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Differential Translation of Mouse Myeloma Messenger RNAs in a Wheat Germ Cell-Free System†

Gail E. Sonenshein‡ and George Brawerman*

ABSTRACT: Translation of the polysomal mRNA of mouse myeloma cells in a wheat germ cell-free system leads to the immunoglobulin (Ig) light-chain precursor as the major product. Excess polysomal RNA causes strong inhibition of polypeptide synthesis, but has little effect of light-chain precursor synthesis. The inhibitory effect of excess RNA is avoided when the poly(A)-containing RNA fraction is used. With nearly saturating amounts of the latter RNA, light-chain precursor synthesis becomes more predominant, possibly as a result of competition between different mRNA species. High levels of potassium acetate cause strong inhibition of overall translation, but do not inhibit light-chain precursor synthesis. Addition of poly(A) to the cell-free system also causes inhibition, presumably through interference with the initiation process. Again, light-chain precursor synthesis is relatively resistant. Ig heavy-chain synthesis is relatively inefficient, but its resistance to the inhibitors tends to be nearly as great as that of the light-chain precursor. The results indicate that the Ig mRNAs are particularly efficient in initiating translation. This characteristic may account for certain features of the regulation of Ig synthesis in intact myeloma cells.

There is little knowledge of the extent to which modulation of mRNA translation contributes to the control of gene expression. It has been shown that the mRNAs for the two polypeptide chains of hemoglobin are translated with different efficiencies in rabbit reticulocytes (Lodish, 1971). The preferential translation of the β-globin mRNA appears to be due to more efficient polypeptide chain initiation with this RNA. The latter characteristic can also be observed in cell-free systems, where the β-globin mRNA is translated preferentially when the two mRNAs are present in excess (Beuzard and London, 1974; McKeehan, 1974). These findings suggest a possibility for modulation based on unique structural features of different mRNA species. The physiological significance of the observed differences between the two globin mRNAs is not clear, since the two polypeptides are produced in equal amounts.

We have approached the problem of modulation of mRNA translation by asking whether the synthesis of specialized proteins that are produced in large amounts for secretion may be subject to a separate regulatory process. We observed that immunoglobulin (Ig[1]) synthesis in mouse myeloma cells is particularly insensitive to treatments that interfere with polypeptide chain initiation (Sonenshein and Brawerman, 1976). This leads to a more predominant synthesis of the heavy and light Ig chains under the restrictive conditions. In the present study, we have examined the translation of the myeloma mRNAs in a wheat germ cell-free system, to see whether the in vivo effects could be accounted for by characteristics of the mRNAs. Conditions that limit the capacity of the system for translation of exogenous RNA led to preferential synthesis of the Ig polypeptides. Thus, the Ig mRNAs, like the β-globin mRNA, appear to be particularly effective in promoting polypeptide chain initiation. The results suggest that mRNAs with high translation efficiency may be of widespread occurrence, and that this characteristic may influence cellular rates of synthesis of individual proteins.

Materials and Methods

Tumor Growth and Polysome Preparation. Mouse myeloma MPC-11 45 cells were grown in culture as described previously (Sonenshein and Brawerman, 1976). Cells were harvested, washed, and resuspended in Hank's buffered salt

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[1] Abbreviations used are: Ig, immunoglobulin; poly(A), poly(adenylic acid); ATP, GTP, adenosine and guanosine triphosphates.

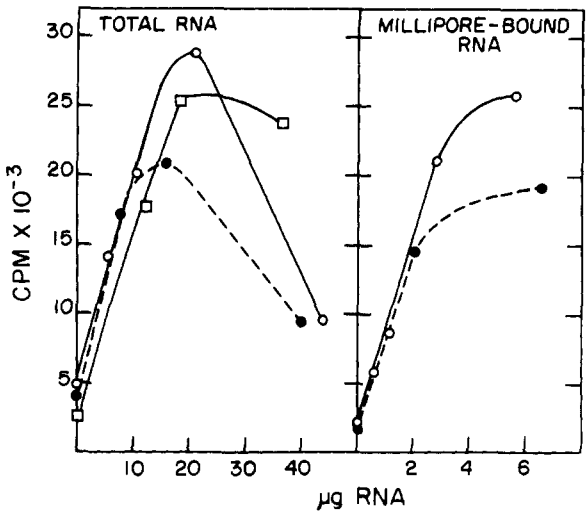

FIGURE 1: Effect of RNA concentration on [^{35}S]methionine incorporation. Indicated amounts of either total or poly(A)-containing RNA, isolated as described under Materials and Methods, were added to a wheat germ cell-free system. Incubations were carried out in presence of 125 mM potassium acetate, in most cases. After ribonuclease digestion, aliquots were removed and Cl_3CCOOH-insoluble radioactivity was measured. Total RNA: (●- - -●) cytoplasmic; (○-○) membrane-bound polysomal; (□-□) membrane bound, incubated at 160 mM potassium acetate. Millipore-bound RNA: (○-○) membrane-bound polysomal; (●- - -●) free polysomal.

