Summary

Cell hybrids were prepared from anucleate fragments of A. cliftonii into which a cell nucleus of A. major was implanted. In such hybrids the isozyme pattern of malic dehydrogenase was studied at different times and in different cell regions. It could be shown that as short a time as 5 days was sufficient for the implanted nucleus to exert a species specific change in the isozyme pattern. Under the influence of the implanted nucleus 3 of the 4 main bands characteristic of A. major were formed in the cytoplasm of A. cliftonii. Instead of the fourth main band of A. major an activity peak appeared with a somewhat reduced electrophoretic mobility. In contrast to two minor bands the main band of A. cliftonii turned out to be at least relatively stable. The new isozyme pattern appeared in both, the apical and basal regions. At least one of the new bands appeared more rapidly in the apical part of the cell. It has to be concluded that at least in the case of malic dehydrogenase all regions of the cell are involved in the synthesis of this enzyme.

1. Introduction

Experiments with heterologous nucleus-cytoplasm combinations (hybrids) of different species of the green alga Acetabularia have shown that there is a determining influence of the cell nucleus on the complex level of developmental patterns, for example, on the species specific morphogenesis of the cap and on a molecular level on the synthesis of species specific proteins of the cytoplasm (Hämmerling 1963, Schweiger, Master, and Werz 1967, Reuter and Schweiger 1969). Little is known about the regulatory mechanism underlying development. So far this process can be described only in common terms concerning temporal and the spatial organization.

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In the case of MDH-distribution it is not clear where in the cell the nucleus determined isozymes are synthesized. It has to be taken into consideration that these proteins are either synthesized in a specific site of the cell or that the synthesis takes place all over the cell.

The problem of spatial organization of the living cytoplasm is of course one of the most exciting problems of cell biology. A suitable object for studying this problem is *Acetabularia*. In this cell the appearance of a new enzyme pattern in the different regions of the cell can be demonstrated after heterologous nucleus implantation.

We describe, in the following, the changes of the MDH isozyme after the implantation of an heterologous nucleus into an anucleate *Acetabularia* cell. The changes were studied in different parts of the hybrid cells and at different times. These experiments have been performed with MDH isozymes since the sensitivity of the method permits investigation of the isozyme pattern in different regions of an individual hybrid cell. Moreover, it has been shown recently that in nucleate as well as in anucleate cells the increase in activity of this enzyme is due to *de novo* synthesis (Schweiger, Apel, and Kloppstech 1972).

In this study we wish to call attention to intracellular topography of gene expression. The complexity of the spatial organization of the *Acetabularia* cell can be seen from the following facts. On the one hand it has been shown that the occurrence of cap specific polysaccharides is confined to the apical part of the cell. On the other hand it is known that nucleus determined proteins, for example, malic dehydrogenase and probably also other proteins are evenly distributed over the whole stalk. There are some indications that the specific so-called “morphogenetic substances” are synthesized in the nucleus and that these substances migrate through the stalk to the tip of the cell where they are accumulated and used for the synthesis, e.g., of specific cap polysaccharides (Hämmerling 1963).

2. Materials and Methods

The experiments have been performed on *Acetabularia (Polyphysa) cliftonii* (*cliff*), *Acetabularia major* (*mai*) in the early stage of cap formation (length of the cells: 80–100 mm), *Acetabularia crenulata* (*cren*) in the stage of small caps with diameters of 1 to 5 mm (length of the cells: 90–120 mm), or with *Acetabularia crenulata* (*cren*) in the stage of small caps with diameters of 1 to 3 mm (length of the cells: 25–30 mm). The cells were grown in Erd-Schreiber medium (Schweiger 1969). The preparation of the nucleus cytoplasm hybrids was performed by means of the modified nucleus implantation technique (Hämmerling 1955). The nuclei were isolated under a binocular magnifier at room temperature in the isolation medium (IM) which contained 0.1 M phosphate buffer, pH 7.4, with 0.3 M sucrose, 0.005 M magnesium acetate and 0.1% serum albumin. The cytoplasm together with the nuclei were squeezed out from the cut off rhizoid, the nucleus was freed from adhering cytoplasm, and was pushed into the stalk of the acceptor cell by means of a thin glass needle with a sphere at its tip (diameter about 0.1 mm). After the introduction of the nucleus the open end of the stalk was tied off. A second ligature was applied