Proc. Nat. Acad. Sci. USA
Vol. 73, No. 3, pp. 857–861, March 1976
Cell Biology

Novel mechanism for translational control in regulation of ferritin synthesis by iron

(liver ferritin mRNA and messenger ribonucleoprotein/response to iron/cordycepin/actinomycin)

J. ZÄHRINGER, B. S. BALIGA, AND H. N. MUNRO

Laboratory of Physiological Chemistry, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass. 02139

Contributed by H. N. Munro, December 29, 1975

ABSTRACT **Poly(A)-containing RNA was isolated from the polyribosomal and post-ribosomal fractions of the livers of normal and iron-treated rats. These RNA fractions were then translated in a wheat germ system to provide a measure of the amount of ferritin mRNA present in each fraction. Following iron administration, there was a 2-fold increase in the amount of ferritin mRNA in the polyribosomal fraction. This increase was not inhibited by prior treatment of the rats with actinomycin D or cordycepin, suggesting a cytoplasmic control mechanism. In normal rats, the post-ribosomal fraction contained an amount of ferritin mRNA equal to that in the polyribosomes. When iron was administered, this untranslated ferritin mRNA became reduced to negligible quantities, thus accounting for the doubling of the ferritin mRNA content of the polyribosomal fraction. A scheme is proposed in which translation of the ferritin mRNA in the post-ribosomal fraction is prevented by adhering ferritin subunits. Iron administration removes this inhibition of the translation of ferritin mRNA by promoting aggregation of these subunits into ferritin.**

Evidence from many tissues shows that not all cytoplasmic mRNA is associated with polyribosomes (1–19). This pool of free untranslated cytoplasmic mRNA constitutes 10–30% of the total cytoplasmic mRNA content of a number of tissues (8, 10, 15, 17–19) and in some others even accounts for 50–80% (1, 3, 4, 11). It is generally believed to represent a storage of precursor form of the polyribosomal mRNA. Recently, several individual mRNAs, coding for specific proteins, have been identified in the post-ribosomal supernatant of a variety of tissues, amongst them the mRNAs coding for actin (10), ferritin (18), the α-chain of hemoglobin (14, 15), myosin (9, 11), and vesicular stomatitis virus proteins (8). Nevertheless, no precise function for any one of these post-ribosomal mRNAs has been demonstrated so far.

Since the early observations of Granick *et al.* (20), it has been shown by many authors that iron increases the synthesis of ferritin in many different tissues (21–30). In rat liver we have recently demonstrated that this iron-induced increase in the synthesis of ferritin is associated with a 2-fold increase in the polysomal ferritin mRNA content (31). This result might be caused by either more extensive transcription of mRNA, increased processing of ferritin mRNA precursor to ferritin mRNA, increased transport of ferritin mRNA from the nucleus to the cytoplasm, the presence of an untranslated pool of ferritin mRNA in the cytoplasm of normal rat liver that becomes available for translation upon iron-treatment, or, finally, increased stability of ferritin mRNA. We report here experiments which distinguish between these possibilities and which suggest that iron administration leads to the mobilization of the ferritin mRNA from the post-ribosomal to the polyribosomal fraction of the liver cell.

Abbreviations: mRNP, messenger ribonucleoprotein; hnRNA, heterogenous nuclear RNA.

MATERIALS AND METHODS

Materials. L-[4,5-^{3}H(N)]Leucine, specific activity 30–50 Ci/mmol, and Protosol were obtained from New England Nuclear oligo(dT)-cellulose, from Collaborative Research; actinomycin D, from Calbiochem; and cordycepin, grade 3, from Sigma Chemical Co. Wheat germ was kindly provided by Dr. B. Roberts (Massachusetts Institute of Technology); purified rat liver ferritin and ferritin antibody, by Dr. Maria Linder (Massachusetts Institute of Technology).

Animals. Male Sprague-Dawley rats (90–100 g) were fasted overnight before each experiment. Ferric ammonium citrate (400 μg/100 g of body weight) was administered intraperitoneally 3 hr before killing. Actinomycin D (150 μg/100 g of body weight) and cordycepin (1.5 mg/100 g) were given intraperitoneally, both at 4 and 2 hr before killing.

