Alterations in Translational Control Mechanisms in Friend Erythroleukemic Cells During DMSO Induced Differentiation

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Presently many laboratories are involved in studies on the regulation of the biosynthesis of proteins at the level of translation [1,2]. We have reported previously that membrane mediated events are involved in the regulation of biosynthesis of proteins [3]. Several physiological conditions (hyperosmolarity of the growth medium, hypertonic salt, DMSO, ethanol) induce a rapid increase in the number of 80S ribosomes and a concomitant decrease in the size and number of polysomes indicating that protein synthesis is affected at the level of initiation [4,5,6]. The quantitative decrease in the amount of protein synthesis is accompanied by an extensive alteration in the pattern of protein synthesis as observed upon pulse labeling of treated and untreated cells with radioactive amino acids.

When overall protein synthesis in cells in culture is inhibited by more than 80% by the hypertonic initiation block (HIB), the synthesis of one major cellular protein, actin, is reduced to a level of 10%. In contrast, the synthesis of some proteins is unchanged or less reduced indicating that the translation of some species of mRNA i.e. actin are more sensitive to and other species of mRNA are more resistant to HIB.

Thus the relative translational efficiencies (RTE) of viral and cellular mRNAs can differ over a wide range, and as such mRNAs can be classified in a hierarchical order according to translational efficiencies. Based on the example of actin stated above, we would assign an RTE of 0,5 to actin and an RTE of 1 to mRNAs from which the relative synthesis of the corresponding protein remains unaltered by HIB conditions.

Friend virus transformed mouse erythroleukemia cell lines have attained widespread use as a model system for the in vitro study of differentiation [7,8]. In this paper we describe studies concerned with the RTEs of several viral mRNAs and selected cellular mRNAs in Friend virus induced erythroleukemia cells. We also report on the effect of induced differentiation on translation control mechanisms in these cells.

Materials and Methods

Cell Cultures

Friend virus producing erythroleukemia cell line FSD-1-F4 was generously provided by W. Ostertag, Max-Planck-Institut für Experimentelle Medizin, Göttingen.

Labeling of Cells and Extraction of Proteins

The synthesis of viral proteins was analyzed by pulse labeling of cells with [^{35}S] methionine, followed by lysis of the cells and selection of virus specific proteins by incubation with antisera directed against viral proteins and subsequent separation by polyacrylamide gel electrophoresis (PAGE), autoradiography and densitometry [9,10,11]. Protein synthesis was quantitated by the method of Mans and Novelli [12].

Results

Identification of Viral and Cellular Proteins and Determination of RTEs of the Corresponding mRNAs

In previous publications [9,10] we reported on the identification by immunoprecipitation of a number of Friend virus specific proteins in several Friend erythroleukemia cell lines isolated by Ostertag, in the Eveline cell line and in the original cell line 745 characterized by Dr. Friend. Our recent investigations were performed with the Ostertag cell line F4–N. Additional virus specific proteins are present in this cell line based upon data obtained by immunoprecipitation with specific antisera against viral proteins.

Cell extracts prepared as described previously [10] from 60 min [^{35}S] methionine pulse labeling experiments were first exposed to normal goat antiserum, then to *Staphylococcus aureus* strain Cowan I (S. aureus), followed by exposure to anti p30 goat serum, again to *S. aureus* and finally to anti gp70 antiserum and to *S. aureus.* Labeled proteins specifically adsorbed to the three *S. aureus* precipitated samples were eluted and separated by PAGE as previously described [10]. Proteins which react preferentially or exclusively with one of the specific sera (see Fig. 1, legend) are considered virus specific. Cellular proteins (especially actin) contaminated all 3 antigen-antibody-*S. aureus* complexes to a comparable extent.

We have determined the RTE of cellular and viral mRNAs by quantitation of the amount of synthesis of corresponding proteins under isotonic and various HIB conditions. Fig. 1 shows densitometer tracings of autoradiographs from Page separations of [^{35}S] pulse labeled proteins from Friend cells after immunprecipitation and adsorption and desorption to *S. aureus.* The apparent molecular weights of the proteins in kilodaltons are indicated above each peak. We have previously shown that a quantitative analysis of the synthesis of individual proteins can be achieved by densitometry of autoradiographs [11]. One can readily see that the synthesis of various proteins in the non-induced cells was differentially affected by HIB. Actin migrates with an apparent molecular weight of 44 K in the present gel system. The synthesis of actin is highly sensitive to HIB indicating that in Friend cells – as in other tissue culture cells – the RTE of the mRNA coding for actin is very low. The mRNAs coding for the cellular proteins with a molecular weight of 19 and 20 K have an RTE of approximately 1, that is, the synthesis of the 19 and 20 K proteins was inhibited by HIB to the same extent as overall cellular protein

