TP synthesis occurs in a circadian fashion, and this enzyme may represent only part of an overall rhythmic pattern in hepatic protein synthesis. Also, intact adrenocortical function may play at least a permissive role in the maintenance of this rhythmicity.

MORTON I. RAPOPORT
RALPH D. FEIGIN
JOSEPH BRUTON, WILLIAM R. BEISEL
U.S. Army Medical Unit,
Walter Reed Army Medical Center,
Fort Detrick, Maryland

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Rate of Protein Synthesis: Regulation during First Division Cycle of Sea Urchin Eggs

Abstract. *Protein synthesis in fertilized sea urchin eggs, or in 12,000g supernatants derived from them, increased linearly during the period preceding prophase of the first mitotic cycle, dropped during metaphase and anaphase, and increased again after telophase. Similar results were observed for whole cells incubated in the presence of colchicine. These changes in the rate of protein synthesis during the mitotic cycle may be regulated at the translational level.*

The overall rate of protein synthesis in the fertilized sea urchin egg increases throughout the early cleavage stages until blastulation (*1*). In addition to this general increase, fluctuations exist in the rate of protein synthesis during the first few division cycles (*2*). Similar variations have been observed in dividing mammalian cells in tissue culture (*3*). We report here that these variations in the rate of incorporation of labeled amino acids into protein occur in vivo, in vitro, and in the presence of colchicine, and they are detectable at the polysome level during the first division cycle of the sea urchin egg.

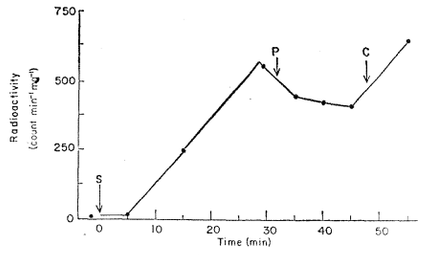

Fig. 1. Incorporation of C^{14}-leucine (231 mc/m*M*) into 5 percent trichloroacetic acid–precipitable protein during the first division cycle. At the indicated time after fertilization the eggs (at 26°C) were "pulse" labeled for 2.5 minutes with C^{14}-leucine (0.03 μc/ml). Eggs were fertilized at zero time. Radioactivity is expressed as counts per minute per milligram of protein; time, as minutes after fertilization. *S* indicates when sperm was added; *P* is prophase; and *C*, cleavage.

Gametes of the sea urchin (*Lytechinus variegatus*) were obtained by the KCl method described by Harvey (*4*). Eggs were washed three times in filtered sea water, washed three more times in Millipore-filtered sea water, and then incubated in Millipore-filtered sea water containing 75 μg of streptomycin sulfate and 300 units of penicillin per milliliter.

The eggs in suspension were fertilized, and portions of the suspension were transferred to separate incubation vessels. After fertilization, eggs were exposed to C^{14}-amino acids for the times indicated in each experiment. Unfertilized control eggs were treated similarly. After incubation an equal volume of 10 percent trichloroacetic acid (TCA) was added and the resultant precipitates were homogenized. These homogenates were then centrifuged and washed three times with 5 percent TCA, heated to 90°C in 5 percent TCA for 30 minutes, again washed three times with 5 percent TCA, and extracted twice with a mixture of ethanol and ether (3 : 1) and once with acetone. Dried precipitates were dissolved in 88 percent formic acid, plated on either preweighed aluminum planchettes or Whatman GF/C glass filter pads, and dried. Radioactivity was measured with a Nuclear-Chicago gas-flow counter or a Packard scintillation counter (efficiencies, 33 percent and 63 percent, respectively). No corrections for self-absorption were required. The amount of protein was determined by weight.

