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Changes in the RNA and Protein Synthesis at the Pre- and Post-fertilization Stages of a Sea Urchin, *Hemicentrotus pulcherrimus*.

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말똥성게(*Hemicentrotus pulcherrimus*)의 수정전과 초기 발생동안 RNA 및 단백질합성의 변화

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요 약

성게의 일종인 *Hemicentrotus pulcherrimus*에서 수정전에 이미 축적되었던 mRNA의 활성화 변화를 알아보기 위하여 초기 발생동안의 RNA와 단백질 합성에 관하여 연구하였다. 미수정란에서는 RNA와 단백질의 합성률이 대단히 낮았다. 그러나 수정과 함께 RNA합성은 크게 변하지 않은 반면, 단백질합성은 크게 활성화되었다. RNA와 단백질 합성율이 병행적으로 변하지는 않지만, 포배와 낭배에서 크게 증가함을 볼 수 있었다. 단백질합성은 양적으로 뿐만 아니라 질적으로도 발생단계에 따라 변하는 사실을 이차원 전기영동에 의한 연구를 통하여 확인할 수 있었다.

INTRODUCTION

Protein synthesis occurs at a very low rate in the unfertilized eggs and abruptly increases in the rate upon fertilization in sea urchins(Epel, 1967; Humphreys, 1969; Rinaldi and Monroy, 1969; Timourian and Watchmaker, 1970; Regier and Kafatos, 1977; Hille and Albers, 1979; Raff *et al.*, 1981). This increase is mostly dependent upon the availability of mRNAs which are synthesized during oogenesis, stored and masked in the unfertilized eggs (Spirin and Nemer, 1965; Wilt, 1970; Gross *et al.*, 1973; Dworkin and Infante, 1978; Jenkins *et al.*, 1978; Kaumeyer *et al.*, 1978; Hough-Evans *et al.*, 1979; Ruderman and Schmidt, 1981). These mRNAs are unmasked upon fertilization and utilized to synthesize proteins at varied rates during the early development (Humphreys, 1971; Brandis and Raff,

1978, 1979; Whiteley and Mizuno, 1981; Davidson *et al.*, 1982; Raff, 1982; Raff and Showman, 1983). Such quantitative change strongly suggested that protein synthesis might have been also qualitatively activated, indicating that the stored mRNAs are differently recruited during the early development. A selective recruitment of mRNAs was reported in mouse (Cascio and Wasserman, 1982), starfish (Rosenthal *et al.*, 1982) and *Spisula* (Rosenthal *et al.*, 1980; Tansey and Ruderman, 1983). Particularly, in *Spisula* the electrophoretic patterns of protein synthesis changes shortly after fertilization and these changes are modulated at the translational level (Rosenthal *et al.*, 1980). It was, however, reported that the quantitative increase in protein synthesis is not accompanied by qualitative change upto blastula stage in sea urchin (Brandhorst, 1976).

Since mRNAs turn over rapidly with a half life of several hours in sea urchin embryos (Davidson *et al.*, 1982), and thus, mRNAs should be continuously supplied from the masked mRNA pool as the development proceeds, a hypothesis of selective recruitment may still be effective in the development of sea urchin. We examined a possibility of the selective recruitment of masked mRNAs in the development of *Hemicentrotus*. In the studies, we determined changes in permeability of the eggs and embryos to an amino acid in synthetic activity and patterns of protein synthesis from the unfertilized throughout the gastrula stage.

MATERIALS AND METHODS

Experimental animal

Hemicentrotus pulcherrimus used in the study was collected from intertidal zones at Seochun, Chung-cheong-nam-do, Korea, from December to March, and maintained in sea water at 10±1°C. Gametes were obtained by intracoelomic injection of 0.5 M KCl, and washed several times with Millipore-filtered sea water (MFSW) before insemination. The eggs were inseminated with an excess of diluted sperm, which was then removed from the cultures. Embryos were cultured at 11±1°C in MFSW, stirring with a 30 rpm clock-motor equipped with paddles.

Observation of developmental stages

The eggs and embryos were observed and photographed with a light microscope, fixing the movement of the embryos after hatching blastula stage in 1% methylcellulose-in-MFSW (Table 1 and Fig. 1).

Uptake and incorporation of ^{3}H-leucine or ^{3}H-uridine

In order to determine the uptake and the incorporation, the eggs or embryos were first labeled with ^{3}H-leucine (New England Nuclear, Sp. Act. 152 Ci/mM) or ^{3}H-uridine (New England Nuclear, Sp. Act. 30 Ci/mM) at a final concentration of 3μ Ci/ml at 11 ±1°C for various times. Immediately after the incubation they were harvested by transferring onto ice and washing twice with 10 volumes of ice-cold MFSW. The labeled eggs

Table 1. Time sequence of the early development in *Hemicentrotus pulcherrimus*

Time after fertilization(hrs.)	Developmental stages
0.0	Fertilization
2.5	1st cleavage
4.0	2nd cleavage
5.3	3rd cleavage
7.0	4th cleavage
23.0	Early blastula
26.0	Hatching blastula
30.0	Mesenchyme blastula
34.0	Early gastrula
47.0	Late gastrula

The temperature was maintained at 11±1°C during the culture.

or embryos were homogenized in precooled 10% trichloroacetic acid (TCA). The homogenate was separated into TCA-soluble and precipitable fractions by centrifugation. The radioactivity in each fraction was counted in a toluene-PPO-POPOP scintillation fluid with a scintillation counter (Beckman LS 6800).

