IS DNA POLYMERASE A CYTOPLASMIC ENZYME?

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One of the most enigmatic results of studies on the enzymic synthesis of DNA is that the polymerase is consistently found in homogenate fractions generally designated as cytoplasmic (1–4). Since an unequivocal answer to the question of intracellular location of the enzyme seemed quite impossible from homogenate studies, we have taken a direct cytological approach to the problem. The experiment consists simply in introducing exogenous DNA[1] into living cells by means of pinocytosis in H^3-thymidine medium. In the presence of the required enzymes (thymidine kinase, thymidylate kinase, and DNA polymerase) and the other substrates required for DNA synthesis, the ingested DNA serves as a primer for DNA synthesis. The site of DNA synthesis is determined by autoradiographic detection of H^3-thymidine incorporated into DNase-sensitive material. The results suggest that DNA polymerase in *Amoeba proteus* may be present throughout the cytoplasm of non-dividing cells.

EXPERIMENTAL

Amebae were washed free of food organisms and starved for 48 hours in inorganic medium (5) before being cut into nucleated and enucleated halves. The halved amebae were then incubated in H^3-thymidine (Schwarz BioResearch; 3.0 c/mM, 25 μc/ml) media with or without DNA (1 mg/ml) in the presence of 0.5 mg/ml egg albumin to stimulate pinocytosis. They were then fixed with alcohol-acetic acid (3:1) and flattened on microscope slides before autoradiography with Kodak NTB2 liquid emulsion.

The marked incorporation of H^3-thymidine that occurs in the cytoplasm of all enucleated and nucleated amebae fed DNA is illustrated in Fig. 1. The radioactivity incorporated in the cytoplasm is not extracted by the acid-alcohol fixative or by a 5 per cent trichloroacetic acid wash at 0°C. More than 95 per cent of the radioactivity is removed by a 5 per cent trichloroacetic acid wash after DNase treatment (Worthington DNase I, 0.2 mg/ml, pH 6.8, 3 hours at 37°C). A low level of H^3-thymidine incorporation occurs in nucleated and enucleated amebae that have *not* been fed with DNA (Figs. 1*C* and *D*). As in the DNA-fed cells, this acid-insoluble activity is almost completely removed by DNase digestion. A similar low level incorporation in ameba cytoplasm was observed earlier by Plaut and Sagan (6).

Amebae obtain nutrients by phagocytosis of microorganisms, and the DNA of the ingested organisms is detectable as Feulgen-positive material in food vacuoles even after many days of starvation. In the present study the density of the autoradiograph was not increased over such vacuoles. While the DNA in the food vacuole apparently does not act as primer, the low level of DNA synthesis observed throughout the cyto-

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[1] Abbreviations: DNA, deoxyribonucleic acid; DNase, deoxyribonuclease.

We recognize the presence of DNA in the cell nucleus, but experiments on carefully fixed cytological preparations lead us to believe that this DNA cannot act as *primer* DNA without first being denatured. (R. C. von Borstel, D. M. Prescott, and F. J. Bollum, unpublished experiments, 1961.)

