MICRONUCLEAR RNA SYNTHESIS IN *PARAMECIUM CAUDATUM*

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ABSTRACT

In a generation time of 8 hr in *Paramecium caudatum*, the bulk of DNA synthesis detected by thymidine-^{3}H incorporation takes place in the latter part of the cell cycle. The micronuclear cycle includes a G_1 of 3 hr followed by an S period of 3–3½ hr. G_2 and division occupies the remaining period of the cycle. Macronuclear RNA synthesis detected by 5′-uridine-^{3}H incorporation is continuous throughout the cell cycle. Micronuclear RNA synthesis is restricted to the S period. Ribonuclease removes 80–90% of the incorporated label. Pulse-chase experiments showed that part of the RNA is conserved and released to the cytoplasm during the succeeding G_1 period.

INTRODUCTION

On the basis of genetic experiments on *Paramecium aurelia*, Sonneborn (11) proposed that the macronucleus in ciliated protozoa is exclusively somatic and that the micronucleus is primarily germinal in function. This idea is supported by the observations that the reproduction of kappa in the cytoplasm of *P. aurelia* (13) is maintained when the K gene is present in the macronucleus but not when it is present only in the micronucleus. Furthermore, certain lines of *Tetrahymena* (4) and *Paramecium busaria* (2, 3), lacking a micronucleus altogether, still grow and divide indefinitely (4). Despite the apparent dispensable nature of the micronucleus, survival of amicronucleates produced in the laboratory is poor (15).

Since contributions of the micronucleus during growth reasonably might be expected to occur via RNA synthesis, at least several laboratories have attempted to demonstrate micronuclear RNA synthesis by radioautographic methods. Kimball (5) and Miller and Prescott[1] failed to find evidence of such synthesis in *P. aurelia* and *Tetrahymena*, although Moses (8), using cytochemical methods, has detected the *presence* of RNA in the micronucleus of *P. caudatum*. We have taken up the question of synthesis, by increasing the resolution and sensitivity of the radioautographic method by isolation of the large micronucleus of *P. caudatum*.

[1] Miller, O. L. and D. M. Prescott. Unpublished data.

MATERIALS AND METHODS

Culture Methods

P. caudatum was grown in lettuce infusion (12) inoculated with *Aerobacter aerogenes* 24 hr before use. The cell cycle time is about 8 hr in this medium at 27°C.

Labeling Procedure

To determine the timing of the synthesis of DNA and RNA, the cell cycle of *Paramecium* was divided into 16 half-hour intervals, and the isotope was administered, to different groups of cells, which were synchronized by selection of dividers, at the beginning of each interval. At the end of the interval the cells

were washed in nonradioactive medium, and macro- and micronuclei were isolated by lysing the cells in a solution of Triton X-100 and spermidine. The details of the isolation technique have been published elsewhere (10).

Although, after isolation, micronuclei generally have a clean appearance in the light microscope, we have not had the opportunity to look at isolated micronuclei with electron microscopy. Electron microscope examination of macronuclei isolated from *Euplotes* (Dr. A. R. Stevens, personal communication) by the same method, however, shows not only that nuclei are free of cytoplasmic contamination but also that much of the nuclear membrane has been lost. It seems unlikely that ribosomes or other cytoplasmic contamination clinging to the isolated micronuclei could be contributing to the measurements of RNA labeling. Perhaps more important in this connection is the discontinuous nature of micronuclear labeling, a condition that would be difficult to reconcile with any consistent contribution of labeling from cytoplasmic contaminants.

For RNA labeling, uridine-^{3}H at 25 μc/ml (20.4 c/mmole, Nuclear Chicago) with tritium in the 5′ position was added directly to the medium. DNA was labeled by feeding *Paramecium* on bacteria labeled with thymidine-^{3}H at 10 μc/ml (15.0 c/mmole, Nuclear-Chicago Corporation, Des Plaines, Ill.) (1). Unincorporated label was eliminated by washing isolated nuclei with 5% trichloroacetic acid for 5 min at 5°C.

Digestions

The specificities of isotope incorporations were tested by treating isolated nuclei, air-dried on slides, with ribonuclease or deoxyribonuclease. Ribonuclease (Worthington Corporation, Harrison, N. J.) was made up in a 0.5% solution (w/v) in 0.01 M phosphate buffer at pH 6.9, and digestion was carried out for 7 hr at 37°C. Deoxyribonuclease (Worthington Corporation) digestion was carried out with an 0.01% (w/v) solution made up in a solution containing 0.003 M Na_2HPO_4, 0.007 M KH_2PO_4, and 0.005 M $MgSO_4$ at a pH of 5.0 for 7 hr at 37°C.