Polyribosomes and pH 5 Enzymes. Rat liver polyribosomes and pH 5 enzymes were prepared essentially as described (32).

Liver Polyribosomal and Post-Ribosomal RNA. Twenty pooled rat livers were homogenized in 2 volume of 0.1 M Tris·HCl, pH 7.6, 0.1 M NaCl, 0.1 mM EDTA, 5 mM $MgCl_2$, 150 μg/ml of heparin, 0.25 M sucrose, and centrifuged at 10,000 × g for 20 min. The resulting post-mitochondrial supernatant was filtered through glass wool and centrifuged at 42,000 rpm for 50–70 min in the Beckman Ti 50.1 rotor. The post-ribosomal supernatant was filtered through glass wool, diluted with 0.5 volume of 0.1 M Tris·HCl, pH 7.4, 0.1 M NaCl, 1 mM EDTA, and 50 μg/ml of heparin and made 1% in sodium dodecyl sulfate and 1 mM in EDTA. The pH was adjusted to 9.0 with 1 M NaOH prior to phenol extraction (33). Analysis on sucrose density gradients showed ribosome subunits to be nearly absent (18), and this was confirmed by sucrose gradient analysis of post-ribosomal RNA.

Total polyribosomal RNA was isolated from the microsomal pellets essentially as described (33). Before adsorption onto oligo(dT)-cellulose, the samples were routinely heated for 5–8 min at 55–60°. Poly(A)-containing RNA was obtained by affinity chromatography on oligo(dT)-cellulose as described (18).

Cell-Free Incubations. Rat liver polysomes were incubated in the presence of pH 5 enzymes and [^{3}H]leucine as described (32). The final concentration of polysomes in the reaction mixture was 1.5 mg/ml. From an incubation volume of 75 μl, 50 μl were used for immunoprecipitation of the synthesized ferritin chains after addition of 7.5 μg of carrier ferritin (31) and 5 μl for assay of total radioactivity in trichloroacetic-acid-precipitable material (34).

Rat liver polyribosomal and post-ribosomal poly(A)-containing RNA were translated in a cell-free system prepared from wheat germ as described (35) except for the omission of the preincubation step. The standard protein synthesis

assay contained in a final volume of 25 μl, 10 μl of wheat germ S_{30}, 28 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes), pH 7.0, 1 mM ATP, 0.2 mM GTP, 8 mM creatine phosphate, 2 mM dithiothreitol, 8 μg/ml of creatine kinase, 25 μM appropriate unlabeled amino acids, 4 μl of [^{3}H]leucine, 80 mM KCl, 3 mM magnesium acetate, 40 μM spermine, and 0.5 μg of mRNA (half-saturating for the system). After incubation at room temperature (21–25°) for 3 hr, radioactivity incorporation into total protein was determined according to Mans and Novelli (34).

Product Analysis. To analyze the products synthesized under the direction of polyribosomal and post-ribosomal mRNA for synthesis of ferritin, wheat germ reaction mixtures were increased to 125 μl. After incubation, 7.5 μg of carrier ferritin were added and immunoprecipitation and purification of the immunoprecipitate were performed as described (31). The purified immunoprecipitate was dissolved in 25 μl of a solution containing 0.062 M Tris·HCl, pH 6.8, 2% sodium dodecyl sulfate, 10% glycerol (vol/vol), and 5% 2-mercaptoethanol, boiled at 100° for 1–5 min and analyzed on a sodium dodecyl sulfate-polyacrylamide gradient (10–15%) gel, which was prepared and run as described by Maizel (36). The gel was stained to show protein bands, sliced into 3.3 mm pieces, which were placed into vials containing 100 μl of H_2O_2, and incubated at 50° overnight. Then 700 μl of Protosol were added and incubation was continued for 1 hr. After cooling, each vial was counted with 10 ml of toluene/2,5-diphenyloxazole (PPO)//(phenyloxazolylphenyl-oxazolylphenyl (POPOP). Alternatively, the H_2O_2-digested gel pieces were counted directly with 10 ml of Aquasol.

