Regulation of Immunoglobulin Synthesis in Mouse Myeloma Cells†

Gail E. Sonenshein‡ and George Brawerman*

ABSTRACT: The rate of synthesis of immunoglobulin (Ig) was compared to that of other proteins in MPC-11 mouse myeloma cells subjected to various treatments. Starvation or exposure to actinomycin D caused a rapid reduction in overall protein synthesis, but affected the heavy and light Ig chains to a lesser extent. Under these conditions, which are known to interfere with polypeptide chain initiation, the synthesis of the light and heavy chains became more predominant. Cycloheximide, which inhibits chain elongation and leads to excess initiation capacity, affected Ig synthesis more severely than that of other polypeptides. The results suggest that the Ig mRNAs are particularly efficient in polypeptides chain initiation, and that this characteristic could influence the rate of Ig synthesis under certain physiological conditions.

A variety of differentiated cells synthesize large amounts of unique proteins related to their specialized functions. These proteins require a major share of the biosynthetic capacity of the cells, yet their contribution to the cells for maintenance and growth must be minimal. Thus, it is possible that their production is regulated by special control processes. This could permit a separate regulation of their production by the cells in different physiological situations. There is suggestive evidence for such specific regulation. It has been shown that the relative rate of immunoglobin synthesis increases in myeloma cells nearing the stationary phase, while the overall rate of protein synthesis decreases markedly (Kimmel, 1971). A similar behavior has been reported for the S-100 protein of brain cells (Labourdette and Marks, 1975).

In the present studies, we have attempted to define the physiological parameters that influence the relative synthesis of immunoglobulin in MPC-11 mouse myeloma cells, in order to gain insight into the mechanism of control. We observed that in cells subjected to starvation or exposed to actinomycin D the synthesis of immunoglobulin became more predominant. These conditions are known to cause an inhibition of polypeptide chain initiation (Lee et al., 1971; Goldstein and Penman, 1973). Treatment with cycloheximide, which inhibits polypeptide chain elongation and results in an increase in the capacity of the cells to initiate (Reichman and Penman, 1973), led to decreased relative synthesis of immunoglobulin. It appears from our results that the immunoglobulin heavy and light chains are synthesized preferentially when the capacity of the cells to initiate polypeptide chains is reduced. Other abundant protein species in these cells showed a similar response. Some species, on the other hand, were particularly sensitive to the restrictive conditions.

Materials and Methods

Cell Culture and Media. Two lines of mouse myeloma MPC-11 cells, the parent clone (45), and a variant (66) which synthesizes only light chain, were kindly supplied by Dr. M. Scharff. They were grown in Dulbecco's modified Eagle's medium (Difco) supplemented with 15% decomplemented horse serum and 0.12 mg/ml of streptomycin, 240 units/ml of penicillin, 6 μg/ml of fungizone, and 72 μg/ml of tylocine, an anti-PPLO agent (Soto et al., 1976). For starvation experiments, medium lacking amino acids was prepared with identical salt and vitamin composition.

Cell Lysates. Cells labeled with [^{35}S]methionine (100–300 Ci/mmol, New England Nuclear) were chilled quickly, pelleted, washed with 50 mM Tris[1] (pH 7.6)–50 mM NaCl (TN) buffer and resuspended in a small volume of the same buffer. Samples were removed for determination of hot Cl_3CCOOH-insoluble radioactivity. Triton X-100 and sodium deoxycholate were added to 1 and 0.5% final volume, respectively, to lyse the cells; lysates were diluted fivefold with TN and nuclei and debris were removed by centrifugation at 1000*g* for 10 min. Sodium dodecyl sulfate was added to a final concentration of 1% and the samples were heated to 100 °C for 1 min. After cooling, they were treated with β-mercaptoethanol at a final concentration of 75 mM for 1 h at 37 °C. To alkylate the reduced proteins, iodoacetamide was added to a concentration of 0.1 M and the incubation was continued for 30 min.

