COMPETITION BETWEEN α AND β GLOBIN MESSENGER RNA

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SUMMARY- Addition to an unfractionated reticulocyte lysate of either α or β globin mRNA or reticulocyte initiation factors does not alter the overall rate of globin synthesis. Addition of β mRNA results in enhanced synthesis of β product and decreased production of α; conversely, addition of α mRNA results in enhanced synthesis of α globin and decreased production of β. We conclude that the amount of any putative α mRNA or β mRNA-specific factor does not normally limit the rate of synthesis of α or β chains; rather, the two mRNAs compete for some non-specific rate-limiting component of chain initiation.

Rabbit reticulocytes are an ideal system for studying the control of protein synthesis at the translational level. They have no nuclei and make no RNA. Over 90% of the proteins synthesized are α and β globin (1). Cell-free extracts of these cells synthesize globin for about one hour at rates comparable to that of the intact cell (2). Further studies showed that there is 1.7 times as much α mRNA as β mRNA. Each α mRNA initiates protein synthesis only 60% as frequently as does each β mRNA (3,4), resulting in synthesis of equal amounts of the two chains (5). The rates of elongation and termination of α and β globin chains are the same (4,6). Consistent with a difference at the level of chain initiation, each α globin mRNA contains, on the average, three ribosomes whilst each β mRNA contains an average of five (3,7,8).

Several groups have reported protein or RNA factors which enhance the translation of certain eukaryotic mRNAs in some cell-free systems. In particular, Nudel, et al., isolated a protein from reticulocyte ribosomes which will preferentially stimulate translation of α mRNA in an extract of ascites cells (9); and Wigle and Smith (10) purified a protein from the supernatant of ascites cells which stimulated translation of β globin mRNA. It is not

yet clear whether these and other message-specific factors (11,12,13) are real entities, artifacts of cell-free systems, or, as we have postulated previously (14), non-specific components which are required for translation of all messenger RNAs. In this paper we shall consider the following question: Do the amounts of any putative α mRNA- or β mRNA-specific factors limit the rate of α or β globin synthesis by reticulocytes? In particular, about 10-20% of the α mRNA (but not β mRNA) in reticulocytes is unattached to polysomes (15,16), and one could imagine that the amount of an α mRNA-specific factor limits the translation of α mRNA. We show here, however, that addition of purified α or β globin mRNA to a reticulocyte extract enhances considerably the synthesis of α or β globin, respectively, and inhibits the synthesis of the opposite chain. We conclude that putative mRNA-specific factors do not limit the rate of α or β globin synthesis.

MATERIALS AND METHODS

Cell-free protein synthesis. Reticulocyte cell-free protein synthesis reactions (0.03 ml) have been described in detail (4,17). They contained 150 μCi/ml [^{35}S]-methionine (100 Ci/m mole; New England Nuclear Corp.). Incubation was at 25°C for 30 min. unless otherwise indicated. Aliquots of 3 μl were taken to measure total incorporation of radioactive methionine into protein. After incubation, about 400,000 cpm [^{3}H]-methionine-labeled rabbit hemoglobin was added. Globin was isolated and digested with trypsin; the methionine-containing peptides were resolved by paper electrophoresis and detected by radioautography (3,4). The measure of α chain synthesis was the ratio [^{35}S] : [^{3}H] in peptide αT5; β synthesis was the ratio [^{35}S] : [^{3}H] in βT5. These peptides are near to the N-terminus, so that synthesis represents predominantly (over 98% in a 30 min. reaction) polypeptide chains initiated during the course of cell-free synthesis.

Polysomes were resolved by centrifugation through 15-30% (w/v) sucrose gradients as detailed previously (3,4).