Radioautography

Radioautographs were prepared with NTB 3 liquid emulsion. The exposure time varied from 10 to 15 days. Isolated nuclei were stained with toluidine blue through the emulsion after development.

RESULTS

DNA Synthesis in the Macronucleus

Incorporation of radioactivity into DNA was slight but definite during the first 2½ hr of the interdivision interval. This radioactivity was removable with deoxyribonuclease digestion. From 2½ to 7 hr the incorporation of thymidine-^{3}H was relatively intense. Labeling appeared to decrease after 7 hr and had ceased when early stages of macronuclear division were recognizable, at 7½ hr. Division events occupied about 30 min.

DNA Synthesis in the Micronucleus

Incorporation of thymidine-^{3}H into the micronucleus began about 3 hr after division and lasted for 3–3½ hr (Fig. 1). DNA synthesis was followed by a clearly recognizable G_2 period of 1 hr or less. Thus, micronuclear DNA synthesis begins at about the time that the rate of macronuclear DNA synthesis accelerates sharply, but ends before macronuclear synthesis is completed.

RNA Synthesis in the Macronucleus

The incorporation of uridine-^{3}H into macronuclear RNA occurs continuously throughout the cell cycle including the period of macronuclear division. Between 80 and 100% of incorporated activity could be removed with ribonuclease.

RNA Synthesis in the Micronucleus

Incorporation of uridine-^{3}H has been demonstrated definitely only in micronuclei of cells incubated with the isotope during the S period (Fig. 2). Ribonuclease digestion removes roughly 70–80% of micronuclear label. The radioactivity not removed by ribonuclease may be due to incorporation of uridine-^{3}H into DNA, although the tritium is located in the 5′ position of the uracil moiety and presumably would be lost in the process of methylation to thymidine. The principal labeling of DNA may occur through conversion to cytidine. In addition, some of the ribonuclease-resistant fraction possibly could represent RNA complexed with DNA.

The intensity of RNA labeling in the most heavily labeled micronuclei is on the average an order of magnitude less that of the macronuclei for the same period of time. There is also considerable variation in the degree of labeling from one micronucleus to another. This could mean that micronuclear RNA synthesis is discontinuous or intermittent within the S period.

In pulse-chase experiments the loss of radioactivity from micronuclear RNA was followed with time. Paramecia were labeled with uridine-^{3}H for 30 min, washed free of isotope, and cultured

