PHYSIOLOGICAL CONTROL OF DNA NUCLEOTIDE SEQUENCE REDUNDANCY IN THE EUKARYOTE, TETRAHYMENA PYRIFORMIS

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SUMMARY: The amount of repeated DNA nucleotide sequences present in the macronuclear genome of the ciliated protozoan, Tetrahymena pyriformis was determined for cells displaying several different cell generation times. Depending on the average cell generation time, the fraction of redundant DNA formed at a given C_0t varies over a five-fold range (from 10 - 50%). Two separate C_0t plots can be distinguished: one which yields a $C_0t1/2$ of 400 for the less repeated fraction (SRF) and combines all the DNA preparations derived from cells whose average cell generation times are greater than 24 hours (infradian mode of growth), and one which has a C_0 t1/2 of 100 for the SRF fraction and which represents all the DNA preparations derived from cells dividing at a rate of more than once a day (ultradian mode of growth).

INTRODUCTION: Studies on the reassociation kinetics of DNA derived from eukaryotic organisms reveal the presence of two categories of DNA nucleotide sequences; a rapidly reassociating fraction (ERRF), composed of families of highly reiterated nucleotide sequences, and a slowly reassociating fraction (SRF) which comprises the nucleotide sequences present in only one or a few copies per haploid genome (Britten and Kohne, 1966,1968). It has been postulated that at least some of the redundant families of genes are primarily of a regulatory character (Georgiev, 1969; and Britten and Davidson,1969) or are involved in some ubiquitous housekeeping function in cell growth and division (Melli and Bishop, 1969; McCarthy and Church, 1970; and Davidson and Hough, 1969). DNA/RNA hybridization studies of chromosomal RNA with the Pea genome redundant DNA (Sivolap and Bonner, 1971) and histone messenger RNA with sea urchin redundant DNA (Kedes and Birnstiel, 1970) are compatible with this notion. Molecular hybridization of ribosomal RNA with wild type

and mutant nucleolar DNA stocks also show that there is considerable variation in rRNA cistron redundancy, that this genetic variation is unstable, and subject to dosage compensation (Ritossa and Scala, 1969; and Tartof, 1970). Gene redundancy is also subject to variations arising during the normal course of embryonic development as far as the number of copies of ribosomal RNA cistrons are concerned (Brown and Dawid, 1968).

In this paper, the reassociation kinetics of Tetrahymena DNA were examined for evidence of nucleotide sequence redundancy, and for possible physiological variations in the amount of repeated DNA present over a wide range of cell growth rates.

MATERIALS AND METHODS:

Growth of T. pyriformis: Cultures of the ciliated protozoan, Tetrahymena pyriformis, the amicronucleate strain, W were grown axenically in two different media to obtain the combination of growth rates needed for this study. In the ultradian mode of growth (Ehret and Wille, 1970), a generation time of 3 hours was obtained by exponential log phase growth on enriched proteose peptone as previously described (Szyszko, et al, 1968); and an exponential log phase growth with a 5 hour generation time can be routinely obtained by growth on yeast-medium (2% yeast extract, difco; 1% dextrose, difco; 0.5% NH_4Cl; 0.1% KH_2PO_4; and 0.1% $MgSO_4.7H_2O$; all dissolved in distilled water). 30 hour and 70 hour generation times here referred to as circadian-infradian mode of growth (Ehret and Wille, 1970) were obtained by allowing batch cultures to proceed through the rapid growth phase, and harvest the culture when the average cell generation times desired were attained in enriched peptone medium.

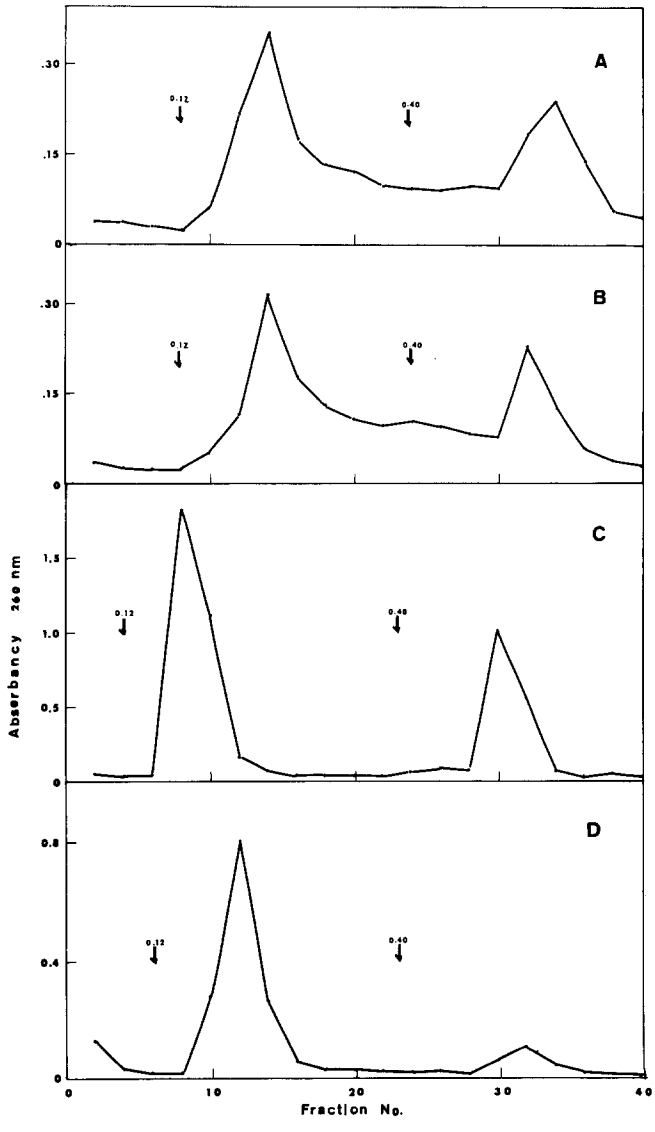
DNA Preparation: Tetrahymena DNA was purified according to the methods previously described (Barnett, Wille, and Ehret, 1971).