Labeling and extraction of proteins

Eggs or embryos were incubated at a concentration of 15% in MFSW containing 250 mg/*l* of streptomycine sulfate, labeling the eggs with 300 μCi/ml of ^{3}H-leucine (New England Nuclear, Sp. Act. 141 Ci/mM) for 4 hours and the embryos with 100 μCi/ml for 2 hours at 11±1°C, respectively. After incubation they were washed twice in ice-cold MFSW and homogenated in boiling sodium dodecyl sulface (SDS) extraction buffer (O'Farrell, 1975). The homogenate was centrifuged at 12,000 rpm (Beckman J2-21 centrifuge, JA-21 roter) at 0±1°C for 10 minutes. The supernatant was collected and precipitated with ice-cold acetone containing 0.2 mM phenylmethylsulfonylfluoride. The pellet was collected by centrifugation at 5,000 rpm (JA-20 roter) at 0±1°C for 5 minutes, and dried by blowing cotton-filtered air. The dried pellet was dissolved in isoelectric focussing lysis buffer (O'Farrell, 1975).

Two-dimensional gel electrophoresis and fluorography

Two-dimensional polyacrylamide gel electrophoresis was performed essentially as described by O'Farrell (1975). The isoelectric focussing (IEF) gels (2.4×128 mm) were electrophoresed at 550V for 15 hours and then at 800V for 1 hour at 22°C. IEF gels were prepared for the second-dimensional electrophoresis by equilibrating in SDS sample buffer for 2 hours at room temperature. An exponential gradient gel of 9~15% polyacrylamide was used for the second-dimensional slab gel electrophoresis (140×90×0.7 mm). After prerunning was performed with high SDS running buffer at 20mA constant current for 20 minutes at room temperature, the gel was run with running buffer at 12.5 mA for 6 hours at 9°C, and

fixed in 7.5% acetic acid. The gel was dehydrated in dimethylsulfoxide (DMSO), changing the DMSO three times, soaked in 20% PPO-in-DMSO for 3 hours, and then in distilled water for 1 hour. After washing several times in distilled water, the gel was dried by aspiration at 55°C for 10 hours. The dried gel was exposed to preflashed X-ray film (Kodak, X-Omat AR) for 144 hours at −70°C (Bonner and Laskey, 1974; Laskey and Mills, 1975).

RESULTS

1. Changes in RNA synthesis

Changes in RNA synthesis were examined at the stages of unfertilized and fertilized egg, blastula and gastrula (Table 2). The radioactivity in the TCA-soluble fraction, which was actually an uptake of ^{3}H-uridine, was considered to be a permeability of the eggs or embryos to ^{3}H-uridine. The uptake was found to remain very low in the unfertilized eggs with a slight increase even after a prolonged incubation upto 90 minutes, but was increased about 10-fold following the fertilization. The uptake was further increased at the blastula stage, even though there was a slight decrease during incubation. The uptake at gastrula stage is slightly lower than at the blastula stage and drastically reduced during the subsequent incubation. With any duration of incubation the uptake is generally increased at the time of fertilization and further increased at the blastula stage but reduced at the gastrula stage, indicating that the permeability of the eggs or embryos to ^{3}H-uridine

Table 2. Changes in radioactivities of ^{3}H-uridine taken up and incorporated into the eggs or embryos of various developmental stages.

stage	Hrs of develop.	Incubation time	TCA-soluble(U) (cpm/10^3 embryos)	TCA-ppt(I) (cpm/10^3 embryos)	I/U
Unfertilized	0	15 min	1,012	17	0.0163
Fertilized	5 min	15 min	11,702	72	0.0061
Blastula	20 hrs	15 min	24,264	851	0.0351
Gastrula	40 hrs	15 min	18,181	1,041	0.0572
Unfertilized	0	45 min	2,439	21	0.0084
Fertilized	5 min	45 min	19,444	88	0.0045
Blastula	20 hrs	45 min	21,447	1,346	0.0628
Gastrula	40 hrs	45 min	13,771	1,838	0.1335
Unfertilized	0	90 min	2,654	14	0.0051
Fertilized	5 min	90 min	17,242	106	0.0061
Blastula	20 hrs	90 min	20,594	1,780	0.0864
Gastrula	40 hrs	90 min	15,889	2,786	0.1753

The eggs or embryos, which had been labelled with ^{3}H-uridine, was homogenized in TCA and the homogenate was fractionated into TCA-soluble and precipitable fractions by centrifugation.

increases upon fertilization and varies during the developmental stages.

The radioactivity in the TCA-precipitable fraction, which was considered to be an incorporation of ^{3}H-uridine into the RNAs of the eggs or embryos, was measured to determine the rate of RNA synthesis (Table 2). The extent of incorporation, which was found to be very low and unchanged in the unfertilized eggs during the incubation period, remained low even after fertilization, but greatly increased at the blastula and gastrula stages.