RESULTS

Effect of Actinomycin D and Cordycepin on the Iron-Induced Increase in the Polysomal Ferritin mRNA Content. One possible cause for the previously observed increase in the polysomal ferritin mRNA content in the liver of iron-treated rats (31) could be that iron specifically increased transcription of heterogeneous nuclear RNA (hnRNA) containing ferritin mRNA from the corresponding DNA region.

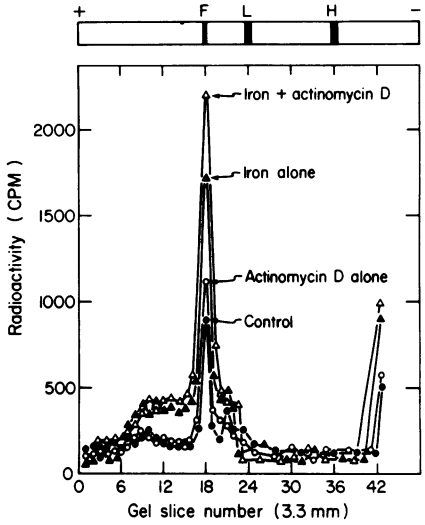

FIG. 1. Effect of actinomycin D on the iron-induced increase in the polyribosomal ferritin mRNA content. Polyribosomal mRNA was isolated from four groups of animals (four rats per group) that had received either saline (controls, ●), actinomycin D (○), iron (▲), or iron plus actinomycin D (△). For doses, injection times, and routes see under *Materials and Methods*. The isolated mRNAs were translated in the wheat germ system and assayed for their ferritin mRNA content as described in *Materials and Methods*. Before immunoprecipitation the incubated wheat germ mixture contained as total trichloroacetic-acid-precipitable radioactivity: 812,000 cpm (controls); 817,000 cpm (actinomycin D alone); 823,000 cpm (iron alone); 831,000 cpm (iron plus actinomycin D). F = ferritin; L = light chain; H = heavy chain of antibody.

Earlier studies employing actinomycin D gave contradictory results (24, 26, 28, 37, 38). We have reexamined this possibility by isolating liver polysomes from normal and iron-treated rats that have received either saline (controls) or actinomycin D and assaying them *in vitro* for their ferritin mRNA content. Fig. 1 and Table 1 show that actinomycin D, when used in concentrations that are known to effectively block the synthesis of mRNA (27, 28), not only failed to prevent the stimulatory effect of iron on the polysomal ferritin mRNA level but even tended to increase it, which may be

Table 1. Effect of actinomycin D and cordycepin on the synthesis of ferritin by rat liver polysomes and polyribosomal mRNA from normal and iron-treated rats

Animal group	Incorporation/incubation: Total protein (cpm)	Incorporation/incubation: Ferritin (cpm)	Ferritin × 100 / Total protein
Polysomes			
Normal	318,000	348	0.11
+ actinomycin	325,000	445	0.14
+ cordycepin	306,000	330	0.11
Iron	330,000	757	0.23
+ actinomycin	336,000	878	0.26
+ cordycepin	328,000	788	0.24
mRNA			
Normal	749,000	715	0.10
+ actinomycin	732,000	929	0.13
+ cordycepin	761,000	680	0.09
Iron	789,000	1499	0.19
+ actinomycin	764,000	1819	0.24
+ cordycepin	737,000	1539	0.21

Administration of saline (normals), iron, actinomycin D, and cordycepin to each animal group (four rats per group) was as described in *Materials and Methods*. Isolation, translation, and assay for ferritin synthesis of polysomes and polyribosomal mRNA were as described under *Materials and Methods*. Each value represents the average of three different polysome or mRNA preparations, with each preparation being assayed four times for its ferritin mRNA content.

