Regulation of Mammalian Protein Synthesis *in vivo*

STIMULATED LIVER RIBONUCLEIC ACID SYNTHESIS *IN VIVO* AFTER CYCLOHEXIMIDE TREATMENT

By JOHN J. CH'IH, LEE M. PIKE* and THOMAS M. DEVLIN
*Department of Biological Chemistry, Hahnemann Medical College and Hospital,
Philadelphia, PA 19102, U.S.A.*

(Received 21 March 1977)

1. As shown by a double-radioisotope technique *in vivo*, at a non-lethal dose of cycloheximide, a stimulation of nuclear RNA synthesis occurred by 12 h after the treatment; the stimulation lasted over 48 h. Analysis of radioactive nuclear RNA by gel electrophoresis demonstrated that most of the cycloheximide-stimulated synthesis could be accounted for by known rRNA precursors (45S, 41S, 32S and 28S). 2. During the inhibitory phase of protein synthesis, 2 h after cycloheximide treatment, synthesis of the poly(A)-containing mRNA isolated from the cytoplasmic ribonucleoprotein complexes with an oligo(dT)-cellulose column was stimulated, whereas the synthesis of rRNA was slightly inhibited. However, during the stimulatory phase of protein synthesis, 24 h after cycloheximide treatment, the syntheses of both poly(A)-containing mRNA and rRNA were enhanced. 3. Kinetic studies revealed that the newly synthesized RNA species were transported from the nuclei, integrated into the ribonucleoprotein complexes, and associated with both free and membrane-bound polyribosomes. 4. These data corroborate our proposal that the stimulated protein synthesis after cycloheximide administration involves gene transcription.

Co-ordination of protein synthesis and RNA synthesis has been a problem of continuing interest over the last decade. By using rapidly growing mammalian cells in tissue culture, changes in the synthesis and processing of rRNA and ribosome maturation have been observed after inhibition of protein synthesis by cycloheximide (Ennis, 1966; Willems *et al.*, 1969; Craig & Perry, 1970; Willis *et al.*, 1974; Chesterton *et al.*, 1975). Muramatsu *et al.* (1970) used lethal doses of cycloheximide (>2.7 mg/kg body wt.) to extend the observations from the tissue-culture systems to intact rats. Incorporation *in vivo* of labelled orotate into nucleolar and nucleoplasmic RNA was inhibited by cycloheximide (10–20 mg/kg) within 3 h of the injection of the antibiotic. Higashinakagawa & Muramatsu (1972) demonstrated that cycloheximide *in vitro* (200–1000 µg/assay) had no effect on the activities of nuclear RNA polymerases I and II, and concluded that the inhibition of nRNA synthesis was not the result of direct action of cycloheximide on the enzymes, but probably was mediated in some way by the cessation of protein biosynthesis. By using similar doses of cycloheximide (up to 30 mg/kg) *in vivo*, Yu & Feigelson (1972) suggested that the observed inhibition of rRNA synthesis was due to the diminution of

nuclear RNA polymerase I activity *in vivo* mediated by a protein with a short half-life. Farber & Farmer (1973), however, reported that nRNA synthesis *in vivo* was unaffected by non-lethal doses of cycloheximide, but was inhibited by lethal doses. They concluded that the inhibition of RNA synthesis caused by lethal doses of cycloheximide may be a direct effect rather than the consequence of the inhibition of protein synthesis. To elucidate the mechanism involved in the observed stimulation of liver protein synthesis after cycloheximide treatment (Ch'ih & Devlin, 1974; Rothblum *et al.*, 1976; Ch'ih *et al.*, 1977), we investigated the synthesis of various RNA species in rat liver during the inhibited and stimulated phases of protein synthesis *in vivo* after administration of a non-lethal dose of cycloheximide.

We report here evidence suggesting that the adaptive mechanism of protein synthesis during and after inhibition of protein synthesis not only involves the translational event, but also is associated with transcriptional activity. Some of the observations described in the present paper have been presented in a preliminary communication (Pike *et al.*, 1975).

