STUDIES ON THE SITE OF SYNTHESIS OF 
SEVERAL SOLUBLE ENZYMES OF THE CELL NUCLEUS

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ABSTRACT

Rats were given radioactive L-leucine intravenously. At various times after injection, the 
livers were removed and separated into nuclear and cytoplasmic fractions by a nonaqueous 
technique. Glyceraldehyde-3-phosphate dehydrogenase, aldolase, and lactic dehydrogenase 
were isolated from each cell fraction by antibody precipitation followed by gel electrophoresis, and the specific radioactivities of the isolated enzymes were determined. In all three 
cases, the onset of labeling and the rate of incorporation were the same for the nuclear 
enzyme as for the corresponding enzyme from the cytoplasm. If we assume that equilibration 
of the enzymes between the cytoplasmic and nuclear pools occurs slowly relative to the 
labeling times employed, we may conclude that the labeled nuclear enzymes either were 
synthesized in the nucleus or moved into the nucleus from a cytoplasmic site of synthesis 
without first passing into the cytoplasmic pool of enzyme. Treatment with puromycin, an 
antibiotic which depresses incorporation into cytoplasmic proteins to a greater extent than 
into nuclear proteins, led to a situation in which the specific activities of the nuclear enzymes 
were several times as high as those of the corresponding cytoplasmic enzymes following a 
short period of incorporation. These data substantiate the assumption that equilibration 
between the cytoplasmic and nuclear enzyme pools occurs slowly and provide further evi-
dence that the labeled nuclear enzymes do not arise from the cytoplasmic enzyme pool.

INTRODUCTION

Little is known concerning the origin of the proteins of the cell nucleus. Of the various classes of 
nuclear proteins, histones have been examined most extensively from this standpoint, but there is 
no general agreement as to the site of synthesis even of these proteins. In a series of elegant studies, 
Robbins and coworkers have identified a class of small cytoplasmic polysomes in HeLa cells which 
is present only during histone synthesis and which incorporates labeled amino acids into a product 
resembling histones (1-3). Similar results have been obtained by others (4, 5), and it has been 
inferrred by these investigators that histones are synthesized in the cytoplasm and then transferred 
to the nucleus. On the other hand, several groups of workers have demonstrated that isolated thymus 
nuclei can incorporate labeled amino acids into histones (6-9); these investigators have concluded 
that histone synthesis occurs within the cell nucleus.

The results obtained by the two approaches 
need not be contradictory, however, since the 
studies are not strictly comparable. Thus, histone 
synthesis by isolated nuclei has been shown only 
for thymus nuclei and only the synthesis of lysine- 
rich histones has been unequivocally demonstrated 
(8), whereas synthesis of histones on cytoplasmic 
polysomes has been shown for HeLa cells and sea 
urchin embryos and, in those cases where the 
histones were separated and identified, incorporation 
was found to have occurred primarily in the 
slightly lysine-rich and arginine-rich fractions. A 
model in which most histone synthesis occurred on 
cytoplasmic polysomes with quantitatively minor
amounts of synthesis in the cell nucleus would accommodate most of the existing data without the need to postulate that the site of formation of histones differs from one cell type to another or from one histone fraction to another.

Allfrey et al. found that isolated thymus nuclei incorporated labeled amino acid into several classes of nuclear proteins in addition to histones, an indication that a number of nuclear proteins might be produced by the nucleus itself (6). Other investigators have provided evidence for the cytoplasmic synthesis of various nuclear proteins. Thus, Zetterberg has concluded from cytophotometric and radioautographic studies that 65% of the nuclear mass of growing mouse fibroblasts is synthesized in the cytoplasm (10), and Byers et al. have shown by nuclear transplantation experiments that, in amoebae, several classes of nuclear proteins are of cytoplasmic origin (11). Transfer of newly synthesized, labeled proteins from the cytoplasm into the nucleus has also been demonstrated in HeLa cells (12) and in sea urchin embryos (5).

