CYCLIC CHANGE IN POLYAMINE CONCENTRATIONS IN SEA URCHIN EGGS RELATED WITH CLEAVAGE CYCLE

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SUMMARY: Spermidine and spermine are found in unfertilized eggs of the sea urchin, Hemicentrotus pulcherrimus. Putrescine becomes detectable and concentrations of spermidine and spermine increase in the eggs upon fertilization. Then, concentrations of these polyamines decrease after respective peaks in polyamine concentrations. The peaks in the concentrations are found at 15 minutes post fertilization for putrescine, at 30 minutes for spermidine and at 30-40 minutes for spermine respectively. Levels of polyamines elevate again and reduce after the 2nd concentration peaks of respective compounds, and then the first cleavage of the eggs takes place. Cyclic change in each polyamine concentration is also observed after the first cleavage, and egg cleavage occurs at decreasing phase of polyamine concentrations.

INTRODUCTION: Intracellular concentrations of polyamines have been reported to be great in animal tissues with high rates in RNA and protein synthesis (1-3). Since protein synthesis in sea urchin eggs, as well as the other physiological functions such as O_2 uptake, glycolysis and so on (cf.4), has been reported to be enhanced upon fertilization, it may be expected that polyamines increase in their concentrations upon fertilization and stimulate several metabolic systems in the eggs, which have been known to be enhanced upon fertilization (cf.4).

In the present paper, we report elevation of polyamine concentrations in sea urchin eggs upon fertilization and cyclic alteration of the concentrations with a relation to the cycle of egg cleavage.

MATERIALS AND METHODS: Eggs of the sea urchin, Hemicentrotus pulcherrimus were obtained by forced spawning with 0.5M KCl injection into the coelom of matured one. Fertilized eggs were kept at 18°C with gentle sterring. Synchronism of the egg cleavage in an egg suspension was examined at the first and third cleavage and the eggs were not used for analysis of polyamines unless all eggs in a suspension finished the cleavage within 5 minutes. 9 batches of the eggs could be used in the present study among 42 batches examined. 10 ml of the egg suspension, which contained about $3x10^5$ eggs, was centrifuged on a hand driven centrifuge every 3 or 6 minutes after fertilization. Egg pellet thus obtained was frozen with liqid nitrogen and homogenized in 1 ml of 0.1N HCl on an ice bath. Then, 1 ml of 10% trichloroacetic acid was added to the homogenate. To 1 ml of the supernatant obtained with centrifugation at 20,000g for 20 minutes, 0.2g of salt mixture (62.5g of anhydrous sodium sulfate and 9g of trisodium phosphate ground together) and 0.1 ml of 5N NaOH were added, and the supernatant was mixed up with 2 ml of n-butanol. Polyamines extracted with n-butanol were separated with paper electrophoresis and estimated according to the method described by Russell (5).

RESULTS: Fig 1 shows change in polyamine concentrations in sea urchin eggs after fertilization. In unfertilized eggs, putrescine was hardly detected and concentration of spermidine was lower than that of spermine. Putrescine concentration increases rapidly following fertilization and then begins to decrease at 15 minutes post fertilization. Levels of spermidine also starts to elevate at just after fertilization and reaches a maximum value at 25-30 minutes after fertilization. Sper-

mine level seems to remain unchanged for 10 minutes, then increases and becomes maximum at 30-40 minutes after fertilization. Polyamine concentrations decrease respectively to minimum values by 50 minutes after fertilization. The minimum concentration of each polyamine in fertilized eggs is still higher than that of unfertilized eggs. Then, polyamines increase again in their concentrations sequentially as found after fertilization and decrease after they reach a little higher values than the maximum ones observed at just after fertilization respectively. The first cleavage of the eggs can be observed after the second peak of spermine. The third peak of each polyamine concentration was also found between the first and second cleavage of the eggs. Since peaks of polyamine concentrations are also observed between the second and third cleavage, change in polyamine concentrations will relate with the mechanism concerning mitosis of sea urchin eggs.

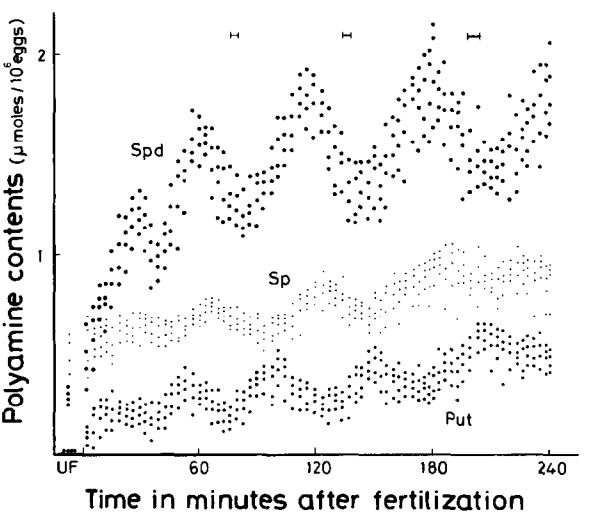

Fig. 1 Cyclic alteration in polyamine contents in sea urchin eggs related with cleavage cycle. Put; putrescine, Spd; spermidine, Sp; spermine. ⊢, ⊢, ⊢ show the 1st, 2nd and 3rd cleavage respectively.

