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Protein synthesis in HL-60 cells treated with DMSO and hypoxanthine

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Short-term treatment of the HL-60 cells with DMSO and hypoxanthine, inducers of granulocytic differentiation, was reported to cause a rapid increase in protein synthesis. This effect was ascribed to the insertion of inosine in the wobble position of the tRNA anticodon and consequently increasing codon recognition potential. In this study we have re-investigated the effects of DMSO and/or hypoxanthine on protein synthesis. In contrast to their findings we were unable to demonstrate stimulated protein synthesis in either short- or long-term treatment with these agents. Polysome analysis under these conditions revealed that polysomes were disaggregated. Finally, the activity of tRNA-hypoxanthine ribosyltransferase, an enzyme responsible for the insertion of inosine in the anticodon, was also relatively low. Under these circumstances, we propose that tRNA modification is not essential in the regulation of protein synthesis.

Protein synthesis; tRNA; DMSO; Hypoxanthine

1. INTRODUCTION

The sequence of events in protein synthesis is now well established for both prokaryotes and eukaryotes. The protein synthesis pathway is commonly differentiated into three major steps, namely initiation, elongation and termination. The basic components needed in the complex process of protein synthesis include, ribosomes, mRNA, tRNA, GTP, ATP, protein factors and various enzymes [1].

Proteins are a major constituent of the cells and therefore their synthesis is controlled under a variety of physiological and pathological conditions. These are growth [2], differentiation [3], starvation [4], stress [5], nutrient, amino acid and serum deprivation [4,6,7], presence of hormones [8] and diabetes [4]. Despite the intense research interest in protein synthesis, the mechanism of regulation of this process is not yet fully understood. The modulation of the level of specific mRNAs, alteration in the number of ribosomes and the rate of polypeptide chain initiation appear to be some of the key regulatory steps [8].

A number of studies have shown that in most of the cases where regulation occurs at the polypeptide chain initiation stage, eIF-2 is central in the process [6,7,9]. Phosphorylation of the α-subunit of eIF-2 results in the inhibition of translation as the catalytic recycling of eIF-2, normally accomplished through the dissociation of the eIF-2 · eIF-2B complex, cannot be achieved with the phosphorylated eIF-2 [10,11]. Another point of regulation of the polypeptide chain initiation is at the step of binding of mRNA to the ribosome. Recent findings show that eIF-4E also undergoes phosphorylation, and in this instance, however, the degree of phosphorylation correlates with translation rates [12]. It has been suggested that this might be a pathway by which growth factors, mitogens and viral oncogenes [12] transmit extracellular signals to the intracellular milieu.

Of particular interest is the fact that tRNA has also been implicated in the regulation of translation. Changes in the profiles of the different isoacceptor tRNAs have been reported in different tissues and cell lines [13–15] and this has given ground for consideration of tRNA as performing a regulatory role. Whilst this observation supported the hypothesis of modulation based largely on codon–anticodon interaction, later evidence showed that base modification far off from the anticodon of tRNA could also affect translation [16]. Recently interest in the role of posttranscriptional modification of tRNA in protein synthesis was aroused by the report of Trewyn et al. [17], who found that short-term treatment of HL-60 cells with DMSO and hypoxanthine simultaneously caused a rapid increase in protein synthesis. The latter effect was thought to be mediated by tRNA modification. In this paper we have re-examined the effects of DMSO and hypoxanthine on protein synthesis in HL-60 cells and report that we were, however, unable to demonstrate the enhanced rate of protein synthesis.

Abbreviations: FBS, fetal bovine serum; DMSO, dimethylsulfoxide; eIF-2, eukaryotic initiation factor 2; TCA, trichloroacetic acid.

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2. MATERIALS AND METHODS

RPMI-1640, hypoxanthine and *E. coli* tRNA were from Sigma (St. Louis). FBS was from Delta Bioproducts (Johannesburg). DMSO from Reidel-deHaen AG (Seelze-Hannover) and all radiochemicals were from Amersham International (UK). The HL-60 cell line was a generous gift from Prof. B. Mendelow (University of Witwatersrand, Johannesburg).