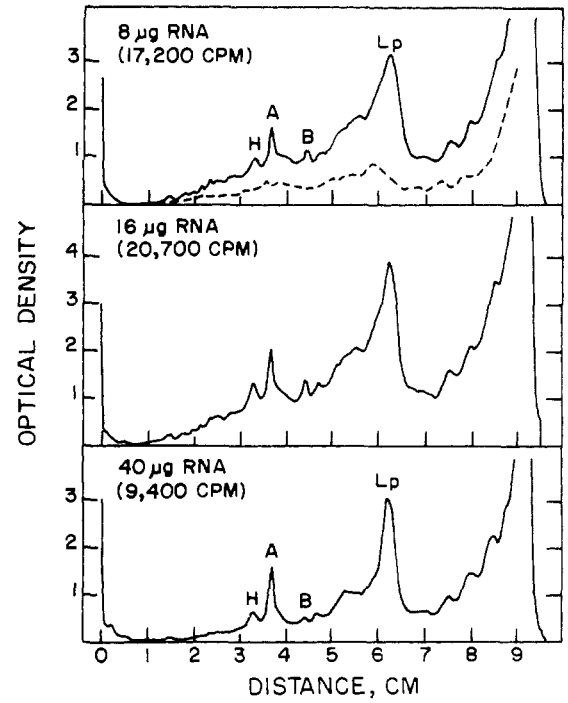

FIGURE 2: Electrophoretic analysis of translation products obtained with increasing concentrations of total cytoplasmic RNA. In vitro products were subjected to electrophoresis on polyacrylamide-sodium dodecyl sulfate slab gels. Autoradiograms of dried gels were scanned as described under Materials and Methods. Amounts of RNA used in the 50-μl reactions are as indicated. Values in parentheses indicate Cl_3CCOOH-insoluble radioactivity incorporated in an aliquot of the reaction mixture. Value for endogenous incorporation (dashed line) was 4100 cpm. H, heavy chain; Lp, light-chain precursor.

solution (Grand Island Biological Co.). Balb/c mice were inoculated subcutaneously with 3×10^6 cells to obtain tumors. Membrane-bound polysomes were isolated essentially by the procedure of Mach et al. (1973). Tumors were disrupted in a volume of buffer A (100 mM KCl–5 mM $MgCl_2$–50 mM Tris-HCl, pH 7.6–0.8 M sucrose) equal to 2.5 times their weight. Gentle homgenization was carried out in a motor-driven Teflon-glass homogenizer (Thomas Co., Philadelphia, Pa.) with seven up and down strokes. The homogenate was centrifuged at 17 000*g* for 10 min. The supernatant was removed, avoiding the fluffy material near the nuclear pellet, and layered over 2 ml of buffer A. A membrane-bound polysome pellet was obtained by centrifugation at 60 000*g* for 20 min. The pellet was resuspended in a small volume of high-speed supernatant from a rat liver extract (which contains an inhibitor of ribonuclease activity and was prepared as described previously by Lee and Brawerman, 1971) and an equal volume of 10 mM Tris-HCl, pH 7.6–10 mM KCl–1 mM $MgCl_2$. Triton X-100 was added at a final concentration of 1% to release polysomes from the membranes. The free-polysome fraction was isolated from the 60 000*g* supernatant by centrifugation at 160 000*g* for 2 h and resuspended in 10 mM Tris-HCl, pH 7.6–10 mM KCl–1 mM $MgCl_2$. Polysome preparations were further purified by precipitation with $MgCl_2$ at a final concentration of 30 mM as described previously (Mendecki et al., 1972).

