altered by the photooxidation, and it is therefore unlikely that guanine residues of the RNA are involved in the inactivation process.

An extensive investigation of the effect of sulfhydryl reagents on ribosomal activity (*11*) showed that the effect of sulfhydryl reagents was primarily that of imposing a conformational change on the ribosomes. Some of the less bulky reagents were found actually to activate, while the largest of the reagents was the most potent inhibitor. The most nearly complete inhibition obtained was 70 percent after 12 hours incubation at 5°C. On the basis of our results (no conformational change; short photooxidation time) we exclude the possibility that dye-sensitized photooxidation of cysteine residues is primarily responsible for the inactivation process.

We feel that these initial photochemical studies provide evidence for the critical involvement of a ribosomal surface protein (or proteins) in the protein-assembly function of the ribosome. Minor modification of the photolabile side chains of the ribosomal protein or proteins *in situ* is sufficient to completely inactivate one or more essential steps of protein synthesis; the extreme rapidity of the inactivation process at very low dye concentrations (concentrations as low as one dye molecule per ribosome were found to be effective) implies an exposed catalytic site.

ROBERT T. GARVIN
GORDON R. JULIAN
SAMUEL J. ROGERS

Department of Chemistry,
Montana State University,
Bozeman 59715

References and Notes

1. S. Osawa, *Annu. Rev. Biochem.* **37**, 109 (1968).
2. For current review of mechanisms of photosensitized oxidation in biological systems, see C. S. Foote [*Science* **162**, 963 (1968)].
3. W. J. Ray and D. E. Koshland, *J. Biol. Chem.* **237**, 2493 (1962).
4. T. R. Hopkins and J. D. Spikes, *Arch. Biochem. Biophys.* **126**, 969 (1968).
5. L. Weil, S. James, A. R. Buchert, *ibid.* **46**, 266 (1953).
6. L. Weil and A. R. Buchert, *ibid.* **34**, 1 (1951).
7. J. D. Spikes and R. Straight, *Annu. Rev. Phys. Chem.* **18**, 409 (1967).
8. J. S. Bellin and C. A. Yankus, *Arch. Biochem. Biophys.* **123**, 18 (1968).
9. J. S. Bellin and L. Grossman, *Photochem. Photobiol.* **4**, 45 (1965).
10. B. Singer and H. Fraenkel-Conrat, *Biochemistry* **5**, 2446 (1966).
11. R. R. Traut and A. L. Haenni, *Eur. J. Biochem.* **2**, 64 (1967).
12. G. R. Julian, *J. Mol. Biol.* **12**, 9 (1965).
13. Contribution No. 955 in the journal series of the Montana Agricultural Experiment Station. R.T.G. is an NIH predoctoral fellow (1-FI-GM-41, 409-01). We thank Dr. J. S. Bellin, Polytechnic Institute of Brooklyn, for her gift of thiopyronin.

11 February 1969; revised 17 March 1969

Preferential Synthesis of Ferritin and Albumin by Different Populations of Liver Polysomes

Abstract. *Free polysomes and a mixture of free and membrane-attached polysomes were isolated separately from rat liver, and each was added to a cell-free, protein-synthesizing system. The free polysomes showed a greater capacity than the mixed polysome population for incorporation of ^{14}C-leucine into ferritin, whereas the reverse was true for ^{14}C-leucine incorporation into albumin.*

The liver cell contains two populations of polysomes, one attached to the membranes of the endoplasmic reticulum and the other free in the cytoplasm. The membrane-bound and free polysomes are thought to be functionally distinct (*1*); the former seem to be active in the biosynthesis of proteins to be secreted by the cell and the latter in the biosynthesis of intracellular protein. Experimental evidence has recently been provided for the preferential synthesis of secreted proteins such as albumin (*2*, *3*) and other serum proteins (*3*) by the membrane-bound ribosomes of liver. However, there is yet no evidence available concerning the site of synthesis of retained proteins and of the function of free polysomes.

Ferritin is a retained liver protein that can be conveniently isolated by chemical and immunological means (*4*). With a cell-free protein-synthesizing system under conditions in which release of completed peptide chains is maximum, we examined the relative capacities of free liver polysomes and of the total liver polysomes (free and attached) to synthesize ferritin; we compared the capacity to synthesize ferritin with the relative activities of the two polysome populations to incorporate labeled amino acids into serum albumin. The results show that free polysomes are more active in the synthesis in vitro of ferritin, whereas the total polysome fraction is more effective for albumin synthesis.

