Inhibition of Polypeptide Chain Initiation by Inducers of Erythroid Differentiation in Friend Erythroleukemic Cells*

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Friend virus transformed erythroid cells can be isolated and maintained serially in cell culture [1,2]. Such erythroleukemic cell cultures can be induced to differentiate along the erythroid pathway by exposure to a variety of compounds including DMSO [1–3], aprotic solvents [4], fatty acids [5], purines and purine analogs [6] and the cardiac glycoside ouabain [7]. Phenotypic changes observed in Friend cells after inducer treatment appear to parallel the expression of genes typical of the normal differentiating cell. A multitude of erythroid functions are induced including the induction of heme pathway enzymes [8], globin mRNA synthesis [2,3,9,10], and the synthesis of erythrocyte specific proteins [11–13]. The extent of hemoglobin synthesis can be as high as 20–25% of the total cellular protein synthesis in induced cells, compared to less than 1% in uninduced cells.

The observation that a variety of inducers could affect a marked alteration in gene expression prompted us to study protein synthesis in Friend cells after treatment with a variety of inducers. We were particularly interested in determining whether there was a common mode of action whereby these inducers could affect the specific synthesis of the unique spectrum of erythrocyte proteins observed during differentiation of erythroleukemic cells.

Materials and Methods

Cell Culture

The origin and maintenance and labeling of Friend erythroleukemia cell cultures is essentially as described in Racevskis and Koch [14]. Protein synthesis was quantitated by the method of Mans and Novelli [15].

Hemoglobin Assay

The hemoglobin content of cell extracts was measured utilizing the benzidine technique described by Luftig et al. [16].

Results and Discussion

In Friend erythroleukemic cell cultures addition of 10 to 15% DMSO to the tissue culture medium results in a rapid, reversible inhibition of cellular protein

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synthesis accompanied by the complete breakdown of polyribosomes. DMSO concentrations effective in inducing cell differentiation (1–2%) cause a sustained inhibition of protein synthesis and a shift in the polyribosome profile wherein there is a substantial decrease in the percentage of mRNA associated ribosomes (Bilello et al., manuscript in preparation). We have observed a similar sustained inhibition of the pattern of protein synthesis with a series of other known inducers (Table 1). Since we have measured protein synthesis by incorporation of radiolabeled methionine into protein, we were concerned that changes in amino acid uptake or pool size may have colored the interpretation of our data. As can be seen in Table 2, amino acid transport was reduced upon the addition of DMSO. The reduction in amino acid transport occured early after addition of inducer, but was not substantial enough to explain the decreased protein synthesis in treated cells. Other inducers appear to affect changes in amino acid and hexose transport and may reflect a general effect of inducers at the cell membrane (17, Ostertag personal communication).

Since we had observed inhibition of initiation in Friend cells treated with 10–15% DMSO we wanted to determine whether initiation was inhibited during the course of Friend cell induction. Polyribosome profiles were analysed in F4–6 cells two hours after the addition of DMSO, HMBA or butyric acid since at this time point inhibition of protein synthesis was established and amino acid uptake was only moderately lowered. After treatment of F4–6 cells more than 10% of the mRNA associated ribosomes were shifted to the

Table 1. Effect of inducers on Friend erythroleukemic cell protein synthesis

Addition	Concentration mM	Hemoglobin[a] μg/10^8 cells	Protein synthesis[b] % Control	
			24 hrs	72 hrs
F4–6				
None	—	14	—	—
DMSO	192	155	34	33
HMBA	5	239	51	34
Butyric Acid	1	178	42	37
Hemin	0.1	189	69	—
Hypoxanthine	2.5	230	65	—
Ouabain	0.1	121	56	—
B8				
None	—	44	—	—
DMSO	192	240	56	41
HMBA	5	341	33	31
Butyric Acid	1	502	56	46
Hemin	0.1	276	107	47
Hypoxanthine	2.5	108	115	48
Ouabain	0.1	406	104	56

[a] Hemoglobin production measured in cell extracts 3 days after addition of the compound as described in the Materials and Methods

[b] Protein synthesis as measured by incorporation of ^{35}S methionine into hot TCA insoluble material 24 or 72 hrs after addition

Table 2. Alteration of amino acid transport subsequent to the addition of DMSO to Friend erythroleukemic cell cultures

Time in culture hrs	Treated cells cpm/mg protein	Untreated cells cpm/mg protein	% $T/_U$
0	13 006	14 213	91,5
2	11 315	14 641	77,2
4	10 426	14 931	69,8
6	10 629	14 490	73,7
12	9 009	15 482	58,3

Cells were labeled with ^3H amino acid mixture for 10 minutes, washed 3 times with Earles Balanced salt solution. Cell extracts were precipitated with 10% TCA and TCA soluble counts were determined. Total cell protein was determined in an aliquot by the Lowry method.

monosome region of the gradient indicative that ribosomes previously associated with mRNA were no longer actively engaged in protein synthesis. Polyribosome profiles indicative of reduced initiation have been observed throughout the course of induction. These results suggested that inhibition of the initiation of protein synthesis is an early event which is sustained during the course of induction.

Table 3 summarizes a series of experiments designed to access whether initiation and/or elongation inhibitors could induce erythroleukemic cell differentiation. Treatment of Friend cells with the elongation inhibitors puromycin, cycloheximide or emetine had no effect upon the induction of hemoglobin synthesis. In contrast hemoglobin synthesis was induced upon exposure to ethanol or hypertonic medium which inhibit initiation [18]. Initiation inhibitors induce higher levels of hemoglobin synthesis in B8 cells,

	Addition	Hemoglobin μg per 10^8 cells at day 3
Exp 1	none	13
	DMSO 192 mM	290
	Sucrose 50–200 mM	13–22
	Emetine 10–50 μg/ml	13–34
Exp 2	none	8
	DMSO 192 mM	310
	Puromycin 0,1–10 μg/ml	2–11
	Cycloheximide 0,1–5 μg/ml	1–19
	Ethanol 1–2%	77–84
Exp 3	none	16
	DMSO 192 mM	127
	Excess KCl 25–75 mM	30–100
	Excess NaCl 25–75 mM	32–81
Exp 4[a]	none	44
	DMSO 192 mM	240
	Excess KCl 50 mM	161
	Excess NaCl 75 mM	100

Table 3. Protein synthesis Inhibitors: Effect upon hemoglobin synthesis in uninduced Friend erythroleukemic cells

[a] Experiment 4 utilized B8 cells which have a high background synthesis of globin and globin mRNA

cells which have a higher background level of globin mRNA in the uninduced state [10]. Inhibition of initiation in such cells leads to a rapid increase in hemoglobin synthesis. Only initiation inhibitors which induced sustained inhibition of cell protein synthesis were capable of induction; sucrose or DEAE dextran were incapable of either induction or sustained inhibition of protein synthesis in Friend cells.

Our findings indicate that inducers of erythroleukemic cell differentiation inhibit protein synthesis at the level of initiation. Different mRNAs often differ in the rate of formation of the initiation complex. Under conditions of lowered initiation, translation of high efficiency mRNAs i.e. globin mRNAs are favored relative to other mRNAs with lower initiation frequencies [18,19]. Other experiments have demonstrated that exposure of erythroleukemic cells to high concentrations of NaCl or KCl increased the synthesis of globin relative to other cell proteins. Thus the ability of inducer moieties to inhibit initiation may serve to provide the optimum conditions for globin synthesis which is the major protein synthesized in the differentiated erythroid cell.

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