Polyacrylamide Gel Electrophoresis. Cytoplasmic extracts were subjected to electrophoresis on 12% polyacrylamide–sodium dodecyl sulfate slab gels with a 5% stacking region according to the procedure of Studier (1973). Wider-spaced combs were used in the case of large samples. The electrophoreses were run for 16 h at 27 V. The gels were stained with 0.1% coomassie blue (in 50% methanol–7.5% acetic acid), destained with a 5% methanol–7.5% acetic acid mixture and dried onto paper. Autoradiograms, produced by exposure of dried gels to Kodak No-Screen x-ray film, were scanned (λ 540 nm) with a Beckman Model DU spectrophotometer adapted with a Gilford gel scanner. The relative amounts of Ig synthesis were determined by comparing the areas under the light and heavy-chain peaks to the total area of the densitometric tracing.

Results

Patterns of Labeling of Myeloma Proteins. The rates of synthesis of individual myeloma cell proteins were estimated by subjecting extracts from pulse-labeled cells to slab gel

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

[1] Abbreviations used are: Ig, immunoglobulin; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol.

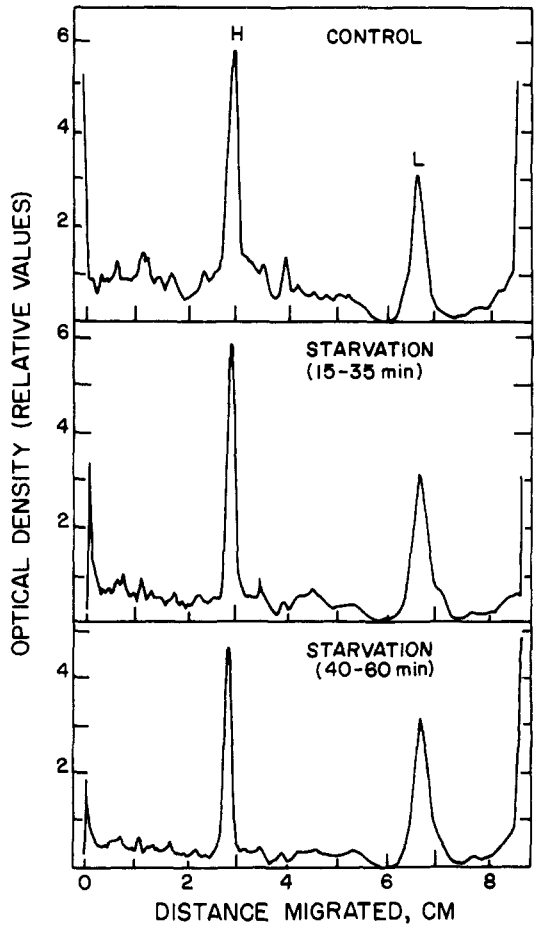

FIGURE 1: Synthesis of cytoplasmic proteins in starved MPC-11 45 cells. Cells of the parent line MPC-11 45, starved by incubation in medium lacking amino acids and serum, were labeled during the indicated time periods with 10 μCi/ml of [^{35}S]methionine. Untreated control cells were similarly labeled for 20 min. Cell extracts were processed as described under Materials and Methods. Autoradiograms of gels were analyzed by densitometric scanning. Scales of recorder tracings were adjusted so as to produce constant height for the light-chain band. The proportions of Ig synthesis for the control cells and for the time periods of starvation were as follows. Light (L) chain: 13, 20, and 27%; heavy (H) chain: 13, 18, and 19%.