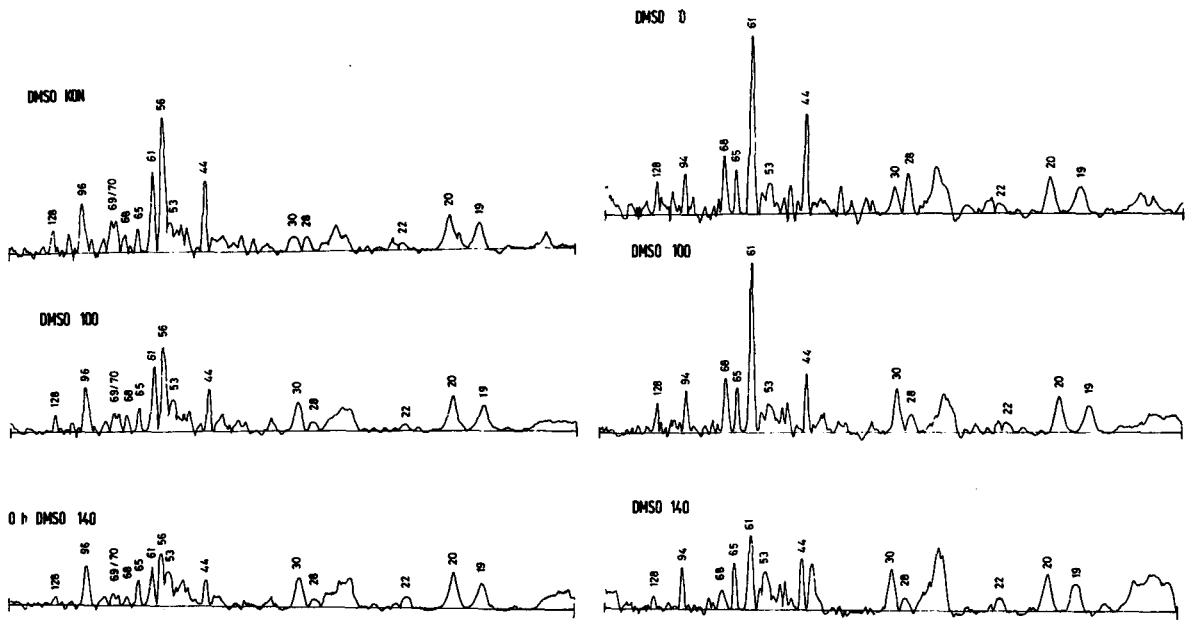

Fig. 1. Densitometry tracings of autoradiographs of SDS gel electrophoresis of S. aureus adsorbed immuncomplexes from friend cell extracts pulselabeled with [^{35}S] methionine. The approximate molecular weights in kilodaltons of the labeled protein peaks is based on the migration of unlabeled molecular weight markers and on the migration of labeled vesicular stomatitis virus proteins. The cells were preincubated for 25 min with medium containing one-twentieth the normal concentration of methionine. The osmolarity of the growth medium was increased by addition of extra NaCl a) 0 mM (0), b) 100 mM (100), c) 140 mM (140) which resulted in an overall inhibition of protein synthesis by a) 0%, b) 50%, c) 80%. Therefore, culture b) and c) received 2 and 10 times more [^{35}S] methionine than culture a) in order to label the proteins in the cell extracts to a comparable extent [11].
Left panel: Cell extract treated with anti gp70 serum
Right panel: Cell extract treated with anti p30 serum

synthesis. We utilized the relative intensity of the labeling of these proteins bands as standards for the estimation of the RTEs of viral and cellular mRNAs from autoradiograms of labeled cell extracts or immune precipitates. The results presented in Table 1 indicate that the RTE of some RNA tumorvirus mRNAs in this system are lower than the RTEs of cellular mRNAs. In fact, the RTE of mRNAs coding for gp70 and gp56 are as low as

Table 1. Relative translational efficiencies of cellular and viral mRNAs in Friend erythroleukemia cells

cellular proteins							
MW (Kd)	128	44	22	20	19		
RTE	0,4	0,5	2,0	1,0	1,0		
gp70 specific proteins							
MW (Kd)	96	70	56				
RTE	0,9	0,3	0,3				
p30 specific proteins							
MW (Kd)	94	68	65	61	53	30	28
RTE	1,0	0,4	1,1	0,4	1,4	1,4	0,4

the RTE of the actin mRNA. This observation posed some very interesting questions concerning the mechanism of translational control in this system, since there is no apparent correlation in the amount of viral mRNAs and the corresponding viral proteins synthesized (Racevskis, Koch and Ostertag, unpublished).