In addition to proteins, such as the histones, whose localization within the cell is almost exclusively nuclear, the nucleus contains a variety of soluble proteins which occur in the cytoplasm as well as the nucleus. These include all of the glycolytic enzymes, which are found in approximately equal concentrations in the nucleus and cytoplasm (13). Several years ago we investigated the kinetics of labeling of one of these proteins (lactic dehydrogenase) following a single injection of a radioactive amino acid into the intact animal (14). Labeling of the nuclear and cytoplasmic lactic dehydrogenase began at about the same time after injection and proceeded at nearly the same rate. On the assumption that movement of the enzyme across the nuclear membrane would occur slowly relative to the labeling times employed, we suggested that incorporation of label into the nuclear lactic dehydrogenase had occurred in the nucleus, although, as will be discussed later, another interpretation of our data is possible.

In the present study we have investigated the kinetics of labeling of nuclear and cytoplasmic glyceraldehyde-3-phosphate dehydrogenase and aldolase and have reinvestigated the labeling kinetics of lactic dehydrogenase at short times following injection of labeled leucine. Experiments have also been done with animals treated with puromycin, an antibiotic which selectively blocks incorporation into the cytoplasmic enzymes. Our data demonstrate that the newly synthesized nuclear enzymes are not derived from the cytoplasmic enzyme pool; either they are synthesized within the nucleus itself, or they pass from a cytoplasmic site of synthesis into the nucleus without first equilibrating with the corresponding cytoplasmic enzymes.

MATERIALS AND METHODS

Radioisotope

L-leucine-4,5-3H (58.2 Ci/mmole) was obtained from New England Nuclear Corp., Boston, Mass.

Enzyme Assays

These were performed as described previously (15).

Antibodies

Preparation of antibodies to lactic dehydrogenase, aldolase, and glyceraldehyde-3-phosphate dehydrogenase from liver has been described, and quantitative precipitation curves for the reaction of each antibody with the corresponding homogeneous enzyme have been presented (15).

In Vivo Labeling of Liver Proteins

Male Holtzman albino rats weighing 250-300 g each were used. Animals were kept on a schedule of alternating periods of light (14 hr) and dark (10 hr) and were given food and water ad libitum until 24-26 hr before injection of radioisotope, at which time they were deprived of food. All injections were given at the same time of day.

Unless otherwise noted, animals were anesthetized with ether and given, via the hepatic portal vein, 1.5 ml/kg body weight of a solution containing, per milliliter: 3.63 mCi (0.097 μmoles) L-leucine-4,5-3H; 5.0 μmoles each of glycine, L-alanine, L-valine, L-isoleucine, L-proline, L-serine, L-threonine, L-aspartic acid, L-glutamic acid, L-asparagine, L-glutamine, L-methionine, L-cysteine, L-phenylalanine, L-tyrosine, L-lysine, L-arginine, and L-tryptophan; 1.0 μmole L-tyrosine; and 150 μmoles NaCl. The pH of the solution was adjusted to 7.35. Following injection, the animals were held under anesthesia until the livers were removed.

Preparation of Subcellular Fractions

Livers were separated into cytoplasmic and nuclear fractions by a nonaqueous technique. The procedure employed was the same as that described previously (14), except that the lyophilized, sieved liver was reduced to subcellular fragments in a colloid mill.
follows: Each 5.0 g of lyophilized liver powder was rather than in a ball mill. This modification greatly reduced the time required for milling and, in addition, improved both the yield and purity of the nuclear fraction. The procedure adopted was as follows: Each 5.0 g of lyophilized liver powder was suspended in 50 ml of hexane; 2.5 ml of 0.12 mm diameter glass beads were added and the resulting suspension was milled for 30 min at rheostat setting 120 and a rotor-stator clearance of 0.040 inches. The sample temperature was maintained at approximately 0°C during the milling.

