Translation Level Control in Normal and Leukemic Cells

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A useful model for consideration of the biochemical aspects of cancer holds the primary lesion to be a block in the normal processes of differentiation. Erythroleukemia induced in susceptible mice by Friend leukemia virus appears to fit this model which is illustrated in Figure 1. This virus blocks the differentiation of

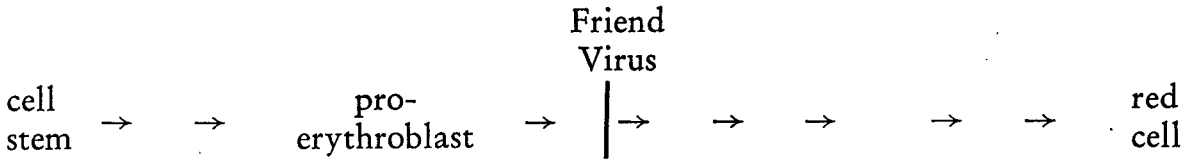

Fig. 1: Leukemia as a block in differentiation.

erythroid cells at the proerythroblast stage (1, 2) before any appreciable synthesis of hemoglobin takes place (3). Friend virus transformed proerythroblasts from leukemic mice can be carried as permanent lines and are easily propagated in suspension culture. Differentiation as reflected by hemoglobin synthesis may be induced *in vitro* by the simple expedient of adding certain aprotonic solvents, such as dimethylsulfoxide (DMSO), to the tissue culture nutrient in which the Friend leukemia cells (FLC) are grown (3–5). Thus, these cell lines provide an excellent system for the study of both transcriptional and translational control mechanisms involved in differentiation leading to the synthesis of hemoglobin. A better understanding of these controls eventually could lead to the manipulation of the regulatory elements involved in leukemia.

The ability of DMSO to overcome the viral induced block in hemoglobin synthesis is a salient feature of the Friend leukemia cell (FLC) system. Approximately 30 hr after the addition of DMSO, globin mRNA becomes detectable in FLC by hybridization with complementary globin DNA, and it reaches a maximal concentration 50–70 hrs after induction (6, 7). Ostertag and coworkers (8) have demonstrated that the rate of cytoplasmic globin mRNA synthesis reaches a maximum 24 hr after induction and decreases rapidly thereafter. Globin synthesis, on the other hand, as measured by the appearance of benzidine positive cells, is not apparent until the third day after induction and reaches a maximum around the fifth day (3, 6, 8). These results, in addition to those obtained by Paul and coworkers (9, 10), suggest the presence of posttranscriptional control elements in these cells. Harrison

et al. (10) have reported that one of their FLC lines, clone 707, contains the same amount of precursor nuclear globin mRNA before and after DMSO induction.

The reticulocyte hemin-controlled repressor

The rabbit reticulocyte system is one of the better characterized protein synthesizing systems and one in which translational level control is well estabished. Globin synthesis in both rabbit reticulocytes (11, 12) and their cell-free lysates (13, 14) is controlled by the availability of hemin. As shown in Figure 2, protein