Experimental

Materials

All chemicals were of the highest purity available commercially. Freshly redistilled phenol was used as

* Present address: Department of Health Sciences, East Tennessee State University, Johnson City, TN 37610, U.S.A.

a routine. Acrylamide and agarose were from Bio-Rad Laboratories (Richmond, CA, U.S.A.); sodium dodecyl sulphate, cycloheximide and yeast RNA were supplied by Sigma Chemical Co. (St. Louis, MO, U.S.A.). Crystalline bovine serum albumin was obtained from Miles Laboratories (Kankakee, IL, U.S.A.), oligo(dT)-cellulose was supplied by Collaborative Research (Waltham, MA, U.S.A.), and ribonuclease-free sucrose was obtained from Schwartz-Mann (Orangeburg, NY, U.S.A.). [^3H]-Orotic acid (17.8–25 Ci/mmol) and [^{14}C]-orotic acid (40–60 mCi/mmol) were purchased from New England Nuclear Corp. (Boston, MA, U.S.A.), and NCS solubilizer was supplied by Amersham/Searle (Arlington Heights, IL, U.S.A.).

Animals

The experiments were performed on male Wistar rats ($210 \pm 10\text{g}$). Maintenance of the animals, treatment with cycloheximide, and removal of livers were carried out as described previously (Ch'ih *et al.*, 1977).

Isolation of subcellular components

Nuclei were isolated by the citric acid procedure as described by Taylor *et al.* (1973). The minced liver was homogenized for 30–60 s in 6–8 vol. of 1.5% (w/v) citric acid with a Tekman Tissumizer at setting 7. The homogenate was passed through three layers of cheesecloth and centrifuged at $600g_{\text{max}}$ for 10 min to pellet nuclei. The supernatant was aspirated and the nuclei were further cleaned by two cycles of homogenization, each in the original volume of 0.25 M-sucrose/1.5% (w/v) citric acid for 20–30 s as described above.

Ribonucleoprotein complexes were precipitated quantitatively with MgCl_2 as described by Palmiter (1974), except that the ribonucleoprotein complexes were collected by centrifuging through 12 ml of 1.0 M-sucrose in 25 mM-Tris/HCl/25 mM-NaCl/5 mM- MgCl_2 buffer, pH 7.5, in a Sorvall SS-34 rotor for 10 min at $27000g_{\text{max}}$. The complexes prepared in this manner had an A_{260}/A_{280} ratio of 1.74–1.85, an A_{260}/A_{235} ratio of 1.40–1.61 and demonstrated normal polyribosomal profiles on centrifugation on continuous 0.5–1.5 M-sucrose gradient as described by Palmiter (1974).

Microsomal fractions, free and membrane-bound polyribosomes were isolated as described previously (Ch'ih *et al.*, 1977).

Isolation and separation of various cellular RNA species

Nuclear RNA was extracted by the procedures described by Wagner *et al.* (1967) and Ro-Choi *et al.* (1970) by using phenol/sodium dodecyl sulphate

containing 0.01% 8-hydroxyquinoline at 55°C . nRNA molecules were separated by the procedure of Dingman & Peacock (1968), in 2% polyacrylamide/0.5% agarose slab gels by electrophoresis at pH 8.3. Cytoplasmic RNA species were extracted from the ribonucleoprotein complexes by the phenol/chloroform method outlined by Palmiter (1974). The isolated RNA species had an A_{260}/A_{280} ratio of 2.06–2.17 and an A_{260}/A_{230} ratio of 1.97–2.12. Poly(A)-containing mRNA was further purified by chromatography on oligo(dT)-cellulose with buffers of low ionic strength, by the method of Aviv & Leder (1972).

Incorporation in vivo of [^3H]orotate into RNA

Groups of two to three animals were injected intraperitoneally with [^3H]orotate ($100\mu\text{Ci}/100\text{g}$ body wt.) 1 h before being killed. At the times indicated, cytoplasmic RNA species were isolated and specific radioactivities (d.p.m./mg of RNA or d.p.m./ A_{260} unit) were determined.

