CONTROL OF GLOBIN SYNTHESIS DURING DMSO-INDUCED DIFFERENTIATION OF MOUSE ERYTHROLEUKEMIC CELLS IN CULTURE

by

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A small fraction of Friend or Stansly Virus (SFFV) transformed erythroleukemic cells of spleen, liver or peripheral blood, if maintained under tissue culture conditions (1–5), continuously differentiate into erythroblasts. Addition of aprotonic solvents, such as DMSO (2) or dimethoxyethane (Ostertag et al. unpublished results), to these cells in culture, stimulates their differentiation along the erythrocytic line much further. We have shown that under favorable conditions the cells are able to synthesize up to 50 % hemoglobin. Usually a 20–30 % globin synthesis is found. Adult α and β globin chains are synthesized (4, 6).

Induced hemoglobin synthesis is a good marker to study the processes involved in cellular differentiation. The differentiation, as evidenced by a 40–100 fold increase in globin synthesis, can be due to a) increased transcription of the mRNA for proteins characteristic for the differentiated state of the cell; b) processes occurring after transcription and leading to translatable globin mRNA (transport and processing of globin mRNA); c) increased translation.

From our experiments published elsewhere (6) it seemed likely that there is an increase in available globin mRNA during induction as evidenced by the appearance of at least two new RNA species in the 8–9 and 11–13S regions. This conclusion was confirmed by other authors using the reverse transcription product of mouse

reticulocyte globin mRNA as a complement for titrating globin mRNA in other strains of mouse erythroleukemic cells (7, 8). However, a positive identification of translatable globin mRNA in these cells has been lacking.

I. Globin mRNA during stimulation with DMSO

In all experiments we used the erythroleukemic cell clone FSD1/F4 since it has very little or no detectable spontaneous globin synthesis in the unstimulated state. In the stimulated state we usually obtain 15–25 % globin synthesis after 5 days of exposure to DMSO (6).

Cells were grown as described (Fig. 1) and 15 min. before harvesting the cells, cycloheximide was added to prevent run-off of monosomes (9). The cells were collected and exposed to 0.5 % NP40. The NP40 cytoplasmic suspension was layered on 12–32 % sucrose gradients. The polysomes were separated from the monosomes and the post-ribosomal supernatant (Fig. 1). The indicated fractions were treated with EDTA, SDS at a high pH and low cation concentration (6, 10) and extracted with a mixture of phenol, cresol, chloroform, isoamyl alcohol (6, 7, 10). The RNA of each fraction was separated on 8–50 % sucrose gradients. Fig. 2 shows the separation of the polysomal RNA of stimulated leukemic cells and Fig. 3 of the poly-

