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Evidence of Gene Diminution During the Formation of the Macronucleus in the Protozoan, *Stylonychia**

(DNA density/melting curves/micronuclear DNA/polytene chromosomes)

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ABSTRACT **The course of events by which a macronucleus is formed from a micronucleus after conjugation in the ciliated protozoan, *Stylonychia*, suggests that genetic diminution may occur. This idea is supported by determinations of the density profiles and melting curves for micro- and macronuclear DNAs. Macronuclear DNA consists of a single density component and melts as if it were a single component. Micronuclear DNA consists of four or more density components and melts as if it were a mixture of several DNAs of different base compositions. These data indicate that at least 60% of the micronuclear DNA components are absent from the macronuclear DNA.**

In the first stage of the formation of the macronucleus from a micronucleus in the hypotrichous ciliate *Stylonychia*, the DNA content increases about 14-fold (1, 2). This increase in DNA results in the formation of polytene chromosomes in the macronuclear anlage. The polytene chromosomes then break up into short lengths by the formation of membraneous partitions between all the bands (chromomeres), such that each band becomes enclosed within a separate vesicle (3). Thus, the macronuclear anlage is converted to a bag containing many hundreds of separate vesicles.

Immediately after the breakup of the polytene chromosomes, 93% of the DNA in the anlage is destroyed (4). Some hours later, the remaining 7% of the DNA in the macronuclear anlage undergoes many rounds of replication (by means of the replication bands that are typical in hypotrichous ciliates), until finally the DNA content is increased to about 65-times the amount in the original micronucleus. At this point the formation of the macronucleus is complete, and the cell begins vegetative reproduction.

We reported (5) that all of the macronuclear DNA is in small pieces, with an average length of 0.78 μm per piece. Micronuclear DNA, by contrast, is obtained as extremely long pieces (the majority being longer than 35 μm). The presence of the short pieces of DNA in the macronucleus is consistent with the observed breakup of the polytene chromosomes.

Concerning the destruction of 93% of the DNA in the macronuclear anlage, there are two possible explanations. Either all of the DNA in most of the vesicles (bands) is destroyed, or 93% of the DNA in each vesicle is destroyed. The first explanation would presumably require a drastic diminution in the genetic complexity (complexity in nucleotide sequences) of the macronuclear anlage (3, 6). The second explanation would presumably maintain the status quo of complexity in nucleotide sequences, but would reduce the amplification represented by polytenization.

In this paper we present melting curves and density determinations for macronuclear and micronuclear DNAs. These data support strongly the hypothesis that genetic (DNA) diminution does occur during the formation of the macronucleus in *Stylonychia*.

MATERIALS AND METHODS

Cultures of Stylonychia. In the initial work the *Stylonychia* were grown on undefined mixtures of bacteria, plus *Tetrahymena*. This method was replaced by cultivation of *Stylonychia* on *Tetrahymena* under bacteria-free conditions. With this culture method, the yield of *Stylonychia* is much higher, the task of preparing micronuclear and macronuclear fractions is simpler, and the possibility of contamination of DNA preparations with various bacterial DNAs is completely avoided.

The lysis of cells and collection of macronuclear and micronuclear fractions were described (5). Fig. 1 shows photomicrographs of the macronuclear and micronuclear preparations.

Preparation of DNA. DNA was prepared from macronuclei and micronuclei by lysis of the nuclei in 0.01 M Tris–0.01 M sodium EDTA (pH = 7.0)–0.1% sodium dodecyl sarcosinate, with subsequent isolation through CsCl as described (5). One preparation of macronuclear DNA was extracted by the Marmur procedure (7), with slight modifications.

