Hydroxymethyluracil in Eukaryote DNA: A Natural Feature of the Pyrrophyta (Dinoflagellates)

Abstract. *Analysis of the DNA of several diverse dinoflagellates and other algae has revealed that the presence of the base hydroxymethyluracil (HOMeU) is a feature common among the DNA's of dinoflagellates; this base has not been found in any other group of eukaryotes that has been examined. Among examined members of the dinoflagellates, the ratio of the base pairs HOMeU · A to T · A, where A is adenine and T is thymine, ranges from 0.14 to 2.13. In addition to hydroxymethyluracil, the DNA of one dinoflagellate contains methylcytosine, and that of another contains methyladenine, while the DNA of other dinoflagellates contains no detectable amounts of either of these two bases.*

The DNA of nearly all prokaryotes and eukaryotes consists of deoxyadenylic, thymidylic, deoxyguanylic, and deoxycytidylic acids in amounts equal to or greater than about 95 percent of the total deoxyribonucleotides. DNA from bacteria may contain small amounts of the minor bases 5-methylcytosine or N^6-methyladenine, and the DNA of eukaryotes commonly contains significant amounts of 5-methylcytosine [about 5 to 20 percent of the cytosine (*1*)]. It has also been found that N^6-methyladenine appears in macronuclear DNA from the protozoan *Tetrahymena pyriformis* to the extent of 0.65 to 0.8 percent of the total adenine (*2*).

When DNA from the dinoflagellate *Crypthecodinium cohnii* (= *Gyrodinium cohnii*) was examined in CsCl density gradients and by thermal denaturation, a large discrepancy was found between the guanine plus cytosine (G + C) content determinations made from buoyant density (ρ) and an evaluation of the temperature (T_m) at which 50 percent of the DNA was denatured in a thermal gradient. The disagreement was resolved with the finding that *C. cohnii* DNA contains a considerable amount of the unusual base hydroxymethyluracil (HOMeU) (*3*). This base has the effect of substantially raising the density and lowering the thermal stability of the DNA in which it is contained. In *C. cohnii*, 11 percent of the DNA nucleotides are hydroxymethyldeoxyuridylate (HOMedUMP), so that 37 percent of the expected thymidylate is HOMedUMP. The only other descriptions of the presence of HOMeU in DNA had come from studies on certain bacteriophages that infect *Bacillus subtilis* (*4*).

I have examined the DNA of several diverse dinoflagellates and of representatives of closely and distantly related algal taxa, and have found that my results, together with data from the literature (*1*) indicate that members of the Pyrrophyta (dinoflagellates) comprise the only group of organisms examined to date (exclusive of some *B. subtilis* phages) wherein hydroxymethyluracil is a natural component of DNA. The data also indicate considerable variation among the Pyrrophyta in terms of both the ratios of HOMedUMP to thymidine monophosphate (TMP) in DNA, and the presence of other rare nucleotides (methylcytosine and methyladenine).

A survey of the buoyant density and thermal denaturation properties of DNA's from several dinoflagellates and other algae is presented in Table 1, from which it is evident that the Pyrrophyta provide the only instances where the percentages of G + C as determined from buoyant density and from T_m disagree markedly. DNA's from members of the other algal groups probably contain no more than very small amounts of bases other than the four normally found (adenine, thymine, guanine, and cytosine), since composition determinations from buoyant density and from T_m agree very closely (*5*, *6*). Were there appreciable amounts of 5-methylcytosine, for example, in any of the DNA's, this would have been reflected in a lower apparent G + C content from buoyant density than from T_m, as has been observed for *Xenopus laevis* chromosomal ribosomal DNA (*7*) and for the DNA of plant species (*8*).