2.1. *Cell culture*

HL-60 cells were grown in RPMI-1640 suspension culture supplemented with 10% FBS and antibiotics (100 μg/ml streptomycin and 100 U/ml penicillin) at 37°C in 5% CO_2/95% air. Cell counts were performed using a Coulter counter (Coulter Electronics) to establish the growth pattern. Viability was estimated using the Trypan blue dye exclusion method. Periodically, HL-60 cells were tested for mycoplasmal contamination using the Hoechst DNA dye.

Experimental cultures were initiated with cells in the exponential growth phase (2–5 × 10^5 cells/ml) and seeded in 25 cm^2 flasks at a cell density of 1 × 10^5 cells/ml. Cultures were treated with 210 mM DMSO, 1 mM hypoxanthine and a combination of the two drugs.

2.2. *Preparation of enzyme extracts*

Following incubation of the HL-60 cells (±10^7) suspended in Hank's solution for 1 h in the presence of different inducers, the cells were harvested in ice-cold phosphate-buffered saline by centrifugation at 300 × *g* for 10 min. The pellet was resuspended in two volumes of extraction buffer: 10 mM Tris-HCl buffer (pH 7.4) containing 10 mM $MgCl_2$, 1 mM EDTA, 0.5 mM dithiothreitol, and 10% glycerol. Lysis of the cells was achieved by using a combination of the freeze–thaw technique in liquid nitrogen and ultimately pushing the suspension three times through a 28-gauge needle. Finally the extract was centrifuged at 30,000 × *g* at 4°C for 10 min and the supernatant was kept for the enzyme assay.

2.3. *Preparation of polysomes*

The method used for preparing polysomes was similar to the above for preparation of the enzyme extract except that the lysis buffer contained 30 mM Tris (pH 7.5), 110 mM KCl, 1.5 mM magnesium acetate, 6 mM mercaptoethanol and 0.1% Triton X-100.

2.4. *Measurement of protein synthesis*

The rate of protein synthesis was assayed by measuring the incorporation of [^{3}H]leucine into TCA-insoluble material.

Briefly, cells were harvested by centrifugation at 300 × *g* for 10 min and washed twice with Hank's solution. Thereafter 200 μl cell suspension were incubated in the presence of 1 μCi [^{3}H]leucine (140 Ci/mmol) for different periods of time. The reaction was terminated with ice-cold 7.5% TCA (10 mM leucine and 10 mM sodium pyrophosphate) and the precipitate was collected onto microglass fiber filters and radioactivity was counted on a Beckman LS 6000IC Scintillation Counter. Protein synthesis was expressed as cpm/10^5 cells.

2.5. *Enzyme assays*

The enzyme assays were carried out according to the method of Elliott and Trewyn [18]. The reaction mixture for tRNA-hypoxanthine ribosyltransferase contained 10 mM Tris-HCl (pH 7.4), 90 mM KCl, 3 mM $MgCl_2$, 0.3 mM 2-mercaptoethanol, 0.01 mM allopurinol, 1.0 A_{260} unit of *E. coli* tRNA, 1 μCi [G-^{3}H]hypoxanthine (3.6 Ci/mmol), and enzyme extract in a total volume of 0.6 ml. The reaction mixture was incubated at 37°C for 1 h and the reaction was terminated by the addition of ice-cold TCA (7.5%). The precipitate was collected onto microglass fiber filters for scintillation counting. A similar assay was carried out for tRNA-guanine ribosyltransferase activity in which [G-^{3}H]hypoxanthine was replaced with [8-^{3}H]guanine (6.0 Ci/mmol).

2.6. *Analysis of polysomes by sucrose density centrifugation*

The postmitochondrial supernatants (100–200 μl) obtained from polysome preparation were layered on 10–40% linear sucrose density gradients prepared in a buffer that contained 25 mM Tris (pH 7.6), 100 mM KCl and 2 mM magnesium acetate. The gradients were centrifuged at 100,000 × *g* (Beckman L8-70M) with a Beckman SW 55 rotor at 4°C. The gradients were analysed from top to bottom using a UV detector at 254 nm (LKB 2238 Unicord SII) and a chart recorder (LKB).

