Differential Translation of Rat Liver Albumin Messenger RNA in a Wheat Germ Cell-Free System†

Gail E. Sonenshein*,‡ and George Brawerman

ABSTRACT: Rat liver polysomal RNA directs the synthesis of albumin in a wheat germ cell-free system. The in vitro product has the same mobility in polyacrylamide-sodium dodecyl sulfate gels as purified rat liver albumin and can be immune precipitated with rabbit anti-rat liver albumin serum. The incorporation of [^{35}S]methionine into total protein in an in vitro system increased linearly with increasing messenger RNA concentration until a saturation plateau was reached. The synthesis of albumin compared to total protein increased continually with increasing RNA concentration. Addition of the polynucleotide poly(A) to the cell-free system inhibited incorporation, presumably through interference with the initiation process, and increased the relative albumin synthesis. Translation of albumin messenger RNA was also resistant to inhibition by high levels of potassium acetate where total incorporation was cut in half. The results suggest that the messenger RNA for albumin, in addition to the mRNAs for the β chain of globin and for the immunoglobulin polypeptides, is particularly efficient in initiating protein synthesis.

Recent studies have indicated that mRNA translation in mammalian cells can be modulated at the levels of polypeptide chain initiation. It has been shown in the case of rabbit reticulocytes that the mRNA for β-globin is translated preferentially when polypeptide chain initiation is rate limiting (Lodish, 1971). A similar effect was observed in vitro when excess globin mRNA is added to a heterologous cell-free system (Beuzard and London, 1974; McKeehan, 1974). These results led to the suggestion that the β-globin mRNA is particularly effective in promoting polypeptide chain initiation, so that it competes effectively with other mRNA species. The concept of a "hierarchy" among mRNAs with respect to initiation efficiency received further support from comparative measurements of rabbit and duck globin mRNA translation (Stewart et al., 1973).

Immunoglobulin synthesis in mouse myeloma cells was also found to be relatively resistant to treatments that interfere with polypeptide chain initiation (Sonenshein and Brawerman, 1976a; Nuss and Koch, 1976a). In vitro studies in this case as well have indicated that the mRNA for the immunoglobulin light chain is particularly potent in promoting chain initiation (Sonenshein and Brawerman, 1976b). It was suggested that high efficiency of initiation might be a common characteristic of mRNAs for abundant proteins specific to particular differentiated cell types. In the present study, we have examined the behavior of the mRNA for rat liver albumin in a wheat germ cell-free system. Its translation shows characteristics similar to that of globin β-chain and immunoglobulin light-chain mRNAs, thus indicating that it is also particularly effective in initiation. This finding provides further support for the hypothesis that efficient initiation may be a significant factor in regulating the overall rate of synthesis of abundant proteins.

Materials and Methods

Preparation of RNA. Polysomes were isolated from rat liver and purified by magnesium precipitation as described previously (Mendecki et al., 1972). The RNA was extracted with phenol in the presence of pH 9.0 Tris-HCl[1] by the procedure of Brawerman et al. (1972). The poly(A)-containing RNA fraction was bound to oligo(dT)-cellulose in the presence of 500 mM NaCl–5 mM $MgCl_2$–50 mM Tris-HCl, pH 7.6–0.5% $NaDodSO_4$, eluted with water, precipitated with ethanol, and prepared for protein synthesis as described previously (Sonenshein, et al., 1976). Approximately 2% of the RNA became bound to oligo(dT)-cellulose.

Protein Synthesis in Wheat Germ Lysates. RNA was incubated in a wheat germ lysate, prepared by the procedure of Roberts and Paterson (1973) as described previously (Sonenshein and Brawerman, 1976b), at a final potassium acetate concentration of 160 mM unless otherwise mentioned. The reaction mixture was treated with pancreatic ribonuclease to release the nascent chains, aliquots were removed, and the level of incorporation was measured (Sonenshein and Brawerman, 1976b).