Relative incorporation rates of orotate into nRNA at various times after cycloheximide administration were measured by using a dual-labelling technique similar to the procedures described by Kano-Sueoka & Spiegelman (1962), Ellem (1967) and Ch'ih & Devlin (1971). Radioactive orotate ([^3H]orotate, $100\mu\text{Ci}/100\text{g}$ body wt.; [^{14}C]orotate, $12.5\mu\text{Ci}/100\text{g}$ body wt.) was injected intramuscularly 1 h before death. The experimental design involved two sets of animals each containing one control and one treated rat. In set I the control animal was injected with [^3H]orotate and the treated animal with [^{14}C]orotate, whereas in set II these radioisotopes were injected in the opposite order. At the end of the labelling period the animals were killed and the livers in each set were pooled; nRNA was prepared and relative labelling rate was determined.

Analytical assays

Protein was determined by the method of Lowry *et al.* (1951), with crystalline bovine serum albumin as the standard. RNA concentration was determined by the orcinol reaction as described by Ch'ih *et al.* (1977).

Samples containing trichloroacetic acid-insoluble radioactivity were assayed as described by Devlin & Ch'ih (1972). The slab gel was cut (1 cm diam.) and sliced into 1 mm slices by using the DE 113 Horizontal Gel Slicer (Hoefer Scientific Instruments, San Francisco, CA, U.S.A.); each slice was solubilized in 0.5 ml of NCS solubilizer at 50°C for 16 h, and 10 ml of scintillation 'cocktail' (Yorktown Research, S. Hackensack, NJ, U.S.A.) was added before radioactivity counting. Radioactivity was determined with a Packard Tri-Carb liquid-scintil-

lation spectrometer. Counting efficiencies were in the range of 25–35% for ³H and 35–45% for ¹⁴C on mutually excluded channels, or 35–40% ³H on its full channel. Channel spillover and quenching were corrected by using an external standard.

Results

Effect on cycloheximide on cellular RNA concentrations and synthesis

Previous studies from this laboratory have shown that non-lethal doses of cycloheximide cause an inhibition of the protein-synthetic activity of rat liver, which is followed by a stimulatory phase. We have been concerned with changes in specific components of the protein-synthesizing system during the recovery period after inhibition. The short- (2h) and long- (24h) term effects of cycloheximide on total liver RNA concentration and rates of [³H]orotate incorporations into different cytoplasmic components were determined (Table 1). There were no significant changes of RNA concentration in the cellular fractions determined 2h after cycloheximide treatment. However, at 24h after cycloheximide treatment, a 2-fold increase of RNA content in the free polyribosomal fraction occurred (*P*<0.05), suggesting a possible alteration of the polyribosomal populations. The microsomal fraction had a significant increase in rate of synthesis, as measured by orotate incorporation, at 2h. At 24h after cycloheximide administration, the syntheses of RNA in the whole homogenate, microsomal fraction, membrane-bound and free polyribosomes were stimulated by 47, 156, 134 and 193% of the control values respectively. The results suggest that RNA synthesis was affected by the non-lethal dose of cycloheximide during both the period of inhibition and the period of stimulation of liver protein synthesis.

Effect of cycloheximide on nuclear RNA synthesis

With the increased content and rate of synthesis of membrane-bound and free polyribosomal RNA at 2 and 24h, it was decided to evaluate the changes in nuclear RNA species that are the precursors of cytoplasmic RNA. The potential problems involved in quantitative isolation of nRNA were avoided by using a dual-labelling technique. The advantage of this technique is that the rRNA of both the experimental

Fig. 1. Relative orotate labelling rate of liver nRNA. Experimental conditions and dual-labelling techniques were as described in the text. Values with ranges shown by bars were the mean±S.E.M. of five experiments; the others represent single experiments. The relative labelling rate was obtained by the equation:

Relative rate = 100 × $\sqrt{\left(\frac{^3\text{H}}{^{14}\text{C}}\right)_{\text{set I}} \times \left(\frac{^{14}\text{C}}{^3\text{H}}\right)_{\text{set II}}}$

Table 1. Effect of cycloheximide on RNA content and synthesis of various subcellular components. Values for RNA content are expressed as mg of RNA/g of tissue; values for RNA synthesis are expressed as orotic acid incorporated (10⁻³×d.p.m./mg of RNA). Each experiment consisted of at least two rats, and data are means ±S.E.M. with the numbers of experiments in parentheses. * indicates *P*<0.05 compared with controls.