RNA Extraction and Purification. RNA from either tumor cytoplasm (17 000*g* supernatant) or from purified polysomes was prepared by phenol extraction in the presence of Tris-HCl, pH 9.0, by the procedure of Brawerman et al. (1972), followed by ethanol precipitation. Where mentioned, the poly(A)-containing fraction was isolated by adsorption to Millipore filters (Lee et al., 1971b). The RNA was eluted from the filters with 50 mM Tris-HCl, pH 9.0–0.5% sodium dodecyl sulfate in the cold. The precipitate of potassium dodecyl sulfate and the filter fragments were removed by centrifugation at 1500*g* for 10 min and the RNA precipitated with ethanol. To remove any sodium dodecyl sulfate in the RNA precipitates, they were washed twice with 95% ethanol–0.1 M NaCl (2 vol/1 vol). Then the ethanol was removed with an ether wash, the RNA was dissolved in water, and the ether was blown off with air.

Protein Synthesis of Wheat Germ Lysates. Reaction mixtures (50 μl total volume) contained 15 μl of wheat germ lysate (prepared by the procedure of Roberts and Paterson, 1973), 26 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) (pH 7.2), 12 μM spermidine, 3 mM magnesium acetate, 2 mM dithiothreitol, 1.8 mM β-mercaptoethanol, 1 mM ATP, 0.2 mM GTP, 8 mM creatine phosphate, 50 μg/ml of creatine phosphokinase, 30 μM of unlabeled amino acids, 5 μCi of [^{35}S]methionine (100–300 Ci/mmol, New England Nuclear), 125 mM potassium acetate (except where stated), and indicated amounts of exogenous RNA. After incubation at 22 °C for 2 h, pancreatic ribonuclease A was added at a final concentration of 200 μg/ml and digestion was carried out at 37 °C for 30 min. After dilution with an equal volume of water, 10-μl aliquots were removed and used for measurements of acid-insoluble radioactivity. Bovine albumin was added to facilitate complete precipitation with trichloroacetic acid containing 3% casamino acids and the precipitates were filtered through glass-fiber filters, washed, dried, and counted as described previously (Lee et al., 1971a).

Polyacrylamide Gel Electrophoresis. Proteins were precipitated with either 10 volumes of acetone or with 10% Cl_3CCOOH containing 3% casamino acids followed by washes with either acetone or 0.1 and 0.01% Cl_3CCOOH, respectively. The precipitates were dissolved in 50 mM Tris-HCl, pH 9.0–2% sodium dodecyl sulfate and heated in boiling water for 1 min. The proteins were treated with β-mercaptoethanol at

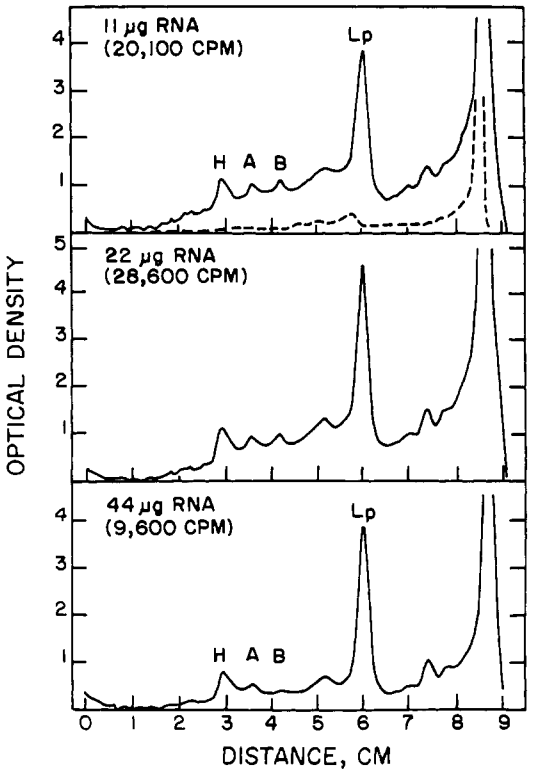

FIGURE 3: Electrophoretic analysis of translation products obtained with increasing concentrations of total membrane-bound polysomal RNA at 125 mM potassium acetate. In vitro products of cell-free reactions directed by various concentrations of total RNA from membrane-bound polysomes were analyzed as described in Figure 2. Value for endogenous incorporation (dashed line) was 4900 cpm. The relative amounts of Ig synthesis varied with increasing concentrations of RNA as follows: light-chain precursor (Lp): 8, 10, and 12%; heavy chain (H): 1.7, 1.9, and 2.5%.

a final concentration of 75 mM for 1 h at 37 °C to reduce disulfide bridges, and alkylated with iodoacetamide (0.1 M) for 30 min at 37 °C. They were subjected to electrophoresis on 12% polyacrylamide–sodium dodecyl sulfate slab gels ($\frac{1}{32}$ inch thick) with a 5% stacking region. The procedures for electrophoresis and autoradiography were as described previously (Sonenshein and Brawerman, 1976).