3. RESULTS AND DISCUSSION

It is well established that both DMSO and hypoxanthine, individually, are capable of inducing HL-60 cell differentiation into mature functional cells [19]. However, in this study DMSO together with a suboptimal concentration of hypoxanthine were evaluated for their effects on the growth and translational activity of HL-60 cells. Fig. 1 illustrates the growth pattern of HL-60 cells in the presence of 210 mM DMSO, 1 mM hypoxanthine and a combination of the two inducers. It is evident that cell growth is inhibited in the presence of the inducers (40 and 18% of control for DMSO and hypoxanthine by day 4, respectively), with a more pronounced inhibition (52% of control by day 4) occurring with the combined treatment. Interestingly, the inhibition of growth was not largely due to the cytotoxicity of the agents as the viability of the cells (unpublished results) under all conditions remained above 96% by day 3, however in the case of DMSO plus hypoxanthine viability declined to about 86% by day 4. It should be noted that the growth patterns obtained with both agents were similar to those reported by Trewyn et al. [17]. They also found that the enhanced inhibition of

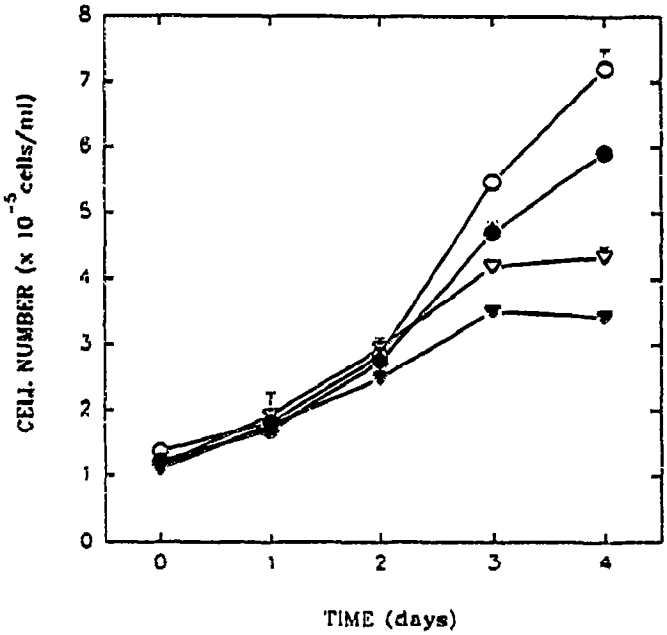

Fig. 1. The growth of HL-60 cells treated with DMSO and/or hypoxanthine. Cells were cultured for a period of up to 4 days in RPMI-1640 plus 10% FBS alone (○), or plus 1 mM hypoxanthine (●), or plus 210 mM DMSO (▽), or plus 210 mM DMSO and 1 mM hypoxanthine (▼). Results represent an average of 3 experiments.

Table I

Leucine incorporation of HL-60 cells in the presence of hypoxanthine and/or DMSO

Time (min)	Conditions			
	Control	Hpx*	DMSO	DMSO + Hpx
	[^{3}H]Leucine incorporation (cpm/10^5 cells)			
0	47 ± 6	47 ± 6	47 ± 6	47 ± 7
30	23,769 ± 276	20,541 ± 171	20,745 ± 1,635	17,951 ± 514
60	32,118 ± 1,317	30,077 ± 826	29,811 ± 1,199	25,859 ± 329
90	45,096 ± 1,201	41,117 ± 442	34,372 ± 46	35,491 ± 329
120	39,910 ± 740	41,186 ± 996	41,731 ± 993	39,893 ± 340

Cells were incubated in Hank's medium plus the different inducers (concentrations as in Fig. 1), and protein synthesis measured as in section 2. Results are means of 3 separate experiments. *Hpx, hypoxanthine.

growth seen in the presence of DMSO plus hypoxanthine was associated with potentiated induction of differentiation of HL-60 cells. Recently, a number of studies have focussed on the ability of different inducers to promote differentiation synergistically [20,21].