Messenger RNAs and initiation factors. α globin mRNA was prepared as in reference 18. By their assay, over 90% of the cell-free product produced was α globin. Unfractionated globin mRNA and β mRNA were prepared as in (19); in the Krebs ascites cell-free system (20), over 90% of the protein whose synthesis was directed by the β mRNA was β globin. Reticulocyte salt wash factors free of RNA (initiation factors) were prepared following the procedure in ref. 21; these stimulated the translation of globin mRNA in the ascites cell-free system ten-fold, precisely as was reported in ref. 22.

RESULTS

Addition to a crude cell-free extract from rabbit reticulocytes of unfractionated rabbit globin mRNA, or purified α or purified β globin mRNA, or a crude preparation of reticulocyte initiation factors, has no effect on the rate of total globin synthesis (Fig. 1, Tables 1 and 2). Tables 1 and 2 show, however, that addition of β mRNA results in an inhibition of α globin synthe-

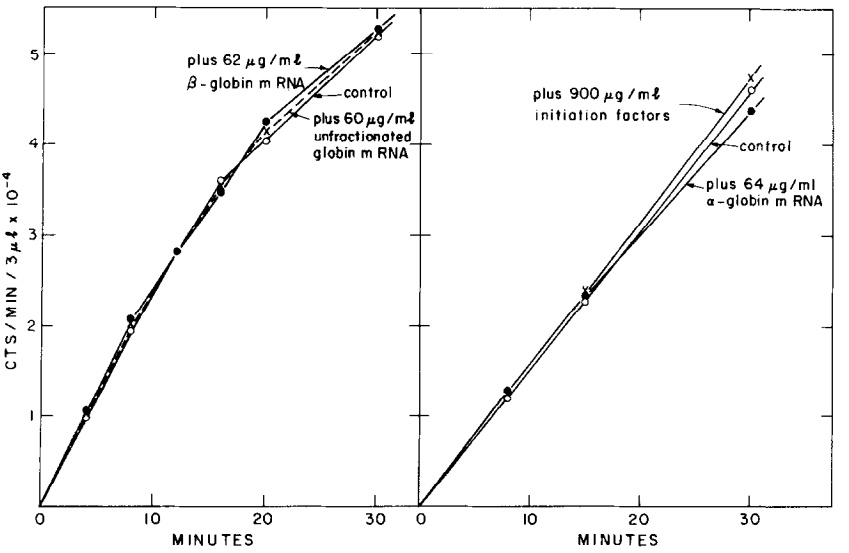

Fig. 1 Effect of mRNA and initiation factors on protein synthesis by cell-free extracts of rabbit reticulocytes. Reactions (30 μl) containing [^{35}S]-methionine were incubated at 25°C; aliquots of 3 μl were taken as noted. Left panel:— O — control reaction;— ● —plus 62 μg/ml β globin mRNA;-- X --plus 60 μg/ml unfractionated globin mRNA. Right panel:— O — control reaction;— X —plus 900 μg/ml reticulocyte initiation factors;— ● —plus 64 μg/ml α globin mRNA.

Table 1

Effect of globin mRNA on reticulocyte cell-free synthesis of α and β globin.

Additions		Total Synthesis Relative to Control Reaction	% α	% β
None [control]		1.00	0.50	0.50
Unfractionated globin mRNA	[17 μg/ml]	1.04	0.48	0.52
	[33 μg/ml]	0.99	0.46	0.54
	[83 μg/ml]	0.99	0.42	0.58
β globin mRNA	[17 μg/ml]	1.03	0.39	0.61
	[33 μg/ml]	1.03	0.33	0.67
	[83 μg/ml]	1.04	0.22	0.78

Table 2

Effect of globin mRNA and initiation factors on synthesis of α and β globin by reticulocyte extracts.

