THE REGULATION OF AMOUNTS AND PROPORTIONS OF GENETIC ELEMENTS IN THE MACRONUCLEI OF TETRAHYMENA THERMOPHILA STRAINS OF DIVERSE KARYOTYPE

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SUMMARY
Measurements of the micronuclear DNA content of clones with assumed different degrees of micronuclear ploidy confirmed the triploid nature of one clone. The micronuclear DNA content of a presumptive haploid clone was found to be slightly higher than expected, whereas one of two aneuploid clones had an unexpectedly low micronuclear DNA content. The macronuclear DNA content of cells with macronuclei which had developed from triploid, diploid and probably haploid and aneuploid macronuclear-Anlagen is very similar. Specifically, the smallest individual macronuclear DNA contents are consistently found within the same size class in all clones tested. Cell volumes, RNA and protein contents are alike in all clones tested. Only the growth rate and maximal density reached of one out of two aneuploid clones is reduced in comparison with the very similar other clones.

The results are discussed with reference to the regulation of macronuclear DNA content, and to the compensation of gene-dosage.

INTRODUCTION
Usually the ciliate macronucleus develops from the fusion product of the pronuclei after cross-fertilization during the process of sexual reorganization. The DNA content of this fusion product, called the synkaryon, increases with time in the holotrich ciliate Tetrahymena thermophila, until the macronuclear DNA content of the vegetative cell is reached (Doerder & DeBault, 1975). Ordinarily, the synkaryon receives a diploid set of chromosomes during fertilization.

In rare cases, however, three instead of two cells mate. In some 'triplet' conjugations one of the mates receives 2 gametic nuclei, which fuse with the stationary pronucleus and another mate receives none, being left with only its own haploid pronucleus (Preparata & Nanney, 1977). Eventually, all 3 mates may give rise to vigorously growing clones, one stemming from a cell with a new macronucleus developed from a triploid synkaryon, the other 2 from cells with macronuclei derived from a diploid...
(i.e. normal) synkaryon, and a haploid pronucleus respectively. Aneuploid cells can be obtained by crossing the haploid and triploid cells in various combinations.

This material offers the opportunity to study the effect of the number of chromosomes originally contained in the synkaryon — and hence in the macronuclear-Anlage — on the constitution and the function of the mature macronucleus of the vegetative cell.

This study was undertaken to determine the amounts of DNA, and some facets of the performance of those macronuclei, derived from macronuclear-Anlagen which had received different numbers of chromosomes, and to compare them with diploid control cells.

MATERIALS AND METHODS

Cells and culture methods

Clones of *Tetrahymena thermophila* (formerly known as *Tetrakymetta pyriformis*, syngen 1; Ref. Nanney & McCoy, 1976) with different degrees of micronuclear ploidy have been used throughout the study. The inbred strain A 1768 II served as the diploid reference. Table 1 (p. 114) displays their designation, the micronuclear karyotypic constitution, based on previous chromosome counts, and the micronuclear DNA contents as they have been measured in this study. All clones had grown without sexual reorganization for about 2 years. Shelf cultures were 'loop' transferred biweekly into test tubes containing 10 ml of 1 % proteose peptone (No 3; Difco). These cultures were kept at room temperature, whereas all experimental cultures were maintained at 29 °C, and adapted at least 3 days prior to an experiment.

The macronuclear DNA contents were measured from cultures in which logarithmic culture growth was sustained for the same period of time.

To determine the rate of RNA synthesis and the RNA and protein contents, as well as to compare the growth rates and distribution of the cell volumes, the cells were grown in defined medium (Elliott, Brownell & Gross, 1954), supplemented with 1 mg/l. cholesterol and 0.04 % proteose peptone. 100-ml cultures in 500 ml Erlenmeyer flasks were inoculated from stationary cultures, kept in defined medium, too, to an initial cell density of 490 ± 20 cells/ml. The cultures were vigorously shaken in a waterbath at 100 rev/min (Braun, Melsungen).

Cell numbers and volumes were determined with a Coulter Counter Model ZB (Coulter Electronics), equipped with a multi-size-channel-analysers ('Coulter Channelyzer'), in Isotone (Coulter Electronics) under standardized conditions.