Increase in Sensitivity to HIB During DMSO Induced Differentiation in Friend Erythroleukemic Cells

Induction of differentiation in Friend erythroleukemic cells causes a severe reduction in the growth rate of the cells [7]. The division time of the cells increases from 13 hours to 24 hours by 3 days after induction with DMSO (Weber and Koch, unpublished). In order to determine and compare the RTEs of mRNAs at various times after induction of differentiation we analyzed the incorporation of ^{35}S methionine into proteins under isotonic and various HIB conditions. The results presented in Fig. 2 confirm previous

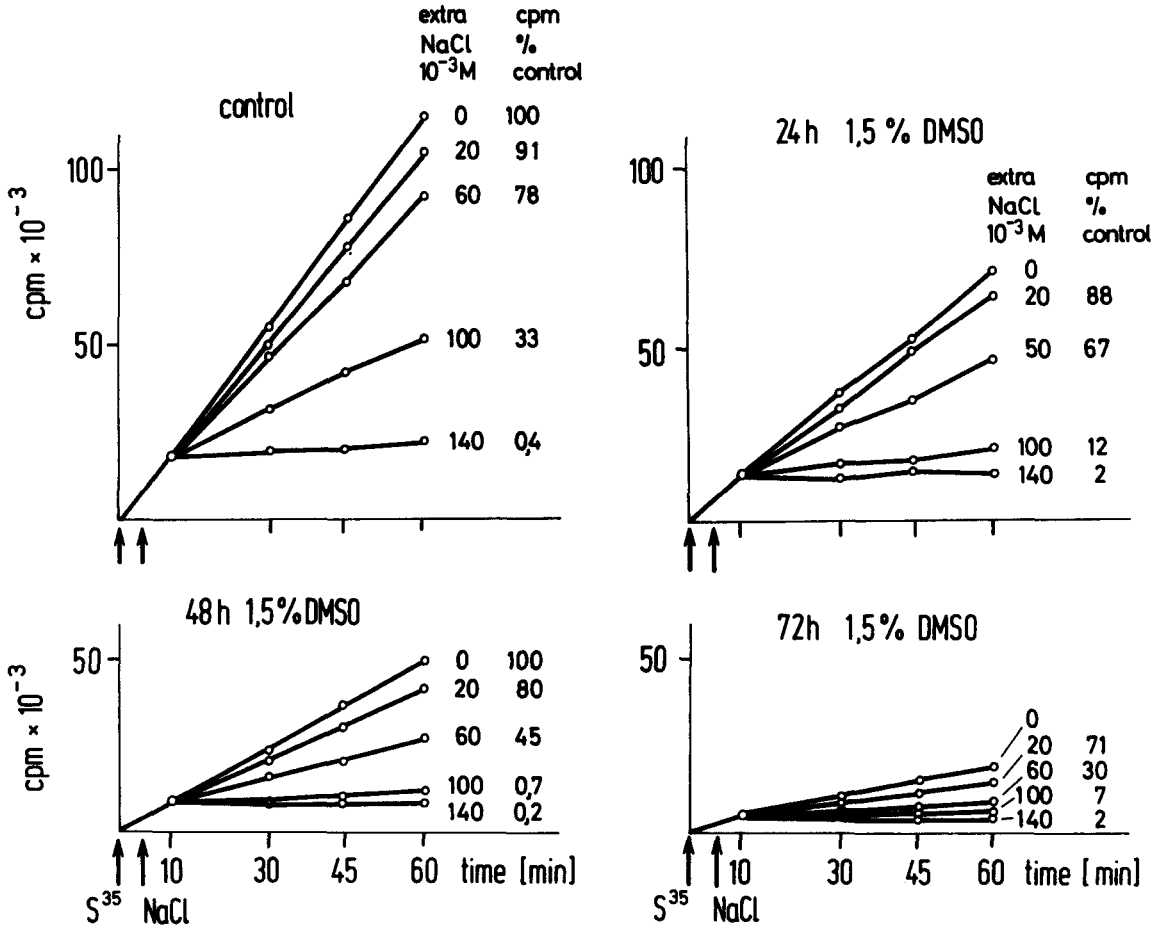

Fig. 2. Effect of HIB on the incorporation of [^{35}S] methionine into proteins at various times during DMSO induced differentiation of Friend-erythroleukemic cells. In order to exclude nutritional effects the cells were fed with fresh medium every 24 hours during the course of the experiments. [^{35}S] methionine was added to several aliquots of the culture (1.0×10^6 cells/ml) and the osmolarity of the growth medium was increased by addition of different amounts of extra NaCl. Incorporation of [^{35}S] methionine into proteins was determined as described [12]

findings on reduced incorporation of amino acids during differentiation [15]. In addition, they indicate that the cells become progressively more sensitive to HIB with increasing time after induction of differentiation, that is, a smaller increase in osmolarity causes a more dramatic inhibition of protein synthesis.