Immune Precipitation. Products of the cell-free reaction directed by rat liver RNA were incubated in the presence of carrier albumin with rabbit anti-rat albumin serum. After 2 h at 4 °C, the immune precipitate was pelleted by centrifugation at 2500 rpm for 10 min, washed extensively with 140 mM NaCl–50 mM Tris-HCl, pH 7.6, then once with 50 mM NaCl–50 mM Tris-HCl, pH 7.6, and 50 mM Tris-HCl, pH 9.0, respectively. The precipitate was resuspended in 50 mM Tris-HCl, pH 9.0–2% sodium dodecyl sulfate, and dissolved by heating in boiling water for 1 min. To prepare samples for electrophoresis, disulfide bridges were reduced by treatment with 2-mercaptoethanol and then alkylated with iodoacetamide as described previously (Sonenshein and Brawerman, 1976b).

Polyacrylamide Gel Electrophoresis. Products of in vitro reactions were precipitated and washed with trichloroacetic acid, dissolved in 50 mM Tris-HCl, pH 9.0–2% sodium dodecyl

† From the Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111. *Received June 23, 1977.* This work was supported by research grants from the United States Public Health Service (GM17973, CA12924) and the American Cancer Society, Massachusetts Division.

‡ Present address: Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass. 02139.

[1] Abbreviations used are: poly(A), poly(adenylic acid); Tris-HCl, 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride.

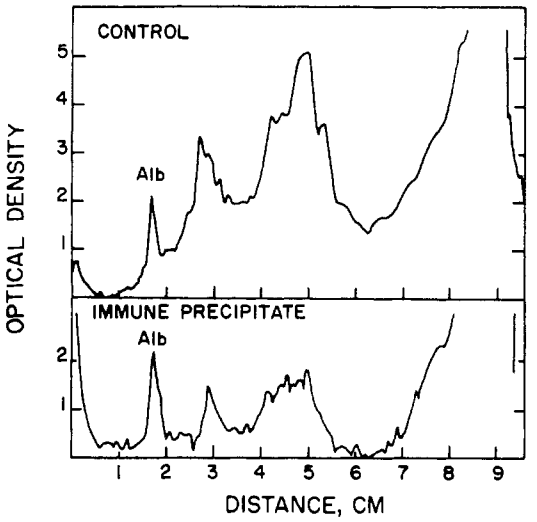

FIGURE 1: Electrophoretic and immunological analysis of translation products obtained with rat liver polysomal RNA. Rat liver polysomal RNA was extracted and used to direct a wheat germ cell-free system at 15 μg/50 μL of reaction. The in vitro products were subjected to electrophoresis on polyacrylamide–sodium dodecyl sulfate slab gels. Autoradiograms of dried gels were scanned as described under Materials and Methods. An aliquot of the cell-free products was subjected to immune precipitation with rabbit anti-rat liver albumin serum. The precipitated proteins were electrophoresed as discussed under Materials and Methods. The position of albumin is labeled "Alb".