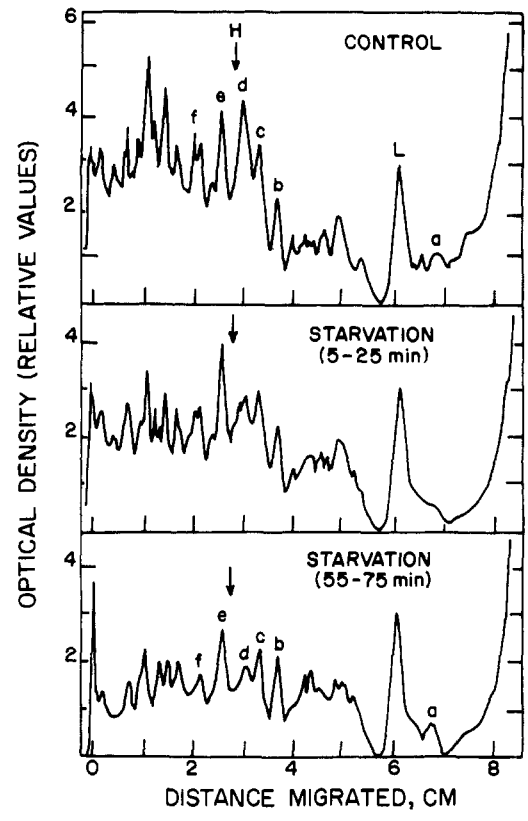

FIGURE 2: Synthesis of cytoplasmic proteins in starved cells deficient in heavy-chain production. Cells of the variant line MPC-11 66 were treated as in Figure 1. The proportions of light-chain synthesis for the control cells and for the time periods of starvation were as follows: 3, 4.5, and 5.5%. H, heavy chain; L, light chain.

electrophoresis in the presence of sodium dodecyl sulfate, followed by autoradiography of the resolved components. The cell suspensions were labeled with [^{35}S]methionine for 20 min or less to avoid release of the immunoglobulin into the medium (Scharff et al., 1967). The proteins of the extract were reduced and alkylated before electrophoresis to separate the light and heavy Ig chains (see Materials and Methods). Autoradiograms were analyzed by densitometry to obtain an objective estimate of the relative amounts of labeling in individual bands. Except where mentioned, the scale of the tracings was adjusted so as to produce a constant peak height for the light chain. By doing so, it was possible to compare more easily the rate of synthesis of individual polypeptides.

The heavy- and light-chain bands were identified by comparison with the mobilities of the Ig polypeptides released into the medium by normal-growing cells. The identity of the latter polypeptides as heavy and light chains was verified by immune precipitation with rabbit antisera to mouse Ig. Under our conditions of electrophoresis, the light chain was well resolved and many individual polypeptide bands were visible over a large background. This background probably represents the labeling of a highly heterogeneous mixture of components present in small amounts in the cell, while the prominent bands represent abundant polypeptide species. The small protein species (<16 000 daltons) migrated with the dye front and were therefore not included in this analysis.

Effect of Starvation on Immunoglobulin Synthesis. Lee et al. (1971) have shown that incubation of mouse sarcoma 180 ascites cells in the absence of nutrients leads to an inhibition of polypeptide chain initiation in these cells. This was evidenced by an accumulation of ribosomal monomers and of messenger ribonucleoprotein particles. Depletion of some amino acids was not the direct cause of the inhibition of protein synthesis, since ribosome runoff was taking place in the starving cells. We observed that the mouse myeloma cells are affected in the same fashion by this treatment. Starvation for 60 min led to nearly complete loss of polysomes (data not shown).

Figure 1 shows the patterns of cytoplasmic protein synthesis in MPC-11 45 cells incubated in complete medium and in the same medium from which amino acids and serum were omitted. Pulse labeling with [^{35}S]methionine was for 20 min in each case. After 15 min of starvation, the cells showed an increased capacity to synthesize heavy and light chains relative to other cellular proteins. This effect was even more pronounced by 40 min. The amount of labeling of the heavy chain relative to the light chain did not seem to change significantly during starvation. The extent of synthesis of Ig chains in control cells tended to vary (compare Figures 1 and 3). The shifts in labeling patterns produced by the various treatments (see below), however, were observed consistently, regardless of the initial level of Ig synthesis in the control cells.

The effect of starvation was also studied with the MPC-11 66 variant cell line deficient in heavy chain production. In these