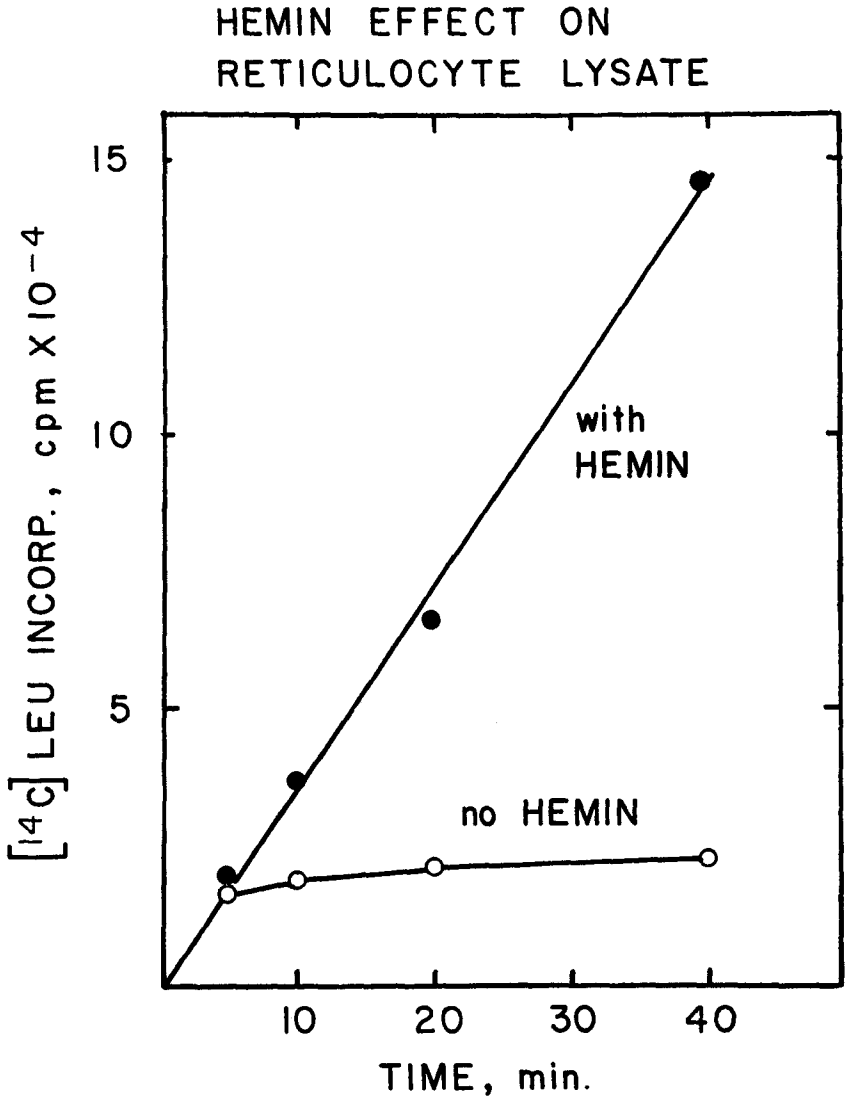

Fig. 2: Effect of hemin on protein synthesis in a rabbit reticulocyte lysate.

Protein synthesis in a reticulocyte lysate 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, 20 µl of rabbit reticulocyte lysate and, where indicated, 20 µg/ml of hemin (bovine, Type I). Incubation was carried out at 34° for the indicated periods of time. Reactions were stopped by diluting the incubation mixture 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 an allowed to stand for 5 min at room temperature. The precipitate formed was collected on nitrocellulose

synthesis in a reticulocyte lysate ceases abruptly after incubation for 5 min at 34° unless hemin is added to the incubation mixture. In the latter case protein synthesis proceeds at a linear rate for at least 40 min. 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 (13–17). Hemin enhances the synthesis of all proteins in reticulocyte lysates, including those programmed by exogenous mRNAs (18, 19), thus negating any specificity in its mode of action. It has been proposed that hemin either prevents the formation of an inhibitor of initiation (17, 20–22) or that it interacts with an initiation factor thus preventing its inactivation (23). Gross and Rabinovitz (21) have isolated an inhibitory protein from reticulocyte postribosomal supernatants incubated in the absence of hemin. This protein, which has been called a translational repressor or the hemin controlled repressor, HCR (21, 22), appears to inhibit protein synthesis by preventing the formation of a stable 40S ribosomal subunit·Met-tRNA$_f^{Met}$ initiation complex (22, 24, 25).