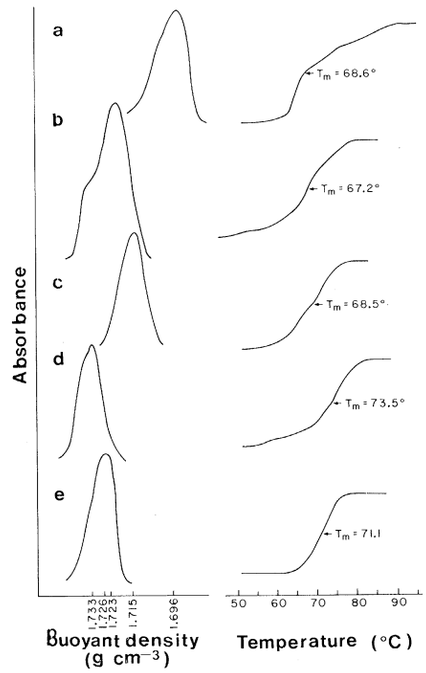

Fig. 1. Buoyant density and thermal denaturation profiles of dinoflagellate DNA's. The conditions are described in Table 1. The DNA's are from (a) *Exuviaella cassubica*; (b) *Amphidinium carterae*; (c) *Crypthecodinium cohnii*: (d) *Peridinium triquetrum*; and (e) *Symbiodinium microadriaticum*. The last organism was isolated by L. Provasoli from the giant clam, *Hippopus hippopus*, with which it has a symbiotic relationship in nature. The other dinoflagellates are free-living.

The conclusion, from a correspondence of buoyant density and T_m, that minor bases are essentially absent from the DNA's of algae exclusive of dinoflagellates depends on the assumption that minor nucleotides with offsetting properties are not present in the DNA's. I will show below that in the dinoflagellate *Exuviaella cassubica* it is possible for a DNA to contain levels of hydroxymethyluracil and 5-methylcytosine such that the effects of these bases on the physical properties of DNA are nearly counteracted one by the other. When nucleotide composition analyses were performed on DNA from the cryptophyte *Chroomonas* sp., the chloromonadophyte *Gonyostomum semen*, and the chrysophyte *Heterosigma akashiwo*, only the four major nucleotides were found, so that in these cases, at least, the CNA's do not contain detectable amounts of two or more antagonistic minor nucleotides.

Among the dinoflagellates, considerable discrepancies are usually noted between the percentages of G + C determined from buoyant density and from T_m (Table 1). In all but one instance (*Exuviaella cassubica*), buoyant densities are much higher, and T_m's are much lower, than could reasonably be expected from consideration of actual nucleotide compositions (Table 2). In all cases, however, chromatography of enzymic hydrolyzates of DNA revealed the presence of HOMedUMP. The nucleotide was identified as such by its position in chromatograms (*3*) and by its ultraviolet absorption spectrum (*4*), and in each instance the molar concentrations of HOMedUMP and thymidine monophosphate essentially equaled those of deoxyadenosine monophosphate (dAMP) in eluates from chromatograms of DNA hydrolyzates (Table 2).

In three of the five dinoflagellates examined, HOMedUMP was present in sufficient quantities to account for buoyant density increases ($\rho_{obs.}$) over values ex-

pected from actual G + C contents ($\rho_{exp.}$) according to the equation established earlier (*3*, *9*).

$$\frac{\rho_{obs.}}{\rho_{exp.}} = 0.0236\left(\frac{\text{HOMedUMP}}{\text{HOMedUMP} + \text{TMP}}\right) + 1 \quad (1)$$

where TMP is thymidine monophosphate. The DNA's from *Exuviaella cassubica* and from *Peridinium triquetrum* are apparent exceptions to this rule. DNA from *E. cassubica* contains substantial amounts of 5-methylcytosine as well as hydroxymethyluracil (Table 2). It may be calculated from data given by Dawid *et al.* (*7*) and by Kirk (*8*) that the effect of the inclusion of 5-methylcytosine (MeC) in DNA on buoyant density is the following

$$\frac{\rho_{obs.}}{\rho_{exp.}} = 1 - 0.0094\left(\frac{\text{MeC}}{\text{MeC} + \text{C}}\right) \quad (2)$$

and a fitting of data from Table 2 with this equation and Eq. 1 shows that *Exuviaella* DNA conforms to the relationship established for the DNA of other dinoflagellates. The buoyant density of DNA which contains a 12 percent substitution of thymine by HOMeU and a 16 percent substitution of cytosine by 5-methylcytosine, and has a G + (C + MeC) content of 36.8 percent, should be 1.698 g cm^{-3}; this is the value found for the midpoint of the buoyant density distribution of *Exuviaella* DNA (*9*).