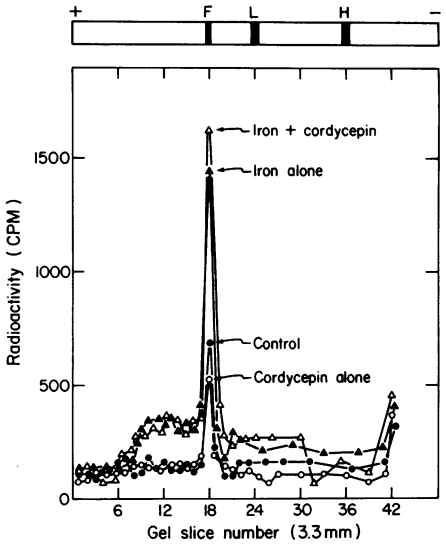

FIG. 2. Effect of cordycepin on the iron-induced increase in the polyribosomal ferritin mRNA content. For experimental details see under *Materials and Methods* and in the legend to Fig. 1 (use of cordycepin instead of actinomycin D). Total acid-precipitable radioactivity before immunoprecipitations: 748,000 cpm (controls, ●); 669,000 cpm (cordycepin alone, ○); 775,000 cpm (iron alone, ▲); 781,000 cpm (iron plus cordycepin, △). Abbreviations as in Fig. 1.

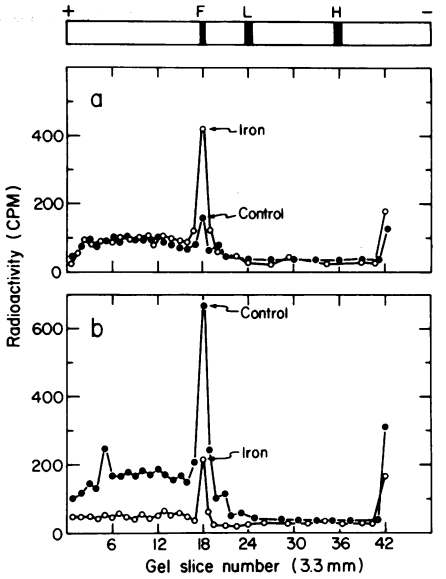

FIG. 3. Synthesis of ferritin by rat liver polyribosomal (a) and post-ribosomal (b) poly(A)-containing RNA from normal and iron-treated rats. For experimental details see under *Materials and Methods*. Before immunoprecipitation the incubated wheat germ mixture contained in a final volume of 30 μl as total acid-precipitable radioactivity: 169,000 cpm (control, a, ● = polyribosomal mRNA from normal rats); 211,000 cpm (iron, a, ○ = polyribosomal mRNA from iron-treated rats); 136,000 cpm (control b, ● = post-ribosomal mRNA from normal rats); 141,000 cpm (iron, b, ○ = post-ribosomal mRNA from iron-treated rats). Abbreviations as in Fig. 1.

attributed to the well-known super-induction effect exerted by actinomycin D (39, 40).

In order to corroborate this further and to evaluate the possibility of increased conversion of hnRNA to ferritin mRNA and increased transport into the cytoplasm, similar experiments were performed using cordycepin as an inhibitor which allegedly inhibits addition of poly(A) to the mRNA while it is still covalently linked to the rest of the hnRNA molecule, thereby preventing the newly synthesized mRNA from appearing in the cytoplasm (41, 42). In concentrations that were shown by Tilghman *et al.* (39) to be adequate to block the appearance of mRNA in the cytoplasm, cordycepin failed to prevent the iron-induced 2-fold increase in the polysomal ferritin-mRNA content (Fig. 2 and Table 1). This result, together with the evidence from the actinomycin D experiment, strongly indicates that the mechanism by which iron exerts its stimulatory action on the synthesis of ferritin is confined to the cytoplasm. Finally, the observed stability of ferritin mRNA over the time period tested (4 hr) makes it unlikely that iron acts through stabilization of otherwise unstable ferritin mRNA.