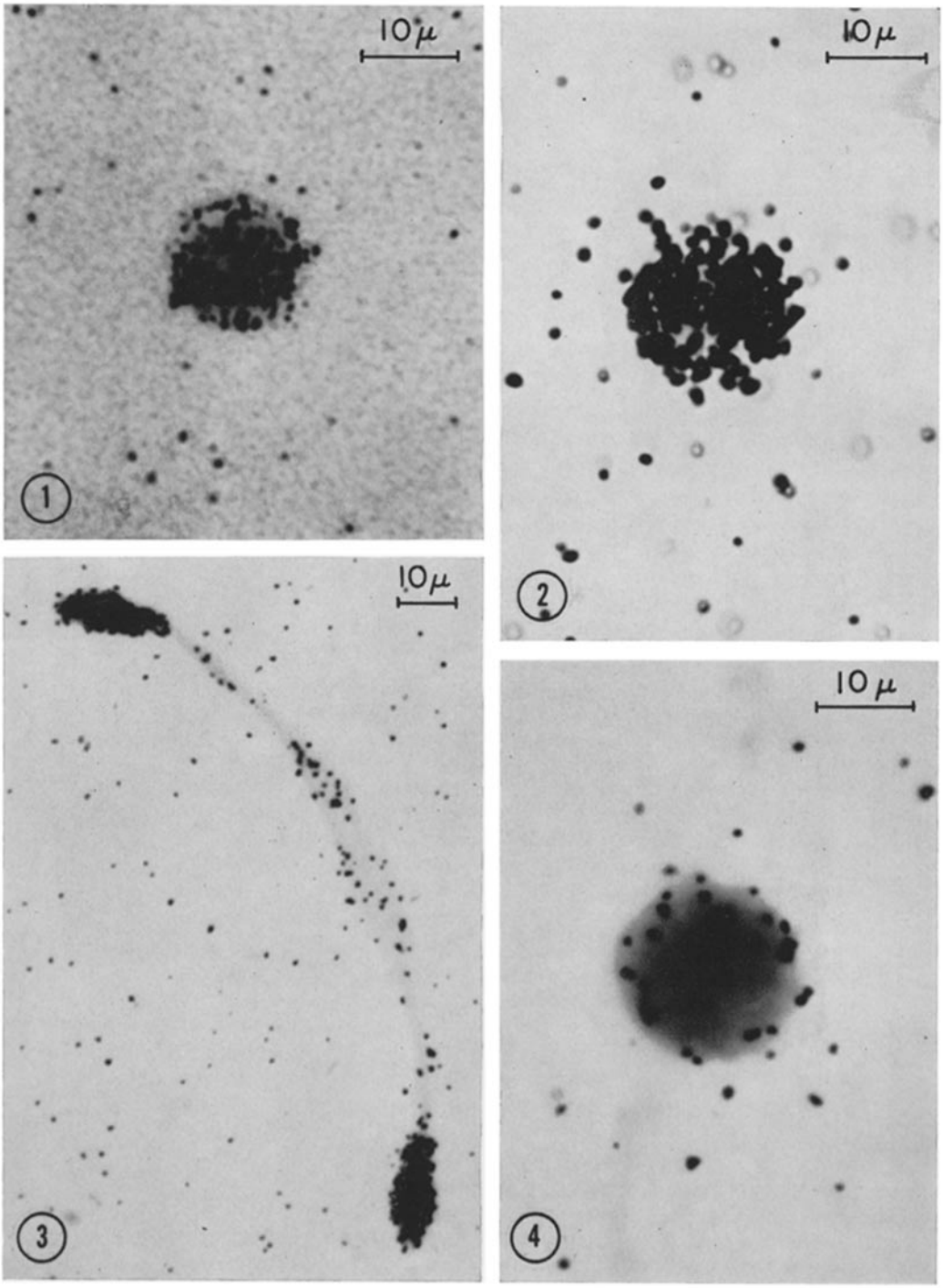

FIGURE 1 Radioautograph of an isolated micronucleus labeled with thymidine-^{3}H for 30 min.

FIGURE 2 Radioautograph of the isolated micronucleus labeled with uridine-^{3}H for 30 min in the S period.

FIGURE 3 Radioautograph of the isolated micronuclear mitotic apparatus. The micronucleus was labeled with uridine-^{3}H for 30 min during the S period, and the cells were grown in nonradioactive medium until the next division.

FIGURE 4 Radioautograph of the isolated G_1 micronucleus. The cell was labeled with uridine-^{3}H for 30 min during the S period of the previous cell cycle and allowed to grow in nonradioactive medium until the next G_1 stage.

in nonradioactive medium. Micronuclei were isolated at regular intervals up to the second division after labeling. There was no clearly apparent loss of radioactivity during the first G_2 or during mitosis (Fig. 3). Although the labeling decreased considerably in the G_1 (Fig. 4) of the subsequent cell cycle, the micronuclei still were labeled at the second division.

DISCUSSION

DNA Synthesis

The S period for the macronucleus is similar to that found for *P. aurelia* (14, 6, 16, 7), to the extent that the bulk of DNA synthesis occurs in the latter part of the interdivision interval. The only significant difference is the slow trickle of DNA labeling in *P. caudatum* at a time that *P. aurelia* is in G_1. The significance of this early labeling is not known.

The S period for the micronucleus in *P. caudatum* is similar to that in *P. aurelia* (16), with the exception that DNA synthesis continues for a much longer time in *P. caudatum*.

RNA Synthesis

The continuous labeling of macronuclear RNA during the cell cycle in *P. caudatum* confirms the previous reports for other ciliates (6, 16, 9). RNA synthesis continues during amitosis, and there is no massive release of RNA to the cytoplasm as there is in cells which divide by a mitotic mechanism.

The relatively small amount of RNA synthesis that occurs in the micronucleus is the possible basis for a contribution of this nucleus to the survival of the cell. The gradual decrease in labeled micronuclear RNA is at least consistent with the interpretation that this RNA migrates to the cytoplasm, but it is nevertheless possible that micronuclear RNA turns over without ever entering the cytoplasm. The restriction of this synthesis to the micronuclear S period may mean that conditions required for such transcription are met only in connection with DNA synthesis.

The more puzzling aspect of any unique contribution of the micronucleus to the cell stems from the evidence that both micro- and macronucleus contain the same genetic elements, but simply with different ploidies. If it is accepted that the micronucleus makes a unique RNA contribution, then it must be supposed either that the particular genetic information for this RNA is lost in the development of the macronucleus from the fusion nucleus after conjugation or that the two nuclei are qualitatively identical in DNA content but are subjected to different gene repressions. The low amount of RNA synthesis and its restriction to a shorter period of the cell cycle perhaps points to the latter explanation.

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