The DNA of *Peridinium triquetrum* proved to be particularly unusual in that its properties did not fit Eq. 1, although nucleotide composition analyses (*3*) revealed no methyldeoxycytidylate (Table 2). The buoyant density of *P. triquetrum* DNA was found to be lower than that expected from its content of HOMedUMP, suggesting the presence of a methylated base other than 5-methylcytosine in this DNA. Since methyladenine was a likely alternative, chromatograms of *Peridinium* deoxyribonucleotides were developed by means of solvents which would reveal the presence of methyldeoxyadenylate (*10*). Indeed, a nucleotide which cochromatographs with N^6-methyldeoxyadenosine monophosphate from ^{32}P-labeled *Tetrahymena* DNA (*2*) comprises 2 to 3 percent of *P. triquetrum* DNA, and such a feature may account for abberant behavior of the DNA in CsCl density gradients. The antagonistic effects of hydroxymethyluracil and methyladenine on the physical properties of DNA would thus be similar to those of hydroxymethyluracil and methylcytosine (that is, methyladenine reduces the buoyant density of DNA in which it is contained).

Figure 1 illustrates the buoyant density and melting profiles of DNA's extracted from the various dinoflagellates considered in the present study. In an earlier study (*3*), asymmetries in such profiles of *Crypthecodinium cohnii* DNA were noted, and it is evident from Fig. 1 herein that the DNA's of other dinoflagellates exhibit similar asymmetries to greater or lesser extents. With *C. cohnii* DNA, it was shown that the heterogeneity in the buoyant density profile was due in part to a nonrandomness in the distribution of HOMeU and thymine in the DNA, and this may be the case in other dinoflagellates as well (*11*).

These data suggest that dinoflagellates are unique among eukaryotes in their having substantial amounts of hydroxymethyluracil in DNA. In the several diverse dinoflagellates examined, between 12 and 68 percent of the nucleotides complementary to deoxyadenylate in DNA are HOMedUMP, while DNA from representatives of the other algal groups gives no indication of the presence of nucleotides other than the common four (that is, DNA's from the algae other than

Table 1. Properties of DNA's from various dinoflagellates and other algae. Algal cells were grown axenically in 1-liter cultures. For the preparation of DNA from algae other than dinoflagellates, cells were pelleted, then lysed during suspension in 0.1*M* EDTA, 0.05*M* tris (*p*H 8), and 2 percent Sarkosyl. After being shaken, the lysate was combined with concentrated CsCl to make a solution having a density of about 1.70 g cm^{-3}. This was centrifuged in a Beckman 60Ti rotor for 20 to 40 hours at 40,000 rev/min and 22°C. Fractions were then collected, and those containing DNA were pooled. DNA was precipitated by the addition of three volumes of 70 percent ethanol, or the pool was diluted and DNA was pelleted by ultracentrifugation. The DNA was then dissolved in one-tenth strength saline sodium citrate (0.1 × SSC) and the solution was either changed to full strength and precipitated with ethanol or was repelleted in 0.1 × SSC. Because dinoflagellates are more difficult to disrupt, DNA was prepared from these organisms either by the method described earlier (*3*), or in the following way: Cells were collected, resuspended in the EDTA-tris solution, then frozen at −40°C. The suspension was then thawed to 4°C, and cells were disrupted in a French press at 5000 p.s.i. (340 atmospheres). The homogenate was made 2 percent with respect to Sarkosyl, then DNA was prepared as described for other algae. For the collection of the buoyant density data, samples of DNA in 0.1 × SSC were centrifuged in CsCl along with *Micrococcus lysodeikticus* DNA (ρ = 1.731 g cm^{-3}) or *Escherichia coli* DNA (ρ = 1.710 g cm^{-3}) in the Beckman model E analytical ultracentrifuge. To obtain the T_m data, samples were melted along with reference DNA from *E. coli* [T_m = 75.2°C in 0.1 × SSC (*6*)] in a Gilford 2000 recording spectrophotometer or a 240 with a 2527 thermoprogrammer, reference compensator, and recorder.