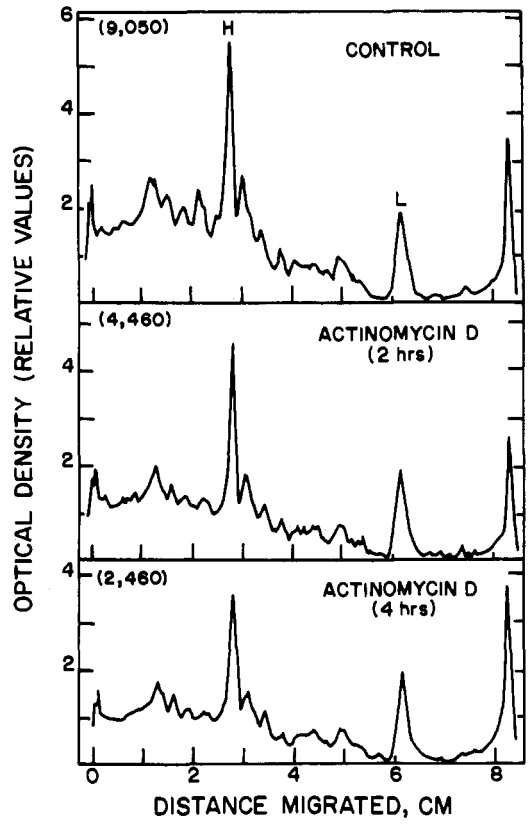

FIGURE 3: Synthesis of cytoplasmic proteins in actinomycin-treated parent cells. Cells of the MPC-11 45 line were incubated in the absence or presence of 4 μg/ml of actinomycin D for the times indicated and then labeled for 20 min with 50 μCi/ml of [^{35}S]methionine. The values in parentheses indicate the hot Cl_3CCOOH-insoluble cpm in aliquots of washed-cell suspension. The proportions of Ig synthesis for the control cells and for the time periods of actinomycin D treatment were as follows. Light (L) chain: 6.2, 7.9, and 8.6%; heavy (H) chain: 6.5, 8.0, and 7.5%.

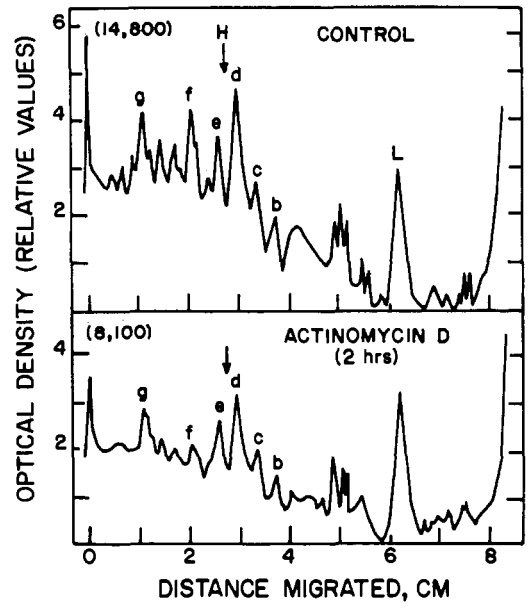

FIGURE 4: Synthesis of cytoplasmic proteins in actinomycin-treated variant cells. MPC-11 66 cells were treated as in Figure 3. The proportions of light-chain synthesis for the control and treated cells were 5.9 and 9.0%, respectively. H, heavy chain; L, light chain.

cells, light-chain synthesis represents a considerably smaller portion of cellular protein synthesis (Figure 2). This, combined with the absence of the prominent heavy band, permitted the analysis of labeling patterns of other polypeptides. Again, the relative amount of light-chain synthesis increased markedly during starvation. Several other abundant protein species seemed equally resistant to the starvation treatment (see bands a, b, c, and e in Figure 2), while the synthesis of some major components was considerably reduced (band f). Band d was nearly eliminated. The background labeling, presumably representing a mixture of minor protein species, was highly sensitive to this treatment.

Protein Synthesis in Actinomycin D Treated Cells. Goldstein and Penman (1973) have shown that the rapid inhibition of protein synthesis in HeLa cells exposed to high levels of actinomycin D is not due to mRNA decay. Their data suggest that polypeptide chain initiation is specifically affected in the treated cells. We examined the effect of actinomycin D (4 μg/ml) on protein synthesis in the myeloma cells and observed changes similar to those produced during starvation. Figure 3 shows the effect of the drug on the parent cells. The capacity of the cells to synthesize protein decreased rapidly. Ig synthesis became gradually more predominant in the course of the treatment. Heavy chain synthesis seemed somewhat more sensitive, as evidenced by the decrease in the heavy/light chains labeling ratio after 4 h of treatment.