Isolation of Enzymes from Labeled Subcellular Fractions

A 50-100 mg portion of each nuclear and cytoplasmic fraction was suspended in 25 vol of 3.0 mM ethylenediaminetetraacetate (EDTA)–0.4 mM diethylenetriaminepentaacetic acid (DTPA)–2.7 mM dithiothreitol–50 mM 2-(N-morpholino)-ethanesulfonic acid (MES) buffer, pH 6.2, held for 15 min at 0°C, and centrifuged for 80 min at 105,000 gavg. The supernatant was dialyzed for 4 hr at 4°C against 50 ml of 0.80 M NaCl–1.0 mM EDTA–0.2 mM DPN+–1.3 mM dithiothreitol–1.0 mM L-leucine–50 mM potassium phosphate buffer, pH 7.8, then centrifuged for 10 min at 25,000 g to remove a small amount of insoluble material. The resulting solution was assayed for glyceraldehyde-3-phosphate dehydrogenase, aldolase, and lactate dehydrogenase; and, from the enzyme activities obtained, the concentration of each enzyme was calculated (15). The three enzymes were precipitated sequentially (glyceraldehyde-3-phosphate dehydrogenase, first; aldolase, second; and lactic dehydrogenase, last), each with the appropriate antibody added as a 10 mg/ml solution in 0.80 M NaCl–1.0 mM EDTA–50 mM potassium phosphate buffer, pH 7.8 (glyceraldehyde-3-phosphate dehydrogenase and aldolase) or in 67 mM sodium phosphate buffer, pH 7.8 (lactic dehydrogenase). For each microgram of enzyme, 67 μg of antibody was added. After each addition of antibody, the solution was incubated for 90 min at 23°C then for 20 hr at 4°C to allow the antigen-antibody precipitate to form. Comparable results were obtained when the three enzymes were precipitated sequentially from a single portion of a liver extract as when each enzyme was precipitated from a separate sample of the extract.

Precipitates were washed, reduced, alkylated, and subjected to electrophoresis on polyacrylamide gels essentially as described earlier (15). Approximately 25 μg of enzyme was run on each gel. Following electrophoresis, the polyacrylamide gels were fixed overnight in 12% trichloroacetic acid. After fixation, the protein bands were visible in oblique light. The enzyme-containing band in each gel was identified by comparison with control gels loaded with reduced, alkylated samples of purified enzyme and/or antibody. A 7.5 mm long segment centered on the enzyme band was sliced from each gel. Additional 7.5-mm segments were then cut on either side of the initial segment.

The corresponding segments from two gels were placed in a counting vial with 3 ml of water. The trichloroacetic acid, which diffused from the gel segments into the aqueous phase, was extracted with four 5-ml portions of ether. A 0.3 ml sample of concentrated NH4OH was added, and the gels were allowed to dissolve. The resulting solution was evaporated to dryness at 60-80°C; the residue was moistened with 0.25 ml of water and dissolved in 20 ml of scintillation solution (4.0 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis-2-(5-phenyloxazoyl)benzene in 1 liter of toluene) containing 1.5 ml of NCS solubilizer (a toluene-soluble, quaternary ammonium base supplied by Amersham-Searle Corp., Arlington Heights, Ill.).

Radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer with automatic external standardization. Peak fractions from the polyacrylamide gels were counted to an error of 6% or less (95% confidence level). Counts obtained were converted to disintegrations by use of external standardization in conjunction with a quench correction curve.

In several experiments extra polyacrylamide gels were prepared, stained for protein, and cut into segments beginning with the enzyme band. The protein concentration in each segment was determined from the intensity of staining as described previously (15).

RESULTS

Quantitative Determination of Enzyme Protein

In the present investigation we have estimated the amounts of enzyme protein used for radioactivity determinations from the enzymatic activity present in the cytoplasmic and nuclear extracts before antibody precipitation. A more direct, but less convenient, method of quantitation involves measurement of the staining intensity of the enzyme bands following electrophoresis. In a previous study, in which the enzymes were isolated from extracts of unfractionated liver, both methods were employed (15). The amounts of glyceraldehyde-3-phosphate dehydrogenase and aldolase were more than twice as great when determined by staining as when estimated from enzyme activity; both methods gave comparable results for lactic dehy-
Comparison of Methods for Estimating Enzyme Protein

Enzyme protein was estimated from activity measurements before antibody precipitation and from the amount of dye bound by the enzyme band following electrophoresis. Values listed are averages from two experiments. The most widely scattered experimental values differed from the average by 22%.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Enzyme activity</th>
<th>Dye binding</th>
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<tbody>
<tr>
<td>Glyceraldehyde-3-phosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dehydrogenase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic</td>
<td>1.0</td>
<td>2.10</td>
</tr>
<tr>
<td>Nuclear</td>
<td>1.0</td>
<td>2.22</td>
</tr>
<tr>
<td>Aldolase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic</td>
<td>1.0</td>
<td>2.81</td>
</tr>
<tr>
<td>Nuclear</td>
<td>1.0</td>
<td>1.34</td>
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<tr>
<td>Lactic dehydrogenase</td>
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<td></td>
</tr>
<tr>
<td>Cytoplasmic</td>
<td>1.0</td>
<td>0.85</td>
</tr>
<tr>
<td>Nuclear</td>
<td>1.0</td>
<td>0.84</td>
</tr>
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</table>