DNA reassociation: Tetrahymena DNA was sheared to equal-sized pieces of 750,000 daltons by passing it through a Ribi cell fractionator (Sorvall,

Inc; Norwalk, Conn.) at 50 k psi. Sheared DNA was concentrated when necessary by addition of dry biogel P-100 (Calbiochem, Los Angeles, Cal.), and the concentrate collected by vacuum filtration. DNA was heated at 100°C for 5 minutes and adjusted to be 0.12 M phosphate buffer (pH=7), and incubated at 44°C for different lengths of time. The amount of reassociation which occurred after incubation to a given Cot was measured in two different ways: for low Cots, the DNA solution was transferred to a 1.0 cm stoppered quartz cuvette, immediately after heat denaturation and the change in absorbancy at 260 nm recorded in a Gilford recording spectrophotometer (De Ley, Cattoir, and Reynaerts, 1970); for high Cots, requiring higher initial DNA concentration, i.e., above 2.0 O.D. units, DNA solutions after heat denaturation were incubated in a water bath at 44°C, and fractionated on hydroxylapatite (HA) into single and double-stranded DNA fractions by step-wise elution first with 0.12 M phosphate buffer, and then by 0.4 M phosphate buffer as described by Bernardi (1965). Tetrahymena DNA will not reassociate appreciably at 60°C under these conditions, as expected from its 70% A+T base composition; its TOR (temperature of optimal reassociation) is 20-30° lower than its T_m (De Ley, et al, 1970). Reassociation will occur to a limited extent at 33°C, but not greater than 20% beyond a Cot of 100. Buoyant density determinations of the ERRF and SRF DNA components separated by HA column fractionation were carried out by cesium chloride density gradient centrifugation in the Model E analytical ultracentrifuge. ERRF DNA in all cases had the same buoyant density (ρ=1.692g/cm) as native Tetrahymena pyriformis W DNA, while SRF DNA had a buoyant density like single-stranded DNA (ρ=1.705g/cm).

RESULTS:

Tetrahymena DNA contains both repeated and non-repeated nucleotide sequences which can be separated by hydroxylapatite column fractionation as shown in figure 1. However, the proportion of the repeated DNA found

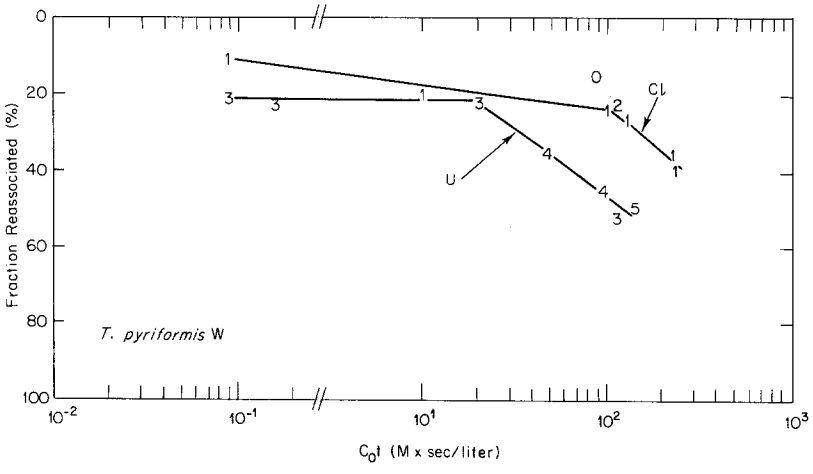


1: Hydroxylapatite column elution profiles for partially reassociated Tetrahymena DNA. In each case denatured DNA was reassociated to a Cot of about 100, and the partially reassociated DNA fractionated by stepwise elution, with first 0.1 M phosphate buffer (indicated by arrows) and then with 0.4 M phosphate buffer. A, DNA from cells with a 3 hr. generation time (GT); B, DNA from cells with a 5 hr. GT; C, DNA from cells with a 30 hr. GT; and D, DNA from cells with a 70 hr GT.

in ERRF fraction (DNA which will reassociate to a Cot of 100) varies directly with the growth rate of the culture from which the DNA was obtained, e.g., with a 3 hour GT (fig. 1A) ERRF is 52% of total, with a 5 hour GT (fig. 1B) ERRF is 40% of total, with a 30 hour GT (fig. 1C)

ERRF is 26% of the total, and with a GT of 70 hours, ERRF is only 17% of total (fig. 1D).

