REGULATION SYSTEM OF PROTEIN SYNTHESIS IN EARLY EMBRYOGENESIS IN THE SEA URCHIN*,**

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In a variety of organisms, it has been suggested that there are cyclic variations in the rate of protein synthesis with the cell division. Recently, several different views have been expressed regarding whether the pattern of protein synthesis after fertilization is cyclic or noncyclic during early development of the sea urchin (1 - 3). Since protein synthesis in the sea urchin in early embryogenesis appears to be independent of nuclear control, the cyclic variation in this early stage in the rate of synthesis must be under cytoplasmic control. The present study was designed to analyze the system controlling protein synthesis in early embryogenesis in the sea urchin (Hemicentrotus pulcherrimus, Pseudocentrotus depressus and Anthocidaris crassispina). The results showed that the regulation of synthesis involves multiple controlling systems and most of them are believed to be under cytoplasmic regulation. Each mechanism has been investigated, and it is concluded that there may be a variety in the course of formation of polyribosomes.

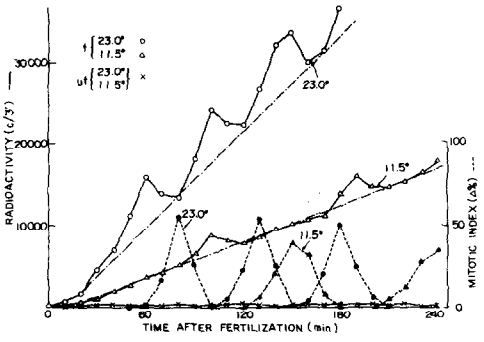

Fig. 1 Rate of amino acid incorporation in intact embryos.

The suspension of eggs or embryos of P. depressus in Millipore (HA 0.45 μ) filtered sea water (2 x 10^4 cells/ml) was allowed to develop at the indicated temperature. At suitable times, 5 ml-aliquots were added to 0.5 μc ^{14}C-amino acids (acid hydrolysate of Chlorella protein, 6.88 mc/mC) in 50 μl and the mixtures were incubated at the same temperature for further 10 min. The reaction was terminated by the additior of an equal volume of 20 % TCA containing 0.2 % casamino acids. Incorporation of the radioactivity was analyzed as described previously (8). The time indicated refers to the starting time of each pulse experiment after fertilization. The synchrony of cell division is expressed as differential mitotic index for 10 min. The line (—·—·—) represents the increase in the basal rate of incorporation. f: fertilized, uf: unfertilized.

* A review including this work has been published (5).

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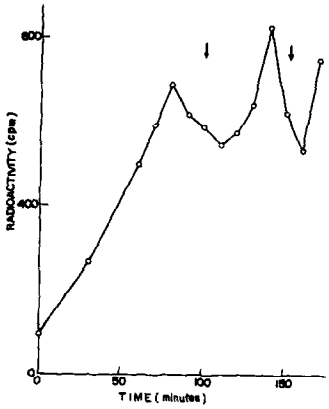

Fig. 2 Amino acid incorporation in anucleated egg fragments.

Anucleated egg fragments were prepared from P. depressus eggs by a modification of the previous method (7). Improved results were obtained by changing the egg concentration to 1.8×10^5 cells/ml per tube, the sucrose concentration to 1.1 M sucrose : sea water in 1.3 : 1 with 4 : 1 as a cushion, and carrying out the centrifugation at 11,500 r.p.m. for 15 min. with RPS 25A rotor of the Hitachi 40PA Ultracentrifuge. Nuclear contamination was less than 1 %. Parthenogenetic activation was effected by 1 min. treatment with 5 mM n-butyric acid in sea water. Each sample contained 1.2×10^5 of the anucleated egg fragments. Incubation was carried out in a volume of 1.0 ml at 20°. Other details of the experiment were similar to those described in Fig. 1.

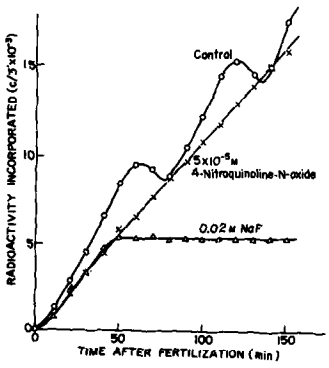

Fig. 3 Effects of 4-nitroquinoline-N-oxide and NaF on amino acid incorporation in intact embryos.

