirement to a higher value (data not shown).

Figure 4 shows the effect of increasing amounts of the inhibitory fraction prepared from FLC lysates on protein synthesis with a lysate from reticulocytes. It exhibits a concentration dependent effect and causes changes in the kinetics of synthesis very similar to those observed with the hemin sensi-

tive inhibitor from rabbit reticulocyte (20) but its formation appears to be unaffected by hemin. The FLC inhibitor preparation also inhibits protein synthesis with FLC lysates but it has no effect on poly(U)-directed synthesis of polyphenylalanine in a system derived from reticulocytes (data not shown).

We interpret these data to reflect a specific inhibitor of natural mRNA translation in unstimulated FLC. Formation of this inhibitor appears to be unaffected by hemin. The mechanism of its inhibition appears to be similar to that of the hemin sensitive inhibitor from reticulocytes.

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