The conclusions drawn in the present study are justified only insofar as the enzymes under investigation have been isolated in a pure form from the labeled cytoplasmic and nuclear fractions. We have, therefore, run several control experiments to assess the purity of the enzymes after immunoprecipitation and gel electrophoresis.

When unlabeled bovine serum albumin was precipitated with antibody from extracts of leucine-3H-labeled rat liver, significant amounts of radioactivity were found in the washed precipitates. Since the antibody to bovine serum albumin does not cross-react with rat serum albumin, this result demonstrated that labeled components from the liver extract had been adsorbed nonspecifically to the antigen-antibody precipitates. When such precipitates were dissolved in detergent solution, reduced, alkylated, and subjected to electrophoresis on polyacrylamide gels, the radioactive material became distributed over the entire length of the gel (Fig. 1A), thus demonstrating the usefulness of electrophoresis for removing material adsorbed nonspecifically to an enzyme-antibody precipitate. The electrophoretic pattern (Fig. 1A) has two major protein peaks. The leading peak contains the antibody light chains; the trailing peak consists of antibody heavy chains and bovine serum albumin.

In a second experiment, aldolase was precipitated from a labeled liver extract with the appropriate antibody, and the resulting precipitate, after being washed, reduced, and alkylated, was subjected to gel electrophoresis. The electrophoretic pattern (Fig. 1B) has three main protein peaks: the leading and trailing peaks represent the light and heavy chains of the antibody molecule, respectively; the middle peak is aldolase. The single major peak of radioactivity coincides with the enzyme peak. This correspondence, together with the absence of any other significant peaks of radioactivity, gives us some confidence in the purity of the enzyme.

In parallel experiments, glyceraldehyde-3-phosphate dehydrogenase and lactic dehydrogenase gave electrophoretic patterns similar to that of aldolase except that the enzyme peaks were not so widely separated from the antibody heavy chain peaks. The livers used in the control experiments described in this section were taken from rats.
Figure 1. Distribution of radioactivity and protein in polyacrylamide gels following electrophoresis of reduced, alkylated antigen-antibody precipitates. A. A 75 μg portion of unlabeled bovine serum albumin was added to 0.80 ml of an extract from the liver of a 1-leucine-3H-labeled rat and precipitated with 5.0 mg of antibody. B. Aldolase was precipitated from a 0.45 ml sample of the labeled liver extract (= 75 μg aldolase) by addition of 5.0 mg of antibody. Extracts were prepared from liver of rats which had received 3.0 mCi/kg of L-leucine-3H 72 hr previously. Techniques for administration of isotope and preparation of extracts were as described in an earlier paper (15). Each antigen-antibody precipitate, after being washed, reduced, and alkylated, was applied to three polyacrylamide gels. After completion of electrophoresis, the gels were stained. One of the stained gels was photographed and a densitometer tracing was made of the photographic negative. All three gels were then cut into 5-mm long segments, starting with the enzyme band. Corresponding segments were combined and prepared for counting.

Sacrificed 72 hr after injection of label. Similar experiments done with livers from animals labeled for only 2 min gave comparable results.

Labeling Kinetics of Nuclear and Cytoplasmic Enzymes

Rats were injected with 1-leucine-3H and sacrificed at various time intervals. The livers were separated into nuclear and cytoplasmic fractions, and the specific activities of the glyceraldehyde-3-phosphate dehydrogenase, aldolase, and lactic dehydrogenase in each of the fractions were determined. The results are presented in Fig. 2. For all three enzymes label appears in both the nuclear and cytoplasmic enzymes within a few seconds after injection of isotope; there is no indication that the enzymes at one locus become labeled at an earlier time than those at the other. Incorporation is approximately linear over the time interval investigated. The rates of labeling of the corresponding nuclear and cytoplasmic enzymes are, within experimental error, the same for all three enzymes.