Amino acid incorporation in the embryos of H. pulcherrimus was examined at 21° with and without 5.0×10^{-5} M 4-nitroquinoline-N-oxide or 0.02 M NaF by the procedures described in Fig. 1. The inhibitors were added 2 min. after fertilization.

Results and Discussions

Protein synthesis, as detected in pulse incorporation of amino acids, in intact embryos of sea urchin in the early cleavage stage showed cyclic variations which were superimposed on an increasing basal rate of synthesis (Fig. 1). The cyclic variations in protein synthesis were apparently independent of any similar cyclic cellular uptake of amino acids as shown by a preloaded cummulative incorporation (4) and by cell-free incorporation which was also cyclic (Fig. 4). These variations seem to correlate with mitotic divisions and may be directly related to the mitotic cycle since each peak coincided with the stage from prometaphase to metaphase of mitotic division. The interval of the cycle in the intact system was in inverse correlation with temperature (Fig. 1). The whole pattern of variation in protein synthesis, which was initiated by fertilization and was not observed in unfertilized eggs, was observed even in the presence of actinomycin D (10 µg/ml) in both chemically anucleated (6) or in anucleated egg fragments prepared by a similar method to that developed by Harvey (7) (less than 1 % nuclear contamination) and acti-

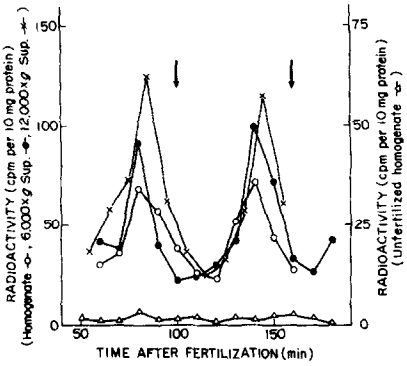

Fig. 4 Cyclic variations in the rate of amino acid incorporation in cell-free systems.

The homogenate or supernatant was prepared from fertilized H. pulcherrimus embryos with Millipore filtered sea water (Fig. 1). The time of centrifugation was 30 min. Assay for amino acid incorporation was carried out at 18.5° with 1 μc ^{14}C-amino acids (7.37 mc/mC) in a total volume of 1.0 ml. Protein content in the experiments with homogenate, 6,000 and 12,000 x g supernatants were 32.3 mg, 28.4 mg and 27.1 mg, respectively. Measurement of the incorporation was followed that described in Fig. 1. Arrows indicate the time of cell division observed in a parallel experiment with intact embryos at 20°. Another parallel experiment with homogenate of unfertilized eggs of the same species (protein concentration, 36.7 mg) is given as a reference.

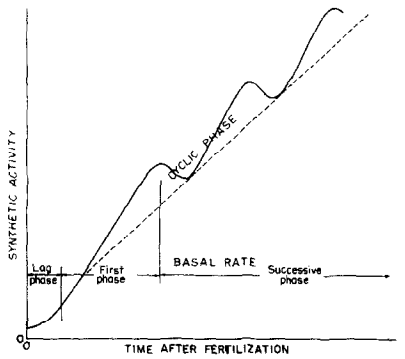

Fig. 5 Scheme illustrating the regulation systems for protein synthesis in early embryogenesis in the sea urchin.

vated by butyric acid parthenogenetically (Fig. 2). It was thus suggested that the cytoplasm may possess regulatory mechanisms for protein synthesis which lead to the mitotic cycle.

The cycles were observed weakly even in the absence of nuclear and cytoplasmic divisions when protein synthesis was inhibited partially by colchicine (5×10^{-5} M), puromycin (2×10^{-3} M) or cycloheximide (2×10^{-3} M). The results together with other general principles in mitotic cycles suggest that the occurrence of the metabolic cycle is a necessary but not sufficient condition for cell division.

Similar experiments with 4-nitroquinoline-N-oxide (2×10^{-5} M) and NaF (0.02 M), where no cell division was observed, showed that both drugs inhibited the occurrence of the cyclic phase. In the presence of 4-nitroquinoline-N-oxide the linear increase in the basal rate of incorporation still occurred while NaF inhibited the further increase in the basal rate after the first cycle (Fig. 3). The absence of the normal increase of the basal rate after the first cycle was noted in the experiments with eggs anucleated, either chemically or mechanically as mentioned above, but the cyclic variation still survived in these cases (e.g., Fig.

