The Effect of Ribonuclease on Protein Synthesis in Nucleated and Enucleated Fragments of *Acetabularia*.

By H. Stich and W. Plaut. (*From the Saskatchewan Research Unit of the National Cancer Institute of Canada, Saskatoon, Canada, and Botany Department, University of Wisconsin, Madison*)*

Recent experiments demonstrating an intimate association between ribonucleic acid (RNA) and protein synthesis (1), the ability of RNA to induce protein specificity (2) and the transfer of nuclear RNA to the cytoplasm (3), have lent support to the hypothesis that RNA may serve as the transfer agent of genetic specificity between nucleus and cytoplasm. The apparent independence of cytoplasmic protein and of RNA synthesis in *Acetabularia*, where enucleate fragments can grow, synthesize proteins and RNA, and differentiate without a nucleus, has been interpreted as an argument against this postulation (4). In order to subject this apparent cytoplasmic independence to more critical examination, we have treated nucleate and enucleate pieces of *Acetabularia* with ribonuclease and examined the subsequent growth, protein synthesis, and differentiation of these fragments.

The experiments were performed on *Acetabularia mediterranea* and *A. crenulata* growing under controlled conditions in the laboratory (for details of culture method see Hämmerling (5)). These unicellular algae possess a single nucleus during their vegetative growth phase and attain a length of 3 to 4 cm. when mature. They are readily cut into nucleate and enucleate fragments. Healthy individuals of 22 to 25 mm. length were selected, placed in a solution consisting of normal culture medium and from 0.10 to 0.12 mg./ml. commercial crystalline ribonuclease (Worthington Biochemical Corporation) or similar concentrations of a highly purified ribonuclease preparation obtained through the courtesy of Dr. M. R. McDonald (Department of Genetics, Cold Spring Harbor, New York). The cells were cut into two or more pieces while in this solution. By this cutting procedure the cell sap is brought into direct contact with the ribonuclease containing medium and the penetration of the enzyme is facilitated. To enhance the significance of the results, the nucleated fragments were kept in the RNase containing solution for 7 days, whereas the application time for the enucleated fragments was shortened to 4 days thus giving them a greater opportunity to recover from this treatment. During this time growth, differentiation, and net increase in protein in the fragments were determined by measuring respectively the increase in fragment length, noting the formation of specific structures such as whorls and caps, and determining the increase in nitrogen content of the 10 per cent TCA precipitate of cell homogenates (6). Both nucleate and enucleate fragments were returned to normal culture medium after the period of treatment with ribonuclease and their subsequent growth, differentiation, and protein synthesis were determined as before.

The results of these experiments are graphically summarized in Figs. 1 to 6. The ribonuclease treatment had a similar effect on the nucleate and enucleate fragments. During ribonuclease treatment there was a cessation of growth (Figs. 1 and 2), protein synthesis (Figs. 3 and 4), and differentiation (Figs. 5 and 6) in nucleate as well as enucleate pieces. When the two types of fragments were returned to normal culture medium, nucleate pieces resumed both normal growth and protein synthesis after a short recovery period and continued to differentiate in the normal pattern. Enucleate fragments, on the other hand, did not resume growth, showed no significant recovery of the capacity for protein synthesis during the time over which measurements were made and did not differentiate. Two and one half months later these treated enucleate fragments were still alive but had shown no increase in length and no differentiation, except the development of some morphological anomalies. Both the Worthington ribonuclease and the highly purified ribonuclease produced the same results.

The similarity in growth and protein synthesis of nucleate and enucleate *Acetabularia* fragments prior to ribonuclease treatment again demonstrates the apparent independence of the *Acetabularia* cytoplasm. There appears to be a cytoplasmic system for protein synthesis and growth which does not require the presence of the nucleus for continued operation. This system can be temporarily stopped by subjecting the fragments to darkness (7) or by treating with trypoflavine (8); and

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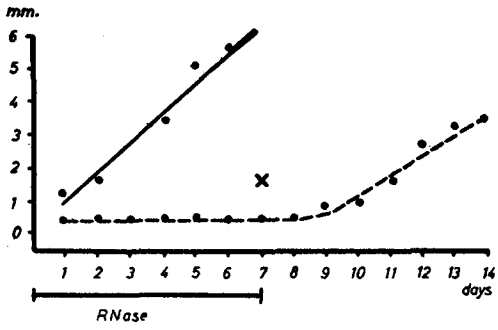

FIG. 1

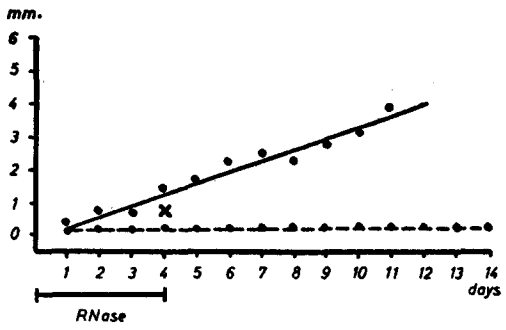

FIG. 2

FIGS. 1 and 2. Growth of nucleated (Fig. 1) and enucleated (Fig. 2) cell fragments of *Acetabularia mediterranea* in a normal culture medium (solid line) and in a RNase-containing medium (dotted line). At the beginning of the experiment the nucleated cell fragments were 3 mm. in length and the enucleated 10 mm. in length. Each line on the graph represents the measurements on 50 cells. The transfer of both cell pieces to a normal medium is marked ×.

