GLOBIN MESSENGER IN MOUSE LEUKEMIC CELLS: ACTIVITY ASSOCIATED WITH RNA SPECIES IN THE REGION OF 8 TO 16 S

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1. Introduction

We have previously shown a correspondence between haemoglobin production and the appearance of new species of "9" and "13 S" RNA's in Friend virus infected mouse leukemic spleen cells in tissue culture stimulated with dimethylsulfoxide [1].

We have now examined the various fractions of polysomal RNA from these cells for their ability to program globin synthesis in frog oocytes and here we show that the globin template activity is associated with RNA species of 3 different size classes ranging between 8 and 16 S.

2. Results

Fig. 1a shows the sucrose density gradient fractionation of polysomal RNA from leukemic cells FSD-1 [1]. Also plotted in the same figure are globin template activities of fractions across the gradient (fig. 1a, table 1). The template activity was assayed in frog oocytes [2] and the product fractionated on carboxymethylcellulose-urea columns [1, 3] using mouse adult globin as carrier. The data show a broad peak of activity in the region of 6 to 16 S and little activity on either side of this region. Although the specific activity is highest for fractions in the region of 8 to 12 S, the total globin template activity of these fractions is approximately equal to those in the region of 12 to 16 S implying a heavier contamination with ribosomal RNA in the latter part of the gradient.

While all the messenger active fractions from the gradient programmed the synthesis of both α- and β-globin chains (fig. 1b, table 1), the fraction in the region of 6 to 9 S showed an enrichment of template activity for α-chains. In general, the ratio of α- to β-chains was low reflecting the unbalanced globin synthesis in FSD-1 cells, approx. 75% β- and 25% α-chains [1]. To clearly establish the point that globin template activity resides in several discrete RNA species, the active fractions in fig. 1a were pooled and fractionated on polyacrylamide gels. ^{32}P-labelled polysomal RNA from the same cells was used as tracer to identify the positions of various bands. The RNA taken from these bands was used in the frog oocyte assay and the synthesis of globin chains was again monitored on CMC urea columns using mouse adult globin as carrier. The data are represented in fig. 2 and table 1.

Fig. 2 shows the autoradiograph of the gel, the diagrams of the autoradiograph and of stained gel, and a histogram of the template activity of each band. Table 1 describes how the template activity data were arrived at and also gives the relative amounts of α- and β-globin chains synthesized in response to the added RNA from each band.