Time after cycloheximide treatment (h)	Whole homogenate		Microsomal fraction		Polyribosomes			
					Membrane-bound		Free	
	Content	Synthesis	Content	Synthesis	Content	Synthesis	Content	Synthesis
(0) (control)	7.73 ± 0.18 (19)	211.8 ± 26.9 (7)	2.50 ± 0.12 (6)	24.5 ± 2.6 (6)	0.77 ± 0.05 (16)	57.0 ± 0.4 (2)	0.22 ± 0.04 (14)	48.0 ± 9 (2)
2	8.50 ± 0.32 (4)	236.9 ± 32.6 (4)	2.51 ± 0.05 (4)	45.0 ± 11.6* (4)	0.87 ± 0.01 (2)	—	0.21 ± 0.01 (2)	—
24	8.45 ± 0.24 (4)	311.6 ± 43.1* (3)	3.16 ± 0.05* (2)	62.9 ± 4.7* (2)	0.76 ± 0.06 (9)	133.2 ± 4* (2)	0.44 ± 0.03* (9)	117.0 ± 24* (2)

and control animals is isolated under identical conditions, thereby minimizing problems of artifacts arising from slight variations in the state of purity of the nuclei or differential losses of rRNA. With this procedure, the effect of non-lethal dose of cycloheximide on the rate of orotate labelling of rRNA is shown in Fig. 1. An immediate 20% inhibition lasting 5–6 h was followed by a period of stimulation. The maximum stimulation, about 60%, was observed by 12 h and persisted for several hours.

Polyacrylamide-gel electrophoresis was carried out to determine the effects of cycloheximide administration on the rate of orotate labelling of rRNA precursors and subsequent processing of intermediates. Fig. 2 shows that, after 1 h of labelling, the radioactivity occurring in the ribosomal precursor region of the gel (28S–45S) represents a much larger proportion of the rRNA from cycloheximide-treated animals than it does for control rRNA. This was true in measurements with rRNA from both set I (results shown in Fig. 2) and set II (results not shown) experiments (see the Experimental section for

description). Nuclear rRNA therefore appears to be one class of RNA whose rate of synthesis is stimulated by prior administration of cycloheximide in non-lethal doses, a result which is different from that with higher, lethal, doses (Muramatsu *et al.*, 1970). The rate of labelling of each nuclear rRNA precursor was next determined at three times selected to cover the range of maximal stimulation of orotate labelling of rRNA after cycloheximide administration. Gels from several samples were used to calculate the relative labelling rates shown in Table 2. All four nuclear rRNA precursors analysed were stimulated by more than 60% at all three times. Cycloheximide administration therefore appears to stimulate the rate of labelling of rRNA to a greater extent than that of most of the mRNA. The rRNA precursors, however, were not equally affected. For example, the rate of labelling of 32S rRNA is elevated 2.3–2.5-fold relative to the control, whereas 28S rRNA labelling is elevated only 1.7–2.0-fold. At least two explanations are possible for this relative increase in 32S rRNA labelling. Either the conversion of 32S into 28S rRNA may be slightly inhibited as the result of cycloheximide administration, or the normal maturation mechanism may not be adequate to process the increased amount of rRNA precursor resulting from stimulated synthesis of rRNA.