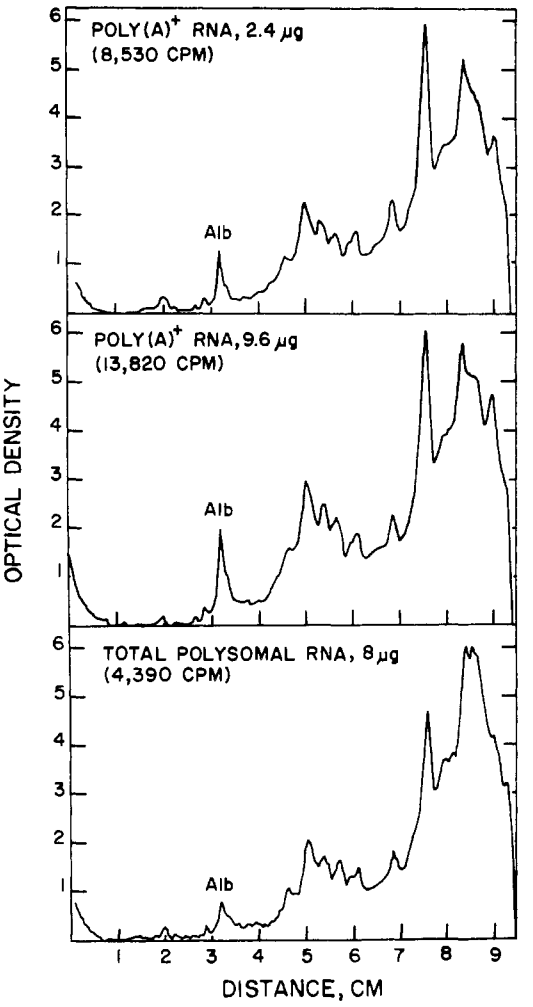

FIGURE 2: Electrophoretic analysis of translation products obtained with varying concentrations of either poly(A)-containing or total polysomal RNA. In vitro products of cell-free reactions were analyzed as described in Figure 1. Values in parentheses indicate acid-insoluble radioactivity incorporated in an aliquot (4 μL) of the reaction mixture.

sulfate, and prepared for electrophoresis with reduction and alkylation as discussed above. Samples were subjected to electrophoresis in 13% polyacrylamide–sodium dodecyl sulfate slab gels with a 5% stacking region according to the procedure of Studier (1973) with the modification of gel dimensions as indicated previously (Sonenshein and Brawerman, 1976a). The electrophoreses were run overnight at 27 V. The gels were stained, destained, and dried onto paper, and autoradiograms were prepared and scanned as reported previously (Sonenshein and Brawerman, 1976a).

Analysis of the Gel Products. The relative quantity of albumin synthesis was measured from the profiles of the scanned autoradiograms. The appropriate areas were cut out and weighed, and the percent of the total area represented by albumin was calculated.

Results

Products of Translation of Rat Liver RNA. Rat liver total polysomal RNA or poly(A)-containing mRNA directs the synthesis of a heterogeneous population of polypeptides in a wheat germ cell-free system (Figure 1). Most of the products of translation of the wheat germ system are relatively small. Polypeptides of molecular weight 10 000–15 000 represent a large peak migrating with the solvent front. The major large molecular weight component has the same mobility as purified albumin. Identification of this band as albumin was made using rabbit antiserum to rat liver albumin. Immune precipitation of the translation products gave the enrichment of this peak shown in Figure 1. The amount of albumin synthesis varied with the RNA preparation (compare Figures 1 and 4). Synthesis of rat liver albumin in a wheat germ lysate has been reported previously (Astell and Ganoza, 1974; Peterson, 1976).

Effect of Saturating Levels of RNA. A comparison of the profiles of polypeptide products obtained with either poly(A)-containing or total polysomal RNA is shown in Figure 2. The same population of polypeptides appears to be synthesized with the two types of RNA, but the proportions differ considerably. The longer separation obtained in the gel enables one to better examine the larger molecular weight region, although the small polypeptides have migrated off the gel.

TABLE I: Effect of Excess Messenger RNA on Albumin Synthesis.[a]

Poly(A)-containing RNA per 50 μL of reaction (μg)	Total methionine incorp	Albumin synthesis (% of total)
	580	
2.4	8 530	0.56
4.8	13 290	0.78
7.2	14 770	0.86
9.6	13 820	0.92

[a] Products of reactions were analyzed by polyacrylamide–sodium dodecyl sulfate gel electrophoresis as shown in Figure 2. The relative albumin synthesis was calculated from the tracings as described under Materials and Methods.

From such profiles one can compute the percentage of albumin synthesis compared to total synthesis using the area under the curves (see Materials and Methods). Total [^{35}S]-methionine incorporation increases with increasing amounts of poly(A)-containing RNA until RNA saturation is reached at approximately 5 μg in a 50-μL reaction, i.e., a concentration

TABLE II: Effect of Poly(A) on Translation of Liver RNA.[a]

Poly(A) (μg)	Methionine incorp Total	Methionine incorp % inhibition	Albumin synthesis (% of total)
0	12 880		1.5
0.5	5 690	56	2.3
1	4 530	65	3.3

[a] The products of reactions were analyzed as described in Figure 3. The relative albumin synthesis was calculated as in Table I. Endogenous incorporation without poly(A) was 1150 cpm.