Since the extent of incorporation was considered to be dependent upon the availability of ^{3}H-uridine triphosphate (uptake), even though they may not always parallel with each other, the rate of RNA synthesis was determined from the ratio of the incorporation to the uptake. The rate is very low in the fertilized eggs as well as in the unfertilized eggs but increases 17-fold in the blastula and 34-fold in the gastrula stage by the incubation time of 90 minutes (Table 2 and Fig. 2).

2. Changes in protein synthesis

The amount of ^{3}H-leucine taken up and incorporated into the eggs and embryos were determined as a TCA-soluble and precipitable fraction, respectively, to examine changes in a relative rate of protein synthesis during the early development of sea urchin. The size of TCA-soluble fraction, representing a permeability of the eggs or embryos to ^{3}H-leucine, is fairly large in the unfertilized eggs, which were labeled for only 30 minutes, and slightly decreases when incubated for a prolonged time, indicating that ^{3}H-leucine is taken up very quickly to the maximum within 30 minutes. Transport of ^{3}H-leucine is slightly increased after fertilization, indicating that the permeability of the eggs to ^{3}H-leucine changes very little by fertilization and decreases at the blastula stage to the level even lower than in the unfertilized eggs. In the gastrulae transport of ^{3}H-leucine occurs very quickly to the maximum within 30 minutes and the amount of free ^{3}H-leucine decreases rapidly during the subsequent incubation upto 2 hours, suggesting that much of free ^{3}H-leucine was incorporated into protein or released out of the embryos. In general, the results indicated that the permeability to leucine is high in the unfertilized eggs and slightly enhanced upon fertilization but greatly reduced during the subsequent development.

The incorporation of ^{3}H-leucine is very low in the unfertilized eggs, in spite of a high permeability to leucine, and increases 10-fold following fertilization, 20-fold at blastula and 45-fold at gastrula stage by the incubation of 30 minutes. The incorporation, which increases very slowly in the unfertilized eggs, increases rapidly in the fertilized eggs throughout the incubation period. Incorporation occurs more rapidly in the blastulae than in the fertilized eggs in the first 30 minutes of incubation but approaches a plateau thereafter in the blastulae. This tendency was also observed in the gastrulae, in which the incorporation drastically increases in the first 30 minutes and then reaches a plateau. Since the change of incorporation did not show a linearity at the blastula and gastrula stages, it was hard to make a generality as to the leucine incorporation during the development. Evaluating the results on the basis of the data of 30 minutes incubation, it was possible

Table 3. Changes in radioactivities of ^{3}H-leucine taken up and incorporated into the eggs or embryos of various developmental stages.

stage	Hrs of develop.	Incubation time	TCA-soluble(U) (cpm/10^3 embryos)	TCA-ppt(I) (cpm/10^3 embryos)	I/U
Unfertilized	0	30 min	16,131	153	0.0095
Fertilized	5 min	5 min	13,208	241	0.0182
		15 min	18,211	771	0.0423
		30 min	24,798	1,613	0.0650
Blastula	20 hrs	30 min	9,246	2,776	0.3002
Gastrula	40 hrs	30 min	19,427	6,666	0.3431
Unfertilized	0	1 hr	13,584	207	0.0152
Fertilized	5 min	1 hr	26,293	3,504	0.1333
Blastula	20 hrs	1 hr	8,720	3,827	0.4389
Gastrula	40 hrs	1 hr	13,288	7,367	0.5544
Unfertilized	0	2 hrs	12,863	273	0.0212
Fertilized	5 min	2 hrs	22,164	5,913	0.2668
Blastrula	20 hrs	2 hrs	8,772	4,566	0.5205
Gastrula	40 hrs	2 hrs	9,650	8,471	0.8778

The eggs or embryos, which had been labeled with ^{3}H-leucine, was homogenized in TCA and the homogenate was fractionated into TCA-soluble and precipitable fractions by centrifugation.

to conclude that the incorporation increases with the development (Table 3 and Fig. 3).

The rate of protein synthesis, which was also evaluated by the ratio of the incorporation to the uptake value at each stage, was found to be very low in the unfertilized eggs. However, when the eggs are fertilized, the rate increases 7-fold within 30 minutes in spite of suppression of the RNA synthesis. The rate continues to increase from the fertilized stage throughout the gastrula stage, even though the change in the rate is not always linearly increasing. This increase in protein synthesis indicated that masked mRNAs are very quickly activated to be utilized to synthesize proteins.

3. Qualitative change in the protein synthesis

Proteins synthesized in the unfertilized and fertilized eggs, blastulae and gastrulae were analyzed by two-dimensional gel electrophoresis, since the quantitative change may reflect a qualitative change in the protein synthesis. Although approximately 200 proteins were detectable from the fluorogram of each stage, we have analyzed only those distinctively identifiable spots, each being compared among the stages with regards to the presence or absence of the particular protein at each stage.