DISCUSSION: The same polyamines as those of mammalian tissues (5), such as putrescine, spermidine and spermine are found in sea urchin eggs. Polyamine concentrations, especially those of putrescine and spermidine are very low in unfertilized eggs and increase after fertilization. Since fertilized eggs will be assumed to be "active" cells with higher rate in protein synthesis than that of unfertilized eggs (cf.4), increase in polyamine concentrations upon fertilization, especially those of putrescine and spermidine, will agree well with the fact that the levels of polyamines are high in active mammalian tissues such as the regenerating rat liver, tissues stimulated with hormones, turmor cells and so on (5-10). Levels of polyamines, however, change very quickly as compared with those of mammalian cells (5). The enzyme activities concerning polyamine synthesis were not estimated in the present study. However, if the same metabolic system for polyamine synthesis as that found in mammalian tissues (1,7,11,12) will operate in sea urchin eggs, activities of ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase will be enhanced upon fertilization and the change in the activity of each enzyme will be more dramatic in sea urchin eggs than that of mammalian cells. These enzymes have been assumed to be stimulated with adenosine 3'5'-cyclic monophosphate (cAMP), since increase in cAMP concentration is correlated with these enzyme activities in mammalian tissues (13-15). It, however, will not be probable that cAMP stimulates the enzyme activities concerning polyamine synthesis in the eggs at just after fertilization, since cAMP level has been found to be as low as that in unfertilized eggs until the first peak of polyamines (16).

After the second peak of polyamine concentrations, however, change in polyamine concentrations seems to keep pace with that

of cAMP content in fertilized eggs and cleavage of the eggs can be observed after the peak of polyamine concentrations. These facts are in well agreement with mammalian cells (17), and it may be supposed that cAMP stimulates polyamine synthesis in the eggs during cleavage as has been assumed in mammalian tissues (17). Further, it will be also probable that cyclic change in polyamine concentrations, as well as cAMP level (16), concerns with the mechanism for egg cleavage.

REFERENCES:

1. Pegg, A.E. and Williams-Ashman, H.G. Biochem. J., 108, 533 (1968)
2. Cohen, S.S., Hoffner, N., Jansen, M., Moore, M. and Raina, A. Proc. Natl. Acad. Sci. U.S.A., 57, 721 (1967)
3. Raina, A., Janne, J. and Siimes, M. Biochim. Biophys. Acta, 123, 197 (1966)
4. Monroy, A. Chemistry and physiology of fertilization. Holt, Rainehart and Winston, New York. (1965)
5. Russell, D.H., Medina, V.J. and Snyder, S.H. J. Biol. Chem., 245, 6732 (1970)
6. Russell, D.H. and Snyder, S.H. Proc. Natl. Acad. Sci. U.S.A., 60, 1420 (1968)
7. Janne, J. and Williams-Ashman, H.G. J. Biol. Chem., 246, 1725 (1971)
8. Cohen, S., O'Malley, B.W. and Stastny, M. Science, 170, 336 (1970)
9. Russell, D.H. and Taylor, R.L. Endocrinology, 88, 1397 (1971)
10. Russell, D.H. Nature New Biol., 233, 144 (1971)
11. Janne, J. and Raina, A. Acta Chem. Scand., 22, 1349 (1968)
12. Williams-Ashman, H.G., Janne, J., Coppoc, G.L., Geroch, M.E. and Schenone, A. Advan. Enzyme Reg., 10, 225 (1972)
13. Beck, W.T., Bellantone, R.A. and Canellakis, E.S. Biochem. Biophys. Res. Comm., 48, 1649 (1972)
14. Aisbitt, R.P.G. and Barry, J.M. Biochim. Biophys. Acta, 320, 610 (1973)
15. Byus, C.V., Guidotti, A., Costa, E. and Russell, D.H. Federation Proc., 33, 1392 (1974)
16. Yasumasu, I., Fujiwara, A. and Ishida, K. Biochem. Biophys. Res. Comm., 54, 628 (1973)
17. Russell, D.H. and Stambrook, P.J. Proc. Natl. Acad. Sci. U.S.A., 72, 1482 (1975)