Effect of cycloheximide on the synthesis of poly-ribosomal RNA

The distinct increases of [^3H]orotate incorporation into both microsomal fraction (Table 1) and nuclear rRNA (Table 2) indicated the potential value of determining whether there would be differences in [^3H]orotate incorporation into different species of RNA, such as polyribosomal RNA, poly(A)-lacking RNA and poly(A)-containing mRNA. RNA was extracted from ribonucleoprotein complexes and separated on an oligo(dT)-cellulose column. The specific radioactivities of these RNA species are presented in Table 3. At 2 h after cycloheximide administration, RNA isolated from the complexes were similar to those of the control, but incorporation into the poly(A)-lacking RNA, which contained most of the rRNA, was inhibited by 20%, and into the

Fig. 2. Radioactivity profile of nuclear RNA separated by electrophoresis on a 2% polyacrylamide slab gel

Experimental conditions and dual-labelling technique were as described in the text. RNA from 24 h-cycloheximide-treated animals was used in the separation. Δ , Control animal received [^3H]orotate; $\circ$, cycloheximide-treated animal received [^{14}C]orotate. S values were obtained by comparison with a known 28S rRNA.

Table 2. Relative incorporation rate of orotate into nuclear rRNA precursors at various times after cycloheximide administration. Experimental condition, dual-labelling technique and separation of rRNA were the same as described in Figs. 1 and 2. Values are means $\pm$ S.E.M. from five separate experiments.

Time after cycloheximide treatment (h)	Nuclear rRNA precursors			
	45S	41S	32S	28S
12	1.76 $\pm$ 0.23	2.07 $\pm$ 0.19	2.37 $\pm$ 0.17	1.65 $\pm$ 0.05
16	1.91 $\pm$ 0.28	2.05 $\pm$ 0.21	2.56 $\pm$ 0.17	1.98 $\pm$ 0.15
24	1.66 $\pm$ 0.18	1.76 $\pm$ 0.16	2.25 $\pm$ 0.15	1.99 $\pm$ 0.28

Table 3. *Effect of cycloheximide on [³H]orotate incorporation into various cytoplasmic RNA species*

Values are means $\pm$ S.E.M.; percentages of control values are given in parentheses; *n* is the number of experiments and each experiment consisted of two or three rats. * *P* < 0.05.

Condition of rat	<i>n</i>	10 ⁻³ $\times$ Incorporation (d.p.m./A ₂₆₀ unit)		
		RNA of the ribo-nucleoprotein complex	Poly(A)-lacking RNA (mainly rRNA)	Poly(A)-containing RNA (mRNA)
Control, 0h	6	2.39 $\pm$ 0.19 (100)	2.05 $\pm$ 0.10 (100)	17.34 $\pm$ 2.2 (100)
Cycloheximide-treated, 2h	2	2.33 $\pm$ 0.14 (98)	1.61 $\pm$ 0.01 (79)	30.93 $\pm$ 6.4* (179)
24h	2	4.88 $\pm$ 0.16* (205)	5.00 $\pm$ 0.56* (244)	26.9 $\pm$ 4.6 (146)

Fig. 3. *Kinetic study of [³H]orotate incorporation in vivo after cycloheximide treatment*

Experimental conditions and radioisotope labelling were described in the text. For nRNA synthesis (●) the dual-labelling technique was used; at 16h after cycloheximide treatment, animals were labelled with radioactive isotope for various times (20–180 min). For cytoplasmic fractions (○, free polyribosomes; □, membrane-bound polyribosomes; △, soluble fraction) 24h-cycloheximide-treated animals were used. Each value represents the average of at least two experiments. Relative rate of orotate incorporation is defined as the ratio of treated/control.

poly(A)-containing mRNA fraction there was a marked stimulation (to 179% of the control). By 24h incorporations into the ribonucleoprotein-complex RNA and poly(A)-lacking RNA were stimulated 2-fold, and that the poly(A)-containing mRNA was 146% of control. Data in Table 3 indicate that with a non-lethal dose of cycloheximide the synthesis and processing of all RNA species were stimulated, implicating gene activation at the transcriptional level.