Estimation of the nuclear DNA contents

To obtain comparable values of macronuclear DNA contents only $G_1$ macronuclei were measured. Either dividing cells were hand-selected from a culture with a constriction pipette, or the whole culture was harvested by low-speed centrifugation, re-suspended in a small volume of medium, and 50 µl of the suspension were spread out onto a clean slide. After evaporation of the fluid in an evacuated desiccator the slides were covered with more or less separated cells. Only macronuclei which just had divided, as judged from their typical appearance, were measured.

Micronuclear DNA contents were measured from either starved cells (longer than 12 h in Dryl's salt medium (Dryl, 1959)) or cells of stationary cultures. Under either condition all micronuclei are in $G_1$ (Doerder & DeBault, 1975; own measurements). After fixation in ethanol/acetic acid (v/v, 3:1, 20 min) the slides were rinsed twice in 95 % ethanol (2 × 10 min), hydrated through a decreasing ethanol series and hydrolysed for 30 min in 5 M HCl for Feulgen staining. Since the macronuclear DNA contents were measured cytofluorometrically, slides for that purpose were stained with a Schiff solution (Kimball, Perdue, Chu & Ortiz, 1971) containing only 0.1 % dye (w/v), whereas the slides from which the micronuclear DNA contents were to be measured by absorption-cytophotometry were stained more heavily with a 0.5 % (w/v)
Amount, proportion and regulation of macronuclear DNA

Dye solution. After staining for 1 h the slides were rinsed in SO\textsubscript{2} water (4 x 5 min) dehydrated in an ethanol series and mounted in Ceadex (Merck).

Macronuclei were measured with a Leitz MPV 2 cytofluorometer (objective 63 x, n.a. 1:30, oil-immersion) using a green filter. The micronuclear DNA content was measured with a Zeiss scanning-absorption-cytophotometer SMP 05 at 550 nm (objective 100 x, n.a. 1:30; diameter of measuring diaphragm, 0.5 \(\mu\)m; width of scanning steps, 0.5 \(\mu\)m). Since the micronuclear values were derived from several experiments they have been normalized and pooled by setting the mean extinction of the co-stained diploid reference group as 1.0 and relating all other values to that. Comparison with co-stained bull sperm allowed in some cases the conversion of measured cytophotometer units into absolute amounts of DNA, by taking the bull sperm mean as \(3.3 \times 10^{-13}\) g of DNA (Leuchtenberger, Leuchtenberger, Vendrely & Vendrely, 1952).

Labelling conditions and biochemical procedures

To determine the rate of RNA synthesis 10 ml of the cell culture were removed and added to 1 ml of labelling medium. In sterile, redistilled water this contained 55 \(\mu\)Ci [\(5\)-\textsuperscript{3}H]uridine (sp. act. 25 Ci/mmole, The Radiochemical Centre, Amersham, U.K.) and 2.75 mg of ‘cold’ uridine. Compared to the growth medium, the concentration of the cold uridine was thus 10 times higher during the labelling period, which assured a constant specific activity of the isotope in all experiments. After 20 min labelling was ended by mixing the suspension with 0.5 ml concentrated perchloric acid (PCA) at 0 \(^\circ\)C.

From this material the RNA and protein contents were estimated according to Munro & Fleck (1966), with some modifications: The cells were harvested in a refrigerated centrifuge (Beckman, J 21, rotor JA 20; 5000 rev/min, 10 min, 4 \(^\circ\)C), washed with 10 ml 0.6 N PCA in the cold and the pellet was hydrolysed (70 \(^\circ\)C, 20 min) by the addition of 1 ml 1 M KOH to \(1.5 \times 10^5\) cells. Adding an equal volume of ice-cold 1.2 N PCA ended hydrolysis. After centrifugation (10000 rev/min, 15 min) the amount of RNA was calculated by converting the absorbance of the supernatant at 260 nm (Beckman spectrophotometer, mod. 25) into pg of RNA by comparison with a standard curve.

The amount of radioactivity incorporated into acid-insoluble RNA was determined by counting 100 \(\mu\)l of this supernatant in a Packard tri-carb liquid scintillation counter in 10 ml Unisolve (Packard).

