transferase based on the *meso*-DAP decarboxylase reaction is currently in progress.

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Nuclear Deoxyribonucleic Acid Characterization of the Marine Chromophyte *Olisthodiscus luteus*†

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ABSTRACT: Nuclear DNA of the marine chromophytic alga *Olisthodiscus luteus* was analyzed in this study. Reassociation kinetics analysis has shown that 440-nucleotide DNA fragments from the genome of this alga contain 4% foldback, 58% repetitive, and 34% single-copy sequences. Precise analysis using isolated single-copy DNA revealed that *Olisthodiscus* has a large haploid DNA content of 1.66×10^{-12} g/cell. For determination of the organization of single-copy and repetitive sequences within this genome, DNA fragments 3000 nucleotides in length were reassociated to C_0t = 100 M·s. At this low C_0t value 89% of the DNA bound to hydroxylapatite, suggesting that single-copy and repetitive elements are interspersed. The lengths of the duplexed repetitive DNA on these 3000-nucleotide fragments were measured by electron microscopy after digestion with S1 nuclease which removed the unreassociated single-copy DNA regions. Of these repetitive sequences, 68% were shorter than 1200 nucleotide pairs in length and had a modal length of 350 nucleotide pairs. A minor class of longer (to 4000 nucleotide pairs) repetitive sequences was also observed.

The Chromophyta represent a major evolutionary group of plants which have only minimal representation in the vast literature concerning eukaryotic nuclear morphology and biochemistry. Members of this taxonomic sequence include the Dinophyceae (dinoflagellates), Bacillariophyceae (diatoms), Chrysophyceae, and Chloromonadophyceae and culminate in the Phaeophyceae (brown algae including the kelps). The Chromophyta (chlorophyll *a,c* line) are thought to have split or to have arisen independently early in evolutionary time (Taylor, 1978) from those plants designated the Chlorophyta (chlorophyll *a,b* line). Unlike the Chromophyta, which only include algal species, the Chlorophyta include algae, ferns, and

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higher land plants.

Two nucleomorphic types are evident among the limited chromophytic taxa studies to date. The first type is represented by the Dinophyceae. Cytological observations have demonstrated (Dodge, 1973) that the chromosomes of these algae remain in a permanently condensed state throughout the cell cycle. For example, the distance (Oakley & Dodge, 1979) between the "spirals" or "penny stacks" of chromatin within an *Amphidinium* chromosome is 82.2 and 95.6 nm when measured in the anaphase and interphase, respectively. On division, the dinophycean nuclear envelope becomes ramified by tunnels (Oakley & Dodge, 1979) containing microtubules. Although chromosomes associate with these tunnel structures, no discrete spindle apparatus is ever formed. DNA of the dinophycean chromosome occurs (Rizzo & Burghardt, 1980) as non-histone-associated smooth fibrils which are 6.5 nm in diameter. The limited complement of basic proteins and the lack (Rizzo & Burghardt, 1980) of nucleosomal subunits seen in the nuclei of these algae are reminiscent of prokaryotic DNA structure, thus placing the dinoflagellates in a "mesokaryotic" position. The fact that as much as 68% of the thymidine in the DNA of dinoflagellates is replaced with 5-(hydroxymethyl)uracil (Rae, 1976) is sufficient to distinctly segregate these organisms from other eukaryotic cell types.

All other algal classes of the chromophytic algal line display a significantly different nuclear morphology than that seen in the dinoflagellates. Dispersed chromatin within the interphase cell condenses (Dodge, 1966) only at mitosis, and the distribution of chromosomes during cell division is associated with the formation of a recognizable spindle apparatus (Slankis & Gibbs, 1972). The nuclear envelope of these algae often (but not universally) disperses during division. Work done with *Olisthodiscus luteus*, the only chromophyte of this nucleomorphic type studied to date with respect to chromatin structure, has shown (Rizzo & Burghardt, 1980) that nuclear DNA is combined with histone protein. The histones of *Olisthodiscus* which comigrate with calf thymus histones H2a, H2b, H3, and H4 are complexed with DNA to form regularly repeating nucleosome particles of 9.1–11.2-nm size. These observations suggest that, despite the isolated taxonomic position of the Chromophyta, these organisms with the exception of the dinoflagellates possess a nuclear structure similar to that seen in most animal and chlorophytic plant cells.