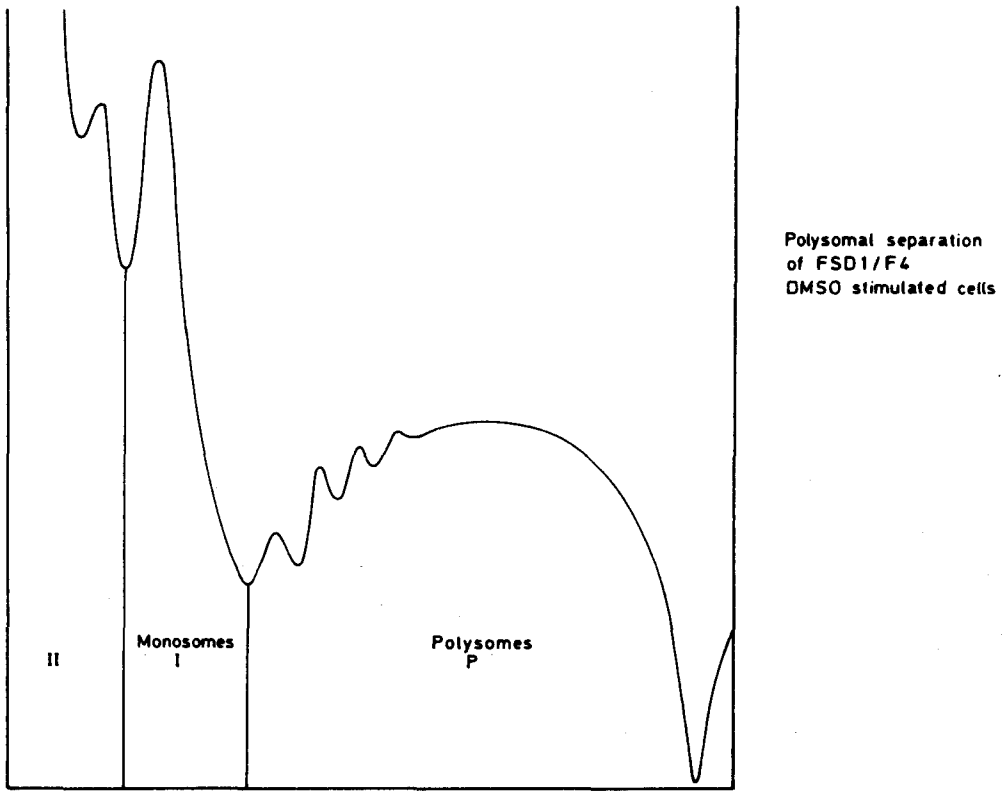

Fig. 1: Polysome profile of stimulated cells. Cells were grown in modified Eagle's medium (6). The stimulated cells were kept in 1.5 % DMSO containing medium for 5 days. Unstimulated and stimulated cells were then exposed to cycloheximide 100 µg/ml for 15 min. at 37 °C. The cytoplasmic NP40 soluble supernatant (6) was centrifuged in a linear 12–32 % sucrose gradient containing 0.05 M Tris-HCl, ph 7.4, 0.025 M KCl, 0.005 M $MgCl_2$ in a Beckman SW 27 rotor at 100,000 g for 2 hours. The fractions indicated as P, I and II were collected and the RNA extracted as in Fig. 2.

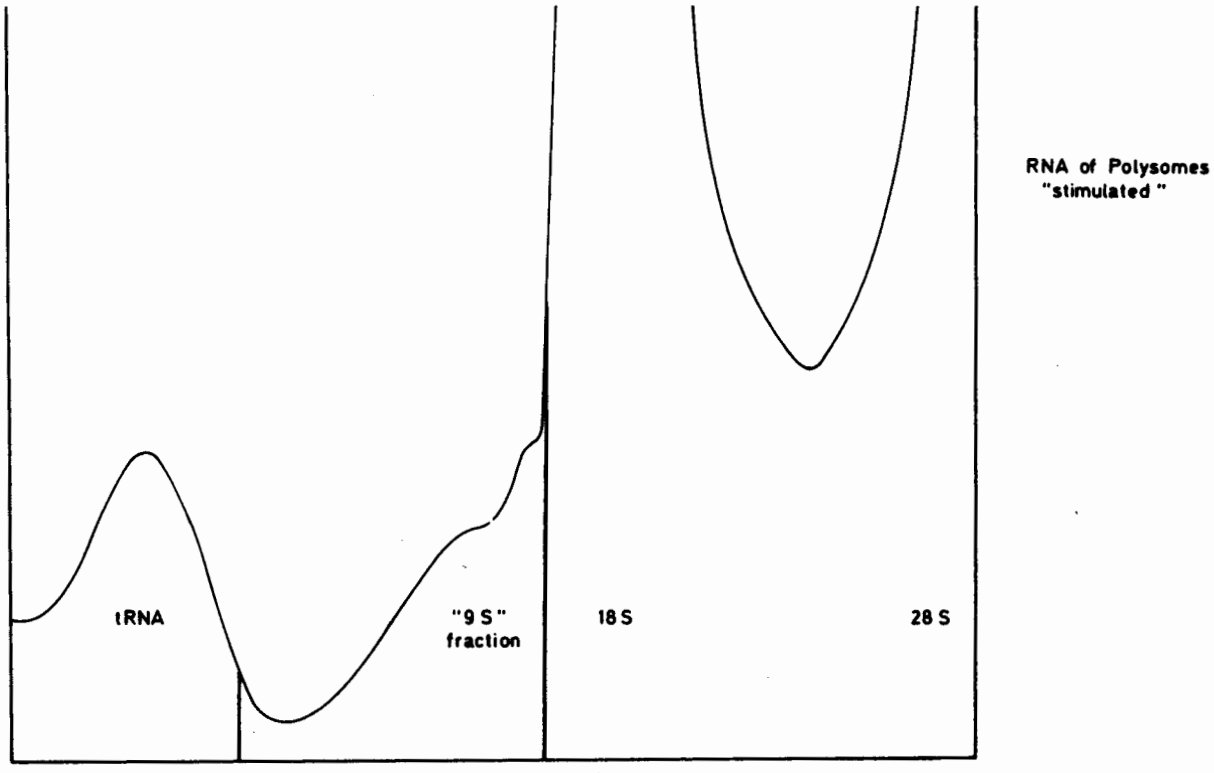

Fig. 2: Polysomal RNA (fractions P of Fig. 1) of stimulated cells separated on a sucrose gradient. The fractions as indicated in Fig. 1 were deproteinized (6, 7, 10). Amoniosalicylate was not used. RNA was applied to 8–50 % sucrose gradients in 0.01 M Tris-HCl, pH 7.4, 0.015 M KCl (SW40, 190,000 g, 16 hours, 4 °C). The 8–16S fraction was used for injection into Xenopus oocytes.