Kinetic studies of orotate incorporation

Data in the Tables and Figures indicated that the synthesis of nRNA and cytoplasmic RNA species was enhanced during the period of cycloheximide-stimulated protein synthesis. To ascertain the events occurring after the synthesis of the RNA, a kinetic study of the labelling pattern of the different RNA species was carried out during the stimulatory phase (Fig. 3). Results obtained by using the dual-radioisotope technique indicated that the maximum labelling of nRNA occurred at 20 and 40 min and declined to almost the normal value by 180 min, indicating a continued higher rate of synthesis, processing and removal from the nuclei; the labelling was carried out at 16h after cycloheximide administration, which is the time of maximum incorporation into nRNA. When cytoplasmic fractions were examined 24h after cycloheximide, the polyribosomal populations (free and membrane-bound) showed a slight decrease in [³H]orotate incorporation at 20 min, then reached a maximum stimulation (3-fold) at 60 min, and decreased to 2-fold stimulation at 180 min. Labelling of the soluble fraction was stimulated by over 40% throughout the 180 min labelling period. Thus the data further suggest that, during the stimulatory phase of protein synthesis, the newly synthesized mRNA and rRNA were transported into membrane-bound as well as into free polyribosomes.

Effect of cycloheximide on the relative amount of poly(A)-containing mRNA

The lower degree of stimulation of poly(A)-containing mRNA synthesis observed 24h after cycloheximide treatment (Table 3) may reflect a dilution of the newly synthesized mRNA (labelled in 1h) by the pre-existing mRNA accumulated throughout cycloheximide treatment (23h). Therefore the relative amounts of these RNA species isolated from control, 2h- and 24h-cycloheximide-treated

Table 4. *Relative amounts of poly(A)-lacking and poly(A)-containing RNA recovered from oligo(dT)-cellulose column chromatography*

RNA isolated from the ribonucleoprotein complexes (30–31 A_{260} units) was applied to the column; the values are expressed as the percentage of total, and are means $\pm$ S.D. of n , the number of experiments, each consisting of two or three rats. * $P < 0.05$.

Time after cycloheximide treatment (h)	n	Poly(A)-lacking RNA (mainly rRNA) (%)	Poly(A)-containing RNA (mRNA) (%)	Total recovery (%)
0 (control)	6	98.4 $\pm$ 0.5	1.5 $\pm$ 0.4	99.9 $\pm$ 0.9
2	2	97.5 $\pm$ 0.2	2.4 $\pm$ 0.2*	99.9 $\pm$ 0.4
24	2	97.3 $\pm$ 0.2	2.7 $\pm$ 0.2*	100.0 $\pm$ 0.4

rat liver were compared (Table 4). Similar amounts of polyribosomal RNA (30–31 A_{260} units) isolated by phenol/chloroform procedure were applied to an oligo(dT)-cellulose column. For most of the rRNA and poly(A)-lacking mRNA eluted from the column with 0.5 M-KCl/0.01 M-Tris/HCl buffer (pH 7.5), there was no difference between the control and the treated animals. The poly(A)-containing mRNA fraction, eluted with low-ionic-strength buffer [0.1 M-KCl in 0.01 M-Tris/HCl buffer (pH 7.5) followed by 0.01 M-Tris/HCl buffer (pH 7.5)] was isolated from the control with a yield of 1.5% of the total RNA applied to the column, which is in full agreement with values reported by Tilghman *et al.* (1976). However, fractions from the 2h- and 24h-cycloheximide-treated animal were obtained in yields of 2.4 and 2.7% respectively, which differ significantly from the control (Table 4).