To date, nuclear DNA sequence organization of many chlorophytes (Walbot & Goldberg, 1979) but only one chromophyte has been examined. The genome of the dinoflagellate *Crypthecodinium cohnii* is composed of 63% repeated and 37% unique DNA sequences which are arranged (Hinnebusch et al., 1980) in a mixed "short"- and "long"-period interspersion (Davidson et al., 1974) pattern.

In an effort to gain further insight on the patterns of genome organization within the chromophytic plant line, the marine alga *Olisthodiscus luteus* was analyzed. This study represents the first data on DNA sequence organization of a chromophytic plant which displays a typical eukaryotic nuclear morphology.

Materials and Methods

Growth and in Vivo Radiolabeling of Cells. Olisthodiscus luteus (Carter) was grown in an artificial sea-water medium on a 12-h light/12-h dark cycle at 20 °C. Details of culture maintenance have been presented elsewhere (Cattolico et al., 1976; McIntosh & Cattolico, 1978). All cultures were stringently monitored for both bacterial and fungal contamination (Cattolico, 1978a). Cells were sampled during the linear growth phase at hour 6 of the synchronous growth cycle. *Olisthodiscus* cells were labeled for 7 days by adding [^{3}H]-adenosine (31 Ci/mmol) to the culture medium at a final concentration of 1.23 μCi/mL while cells were in the logarithmic growth phase.

Escherichia coli, strain MRE 600, was grown at 37 °C on a glucose-supplemented medium (Mahler, 1967). Cells were labeled for 2 h during the logarithmic growth phase with 0.11 μCi/mL [^{14}C]thymidine-supplemented (55.7 mCi/mmol) liquid medium.

DNA Extraction. Pelleted *Olisthodiscus* cells were lysed in pH 7.0 SSC buffer (150 mM NaCl and 15 mM trisodium citrate) containing 2.5% *N*-lauroylsarcosine. The lysate was phenol extracted as described by Cattolico (1978b). For removal of contaminating polysaccharides, the DNA was dissolved at a concentration of 100 μg/mL in a pH 8.0 "storage buffer" containing 1 mM Tris[1] and 1 mM EDTA. The solution was brought to 1.0 M NaCl concentration, followed by the addition of cetyltrimethylammonium bromide (CTAB) to a final concentration of 1.0% (Darby et al., 1970). The precipitate formed at this stage was removed by centrifugation at 15000*g*. The supernatant was diluted to 400 mM NaCl, and the DNA which precipitated from this solution was caught on a glass rod. The DNA was then washed twice with fresh 400 mM NaCl. After solubilization of the DNA in 1.0 M NaCl, the solution was adjusted to 400 mM NaCl concentration, and an equal volume of chloroform–isoamyl alcohol (24:1) was added. The aqueous phase was recovered by centrifugation at 10500*g*. This extraction procedure was repeated until the interphase disappeared. The solution was desalted by dialysis against storage buffer.

Alternatively, DNA was isolated from lysed cells by the Hoechst dye 33258–CsCl gradient method developed by Aldrich et al. (1981). Nuclear DNA was separated from the chloroplast component by fractionation of the CsCl–dye gradients. The Hoechst dye was removed from the DNA by extraction with CsCl-saturated isopropyl alcohol. This DNA was further purified by treatment with RNase, followed by CTAB precipitation as described above.

Tritium-labeled nuclear DNA from *Olisthodiscus* cells labeled in vivo was extracted by the Hoechst dye–CsCl gradient procedure. The specific activity of this DNA was determined to be 5×10^3 cpm/μg.

DNA ^{14}C-labeled in vivo from *E. coli* was extracted by using the method of Marmur (1961). The specific activity of the DNA was determined to be 1.7×10^3 cpm/μg.

The purity of the DNA preparations obtained by these isolation methods was determined by a number of criteria including UV spectral quality, microfluorometric assay (Cattolico, 1978b), and thermal melt hyperchromicity in 2.4 M tetraethylammonium chloride (Murray & Thompson, 1977).

Double-stranded SV40 DNA was a gift from G. Lamppa (University of Washington).