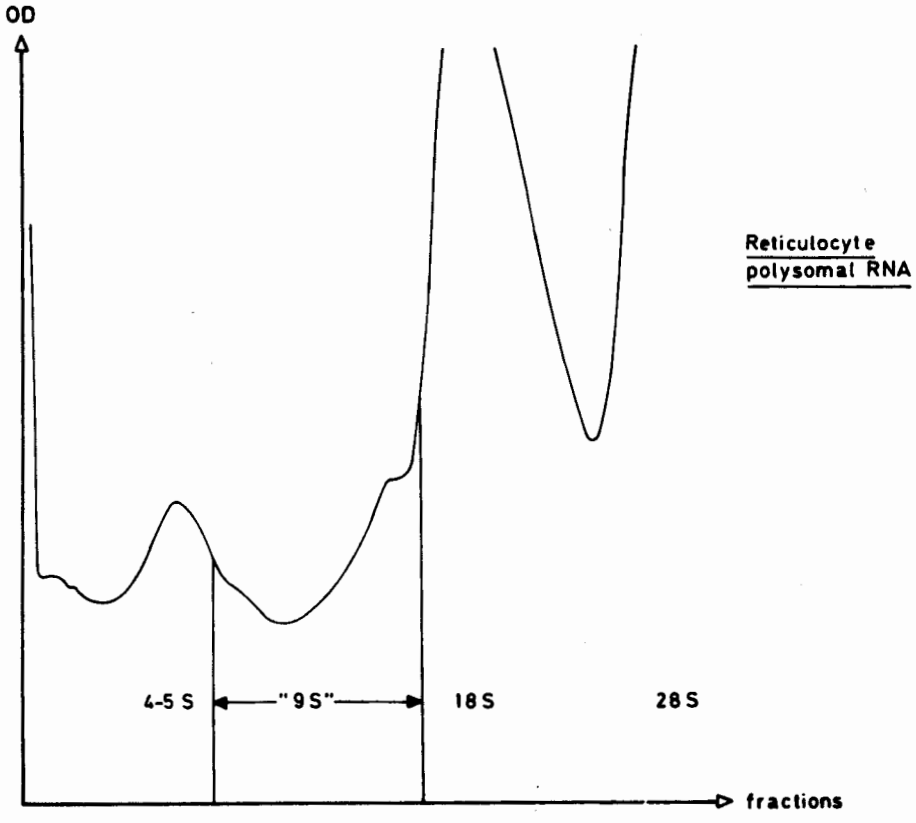

Fig. 3: Polysomal RNA of mouse reticulocytes separated on a sucrose gradient as described in

somal RNA of mouse reticulocytes as a control. It can be seen that the polysomal RNA extracted from reticulocytes, stimulated erythroleukemic cells and nonstimulated cells has a similar distribution profile in the sucrose gradient. However, much more material in the 12–16S region is present in the stimulated post-ribosomal fraction as compared to the same fraction extracted from nonstimulated cells (Fig.4, 5). The recovery of the RNA during the isolation procedure was monitored at each step to allow an estimation of globin mRNA activity per total RNA of an average leukemic cell.

The RNA indicated as „9S" RNA was collected and injected into frog oocytes (10–13). The proteins synthesized by the Xenopus oocytes were applied to carboxymethyl cellulose columns (6) using as carrier proteins either adult mouse globin or alternatively ^{14}C labelled β and α chains. The latter were isolated from DMSO stimulated erythroleukemic cells. Table 1 shows that most of the globin mRNA activity in stimulated cells is in the polysomal fraction (70–80 %), whereas the other two fractions contain much less globin mRNA activity. In all corresponding fractions of the unstimulated cells we found virtually no globin mRNA activity.

Fig. 6 shows the relative increase of synthesis of globin chains in the oocytes after injection of equivalent amounts of polysomal RNA of stimulated and unstimulated cells. The increase in translatable globin mRNA activity confirms our previous conclusions that DMSO induces an increase in translatable globin mRNA in step with the increase in globin synthesis. This increase does not start before the