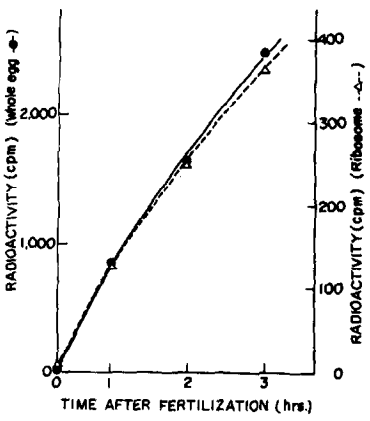

Fig. 6 Parallelism in the incorporation activity of intact embryos and isolated ribosomes.

Amino acid incorporations by intact embryos of P. depressus and by ribosomes isolated from the same embryos were compared without the addition of exogenous template RNA according to the procedures described previously (8). For the incubation with ribosomes, unfertilized P. depressus 144,000 x g supernatant (28.8 mg protein) was supplemented. Ribosomes were prepared as described (8) at the stage of development indicated. Values presented are for the incorporation by 1 x 10^5 of intact embryos and by ribosomes containing 120 µg of protein. To make sure that the ribosomes were isolated from cells at the proper phase, low points of the cycle were determined in a parallel experiment with intact embryos.

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2). On the other hand, cell-free systems (homogenate, 6,000 and 12,000 x g supernatant) of fertilized eggs in various stages have been shown to carry out protein synthesis at rates which varied in a cyclic fashion without an increase in the basal level of synthesis (Fig. 4). Such changes were observed neither in intact nor cell-free system of unfertilized eggs. As shown in Fig. 1, a lag phase observed during about 10 minutes after fertilization may be controlled by mechanism other than above as discussed earlier (8 - 10). These findings may imply that the cyclic and the basal rate of protein synthesis are controlled by different mechanisms. The latter is thought to be controlled further by at least three different systems depending upon the developmental stage: that of the initial stage of activation expressed as the lag phase, that of between the lag phase and the first cycle expressed as the first phase, and that of the succeeding stage expressed as the successive phase as illustrated in Fig. 5. Three of them except for the successive phase are at least believed to be under cytoplasmic control. This idea may be a counterpart to that of genic control in the mechanism of mitotic cycle (11 - 17). The cytoplasmic control of such cyclic activity has been suggested in DNA synthesis (18, 19). Cyclic expressions of metabolism observed in somatic cell division is thus considered to be a combination of genic and cytoplasmic cycles.

The increase in the basal rate of synthesis appeared to relate linearly to an increase in activity of polyribosomes prepared from the stages of the same phase

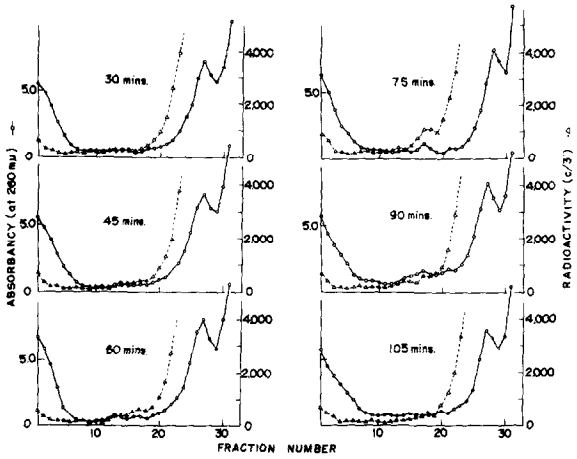

Fig. 7 Periodic formation of polyribosomes in the 12,000 x g supernatant system.

One ml of the 12,000 x g (centrifuged for 10 min.) supernatant (80.4 mg protein in 2.0 ml) was incubated with 0.5 μc of ^{14}C-amino acid mixture (9.4 mc/mC) for 15 min. at each stage at 21° as in Fig. 4. The reaction was terminated by the addition of NaF to a final concentration of 0.05 M. Then, 0.2 ml-aliquot was layered on a linear sucrose density gradient (15 - 30 % gradient containing 0.01 M Mg-acetate and 0.05 M Tris-acetate buffer pH 7.8) and analyzed by centrifugation at 130,580 x g for 150 min. as described previously (9). The time indicated is the starting point of the incubations with the isotope.