The FLC repressor

We have observed that hemin has a relatively low but detectable stimulatory effect on protein synthesis in lysates from uninduced FLC (26). The stimulation of protein synthesis by hemin is comparable to that observed in intact Krebs II ascites tumor cells or their lysates (27), but it is an order of magnitude lower than that observed in reticulocyte lysates (26). Moreover, mixing of equal volumes of reticulocyte and FLC lysates results in the less than additive synthesis of proteins. These results led us to examine the products formed in reticulocyte, FLC and mixed lysates by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (28). As seen in Figure 3A, hemoglobin comprises approximately 90 % of the protein synthesized in the reticulocyte lysate as indicated by summating the counts in the portion of the gel within the limit bars. Authentic rabbit globin migrates into this portion of the gel under the conditions used. On the other hand, there is no detectable globin synthesis in lysates from uninduced FLC. Higher molecular weight proteins characteristic of FLC were synthesized (Figure 3B). As shown in Figure 3C, synthesis of rabbit globin is reduced by 60 % in the mixed lysate system while there appears to be no decrease in the synthesis of FLC proteins.

Subsequently we have purified partially by chromatography on DEAE-cellulose and Sephadex G-200 an inhibitory protein present in lysates from uninduced FLC which may be responsible for the differential inhibition of globin synthesis observed in the mixed lysate system. This repressor protein does not affect poly(U) directed synthesis of polyphenylalanine at a concentration twice as high as that necessary for maximal inhibition of reticulocyte mRNA translation (29), nor does it interfere with the completion and release of nascent globin chains initiated in intact reticulocytes (see Table I).

The FLC repressor, however, promotes the protein synthesis dependent breakdown of polysomes in a reticulocyte lysate. This is shown in Figure 4C. In the

filters (0.45 μm pore size, type HAWG, Millipore Corp., Bedford, MA), washed with three 5 ml portions of 5 % trichloroacetic acid and counted by liquid scintillation. (○) no hemin added; (●) hemin (20 μg/ml) added. [This figure is from (26)].