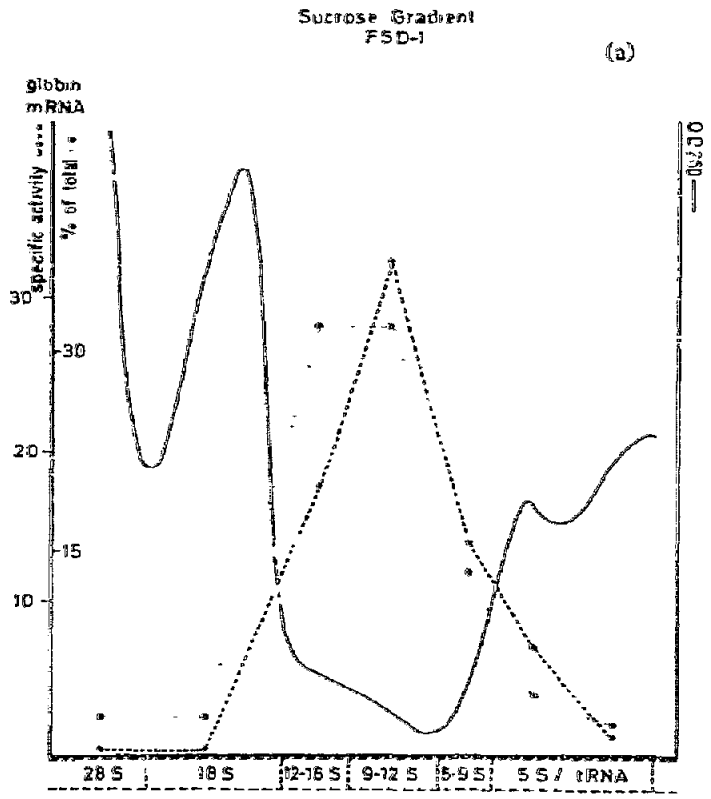
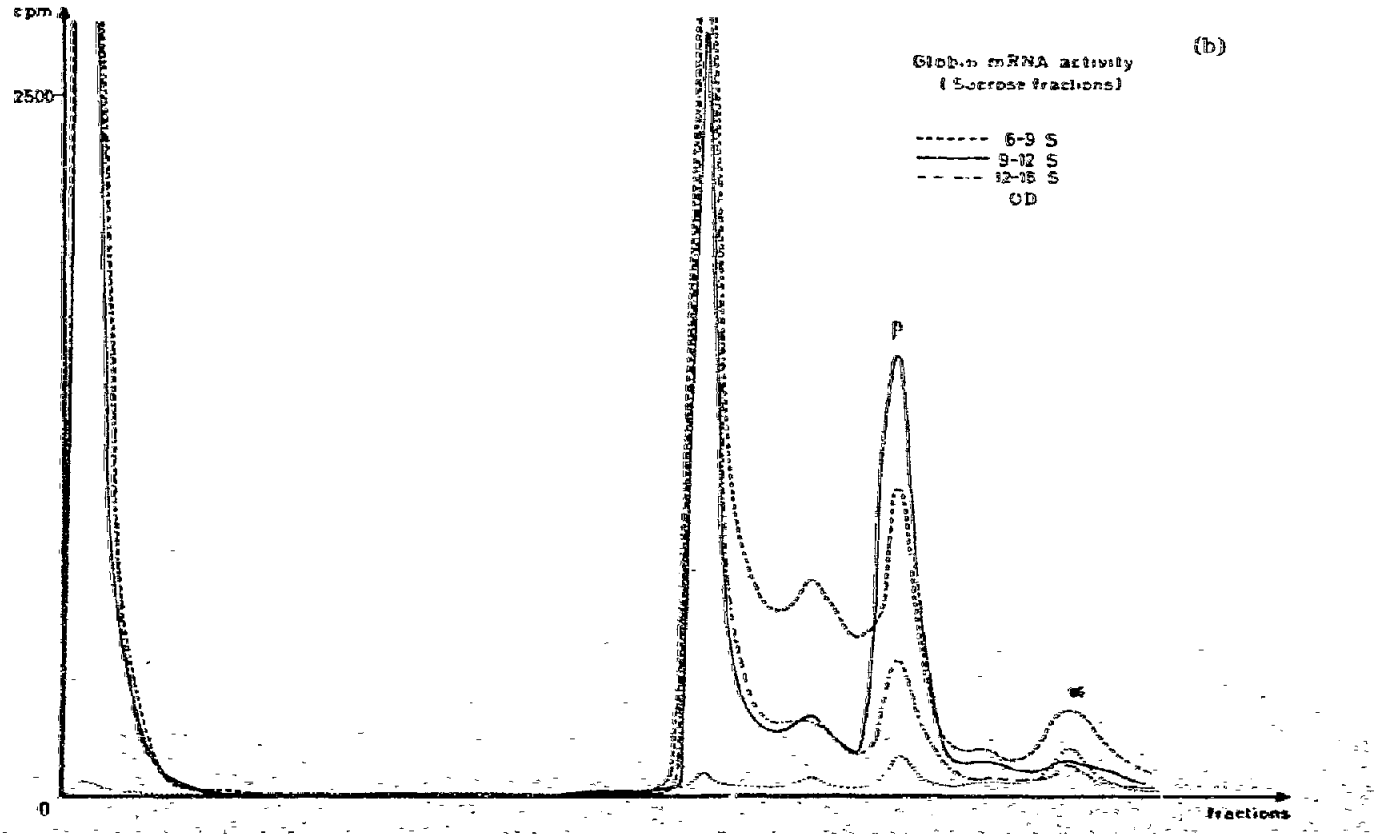