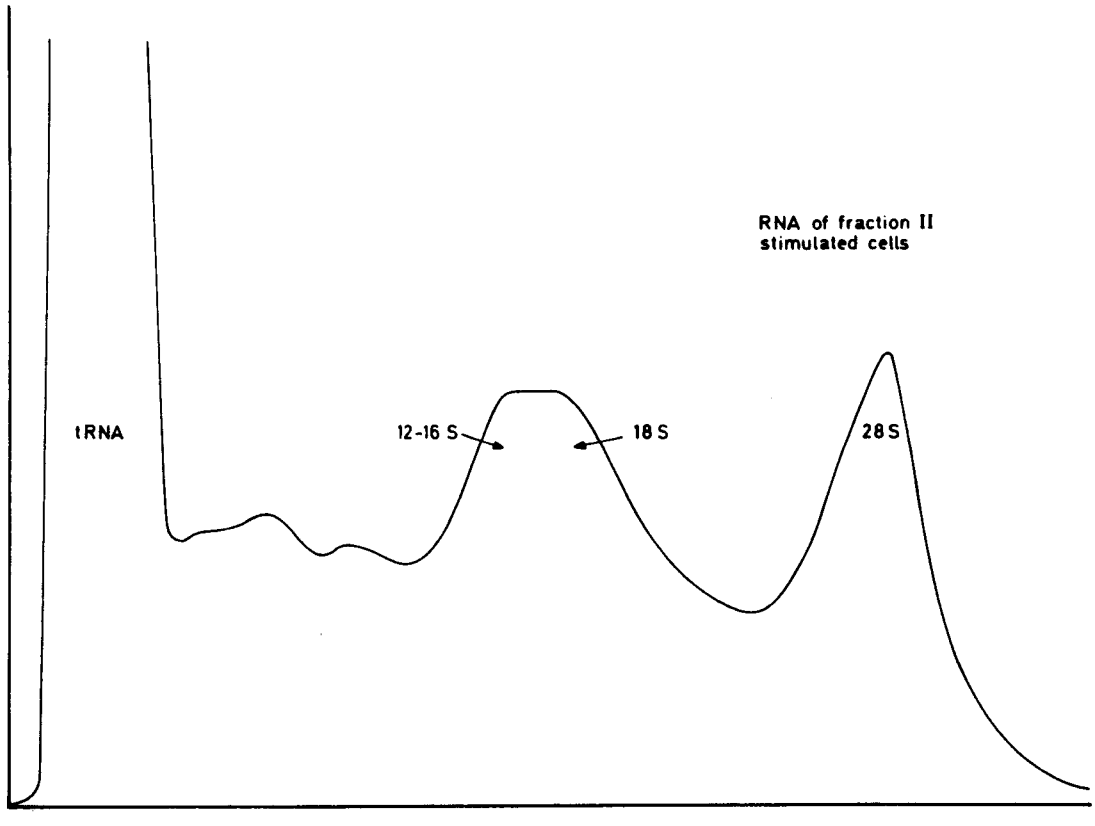

Fig. 4: Stimulated cells. RNA of fraction II of Fig. 1 (post-ribosomal fraction) separated on a sucrose gradient as described in Fig. 2. The fraction in the 8–16S region was again pooled and injected into oocytes.