Tracer DNA Preparation. Tritium-labeled *Olisthodiscus* DNA was prepared (Maniatis et al., 1975) by nick translation at 15 °C with [^{3}H]thymidine 5′-triphosphate (dTTP) (55.1 Ci/mmol) as the labeled nucleotide. "Short tracer" DNA was

[1] Abbreviations used: nt, nucleotide(s); ntp, nucleotide pair(s); Tris, tris(hydroxymethyl)aminomethane; EDTA, (ethylenedinitrilo)tetraacetic acid; NaP–NaDodSO$_4$, sodium phosphate buffer (pH 6.8) containing 0.1% sodium dodecyl sulfate; T_m, temperature at which half the DNA has been thermally denatured (the melting point of DNA); HAP, hydroxylapatite; C_0t, product of molar concentration of DNA nucleotides and time of incubation (M·s); K, the observed second-order reassociation rate constant ($M^{-1}\cdot s^{-1}$); Pipes, 1,4-piperazinediethanesulfonic acid.

obtained by labeling whole-cell DNA for 60 min in the presence of 0.005 μg/mL DNase I (Worthington). This treatment resulted in DNA of approximately 1200 ntp in length. The DNA was then sonicated (see below) to give a final 440-nt length. "Long tracer" DNA was prepared by labeling unsheared nuclear DNA for 15 min without added DNase. DNA fragments of 3000-nt weight-average length were obtained by this preparation method. We attribute this final fragment size to be a result of very low levels of contaminating DNase in the nick-translation mixture. These fragments were suitable without further treatment for long tracer studies.

Shearing DNA. "Short DNA fragments": The DNA was dissolved in a pH 8.0 buffer containing 100 mM EDTA, 10 mM Tris, and 200 mM NaCl. Samples of 2.0-mL volume were sonicated (Lamppa & Bendich, 1979) with a Braunsonic 1510 apparatus. The resulting DNA fragments were sized by alkaline agarose gel electrophoresis (McDonnell et al., 1979), and the gels were fluorographed (Chamberlain, 1979) for visualizing radioactive DNA bands. Sonication produced fragments with a weight-average single-stranded length of 440 nt. The calculations of all weight-average single-stranded DNA fragment lengths were performed as detailed by Hinnebusch et al. (1978). *"Long DNA fragments"*: Samples (1 mL) containing 100–200 μg of unlabeled nuclear DNA dissolved in 200 mM sodium acetate buffer (pH 6.0) were sheared for 60 min at 0 °C in a Virtis 45 homogenizer equipped with a microattachment. An empirically determined power setting was used to produce DNA fragments with a weight-average single-stranded length of 3000 nt.

DNA Reassociation. "Tracer" *Olisthodiscus* DNA was mixed with unsheared cold "driver" *Olisthodiscus* DNA and sonicated. For removal of polyvalent cations, the DNA mixture was passed over a 200 mM sodium acetate (pH 6.0) equilibrated Chelex 100 (Bio-Rad Laboratories) column. The tracer–driver mixture was concentrated by ethanol precipitation. Reassociations were performed in a Chelex-treated buffer containing 1.0 M sodium perchlorate, 0.1 mM EDTA, and 30 mM Tris (pH 8.0). Concentrations of DNA in the reaction mixtures (see figure legends) were determined by microfluorometric analysis (Kapp et al., 1974). Samples (10 μg) of the *Olisthodiscus* tracer–driver mixture plus 1 μg of sonicated ^{14}C-labeled *E. coli* internal standard DNA were incubated in sealed capillary pipets (total volume 1–100 μL). Alternatively, the samples were prepared in 1-mL aliquots of 140 mM NaP–NaDodSO$_4$ buffer, placed in small glass vials, and overlaid with mineral oil for incubation. The reassociation incubation temperature (T_m = 25 °C for *Olisthodiscus* DNA) was dependent on the buffer system used: that is, 64.5 °C for sodium perchlorate–EDTA–Tris and 63 °C for 140 mM NaP–NaDodSO$_4$.

For determination of the percent single-stranded vs. double-stranded DNA present in each C_0t point, the samples were fractionated on HAP microcolumns (Bendich & Anderson, 1977; Lamppa & Bendich, 1979). Radioactivity in column eluents was determined as described by Bendich & Anderson (1977). Count recoveries were routinely 95–105%. The data were plotted in C_0t curve forms and analyzed in terms of ideal second-order reassociation components by using the nonlinear least-squares program developed by Britten et al. (1974), supplied to us by Dr. R. B. Goldberg, UCLA. The program was run on a CDC 6400 computer at the University of Washington.