In the experiments on in vitro synthesis of protein, eggs were first incubated at 30°C to the appropriate stage, rapidly cooled to 5°C, washed three times with a mixture of cold isotonic NaCl and KCl (19 : 1) and once with cold homogenizing medium (0.01*M* $MgCl_2$, 0.24*M* KCl, and 0.01*M* tris-HCl, *p*H 7.6), resuspended in four

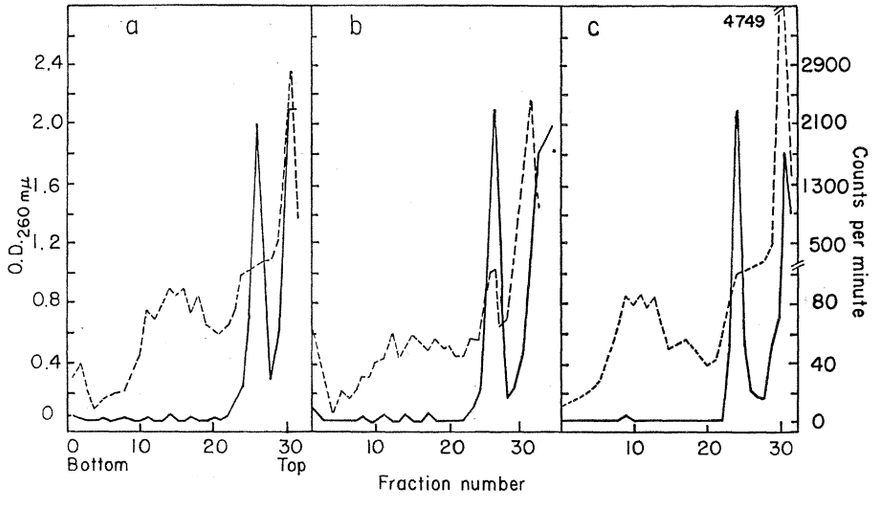

Fig. 2. Sucrose-gradient analysis of the 12,000*g* supernatant from eggs homogenized after exposure to C^{14}-leucine (231 mc/m*M*) at different stages during the first division cycle. Eggs were incubated in the presence of C^{14}-leucine from (a) 17 to 22 minutes, (b) 28 to 33 minutes, and (c) 43 to 48 minutes after fertilization. After fertilization eggs (at 30°C) were "pulse" labeled at the indicated times with C^{14}-leucine (0.025 μc/ml), then homogenized and centrifuged at 12,000*g* for 30 minutes; 0.25 ml of the supernatants of equal $O.D._{260}$ were layered on 29 ml of 15 to 50 percent sucrose gradients. The gradients were centrifuged for 3 hours at 24,000 rev/min in a SW-25 rotor. The polysome region lies between fractions 0 and 20. Solid line is $O.D._{260}$; dotted line is counts per minute.

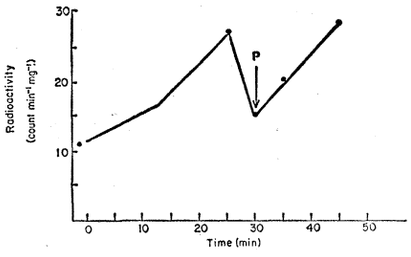

Fig. 3. Incorporating capacity in vitro of the 12,000*g* supernatant derived from eggs homogenized at different times after fertilization. Radioactivity is expressed as counts per minute per milligram of protein; time, as minutes after fertilization. *P* is prophase.

volumes of homogenizing medium, and homogenized gently in a Duall tissue homogenizer fitted with a Teflon pestle. Homogenates were centrifuged at 12,000*g* for 30 minutes, and the supernatants were used for incubation. One milliliter of the cell-free reaction mixture contained 20 μM phosphoenolpyruvate, 0.4 μM guanosine triphosphate, 0.1 mg of pyruvate kinase, 0.3 μc of C^{14}-leucine (specific activity, 67.4 mc/mM), 2 μM adenosine triphosphate, and approximately 2 mg of supernatant protein. After incubation for 45 minutes at 30°C, an equal volume of 10 percent TCA was added. The precipitates were washed and analyzed as above.

The experiment designed to show differences in polysomes at various times after fertilization was performed as previously reported (*5*). The $O.D._{260}$ was measured in a Gilford multiple sample absorbance unit with the use of a 1-cm flow-through cell.