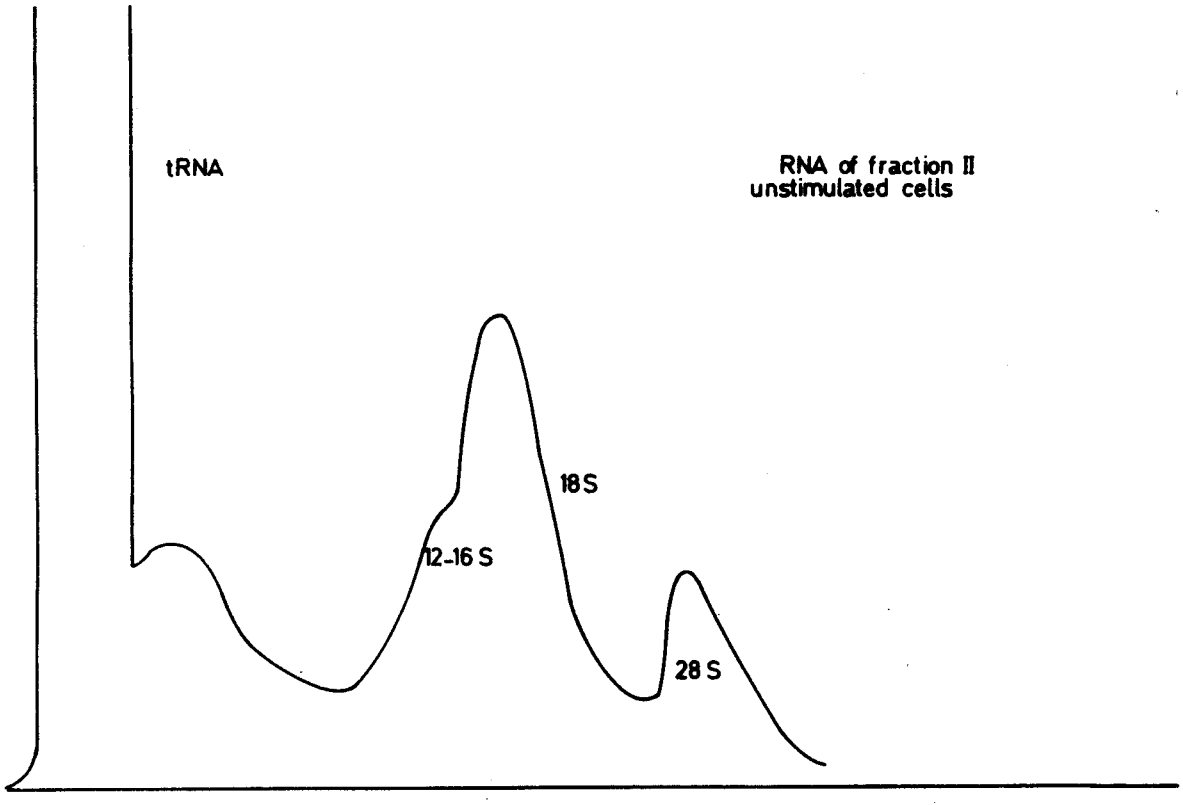

Fig. 5: Unstimulated cells. RNA of fraction II (post-ribosomal fraction) separated on sucrose gradients as in Fig. 2.

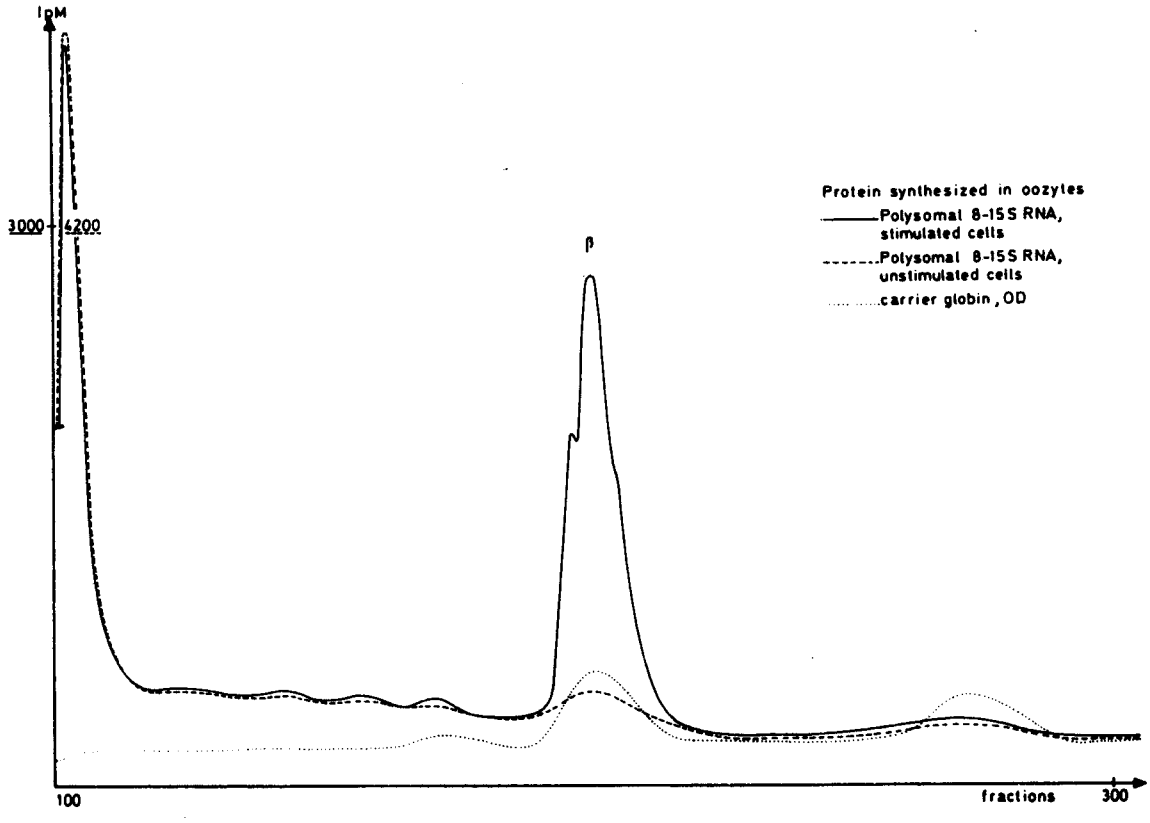

Fig. 6: Relative amounts of globin synthesized in frog oocytes in response to 8—16S RNA extracted from stimulated and unstimulated cells. The experimental details were described previously (10).