The pellet was resuspended in 1 M NaOH (1 ml/10\(^6\) cells) overnight at room temperature, and the protein content was determined with the Folin test (Lowry, Rosebrough, Farr & Randall, 1951) calibrated with bovine-serum-albumin as reference.

RESULTS

Nuclear DNA contents

The micronuclear DNA contents were determined to check with a different method the assumed different degrees of micronuclear ploidy, which had previously been assessed by somewhat cumbersome countings of micronuclear chromosomes. With 1.06 pg of DNA the micronuclear DNA content of the diploid strain A II (Table 1) was found to be slightly higher than in previous determinations of \(T.\) thermophila micronuclei (Gibson & Martin, 1971; Seyfert, 1979; Weidenruch & Doerder, 1975; Woodard, Kaneshiro & Gorovsky, 1968). The triploid clone 8d had about 1.5 times as much DNA, as expected. The observed mean of 1.53 does not differ significantly from the expected 1.50 (t-test, \(P > 0.05\)). The haploid clone 4b has approximately half the diploid micronuclear DNA content. The observed mean of 0.56 for the pooled data has been found independently in 3 separate comparisons, one involving
measurements of the micronuclear DNA content of mating cells in a cross between A II and 4b. The difference from the expected 0.50 is significant ($P < 0.001$).

The aneuploids used were all derived by crossing haploid x haploid cells, and were thus 'hypodiploid' (Nanney & Preparata, 1979), i.e. had less than $2n = 10$ chromosomes. Considering their chromosome number they should have a higher micronuclear DNA content than the haploid cells. One clone (112c) has the same DNA content, whereas clone 80a clearly has a lower DNA content. In the latter case the difference is so large that it is beyond any experimental doubt.

Table 1. Micronuclear constitution and DNA contents of the experimental clones

<table>
<thead>
<tr>
<th>Clone designation</th>
<th>Degree of ploidy</th>
<th>Chromosome no.</th>
<th>AU</th>
<th>n</th>
<th>CV</th>
<th>DNA, pg</th>
</tr>
</thead>
<tbody>
<tr>
<td>8d</td>
<td>Triploid</td>
<td>15</td>
<td>1.53</td>
<td>58</td>
<td>8.9</td>
<td>1.62</td>
</tr>
<tr>
<td>All</td>
<td>Diploid</td>
<td>10</td>
<td>1.00</td>
<td>80</td>
<td>8.2</td>
<td>1.06</td>
</tr>
<tr>
<td>4b</td>
<td>Haploid</td>
<td>5</td>
<td>0.56</td>
<td>62</td>
<td>12.6</td>
<td>0.39</td>
</tr>
<tr>
<td>112c</td>
<td>Aneuploid</td>
<td>6 or 7†</td>
<td>0.56</td>
<td>8</td>
<td>13.4</td>
<td>0.39</td>
</tr>
<tr>
<td>8oa</td>
<td>Aneuploid</td>
<td>6 or 7†</td>
<td>0.35</td>
<td>36</td>
<td>10.9</td>
<td>0.37</td>
</tr>
<tr>
<td>57-I-g</td>
<td>Aneuploid</td>
<td>8†</td>
<td></td>
<td>31</td>
<td>5.6</td>
<td>3.32†</td>
</tr>
</tbody>
</table>

Au = Arbitrary units, diploid mean set at 1.00; n = number of specimen; CV = coefficient of variation.
† The chromosome numbers of the aneuploid clones are uncertain due to counting problems in the cytological preparations.
‡ Taken from Leuchtenberger, Leuchtenberger, Vendrely & Vendrely (1952).

The micronuclear DNA contents (Fig. 1, Table 2) are almost identical, ranging between 10 and 12 pg of DNA. Only the macronuclei of the haploids have a little less DNA than the others. This difference, though at the limit of statistical significance, has been found in all experiments. Indeed, experiment 3 (Table 2) was designed only to test this difference: All hand-selected specimens had been placed on one slide to minimize systematic errors. The 9.4% lower DNA content observed in this case agrees well with the mean of 13.5% less DNA found in the macronuclei of the haploid cells in comparison with the rest of the clones if all 3 experiments are taken into account.