Reaction	Time [min.]	Total Synthesis Relative to Control Reaction	% α	% β
Control	10		0.50	0.50
	25		0.50	0.50
	40		0.51	0.49
Plus 64 μg/ml α globin mRNA	10	1.04	0.66	0.34
	25	1.00	0.69	0.31
	40	0.95	0.68	0.32
Plus 900 μg/ml reticulocyte initiation factors	10	1.03	0.48	0.52
	25	1.02	0.49	0.50
	40	0.96	0.50	0.50
Plus 62 μg/ml β globin mRNA	4	1.03	0.28	0.72
	8	1.08	0.28	0.72
	12	1.00	0.29	0.71
	16	0.96	0.30	0.70
	20	1.06	0.30	0.70
	30	1.00	0.29	0.71

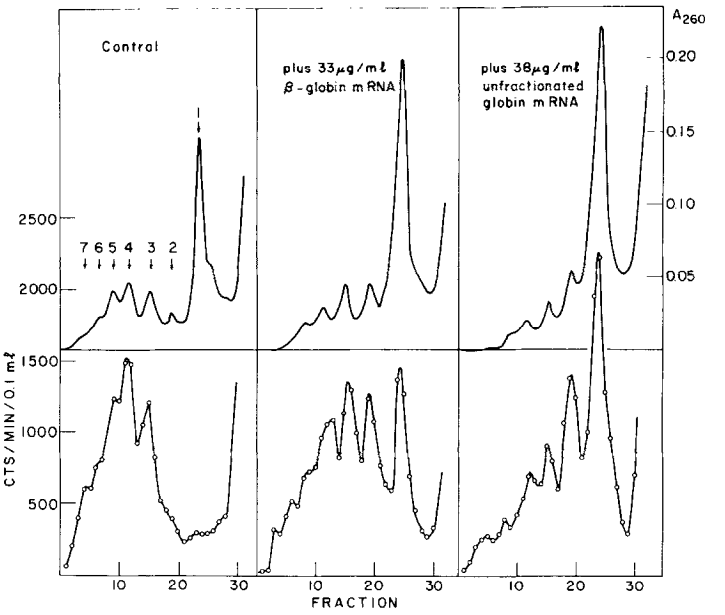

Fig. 2 Effect of addition of mRNA on size and labeling of reticulocyte polysomes. Reactions (120 μl) contained [^{35}S]-methionine with: (left) no added RNA; (middle) 33 μg/ml β globin mRNA; (right) 38 μg/ml unfractionated globin mRNA. Incubation was at 25°C for 8 min.; the reactions were chilled and layered on a 16.5 ml 15-30% sucrose gradient (3,4). Centrifugation was for 3.5 hr. at 26,500 rpm in the Beckman SW27.1 rotor. The gradient was collected through a Gilford flow cell and the absorbance at 260 nm recorded (top panels); fractions of 0.5 ml were collected and protein radioactivity in 0.1 ml was measured.

sis; the ratio of β : α globin produced changes from 1.0 to 3.5 at high concentrations of β mRNA. Likewise, addition of α mRNA results in enhanced synthesis of α and decreased synthesis of β globin (Table 2). Unfractionated globin mRNA reproducibly resulted in a slight inhibition of α globin synthesis and a corresponding increase in β globin production (Table 1). Reticulocyte initiation factors had no effect on the protein product produced (Table 2).

The addition of 30-40 μg globin mRNA resulted, as expected, in a reduction in the average size of the polysomes (Fig. 2). The total amount of nascent globin chains was only slightly increased (Fig. 2 and Table 3). A simple calculation (Table 3) shows that there is now twice as much globin mRNA on polysomes and that the average number of ribosomes per mRNA is reduced two-fold.

Table 3

Exogenous globin mRNA is found on reticulocyte polysomes.

