NUCLEO-CYTOPLASMIC INTERACTIONS IN THE SYNTHESIS OF SPECIES-SPECIFIC PROTEINS IN ACETABULARIA

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Received July 8, 1960

It is a well established fact that enucleated parts of the green alga Acetabularia are capable of synthesizing relatively large amounts of total protein (Vanderhaege, 1954; Hämmerling, Clauss, Keck et al., 1959). There is also good evidence that enzymes, i.e., highly specific proteins are synthesized in the absence of the cell nucleus. Thus the activities of aldolase (Baltus, 1955), invertase (Keck and Clauss, 1958), and phosphorylase (Clauss, 1959) were reported to increase from 40% to 600% respectively, in the plants after amputation of the nucleus-containing rhizoid. Acid phosphatase differs from the other investigated enzymes in that the synthesis of this enzyme ceases shortly after enucleation of the algae (Keck and Clauss, 1958). Studies of this nature indicate a rather indirect control of protein synthesis by the cell nucleus. It seems that the cytoplasm per se has a high capacity to maintain genetical information originally derived from the nucleus.

Experiments designed to analyze both nuclear and cytoplasmic factors which might possibly be involved in determining the specific structure of an enzyme protein, would require analytical separation of species specific proteins. Recently the separation of species and even tissue-specific enzymes of animals has been achieved with great resolving power by starch gel electrophoresis (Hunter and Markert, 1957; Dixon and Smithies, 1957; Markert and Møller, 1959). Preliminary experiments with Acetabularia have

shown that acid phosphatase of two species of this alga, namely Acicularia Schenckii and Acetabularia mediterranea is present in two forms differing significantly in their electrophoretic mobility. Since, furthermore, successful grafts between these two species have been reported in the literature (Beth, 1943) a system has become available to investigate nuclear and cytoplasmic factors involved in expressing the specificity of an enzyme protein.

Methods

Acetabularia mediterranea and Acicularia Schenckii both were grown in "Erdschreiber"-medium according to methods described by Hämmerling (1944) and Beth (1953). The plants were illuminated with 2500 lux in 12-hour light-dark rhythms. Grafts between both species were made in the conventional way by dissecting corresponding parts with Wecker-scissors and pushing the stalk ends together to form telescope-like joints. Five to 8 algae or parts thereof were ground in a 0.1 ml glass homogenizer without the addition of any liquid. The total homogenate was transferred to a small piece of filter paper which was inserted then in the starch strip. In some experiments the contents of individual stalks was squeezed out onto a 2 x 5 mm filter paper and this inserted.

The techniques described by Smithies (1955) for starch gel electrophoresis were applied. The samples were run for 6 hours at room temperature (23° C) in a voltage gradient of 3 V / cm. Tris(hydroxymethyl)aminomethane buffer, pH 8.7, 0.05 M was used in making the starch gel; the same buffer at a higher concentration (0.25 M) was used as bridge solution.

Results and Discussion

When homogenates of Acetabularia mediterranea (med) or of Acicularia Schenckii (acic) were subjected to starch gel electrophoresis all acid phosphatase activity, as detected histochemically, was found localized within one band in the starch strip. The electrophoretic mobility of the med-type phosphatase was significantly greater than the mobility of the

acic-type phosphatase under comparable conditions. The existence of multiple forms of this enzyme, or "isozymes" (Markert and Møller, 1959) could not be observed in these algae. Both types of acid phosphatase could be separated by starch gel electrophoresis when a mixture of acic and med homogenates was tested. In the mixture both components retained their characteristic mobility. The species specific mobility of either type enzyme proved to be constant throughout the entire life cycle of the algae. Plants of both species were tested at various stages of development, ranging from small 3 mm long algae to full-grown plants with caps. In each of the investigated groups of plants the same, species specific mobility of acid phosphatase was encountered.

It has been shown in previous experiments (Keck and Clauss, 1958) that the synthesis of acid phosphatase is strongly affected by enucleation. The synthesis of this enzyme ceases in stalks shortly after rhizoid amputation. It still remained to be investigated whether phosphatase synthesized in the cytoplasm during a short period after enucleation is structurally "normal". Homogenates of rhizoid amputated algae were prepared at various intervals (5 to 25 days) after dissection and tested in starch gel electrophoresis. No changes could be detected in the mobility of acid phosphatase of any of the anucleate plants as compared with nucleate controls of the same species; nor was there any evidence for other enzymatically active components. We may conclude from these findings that a structurally and functionally "normal" enzyme protein can be produced, at least for a short period after enucleation in the absence of the cell nucleus.

A separation of cytoplasmic from nuclear factors involved in determining the structural specificity of a protein molecule was attempted by studying the behavior of both types of this enzyme in interspecific nuclear grafts. A number of full-grown acic plants were enucleated by rhizoid amputation and med rhizoids implanted immediately afterwards ($acic_0$-med_1). Algae were taken in 24 hour intervals after the operation, the implanted rhizoids were removed and the remaining acic stalks were homogenized and tested in the starch. One day after the grafting only the acic-type phosphatase is recognizable. On the

second day a faint med-type phosphatase band becomes visible and increases in intensity during the following days. At the same time the acic-type band decreases in intensity and finally disappears around the fifth day. No further changes can be observed when $acic_0$-med_1 grafts are kept for 20 more days.