Figure 2 and Table 1 present Cot plots and comparisons of the degrees of sequence redundancy encountered for DNA preparations derived from cultures dividing more than once a day (U), or less than once a day (CI). The U cot plot differs from the CI cot plot in the rapid region of the kinetic plot (Cot less than 0.1) in that it has 20% of the genome with an average sequence reiteration of 4.3×10^3 copies, while CI has only 15% of the genome in 2.6×10^3 copies. There is virtually no intermediate region (cot between 100 and 0.1) in either U or CI Cot plots. The slow region of the CI plot has 79% of the DNA which is present in only one gene copy per haploid genome, while in the U plot of this region those same sequences are now present four-to-five times per haploid genome.



2: Cot plots for Tetrahymena DNA. Two different curves represent the grouping of data on the kinetics of reassociation of different DNA preparations according to whether the cell generation times of the cultures which served as source for the DNA were ultradian (U) or circadian-infradian (CI). DNA preparations 0,1, and 2, came from cells with 70, 30, and 32 hours GTs, respectively; while DNA preparations 3,4, and 5 came from cells with 3,5, and 2.5 hours GTs, respectively.

DISCUSSION:

Previous reports in the literature (Britten and Kohne, 1968; Britten, 1969; and McCarthy and Church, 1970) have dealt with the generality of

TABLE I

Composition of Tetrahymena genome with respect to components of various degrees of sequence redundancy.

Category of DNA	Amount per Genome (g)	Cot1/2	Genome Size relative to E. coli	Cot1/2 if all sequences expected unique	Redundancy (Cot1/2 exp/Cot1/2 found)
CI mode: rapid	3.8×10^{-14}	0.03	10	80	2.6×10^{3}
Intermediate	1.5×10^{-14}	10	4	32	3
Slow	1.97×10^{-13}	400	50	400	1
U mode: rapid	5.0×10^{-14}	0.03	16	128	4.3×10^{3}
Slow	2.0×10^{-13}	100	60	480	4.8

Calculated from data of figure 2, and based on facts, 1) haploid Tetrahymena genome consists of 2.5×10^{-13}g (60 ploid macronucleus, having 1.5×10^{-11}g DNA/cell in CI mode, Scherbaum, 1957), and that the Cot1/2 for reannealing E. coli DNA under these conditions is 8.

repetitious DNA sequences in the genome of most eukaryotes, and the virtual absence of gene redundancy in prokaryotes (Gillis and DeLey, 1970). This paper reports for the first time the presence of repeated DNA sequences in the ciliate macronucleus. As Tetrahymena has been employed extensively in physiological studies of cell growth and development (Prescott, 1970), its possession of repeated DNA sequences in the macronucleus permit and encourage investigations of the significance and role of repeated and non-repeated DNA in cell development and heredity. The results presented above do in fact indicate that a correlation exists between cell generation time and "genomic" redundancy, i.e. rapidly dividing cells possess at least four to five times as many copies of each gene present only once in slowly dividing cells. Moreover, the seemingly arbitrary division of cell growth rates into ultradian (cells dividing more than once a day) and circadian-infradian (cells dividing less than once a day) is shown to be justified by the separation of all kinetics of reassociation data into two clearly discontinuous categories, the ultradian, (U) - Cot plot, and the circadian-infradian, (CI) - Cot plots (see figure 2). The genetic basis of DNA organization in the ciliate macronucleus is at present an enigma, and we can only speculate as to how genomic redundancy might arise. Although polyploidization of identical haploid genomes in the polyenergid macronucleus cannot be ruled out, the complete absence of chromosomes in the vegetative macronucleus argues against it. A more attractive hypothesis, one compatible with the phenomenon of gene amplification (Brown and Dawid, 1968) is that the macronucleus is composed entirely of replicons dissociated from any linear chromosomal backbone, i.e., a replicon "soup", that these units in turn can be not only independently replicated, but independently amplified and that the regulation of rounds of replication is governed by growth rate mediated feedback control. This hypothesis accounts for the recent finding that nucleolar DNA is replicated in the G2 period of the cell cycle in

Tetrahymena (Charret, 1969), and that the number of peripheral nucleoli in the macronucleus undergoes considerable amplification when stationary phase cells are refed (Cameron, Padilla, and Miller, 1966; Nilsson and Leick, 1970). As the ERRF DNA fraction contains all the templates for in vivo transcription of rRNA (Wille, Barnett, and Ehret, 1971), and can be selectively replicated during heat shock treatment of cells in the infradian mode of growth, (Wille, 1971) genomic amplification may be the rule for Tetrahymena DNA replication during the ultradian mode of growth.

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