JOURNAL OF BACTERIOLOGY, Sept. 1978, p. 1156-1157
0021-9193/78/0135-1156$02.00/0

Vol. 135, No. 3
Printed in U.S.A.

Comparative Study of DNA Methylation in Three Unicellular Eucaryotes

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Received for publication 17 April 1978

We have analyzed the nature/content of methylated bases in the nuclear DNA of three unicellular eucaryotes. The pattern of methylation was different for each of the three organisms studied: *Saccharomyces cerevisiae* contained only 5-methylcytosine; *Tetrahymena pyriformis* contained only N^6-methyladenine; and *Chlamydomonas reinhardi* contained both modified bases.

The occurrence of methylated bases in cellular DNA appears to be universal (5, 17). In procaryotes both N^6-methyladenine (MeAde) and 5-methylcytosine (MeCyt) have been observed; in contrast, in plants and higher animals only MeCyt has been detected with certainty. However, in a study of green algae, Pakhomova et al. (13) reported the presence of very low levels of MeAde also (approximately 1 MeAde per 500 Ade residues). The methods used in that study relied on measurement of differences in spectral absorption ratios. Using radioisotope labeling, we demonstrated that the ciliated protozoan, *Tetrahymena pyriformis*, contains MeAde in its macronuclear DNA (1 MeAde per 120 Ade residues), whereas micronuclear DNA has little or no MeAde (6). MeAde has also been found in the DNA of *Paramecium aurelia*, but unlike other eucaryotes *Paramecium* DNA is devoid of MeCyt (4). The DNA of the dinoflagellate, *Peridinium triquetrum*, has also been observed to contain MeAde (14). The present communication describes a comparative analysis of the nature and level of methylated bases in the DNA of several unicellular eucaryotes (a preliminary account of part of these studies has been reported [C. Kenny and S. Hattman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, Abstr. no. K245, p. 227]).

The results of these studies are summarized in Table 1. We repeated the observation that *Tetrahymena* macronuclear DNA contains MeAde. In contrast, *Tetrahymena* DNA appears to lack MeCyt; i.e., there is less than 1 MeCyt per 5,000 Cyt residues (Table 1). This conclusion was confirmed by examining DNA from cells labeled in the presence of [*methyl*-^{3}H]methionine. We observed 49,000 cpm in [^{3}H]MeAde and no detectable radioactivity in MeCyt (data not shown). This pattern of DNA methylation is similar to that of another ciliated protozoan, *P. aurelia* (4); we have also confirmed that MeAde (1.5 mol%) is present in *P. aurelia* macronuclear DNA (data not shown).

It has been reported that yeast DNA lacks modified bases (8, 17). On the contrary, we were able to show that MeCyt is present in the main-band DNA of the haploid strain, D273-10b, of *Saccharomyces cerevisiae* (Table 1); however, there appears to be no MeAde (less than 1 MeAde per 2,000 Ade residues). These conclusions were confirmed by analyzing DNA from yeast labeled in the presence of [*methyl*-^{3}H]methionine. The precise level of MeCyt in yeast main-band DNA was not clearly established; in two independent investigations the average MeCyt composition was 0.3 and 1.0 mol%, respectively. Although the cause for this discrepancy remains undetermined, it is clear that MeCyt is present in yeast main-band DNA. We have not yet investigated methylation in mitochondrial DNA.

As shown in Table 1, both MeCyt and MeAde are present in the nuclear DNA of the green alga, *Chlamydomonas reinhardi*. The level of each modified base was similar for the mt^+ and the mt^- mating type (data not shown). In contrast, chloroplast DNA appears to be devoid of MeAde (<1 MeAde per 2,000 Ade residues); we were unable to obtain a sufficient quantity of radioactively labeled chloroplast DNA to determine the MeCyt content.