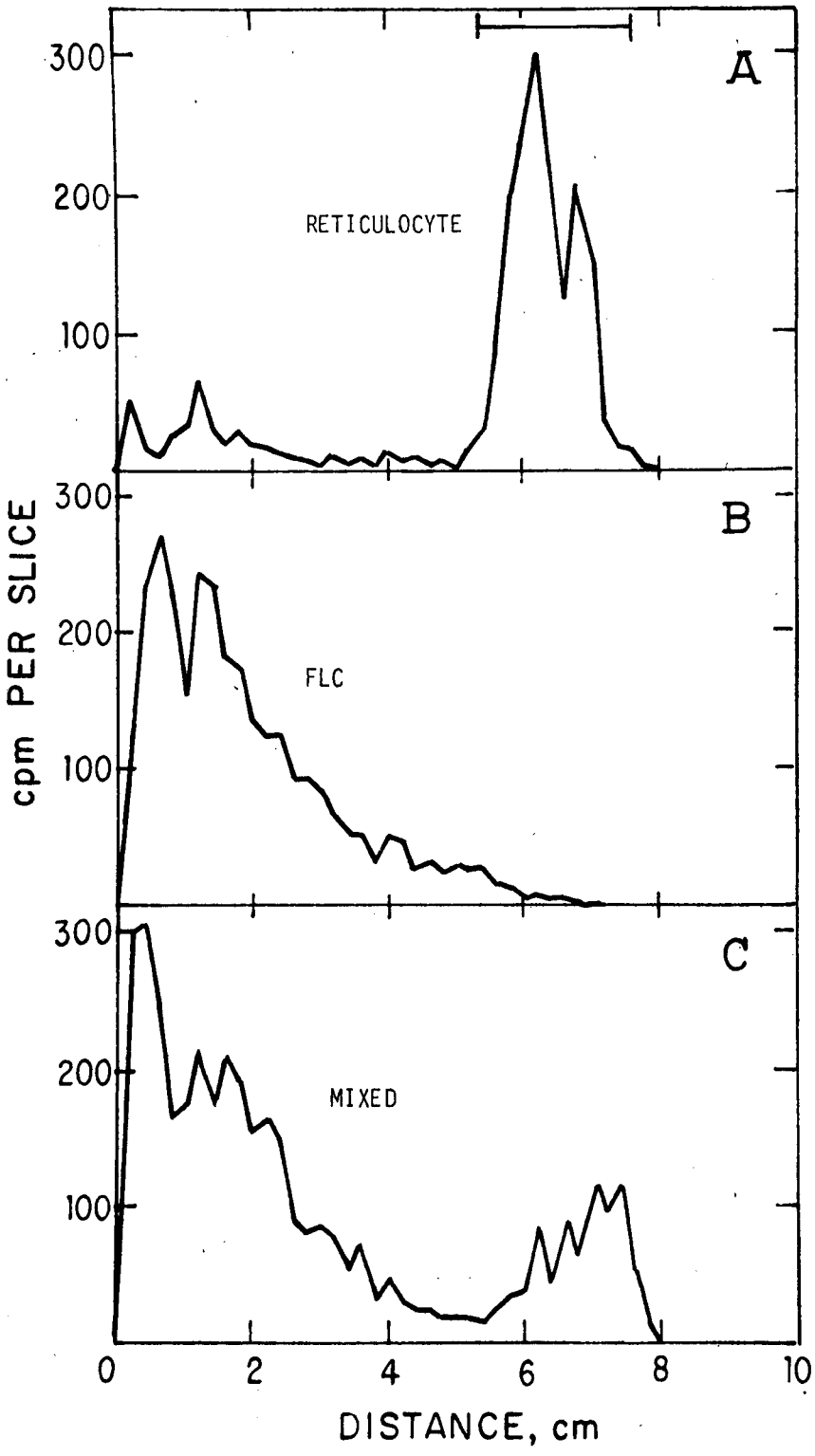

Fig. 3: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the products synthesized in rabbit reticulocyte, FLC and mixed lysate systems. Incubation conditions were as described in Figure 2 except that [^{14}C]leucine (320 Ci/mol) was used and no hemin was added. Aliquots of 10 µl were analyzed under the conditions described by Weber and Osborn (28). After staining with Coomossie 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 %. The limit bars indicate the position of authentic rabbit globin. A, reticulocyte lysate; B, FLC lysate; C, mixed lysate system. [This figure is from (26).]

Table I: Effect of FLC Repressor on Release of Nascent Peptide Chains

	[^{14}C]Leucine, cpm		
	Total	Ribosome-Free Fraction	Release %
Control, 0°	10,566	1,938	18
–FLC repressor	11,153	8,166	73
+FLC repressor	9,856	7,520	76

Table 1: Release of nascent chains labeled with [^{14}C]leucine in intact reticulocytes was measured in incubation mixtures containing the following in a final volume of 500 µl: 20 mM Tris-HCl (pH 7.5), 80 mM KCl, 2.5 mM $MgCl_2$, 5 mM reduced glutathione, 1 mM ATP, 0.4 mM GTP, 5 mM creatine phosphate, 9 units/ml creatine phosphokinase, 25 µg rabbit