Growth parameters and macromolecular composition

To determine growth rates, maximum cell densities, and the RNA and protein contents of the cells, duplicate 100-ml subcultures were inoculated from stationary cultures of each clone. Starting from initial cell densities of 490 ± 20 cells/ml the duplicate subcultures developed so similarly that the 2 subcultures of each clone could only be represented graphically by one line (Fig. 2). Furthermore, the growth rates of the triploid clones were similar, so they have been pooled (solid line, Fig. 2). The values differing the most from the means are represented by short horizontal lines only in those cases in which deviations from the mean were larger than the size of the symbol.

The growth rate of the diploid clones throughout the logarithmic phase of culture
Amount, proportion and regulation of macronuclear DNA

growth was found to be 10% less than that in the triploid and haploid clones (Table 3), but from many comparisons between strain A II and the haploid clone 4b this difference is judged to be insignificant (mean GT of 10 comparisons: GT_{haploid} = 194.7 min, s = 30.3; GT_{diploid} = 187.9 min, s = 25; P > 0.05). The growth rates of 2 aneuploid clones were measured (clones 57-I-g; 80a). They are different in that 57-I-g is entirely similar to the euploid (data not shown), whereas 80a has a clearly reduced growth rate. In addition, the final cell density attained by this clone is lower (420000 cells/ml) than those of the others (570000 cells/ml).

The similarity of the growth curves (except 80a) is matched by that of the histo-

Fig. 1. Distribution of the $G_4$ macronuclear DNA contents. The values of experiments 1 (solid lines) and 2 (broken lines) are shown (cf. Table 2). The means from each experiment are indicated (1, arrow; 2, arrowhead). A, triploid clone 8d; B, diploid strain A II; C, haploid clone 4b; D, aneuploid clones 112c (solid line) and 80a (broken line).
Table 2. DNA content of G, macronuclei

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Clone</th>
<th>AU</th>
<th>n</th>
<th>CV</th>
<th>DNA, pg</th>
<th>AU</th>
<th>n</th>
<th>CV</th>
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<tr>
<td>8d</td>
<td>AU</td>
<td>54.15</td>
<td>25</td>
<td>27.9</td>
<td>11.5</td>
<td>50-56</td>
<td>33</td>
<td>16.1</td>
</tr>
<tr>
<td>All</td>
<td>AU</td>
<td>57.50</td>
<td>28</td>
<td>20.2</td>
<td>12.2</td>
<td>48-55</td>
<td>33</td>
<td>16.1</td>
</tr>
<tr>
<td>4b</td>
<td>AU</td>
<td>47.25</td>
<td>29</td>
<td>17.9</td>
<td>10.0</td>
<td>42-11</td>
<td>35</td>
<td>19.8</td>
</tr>
<tr>
<td>112c</td>
<td>AU</td>
<td>54.2</td>
<td>25</td>
<td>15.6</td>
<td>11.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>80a</td>
<td>AU</td>
<td>15-18</td>
<td>25</td>
<td>5.9</td>
<td>3.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Bull</td>
<td>AU</td>
<td>33</td>
<td>33</td>
<td>35</td>
<td>33</td>
<td>52-52</td>
<td>33</td>
<td>23.8</td>
</tr>
</tbody>
</table>

AU = arbitrary units; n = number of specimen; CV = coefficient of variation.

Fig. 2. Comparison of culture growth in triploid, haploid (+ - - +), diploid (● - - ●) and aneuploid (clone 80a, ■ - - ■) subclones. At time zero 2 subcultures from each clone were inoculated to an initial cell density of 490 ± 20 cells/ml (large cross). The 2 subcultures from each clone have been pooled, as has been done with the triploid and haploid cultures. The maximal deviations from the respective means are indicated by short horizontal lines if they were larger than the size of the symbol. 42 h after inoculation the cell densities of the triploid and diploid cultures were so similar that they were pooled (open circles). At times 1 and 2 (arrowheads) samples were removed to determine the RNA and protein contents as well as the transcription rate (Table 3). The arrow specifies the time at which the cell volume distributions were measured (Fig. 3). Ordinate = logarithm of the cell no./ml.
grams of the distribution of the cell volumes (Fig. 3), measured at the beginning of the stationary phase of culture growth (Fig. 2, arrow): the triploid, diploid and haploid clones are indistinguishable. Once again the duplicate cultures were virtually identical, so that for each clone only one is shown. However, the aneuploid clone 80a might be different from the others. But since these cultures were not completely stationary at the time the volume histograms were taken, they may not be truly comparable.

