COMPARISON BETWEEN THE TIMING OF MICRONUCLEAR AND MACRONUCLEAR DNA SYNTHESIS IN *EUPLOTES EURYSTOMUS*

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We previously showed (5) that in double animals of *Euplotes* the two separate macronuclei begin DNA synthesis simultaneously, demonstrating that the cytoplasm plays a role in initiating DNA synthesis. By studying the timing of DNA synthesis in both the micronucleus and the macronucleus, we have obtained evidence that the initiation of DNA synthesis depends not only upon cytoplasmic conditions but also upon the properties of the particular nucleus.

METHODS

The same strain of the ciliated protozoan *Euplotes eurystomus* was used as in our earlier work.

DNA synthesis in the macronucleus is easily followed by observing in aceto-carmine preparations the initiation and progress of the replication bands, which have been shown to represent waves of DNA synthesis (1, 9). The bands normally originate at the two tips of the elongated macronucleus and move towards the center, fuse, and disappear. Immediately after this, the macronucleus shortens, then elongates and divides amitotically.

The determination of the doubling of micronuclear DNA was accomplished by autoradiographic studies of H^3-thymidine incorporation and microspectrophotometric measurements of Feulgen-positive material. For the autoradiography, tritiated thymidine (3 c/mM, Schwarz BioResearch) was added to a rapidly proliferating culture to a final concentration of 25 μc/ml. After 10 to 30 minutes in the isotope medium, cells were withdrawn, fixed and flattened in alcohol:acetic acid (3:1), Feulgen-stained, and autoradiographed with Kodak NTB-2. The microspectrophotometric measurements were made by the two-wavelength method with a Canalco microspectrophotometer.

RESULTS

The generation time of *Euplotes eurystomus* cultured in lettuce infusion containing *Aerobacter aerogenes* and *Tetrahymena pyriformis* is about 14 hours at 24°C. The bands first appear at the tips of the macronucleus about 3 hours after cytokinesis (Table I). They meet in the middle at about 13 hours after cytokinesis and cell division is completed about 1 hour later. Thus macronuclear G1 (preduplication interphase) is 3 hours, S (DNA synthesis period) is 10 hours, and G2 (postduplication interphase) plus D (division) is 1 hour.

Micronuclear synthesis has a very different pattern, however, as can be seen from the data in Table I. The microspectrophotometric measurements show that Feulgen-positive material doubles between anaphase and very early interphase. Micronuclear doubling thus starts after the macronuclear duplication has ended and is complete or nearly complete before the amitosis of the macronuclear duplication begins. This situation is confirmed by the autoradiographs, which show labeling of the micronucleus only during late mitotic stages.

DISCUSSION AND SUMMARY

These studies demonstrate that two different kinds of nuclei in a common cytoplasm may undergo DNA synthesis at totally different parts of the cell cycle. This asynchrony between macronucleus and micronucleus contrasts with the synchrony of DNA synthesis in the two macronuclei of double *Euplotes* (5). Although the latter observation shows that the cytoplasm exerts some control over the initiation of DNA synthesis in this organism, the position of this synthesis within the cell cycle is a characteristic of the nucleus. Only nuclei of a certain kind can respond to a particular set of cytoplasmic conditions at a given point of the cycle.

Tetrahymena pyriformis and *Paramecium aurelia* demonstrate two additional temporal relations between macronuclear and micronuclear DNA synthesis. In *T. pyriformis* micronuclear synthesis occurs between late anaphase and early interphase as in *Euplotes*, but macronuclear S occupies the early part of the interdivision interval (6–8). In *P. aurelia*, micronuclear S normally occurs midway in the interdivision interval and nearly coincides with the beginning of macronuclear S (3, 4, 10). Under special conditions micronuclear S is delayed, whereas the beginning macronuclear

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TABLE I

Micronuclear and Macronuclear Stages in Euplotes

Micronuclear stage	Macronuclear stage	Duration	Micronuclear Feulgen-postive material (Relative units) No.	Mean	95 per cent CL	H^3-thymidine labeling grains/micronucleus
		hr				
Interphase	Just divided	3	7	1.93	2.09, 1.77	None above background
	Older but no bands		14	1.99	2.07, 1.92	
	Bands at tip to 1/8 way to middle	10	11	2.00	2.07, 1.94	
	Bands 1/8 to 1/4 way to middle		18	2.02	2.08, 1.95	
	Bands 1/4 to 1/2 way to middle		12	2.00	2.11, 1.89	
Prophase	Bands 1/2 way or more		10	2.14	2.28, 2.01	
Metaphase and early anaphase	Bands meeting at middle		13	2.02‡	2.11, 1.91	
Mid to late anaphase*	Condensation and amitosis	1	6	1.05§	1.12, 0.98	n = 10 (5 pairs) mean = 30 range = 4 to 21 background = 0 to 2
Early telophase*			11	1.22§	1.31, 1.13	
Mid to late telophase* and early interphase			19	1.55§	1.69, 1.41	

* Nuclei were scored (*a*) as anaphase when elongated but not constricted, (*b*) as telophase when constricted, and (*c*) as interphase when the daughter nuclei were no longer attached to each other.
‡ Early anaphase nuclei were measured as single entities.
§ The two groups of daughter chromosomes were measured as separate entities.

S is not (2). It is obvious that there are no set rules in ciliates either for the relation between the timing of DNA synthesis in the two nuclear types or for the relation between the cell cycle and DNA synthesis in either nuclear type.

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