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of the cycles (Fig. 6) but not to the supernatant (S-105) activity. The polyribosomal activity seemed irrelevant to the amount and the template activity of ribosome-bound m-RNA, as prepared and measured as described (20), which holds constant level. The increase in the polyribosomal activity may in part be due to an increase in the amount of heavy polyribosomes ranging 200 - 300 S (r-polysomes (21)), but a linear relationship was not always observed between the amount and the activity of polyribosomes. Thus, the increase of the basal rate of synthesis may in part be due to some structural changes in polyribosomes as discussed previously (22).

Data obtained in the cell-free system indicate the cyclic variations may also be regulated by periodic changes in the level of polyribosomes (r-polysomes) (Fig. 7), the cyclic variation is apparently associated with the mitotic cycle. The result is in accord with other findings (23 - 25). The genesis of the cyclic variations of the synthesis will be treated with in a separate paper (26). These findings suggest that there may be a variety in the course of formation of polyribosomes as has been suggested by other workers (27 - 30).

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References

1) Gross, P. R., and Fry, B. J., Science, 153, 749 (1966)
2) Sofer, W. H., George, J. F., and Iverson, R. M., Science, 153, 1644 (1966)
3) Timourian, H., Science, 154, 1055 (1966)
4) Epel, D., Proc. Natl. Acad. Sci. U. S., 57, 899 (1967)
5) Mano, Y., J. Jap. Biochem. Soc., 40, 273 (1968) (in Japanese)
6) Gross, P. R., J. Exp. Zool., 157, 21 (1964)
7) Harvey, E. B., The American Arbacia and Other Sea Urchins, p. 138, Princeton University Press, Princeton, N. J. (1956)
8) Mano, Y., and Nagano, H., Biochem. Biophys. Res. Commun., 25, 210 (1966)
9) Mano, Y., Biochem. Biophys. Res. Commun., 25, 216 (1966)
10) Mano, Y., Symp. Cell Chem., 17, 99 (1966) (in Japanese)
11) Kuempel, P. L., and Pardee, A. B., J. Cell. Comp. Physiol., 62, Suppl. 1, 15 (1963)
12) Masters, M., Kuempel, P. L., and Pardee, A. B., Biochem. Biophys. Res. Commun., 15, 38 (1964)
13) Masters, M., and Pardee, A. B., Proc. Natl. Acad. Sci. U. S., 54, 64 (1965)
14) Kuempel, P. L., Masters, M., and Pardee, A. B., Biochem. Biophys. Res. Commun., 18, 858 (1965)
15) Mitchison, J. M., and Vincent, W. S., Nature, 205, 978 (1965)
16) Bostock, C. J., Donachie, W. D., Masters, M., and Mitchison, J. M., Nature, 210, 808 (1966)
17) Mitchison, J. M., Abstr. 7th Internatl. Congr. Biochem., 363 (1967)
18) Prescott, D. M., and Goldstein, L., Science, 155, 469 (1967)
19) Gurdon, J. B., and Woodland, H. R., Biol. Rev., 43, 233 (1968)
20) Ogata, K., Ishikawa, K., Tominaga, H., Watanabe, I., Morita, T., and Sugano, H., Biochim. Biophys. Acta, 76, 630 (1963)
21) Infante, A. A., and Nemer, M., Proc. Natl. Acad. Sci. U. S., 58, 681 (1967)
22) Mano, Y., Protein, Nucleic Acid, Enzyme, 13, 11 (1968) (in Japanese)
23) Penman, S., Sherrer, K., Becker, Y., and Darnell, J., Proc. Natl. Acad. Sci. U. S., 49, 654 (1963)
24) Vincent, W. S., Biol. Bull., 129, 388 (1965)
25) Scharff, M. D., and Robbins, E., Science, 151, 992 (1966)
26) Mano, Y., J. Biochem., in press.
27) Hultin, T., Exp. Cell Res., 34, 608 (1964)
28) Hultin, T., Develop. Biol., 10, 305 (1964)
29) Marcus, A., and Feeley, J., Proc. Natl. Acad. Sci. U. S., 56, 1770 (1966)
30) Dresden, M. H., and Hoagland, M. J., J. Biol. Chem., 242, 1065 (1967)