Equilibrium Density Centrifugation. The DNA solutions were stirred for 10 min at 1600 rpm at 4°C with a Precision adjustable stirrer, in order to shear the DNA. Solid CsCl was added to 0.7 ml of 0.01 M Tris·HCl buffer (pH = 8.4), containing about 3 μg of 2C phage DNA (to serve as a density marker), to give an initial density of 1700 g cm^{-3}, and the density was checked refractometrically (8). The resulting solutions were centrifuged in the Spinco model E centrifuge at 40,000 rpm at 20°C using double-sector cells with Kel F centerpieces in an AnG rotor. Banding patterns were traced with the ultraviolet scanning attachment and the buoyant densities of the peaks was determined (9). Centrifugation was continued until equilibrium was reached. The establishment of equilibrium was judged by the absence of any further change in the ultraviolet scanner tracings over a 4-hr interval.

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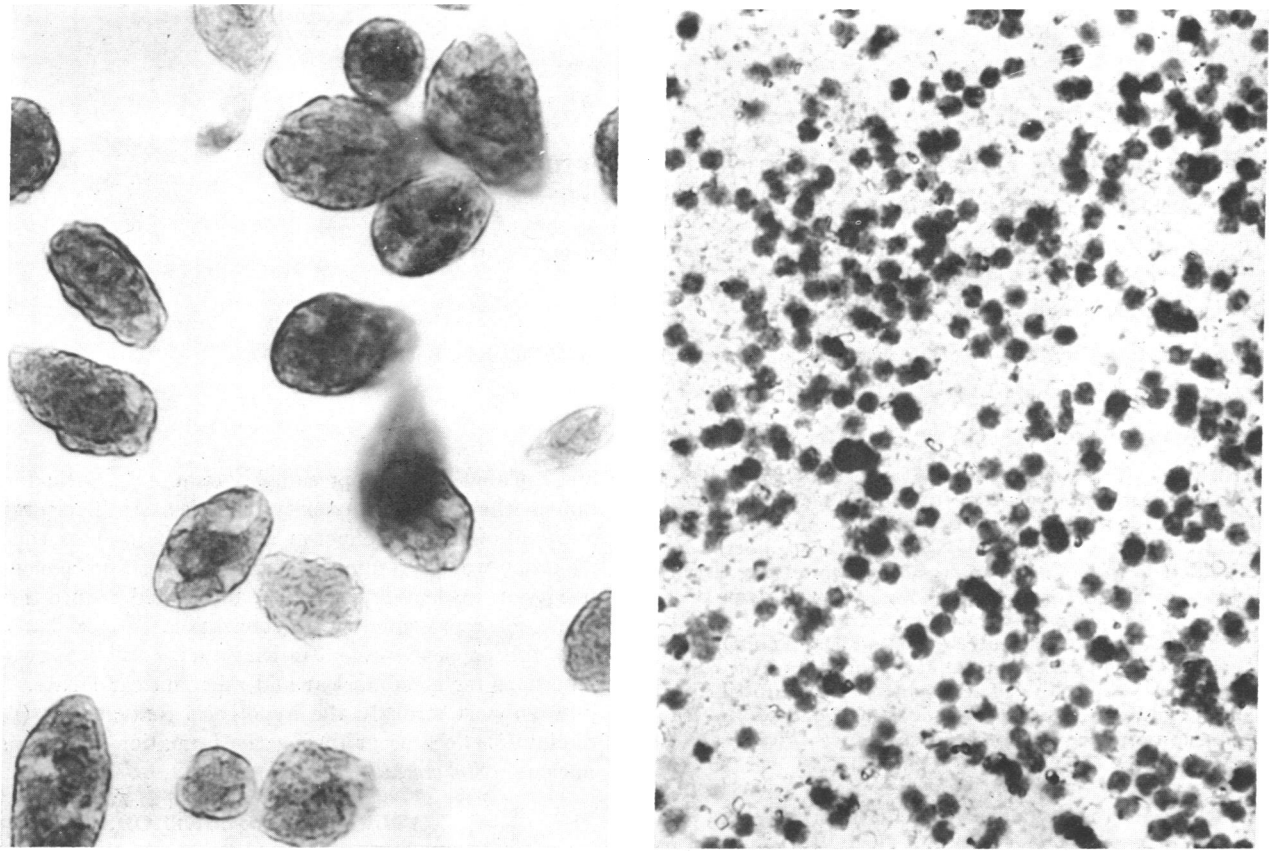