All C_0t values reported in this paper have been corrected to the equivalent C_0t values obtained in 180 mM Na^+ at 60 °C (Britten et al., 1974). Reassociations were accelerated by a factor of 10.0 in 1.0 M sodium perchlorate–EDTA–Tris buffer as determined for *E. coli* DNA at T_m = 25 °C.

Preparation of Single-Copy DNA. Sonicated *Olisthodiscus* nuclear DNA was HAP fractionated at equivalent C_0t = 1000 M·s, and the single-stranded DNA was collected (Britten et al., 1974). The single strands were Chelex treated, incubated to equivalent C_0t = 30000 M·s, and HAP fractionated. The double strands collected were tritium labeled in vitro (Galau et al., 1976) with *E. coli* DNA polymerase I (Worthington). Foldback sequences were removed from the labeled DNA by HAP fractionating at equivalent $C_0t = 1.1 \times 10^{-5}$ M·s. The resulting single-stranded material (3.4×10^5 cpm/μg of DNA) which had a weight-average length of 380 nt was mixed with a 10000-fold excess of sonicated nuclear driver DNA and a trace amount of sonicated ^{14}C-labeled *E. coli* DNA. This mixture was subjected to reassociation analysis as described above.

S1 Nuclease Digestion. Unlabeled samples of 3000-nt nuclear DNA fragments, resuspended in a pH 6.7 buffer containing 300 mM NaCl and 10 mM Pipes, were reassociated in sealed capillaries at 69 °C (T_m = 25 °C) to equivalent C_0t = 100 M·s. The samples were treated directly with S1 nuclease under the mild digestion conditions defined by Britten et al. (1976). S1 nuclease resistant DNA was collected by HAP fractionation at 60 °C. A level of 3 units of S1 nuclease/μg of DNA was selected from a series of digestions performed with increasing S1:DNA ratios. Under our conditions, 3 units of S1 nuclease (Bethesda Research Laboratories)/μg of DNA was the minimum amount of enzyme needed to degrade denatured DNA to oligonucleotides which were shorter than 40 nt in length. Native DNA retained full size and thermal hyperchromicity under these digestion conditions.

Electron Microscopy. S1 nuclease resistant *Olisthodicus* nuclear DNA and double-stranded SV40 DNA were prepared for electron microscopy by using the formamide spreading technique (Davis et al., 1975). Grids were viewed in a Philips 201 electron microscope. *Olisthodiscus* DNA fragments and SV40 DNA contour lengths were measured with a Numonics digital graphics calculator from projections of negatives (final magnification 117000×).

Results

Reassociation Kinetics of Whole Cell Olisthodiscus DNA. The reassociation kinetics of 440 nt length total cellular DNA of *Olisthodiscus* were examined by using ^{3}H-labeled whole-cell DNA tracer prepared by nick translation (Maniatis et al., 1975) mixed with a 10000-fold excess of unlabeled driver DNA. The data obtained from two experiments were computer analyzed (Britten et al., 1974) for the best nonlinear least-squares fit, assuming two second-order kinetic components. Results of this experiment are presented in Figure 1. A zero-time binding fraction, assayed with ^{3}H-labeled DNA labeled in vivo at $C_0t = 6 \times 10^{-6}$ M·s, represented 4.0% of the total reassociation reaction. This fraction is characteristically seen in eukaryotic nuclear DNA samples and probably represents (Schmid & Deininger, 1975) intramolecular hybridization of inverted repeat (palindromic) sequences.