Again, the use of the MPC-11 66 cells permitted a more detailed analysis of protein species other than the Ig polypeptides. The changes in labeling pattern after a 2-h exposure to actinomycin D are shown in Figure 4. A 4 h exposure to the drug caused a further reduction in the rate of protein synthesis, but produced very little additional change in the labeling pattern. The labeling of bands b, c, and e, which was relatively resistant to the starvation treatment, showed a similar response to the actinomycin D treatment. Band d, however, was highly sensitive to starvation but showed substantial resistance to the actinomycin treatment. Band f was equally sensitive to both treatments.

The response of the cells to actinomycin D proved to be highly dependent on the temperature of incubation. Treatment at temperatures slightly below 37 °C had almost no effect on overall protein synthesis and caused little or no change in the labeling profiles. Inhibition of protein synthesis and relative increase in light- ane heavy-chain labeling were obtained at temperatures between 37 and 39 °C. This dependence of inhibition of protein synthesis on temperature of incubation had been observed by Goldstein and Penman (1973).

Effect of Cycloheximide. Reichman and Penman (1973) have shown that extracts of HeLa cells previously subjected to cycloheximide acquire an enhanced capacity for polypeptide chain initiation. Thus, immediately after recovery from this treatment there should be a reduction in the relative rate of synthesis of polypeptides normally initiated with high efficiency. This was observed in the case of immunoglobulin synthesis in the myeloma cells.

Incubation of cells with 0.75 μg/ml of cycloheximide for 90 min caused a 70–80% reduction in the rate of protein synthesis. The pattern of labeled bands was altered drastically (Figure 5). Removal of the drug caused a rapid increase in protein synthesis to levels somewhat higher than in control cells. In the recovering cells from the parent line, there appeared to be a slight reduction in the relative labeling of the heavy and light bands (data not shown). The effect of recovery on the labeling pattern was more pronounced in the case of the MPC-11 66 cells (Figure 5). There was a marked increase in the "heterogeneous" labeling, as well as in the amount of several major components. The enhancement was particularly striking in the

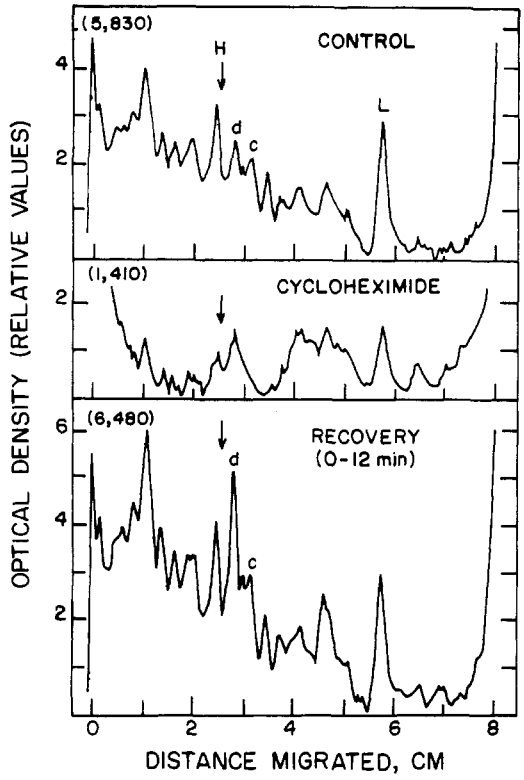

FIGURE 5. Synthesis of cytoplasmic proteins in cycloheximide-treated and recovering variant cells. MPC-11 66 cells were incubated in the presence of 0.75 μg/ml of cycloheximide for 90 min. A portion of the cells was then washed and resuspended in fresh medium. Labeled methionine (50 μCi/ml) was added to the cells after the initial period of cycloheximide treatment and to the washed cells resuspended in fresh medium. Labeling was for 12 min. Control cells were incubated for 90 min in medium lacking cycloheximide, then subjected to the same washing procedure and labeled under the same conditions as the cycloheximide-treated recovering cells. Values in parentheses indicate hot Cl_3CCOOH-insoluble counts in aliquots of suspensions of washed cells. Scales of recorder tracings were adjusted as in other figures, except for samples of cycloheximide-treated cells. The proportions of light-chain synthesis for the control and recovering cells were 7.2 and 5.1%, respectively. H, heavy chain; L, light chain.

case of the d band, which was greatly reduced during the starvation treatment. An additional band between c and d became visible. The patterns of labeling became nearly normal after 30 min of recovery, except for the d band which was still unusually high (data not shown).