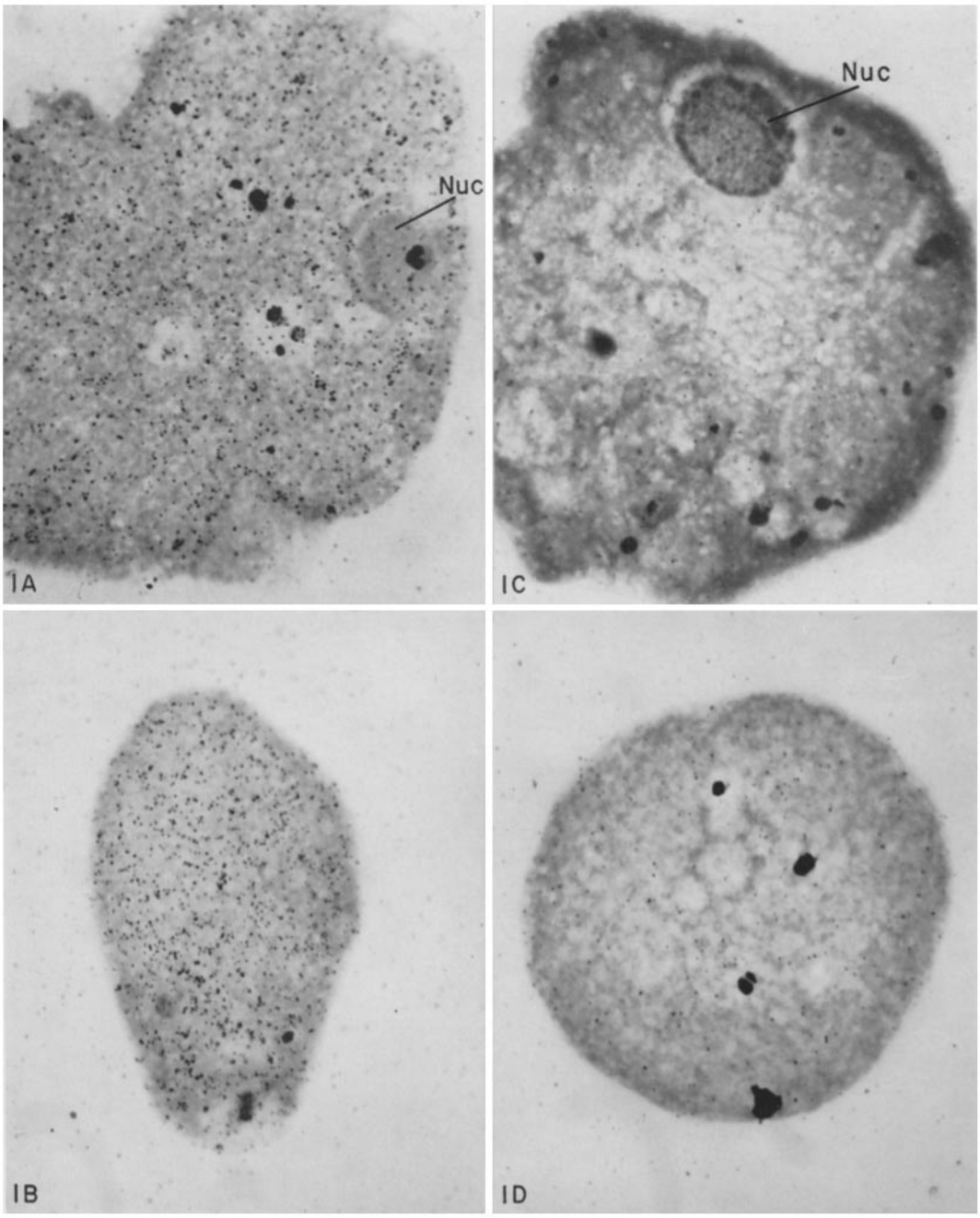
FIGURES 1 *A* AND *B*

Autoradiographs of a nucleated (*A*) and an enucleated (*B*) ameba incubated for 3 hours in a medium of H^3-thymidine and DNA primer. × 800.

FIGURES 1 *C* AND *D*

Autoradiographs of a nucleated (*C*) and an enucleated (*D*) ameba incubated for 3 hours in a medium of H^3-thymidine without DNA primer.

The amebae were stained with toluidine blue. Food vacuoles are in most cases very heavily stained.


Nuc
1A
Nuc
1C
1B
1D

plasm of amebae *not* fed DNA might be due to priming action of partially degraded DNA diffusing out from the food vacuoles. Increasing the DNA content of the ingested food material may be presumed to increase the DNA level of the cytoplasm and, therefore, increase the intensity of the autoradiograph. The critical analysis in the present experiment is, then, the change in cytoplasmic grain count following feeding of denatured DNA. The data in Table I demonstrate that cytoplasmic grain count is increased sixfold in DNA-fed amebae. The grain count is significantly higher over enucleates than over nucleated amebae, suggesting an effect of the nucleus on cytoplasmic polymerase activity.

TABLE I

Grain Counts of Cytoplasmic H^3-Thymidine Incorporation

Twenty standard areas of cytoplasm of several nucleated and enucleated amebae were counted after incubation in H^3-thymidine with or without exogenous DNA for 3 hours. The means have been corrected for background and the ± indicates 95 per cent confidence limits.

Amebae Fragments	With Primer	Without Primer
Nucleated	168 ± 22	29 ± 6
Enucleated	261 ± 27	43 ± 9

These preliminary experiments, while themselves not immediately amenable to rigorous interpretation, do raise questions about the nature of H^3-thymidine incorporation and the intracellular localization of the enzymes required for such incorporation. First of all, we cannot rule out the possibility that exogenous DNA stimulates DNA synthesis in the cytoplasmic "endosymbionts" described by Roth and Daniels (7). We do know, however, that exogenous deoxyribonucleosides *do not* stimulate cytoplasmic H^3-thymidine incorporation. In addition, it may be pointed out that several types of deoxyribonucleotide incorporation are known, for example: end addition to deoxyribopolynucleotide chains as well as replicative DNA synthesis. Since cytological observations do not permit distinction of these two reaction types, it should be obvious that the detailed nature of both "nuclear" and "cytoplasmic" autoradiographs from H^3-thymidine remains undetermined and cannot be decided simply by vocal agreement. It is rather unusual that the end-addition reaction has been described in extracts from nuclear preparations (8), whereas replication enzymes are commonly isolated from "cytoplasmic" material (9).

The present observations simply emphasize that prejudice is not an adequate criterion for determining fact and that it is well to keep an open mind regarding the localization of enzymes and the site of replicative DNA synthesis.

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