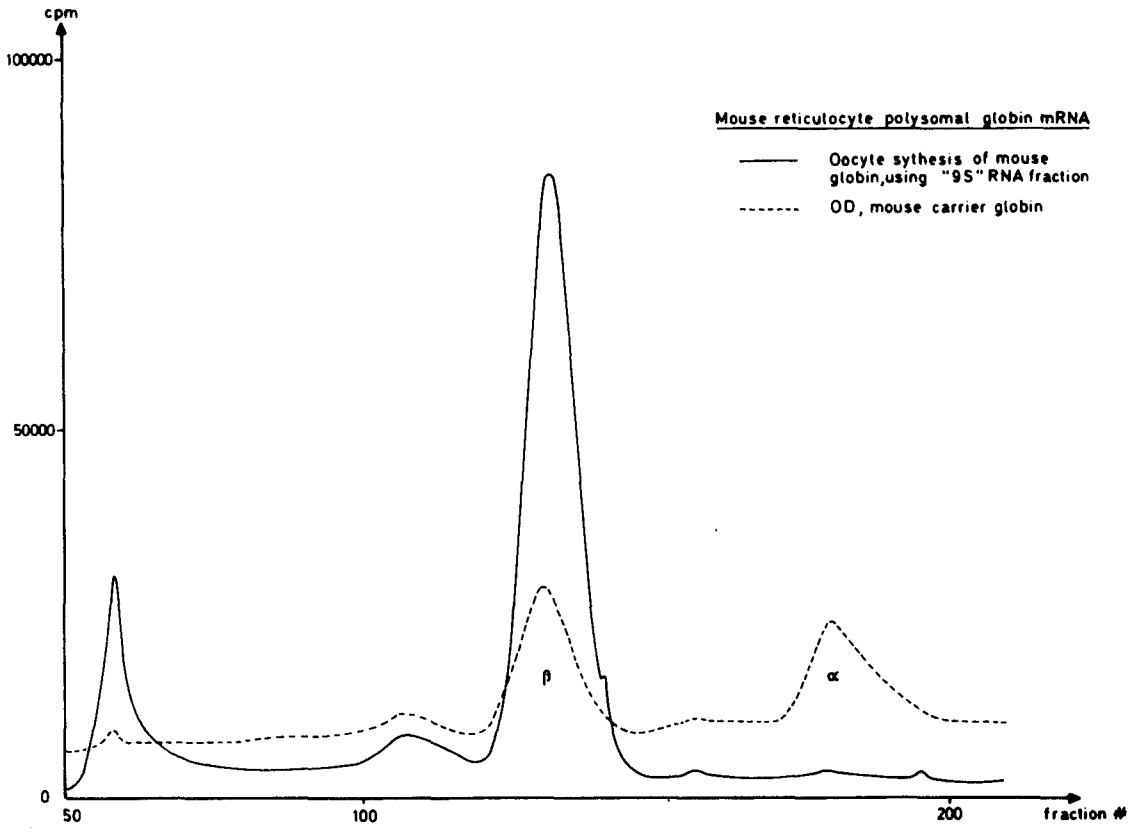

Fig. 7: Globin synthesized in frog oocytes in response to "9S" RNA from mouse reticulocytes.

second day of stimulation with DMSO. It is tempting to conclude that DMSO induces a change in the transcriptional pattern of the cell, although on the base of our evidence, post-transcriptional control mechanisms cannot be ruled out.

Stimulated erythroleukemic cells synthesizing 15–20 % globin contain about 80 % of the amount of globin messenger found in mouse reticulocytes. Leder et al. (14) indicate that only about 1/3 as much globin mRNA can be found in a similar stimulated erythroleukemic cell line. If mouse globin mRNA of reticulocytes and erythroleukemic cells is injected into oocytes we recover very little α globin chains (Fig. 6, 7). The specific messenger activity per RNA fraction per oocyte of mouse 8–12S RNA fractions in our experiments is higher than that of the rabbit reticulocyte RNA described by MOAR et al. (12). It is therefore probable that very few α chains are synthesized, and not that the chains are synthesized, degraded and lost. Another possibility is that the α chain messenger is larger than the messenger for β chain synthesis and lost during isolation on sucrose gradients. However, if we analyze the different size classes of mRNA separated on sucrose gradients or acrylamide gels, we find the highest enrichment of α globin mRNA in the lowest molecular weight region (10). This result argues against the interpretation of a loss of large size mRNA.