Fig. 3. Histogram of the cell volume distributions. ●—●, triploid clone 8 d; +--+, diploid strain A11; ○⋯○, haploid clone 4b; ---−−−, aneuploid clone 80a. At the beginning of stationary phase of culture growth (Fig. 2, arrow) cell volume distributions were recorded with a Coulter Counter. The total cell number contributing to each curve equals that from 0.83 ml of culture fluid. Because of their similarities only one subculture from each clone is shown.

The mean RNA and protein contents, as well as the relative amounts of [3H]uridine incorporated during 20-min pulses at the times 1 and 2 (Fig. 2, arrowheads) are displayed in Table 3. Each value shown represents the mean of 4 determinations (2 subcultures from each clone, measured at times 1 and 2).

The protein contents of all cells are very similar. The only statistically significant difference is that the haploid cells have less protein than the triploid (P < 0.05).
Since, however, those have the same protein content as the diploids and aneuploids, and these are not significantly different from the others at the same level of discrimination, the observed difference is within the confidence limits of the method.

Differences in RNA contents are small: of the 4 clones examined 3 (A II, 4b, 80a) have almost identical RNA contents, whereas that of the triploid clone was found to be about 13% higher. Since the only statistically significant difference ($P < 0.05$) exists between the triploid and diploid cells, and the latter have the same RNA content as the other 2 clones (4b and 80a), the extent of this difference, too, is within the methodological confidence limits.

**Table 3. Cell growth parameters**

<table>
<thead>
<tr>
<th>Clone</th>
<th>GT</th>
<th>(A) Values measured (± S.E.M.)</th>
<th>(B) Relative values, related to A II = 1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RNA, µg*</td>
<td>Protein, µg*</td>
</tr>
<tr>
<td>8d</td>
<td>177 ± 1.3</td>
<td>307.7 ± 12.1</td>
<td>344.8 ± 11.2</td>
</tr>
<tr>
<td>A II</td>
<td>203 ± 1.3</td>
<td>271.2 ± 2.4</td>
<td>345.3 ± 38.7</td>
</tr>
<tr>
<td>4b</td>
<td>179 ± 1.3</td>
<td>269 ± 16.5</td>
<td>279 ± 23.3</td>
</tr>
<tr>
<td>80a</td>
<td>256 ± 6.8</td>
<td>276 ± 17.1</td>
<td>339 ± 34.1</td>
</tr>
</tbody>
</table>

(A) Values measured (± S.E.M.)

(B) Relative values, related to A II = 1.0

* Amounts per 10⁶ cells.

In order to describe the transcriptional activity of the cells, the relative amounts of [³H]uridine incorporated into acid-insoluble RNA during 20-min pulses (at times 1 and 2, Fig. 3) were determined and expressed as cpm/10⁶ cells. The values vary more than 2-fold, with the aneuploids having the highest rate of incorporation and the haploids having the least; they incorporated only 54% of the activity of the diploid cells. Except for the pair diploid/triploid the differences are highly significant.

Yet, in the evaluation of those differences one has to consider the following. (i) If the values were strictly comparable, then the RNA of the haploids would have to be at least twice as stable as that of the aneuploid cells, since with a much higher growth rate the cells have almost identical RNA contents, gained with less than half the rate of RNA synthesis. (ii) The cell density in the haploid culture was 4 times higher than in the aneuploid subcultures and 60% higher than in the diploid cultures. Furthermore, it is obvious from Fig. 2 that, unlike the aneuploids, the haploid and triploid clones had already approached the transition phase of culture growth. Since in this stage of culture growth cell cycle parameters become changed (W. O. Reuter, in preparation) with an enrichment of cells with a $G_1$ macronuclear DNA content, as has been observed in starved cultures (Salamone & Pearlman, 1977; Seyfert, 1979), and the rate of RNA synthesis is low in $G_1$ (Cleffmann, 1965; Jauker, Seyfert & Sgonina 1975; Kneiding & Andersen, 1978), the rate of RNA synthesis thus becomes affected to a large extent.