Discussion

The results in this study indicate that the relative rates of synthesis of individual proteins can be made to vary by altering the physiological state of the cell. The effects described here are most probably at the level of mRNA translation. It is unlikely that differential mRNA stability plays a significant role in the observed variations in rates or protein synthesis. The effects produced by starvation appear very rapidly. Moreover, starvation does not lead to any obvious mRNA destruction. The mRNA released from polysomes is stored as nucleoprotein particles, and refeeding leads to rapid and complete reconstitution of polysomes. This recovery takes place even in cells pretreated with high levels of actinomycin D to prevent new mRNA synthesis during starvation (Lee et al., 1971). In the actinomycin D treatments described in the present report, significant mRNA decay could have conceivably taken place, particularly among species of low metabolic stability. If this were a significant factor in the differential effects on protein synthesis observed here, then the effects should have become gradually more pronounced with increasing time of exposure. This was not observed in the experiment described in Figure 4. Also, the differential actinomycin effect was highly dependent on the temperature of incubation. Such a temperature sensitivity has not been described for the effect of actinomycin D on transcription. Since starvation and actinomycin D treatment had a similar effect on immunoglobulin synthesis, and since both treatments are known to affect polypeptide chain initiation, it seems reasonable to deduce that the changing patterns of protein synthesis were due to differential inhibition of the initiation step.

The above effects can be explained by assuming that the immunoglobulin mRNAs are translated more effectively than other mRNAs, owing to a particularly high efficiency of initiation. Under conditions unfavorable for initiation, the Ig RNAs would be affected less seriously than other RNAs with lower initiation efficiency. The high efficiency of the Ig mRNAs could be due either to the existence of special initiation factors, or to an inherent property of the RNA molecules. The latter possibility appears more likely, since the Ig mRNAs also show a greater potency for initiation in wheat germ extracts (Sonenshein and Brawerman, 1976). Other examples of mRNA with high initiation potency have already been described (Lodish, 1971; Stewart et al., 1973).

The effects of cycloheximide are compatible with the above interpretation. Cells recovering from exposure to this drug have been shown to be enriched in initiation capacity (Reichman and Penman, 1973). This should tend to favor the less effective mRNAs. This effect was clearly evident in the variant cell line, where the relative rate of light-chain synthesis was markedly reduced after removal of cycloheximide (Figure 5). In the case of the parent line, however, the effect was marginal.

Differential effects on the rates of protein synthesis were also observed in the present study with other abundant polypeptide species. Some were as resistant as the Ig polypeptides to the inhibitory effects, while others were particularly sensitive. In most cases, the effects of starvation and actinomycin were parallel. One major band, however, showed high sensitivity to starvation but was resistant to actinomycin. Thus, factors other than differential initiation efficiency must govern the synthesis of some proteins.

It is not clear whether the response of immunoglobulin synthesis to treatments that restrict initiation is indicative of a physiologically meaningful process, or is merely fortuitous. While continued production of proteins for secretion under unfavorable conditions is not to the cell's advantage, it may be of benefit to the organism as a whole. We have observed that the mRNA for rat liver albumin is also particularly potent in initiation in vitro. It could be that this is a general feature of the synthesis of proteins for specialized functions. More studies will be required to verify this possibility.

Note Added in Proof

It was reported recently that the relative rate of immunoglobulin synthesis is enhanced when polypeptide chain initiation is inhibited by incubation of mouse myeloma cells in high salt (Nuss, D. L., and Koch, G. (1976), *J. Mol. Biol. 102*, 601). The authors conclude, as we do, that the immunoglobulin mRNA is particularly effective in translation.

Acknowledgments

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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

† From the Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111. *Received April 28, 1976.* This work was supported by research grants from the United States Public Health Service (GM 17973, CA 12924) and the American Cancer Society, Massachusetts Division.

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

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