The predominant component observed in the reassociation of *Olisthodiscus* DNA occurs with a second-order rate constant of 0.172 $M^{-1}\cdot s^{-1}$ or a $C_0t_{1/2}$ of 5.81 M·s and represents 58% of the total reassociation reaction. This kinetic class of "middle repetitive sequences" (Holland & Skinner, 1977) occurs with an average reiteration frequency of 338 per haploid *Olisthodiscus* genome. The reassociation of this component

Table I: Summary of *Olisthodiscus* DNA Reassociation Kinetics Experiments

reassociation curve	component	fraction of reassociation	K^a	$C_0t_{1/2}{}^a$
440-nt tracer	foldback[b]	0.04		
	repetitive	0.58	0.172	5.81
	single copy	0.34	0.000 443	2257
380-nt single-copy tracer	repetitive[c]	0.05		
	single copy	0.83	0.000 651[d]	1536
3000-nt tracer HAP	foldback[b]	0.14		
	repetitive	0.75	0.639	1.56
	single copy[e]	0.07		
3000-nt tracer S1 nuclease	foldback	0.02		
	repetitive	0.48	0.639	4.20

[a] K is the second-order rate constant observed for the component in the whole reassociation reaction ($M^{-1}\cdot s^{-1}$). $C_0t_{1/2} = 1/K$ in the HAP assay reactions (M·s). The S1 nuclease reaction $C_0t_{1/2}$ was taken from Figure 3 as the point where one-half of the renaturation component shown attained resistance to digestion. [b] Zero-time binding of nuclear DNA labeled in vivo. HAP assays were performed according to Ersland (1980). [c] Residual repetitive DNA remaining after 380-nt tracer DNA was stripped of zero time binding fraction. [d] K value was corrected for tracer length to allow direct comparison with the single-copy component of the 440-nt tracer curve. [e] Assumed to be single-copy fragments not containing a repetitive sequence. Any fragments containing repetitive sequences would bind to HAP by $C_0t = 500$ M·s.

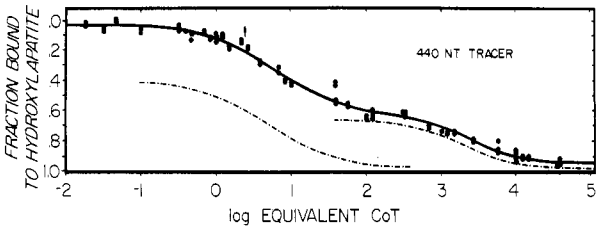

FIGURE 1: Reassociation of 440-nt *Olisthodiscus* DNA. The specific activity of the ^{3}H-labeled 440-nt tracer was 1.0×10^6 cpm/μg of DNA. Reassociations of sonicated total DNA were carried out at $T_m = 25$ °C criteria in either (a) 140 mM NaP–NaDodSO$_4$ to $C_0t = 0.10$ M·s, at 10 μg of DNA/mL, or (b) 1 M sodium perchlorate buffer to C_0t values ranging from 0.085 to 35 500 M·s, at DNA concentrations of 100–2070 μg/mL. The solid line is the best computer fit of the data to two ideal second-order reassociation components. The dashed lines are the individual components that sum to produce the whole curve. The root mean square of the fit was 0.0304. The arrow is the expected $C_0t_{1/2}$ of chloroplast DNA reassociation (5% reassociation component).

was complete by $C_0t = 100$ M·s. The repetitive component contains both chloroplast and mitochondrial DNA since whole-cell DNA was used in this experiment. Isolated chloroplast DNA reassociates (Ersland, 1980) with a second-order rate constant of 8.33 $M^{-1}\cdot s^{-1}$ or a $C_0t_{1/2}$ of 0.12 M·s. The kinetic complexity of chloroplast DNA is approximately 92×10^6 d (Ersland, 1980) and represents 4.85% of the total DNA of the cell (Cattolico, 1978c); for this reason the reassociation rate of these sequences would be decreased by a factor of 20 in the total DNA reassociation reaction. As a result, a $C_0t_{1/2}$ of 2.4 M·s for chloroplast DNA is expected (Figure 1) in the whole-cell DNA reaction. The contribution of *Olisthodiscus* mitochondrial DNA to the middle repetitive component is presently unknown.