The latter explanation is supported by an additional experiment in which the hap-
loid cells incorporated 27% more [H]uridine within 20 min than the diploid controls. This time both cultures were fully exponential, with cell densities at the time the labelling began of 15,000 (haploids) and 21,000 (diploids) cells/ml. Thus this parameter seems to vary greatly with the cell density, and as indicated by other studies in our laboratory, it varies with the history of any particular culture.

DISCUSSION

The micronuclear DNA content of the diploid strain A II was some 10% higher than in other determinations of the same species (Gibson & Martin, 1971; Seyfert, 1979; Weidenruch & Doerder, 1975; Woodard, Gorovsky & Kaneshiro, 1968; Woodard et al. 1972). Yet, the calculated macronuclear copy ('c') volume of the micronuclear genome is 45, and agrees with previous determinations. The assumed triploid nature of clone 8d is confirmed, since its micronuclear DNA content is 1.5 times higher than in the diploid control. On the other hand the presumptive haploid cells have consistently a 12% higher DNA content than expected. The amount of this difference is in the range of the DNA content of the smallest chromosome (Seyfert, 1979), and suggests that this chromosome may be present twice in the karyotype of this micronucleus. If so, this clone will have to be considered as aneuploid. The problem that the number of chromosomes counted a few weeks after establishing a clone does not agree with the determination of the micronuclear DNA content measured almost 2 years later arises with the aneuploid clone 80a, too. Here we are sure that it has a much lower micronuclear DNA content than expected on the basis of the 6 or 7 chromosomes counted. Conceivably, these discrepancies are related to karyotypic micronuclear instabilities arising during culturing T. thermophila over longer periods. It is known that micronuclei may lose DNA (Nanney, 1957; Nanney & Nagel, 1964), and this phenomenon led to the construction of the T. thermophila 'star' lines (Allen & File, 1967; Allen & Weremink, 1971; Weidenruch & Doerder, 1975).

Nevertheless, the measurements prove that the micronuclear karyotypes of all the clones tested must be different. Thus, the input of DNA into the macronuclear-Anlagen was probably different when the macronuclei of those cells developed. Yet, the macronuclear DNA contents of the mature cells are similar (Fig. 1). This means that the initial differences in the DNA contents have been compensated. At present we do not know when during the development of the mature macronucleus this compensation occurs. If it were during the Anlage stage, then the DNA of the haploid pronucleus – the source of the macronuclear Anlage in the haploid cell – would have to be replicated at least once more than the DNA originally present in the triploid Anlage. Thus, the number of the rounds of replication during macronuclear development would not be fixed; one possible mechanism for establishing the mature macronuclear DNA content would be eliminated.

Alternatively it is possible that the early postzygotic cell with different micronuclear karyotypes may have different macronuclear DNA contents. These could be regulated to a common threshold of vegetative macronuclear DNA content by those
mechanisms regulating macronuclear DNA content during vegetative growth (Cleffmann, 1965; Doerder & DeBault, 1978). The observation in diploid *T. thermophila* cells that the macronuclear DNA content early after macronuclear reorganization (i.e. within 50–100 fissions after conjugation) is different from that of cells of greater clonal age (Doerder, Lief & DeBault, 1977) is compatible with the latter assumption.

With respect to the total macronuclear DNA content the compensation is complete in all clones studied: the means are nearly alike. The significance of slightly lower DNA always observed in the haploid cells is not obvious. In experiments 1 and 3 (Fig. 1; Table 2) the smallest macronuclei of all clones are found in the same size class. Thus a lower mean DNA content is apparently brought about by a different distribution of the individual DNA contents rather than by a changed range of DNA content. The frequency distribution of the macronuclear DNA contents, however, reflects the frequency of regulatory steps. These include the extrusion of small portions of chromatin during almost every macronuclear division (Cleffmann, 1968, 1975), the skipping of S-phases (Doerder & DeBault, 1978), and the rarely encountered extra S-phases. These latter events increase the macronuclear DNA content 4-fold (Cleffmann, 1968, 1975; Cleffmann & Jauker, 1968) during one cell-cycle. Thus the differences between haploid and diploid cells can reflect differences in the thresholds and frequencies of those events.