With fasting adult male rats, free and total polysome populations were isolated from the homogenates of liver from which the mitochondria were removed (*5*). Each polysome population was incubated for 1 hour along with a mixture of ^{14}C-amino acids and activating and transferring enzymes in the form of the *p*H 5 fraction prepared from liver cell sap (*6*). The amount of the *p*H 5 enzyme fraction was, however, increased by five times, since it has been found (*7*) that this favors maximum release of completed peptide chains in a cell-free system. At the end of incubation, rat liver homogenate was added to provide unlabeled carrier ferritin, and the ferritin was isolated (*4*) from the incubation mixture and

Table 1. Relative incorporation of ^{14}C-leucine into ferritin and into serum albumin by free polysomes and total polysomes isolated from rat liver.

Exp. No.	Uptake into protein (% of total peptide incorporation)		Difference (%)
	Total	Free	
	*Ferritin**		
1	0.09	0.12	+33
2	.08	.14	+75
3	.10	.15	+50
4	.08	.15	+85
5	.06	.11	+80
Mean	.08	.13	+65
	Albumin†		
1	0.30	0.10	−67
2	.39	.21	−46
Mean	.35	.16	−56

* At the end of incubation, rat liver homogenate was added to provide ferritin as unlabeled carrier. The ferritin was then isolated by the procedure of Drysdale and Munro (*4*) with omission of the carboxymethyl-cellulose step, which reduces recovery of ferritin by about 20 percent. The final product was then precipitated by addition of antibody to ferritin, the precipitate was washed with cold 0.4*N* $HClO_4$, and the radioactivity was counted. The total incorporation of ^{14}C-leucine into peptides was also measured by precipitating a small amount of the same incubation mixture with 5 percent trichloroacetic acid (TCA), then heating the solution to 90°C to remove nucleic acids, washing with 0.4*N* $HClO_4$, and counting the radioactivity. † At the end of incubation, 100 μg of rat serum albumin was added to the incubation medium, and the protein was precipitated with an equal volume of 10 percent TCA. The precipitate was washed three times with 5 percent TCA and was then extracted with 1 ml absolute ethanol (*9*). After 30 minutes standing, the sample was centrifuged and the supernatant was then dialyzed for 12 hours against water and for 2.5 hours against 10 m*M* phosphate buffer, *p*H 7.5. To remove nonspecific protein that might coprecipitate, chicken albumin was added to the extract, followed by its antiserum (*10*). Rat albumin antiserum was then added to the supernatant; it was incubated for 2 hours at 38°C and for 18 hours at 4°C. The albumin-antiserum complex was then centrifuged, 5 percent TCA was added, and the solution was heated for 15 minutes at 90°C. The precipitate was washed with 0.4*N* $HClO_4$ on a Millipore filter and counted by scintillation.

the radioactivity was counted for incorporation of ^{14}C. Usually incorporation of ^{14}C into ferritin ranged from 50 to 120 counts per minute per milliliter of incubation mixture, whereas radioactivity incorporated into total liver protein was between 30,000 and 100,000 counts per minute per milliliter of incubation mixture. In order to eliminate variations of incorporating capacity from one polysome preparation to another, the uptake into ferritin has been expressed as a percentage of total incorporation of amino acid radioactivity into peptides (Table 1). The difference in ferritin labeling between the two polysome populations is consistent in all five comparisons of free and total polysome populations and averages 65 percent greater activity with the free population. This is not due to a difference in release of completed peptide chains from the two polysome populations; examination of peptides released under the conditions of incubation (*7*) showed that 39 percent of the labeled peptide was released from the free polysomes during incubation, and 43 percent from the total polysome preparation.

In order to verify that the activity observed in the ferritin fraction is not due to a radioactive contaminant, ferritin samples were isolated by the unmodified method of Drysdale and Munro (*4*) after incubation of the cell-free system with free polysomes. The ferritin was then treated with 1 percent sodium lauryl sulfate at 70°C in order to dissociate ferritin into its subunits. The free subunits were separated from the parent ferritin with a polyacrylamide gel (*8*). The protein bands were then eluted and examined for radioactivity. This treatment resulted in dissociation of a substantial part of the original ferritin into subunits which were found to contain a considerable proportion of the radioactivity of the ferritin applied to the gel. Because of the difficulty of measuring the protein content of the subunits in the gel, it was not possible to obtain more than a rough indication that the amount of radioactivity migrating with the subunits was proportional to the amount of protein in this fraction. The presence of activity in the separated subunits strongly suggests that the radioactivity associated with the original isolated ferritin must have taken the form of ferritin protein.