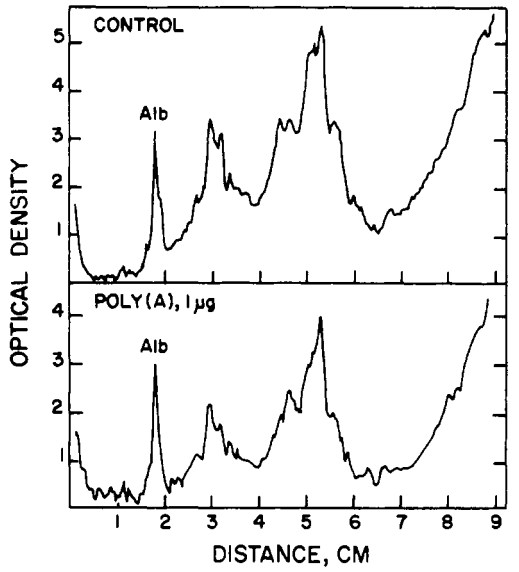

FIGURE 3: Electrophoretic analysis of the effect of addition of poly(A) on the translation of total polysomal RNA. Products of reactions directed by 28 μg/50 μL of reaction of total polysomal RNA in the absence or presence of poly(A) were analyzed. Tracings have been adjusted to the same Alb peak height for ease of comparison. Further details are given in Table II.

of 0.1 mg/mL. The relative albumin synthesis increases with increasing RNA concentration even up to 0.19 mg/mL (Table I). The profiles of the products synthesized with either 2.4 or 9.6 μg in the reaction mixture are shown in Figure 2. Thus, under conditions of mRNA excess, where presumably there is competition of the RNA for ribosomes, albumin synthesis increases.

Effect of Poly(A) on Translation. Lodish and Nathan (1972) have shown that addition of polynucleotides, such as poly(A), to an in vitro protein-synthesizing system results in an inhibition of protein synthesis caused by a block in initiation. Further evidence suggests that the step of binding of the mRNA to the 30S $tRNA^{Met}$ complex is specifically inhibited (T. Hunt, personal communication). Low levels of poly(A) reduce the incorporation of [^{35}S]methionine in a wheat germ system directed with rat liver mRNA (Table II), as has been found with mouse myeloma mRNA (Sonenshein and Brawerman, 1976b). The effect of poly(A) addition on the translation products is shown in Figure 3. The relative albumin synthesis doubles with respect to the control reaction in the presence of 1 μg of poly(A) (Table II).

Effect of Salt Concentration. Translation of the immunoglobulin light-chain mRNA, an efficient initiator of protein synthesis, was found to be resistant to increased levels of potassium acetate even at a concentration which caused a 40% inhibition of total polypeptide synthesis (Sonenshein and Brawerman, 1976b). A similar pattern is found with rat liver albumin synthesis (Figure 4). As the salt concentration is