Upon fertilization the rate of incorporation of C^{14}-leucine into protein increased linearly (Fig. 1) until about the time of nuclear membrane rupture. At that time, the rate of synthesis declined and remained constant to approximately the metaphase or anaphase stage, after which it again increased. Similar patterns occurred in the incorporation of C^{14}-valine and C^{14}-phenylalanine into protein (*6*). This pattern of synthesis was also discernible by determining the amount of labeled protein associated with the polysomes (Fig. 2). At metaphase (Fig. 2b) the amount of radioactivity associated with the polysome region was lower than in comparable regions isolated from eggs before (Fig. 2a) or after (Fig. 2c) division.

Experiments were conducted to eliminate the possibility that observed changes in rates of synthesis were due to differences in permeability of the eggs to added amino acids at various times during the division cycle. Therefore, we measured the capacity of 12,000*g* supernatant fractions, derived from eggs that had been homogenized at different stages of division, to incorporate amino acids in vitro. The patterns observed in vitro (Fig. 3) were similar to those seen in vivo (Fig. 1).

Other experiments demonstrated that these fluctuations in protein synthesis occurred even in the absence of cytokinesis. Even though the cells do not divide in the presence of colchicine (*7*), they exhibited a pattern of incorporation of amino acids (Fig. 4) similar to that of the controls (Fig. 1). Experiments represented in Figs. 1 and 4 cannot be quantitatively compared because eggs, incubation temperature, and specific activity of C^{14}-leucine were different.

Fluctuations in the rate of incorporation of labeled amino acids into protein during the first division cycle of sea urchin eggs are apparently due to some regulatory mechanism affecting the rate of protein synthesis. Observed findings do not appear to have been due to variations in permeability of C^{14}-leucine during the division cycle (Fig. 3). Nor can the results be attributed to a lack of either adenosine or guanosine triphosphate—required for protein synthesis—since an excess of nucleotide triphosphate and a regenerating system were added to each in vitro incubation mixture. Both the size of the amino acid pool (*8*) and the amounts of total protein (*9*) have been observed to be relatively constant during division.

Furthermore, the colchicine experiment (Fig. 4) suggests that neither the normal assembly and disassembly of the mitotic apparatus nor the cytokinetic process itself is solely responsible for regulating protein synthesis. In addition, the in vitro experiment (Fig. 3) demonstrates that structural integrity of the whole egg is not required for regulation.

Inhibition of RNA synthesis after fertilization in the sea urchin egg either by enucleation (*10*) or by treatment with actinomycin D (*11*) does not appear to limit the rate of protein synthesis during the early cleavage stages. Therefore, differences in rate of protein synthesis that we observed during the first division cycle may be due to variations in the rate of translation of the mRNA message or to some undescribed mechanism. Thus, changes in rate of protein synthesis during division may be additional examples of translational control of gene function (*12*).

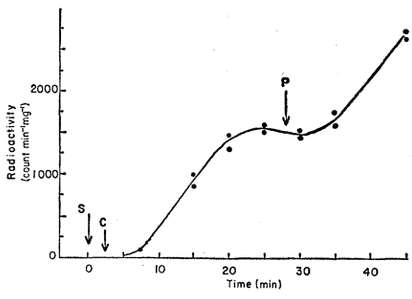

Fig. 4. Effects of colchicine on the incorporation of C^{14}-leucine (67.4 mc/mM) into precipitable protein during the first division cycle. Colchicine ($1 \times 10^{-4} M$) was added 2 minutes after fertilization. At the indicated times after fertilization the eggs (at 30°C) were "pulse" labeled for 2.5 minutes with C^{14}-leucine (0.025 μc/ml). Radioactivity is expressed as counts per minute per milligram of protein; time, as minutes after fertilization. *S* indicates when sperm was added and *C*, when colchicine ($1 \times 10^{-4} M$) was added; *P* is prophase.

W. H. Sofer
J. F. George
R. M. Iverson

Department of Biology,
Laboratory for Quantitative Biology,
University of Miami,
Coral Gables, Florida 33124

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