Results

Translation of Total RNA from Myeloma Cells. Addition of total cytoplasmic RNA to a wheat germ cell-free system causes a substantial stimulation of polypeptide synthesis (Figure 1). A heterogeneous population of polypeptides is produced, as measured by slab-gel electrophoresis of products labeled with [^{35}S]methionine, followed by autoradiography and densitometry tracing of the developed bands. Several major peaks are observed, together with a large background which probably represents a highly heterogeneous mixture of minor polypeptides (Figure 2). The large amount of material at the leading edge represents small polypeptides of the order of 10 000–15 000 daltons. The very abundant component is the immunoglobulin light-chain precursor, which migrates more slowly than the mature light chain (Mach et al., 1973; Kuehl et al., 1975; Blobel and Dobberstein, 1975). The peak labeled H is the heavy immunoglobulin chain. It has approximately the same mobility as heavy chain synthesized in vivo, and is missing when the RNA used for translation is derived from a variant MPC-11 myeloma cell line defective in heavy chain synthesis. The identity of the heavy and light

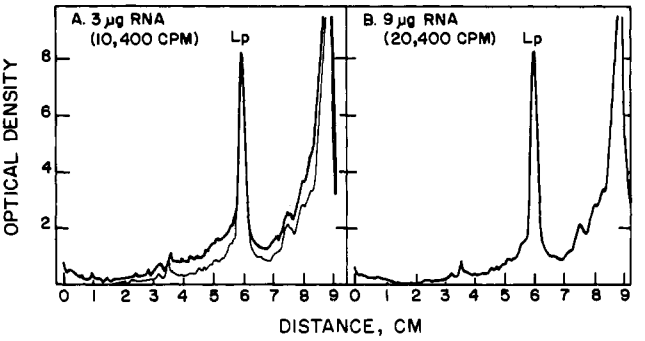

FIGURE 4: Electrophoretic analysis of translation products of poly(A)-containing RNA from membrane-bound polysomes. The in vitro products of a cell-free reaction directed by 3-μg Millipore-bound RNA are compared with those from a reaction with excess RNA (9 μg, see Figure 1). Scale of tracings has been adjusted to obtain the same light-chain precursor peak height for ease of comparison. The 9-μg RNA profile (panel B) is reproduced in panel A (thin line). Endogenous incorporation was 1700 cpm. The proportions of light-chain precursor increased from 11.2% with 3 μg of RNA to 13.7% in the 9-μg RNA reaction. Lp, light-chain precursor.

precursor chains was verified by immune precipitation using a rabbit anti-mouse Ig antiserum (data not shown). The low amount of heavy chain synthesized in vitro is probably due to the low efficiency of the wheat germ system for translation of large mRNAs (Benveniste et al., 1976).

When increasing amounts of RNA are used in the cell-free system, most of the peaks become better defined. The highest concentration of RNA used in these experiments (40 μg/reaction) causes a marked reduction in total amino acid incorporation (Figure 1). This inhibition affects primarily the "background" components (Figure 2). Immunoglobulin light-chain synthesis does not seem to be inhibited. The heavy-chain peak increases at first with RNA concentration, but tends to be somewhat reduced under the inhibitory conditions. One polypeptide, labeled B (see Figure 2), seems particularly affected while another, A, appears to be refractory to the inhibitory effect. The overall electrophoretic profile is much simpler under the inhibitory conditions, with light-chain precursor and A representing a major share of the total synthetic activity.

Similar effects are observed with the RNA of membrane-bound polysomes as template (Figures 1 and 3). The overall distribution of labeled polypeptides is less heterogeneous. In particular, the light-chain precursor peak is better defined, even at relatively low concentrations of RNA. There is also enrichment in peak H, and a considerable reduction in peak A, an indication that the latter polypeptide is not synthesized on membrane-bound polysomes. At inhibitory RNA concentrations, the synthesis of heavy and light-precursor chains is hardly affected and becomes predominant. There is a very sharp reduction in the background polypeptides and in the smaller components running with the dye front. Peak B is eliminated in this case.