Other green algae have been studied with respect to DNA methylation; e.g., the nuclear DNA of *Euglena gracilis* has been shown to contain only MeCyt (1, 2, 15), whereas chloroplast DNA lacks MeCyt. It is interesting to note that chloroplast DNA from higher plants has never been observed to contain MeCyt, even though the nuclear DNA may contain high levels of this base.

The results presented in this paper show that

TABLE 1. *Content of methylated bases in the DNAs of unicellular eucaryotes*[a]

Organism	DNA	mol% MeAde[b]	mol% MeCyt[b]
T. pyriformis	Macronuclear	0.80	<0.02
C. reinhardi	Nuclear	0.50	0.70
C. reinhardi	Chloroplast	<0.05	Not analyzed
S. cerevisiae	Main band	<0.05	0.3–1.0

[a] *T. pyriformis* (B-1868-VII) was grown axenically at 28°C in enriched protease peptone supplemented with [6-^{3}H]uridine or [2-^{3}H]adenosine. The DNA was isolated from purified macronuclei (7) essentially according to the method of Kavenoff and Zimm (11). *C. reinhardi* (*mt*$^+$ and *mt*$^-$ mating types of strain 137c) was grown at 25°C in minimal medium (12) under continuous illumination with cool-white fluorescent light; radioisotope labeling was with [2-^{3}H]adenine or [6-^{3}H]uracil during growth through late log phase. The DNA was isolated from detergent-lysed cells by phenol extraction and ethanol precipitation. After extensive pancreatic and T1 ribonuclease digestion, the DNA was centrifuged to equilibrium in a CsCl gradient. The nuclear and chloroplast DNA peaks (ρ = 1.721 and ρ = 1.691 g/cm^3, respectively) were purified by a second centrifugation. *S. cerevisiae* (haploid strain D273-10B) was grown at 30°C in Difco yeast nitrogen base (without amino acids) containing 0.6% (wt/vol) glucose and 40 μg of streptomycin sulfate per ml. Radioisotope labeling was with [2-^{3}H]adenine or [6-^{3}H]uracil. The DNA was isolated by phenol extraction of lysed spheroplasts prepared essentially as described by Cryer et al. (3). After pancreatic and T1 ribonuclease digestion, nuclear DNA was separated from mitochondrial DNA by centrifugation to equilibrium in a CsCl gradient. We estimate that there was less than 10% mitochondrial DNA contamination of the nuclear DNA preparation. Since the nuclear DNA was not separated from the cytoplasmic 2-μm DNA circles (16), which comprise about 5% of the total DNA, this fraction is referred to as main-band DNA.

The various labeled DNAs were made 0.5 N in NaOH, and any traces of remaining RNA were hydrolyzed at 37°C for 18 h. The acid-precipitable DNA was collected and hydrolyzed in 1 N HCl (for MeAde determination) or 70% perchloric acid; the bases were separated by descending paper chromatography as described earlier (9, 10).

[b] mol% MeAde and mol% MeCyt are calculated on the basis of total Ade and Cyt residues, respectively. The values shown are the averages of analysis of several independent preparations; except for the MeCyt content of yeast, the range was not greater than ±10% of the mean.

each of the three organisms studied has a different pattern of modified bases in DNA. Thus, among other unicellular eucaryotes there is likely to be diversity in DNA methylation similar to that found here. In procaryotes some of the DNA methylation is known to be involved in host-specific restriction/modification; however, in eucaryotes a biological function has not yet been definitively established.

We thank M. Gorovsky for providing the *Tetrahymena* strain, U. Goodenough for providing the *Chlamydomonas* strains, F. Sherman for providing the *Saccharomyces* strain, and A. T. Soldo for *Paramecium aurelia* and procedures for their axenic cultivation.

L.B. was a predoctoral trainee supported by a Public Health Service Training Grant no. GM-07102 from the National Institute of General Medical Sciences. This work was supported by a Public Health Service Grant no. AI-10864 and a Research Career Development Award no. AI-28022 (to S.H.), both from the National Institute of Allergy and Infectious Diseases.

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