Synthesis of Ferritin by Liver Polyribosomal and Post-Ribosomal mRNAs from Normal and Iron-Treated Rats. Since the early experiments of Spirin and coworkers (1), many authors have demonstrated that in a variety of tissues 10–30% of the total cytoplasmic poly(A)-containing RNA can be found in untranslated form in the post-ribosomal supernatant (8, 10, 15, 17–19). In some special tissues, such as loach embryos, embryonic chick muscle, and cultured animal cells, this may even be as much as 50–80% (1, 3, 4, 11). No specific function of any one of these specific untranslated mRNAs has been demonstrated so far.

Since our experiments with actinomycin D and cordycepin indicate a cytoplasmic mechanism in the regulation of ferritin synthesis, we isolated polyribosomal and post-ribosomal poly(A)-containing RNA from the livers of normal and iron-treated animals and assayed them in the wheat germ system for their ferritin mRNA content. As seen in Fig. 3 and Table 2, post-ribosomal mRNA from normal rat liver directed the incorporation of 4–6 times as much radioactivity into ferritin, if expressed as a percentage of the incorporation into total liver protein, as did mRNA from the polyribosomal fraction of normal rat liver. In iron-treated animals, this ratio dropped to 0.6–0.7 times. Despite the considerable variation in the template activity of the post-ribosomal mRNA preparations (normal rat livers: 189,000–695,000 cpm; iron-treated rat liver: 261,000–554,000 cpm), very little variation was observed in the ratio: incorporation of radioactivity into ferritin/incorporation of radioactivity into total protein (Table 2, last column) within the two types of post-ribosomal mRNA preparations (normal rat liver: 0.39–0.56; iron-treated rat liver: 0.11–0.13). Bester *et al.* (43) have demonstrated the presence of translational control RNA (tcRNA) in the post-ribosomal supernatant from embryonic chick muscle and have shown that it inhibits the translation of the corresponding messenger ribonucleoprotein (mRNP). We have preliminary evidence that some of our post-ribosomal mRNA preparations contained variable amounts of translational control RNA which would explain the observed differences in their activities in the wheat germ system.

Distribution of Ferritin mRNA in the Cytoplasm. We have previously shown (18) that the post-ribosomal supernatant of normal rat liver contained 15% of the total poly(A)-containing RNA present in the cytoplasm, while the remaining 85% was contributed by the polysomal mRNA pool. Similar values (14% versus 86%) were obtained for the distribution of the total poly(A)-containing RNA in the cytoplasm of the liver of iron-treated rats. Using these data and the data given in Table 2, it can be calculated that the polyribosomal fraction in the livers of normal rats contains 56% of the total cytoplasmic ferritin mRNA while the post-ribosomal supernatant fraction contains 44% (Table 3). In the livers from iron-treated rats, those values change dramatically to 91%

Table 2. Synthesis of ferritin by rat liver polyribosomal and post-ribosomal mRNA

Type of mRNA	Incorporation/incubation: Total Protein (cpm)	Incorporation/incubation: Ferritin (cpm)	Ferritin × 100 / Total protein
Polyribosomal			
Normal	749,000	715	0.10 ± 0.01
Iron	769,000	1499	0.19 ± 0.02
Post-ribosomal			
Exp. 1–3: Normal	248,000	967	0.39
Iron	261,000	339	0.13
Exp. 4–5: Normal	695,000	2919	0.42
Iron	513,000	561	0.11
Exp. 6: Normal	189,000	1058	0.56
Iron	554,000	730	0.13

Rat liver polyribosomal and post-ribosomal mRNA were isolated from normal and iron-treated rats, translated in the wheat germ system, and assayed for their ferritin mRNA content as described in *Materials and Methods*. Incubation volumes were 125 μl. Values for the polyribosomal mRNAs are the average of six different preparations for each type of mRNA with all 12 mRNA preparations directing the incorporation of 730,000–790,000 cpm into total protein. Background incorporation (= no exogenous mRNA added) was generally between 19,000 and 27,000 cpm. Since the template activity of the corresponding six post-ribosomal preparations varied from preparation to preparation, these values were grouped into three groups according to their capacities to stimulate radioactivity incorporation into total protein. The given values are the average of the indicated preparations. All preparations were assayed four times for their ferritin mRNA content. Incubations were done with half-saturating mRNA amounts (based on assays of each mRNA sample at various concentrations in the wheat germ system). Similar results were obtained using saturating mRNA amounts.

for the ferritin mRNA found in the polyribosomal fraction and 9% for the ferritin mRNA present in the post-ribosomal fraction. Thus, in the liver of iron-treated rats, a redistribution of the cytoplasmic ferritin mRNA takes place with the disappearance of ferritin mRNA from the post-ribosomal fraction and a corresponding increase in the polyribosomal ferritin mRNA content.