	REACTION		
	Control	Plus 33 μg/ml β globin mRNA	Plus 38 μg/ml Unfractionated globin mRNA
Total cts/min in nascent polypeptides $C = \sum_i C_i$	16,385 [1.00]	18,896 [1.15]	18,062 [1.10]
Relative amount of mRNA in polyribosomes $M = \sum_i \frac{C_i}{i}$	4,743 [1.00]	9,284 [1.96]	11,265 [2.38]
Average number of ribosomes/mRNA $= \frac{C}{M}$	3.24	2.03	1.60

The data used is taken from Fig. 2. Radioactivity in nascent chains is the sum of fractions 1-28 in each of the three sections of Fig. 2. We assume that, on the average, ribosomes in all sizes of polysomes contain nascent chains of the same length. Hence the amount of [^{35}S] radioactivity, Ci, in a polysome containing i ribosomes, is proportional to the number of ribosomes in polysomes of this size. Since each mRNA in a polysome of size i contains i ribosomes, the number of mRNA molecules in a polysome of size i, M_i, is proportional to C_i/i. Hence the total amount of mRNA in polysomes is $M = \sum_i C_i/i$. This is the number tabulated in line 2. Since the total radioactivity in nascent chains $C = \sum_i C_i$ is proportional to the total number of ribosomes in polysomes, the average number of ribosomes per mRNA is simply C/M (line 3).

DISCUSSION

It is important to point out one ambiguity in the present studies. We do not know what percentage of the mRNA in our preparation actually participates in *in vitro* protein synthesis, nor do we know the exact concentration of endogenous mRNA in our extracts. Hence, it is unclear whether the added

mRNA initiates protein synthesis at the same efficiency as does the endogenous mRNA. At least some of the added α or β mRNA is translated (Tables 1 and 2) and is found on polysomes (Fig. 2, Table 3).

About 30 per cent of the ribosomes in rabbit reticulocytes are monosomes (Fig. 2). Experiments by Howard, et al., (23) showed that these are essentially inactive in protein synthesis and equilibrate with polysomes and ribosome subunits only after a long (15 minute, 30°C) incubation. Our data suggest that the overall rate of protein (i.e., globin) synthesized by reticulocytes is limited only by the amount of active ribosomes. Addition of neither initiation factors nor globin mRNA (Fig. 1), nor ribosome-free supernatant (unpublished data) has any effect on the total rate or amount of protein produced. Addition of polyribosomes will result in an enhanced rate of protein synthesis (data not shown).

Our results make it very unlikely that there is heterogeneity of reticulocyte ribosomes such that some could translate only α mRNA and others only β mRNA. Addition of β mRNA results in enhanced β globin synthesis within 4 minutes (at 25°C) (Table 2). This is only slightly longer than the time required for complete synthesis of one globin chain. The rapid enhancement of β globin synthesis observed after the addition of β mRNA indicates that ribosomes released from α mRNA are rapidly recruited to translate the newly added β mRNA (and conversely for the addition of α mRNA). Furthermore, since reticulocyte extracts, when supplemented with the appropriate α or β mRNA, can synthesize up to fifty per cent more α or β globin chains, it appears that any putative α or β mRNA-specific factor does not normally limit the rate of synthesis of α or β globin chains.

Addition of unfractionated globin mRNA to a reticulocyte lysate reproducibly results in an enhanced synthesis of β globin chains (Table 1). Similarly, McKeehan showed that increasing the concentration of globin mRNA in a reconstituted cell-free system caused an increasing ratio of β to α product (24). This appears also to be a reflection of the higher affinity

of β mRNA for ribosome subunits or other rate-limiting components of chain initiation (3,4,14); under conditions where the concentration of messenger RNA is increased relative to that of the non-specific components of chain initiation, translation of mRNAs with a higher rate constant for polypeptide chain initiation are favored (14).

We conclude that α and β globin mRNAs compete for the same rate-limiting component, a component acting presumably at the level of polypeptide chain initiation. Considering that total globin synthesis is probably limited by the concentration of active ribosomes, we feel it is likely that this rate-limiting component is the [met-$tRNA_f$: 40s ribosome] pre-initiation complex (see 14). Other possibilities, such as a (non-specific) mRNA binding factor, cannot be excluded. Therefore, the relative amounts of α and β globin synthesized appear to be the consequence of primarily two factors, the relative affinities of the α and β mRNAs for the rate-limiting component, and the relative amounts of α and β mRNAs present in the lysate.

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