The results of the analyses are presented in Figs. 4, 5, 6 and 7 and Table 4. Sixty nine spots, which were analyzed throughout the four stages, are mostly stage-specific except for 5 proteins (1-5), which were found to be synthesized at all stages. In the unfertilized eggs a group of proteins were continuously synthesized even after fertilization

Table 4. Changes in the patterns of protein synthesis during the early development of *Hemicentrotus pulcherrimus.*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Unfertilized	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
Fertilized	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
Blastula	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
Gastrula	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+

	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
Unfertilized	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	
Fertilized	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	
Blastula	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
Gastrula	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	

	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
Unfertilized	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Fertilized	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Blastula	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Gastrula	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Numerals 1 to 69 are the spot numbers in Figs. 4~7.

but the other specifically in the unfertilized eggs with no further synthesis after fertilization. We found that 15 proteins (6-20) were synthesized after the fertilization and 3 proteins (6-8) even through the blastula stage, but 16 proteins (28-43) were synthesized only in the unfertilized eggs but not after fertilization. However, 11 proteins (44-54), which were found to be newly synthesized in the fertilized eggs, were specific to this stage. At the blastula stage synthesis of stage-specific proteins was not observed, but some proteins were found to begin to be synthesized. Many proteins (9-20, 28-54), which have been synthesized in the previous stages, were observed not to be synthesized in the blastulae, even though the rate of the protein synthesis is higher at the blastulae than at the previous stages. At the gastrula stage most of those proteins, which were previously synthesized, were no longer synthesized. Except for those conservative 5 proteins several proteins were common to the blastula stage and many of proteins seemed to be synthesized specifically to the gastrula stage. The results indicated that the kind of proteins synthesized at each stage is not consistent but changing during the early development and even at the time of fertilization.

DISCUSSION

The present studies demonstrate that protein synthesis varies quantitatively and qualitatively from the unfertilized to the gastrula stage, but it is difficult to interpret the changes

as an expression of a selective recruitment of masked mRNAs during the course of the development, because these two changes may not parallel with each other. A big increase in the rate of protein synthesis previously demonstrated, in spite of low synthetic activity of RNA in the fertilized eggs by several investigators (Epel, 1967; Deworkin and Infante, 1978; Humphreys, 1971; Jenkins *et al*,. 1978; Raff *et al.*, 1981, 1982; Timourian and Watchmaker, 1970) may not be always accompanied with a change in kind of protein (Brandhorst, 1976). This increase upon fertilization may reflect an increase in the newly-recruited mRNAs and/or templation activites of previously activated mRNAs. Even though Brandhorst (1976) reported that there occurs only quantitative change in protein synthesis at the time of fertilization of *Lytechinus pictus*, it is unlikely to exclude that no qualitative change occurs after fertilization, since it was observed that in *Spisula* new proteins are synthesized after fertilization (Rosenthal *et al.*, 1980; Tansey and Ruderman, 1983). Qualitative changes in a small number of proteins were observed at the time of fertilization in *Hemicentrotus*. This change was actually observed in two ways; a disappearance of synthesis of the proteins, which had been actively synthesized before fertilization and an appearance of new protein synthesis. The disappearance of the synthetic activity does not seem to be due to time-specific degradation of the active mRNAs, but due to a low templation activities of these mRNAs, since the unfertilized eggs were labeled for longer period with higher concentration of ^{3}H-leucine than the fertilized eggs. However, a possibility of the degradation of the active mRNAs may not be excluded, because it is difficult to expect that the templation activities of these particular mRNAs do not change, while the activities increase in the rest of mRNAs. Whether the active mRNAs are equally conserved in templation activity even after fertilization or totally degraded can not be yet concluded. However, the appearance of 11 proteins (44-54), which are newly synthesized upon ferti lization but do not persist upto the blastula stage, suggested that masked mRNAs might have been newly recruited right after fertilization and degraded during the subsequent stages.

Newly synthesized proteins appeared at the blastula stage, but it was hard to decide whether these proteins are synthesized by the mRNAs synthesized or recruited after fertilization. Because these proteins are also synthesized in the gastrula, they seem to be synthesized from the mRNAs synthesized after fertilization.

The difference between the result of Brandhorst (1976) and the present study seems to be due to difference in species, because it takes only 7 hours for the *Lytechinus* embryos to reach the blastula stage but 20 hours for the *Hemicentrotus* embryos. We were not able to conclude yet whether masked mRNAs are sequentially recruited during development, even though a selective activation was suggested in our studies. A positive approach to confirm a sequential recruitment of masked mRNAs would be to study with transcriptionally inhibited embryos of sea urchins.

SUMMARY

Syntheses of RNA and protein were studied to examine changes in activating stored mRNAs during the early development of a sea urchin, *Hemicentrotus pulcherimus*. The rates of RNA and protein syntheses are very low in the unferilized eggs but the protein synthesis is activated upon the fertilization, while RNA synthesis remains still inactive at the same stage. These rates increase drastically at the blastula and gastrula stages, although the increases are not exactly in parallel. The protein synthesis was found to be also changing in quality during the early development from the studies by the two-dimensional gel electrophoresis.

REFERENCES

Bonner, W.M. and R.A. Laskey, (1974). A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* **46**:83-88.

Brandhorst, B.P. (1976). Two-dimensional gel patterns of protein synthesis before and after fertilization of sea urchin eggs. *Develop. Biol.* **52**:310-317.

Brandis, J.W. and R.A. Raff, (1978). Translation of oogenetic mRNA in sea urchin eggs and early embryos: Demonstration of a change in translational efficiency following fertilization. *Develop. Biol.* **67**:99-113.