Species	Buoyant density*	T_m (°C)*	Actual % (G + C)†	Ratio of HOMedUMP to HOMedUMP + TMP‡
Rhodophyta				
Porphyridium aerugineum	1.707 (48.0)	73.5 (47.9)	48	
Cryptophyta				
Chroomonas sp.	1.719 (60.2)	78.9 (61.0)	61	0
Rhodomonas lens	1.718 (59.2)	78.1 (59.0)	59	
Pyrrophyta				
Exuviaella cassubica	1.696 (37.7)	68.6 (35.9)	36.8	0.12
Amphidinium carterae	1.723 (64.3)	67.2 (32.5)	39.8	0.62
Crypthecodinium cohnii	1.715 (56.1)	68.5 (35.6)	41.3	0.37
Peridinium triquetrum	1.733 (74.5)	73.5 (47.8)	52.7	0.68
Symbiodinium microadriaticum	1.726 (67.7)	71.1 (42.0)	46.2	0.53
Chloromonadophyta				
Gonyostomum semen	1.694 (34.7)	68.2 (34.9)	35	0
Vacuolaria virescens	1.693 (33.7)	67.9 (34.2)	34	
Bacillariophyta				
Cyclotella nana (strain 3H)	1.705 (45.9)	72.6 (45.6)	46	
Chrysophyta				
Heterosigma akashiwo	1.703 (43.9)	71.9 (43.9)	44	0
Euglenophyta				
Euglena gracilis (strain Z)§	1.708 (49.0)	89 to 91 (50.5)	50	
Chlorophyta				
Chlamydomonas reinhardtii	1.723 (64.3)	80.2 (64.2)	64	

*Values in parentheses are G + C contents determined according to formulas given by Mandel *et al.* (*5*) for buoyant density, and by Mandel and Marmur for T_m (*6*). Buoyant density values are those of the peaks of profiles; T_m values were determined in 0.1 × SSC, and the values are temperatures at the midpoints of hyperchromic shifts (T_m). †In the cases of the Pyrrophyta (dinoflagellates), the actual percentages of G + C are taken from data in Table 2; otherwise, values from buoyant density and from T_m are averaged to the nearest percentage. ‡In the cases of the Pyrrophyta, ratios were obtained from data in Table 2. Where no result is given, the absence of HOMedUMP is presumed on the basis of an agreement between G + C content determinations from buoyant density and from T_m. A zero signifies the absence of detectable amounts of HOMedUMP in chromatograms, as well as an agreement between buoyant density and T_m. §The data for *E. gracilis* is taken from Brawerman and Eisenstadt (*19*). For their melting experiments they used 1 × SSC as a solvent. Ray and Hanawalt (*20*) reported that *E. gracilis* nuclear DNA contains 2.3 percent 5-methylcytosine.

Table 2. Nucleotide compositions of DNA's from various dinoflagellates. dAMP, deoxyadenosine monophosphate; dGMP, deoxyguanosine monophosphate; dCMP, deoxycytidine monophosphate; MedCMP, methyldeoxycytidine monophosphate. Classification follows the scheme of Loeblich (*21*). Composition values are the averages of three or more determinations, which were performed essentially as described earlier (*3*).

Species	Nucleotide composition (%)					
	dAMP	TMP	HOMedUMP	dGMP	dCMP	MedCMP*
Prorocentrales						
Prorocentraceae						
Exuviaella cassubica	31.7	27.7	3.8	18.6	15.3	2.9
Gymnodiniales						
Gymnodiniaceae						
Amphidinium carterae	29.6	11.8	18.9	20.7	19.1	ND
Peridiniales						
Crypthecodiniaceae						
Crypthecodinium cohnii	29.6	18.1	11.1	20.9	20.4	< 0.75
Peridiniaceae						
Peridinium triquetrum	24.1†	7.4	16.4	25.7	27.0	ND
Zooxanthellales						
Zooxanthellaceae						
Symbiodinium microadriaticum	26.9	12.7	14.2	21.9	24.3	ND

*The absence of detectable amounts (ND, not detected) of this nucleotide is consistent with the fact that the buoyant density of the DNA is that expected from the G + C + HOMeU content (see text for discussion of *E. cassubica* DNA). †This value includes methyldeoxyadenylate, which is present in DNA of this species to the extent of about 2 to 3 percent of the total nucleotides.

dinoflagellates have buoyant densities and T_m values which both reflect a particular G + C content; Table 1). The equivalence of the percentage of G + C determinations from buoyant density and T_m for DNA from the algae other than dinoflagellates indicates that no more than very small amounts of a minor base such as methylcytosine, methyladenine, or hydroxymethyluracil are present in the DNA's. Similarly, an examination of the data compiled by Shapiro (*1*) on G + C content determinations from buoyant densities and T_m values, and on base compositions of DNA's from a great variety of prokaryotes and eukaryotes, permits the conclusion that detectable amounts of hydroxymethyluracil are absent from the DNA's of the organisms included in his survey.