DNA duplex formation after $C_0t = 100$ M·s produces a single component which has a $C_0t_{1/2}$ of 2257 M·s and represents (Table I) 34% of the total reassociation reaction. When a complexity of 4.24×10^6 ntp (Cairns, 1963) for the *E. coli* genome was used, the *Olisthodiscus* genome was shown to have a kinetic complexity of 2.43×10^{-12} g of DNA (2.22×10^9 ntp). The analytical complexity of *Olisthodiscus* whole-cell DNA (Cattolico, 1978c) is 2.17×10^{-12} g of DNA. On the assumption that 4.85% of the total DNA is of chloroplast origin, then 2.06×10^{-12} g of DNA is present per nucleus. Cytophotometric determination of *Olisthodiscus* nuclear DNA has shown that G_1-phase populations of *Olisthodiscus* cells occur with a normal distribution of DNA per nucleus and that the amount of DNA per nucleus has a mean value of 1.83×10^{-12} g (Ersland, 1980; D. Therrien, personal communication). If the analytical complexity of *Olisthodiscus* nuclear DNA is 2.06×10^{-12} g (1.88×10^9 ntp), then the single-copy sequences observed in the whole-cell reassociation profile are represented approximately 0.85 times per genome. More extensive analysis of the reassociation kinetics of this single-copy DNA component is discussed below.

At $C_0t = 35\,500$ M·s, 4% of the DNA remained unbound to HAP. Zimmerman & Goldberg (1977) determined that tobacco DNA which failed to bind to HAP at the highest C_0t values was extensively degraded, and we therefore assume that the final unbound portion of the whole-cell *Olisthodiscus* DNA reaction is composed of very small DNA fragments.

It should be noted that ^{14}C-labeled *E. coli* DNA, cosonicated with *Olisthodiscus* DNA to 440-nt size, was included as an internal kinetic standard in these experiments. This bacterial DNA reassociated in the reaction mixture with the same kinetics as those observed when *E. coli* DNA was reassociated alone. This control demonstrates that no contaminating factors (Kemp & Merlo, 1975; Murray & Thompson, 1977) capable of altering DNA reassociation rates were present in the *Olisthodiscus* driver DNA preparations. In addition, no viscosity effect (Murray et al., 1978) on the *E. coli* internal control DNA reassociation rate was observed when *Olisthodiscus* driver DNA concentrations as high as 2.4 mg/mL were employed.

Single-Copy Reassociation Kinetics and Calculation of Nuclear Ploidy. For a more definitive determination of the complexity of *Olisthodiscus* single-copy DNA, single-copy tracer was made from nuclear DNA purified by the Hoechst dye separation technique. The DNA was initially fractionated by selecting that DNA which remained unbound to HAP at $C_0t = 1000$ M·s. The final DNA sample which was used in this experiment was 380 nt in length, bound to HAP at $C_0t = 30\,000$ M·s, and represented 4.3% of the total nuclear DNA of the cell. It should be noted that approximately half of the single-copy sequences (17% of the total nuclear DNA) should have been isolated (Figure 1) with this preparative fractionation scheme. However, a larger amount of the DNA was degraded during the $C_0t = 30\,000$ M·s incubation. This degradation is assumed to be random, and the isolated tracer should therefore be representative of single-copy sequences found in the *Olisthodiscus* nuclear DNA. Since the procedure for single-copy sequence isolation resulted in tracer fragments shorter than the 440 nt length fragments which were used in the whole-cell DNA reassociation analysis, the K value obtained from the data of Figure 2 was corrected for tracer length by rearrangement of the Wetmur & Davidson (1968) equation

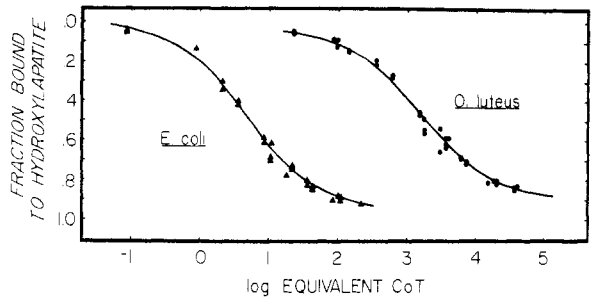

FIGURE 2: Reassociation of *Olisthodiscus* single-copy tracer DNA. (●) Single-copy tracer (3.4×10^5 cpm/mg of DNA). Reassociations were performed in 1 M sodium perchlorate buffer at T_m = 25 °C. The DNA concentrations ranged from 240 to 2425 μg/mL. The data were fitted to ideal kinetics by computer (solid line). The root mean square was 0.0339. The curve shown has been corrected for the dependence of reassociation rate on fragment length to compare with the reassociation curve shown in Figure 1 by the method given in the text. (▲) Reassociation of 440-nt ^{14}C-labeled *E. coli* DNA included as an internal standard. The root mean square of the computer fit (solid line) of these data was 0.0256. Reassociations were carried out at DNA concentrations of 0.01–1.0 μg of DNA/mL.