liver tRNA, 500 µg of the 40–70 % ammonium sulfate enzyme fraction from the postribosomal supernatant of rabbit reticulocytes, 0.1 mM all [^{12}C]amino acids and 1.0 mg of ribosomes containing [^{14}C]leucine labeled nascent peptides. Where indicated, FLC repressor (147 µg of protein) was added. Incubation was for 8 min at 37°. Samples of 200 µl were made 1 mM in cycloheximide and layered on top of 10–44 % (w/v) linear sucrose density gradients containing 50 mM Tris-HCl (pH 7.5), 100 mM KCl, 8 mM $MgCl_2$ and 0.1 mM cycloheximide. Centrifugation was for 90 min at 35,000 rpm in a SW 41 rotor (Spinco Division, Beckman Instruments, Inc., Palo Alto, CA). The gradients were fractionated from the top using an ISCO Model D density gradient fractionator and absorbance at 260 nm was recorded using an ISCO Model UA-2 ultraviolet analyzer. Fractions of 0.5 ml were collected and hot trichloroacetic acid precipitable radioactivity was determined. [This table is from (29).]

absence of the inhibitor (Figure 4B) the polysome profile obtained resembles that of the unincubated control (Figure 4A). These data suggest an effect of the FLC repressor on reattachment of ribosomes to mRNA during the initiation process.