Thus, the only known natural occurrence of hydroxymethyluracil in DNA other than that of dinoflagellates is in the DNA of certain bacteriophages that infect *Bacillus subtilis* (*4*). Dinoflagellate chromosomes are different from those of eukaryotes in several other respects: There may be up to 100 or more chromosomes in a nucleus, and each has the appearance of an ellipsoid containing a regularly coiled arrangement of DNA-containing fibrils (*12*). Histones have not been detected in dinoflagellate nuclei, although there is a report that a basic protein can be extracted from dinoflagellate "chromatin" in a mass ratio to DNA of about 0.1 (*13*). It has frequently been concluded from such studies on the structure and gross composition of dinoflagellate chromosomes that they are vestiges of a primeval transition from a prokaryote organization of genetic material to the eukaryote organization. Supporting this contention are observations on the prokaryote-like mechanism of chromosome segregation during division (*14*), and on the unusual and presumably primitive structure and function of the nuclear envelope in *Noctiluca* (*15*). The organization of DNA sequences in dinoflagellate chromosomes is clearly eukaryotic, however, in that (i) "satellite" DNA, amounting to about 10 percent of the total, is present in the DNA of *Crypthecodinium cohnii* (*3*) and (ii), a large fraction of the DNA of this species is comprised of "repeated" sequences, many of which are interspersed among sequences represented once or a few times in the genome (*16*).

Data given here on the presence of hydroxymethyluracil in dinoflagellate DNA have no clear bearing on the question of the primitiveness of dinoflagellate nuclei. A correlation may be drawn between the presence of this unusual base in DNA and the appearance and composition of dinoflagellate chromosomes, but this is likely to be fortuitous since there is a great variation among dinoflagellate species in HOMeU content but not in chromosome fine structure (*17*). The nonrandomness of the distributions of HOMeU and thymine in *Crypthecodinium* DNA and the interspecific variability suggest that the presence of HOMeU is the consequence of modification of thymine in specific short sequences in DNA, as is the case with the methylation of cytosine in the DNA of other eukaryotes (*18*).

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References and Notes

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9. Since buoyant density profiles of dinoflagellate DNA's are asymmetric, peak values, as given in Table 1, are not necessarily reflections of average G + C contents. Thus, for the purpose of comparison with chemically determined nucleotide compositions, the mean densities of the various dinoflagellate DNA's were determined through integration of the ultraviolet absorption profiles illustrated in Fig. 1. The mean buoyant densities of the DNA's are: *E. cassubica*, 1.698; *A. carterae*, 1.724; *C. cohnii*, 1.715; *P. triquetrum*, 1.732; and *S. microadriaticum*, 1.729 g cm^{-3}. It may be noted that Franker [C. Franker, *J. Phycol.* **6**, 299 (1970)] detected a small amount of methylcytosine in the DNA of a zooxanthella separated from the sea anemone *Anthopleura elegantissima*.
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22. Supported in part by NSF (GB-43821). *Porphyridium aerugineum* was obtained from J. Ramus (Yale University); *Gonyostomum semen* and *Vacuolaria virescens* were provided by R. Guillard (Woods Hole Oceanographic Institute); and DNA from *Chlamydomonas reinhardtii* was a gift from J.-D. Rochaix. Starter cultures of other algae and the dinoflagellates were provided by I. J. Pintner and L. Provasoli (Haskins Laboratories). I thank D. Babbitt for technical assistance, T. Barnett and R. Steele for discussions, and J. Gall, A. Loeblich III, L. Provasoli, and D. Söll for critically reading the manuscript. In a recent abstract, L. A. Loeblich, V. E. Clark, L. C. Klotz, and A. R. Loeblich III [*J. Protozool.* **23(2)**, 19A (1976)] reported that the buoyant density and T_m of DNA from the freshwater dinoflagellate, *Gymnodinium* sp., disagree in such a way that it is likely the DNA contains a significant amount of hydroxymethyluracil.

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