FIG. 1. Photographs of macronuclei (*left*) and micronuclei (*right*) isolated from *Stylonychia* and stained with aceto-carmine. Macronuclear preparations are free of micronuclei and cellular debris. Micronuclear preparations are free of macronuclei, but contain polysaccharide particles. ×1000.

Melting Curve Determinations. Analysis of the melting profiles of macronuclear and micronuclear DNA was done by the procedure described by Mandel and Marmur (10). DNA samples were dialyzed against four changes of standard saline citrate (SSC: 0.15 M NaCl–0.015 M Na_3 citrate, pH = 7.0). Absorbance measurements were made in a Gilford Recording Spectrophotometer fitted with a temperature-recording attachment. The melting profiles shown in Fig. 3 have been corrected for thermal expansion.

RESULTS

The densities of macronuclear and micronuclear DNAs

Equilibrium density centrifugation of DNAs from six different macronuclear preparations and six different micronuclear preparations gave essentially the same results. Density profiles for micro- and macronuclear DNAs are shown in Fig. 2. Macronuclear DNA forms a single, symmetrical band, with a peak density of 1.701 g cm^{-3}. This is the case both for macronuclear DNA isolated by the CsCl method or by the Marmur procedure. Micronuclear DNA is resolved into four or more separate components of different densities. Definite peaks occur at 1.699, 1.704, and 1.709 g cm^{-3}. A fourth component must be present at about 1.701 g cm^{-3} to account for the failure of the density profile to dip between the peaks at 1.699 and 1.704 g cm^{-3}. Additional minor components may be present. The DNA density profiles for all six micronuclear preparations show a small bump at 1.695 g cm^{-3}, and the peak at 1.704 g cm^{-3} is asymmetrical, indicating an additional component with a density slightly less than 1.704 g cm^{-3}.

The data in Fig. 2 were obtained from micronuclear DNA prepared from *Stylonychia* fed partly on bacteria. Essentially the same tracing was obtained for DNA of micronuclei obtained from *Stylonychia* grown on *Tetrahymena* alone under bacteria-free conditions. In the latter case, the density peak at 1.709 g cm^{-3} was not as well resolved as it is in Fig. 2*a*. In any case, none of the components in the density profile in Fig. 2*a* can be due to bacterial DNA.

The possibility that any of the peaks shown in the micronuclear DNA preparation could be due to either contaminating *Tetrahymena* DNA in residual food vacuoles, or to polysaccharides, which band in CsCl density gradients, can also be ruled out. Fig. 2*c* shows that the density of *Tetrahymena* DNA is 1.690 g cm^{-3}, which is considerably lighter than the lightest component of micronuclear DNA of *Stylonychia*. Fig. 2*d* shows the ultraviolet-scanner tracing obtained when a DNA preparation, from which the polysaccharide had not been removed by prior centrifugation, is treated with deoxyribonuclease and centrifuged to equilibrium in CsCl. The band observed at a density of 1.686 g cm^{-3} is polysaccharide; all the bands at densities corresponding to those observed in pure micronuclear DNA preparations are removed by deoxyribonuclease treatment. Furthermore, the bands observed in pure macronuclear and micronuclear preparations reach equilib-

rium slowly, whereas the polysaccharide peak is detectable after a few hours of centrifugation.

The observed heterogeneity in the buoyant density of micronuclear DNA could possibly reflect the presence of unusual bases in micronuclear DNA, without producing any basic difference in the nucleotide sequence composition between micronuclear DNA and macronuclear DNAs. This is unlikely because the melting curve for micronuclear DNA (Fig. 3) shows that there are several DNA components of different G-C content in micronuclei, and these approximately correspond to the different components observed in CsCl density gradient centrifugation.