The FLC repressor appears to block initiation of protein synthesis at a point before the NaF sensitive reaction of peptide initiation. This is shown in Figure 5. Ribosomes obtained from reticulocytes incubated with NaF are almost entirely monomeric and appear to be synchronized at a late stage of peptide initiation after attachment to mRNA (30). Edeine has been shown to inhibit protein synthesis on these ribosomes only after one round of translation has taken place, thus suggesting that the edeine sensitive step precedes the reaction inhibited by NaF (30). As can be seen in Figure 5, the kinetics of polypeptide synthesis in the presence of FLC translational repressor resemble those observed in the presence of edeine. Moreover, simultaneous addition of edeine and FLC repressor to the incubation mixture does not result in greater inhibition than that observed with edeine alone.

Comparison of HCR and FLC repressor

An obvious similarity between the HCR and the FLC repressor is that both inhibit natural mRNA translation at a step of peptide chain initiation. An obvious difference between these two repressors is that while formation of HCR in reticulocytes is controlled by the availability of hemin (17, 20–22), the FLC repressor does not appear to be controlled by this effector (26). Furthermore, functional differences clearly differentiate the HCR from the FLC repressor. The rabbit re-

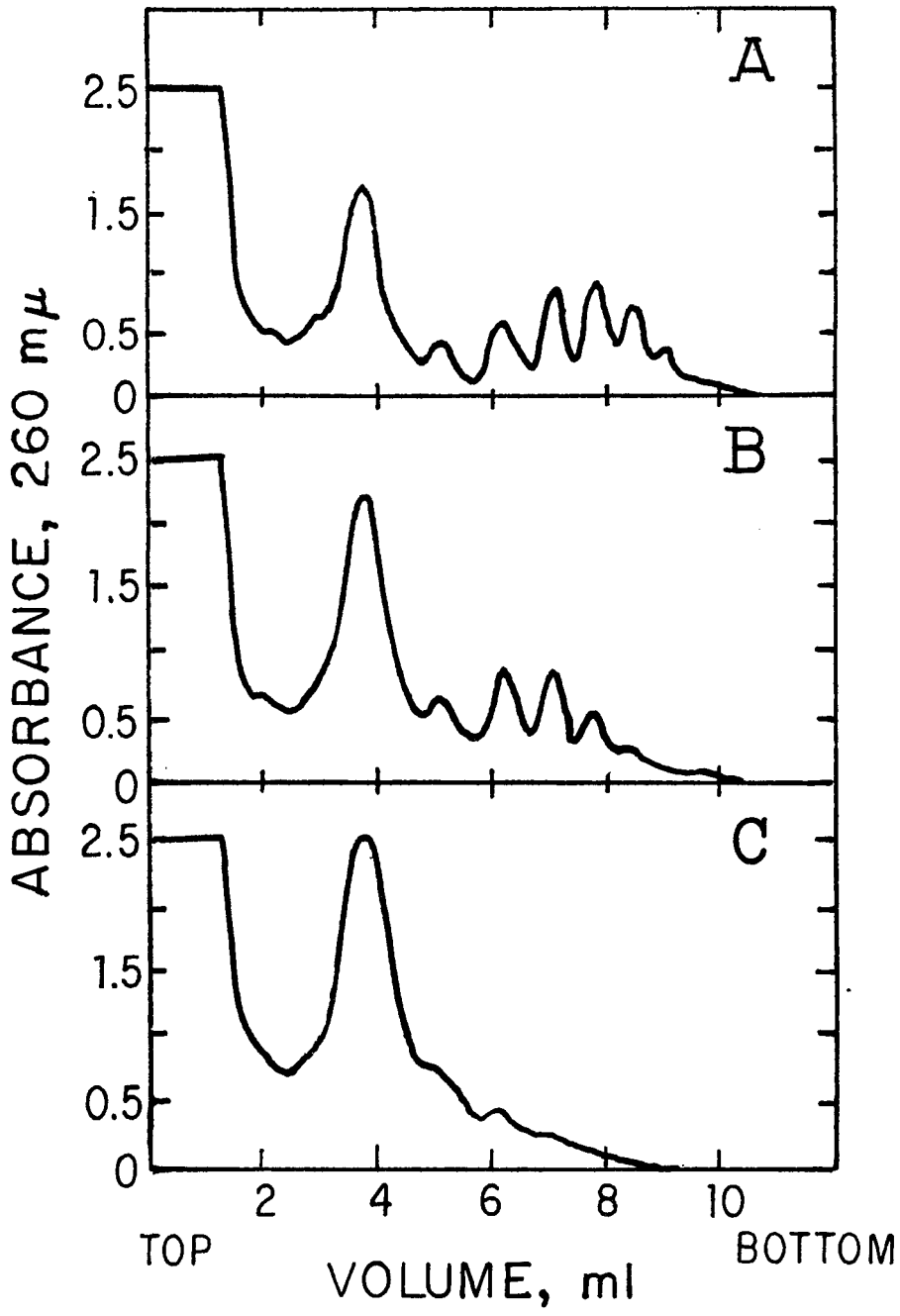

Fig. 4: Effect of FLC repressor on the polysome profile of rabbit reticulocyte lysates.
Reticulocyte lysates were incubated for 15 min at 34° in the presence of 20 μg/ml hemin under the conditions described in Figure 2. Reaction mixtures, 200 μl, were made 1 mM in cycloheximide and analyzed in 10–44 % (w/v) linear sucrose density gradients as described in Table I.

ticulocyte HCR has been shown to inhibit initiation factor dependent formation of methionylpuromycin with reticulocyte ribosomal subunits (25). As shown in Table II, the FLC repressor, unlike HCR or edeine, fails to inhibit methionylpuromycin synthesis even though it efficiently blocks globin mRNA-dependent synthesis of methionylvaline (29).

Also, it has been demonstrated that the inhibition of protein synthesis exerted by HCR in reticulocyte lysates may be overcome by an initiation factor which