Translation of Poly(A)-Containing RNA. Messenger RNAs with a poly(A) sequence are adsorbed on Millipore filters at high-ionic strength (Lee et al., 1971b). The myeloma RNA components retained on Millipore are highly active in promoting polypeptide synthesis in the wheat germ system (Figure 1). Moreover, the inhibitory agent has been removed. No inhibition was observed at the highest concentrations of RNA used in this study. The translation products obtained with the Millipore-bound fraction of membrane-bound polysomal RNA are shown in Figure 4. The light chain is, by far,

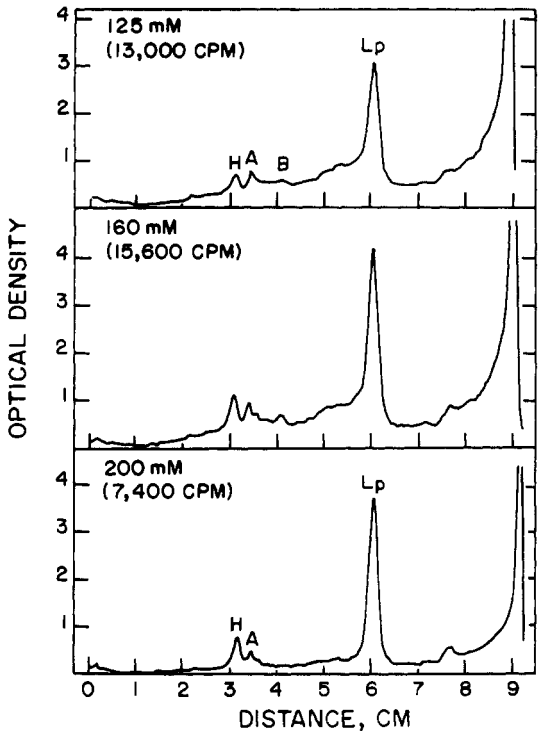

FIGURE 5: Electrophoretic analysis of translation products of total membrane-bound polysomal RNA obtained at various potassium acetate concentrations. Products of reactions obtained after incubation in the absence or presence of 17 μg of RNA were subjected to electrophoresis and autoradiography as in Figure 2. Endogenous incorporation was 1600 cpm for 125 mM, 1200 cpm for 160 mM, and 700 cpm for 200 mM potassium acetate. With increasing salt concentration, the proportions of Ig synthesis changed as follows. Light-chain precursor (Lp): 9, 13, and 22%; heavy chain (H): 0.8%, 1.8% and 2.5%.

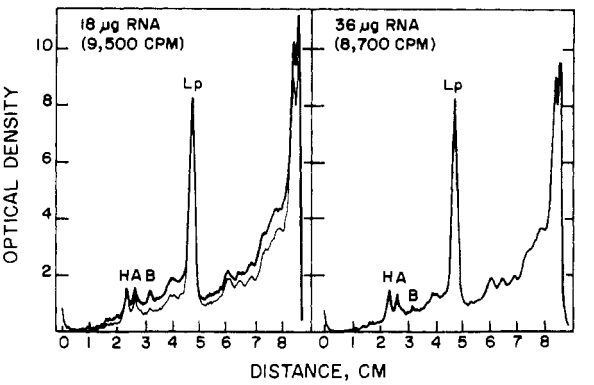

FIGURE 6: Electrophoretic analysis of translation products of total membrane-bound polysomal RNA at 160 mM potassium acetate. Tracings have been adjusted to the same light-chain precursor peak height, as in Figure 4. Endogenous incorporation was 900 cpm. The proportions of Ig synthesis at the low and high RNA concentrations were as follows: light-chain precursor (Lp): 8.8 and 11%; heavy chain (H): 0.7 and 1.1%.

the most prominent product. Heavy-chain synthesis was very low in this case, but this may be due to a particularly low efficiency of translation of the heavy-chain mRNA in this experiment, rather than to failure of this component to bind to Millipore. The light chain is further enhanced at higher RNA concentrations. The scales of the densitometry tracings in Figure 4 were adjusted so as to produce the same magnitude for the light-chain precursor peak. This permits a better comparison of the relative amounts of light chain produced at the two RNA concentrations. The basis for the differential effect in this case may be competition between different mRNAs for limiting amounts of translation factors, since nearly saturating amounts of RNA were used. Similar differential effects were observed with the Millipore-bound RNA derived from free myeloma polysomes (data not shown).