DISCUSSION

Ferritin, the major intracellular iron storage protein, is found in many tissues and organisms (20–30). Its level in the liver can be substantially increased by raising the intracellular concentration of iron (28). We have previously shown that iron increases the polysomal ferritin mRNA content (31) and have concluded that iron stimulates ferritin synthesis by increasing the amount of ferritin mRNA in polyribosomes.

Here, we show that both actinomycin D and cordycepin fail to prevent this iron-induced increase in the polysomal ferritin-mRNA content, suggesting the operation of a cytoplasmic control mechanism. We were able to positively identify this mechanism by demonstrating that iron treatment causes a dramatic decrease in the post-ribosomal ferritin mRNA pool with a corresponding increase in the polyribosomal ferritin mRNA pool. Thus, it appears that a large pool of repressed ferritin mRNA (equal to almost 50% of the total cytoplasmic ferritin mRNA content) is present in the post-ribosomal supernatant of normal rat liver. This pool becomes derepressed in the liver of iron-treated animals and then becomes available for initiation, polysome formation, and ferritin synthesis. A model (Fig. 4) based on these experiments is proposed to explain the manner by which iron specifically increases the amount of polysomal ferritin mRNA and thereby stimulates ferritin synthesis. We suggest that in

Table 3. Distribution of ferritin mRNA between polyribosomes and post-ribosomal supernatant

Animal group	Percentage of ferritin mRNA found in: Polyribosomes	Post-ribosomal supernatant
Normal	56	44
Iron	91	9

The values shown were calculated on the basis that, in normal rat liver, 15% of the total cytoplasmic poly(A)-containing RNA is present in the post-ribosomal supernatant and 85% in the polysomal fraction (18). The proportions were 14% and 86% for the iron-treated animals. From these percentages and the data given in Table 2 for the proportion of ferritin mRNA of the total translated mRNA in each fraction, we calculated the total amount of ferritin mRNA in each fraction. Thus, for the liver of normal rats, the polyribosomal fraction contained 56% of the total cytoplasmic ferritin mRNA [(0.10 × 85%)/(0.10 × 85% + 0.46 × 15%)] while the post-ribosomal fraction contained 44% [(0.46 × 15%)/(0.10 × 85% + 0.46 × 15%)].

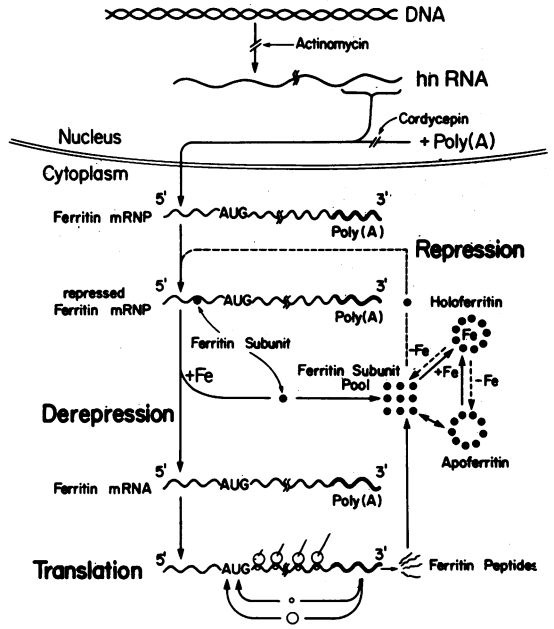

FIG. 4. A proposed model for the translational control mechanism by which iron specifically regulates the synthesis of ferritin.