The paradox that a precisely controlled level of macronuclear DNA content exists in spite of individually tolerated differences larger than 2-fold (Fig. 1; Doerder & DeBault, 1975; Seyfert, 1977) can be resolved by the assumption of relatively fixed upper and lower limits of macronuclear DNA contents (Doerder & DeBault, 1975). Apparently, the macronuclear DNA content is stable under a given growth condition, but varies with environmental changes (Seyfert & Cleffmann, 1979), and can be increased by heat shock treatment and actinomycin D exposure (Cleffmann, 1966, 1975; Jeffrey, Stuart & Frankel, 1970; Jeffrey, Frankel, DeBault & Jenkins, 1973).

The organization of the chromatin within the macronucleus of *Tetrahymena* is unresolved. Several models (see Raikov, 1969, 1976) have been proposed for the organization of this polygenomic macronucleus (Doerder et al. 1977; Nanney, 1964; Orias & Flacks, 1975; Seyfert, 1979; Woodard et al. 1972; Yao & Gorovsky, 1974), but at least the rDNA is an independent molecule (Engberg, Christiansen & Leick, 1974b; Engberg, Anderssen, Leick & Collins, 1976; Gall, 1974; Karrer & Gall, 1976), and since it is under a separate replication control (Engberg, Mowat & Pearlman, 1972; Engberg, Nilsson, Pearlman & Leick, 1974a), it might also be independent of the total macronuclear DNA content. This uncertainty concerning macronuclear organization is a reason not to take the observed equality of the total macronuclear DNA content to mean necessarily that macronuclear gene-dosage has been completely compensated. Since, furthermore, cytophotometric determinations of the macronuclear DNA contents cannot possibly reflect even great differences in the amount of rDNA (due to its small total amount (Engberg & Pearlman, 1972; Marcaud, Protier & Milet, 1976)) we attempted to test some facets of the overall performance of the macronuclei. Thus growth rates, cell volumes, RNA and protein contents (Figs. 2, 3; Table 3) and the transcriptional activity of the cells have been measured. With the
exception of the transcriptional observation, the similarities among the triploid, diploidy, and haploid clones strongly suggest that differences in the original DNA content have not affected the performance of these macronuclei. The observed variations in the transcriptional activity, though a direct measure of the function of the macronuclear chromatin, cannot invalidate this conclusion because of the experimental difficulties apparent in measuring this parameter in a meaningful way.

Yet, the aneuploid clone 80a is clearly different from the others with respect to its growth parameters. Although having virtually the same macronuclear DNA content, these cells have a reduced growth rate and do not reach the same maximal cell density reflecting impairments of these cells in coping with stresses of non-optimal environments. The growth impairment of this clone suggests that the same macronuclear DNA content does not necessarily mean the same functioning of the genetic material.

There are several possibilities why this aneuploid clone 80a may be different. (i) The imbalance of the original input of the number of chromosomes into the macronuclear-Anlage has not been compensated for in the mature macronucleus. The low frequency (20%) of ‘normal’ clones emerging from crosses between haploid and diploid cells (Nanney & Preparata, 1979) may be an expression of the limited re-balancing capabilities of the cells. If such imbalances persisted within the mature macronucleus this would offer important clues concerning macronuclear organization. (ii) The genetic elements are proportionally balanced within the macronucleus of this aneuploid, but one or several mutations covered in all other clones became expressed, reducing the growth rate. This interpretation is favoured since aneuploid ‘hypodiploid’ clone 57-I-g has an entirely normal growth rate. Other aneuploid clones need to be examined before a firm decision is possible.

On balance the results suggest that T. thermophila efficiently compensates for large differences in the micronuclear karyotype and DNA content, as long as the variations are euploid, a similar situation to that described in some wasps (Rasch, Cassidy & King, 1977). Whether or not this ‘quantitative accommodation’ (term proposed by D. L. Nanney, personal communication) of the amounts of the genetic material in the macronucleus results in gene-dosage compensation – i.e. adjustment of unbalanced gene dosage by regulating the amounts or the activities of particular genetic elements, too, cannot at present be decided.

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