Discussion

The co-ordination of protein and RNA synthesis is necessary for normal growth and function of both prokaryotic and eukaryotic cells. The dependence of rRNA synthesis and processing on concomitant protein synthesis is well established (Ennis, 1966; Soeiro *et al.*, 1968; Willems *et al.*, 1969; Craig & Perry, 1970; Willis *et al.*, 1974; Chesterton *et al.*, 1975). The coupling of protein synthesis with RNA synthesis (especially rRNA synthesis) has been studied in animal tissues *in vivo*, by using cycloheximide as an inhibitor of protein synthesis. It is difficult, however, to draw comparisons and conclusions with the variety of different experimental designs used, including lethal doses versus non-lethal doses of cycloheximide, short- and long-term observations, studies *in vivo* versus *in vitro*, and the extent and duration of inhibition of protein synthesis. When lethal doses of cycloheximide are used in the intact rat, many biochemical activities are altered, not only the protein-synthetic ability (Ch'ih *et al.*, 1976), but also ultrastructural changes (Daska *et al.*, 1975), alterations of blood parameters (Young *et al.*, 1963) and other activities whose alteration leads to the demise of the animal (Young *et al.*, 1963; Mura-

matsu *et al.*, 1970). The inhibition of extranuclear and nucleoplasmic RNA synthesis observed with lethal doses of cycloheximide in a number of laboratories is apparently due to a metabolic disturbance other than inhibition of protein synthesis by the antibiotic (Muramatsu *et al.*, 1970; Farber & Farmer, 1973).

The increase of poly(A)-containing mRNA during the inhibitory as well as the stimulatory phases of protein synthesis may indeed be due to stimulated transcription, possibly accompanied by a decrease in degradation of the RNA. A possible explanation would be that there is an extension of the half-life of liver mRNA because of cycloheximide treatment and that mRNA could have been dissociated from polyribosomes and could exist not in the free form but in the form of a ribonucleoprotein complex (J. J. Ch'ih & T. M. Devlin, unpublished work). This complex may be a temporary, non-translatable, form of mRNA that is resistant to the attack of nuclease. As protein synthesis is resumed after the inhibitory period, the ribonucleoprotein complex could combine with existing ribosomes to form functional polyribosomes.

Accumulation of mRNA in the course of inhibition of protein synthesis in bacteria (Artman & Ennis, 1972; Ennis & Kievett, 1976) and in rat liver by ethionine (Endo *et al.*, 1975) is known. Our present report further substantiates the notion that liver mRNA molecules accumulate during the period of inhibition of protein synthesis by cycloheximide and that cycloheximide does not prevent newly synthesized poly(A)-containing mRNA from entering polyribosomal fractions (Van Venrooij *et al.*, 1975; Zlatopolskii, 1976).

In contrast with cycloheximide at lethal doses, we believe that cycloheximide at non-lethal doses is a good tool for studying coupling between RNA and protein synthesis *in vivo*, because we have demonstrated that non-lethal doses (1) are sufficient to inhibit protein synthesis *in vivo* by 80–95% within 2–3 h (Farber & Farmer, 1973; Ch'ih & Devlin, 1974; Hwang *et al.*, 1974; Rothblum *et al.*, 1976), (2) avoid severe metabolic and structural alterations, and the death of the organism (Hwang *et al.*, 1974; Ch'ih

et al., 1976), (3) allow qualitative and quantitative differences in synthesis of specific proteins during the recovery and stimulatory phases of protein synthesis after the initial inhibition (Ch'ih *et al.*, 1977), and (4), as shown in the present paper, enhance synthesis and accumulation of poly(A)-containing mRNA during the inhibitory phase of protein synthesis and the stimulation of rRNA and mRNA synthesis and their integration into polyribosomes during the period after inhibition. Future studies of the structural and functional state of polyribosomal classes and the identification of a specific mRNA should shed some light on our understanding of the underlying mechanism involved.

We are indebted to Mrs. Linda Faulkner, Mrs. Frances Taylor and Ms. Sheila Brown for their excellent technical assistance.