Fig. 1a. Sucrose gradient fractionation and globin template activity of polysomal RNA from erythroleukemic tissue cultures. 40 ℓ of FSD-1 cells were grown in Roller type flasks and Spinner bottles at a density of $1-2 \times 10^6$ cells/ml. They were fed every 24 hr for 5–6 days with medium containing 1.5% DMSO [1, 6]. For cells to be labelled with ^{32}P 0.75% DMSO was used for stimulation since higher concentrations were found to inhibit RNA synthesis. During this stimulation schedule the cells were diluted 1:1 at each feeding except on the last 2 days. Cells were lysed in 0.5% NP40 [1]. The nuclei and particulate cytoplasm were removed by centrifugation at 18,000 *g* for 10 min. The polysomes in the supernatant were pelleted 2 hr at 100 000 *g* in Spinco SW 27 rotor. The pellet was dissolved in 0.1 M Tris-HCl pH 9 made 1% with SDS. The extraction of the RNA was as described [1]. The labelled RNA was used without further fractionation (see fig. 2). The non-labelled RNA was further fractionated on 10–35% sucrose gradients (0.01 M Tris, 0.015 M KCl, pH 7.4). The RNA was recovered by precipitation, dissolved in the oocyte injection buffer and 50 nl of the solution containing 3–100 A_{260} units RNA/ml were injected into each of 15–20 *Xenopus laevis* oocytes. (The frogs were injected with 1000 units of human chorion gonadotropin 15–20 hr prior to the use of their oocytes [2]). Anaesthetization was performed by putting the frog into a solution containing Sandoz MS-222 (1 g/ℓ). The oocytes were incubated for 15 hr at 20° in 0.5 ml incubation mixture [2] with 50 or 500 μCi [^3H] leucine (38 Ci/mM). The oocytes were then washed and stored frozen at −30°. Usually 5 oocytes were homogenized at 4° with 2 ml of incubation buffer [2]. 10–20 mg of DBA-2 mouse haemoglobin was added and the globin precipitated at once in acid-acetone. The globin chains were separated on CMC urea columns [1, 3]. (——) OD pattern of polysomal RNA as separated by sucrose gradient centrifugation; (– – –) specific activity of the indicated RNA fractions in translating globin in frog oocytes (see also legend to table 1); (·····) globin mRNA template activity expressed in % of the total. (b) α- and β-globin mRNA template activities of RNA fractions separated on sucrose gradient of fig 1, table 1. The globin chains synthesized in oocytes were separated on CMC urea columns. The figure shows the relative proportions of α and β chain synthesis.