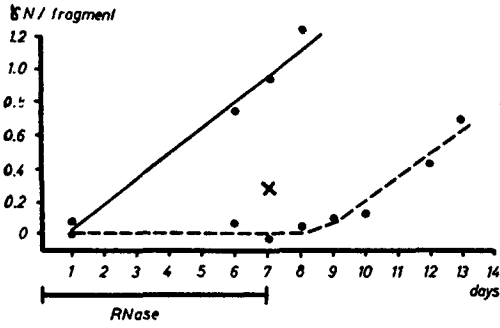

FIG. 3

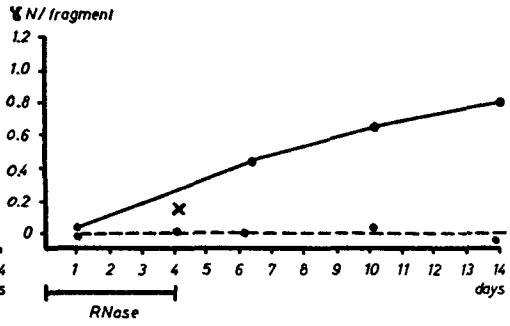

FIG. 4

FIGS. 3 and 4. Protein synthesis of nucleated (Fig. 3) and enucleated (Fig. 4) cell fragments of *Acetabularia mediterranea* in a normal culture medium (solid line) and in a RNase-containing medium (dotted line). At the beginning of the experiment the stalks of the nucleated pieces (without rhizoids) had a protein content of 0.58 γN and the enucleated ones of 1.6γN. Each line on the graph represents the measurements on 96 cells. The transfer of both cell pieces to a normal medium is marked ×.

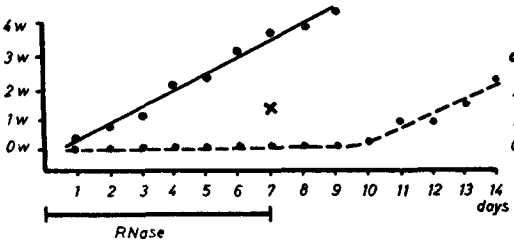

FIG. 5

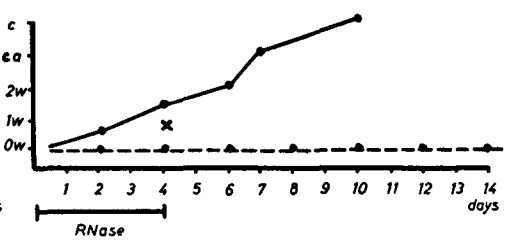

FIG. 6

FIGS. 5 and 6. Differentiation of nucleated (Fig. 5) and enucleated (Fig. 6) cell fragments of *Acetabularia mediterranea* in a normal culture medium (solid line) and in a RNase-containing medium (dotted line). Each line on the graph represents the measurements on 50 cells. The transfer of both cell pieces to a normal medium is marked ×. W = whorls, ca = cap anlage, c = cap.

the system resumes operation when the fragments are returned to light or the trypoflavine is removed. The fact that this cytoplasmic independence is limited is indicated by the effect of ribonuclease treatment: the enucleate cytoplasm is incapable of resuming normal growth, protein synthesis, and differentiation. The ribonuclease treatment constitutes a permanent block to the normal operation of the cytoplasmic system for protein synthesis and it is therefore different from those produced by darkness and trypoflavine, both of which are reversible and presumably result from interference with supply processes. The resumption of normal growth, protein synthesis, and differentiation after ribonuclease treatment in nucleate fragments indicates that the system can be started again when a nucleus is present. Although the ribonuclease treatment resulted in a modification of the nucleolar shape and in a decrease of nucleolar size and basophilia, it apparently had no irreversible effect on the nuclear function.

A simple interpretation of these experimental findings would be that the enucleate cytoplasm has been deprived of its RNA by the enzyme, that in the absence of RNA there is no further protein synthesis, and that no new RNA capable of functioning in protein synthesis is made. The normal recovery of the nucleate fragments suggests that the nucleus provides the cytoplasm with one or more substances which are capable of starting new protein synthesis. As a working hypothesis we would like to suggest that the substance provided by the nucleus is RNA. This hypothesis derives some support from the experimental demonstration that RNA is transferred from nucleus to cytoplasm in *Amoeba proteus* (3). The validity of the hypothesis hinges on the reasonable but unproven assumption that the operative effect of ribonuclease was the deactivation of RNA of the cytoplasm. The suggestion that nuclear RNA is necessary to initiate the protein-synthesizing system in the *Acetabularia* cytoplasm should not be extended to mean that all cytoplasmic RNA in *Acetabularia* is of nuclear origin. This is clearly not the case. It would mean that RNA cannot be synthesized independently in the cytoplasm unless an unspecified but probably small amount of nuclear RNA is present.

SUMMARY

Ribonuclease treated nucleate and enucleate fragments of *Acetabularia* were investigated for their ability to grow, synthesize protein, and differentiate. It was found that, while nucleate fragments recover their capacity in these respects, enucleate fragments do not. It is suggested that the nuclear product which effects the recovery is RNA.

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