Equilibration of the enzymes between the cytoplasmic and nuclear pools is slow relative to the labeling times employed (reference 14 and data presented below). The above results, therefore, exclude the possibility that the newly synthesized enzyme molecules in the nucleus are derived from the cytoplasmic pool of enzyme, for if this were the case, we would expect the specific radioactivities of the cytoplasmic enzymes to be higher than those of the nuclear enzymes at short labeling times. Rather, our results indicate either that synthesis of the enzymes under investigation occurs in the nucleus as well as the cytoplasm or that enzyme synthesized in the cytoplasm can move into the nucleus without passing through the cytoplasmic enzyme pool. The fact that the specific activities are always about the same for the corresponding nuclear and cytoplasmic enzymes could reflect a common origin of the enzymes at the two loci, thus supporting the second of the above alternatives.

Effect of Puromycin on Labeling of Nuclear and Cytoplasmic Enzymes

Recently we reported that puromycin inhibited amino acid incorporation into total cytoplasmic protein to a greater extent than into total nuclear protein in rat liver (16). To determine whether this differential inhibition of synthesis extends to the nuclear and cytoplasmic enzymes being studied in the present work, we have measured the level of incorporation into these enzymes in puromycin-treated rats after a single injection of labeled leucine. The results (Table II) indicate that incorporation into the cytoplasmic enzymes is inhibited to a significantly greater degree than into the corresponding enzymes in the nucleus. Whereas the specific radioactivities of the nuclear and cytoplasmic enzymes are similar in the absence of the antibiotic, in its presence the specific ac-

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activities of the nuclear enzymes are several times as great as those of their cytoplasmic counterparts. We have been able to reproduce this effect under several variations of the experimental procedure used to obtain the data given in Table II. The effect is not an artifact due to the low level of incorporation in the puromycin-treated animals. Control animals incorporate about the same amount of radioactivity in 1 min as the puromycin-treated animals do in 5 min; yet in control animals labeled for 1 min the specific activities of the nuclear and cytoplasmic enzymes are about equal.

The following experiment suggests that puromycin acts by inhibiting incorporation into cytoplasmic enzymes to a greater extent than into the corresponding nuclear enzymes, rather than by affecting the rate of movement of newly labeled molecules between the nucleus and the cytoplasm. Rats were given an intravenous injection of labeled leucine. 20 min later, when net incorporation had nearly ceased, the animals were given intraperitoneally 25 mg of puromycin/kg body weight. 20 min following injection of the drug, the animals were sacrificed. The specific activities of the nuclear and cytoplasmic enzymes were about equal, demonstrating that the puromycin does not alter appreciably the intracellular distribution of the recently synthesized enzyme molecules.

The results obtained with puromycin-treated
TABLE II
Effect of Puromycin on Labeling of Nuclear and Cytoplasmic Enzymes

Rats were given intraperitoneally 25 mg/kg body weight of puromycin as a neutral solution in 0.15 M NaCl. 15 min later, 9.0 mCi of L-leucine-4,5-3H/kg body weight was injected into the hepatic portal vein as described under Materials and Methods. 5 min after injection of isotope, livers were taken and separated into nuclear and cytoplasmic fractions. The specific radioactivity of the glyceraldehyde-3-phosphate dehydrogenase, aldolase, and lactic dehydrogenase in each of the subcellular fractions was determined. Control animals were treated in a similar manner but received no puromycin. Values given are averages from two separate experiments.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>Puromycin</th>
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<tbody>
<tr>
<td></td>
<td>Disintegrations/min per mg protein</td>
<td></td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasm (C)</td>
<td>57,500</td>
<td>3550</td>
</tr>
<tr>
<td>Nucleus (N)</td>
<td>57,600</td>
<td>8530</td>
</tr>
<tr>
<td>C/N</td>
<td>1.00</td>
<td>0.42</td>
</tr>
<tr>
<td>Aldolase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>61,200</td>
<td>2120</td>
</tr>
<tr>
<td>Nucleus</td>
<td>64,100</td>
<td>12150</td>
</tr>
<tr>
<td>C/N</td>
<td>0.95</td>
<td>0.17</td>
</tr>
<tr>
<td>Lactic dehydrogenase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>83,000</td>
<td>4210</td>
</tr>
<tr>
<td>Nucleus</td>
<td>93,500</td>
<td>14650</td>
</tr>
<tr>
<td>C/N</td>
<td>0.89</td>
<td>0.29</td>
</tr>
</tbody>
</table>