normal liver one or more ferritin subunits are attached to a sequence near or at the 5′-end of the ferritin mRNP which can be specifically recognized by ferritin subunits, thus preventing the ferritin mRNP from accepting ribosomal subunits and engaging in ferritin synthesis. Upon iron treatment, this(these) ferritin subunit(s) becomes freed from the mRNA by conversion into holoferritin and the derepressed ferritin mRNP can now engage in initiation and ferritin synthesis. Additional evidence in support of the model is: (*a*) The finding by Chu and Fineberg (26) that iron stimulates ferritin synthesis in HeLa cells almost immediately (within 10 min), thus requiring a mechanism that is able to respond very fast to added iron. Induction of other proteins via transcriptional control mechanisms generally requires much more time. (*b*) The finding that cycloheximide completely abolishes the stimulating effect of iron on ferritin synthesis (26, 27), thus demonstrating that protein synthesis is required for the iron effect. (*c*) The identification by Lee *et al.* (44) of free ferritin subunits in the cytoplasm of hepatoma cells in culture and their aggregation into ferritin on addition of iron to the medium. (*d*) The observation by Drysdale and Shafritz (45), that, *in vitro*, iron stimulates the conversion of ferritin subunits into holoferritin, which in our model would exert a trigger function by converting free cytoplasmic ferritin subunits into holoferritin, thus favoring the detachment of the ferritin-mRNA-bound ferritin subunit which is in equilibrium with the free ferritin subunit pool. (*e*) The reported nucleotide sequence heterogeneity of the 5′-terminus of viral mRNA (46) and myeloma cell mRNA (47) that could provide the specific structural basis which is necessary for the specific interaction between ferritin mRNA and ferritin subunits. The location of this binding site at the 5′-terminus and prior to the AUG codon would probably provide the blocking effect on initiation. (*f*) The demonstration by Jay and Kaempfer (48) that an *Escherichia coli* host protein specifically binds to the viral R17 RNA, thereby preventing it from initiation and translation.

This investigation was supported by Grant no. 15364-05 from the U.S. Public Health Service and by a research fellowship from the Deutsche Forschungsgemeinschaft to J.Z.