Brandis, J.W. and R.A. Raff, (1979). Elevation of protein synthesis is a complex response to fertilization. *Nature* (London) **278**:467-469.

Cascio. S.M. and P.M. Wasserman, (1982). Program of early development in the mammal: Post-transcriptional control of a class of proteins synthesized by mouse oocytes and early embryos. *Develop. Biol.* **89**:397-408.

Davidson, E.H., B.R. Hough-Evans, and R.J. Britten, (1982). Molecular biology of the sea urchin embryo. *Science* **217**:17-26.

Dworkin, M.B. and A.A. Infante, (1978). RNA synthesis in unfertilized urchin eggs. *Develop. Biol.* **62**:247-257.

Epel, D. (1967). Protein synthesis in sea urchin eggs: A "late" response to fertilization. *Proc. Nat. Acad. Sci.* USA **57**:899-906.

Gross, K.W., M. Jacobs-Lorena, C. Baglioni, and P.R. Gross, (1973). Cell-free translation of maternal messenger RNA from sea urchin eggs. *Proc. Nat. Acad. Sci.* USA **70**:2614-2618.

Hille, M.B. and A.A. Albers, (1979). Efficiency of protein synthesis after fertilization of sea urchin eggs. *Nature* (London) **278**:469-471.

Hough-Evans, B.R., S.G. Ernst, R.J. Britten, and E.G. Davidson, (1979). RNA complexity in developing sea urchin oocytes. *Develop. Biol.* **69**:258-269.

Humphreys, T. (1969). Efficiency of translation of messenger RNA before and after fertilization in sea urchins. *Develop. Biol.* **20**:435-458.

Humphreys, T. (1971). Measurements of messenger RNA entering polysomes upon fertilization of

sea urchin eggs. *Develop. Biol.* **26**:201-208.

Jenkins, N.A., J.F. Kaumeyer, E.M. Young, and R.A. Raff, (1978). A test for masked message: The template activity of messenger ribonucleoprotein particles isolated from sea urchin eggs. *Develop. Biol.* **63**:279-298.

Kaumeyer, J.F., N.A. Jenkins, and R.A. Raff, (1978). Messenger ribonucleoprotein particles in unfertilized sea urchin eggs. *Develop. Biol.* **63**:226-278.

Laskey, R.A. and A.D. Mills, (1975). Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. *Eur. J. Biochem.* **56**:335-341.

O'Farrell, P.H. (1975). High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250**:4007-4021.

Raff, R.A., J.W. Brandis, C.J. Huffman, A.L. Koch, and D.E. Leister, (1981). Protein synthesis as an early response to fertilization of the sea urchin egg: A model. *Develop. Biol.* **86**:265-271.

Raff, R.A. (1982). Maternal mRNA utilization in the control of protein synthesis by sea urchin embryos. *Cell Differ.* **11**:305-307.

Raff, R.A. and R.M. Showman, (1983). Maternal messenger RNA: Quantitative, qualitative and spatial control of its expression in embryos. *In*: The biology of fertilization, Ed. C.B. Mets and A. Monroy.

Regier, J.C. and F.C. Kafatos, (1977). Absolute rates of protein synthesis in sea urchins with specific activity measurements of radioactive leucine and leucyl-tRNA. *Develop. Biol.* **57**:270-283.

Rinaldi, A.M. and A. Monroy, (1969). Polyribosome formation and RNA synthesis in the early post-fertilization stages of the sea urchin egg. *Develop. Biol.* **19**:73-86.

Rosenthal, E.T., T. Hunt, and J.V. Ruderman, (1980). Selective translation of mRNA controls the pattern of protein synthesis during early development of the surf clam, *Spisula solidissima*. *Cell* **20**:487-494.

Rosenthal, E.T., B.P. Brandhorst, and J.V. Ruderman, (1982). Translationally mediated changes in patterns of protein synthesis during maturation of starfish oocytes. *Develop. Biol.* **91**:215-220.

Ruderman, J.V. and M.R. Schmidt, (1981). RNA transcription and translation in sea urchin oocytes and eggs. *Develop. Biol.* **81**:220-228.

Spirin, A.S. and M. Nemer, (1965). Messenger RNA in early sea urchin embryos: Cytoplasmic particles. *Science* **150**:214-217.

Tansey, T.R. and J.V. Ruderman, (1983). Differential mRNA accumulation and translation during *Spisula* development. *Develop. Biol.* **99**:338-351.

Timourian, H. and G. Watchmaker, (1970). Protein synthesis in sea urchin eggs: Ⅱ. Changes in amino acid uptake and incorporation at fertilization. *Develop. Biol.* **23**:478-491.

Whiteley, A.H. and S. Mizuno, (1981). Electron microscope visualization of giant polysomes in sea urchin embryos. *Wilhelm Roux's Archives* **190**:73-82.

Wilt, F.H. (1970). The acceleration of ribonucleic acid synthesis in cleaving sea urchin embryos. *Develop. Biol.* **23**:444-455.