On the basis of these facts, we conclude with confidence that none of the components in the tracing for micronuclear DNA (Fig 2*a*) can be due to contamination by bacterial DNA, *Tetrahymena* DNA, or polysaccharide.

Fig. 2*e* shows the tracing of micronuclear DNA, with a gaussian curve drawn in at a density of 1.701 g cm^{-3}, which is the density of macronuclear DNA. If we assume that the material at 1.701 g cm^{-3} in the micronuclear DNA tracing corresponds to macronuclear DNA, then an absolute maximum of only 40% of micronuclear DNA is represented in macronuclear DNA. This says that there is at least a 60% diminution in the macronuclear DNA components during the formation of the macronucleus. The actual diminution is undoubtedly greater than 60%, since much of the ultraviolet-absorbing material encompassed by the gaussian curve in Fig. 2*e* consists of material from the two adjacent peaks.

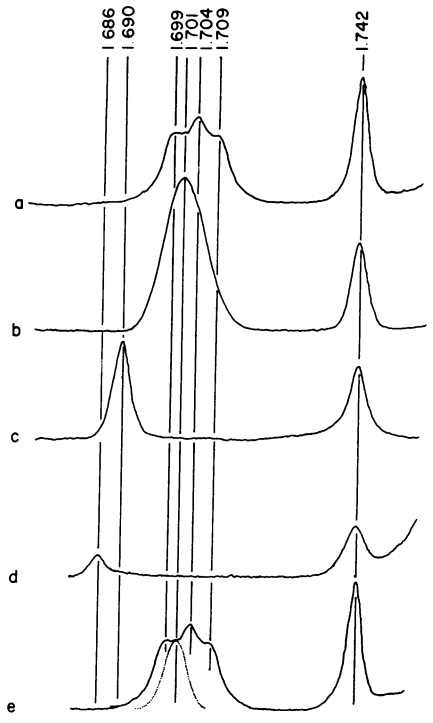

FIG. 2. Ultraviolet-scanner tracings of the banding patterns of DNA in neutral CsCl density gradients centrifuged to equilibrium in the Spinco model E analytical centrifuge. (*a*) *Stylonychia* micronuclear DNA; (*b*) *Stylonychia* macronuclear DNA; (*c*) *Tetrahymena* total DNA; (*d*) DNase-treated *Stylonychia* micronuclear DNA containing polysaccharide; (*e*) a tracing of *Stylonychia* micronuclear DNA, to which has been added a gaussian curve at a density of 1.701 g cm^{-3}. The band at a density of 1.742 g cm^{-3} represents 2C phage DNA, which was used as a density marker.

Melting curves for macro- and micronuclear DNAs

These are shown in Fig. 3. Macronuclear DNA melts as if it were a single component. In contrast, the melting curve for micronuclear DNA is complex. The curve reflects the presence of several components, which probably correspond to the four components observed in the buoyant density profiles. Thus, the melting curves indicate that micronuclear DNA is substantially different in base composition from macronuclear DNA.

DISCUSSION AND CONCLUSIONS

Micronuclear DNA contains at least four components of different buoyant densities. Macronuclear DNA contains a single density component. These differences in density between micro- and macronuclear DNAs are borne out by differences in the melting curves for the two DNAs. We assume that the differences arise by the destruction of DNA after the polytene chromosome stage in the formation of a macronucleus from a micronucleus.