Effect of Potassium Acetate on Translation. Translation of the RNA from membrane-bound polysomes was enhanced when the potassium acetate concentration was raised from 125 to 160 mM (Figure 5). The synthesis of both immunoglobulin chains, as well as that of polypeptide B, was increased preferentially (Figure 5). A further rise in the salt concentration to 200 mM caused a considerable reduction in overall polypeptide synthesis, but the heavy and light chains were not affected. The amount of radioactivity in the heterogeneous components and in the small polypeptides was sharply reduced, and peak B was eliminated. Thus, immunoglobulin synthesis represented the great majority of total polypeptide synthesis at 200 mM potassium acetate.

Translation of Polysomal RNA at High Concentrations of Potassium Acetate. The inhibitor present in polysomal RNA, evident at high RNA concentrations, was ineffective at 160 mM potassium acetate (Figure 1). Overall polypeptide synthesis increased with RNA concentrations up to about 20 μg/reaction and then leveled off, instead of falling off sharply. The polypeptide distribution was altered when the amount of membrane-bound polysomal RNA appeared to be saturating (Figure 6). Again, the labeling of the heterogeneous components and of the small polypeptides was reduced when compared to that of the heavy, light-chain precursor, and A bands. Band B was almost eradicated.

Effect of Poly(A) on Translation. Small amounts of poly(A), which inhibit polypeptide synthesis in a rabbit reticulocyte lysate by blocking chain initiation (Lodish and Nathan, 1972), had a similar effect on the wheat germ system. The synthesis of light-chain precursor became more predominant with increasing concentration of poly(A) (Figure 7). The relative synthesis of both heavy and A bands was reduced in this case. Translation of the B band was eliminated at the highest poly(A) concentration.

Discussion

In the present study, we have used total cytoplasmic and polysomal RNA preparations as sources of mRNA for translation. This approach permitted the direct comparison of various mRNA species simply by measuring relative amounts of different radioactivity peaks. One serious disadvantage is that most of the polypeptides produced in the cell-free system are not identified as specific translation products. For instance, we attributed the large background radioactivity in the slab gels to a highly heterogeneous mixture of minor myeloma polypeptides. While this seems like a reasonable interpretation, it is not possible to assess precisely the contribution of endogenous wheat germ products to the heterogeneous component. Slab gel profiles of the endogenous products showed far less heterogeneous radioactivity, but the possibility of nonspecific stimulation of the endogenous incorporation by the added RNA cannot be ignored. The majority of the resolved bands could be safely attributed to specific myeloma proteins, since a variety of different RNA preparations (including mouse myeloma free polysomal RNA and rat liver RNA, data not shown) produced different band patterns.

Four sets of conditions were identified which cause a serious distortion in the distribution of translation products from a given RNA preparation. A very pronounced effect was pro-

duced by an inhibitory substance present in total polysomal RNA. The existence of this inhibitor was first suggested by the fact that high concentrations of polysomal RNA in the wheat germ system cause a strong inhibition of polypeptide synthesis. The active agent, present in rat liver as well as mouse tumor cells, isbeing characterized in our laboratory. Partially purified preparations, when added to reactions with noninhibitory amounts of polysomal RNA, led to the same effect as that observed with high amounts of polysomal RNA. The synthesis of the heavy and light chains, as well as that of polypeptide A, was resistant to the inhibitor, while that of the heterogeneous component and of polypeptide B was strongly affected. Endogenous wheat germ synthesis does not appear to be sensitive to the inhibitor. Addition of low levels of poly(A), which has been shown to block initiation in a reticulocyte system (Lodish and Nathan, 1972), caused a very pronounced increase in relative light-chain precursor synthesis. High potassium acetate concentrations also had a strong effect on myeloma mRNA translation. While the synthesis of most polypeptides was drastically reduced at a concentration of 200 mM, that of the light-precursor, heavy, and A components seemed unaffected. The fourth condition may be tentatively identified as competition between different mRNA species. This was achieved with preparations of poly(A)-containing RNA used in almost saturating amounts, or with an excess of total polysomal RNA used at 160 mM potassium acetate. In these cases, the inhibitor is either missing or its activity greatly reduced. Again, the synthesis of the heterogeneous polypeptides and of B was reduced preferentially.