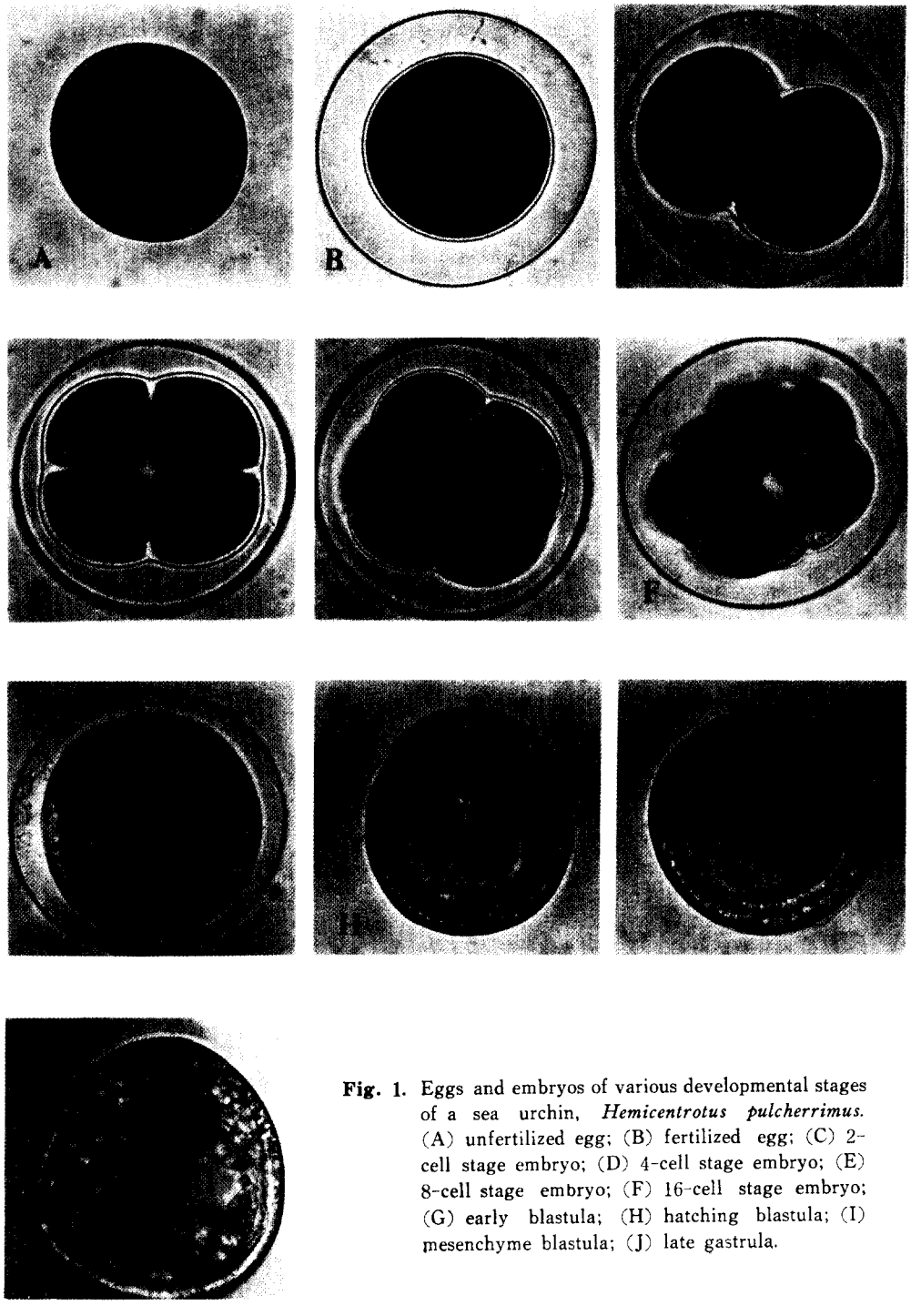
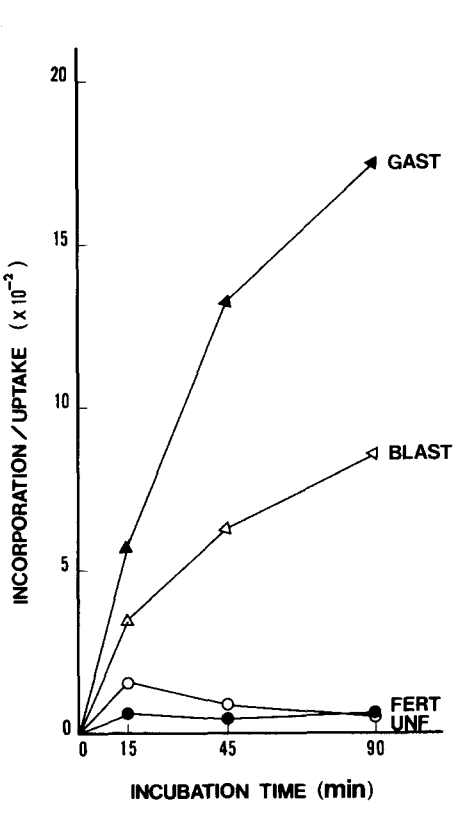
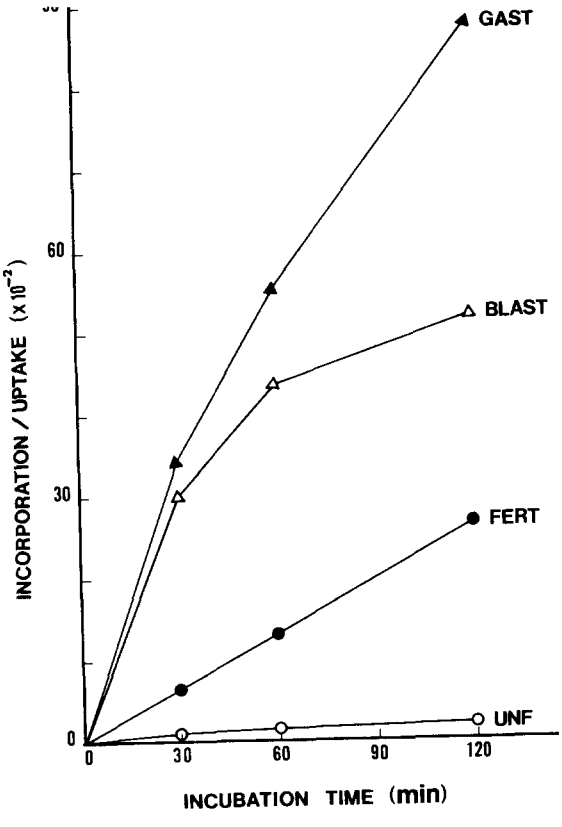