The process of growth and differentiation of cells is normally accompanied by changes in the rates of macromolecular synthesis. We studied the effects of short-term treatment of HL-60 cells with agents inducing differentiation on protein synthesis (Table I). With incubation periods of up to 120 min, protein synthesis increased in a linear fashion. However, more important was that under all conditions used there was no significant difference in the rates of protein synthesis. In experiments carried out (unpublished results) where the cells were incubated in either Hank's plus 10% FBS or RPMI-1640 or RPMI-1640 plus 10% FBS similar results were obtained, except that the counts were lower because RPMI-1640 and FBS contained appreciable amounts of leucine. These results are in contrast to the findings of Trewyn et al. [17] who found that simultaneous treatment of cells with DMSO and hypoxanthine increased protein synthesis by 100% (above control) after 60 min. The reason for this apparent discrepancy is not clear. One possibility is that, despite similar growth patterns in the two HL-60 sublines, there might be subtle intrinsic differences in their biochemical repertoire.

In order to confirm the above observations we determined polysome distribution by sucrose density gradient under the same conditions (Fig. 2). The polysome profiles were all similar, that is they were characterized by a large proportion of monosomes to polysomes. Morgan et al. [22] demonstrated the dependency of the rate of protein synthesis on the levels of amino acids in perfused hearts, whereas van Venrooij et al. [23], in Ehrlich ascites tumor cells, found that amino acid dep-

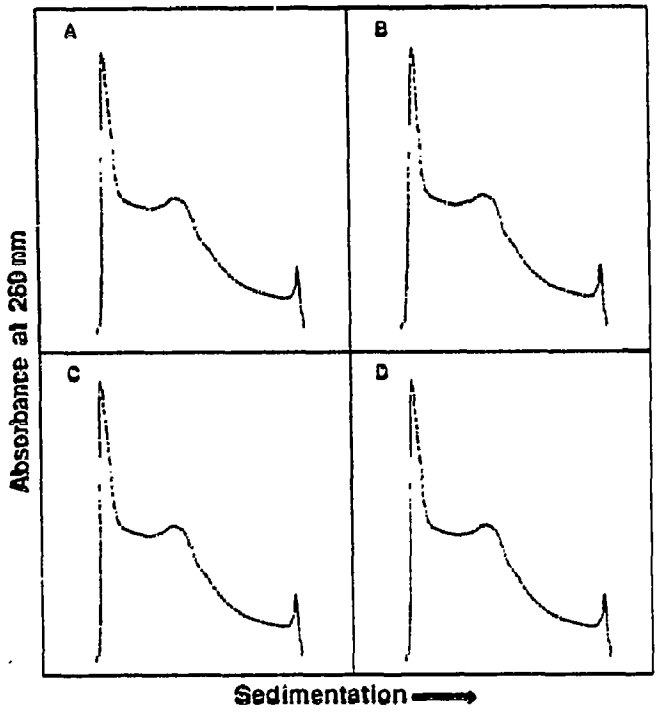

Fig. 2. The effects of DMSO and/or hypoxanthine on the distribution of polysomes. The cells were incubated for 1 h in Hank's solution only (a), or plus 1 mM hypoxanthine (b), or plus 210 mM DMSO (c), or plus 210 mM DMSO and 1 mM hypoxanthine (d). The preparation of polysomes and their analysis was carried out as described in section 2.

Table II

Enzyme activities in HL-60 cells treated with DMSO and/or hypoxanthine

Conditions	Enzyme activity (cpm/10^6 cells/hour)	
	THRT**	TGRT***
Control	47 ± 7	129 ± 23
Hpx*	42 ± 8	125 ± 13
DMSO	46 ± 9	128 ± 37
DMSO + Hpx	38 ± 9	122 ± 5

Cells were incubated in Hank's medium plus the different inducers (concentrations as in Fig. 1), and the enzyme activities determined as in section 2. Results represent a mean of 3 separate experiments. *Hpx, hypoxanthine; **THRT, tRNA-hypoxanthine ribosyltransferase; ***TGRT, tRNA-guanine ribosyltransferase.

