RIBOSOMAL RNA GENE CONTENT IN MICRONUCLEATE AND AMICRONUCLEATE STRAINS OF *TETRAHYMENA PYRIFORMIS*

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1. Introduction

Engberg and Pearlman [1] have observed cell growth rate dependent variation in the ribosomal RNA gene content of purified macronuclear DNA isolated from the amicronucleate strain GL of *Tetrahymena pyriformis*. They report that under saturating conditions 25S rRNA hybridizes to 0.23% of the macronuclear DNA of exponentially growing cells and to 0.13% of that of stationary phase cells.

Using different procedures for purification of DNA and for DNA–RNA hybridization, we have observed the same phenomenon in *Tetrahymena pyriformis* GL. Our results, however, reveal a wider range of 25S rRNA gene contents than that reported by Engberg and Pearlman since we find hybridization saturation values for 25S rRNA varying from 0.12% to 0.46% of macronuclear DNA.

On the other hand, we did not observe cell growth rate dependent variation in the ribosomal RNA gene content of purified macronuclear DNA isolated from the micronucleate strain B1868 III (syngen 1). As will be discussed in the last part of this paper this result suggests that in micronucleate strains of *T.pyriformis* the micronucleus may be involved in the regulation of macronuclear DNA replication.

2. Materials and methods

2.1. *Cell culture conditions*

Tetrahymena pyriformis strains GL (amicronucleate) and B 1868 III (micronucleate) [2], kindly provided by R. Charret and D. Orias respectively, were grown axenically at 28°C. Cultures in medium PPY [3] were incubated in shallow layers in toxin flasks (250 ml per 2000 ml flask) with gentle shaking when fast growth was desired (generation time 2½ h) or without shaking for slower growth (generation time about 4 h). Stationary phase cells were harvested from cultures grown in medium PPY diluted 10-fold with 50 mM NaCl, 1 mM $MgCl_2$, 5 mM Na-phosphate pH 6.8 [4].

2.2. *Isolation of macronuclei and purification of DNA*

Macronuclei were prepared from strain GL according to Gorovsky [5]. Preparation of macronuclei from strain B 1868 III by this procedure in which cells are disrupted by mechanical agitation (Waring Blendor) was found to cause extensive loss of their ribosomal RNA gene content. Cells of this strain were therefore broken by suspension in 10 mM Tris-HCl, pH 7.5; 3 mM $CaCl_2$; 1 mM $MgCl_2$; 0.25 M sucrose; 10% (w/v) Nonidet P40, for 3 min at 0°C. As soon as the purification of macronuclei was completed they were suspended in 0.15 M NaCl, 0.1 M EDTA pH 8 and lysed by addition of SDS to a final concentration of 0.5 per cent. One volume of chloroform–isoamyl alcohol (24/1, v/v) was added to the lysate and the mixture was shaken gently for 20 min at 4°C and centrifuged at 3000 *g* for 5 min. The aqueous phase was recovered and treated twice more with an equal volume of chloroform–isoamyl alcohol as before, after which its NaCl concentration was adjusted to 1 M and it was added slowly to an equal volume of 95 per cent ethanol. Fibers of precipitated DNA were collected and dissolved in a small volume of 0.1 SSC and the resulting solution was diluted to an A_{260} nm of 2.0 by addition of 0.15 M Na-phosphate buffer pH 6.8 and adsorbed to a 27 × 2 cm column of hydroxyapatite which had previously been equilibrated with

the same buffer [6]. The column was then eluted at a flow rate of 20 ml/h with a linear concentration gradient generated from 250 ml of 0.15 M and 250 ml of 0.5 M Na-phosphate buffer pH 6.8. RNA and DNA were eluted in two completely separated peaks at buffer concentrations of 0.18 M and 0.27 M respectively. DNA containing fraction were pooled, dialysed against 0.01 SSC, concentrated in the dialysis bag by ventilation at room temperature and the concentrate was stored at −20°C. The DNA so obtained is free of RNA (negative orcinol reaction, no loss of radioactivity when DNA prepared from labelled cells is subjected to alkaline hydrolysis) and does not contain the orcinol reacting impurity previously observed by Allen and Gibson [7].

2.3. *Preparation of radioactive RNA*

Cells were labelled by growing them for six generations in the presence of [^{3}H]uracil (0.1 mCi/ml) in medium PPY diluted ten-fold with 50 mM NaCl, 1 mM $MgCl_2$, 5 mM Na-phosphate buffer pH 6.8 and RNA was extracted as described by Yuyama and Zimmermann [4]. Total RNA prepared in this way had a specific activity of about $5 \cdot 10^5$ cpm per μg.

25S rRNA was isolated by electrophoretic fractionation of total RNA on 2.7 per cent polyacrylamide gels as described elsewhere [8]. After electrophoresis gels were frozen and cut into 2 mm slices and RNA was eluted by incubation of each slice with 1 ml of 0.04 M Tris-acetate pH 7.2 at 60°C overnight. 25S rRNA was precipitated by addition of NaCl (final concentration 0.1 M) and two volumes of ethanol to pooled eluates containing this species and redissolved in 0.01 M Tris pH 7.2.

2.4. *Hybridization*

DNA was denatured by incubation for 1 h at 0°C in 0.5 N NaOH, after which the solution was neutralized with N HCl. Hybridization was carried out by the liquid phase procedure described by Bolle et al. [9]. Membrane filters containing hybridized radioactivity were dried and counted in a liquid scintillation spectrometer.