Fig. 1. Eggs and embryos of various developmental stages of a sea urchin, *Hemicentrotus pulcherrimus*. (A) unfertilized egg; (B) fertilized egg; (C) 2-cell stage embryo; (D) 4-cell stage embryo; (E) 8-cell stage embryo; (F) 16-cell stage embryo; (G) early blastula; (H) hatching blastula; (I) mesenchyme blastula; (J) late gastrula.



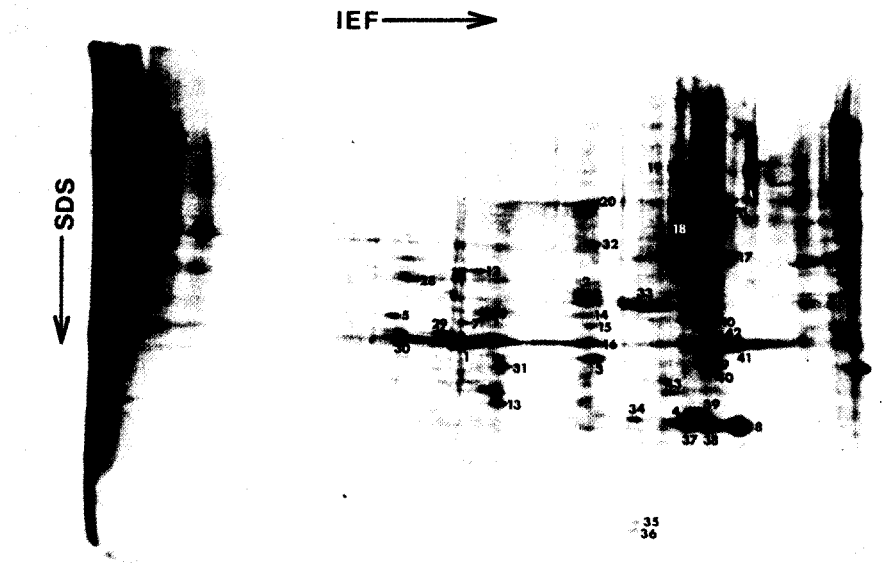
(2)



(3)

Fig. 2. Relative rates of RNA synthesis during the early development of *Hemicentrotus pulcherrimus*. Refer to the legend in Table 2.

Fig. 3. Relative rates of protein synthesis during the early development of *Hemicentrotus pulcherrimus*. Refer to the legend in Table 3.