References

- Artman, M. & Ennis, H. L. (1972) *J. Bacteriol.* **110**, 652-660
- Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1408-1412
- Chesterton, C. J., Coupar, B. E. H., Butterworth, P. H. W., Buss, J. & Green, M. H. (1975) *Eur. J. Biochem.* **57**, 79-83
- Ch'ih, J. J. & Devlin, T. M. (1971) *Biochem. Biophys. Res. Commun.* **43**, 962-967
- Ch'ih, J. J. & Devlin, T. M. (1974) *J. Cell Biol.* **63**, 59
- Ch'ih, J. J., Olszyna, D. M. & Devlin, T. M. (1976) *Biochem. Pharmacol.* **25**, 2407-2408
- Ch'ih, J. J., Procyk, R. & Devlin, T. M. (1977) *Biochem. J.* **162**, 501-507
- Craig, N. C. & Perry, R. P. (1970) *J. Cell Biol.* **45**, 554-564
- Daska, I., Merski, J. A., Hughes, J. B. & Busch, H. (1975) *Exp. Cell Res.* **93**, 395-401
- Devlin, T. M. & Ch'ih, J. J. (1972) *Arch. Biochem. Biophys.* **152**, 521-530
- Dingman, C. W. & Peacock, A. C. (1968) *Biochemistry* **7**, 659-668
- Ellem, K. A. O. (1967) *Biochim. Biophys. Acta* **149**, 74-87
- Endo, Y., Tominaga, H. & Natori, Y. (1975) *Biochim. Biophys. Acta* **383**, 305-315
- Ennis, H. L. (1966) *Mol. Pharmacol.* **2**, 543-557
- Ennis, H. L. & Kievet, K. D. (1976) *J. Biol. Chem.* **251**, 2854-2860
- Farber, J. L. & Farmer, R. (1973) *Biochem. Biophys. Res. Commun.* **51**, 626-630
- Higashinakagawa, T. & Muramatsu, M. (1972) *Biochem. Biophys. Res. Commun.* **47**, 1-6
- Hwang, K. M., Yang, L. C., Cerrico, C. K., Schultz, R. A., Shenkman, J. B. & Sartorelli, A. C. (1974) *J. Cell Biol.* **62**, 20-31
- Kano-Sueoka, T. & Spiegelman, S. (1962) *Proc. Natl. Acad. Sci. U.S.A.* **48**, 1942-1949
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Muramatsu, M., Shimada, N. & Higashinakagawa, T. (1970) *J. Mol. Biol.* **53**, 91-106
- Palmiter, R. D. (1974) *Biochemistry* **13**, 3606-3615
- Pike, L. M., Ch'ih, J. J. & Devlin, T. M. (1975) 170th *Natl. Meet. Am. Chem. Soc. Abstr. no. 77*
- Ro-Choi, T. S., Moriyama, Y., Choi, Y. C. & Busch, H. (1970) *J. Biol. Chem.* **245**, 1970-1977
- Rothblum, L. I., Devlin, T. M. & Ch'ih, J. J. (1976) *Biochem. J.* **156**, 151-157
- Soeiro, R., Vaughan, M. H. & Darnell, J. E. (1968) *J. Cell Biol.* **36**, 91-101
- Taylor, C. W., Yeoman, L. C., Daskal, I. & Busch, H. (1973) *Exp. Cell Res.* **82**, 215-226
- Tilghman, S. M., Fisher, L. M., Reshef, L., Ballard, F. J. & Hanson, R. W. (1976) *Biochem. J.* **156**, 619-626
- Van Venrooij, W. J., Gielkens, A. L. J., Janssen, A. P. M. & Bloemendal, H. (1975) *Eur. J. Biochem.* **56**, 229-238
- Wagner, E. K., Katz, L. & Penman, S. (1967) *Biochem. Biophys. Res. Commun.* **28**, 152-159
- Willems, M., Penman, M. & Penman, S. (1969) *J. Cell Biol.* **41**, 177-187
- Willis, M. V., Baseman, J. B. & Amos, H. (1974) *Cell* **3**, 179-184
- Young, C. W., Robinson, P. F. & Sacktor, B. (1963) *Biochem. Pharmacol.* **12**, 855-865
- Yu, F. L. & Feigelson, P. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2833-2837
- Zlatopolskii, A. D. (1976) *Biokhimiya* **41**, 304-307