We estimate that more than 60% of the DNA sequences in the micronucleus are not present in the macronucleus. This does not necessarily represent an equivalent reduction in the complexity of nucleotide sequences, since some of the micronuclear DNA that is absent in the macronucleus may be composed of repeated sequences. An estimate of the amount of sequence diminution could be obtained by comparing the rates of renaturation of micro- and macronuclear DNAs according to the method of Britten and Kohne (11). We have not yet obtained a sufficient quantity of micronuclear DNA

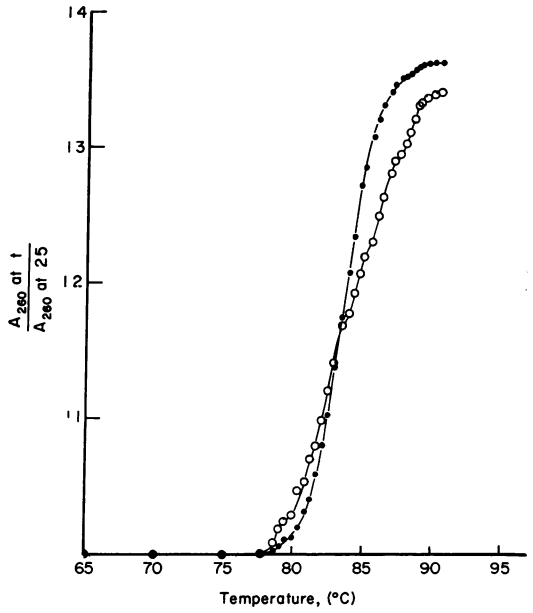

FIG. 3. Thermal denaturation curves of DNA isolated from macronuclei (●——●) and micronuclei (○——○) of *Stylonychia*. Both samples were at a concentration of about 15 μg/ml in SSC. The absorbance at elevated temperatures has been corrected for thermal expansion. The increase in hypochromicity is expressed as the ratio of absorbance at 260 nm at the elevated temperature relative to the absorbance at 260 nm at 25°C.

for this purpose. [Macronuclear DNA renatures 20-times more slowly than *Escherichia coli* DNA (unpublished data), which indicates that the macronucleus is genetically quite simple for a eukaryotic cell of such size and complexity.]

The function of the DNA that is eliminated from the original micronuclear genome during formation of the macronucleus is unknown. Such DNA might have a role in the various steps of conjugation (meiosis, mating, crossing-over, etc.), since the macronucleus does not participate in conjugation, but it seems unlikely that such a large amount of micronuclear DNA is necessary for these functions. We can only comment, rather vaguely, that there are probably functional properties of the DNA in eukaryotic cells that are not yet understood.

Finally, nothing is yet known at the molecular level about the mechanism of destruction of DNA sequences. Presumably, the transection of the polytene chromosomes involves at least one enzyme with a specificity that restricts its action to interband regions of the chromosome. Those DNA sequences that are subsequently destroyed must be marked in some way that permits recognition by one or more nucleases. Alternatively, the nucleotide sequences that are retained must have a property that protects them against digestion.

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1. Ammermann, D. (1965) *Arch. Protistenk.* **108,** 109–152.
2. Ammermann, D. (1968) *Chromosoma* **25,** 107–120.
3. Kloetzel, J. A. (1970) *J. Cell Biol.* **47,** 395–407.
4. Ammermann, D. (1969) *J. Cell Biol.* **40,** 576–577.
5. Prescott, D. M., Bostok, C. J., Murti, K. G., Lauth, M. R. & Gamow, E. (1971) *Chromosoma* **34,** 355–366.
6. Ammermann, D. (1971) *Chromosoma* **33,** 209–238.
7. Marmur, J. (1961) *J. Mol. Biol.* **3,** 208–218.
8. Ifft, J. B., Voet, D. H. & Vinograd, J. (1961) *J. Phys. Chem.* **65,** 1138–1145.
9. Mandel, M., Schildkraut, C. L. & Marmur, J. (1968) in *Methods in Enzymology,* ed. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 12B, p. 184–195.
10. Mandel, M. & Marmur, J. (1968) in *Methods in Enzymology,* ed. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 12B, p. 195–206.
11. Britten, R. J. & Kohne, D. E. (1968) *Science* **161,** 529–540