Alterations in Translational Control Mechanisms in Friend Erythroleukemic Cells During DMSO Induced Differentiation

The question arose as to whether the inhibition of cellular protein synthesis and the intracellular concentration of a given polypeptide changes coordinately after induction of differentiation and also whether coordinate changes in RTE and the yield of the corresponding proteins occur.

The experiments described in Fig. 1 were repeated at daily intervals after induction of differentiation with DMSO, and the results are summarized in Table 2. RTEs of mRNAs can remain constant (i.e. for cellular proteins 20 and 19 K), decrease (i.e. cellular 128 K) or increase (mRNA for some viral proteins, and most dramatically for a cellular protein with a molecular weight of 22 K). An RTE over 1 indicates a relative increase in the amount of synthesis of a given protein under restricted condition of polypeptide chain initiation. The cellular protein 22 K with an RTE of 4 at 72 hours after induction accumulates under conditions of 80% inhibition of overall protein synthesis at a rate of 80% of the normal rate. An actual increase in the synthesis of a given protein under HIB conditions was observed for the synthesis of globin in Friend cells by utilizing two different experimental approaches both the quantitative determination of the hemoglobin content by the ben-

MW (Kd)	RTE		% Change in yield of intracellular protein from 0–72 h
	0 h	72 h	
cellular proteins			
128	0,4	0,3	180
44	0,5	0,4	130
22	2,0	4,0	100
20	1,0	1,0	100
19	1,0	1,0	100
gp70 proteins			
96	0,9	1,0	160
70	0,3	0,6	110
56	0,3	0,6	120
p30 proteins			
94	1,0	0,8	70
68	0,4	0,9	230
65	1,1	1,2	130
61	0,4	0,8	60
53	1,4	2,4	110
30	1,4	2,5	160
28	0,4	0,4	130
25	1,8	2,8	110

Table 2. Changes in RTE of mRNAs and in the relative amounts of corresponding proteins during DMSO induced differentiation

zidine technique (Bilello, unpublished) and from autoradiographs of HIB treated induced cells. It is important to mention that in spite of the dramatic increase in the RTE of the mRNA coding for the cellular protein 22 k during differentiation, this protein does not accumulate at a higher rate under isotonic conditions in differentiated cells.

Summary

Induction of erythroid differentiation in Friend erythroleukemia cells causes a reduction in the rate of protein synthesis and a prolonged growth cycle of the cells. A study of the relative translational efficiencies (RTE) of viral mRNAs and selected host cell mRNAs revealed unexpected low RTEs of the mRNA coding for the gp70 protein and RTE of the mRNA coding for the p30 protein. The RTEs of viral and of some host mRNAs show significant changes during differentiation. The rate of synthesis of corresponding proteins are altered non-coordinately, indicating transcriptional and/or posttranscriptional regulation of mRNA, as well as amplified regulation at the level of translation of mRNA.

Discussion

A great number of laboratories are engaged in studies to delineate the pleiotropic effects which are triggered by inducers of erythroid differentiation in Friend erythroleukemia cells. Nevertheless, little is known so far on the role of the regulation of protein synthesis at the level of translation and on the fate of newly synthesized proteins (post translational modification, cleavage and degradation). We have previously reported on alterations in the processing pathway and in the rate of processing of viral precursor proteins in the Friend cell line 745 [10]. The experiments presented here show that the decreased rate of overall protein synthesis observed during differentiation is accompanied by drastic alterations in the rate of synthesis of some proteins and in the RTE of mRNAs. Changes in the RTE of an mRNA and in the rate of synthesis of the corresponding proteins are not coordinate, indicating that a lowered RTE may be counteracted by an increased availability of active mRNA. Likewise the effect of an increase in the RTE of mRNAs might be reduced by a decreased amount of availability of an mRNA. Regulation of protein synthesis at the level of translation might also involve other factors than amount of mRNA and RTE of mRNA. This view is supported by the observation that early vaccinia virus mRNAs with high RTEs are present but not translated at later times in the replicative cycle [13]. Comparable results have been obtained in frog virus infected cells [14]. In this study we were able to demonstrate both alterations in the RTEs of several mRNAs and a non-coordinate change in the accumulation of corresponding proteins. While other investigators have reported on the existence of specific regulatory mechanisms for the translation of a given mRNA it is difficult for us to imagine how such a model could

be consistent with our findings in regard to the multitude of changes. Thus we would like to suggest that more generalized alterations in the translational machinery must occur during differentiation and the ultimate alteration in the pattern of proteins synthesized is dependent upon multiple factors at both the transcirptional and post transcriptional levels.

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