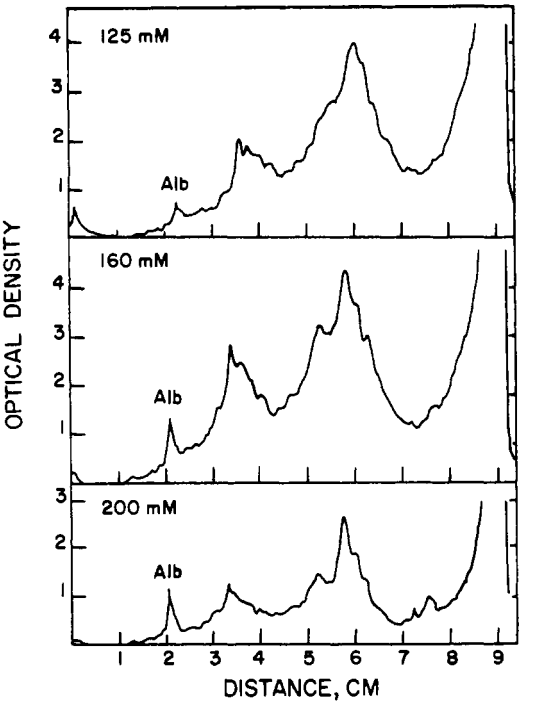

FIGURE 4: Electrophoretic analysis of translation products obtained at various potassium acetate concentrations. Products of reactions were obtained in the presence of 13 μg of total polysomal RNA. Further details are given in Table III.

TABLE III: Effect of Potassium Acetate Concentration on Liver RNA Translation.[a]

Potassium acetate concn (mM)	Methionine incorp Endogenous	Methionine incorp Plus RNA	Albumin synthesis (% of total)
125	2150	9 450	0.2
160	1530	11 900	0.6
200	950	4 670	1.7

[a] The products of reactions described in Figure 4 were analyzed as discussed in Table I.

raised there is an increase in both the relative and total synthesis of albumin, the latter despite a 50% inhibition of [^{35}S]methionine incorporation (Table III). This increase does not appear to be due simply to a faster elongation rate caused by the high salt (Mathews and Osborn, 1974), since no significant switch to synthesis of larger polypeptides is observed. This salt effect on relative albumin translation has also been reported recently by Peterson (1976). Also a similar observation has been reported for the in vitro synthesis of rabbit β-globin, an efficient initiator of protein synthesis, compared with α-globin (McKeehan, 1974).

Discussion

The translation of albumin mRNA in a wheat germ system has been compared to that of the total polysomal mRNA population. Under conditions of mRNA excess in the reaction mixture the relative synthesis of albumin increases. Thus, at high RNA concentrations, the albumin mRNA is more effective in competing for the limiting initiation components. Furthermore, if one inhibits the initiation step directly by addition of poly(A), the relative synthesis of albumin also increases, and, although the mechanism of action of high salt inhibition is not well understood, albumin synthesis is more resistant to this treatment as well. Thus, by three criteria, the

characteristics of translation of albumin mRNA in vitro are similar to those of immunoglobulin light-chain mRNA. That is, under conditions that restrict or limit initiation of protein synthesis the relative translation of these two proteins is enhanced. Studies on the synthesis of immunoglobulin light chain in mouse myeloma cells have indicated that its mRNA is able to initiate protein synthesis preferentially in vivo as well (Sonenshein and Brawerman, 1976a; Nuss and Koch, 1976a).

Thus, there may exist a class of mRNAs able to initiate protein synthesis more efficiently than others. Included in this group would be the mRNAs for rabbit β-globin and, to a lesser extent, for α-globin (Lodish, 1971; Beuzard and London, 1974; Stewart et al., 1973), for rat albumin, and for the light and heavy chains of mouse immunoglobulin (Sonenshein and Brawerman, 1976b). This group codes for major proteins that are specifically related to particular functions of differentiated cell types. They fall into the category often referred to as "nonhousekeeping". It is possible that mRNAs for all such proteins are better able to initiate protein synthesis. It would be interesting to investigate this possibility further as well as to compare their translation capability with that of a mRNA for an abundant "housekeeping" protein.

One final point that should be noted is that in this as well as in the previous study (Sonenshein and Brawerman, 1976b) the relative translation of the various in vitro products varied greatly with the conditions of incubation. This indicates that caution should be taken when in vitro synthesis is used to measure relative mRNA quantities and in particular when it is used as a criterion of mRNA purity.

Acknowledgments

We thank Dr. B. David Stollar for his help in preparing rabbit anti-rat albumin serum. The excellent technical assistance of Edwin Richard and Mary Geisz is gratefully acknowledged.

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