A much larger amount of 8–16S material is present in the postribosomal fraction of stimulated erythroleukemic cells as compared to the same fraction of unstimulated cells (Fig. 4, 5). This RNA has very little globin mRNA activity as compared to the polysomal RNA of the 8–16S region. Probably during DMSO stimulation some

ribosomal RNA is degraded, which appears in the post-ribosomal fraction, mainly as 12–16S material. This agrees with observations of ribosomal RNA breakdown during normal reticulocyte maturation (15).

II. Induction of differentiation

In previous experiments we have shown (Kluge et al. unpublished data) that DMSO induces differentiation in erythroleukemic cells grown in serum-free medium. It therefore seems likely that DMSO acts directly on the target cell.

The intention of the experiments which are described below was to show that DMSO induces cellular changes, especially of the transport of small molecules into the erythroleukemic cell. This occurs before the increase of globin mRNA is observed. Table 2 shows that the cell number increases 6.4 fold after stimulation. The unstimulated control cells increase 25 fold. We compared that to the total amount of cytoplasmic RNA recovered in the stimulated cells. There is only a 30 % increase

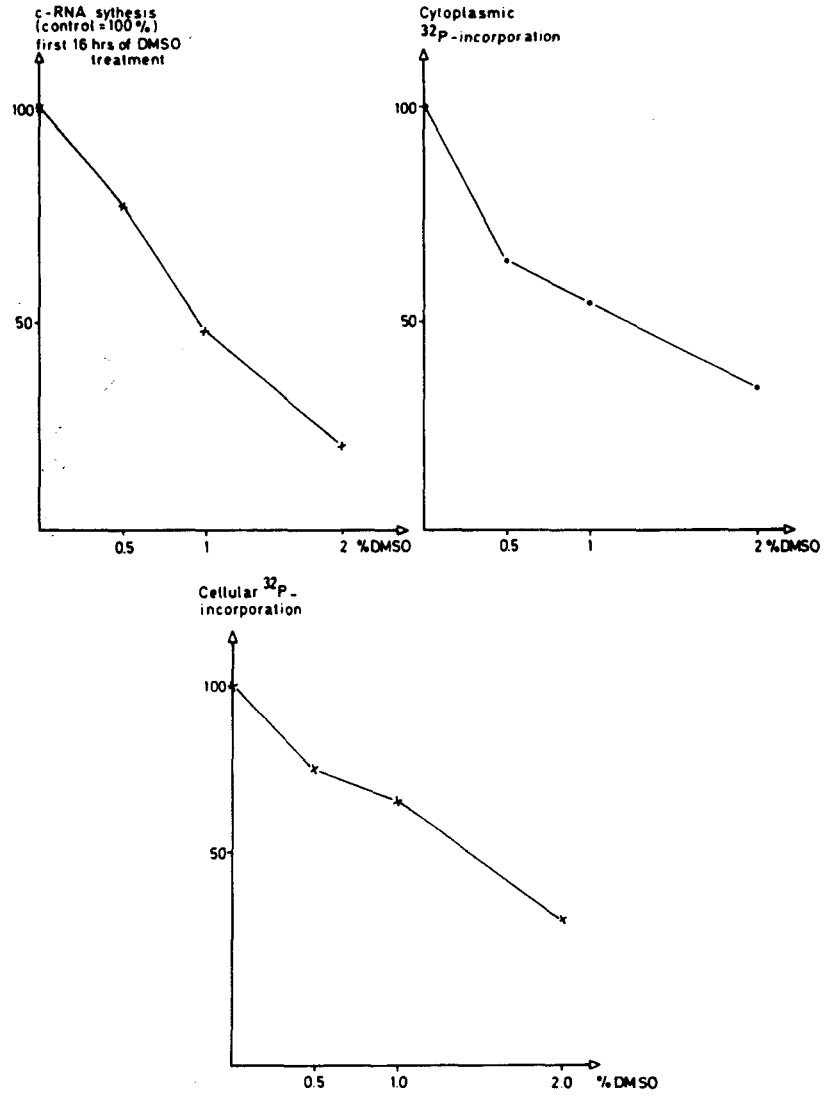

Fig. 8: Decrease of synthesis of cytoplasmic RNA (cRNA), decreased incorporation of $^{32}PO_4$ in cytoplsm and cells the first 16 hours of treatment with DMSO as indicated.

of total RNA, although cells do multiply by a factor of 6.4 (Table 2). This results in an average decrease of RNA content/cell in the stimulated state (Table 2), although globin mRNA activity increases at least 25 fold uring the same time.