This evidence of preferential synthesis of ferritin by free polysomes prompted us to examine the relative efficiencies of free and total polysome populations in the synthesis of serum albumin, a secreted protein. The conditions of incubation of free and total polysomes were similar to those used for studying ferritin biosynthesis. After an hour of incubation, albumin was isolated with acid ethanol followed by immunological precipitation; coprecipitation of nonspecific proteins was minimized by initial treatment with chick serum albumin and then antiserum to chick albumin. Incorporation of ^{14}C-amino acids into albumin was expressed as a percentage of total counts of radioactivity incorporated into peptides by the cell-free system. The results of two such experiments (Table 1) show that, in contrast to ferritin, albumin is less effectively synthesized by free polysomes than by the mixture of free and attached polysomes. Consequently, our evidence favors the view that free polysomes and membrane-attached polysomes synthesize different types of protein, and is compatible with the hypothesis that the free polysomes are associated with formation of proteins retained in the cell, whereas the attached polysomes make secreted proteins.

SONJA J. HICKS
J. W. DRYSDALE
H. N. MUNRO
Physiological Chemistry Laboratories, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge 02139

References and Notes

1. P. Siekevitz and G. Palade, *J. Biophys. Biochem. Cytol.* **7**, 619 (1960); M. S. C. Birbeck and E. H. Mercer, *Nature* **189**, 558 (1961); T. Peters, Jr., *J. Biol. Chem.* **237**, 1186 (1962); J. R. Sargent and P. N. Campbell, *Biochem. J.* **96**, 134 (1965).
2. C. M. Redman, *Biochem. Biophys. Res. Commun.* **31**, 845 (1968); M. Takagi and K. Ogata, *ibid.* **33**, 55 (1968).
3. M. C. Ganoza and C. A. Williams, private communication.
4. J. W. Drysdale and H. N. Munro, *Biochem. J.* **95**, 851 (1965).
5. G. Blobel and V. R. Potter, *J. Mol. Biol.* **26**, 279 (1967).
6. B. S. Baliga, A. W. Pronczuk, H. N. Munro, *ibid.* **34**, 199 (1968).
7. S. J. Hicks and J. W. Drysdale, *Biochim. Biophys. Acta*, in press.
8. J. W. Drysdale, in *Regulation of Protein Synthesis in Animal Cells*, A. San Pietro, M. H. Lamborn, F. J. Kenny, Eds. (Academic Press, New York, 1968).
9. A. Korner and J. R. Debro, *Nature* **178**, 1067 (1956).
10. T. Peters, Jr., *J. Biol. Chem.* **237**, 1181 (1962).
11. We thank Dr. Peters, Jr., of Cooperstown, N.Y., for his gifts of chick and rat antialbumin and his advice in albumin isolation. Supported by NIH grant No. CA 08893-03.

25 November 1968; revised 17 February 1969

Alcohol Dehydrogenase in Maize: Genetic Basis for Multiple Isozymes

Abstract. *There are two distinct sets of alcohol dehydrogenase isozymes from maize, but they have subunits in common. Induced mutations at the* (Adh_1) *locus altered the migration rate of both Set I and Set II isozymes.*

Maize zymograms show two distinct well separated sets of alcohol dehydrogenase (ADH) isozymes (*1*). Intense slow migrating isozymes have been designated as the Set I bands and the less intense bands which migrate to a more anodal position as Set II. Inheritance of the Set I and Set II ADH isozymes is completely correlated. In homozygotes, single ADH bands appear in one of three positions in the Set I and Set II regions. The position of

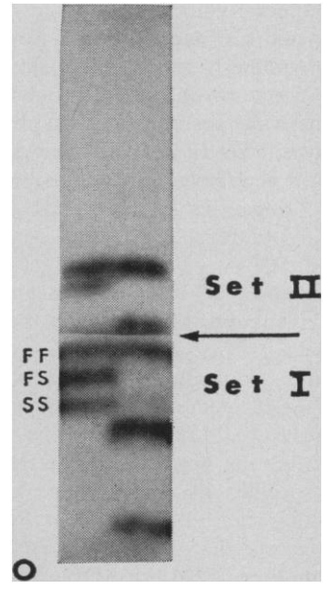

Fig. 1. Zymograms of Adh_1^F heterozygous with Adh_1^S (left) and with a mutant allele obtained by EMS treatment of Adh_1^S (right). Note the altered positions of both the Set II and Set I bands in the EMS-induced mutant. The Set II bands are much fainter than the Set I bands. In order to show all the bands clearly in a single photograph the gel was cut in two in the region between the two sets of bands (indicated by the arrow), and the upper half of the gel was developed for a longer period to intensify the Set II bands; *O* designates the origin, and the anodal pole is at the top. Materials used for electrophoresis were scutella from kernels which had been soaked in water for 48 hours, germinated 24 hours, and then soaked again in water for another 24 hours. The starch-gel electrophoretic and zymographic procedures were as described (*4*).