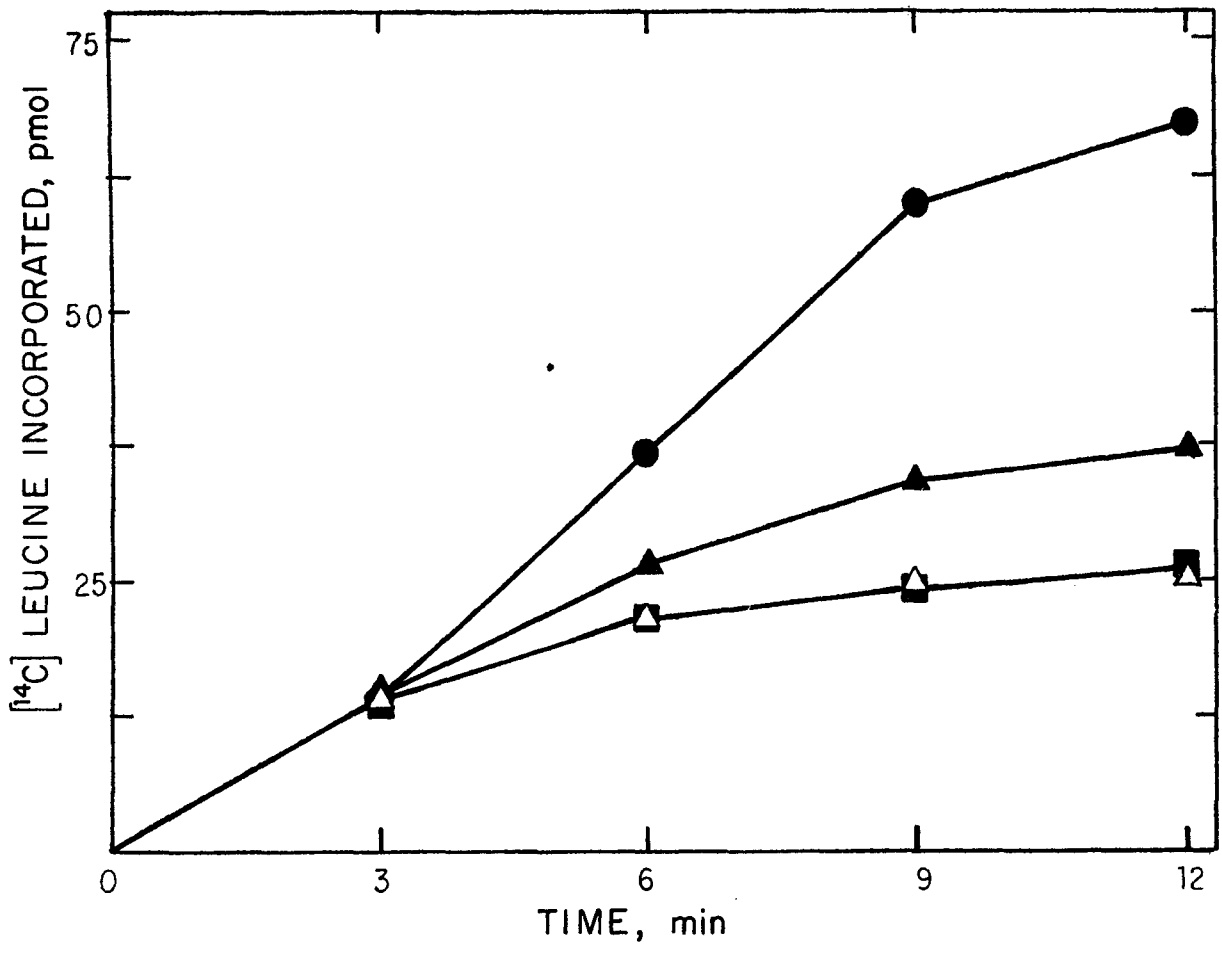

Fig. 5: Effect of FLC repressor and edeine on protein synthesis with NaF ribosomes.
Peptide synthesis with unwashed ribosomes from rabbit reticulocytes previously incubated in the presence of NaF was measured in incubation mixtures containing the following in a final volume of 100 µl: 20 mM Tris-HCl (pH 7.5), 80 mM KCl, 2.5 mM $MgCl_2$, 5 mM reduced glutathione, 1 mM ATP, 0.4 mM GTP, 5 mM creatine phosphate, 9 units/ml creatine phosphokinase, 20 µM [^{14}C]leucine (40 Ci/mol), 50 mM all other [^{12}C]amino acids, 5 µg rabbit liver tRNA, 100 µg of protein of the 40–70 % post-ribosomal enzyme fraction from rabbit reticulocytes, and 100 µg of ribosomes. FLC repressor (147 µg of protein) and/or edeine (1 µM) were added as the first components of the mixture. Incubation was at 37° for the indicated periods of time. Reactions were terminated by the addition of 100 µl of 1.0 N NaOH and treated as described in Figure 2. (●) no inhibitor; (▲) FLC repressor; (■) edeine; (△) FLC repressor and edeine. [This figure is from (29).]

Table II: Effects of HCR and FLC Repressor on Methionylpuromycin Formation

Additions	Methionylpuromycin (pmol)
Control	2.72
+ HCR	1.36
+ FLC repressor	2.66
+ edeine	0.06

Table 2: Initiation factor dependent formation of methionyl-puromycin with reticulocyte ribosomal subunits was determined by a modification (29) of the stepwise incubation procedure described by Levin *et al.* (31).