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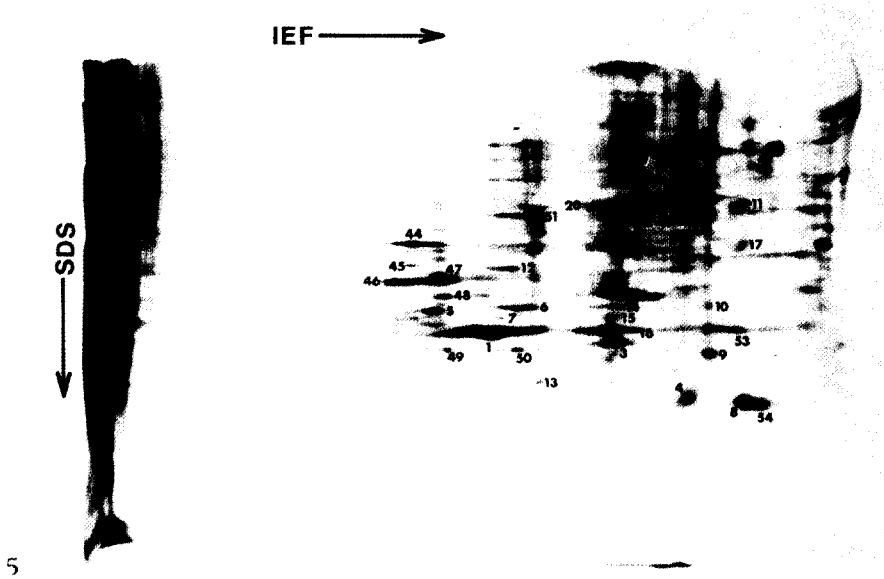
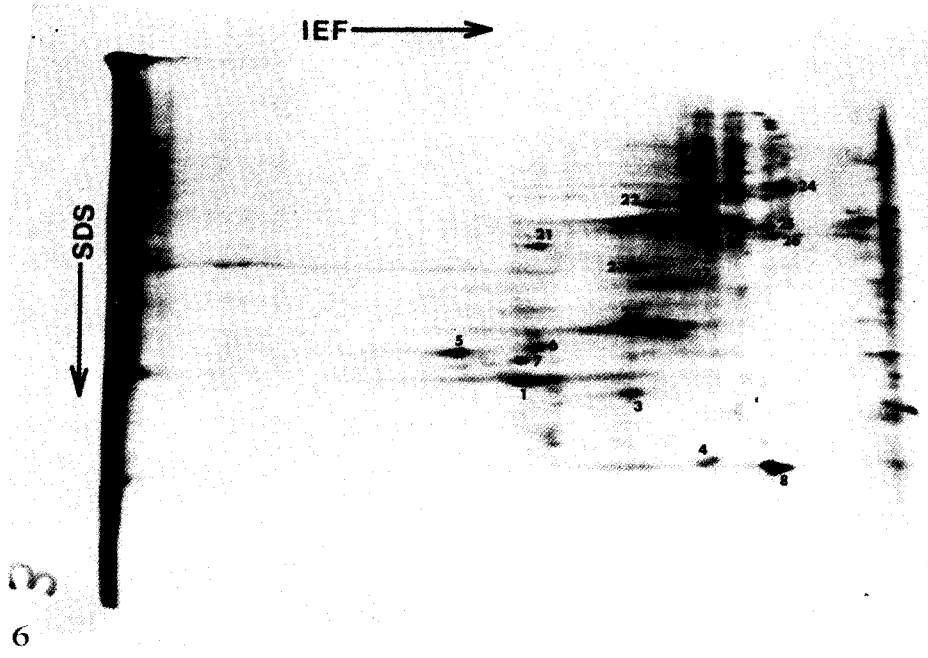


5

Fig. 4. Fluorogram of the proteins synthesized in the unfertilized eggs of *Hemicentrotus pulcherrimus*. The proteins were labeled with ^{3}H-leucine and analyzed by two-dimensional gel electrophoresis and fluorography. The proteins (28~43) are found to be specific to the unfertilized eggs, whereas the proteins (6~8) are appeared up to the blastulae, but not in the gastrulae. The proteins (9~20) seem to be synthesized in both the unfertilized and fertilized eggs.

Fig. 5. Fluorogram of the proteins synthesized in the fertilized eggs of *Hemicentrotus pulcherrimus*. The proteins (44~54) appear to be specific to the fertilized eggs.



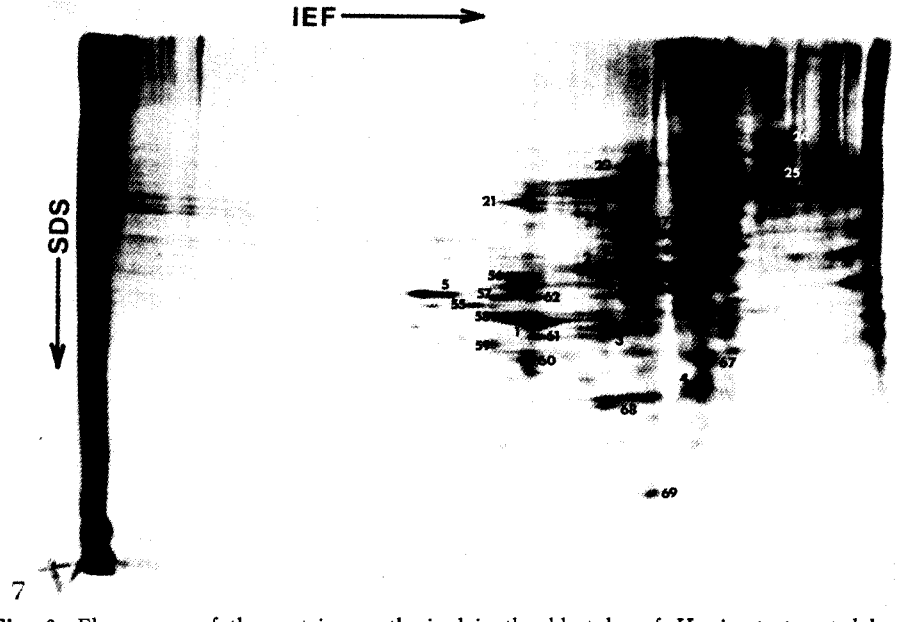


Fig. 6. Fluorogram of the proteins synthesized in the blastulae of *Hemicentrotus pulcherrimus*. The proteins (21~27) are synthesized specifically in the blastulae and gastrulae.

Fig. 7. Fluorogram of the proteins synthesized in the gastrulae of *Hemicentrotus pulcherrimus*. The proteins (55~69) are specifically synthesized in the gastrulae.