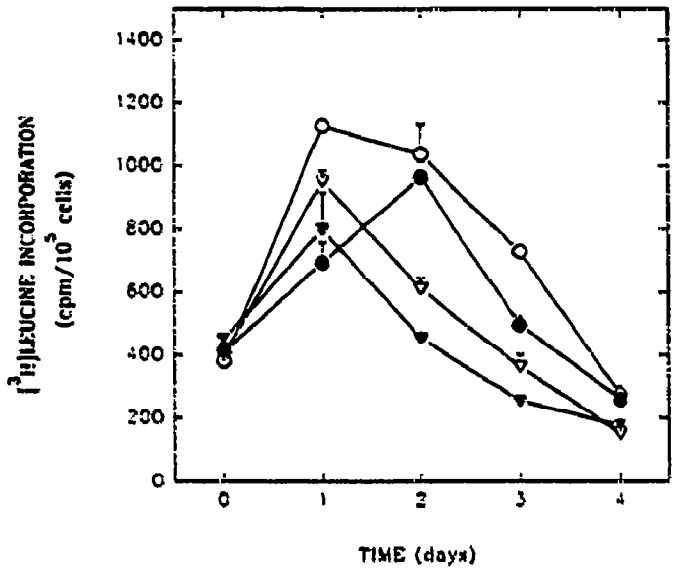

Fig. 3. Protein synthesis in HL-60 cells treated with hypoxanthine and/or DMSO. Conditions were the same as in Fig. 1.

rivation resulted in an immediate decrease in protein synthesis. The latter effect was accompanied by a marked disaggregation of polysomes. It is therefore not surprising that the polysomes obtained are disaggregated since the cells were incubated in Hank's medium, which contains only glucose besides basal salts.

Trewyn et al. [17] postulated that the observed induction of protein synthesis following charging cells with DMSO and hypoxanthine was a result of insertion of inosine, derived from hypoxanthine, into the anticodon of tRNA catalyzed by the enzyme tRNA-hypoxanthine ribosyltransferase. In accordance with the wobble hypothesis such an insertion would seemingly increase the anticodon reading potential and consequently accelerate peptide chain elongation. This led us to measure the activities of both tRNA-hypoxanthine and tRNA-guanine ribosyltransferases in cells exposed to DMSO and hypoxanthine (Table II). In general the activity of tRNA-hypoxanthine ribosyltransferase was almost undetectable (±40 cpm/10^6 cells/h), whereas that of tRNA-guanine ribosyltransferase was also relatively low. Of significance was the fact that there were no differences in activities of the enzymes in the presence of the different agents. The occurrence of low levels of these enzymes might explain failure to demonstrate enhanced protein synthesis in this study. Furthermore, this may be one of the biochemical features that is different in the HL-60 sublines used in the two studies. One cannot exclude the other possibility that the low levels of the enzymes might indicate that they are not critical to the process of protein synthesis. In a recent study Kretz et al. [24] found that both thioguanine and queuine participated in the modification of tRNA anticodon, and queuine also reversed differentiation induced by thioguanine. Of particular interest was that in our system upon incubating cells in either Hank's or RPMI-1640 plus FBS (the latter contains queuine) there was still no increase in the capacity of the cells to synthesize protein (results not given). This observation seems to underscore the need for evaluating the precise role of hypomodification and/or modification of tRNA on protein synthesis.

Since short-term treatment of cells with DMSO and hypoxanthine did not stimulate protein synthesis, we therefore monitored protein synthesis in cells cultured in RPMI-1640 plus 10% FBS over a period of 4 days (Fig. 3). In all cases protein synthesis increased to a peak between day 1 and 2 and thereafter declined. Consistent with growth patterns substantially high rates of protein synthesis were observed in controls and cells treated with 1 mM hypoxanthine whilst that for DMSO and DMSO plus hypoxanthine were relatively low throughout The latter observation suggests that even the long-term exposure of cells to DMSO and hypoxanthine does not appear to support the notion that modification of tRNA is important in protein synthesis.

In conclusion, we have shown that short-term treatment of HL-60 cells with DMSO and hypoxanthine does not enhance protein synthesis in HL-60 cells. Whilst changes in protein synthesis occurred during long-term treatment, these also do not lend support to the view that modification of tRNA regulates protein synthesis.

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