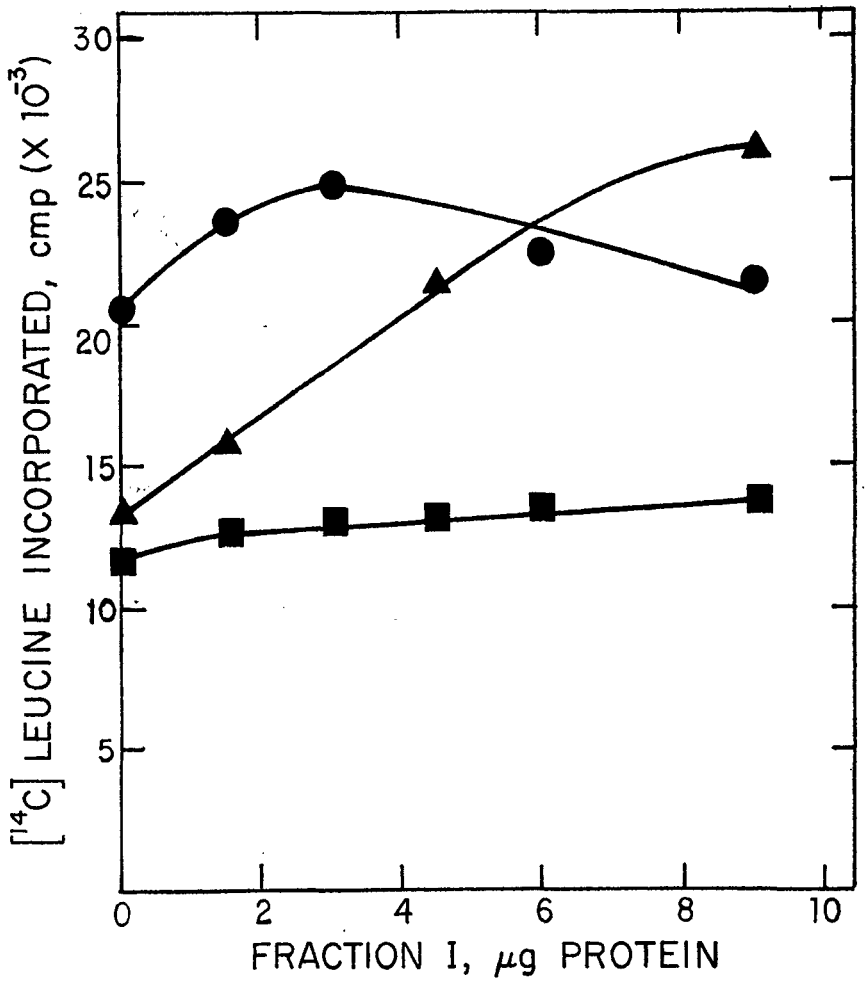

Fig. 6: Reversal of inhibition of protein synthesis by a rabbit reticulocyte initiation factor fraction.

Rabbit reticulocyte lysates were incubated under the conditions described in Figure 2. Where indicated, FLC repressor (147 μg of protein) or HCR (0.28 μg of protein) were added. The indicated amounts of protein of an initiation factor fraction obtained by chromatography of a reticulocyte ribosomal salt wash preparation on DEAE-cellulose, Fraction I (29), was added as indicated. (●) no inhibitor; (▲) HCR; (■) FLC repressor.

forms a ternary complex with GTP and Met-tRNA$_f^{Met}$ (25). Similar results are shown in Figure 6. Addition of increasing amounts of a protein fraction, obtained upon fractionation of the rabbit reticulocyte ribosomal salt wash fraction on DEAE-cellulose (29), that contains the ternary complex formation activity (Fraction I) results in the reversal of inhibition exerted by HCR. However, as seen also in this figure, the inhibition produced by the FLC repressor is impervious to increasing amounts of Fraction I.

Discussion

We have presented evidence for the presence of a translational repressor in lysates from uninduced FLC. Formation of this repressor, unlike formation of HCR in reticulocytes, is not controlled by the availability of hemin. Moreover, the FLC repressor appears to inhibit peptide chain initiation at a different step than HCR.

Paul and coworkers (in this volume) have described a lymphoma-FLC hybrid

cell line which may be induced by DMSO to synthesize globin mRNA without any detectable synthesis of hemoglobin. The data presented by these workers may reflect phenomena comparable to those involved in the inhibition of hemoglobin synthesis obtained in mixtures of lysates from FLC and reticulocytes. The existence of translational repressors in FLC suggests the possibility that virus-induced modification of translational control mechanisms might be involved in other leukemias. However, the relation of the FLC repressor to the primary biochemical lesion of this leukemia remains to be established.

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