Of particular interest is the rather rapid disappearance of the acic-type enzyme, which cannot be explained alone by the absence of the acic nucleus; the acic-type phosphatase can be detected in enucleated acic plants as late as 35 days after rhizoid amputation with little diminished intensity. Inactivation, of some nature, of the acic-type enzyme caused by the med nucleus appears very unlikely since it would involve a rather specific mechanism. It seems justified to assume, however, that the remaining acic-type enzyme in the stalks is gradually converted into the med-type enzyme under the control of the med nucleus, probably in addition to a net synthesis of new med-type enzyme.

If we assume that the cell nucleus contains the only system controlling the structural specificity of this enzyme protein then one would expect to find the opposite conversion from one to another phosphatase type in reciprocal grafts of acic rhizoids into med stalks ($acic_1$-med_0). Such grafts are rather difficult to make because of differences in the stalk diameters unfavorable to the acic rhizoid implantation. Several successful grafts were produced, however, and the med stalks were tested 17 days after operation. In all the stalks tested the med-type phosphatase was the only component observed.

These results may be interpreted in 2 ways: 1. In the $acic_1$-med_0 graft the acic nucleus is rendered inactive with respect to initiating the synthesis of its own type phosphatase; the enzyme present is a remainder of the med moiety. 2. In the combination med cytoplasm and acic nucleus the med-type phosphatase is synthesized de novo because of some controlling factors present in the med cytoplasm.

In an attempt to find an explanation for these puzzling results interspecific binucleate grafts between acic and med rhizoids were made ($acic_1$-med_1). Stalks regenerated from these binucleate grafts within 4 weeks were collected and homogenized. Individual stalks as well as homogenates obtained by pooling

several stalks were tested for phosphatase mobilities. Without any exception the existence of only one phosphatase band was observed in the starch strips. This phosphatase band was identical with the med-type enzyme. With respect to the morphology of the regenerated stalks and caps, the acic character, however, was prevalent in the hybrid regenerates thus indicating certain activities of the acic nucleus. Moreover, after harvesting the stalks the remaining rhizoids were separated from each other and each one allowed to regenerate. Successful regeneration of the separated rhizoids demonstrated the survival of both nuclei of each graft.

In both types of experiments, i.e., in interspecific nuclear transplants ($acic_1$-med_0 and $acic_0$-med_1) as well as in the interspecific binucleate grafts ($acic_1$-med_1) a "dominance" of the med-type enzyme over the acic-type enzyme was observed. Nuclear and cytoplasmic factors seem to be responsible for the expression of the protein structure in a common system. It may be assumed that the postulated conversion from the acic-type phosphatase to the med-type is brought about by rather small changes in fundamentally similar molecular structures.

References

Baltus, E. (1955). Influence du noyau sur le maintien de l'aldolase dans le cytoplasme. Congr. internat. Biochem. Résumés communs 3e Congr. Brussels. P. 76.

Beth, K. (1943). Ein- und zweikernige Transplantate zwischen Acetabularia mediterranea und Acicularia Schenckii. Zeitschr. Vererbungslehre 81:271-312.

Beth, K. (1953). Experimentelle Untersuchungen über die Wirkung des Lichtes auf die Formbildung von kernhaltigen und kernlosen Acetabularia-Zellen. Zeitschr. f. Naturforsch. 8b:334-342.

Clauss, H. (1959). Das Verhalten der Phosphorylase in kernhaltigen und kernlosen Teilen von Acetabularia mediterranea. Planta 52:534-542.

Dixon, G.H., and Smithies, O. (1957). Zone electrophoresis of cabbage enzymes in starch gels. Biochim. Biophys. Acta 23:198-199.

Hämmerling, J. (1944). Zur Lebensweise, Fortpflanzung and Entwicklung verschiedener Dasycladaceen. Arch. Protistenk. 97:7-56.

Hämmerling, J., Clauss, H., Keck, K., Richter, G., and Werz, G. (1959). Growth and protein synthesis in nucleated and enucleated cells. Exptl. Cell Res., Suppl. 6:210-226.

Hunter, R. L., and Markert, C. L. (1957). Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. Science 125: 1294-1295.

Keck, K., and Clauss, H. (1958). Nuclear control of enzyme synthesis in Acetabularia. Bot. Gazette 120:43-49.

Markert, C. L., and Møller, F. (1959). Multiple forms of enzymes: Tissue, ontogenetic, and species specific patterns. Proc. Natl. Acad. Sci. 45:753-763.

Smithies, O. (1955). Zone electrophoresis in starch gels: Group variations in the serum proteins of normal human adults. Biochem. J. 61:629-641.

Vanderhaege, F. (1954). Les effets de l'énucléation sur la synthèse des protéines chez Acetabularia mediterranea. Biochim. Biophys. Acta 15:281-287.