3. Results and discussion

The figure shows typical curves obtained by hybridizing increasing amounts of 25S rRNA to DNA extracted from macronuclei of rapidly growing (generation time 2 1/2 h), of slowly growing (generation time about 4 h) and of stationary phase cells for the amicronucleate strain GL (A) and for the micronucleate strain B 1868 III (B). The percentage of macronuclear DNA complementary to 25S rRNA is calculated from the amount of DNA used in hybridization mixtures, the known specific activity of [^{3}H] 25S rRNA, and the measured amount of hybridized radioactivity. The numbers of 25S rRNA genes per haploid genome equivalent of macronuclear DNA are

Table 1

Generation time	DNA origin	Percentage of DNA saturation with 25S rRNA	Number of 25S rRNA genes per haploid genome
Stationary phase	GL macronuclei syngen I	0.13	130
	B 1868 III macronuclei	0.17	170
4 h	GL macronuclei syngen I	0.24	240
	B 1868 III macronuclei	0.17	170
2½ h	GL macronuclei syngen I	0.46	460
	B 1868 III macronuclei	0.17	170

calculated according to Yao et al. [10] using 1.3×10^6 as the mol. wt. of 25S rRNA and 1.3×10^{11} daltons as the mass of the *T. pyriformis* haploid genome [10] (see table 1).

While confirming the previous findings of Engberg and Pearlman [1] these results show that the variation in the rRNA gene content of *T. pyriformis* GL macronuclear DNA can be greater than they observed. Under the culture conditions used by these authors the 25S rRNA gene content of macronuclear DNA of exponentially growing cells (generation time: 170 min) is about twice that of the DNA of stationary phase cells (saturation values of 0.13% and 0.23% respectively). Using slightly different culture conditions we find the same 25S rRNA gene content in macronuclear DNA of stationary phase cells but a two-fold higher value in cells growing exponentially with a generation time of 150 min (saturation value of 0.13% and 0.46% respectively). The reasons for this difference are not known but it may be related to the use of different cell growth conditions in the two series of experiments, and perhaps to the different origins of the GL strains. Since the 25S rRNA gene content of GL macronuclear DNA varies as a function of the physiological state of the cell, replication of the DNA containing these genes must be independent of that of bulk macronuclear DNA and a mechanism must exist for the elimination of superfluous copies of these genes when cells enter the stationary phase of growth. The expulsion of nucleoli from the macronucleus of starved or ageing *T. pyriformis* in the form of particles called RNA blebs is in fact well known [13]. In addition two recent reports [14,15] show that the genes for 17S and 25S rRNA are located in a class of extra-chromosomal low mol. wt. DNA molecules in the macronucleus of GL *T. pyriformis*, a finding which is consistent with the autonomous replication of these genes.

In contrast to the results observed with the amicronucleate strain GL we do not find a growth rate dependent variation in the amount of rRNA genes in the macronucleus of the micronucleate strain B1868 III syngen I (see fig.1). Comparison of these two sets of results suggests that the micronucleus may have some influence on macronuclear DNA replication and particularly on rDNA replication and that the variability of the macronuclear content of rRNA genes in amicronucleate strains could be the consequence of the absence of a micronuclear control function. Although at present there is no direct evidence for micronuclear activity during the vegetative growth of *T. pyriformis* it is known that loss of the micronucleus by micronucleate strains is often followed by cell death, a fact which strongly suggests that the micronucleus contributes information in some way to the

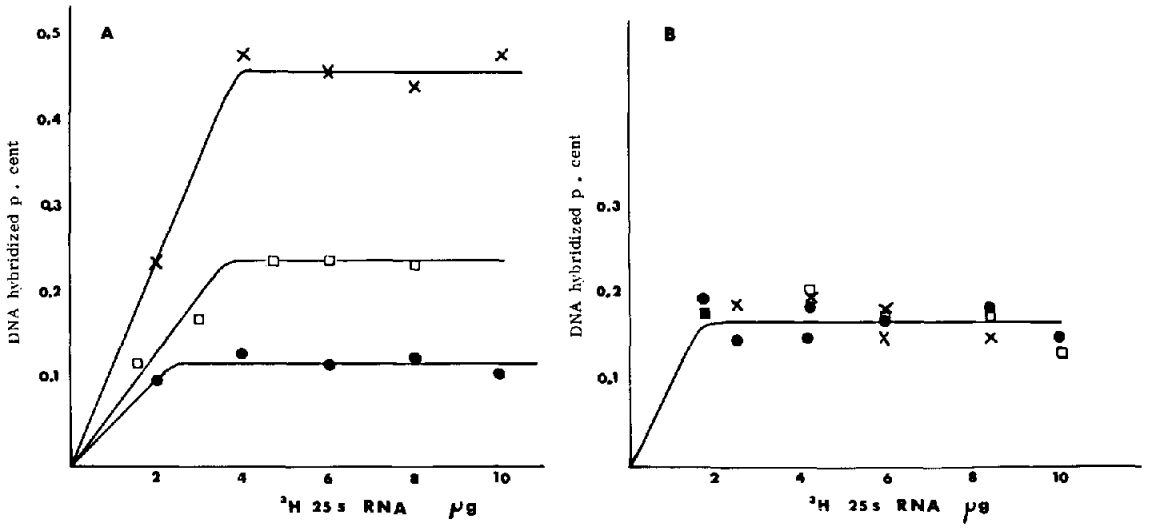

Fig.1. Hybridization saturation curves of ^{3}H-labelled 25S rRNA and macronuclear DNA of *T. pyriformis*. (A): GL macronuclear DNA. (B) Syngen 1 B 1868 III macronuclear DNA. (×–×) 2.5 µg DNA from rapidly growing cells. (□–□) 2.5 µg DNA from slowly growing cells. (●–●) 2.5 µg DNA from stationary phase cells.

cell during asexual growth [16]. Proof that the micronucleus participates in the control of macronuclear ribosomal DNA replication would provide direct evidence for an active role of the micronucleus during vegetative cell growth.

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