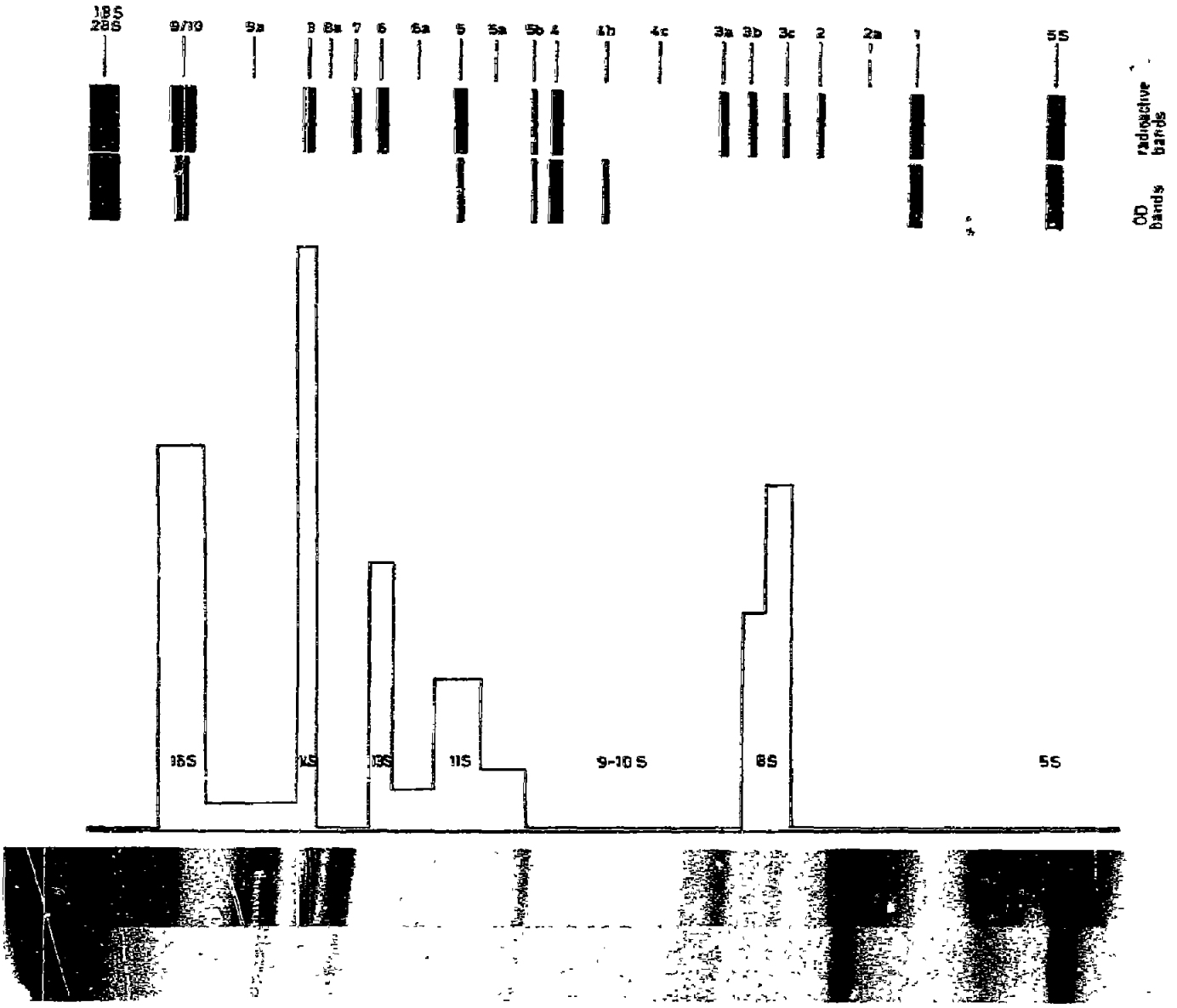


Fig. 2. Polyacrylamide gel fractionation and globin template activity of polysomal RNA. The RNA fractions of fig. 1a with sedimentation coefficient below 18 S were pooled, mixed with ^{32}P-labelled RNA and subjected to polyacrylamide gel electrophoresis on 4.25% slab gels [1]. The labelled RNA was used as a tracer to locate the positions of various bands by autoradiography [1]. The gel sections corresponding to the bands and interbands (regions between two bands) were cut out and the RNA eluted by repeated homogenization with tight fitting teflon homogenizers in 0.01 M Tris, 0.015 M KCl, pH 7.4 buffer. The gel was removed by centrifugation at 4° at 14 000 *g* for 60 min. Several re-extractions were done until at least 90% of the RNA as judged by Cerenkov counting of the ^{32}P was recovered. The supernatant was then spun at 100 000 *g* for 10–20 min, to remove some more finely suspended gel. The supernatant was precipitated in 70% ethanol, dissolved in Tris-KCl buffer and applied to 10–50% sucrose gradients at 200 000 *g* for 8 hr. The radioactive fractions were collected, precipitated in ethanol and dissolved in oocyte injection buffer as in fig. 1a. The A_{260} was determined. The samples were reprecipitated in ethanol and redissolved in 5–20 µl injection buffer. The repeated purification steps were necessary to obtain RNA fractions reasonably free of gel. The RNA obtained from each band and interband was checked for its template activity by injection into frog oocytes and fractionation of α- and β-globin chains on CMC urea columns as in fig. 1a. a) Diagram showing the absorbance pattern of RNA separated on acrylamide gel as determined by staining of one part of the gel with methylene blue [7] (upper row) and of band pattern of ^{32}P-labelled RNA as determined by autoradiography (lower row) as shown in fig. 2c. b) Histogram of globin mRNA activity of acrylamide fractions of fig. 2a. The data of the last column of table 1 were used to draw the histogram. c) Actual autoradiograph of ^{32}P-labelled RNA (shown diagrammatically in fig. 2a). d) Autoradiograph at shorter exposure time to show more clearly the bands 9/10.