Differential mRNA translation has been well documented in the case of the rabbit reticulocyte globin RNAs. High mRNA concentrations lead to preferential β-globin synthesis (Beuzard and London, 1974; McKeehan, 1974). Increases in salt concentration (McKeehan, 1974) or addition of inhibitors of initiation, such as poly(A) (Lodish and Nathan, 1972) have similar effects. This behavior has led to the conclusion that the β-chain mRNA is more effective in initiating translation than the α-chain RNA. Thus, the Ig mRNAs in our studies (particularly the light-chain RNA) behave like the β-globin mRNA.

Precise interpretation of our results would require better understanding of the nature of the inhibitory effects. It can be concluded safely at this stage that various conditions that cause an overall reduction of exogenous polypeptide synthesis have relatively little effect on the translation of the Ig mRNAs. In this sense, the in vitro results described here are analogous to the effects observed in intact myeloma cells (Sonenshein and Brawerman, 1976). Thus, the enhancement in relative Ig synthesis observed in cells exposed to unfavorable conditions for protein synthesis can probably be attributed to some structural feature of the Ig mRNAs. An effect of specific initiation factor, although not excluded in the case of the in vivo experiments, is highly unlikely in a cell-free system derived from a plant source. The Ig mRNAs share the above characteristic with some other myeloma mRNA species, derived from free polysomes (data not shown). The studies of protein synthesis in intact cells also showed that several major polypeptides other than th heavy and light chains were relatively enhanced under the restrictive conditions (Sonenshein and Brawerman, 1976). Thus, it appears that a variety of cellular mRNAs may be particularly potent in promoting polypeptide chain initiation. One possibility that deserves further study is that this property may be a common feature of abundant proteins with specialized functions. In this respect, we have observed that the cell-free translation of the mRNA for rat liver albumin is also refractory to inhibitory conditions.

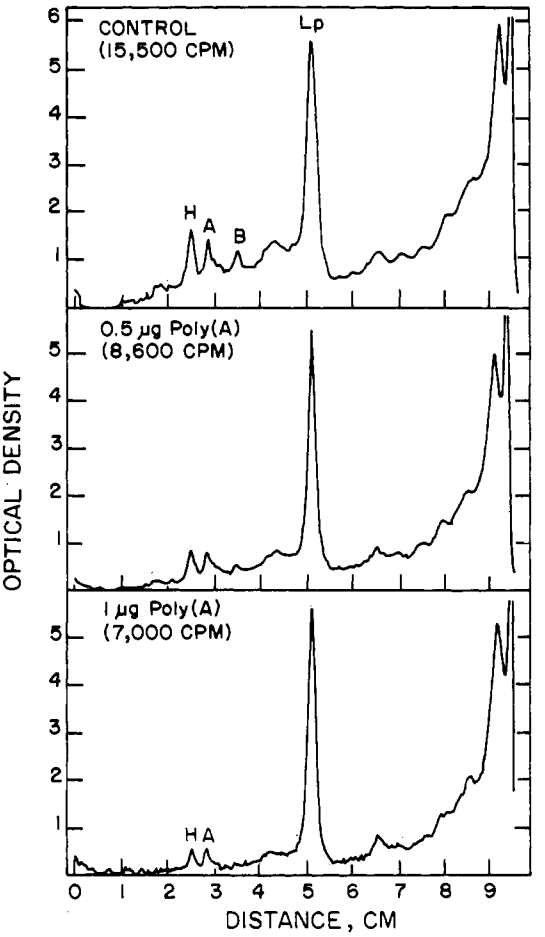

FIGURE 7: Electrophoretic analysis of the effect of poly(A) on the translation of total membrane-bound polysomal RNA. Products of reactions directed by 25 μg of membrane-bound polysomal RNA at 160 mM potassium acetate in the absence or presence of poly(A). Tracings have been adjusted to the same light-chain precursor peak height. Endogenous incorporation was 1600 cpm. With increasing poly(A) concentrations, the relative amount of Ig synthesis changed as follows: light-chain precursor (Lp): 9.8, 12.4, and 14.8%; heavy chain (H): 1.5, 1.0, and 0.7%.

Acknowledgments

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Isolation and in Vitro Translation of Zein Messenger Ribonucleic Acid†

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ABSTRACT: Zein messenger RNA was isolated from membrane-bound polyribosomes of developing maize kernels by oligo(dT)-cellulose chromatography. Translation of the mRNA in vitro yielded protein similar to native zein in amino acid content, ethanol solubility, and mobility on sodium dodecyl sulfate-polyacrylamide gels. The zein mRNA sedimented as a homogeneous peak on sucrose gradients and contained a poly(A)-rich region based upon hybridization to [^{3}H]poly(U). The mRNA had an apparent molecular weight of 540 000 on agarose-acrylamide gels. It synthesized both 21 800 and 19 000 molecular weight zein components in the wheat-germ cell-free protein synthesis system. The possibility of a polycistronic mRNA or two mRNAs of similar molecular weight is considered.