1. Spirin, A. S. (1969) *Eur. J. Biochem.* **10**, 20–35.
2. Lee, S. Y., Krsmanovic, V. & Brawerman, G. (1971) *Biochemistry* **10**, 895–900.
3. Enger, M. D., Campbell, E. W. & Hanners, J. L. (1975) *FEBS Lett.* **55**, 194–197.
4. Spohr, G., Granboulan, N., Morel, C. & Scherrer, K. (1970) *Eur. J. Biochem.* **17**, 296–318.
5. Schochetman, G. & Perry, R. P. (1972) *J. Mol. Biol.* **63**, 577–590.
6. Schultz, G. A., Chen, D. & Katchalski, E. (1972) *J. Mol. Biol.* **66**, 379–390.
7. Knöchel, W., Tiedemann, H. & Fellmann, I. (1972) *Biochim. Biophys. Acta* **269**, 104–117.
8. Morrison, T. G. & Lodish, H. F. (1975) *J. Biol. Chem.* **250**, 6955–6962.
9. Buckingham, M. E. & Gros, F. (1975) *FEBS Lett.* **53**, 355–359.
10. Bag, J. & Sarkar, S. (1975) *Biochemistry* **14**, 3800–3807.
11. Heywood, S. M., Kennedy, D. S. & Bester, A. J. (1975) *FEBS Lett.* **53**, 69–72.
12. Gander, E. S., Stewart, A. G., Morel, C. M. & Scherrer, K. (1973) *Eur. J. Biochem.* **38**, 442–452.
13. Spohr, G., Kayibanda, B. & Scherrer, K. (1972) *Eur. J. Biochem.* **31**, 194–208.
14. Bonanou-Tzedaki, S. A., Pragnell, I. B. & Arnstein, H. R. V. (1972) *FEBS Lett.* **26**, 77–82.
15. Jacobs-Lorena, M. & Baglioni, C. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 1425–1428.
16. Endo, Y., Tominaga, H. & Natori, Y. (1975) *Biochim. Biophys. Acta* **383**, 305–315.
17. Hemminiki, K. (1975) *Mol. Cell Biochem.* **8**, 123–128.
18. Zähringer, J., Baliga, B. S. & Munro, H. N. (1976) *Biochem. Biophys. Res. Commun.*, in press.
19. Mullock, B. M. & Hinton, R. H. (1975) *Biochem. J.* **152**, 51–56.
20. Granick, S. (1943) *J. Biol. Chem.* **149**, 157–167.
21. Richter, G. W. (1961) *Nature* **190**, 413–415.
22. Richter, G. W. (1965) *Nature* **207**, 616–618.
23. Fineberg, R. A. & Greenberg, D. M. (1955) *J. Biol. Chem.* **214**, 97–106.
24. Yu, F. L. & Fineberg, R. A. (1965) *J. Biol. Chem.* **240**, 2083–2087.
25. Saddi, R. & Von der Decken, A. (1965) *Biochim. Biophys. Acta* **111**, 124–133.
26. Chu, L. L. H. & Fineberg, R. A. (1969) *J. Biol. Chem.* **244**, 3847–3854.
27. Millar, J. A., Cumming, R. L. C., Smith, J. A. & Goldberg, A. (1970) *Biochem. J.* **119**, 643–649.
28. Drysdale, J. W. & Munro, H. N. (1966) *J. Biol. Chem.* **241**, 3630–3637.
29. Linder-Horowitz, M., Ruettinger, R. T. & Munro, H. N. (1970) *Biochim. Biophys. Acta* **200**, 442–448.
30. Linder, M., Munro, H. N. & Morris, H. P. (1970) *Cancer Res.* **30**, 2231–2239.
31. Zähringer, J., Konijn, A. M., Baliga, B. S. & Munro, H. N. (1975) *Biochem. Biophys. Res. Commun.* **65**, 583–590.
32. Baliga, B. S., Pronzcuk, A. W. & Munro, H. N. (1968) *J. Mol. Biol.* 34, 199–218.
33. Aviv, H. & Leder, P. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 1408–1412.
34. Mans, R. J. & Novelli, G. D. (1961) *Arch. Biochem. Biophys.* **94**, 48–53.
35. Roberts, B. E. & Paterson, B. M. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2330–2334.
36. Maizel, J. V. (1971) in *Methods of Virology*, eds. Maramorosch, K. & Koprowski, H. (Academic Press, New York), Vol. 5, pp. 179–246.
37. Yoshino, Y., Manis, J. & Shachter, D. (1966) *Nature* **210**, 538–539.
38. Yoshino, Y., Manis, J. & Shachter, D. (1968) *J. Biol. Chem.* **243**, 2911–2917.
39. Tilghman, S. M., Hanson, R. W., Reshef, L., Hopgood, M. F. & Ballard, F. J. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 1304–1308.
40. Steinberg, R. A., Levinson, B. B. & Tomkins, G. M. (1975) *Cell* **5**, 29–35.
41. Penman, S., Rosbash, M. & Penman, M. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 1878–1885.
42. Darnell, J. E., Philipson, L., Wall, R. & Adesnik, M. (1971) *Science* **174**, 507–510.
43. Bester, A. J., Kennedy, D. S. & Heywood, S. M. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 1523–1527.
44. Lee, J. C. K., Lee, S. S. C., Schlesinger, K. J. & Richter, G. W. (1975) *Am. J. Pathol.* **80**, 235–244.
45. Drysdale, J. W. & Shafritz, D. A. (1975) *Biochim. Biophys. Acta* **383**, 97–105.
46. Rottman, F., Shatkin, A. J. & Perry, R. P. (1974) *Cell* **3**, 197–199.
47. Adams, J. M. & Cory, S. (1975) *Nature* **255**, 28–33.
48. Jay, G. & Kaempfer, R. (1975) *J. Biol. Chem.* **250**, 5749–5755.