Table 1
Globin template activity of RNA fractions from sucrose gradient and polyacrylamide gels.

RNA fraction from	Total protein synthesis by frog oocytes in 10^6 cpm	Globin synthesis					Globin template activity/ A units RNA*	% of total globin template activity**
		β-chain in 10^2 cpm	α-chain in 10^2 cpm	α-chain (%)	Total in 10^2 cpm	in % of total protein synthesis		
Sucrose gradient:								
28 S	4.2	2.3	–	–	2.3	0.5	0.5	3
18 S	3.2	1.4	–	–	1.4	0.4	0.5	3
12–16 S	14	62	10	14	72	5.3	17.5	33
9–12 S	15	230	17	7	250	17	32	34
6– 9 S	6.5	42	12	22	53	8	18	17
5 S, tRNA	7	21	–	–	21	2.9	4.1	8.5
tRNA	8.3	8	–	–	8	0.9	1.9	1.5
Polyacrylamide gel:								
9–11 (16–17 S)	31	160	17	10	180	5.8	73	28.4
9a	30	2.5	–	–	30	0.8	6.1	3.9
8	110	270	49	16	320	2.9	32	16.1
8a	160	–	–	–	–	–	–	–
7	150	–	–	–	–	–	–	–
6 (12 S)	100	280	30	10	310	3.2	52	9.8
6a	170	140	15	10	155	0.85	34	2.7
5	120	280	49	15	330	2.7	54	11.3
5a	62	74	16	17	90	1.5	48	4.6
5b + 4 (10 S)	110	16	–	–	16	0.1	0.5	0.2
4abc	50	–	–	–	–	–	–	–
3a	110	10	–	–	10	0.1	10	–
3b	95	150	89	38	240	2.5	50	8.1
3c	66	130	30	20	160	2.3	170	12.5
2a + 2 (7 S)	115	43	9	17	52	0.4	5	2.4
1 (5 S)	44	–	–	–	–	–	–	–

The details of the fractionation and template activity determination are given in figs. 1 and 2.

* These are relative values. They are based on the assumption that the different oocytes respond equally well to exogenous injected RNA and that the pool of available leucine is roughly the same in different oocytes.

** For the total globin template activity the amount of RNA recovered was multiplied with the specific activity (in the preceding column). All of the values thus obtained were summed up and the total template activity of each sample was determined as a fraction (%) of the total.

3. Discussion

Several points emerge from these data: i) the globin template activity is associated with RNA species which resolve into 5 discrete bands on polyacrylamide gels and which fall into 3 different size classes eg. 8–9 S, 11–13 S, 14–17 S; ii) the RNA from each of the five bands programs the synthesis of both α- and β-globin chains; iii) the RNA in the smallest size class eg. 8–9 S, is highly enriched for α-chain template activity; iv) the RNA in the 9–10 S region, which is seen as a pair of discrete bands both in the stained gel and in the autoradiograph, is essentially devoid of globin template activity. This RNA has also been found in polysomal

RNA preparations from several species of mammalian cells including mouse reticulocytes and the fingerprints of this RNA from various sources have been found to be remarkably similar, thus ruling it out to be globin mRNA [4].

Whether the RNA species in the various bands represent different maturation products obtained by cleavage from a single precursor RNA [5] remains to be seen. In any event these data not only draw attention to the considerable heterogeneity of globin mRNA but taken together with those presented in [4] also suggest that the various results obtained with globin mRNA might well be interpreted with caution.

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