Zein, the principle storage protein in maize endosperm, is an ethanol soluble protein localized in structures called protein bodies (Wolf et al., 1967). We reported that this protein is synthesized primarily by membrane-bound polyribosomes in the developing endosperm (Larkins and Dalby, 1975), and similar results were obtained using polysomes associated with a protein body fraction (Burr and Burr, 1976).

Mutation at the *opaque*-2 locus causes a substantial reduction in zein levels and results in an increased proportion of nutritionally superior nonzein protein (Mertz et al., 1964). In addition to the reduction in total zein, the *opaque*-2 mutant is deficient in one of the major zein proteins, Z-1 (Lee et al., 1976). We reported that certain unique size classes of membrane-bound polyribosomes present in normal maize were absent in the *opaque*-2 mutant, and the Z-1 component was not synthesized in vitro by membrane-bound polyribosomes of *opaque*-2 (Jones et al., 1976). These results suggested there may be differences in the amount and type of mRNA associated with the membrane-bound polysomes of the mutant and (or) differences in the rate of mRNA translation.

We now report the successful isolation and in vitro translation of an mRNA which synthesized a protein similar to native zein. This is the first report of the purification of an mRNA coding for a seed storage protein.

Materials and Methods

Materials. [^{3}H]Poly(U)[1] (7.8 mCi/mmol) was obtained from Schwarz/Mann; [^{14}C]leucine (324 and 150 mCi/mmol), [^{14}C]lysine (150 mCi/mmol), [^{14}C]proline (125 mCi/mmol), [^{14}C]glutamic acid (125 mCi/mmol), and [^{14}C]glutamine (52 mCi/mmol) from Amersham/Searle. Ribonuclease-free sucrose and diethyl pyrocarbonate were from Sigma Chemical Co. Creatine phosphate kinase was from Calbiochem. Oligo(dT)-cellulose (type 7) was from P-L Biochemicals Inc. Omnifluor, Protosol, and Aquasol were from New England Nuclear. Hard red wheat, cv. *Centurk,* was a gift from Dr. Don M. Huber, Purdue University.

Polysome Isolation. Kernels from the maize inbred line W22 were frozen in liquid nitrogen 22 days after pollination and stored at −80 °C (Larkins et al., 1976). Groups of 30 kernels were ground in 25 ml of buffer A (200 mM Tris-HCl, pH 8.5, 200 mM sucrose, 50 mM $MgCl_2$, 60 mM KCl, and 1 mM dithiothreitol) with a chilled mortar and pestle. The extract was strained through four layers of cheesecloth and centrifuged at 500*g* for 5 min. The supernatant fraction was centrifuged at 37 000*g* for 10 min to pellet the membrane-bound polyribosomes. The 37 000*g* pellet was suspended in 5 ml of buffer A containing 1% Triton X-100 with a glass tissue homogenizer and centrifuged at 37 000*g* for 10 min. The supernatant fraction was layered over 4 ml of 2 M sucrose in buffer B (40 mM Tris-HCl, pH 8.5, 10 mM $MgCl_2$, and 20 mM KCl) and the polysomes were pelleted by centrifuging for 3 h at 229 000*g* in the 65 rotor of a Beckman L2-65 ultracentrifuge. The polysome pellet was suspended in buffer B, layered on 150–600 mg/ml sucrose gradients, and centrifuged for 40 min at 189 000*g* in a Beckman SW 50.1 rotor. Gradients were scanned at 254 nm with an ISCO Model UA-5 absorbance monitor.

Isolation of Poly(A)-Containing RNA from Polyribosomes. All solutions were autoclaved and glassware was washed with 0.1% diethyl pyrocarbonate and boiled briefly to destroy ribonuclease activity.

Poly(A)-containing mRNA was isolated by a modification of the procedure of Krystosek et al. (1975). Membrane-bound polyribosome pellets were dissolved in 10 mM Tris-HCl, pH

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[1] Abbreviations used: poly(U), poly(uridylic acid); poly(A), poly(adenylic acid); Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.