The first observed change in cells after adding DMSO before onset of globin synthesis is, however, an immediate decrease of synthesis of cytoplasmic RNA (Fig. 8). Even more interesting, a decrease of ^{32}P incorporation into the cell is found. By adding cAMP to 3T3 cells KRAM et al. (16) observed a similar effect for leucine, deoxyglucose and uridine transport. The similarity of the action of DMSO on inducing differentiation to cAMP found in other systems is further emphasized by the effect of DMSO on decreasing the cell division rate. We have also checked cAMP on the induction of differentiation in erythroleukemic cells. cAMP induces differentiation, however, to a much lower degree as DMSO. A similar degree of stimulation is obtained with the steroid hormone etiocholanolone (Fig. 9).

Summary DMSO induces erythroid differentiation in Friend virus (SFFV) transformed spleen cells in culture. This differentiation results in the appearance of globin synthesis (20 % of the total protein synthesis) and in a correlated increase of globin mRNA. Two days after adding DMSO the first increase of globin mRNA and globin synthesis is observed. In the first 16 hours after addition of DMSO the transport of small molecules (phosphate) into the cells is reduced. Synthesis of cytoplasmic RNA is decreased. Cell division rate is much less reduced after stimulation. The amount of RNA per cell is decreased to 20 % of that of the nonstimulated cells. The effect of DMSO on differentiation shows similarities to hormone and cAMP action.

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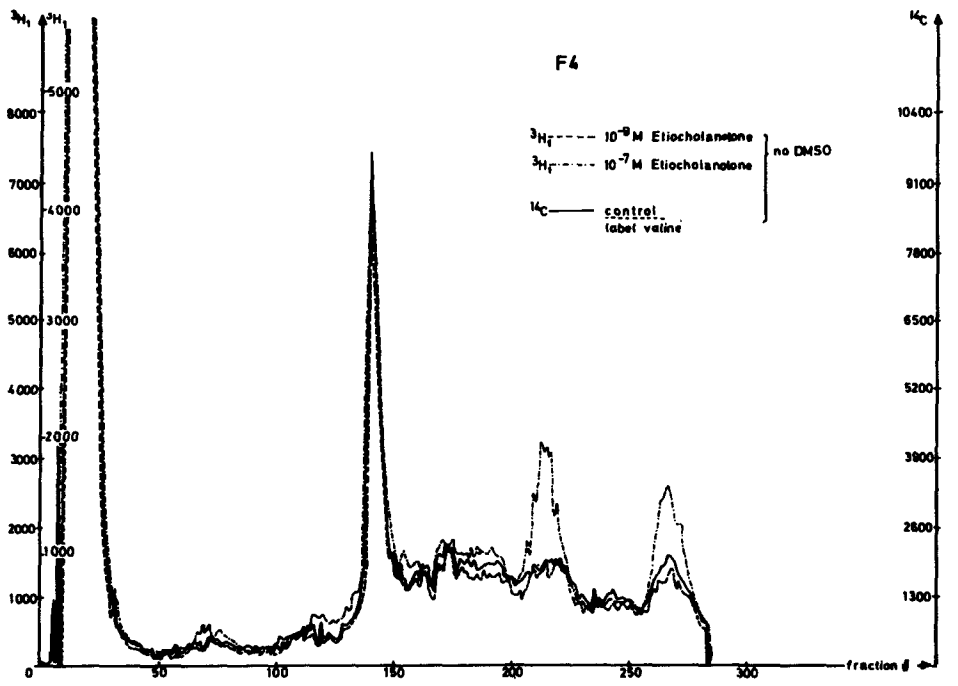

Fig. 9: Stimulation of SFFV-transformed erythroleukemic cells by etiocholanolone. CMC-column chromatography (10).

		cellular globin synthesis in %	relative increase during stimulation	relative globin m-RNA activity corr. for OD	relative increase of globin m-RNA (oocyte test)
unstimulated	polysomal fraction	—	—	‹225	1
	monosomal fraction	—	—	‹1100	1
	postribosomal fraction	—	—	‹150	1
	total	‹0.5	1	‹1500	1
stimulated	polysomal fraction	—	—	25600 (74%)	›100
	monosomal fraction	—	—	5300 (16%)	›5
	postribosomal fraction	—	—	3800 (11%)	›25
	total	20%	›40	34700	› 23
	mouse reticulocyte polysomes	98%	—	29000	—

Table 1: Distribution of globin mRNA activity in different RNA fractions of stimulated, unstimulated cells and mouse reticulocytes.

	unstimulated cells	DMSO stimulated cells
increase in cell number (4 days)	25	6.4
cytoplasmic RNA content per cell in 10^{-11}g	2.0	0.4
total cytoplasmic RNA of all cells in OD units day 0	9	
total cytoplasmic RNA of all cells day 4	225	11.5

Table 2: Cell division and RNA content/cell during stimulation of erythroleukemic cells.

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