Spicule formation and pigment cell differentiation in primary cell cultures of sea urchin embryos. Cryopreservation of the cultures

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Keywords: *cell culture, sea urchin, embryos, spicule formation, lectin, pigment differentiation, echinochrome, cryopreservation*

Marine organisms passed through the long path of evolution and adaptations; this is duly reflected in the peculiarities of their biosynthesis and metabolism. The purpose of the study is to reveal exogenous factors that influence the implementation of the spicule- and pigment-formation program in a culture of sea urchin embryonic cells and to estimate the effect of these factors on cell differentiation. As shown by Okazaki (1975), isolated sea urchin micromeres can under certain conditions differentiate into cells capable of forming spicules. We have found that the process of spicule formation depends on the substrate type and the medium composition. The serum required for spicule formation *in vitro* can be replaced by a complex of factors, including insulin, transferrin and lectins. In addition, specific conditions including the use of natural matrix proteins have been developed to promote pigment cell differentiation in the sea urchin cell culture. Shikimic acid, the precursor of naphthoquinone pigments, has been found to affect the expression of some pigment cell-specific genes in the cell culture. After cryopreservation of embryonic sea urchin cells in the medium containing trehalose, echinochrome and total lipid extract of mussel tissues, the output of viable cells reached 75–80%. Our results have demonstrated synergistic activity of these components in cryoprotective mixtures. The thawed cells attached to the substrate, some synthesized pigment granules and spicules, a part of the cells aggregated to form embryo-like structures that moved actively during 5–21 days. The results of this study may be valid to solve practical problems in marine biotechnology, such as the establishment of cell cultures capable of producing mineral structures and biologically active substances. (This work is supported by the Program of Far Eastern Federal University (11 G34.31.0010), Presidium of FEB RAS (12-I-0-02-027, 12-I-0-06-015, 12-III-A-06-005) and RFBR (10-04-01319-a)).