animals support the key assumption made in interpreting the labeling kinetics of the nuclear and cytoplasmic enzymes; namely, that movement of the enzymes between the nuclear and cytoplasmic pools occurs slowly. If movement were very rapid, there would not have been a significant difference between the specific radioactivities of the nuclear and cytoplasmic enzymes.

In addition, these results provide independent evidence that the enzymes of the cytoplasmic pool are not precursors to those in the nucleus, at least in the puromycin-treated animals. If such were the case, the specific radioactivities of the cytoplasmic enzymes could never be lower than those of the corresponding nuclear enzymes.

DISCUSSION
We have ruled out the possibility that the nuclear enzymes investigated in this study originated via synthesis at a cytoplasmic locus, release into the soluble portion of the cytoplasm, and subsequent migration across the nuclear envelope. The simplest alternative is that these enzymes are synthesized within the nucleus, and we have previously sought to explain the labeling kinetics of nuclear lactic dehydrogenase on this basis (14). Still another alternative is that the nuclear enzymes are synthesized at a cytoplasmic locus, but move into the nucleus with no intervening sojourn in the soluble portion of the cytoplasm. A mechanism by which this might occur is suggested by studies on several proteins which are exported from the cells in which they are formed. Thus, digestive enzymes produced by the acinar cells of the guinea pig pancreas are synthesized by membrane-bound ribosomes, move vectorially into the lumen of the endoplasmic reticulum, then pass to the Golgi apparatus, where they are packaged into secretory granules in which form they are transported to the margin of the cell (17-20). Serum albumin synthesized by rat hepatocytes and immunoglobulins produced by mouse plasma cells are transported in a similar fashion (21, 22).

By a mechanism analogous to that operating in the case of secretory proteins, nuclear proteins might be synthesized by cytoplasmic ribosomes and move into the cell nucleus without equilibrating with the soluble space of the cytoplasm. The lumen of the endoplasmic reticulum is contiguous with the perinuclear space; therefore, any protein which, like the secretory proteins, moved directly from the site of synthesis into the lumen of the endoplasmic reticulum might enter the nucleus without passing through the soluble space of the cytoplasm. It should be noted that the outer nuclear membrane is studded with polysomes, and that they differ from other cytoplasmic polysomes with respect to the kinds of protein which they synthesize (23) and with respect to their labeling kinetics following a pulse of a radioactive amino acid (23) or RNA precursor (24, 25). Studies by Gorovzky using *Tetrahymena* indicate that the outer membrane ribosomes may be responsible for synthesis of nuclear proteins (23). Both the kinetics of amino acid incorporation into outer membrane ribosomes in vivo and the fate of labeled polypeptides released from the outer membrane ribosomes by puromycin in vitro are consistent with this hypothesis. If the outer membrane polysomes are specifically involved in synthesis of nuclear proteins, we might have an explanation not only for...
our labeling results, but also for the differential effect of puromycin on amino acid incorporation into nuclear and cytoplasmic proteins if the outer membrane polysomes should prove to be less sensitive to this drug than other cytoplasmic polysomes.

Nuclear protein synthesis has recently been the subject of an extensive and critical review by Goldstein (26). He has concluded that, except for several highly specialized and possibly atypical kinds of cells, there is no convincing evidence that proteins are synthesized in the nucleus. In our own laboratory we have, for the past several years, been engaged in experiments designed to indicate whether or not the liver cell nucleus is capable of protein synthesis. On the basis of preliminary results, we are inclined to doubt that the nuclear enzymes which we have investigated are synthesized in the cytoplasm—possibly by the outer membrane polysomes—and move directly into the nucleus without being released into the soluble portion of the cytoplasm.

We wish to acknowledge the excellent technical assistance of Miss Claudia W. Thorpe, who prepared all of the subcellular fractions used in this study.

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