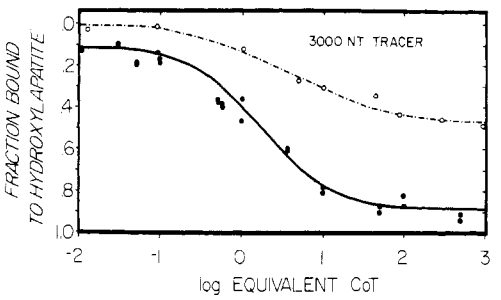

FIGURE 3: Reassociation of long tracer *Olisthodiscus* DNA. Nuclear DNA, labeled in vitro (2.5×10^5 cpm/μg of DNA), was reassociated with excess 440-nt driver DNA at T_m = 25 °C in each buffer used. All C_0t points were HAP fractionated. (●) Samples up to C_0t = 0.05 M·s were reassociated in 140 mM NaP–NaDodSO$_4$ (10 μg of DNA/mL). Sodium perchlorate buffer was used for C_0t values of 0.01–500 M·s at 230–2300 μg of DNA/mL. The zero-time binding fraction was 13.6% as determined from in vitro and in vivo labeled DNA fragments. The HAP binding data were corrected for zero-time binding by the formula $(B - 0.136)/(1.0 - 0.136)$, where B is the fraction of DNA bound to HAP at any C_0t value (Davidson et al., 1973). The root mean square for the computer fit (solid line) was 0.0423. (○) Reassociations were performed with 10 mM Pipes (pH 6.7) and 300 mM NaCl at concentrations of 32–2900 μg of DNA/mL. Each point was then treated directly with S1 nuclease. The data were hand fitted to a 48% ideal S1 nuclease reassociation component generated by using the equation $C/C_0 = [1/(1 + KC_0t)]^{0.44}$ (Smith et al., 1975).

where (440 nt/380 nt)$^{0.5} \times 6.03 \times 10^{-4}$ M^{-1}·s^{-1} = K. The corrected single-copy K value obtained from this experiment (Table I) was then used for the reanalysis of the data of Figure 1. When the single-copy K value was fixed and the total reassociation reaction reanalyzed, the following kinetic parameters were obtained: 58% repetitive (K = 0.152 M^{-1}·s^{-1}), 32% single copy (K = 0.000651), and 6% unbound at the completion of the reassociation reaction. The root mean square for the new solution to the whole-cell reassociation profile from the data of Figure 1 was 0.0305. This result is significant because it demonstrates that, even though the single-copy component is small in whole-cell DNA preparations, the reaction kinetics which were observed occur at a rate similar to those obtained for the isolated single-copy sequences. The genome size of *Olisthodiscus*, using the K value obtained from the single-copy tracer experiment, was calculated to be 1.66×10^{-12} g (1.52×10^9 ntp), which is consistent with *Olisthodiscus* being haploid under our culture conditions.

Organization of the Olisthodiscus Genome. Reassociation Kinetics of 3000-nt Nuclear DNA Fragments. Comparison of the reassociation kinetics profile which results when either long (3000-nt) or short (440-nt) tracer fragments are used in a DNA reassociation reaction provides a simple test to determine whether repetitive and single-copy sequences are interspersed. If long tracer DNA contains contiguous single-copy and repeat sequences on the same fragment, reassociation of the repeat sequences alone would be sufficient to effect binding of these long fragments to HAP. An increase in the apparent proportion of the repeat kinetic component should result from this experiment since unreacted single-copy DNA linked to repeat DNA would be attached to the HAP columns. In application of this technique to the *Olisthodiscus* system, ^{3}H-labeled tracer nuclear DNA of 3000-nt length prepared by nick translation without DNase I (Maniatis et al., 1975) was reassociated to C_0t = 500 M·s with a large excess of unlabeled 440-nt driver DNA. It was immediately apparent (Figure 3) that the repeated sequence (fast) component of the reaction significantly increased in amount. Eighty-nine percent of the 3000-nt tracer binds to HAP by C_0t = 100 M·s in the foldback and fast reassociating components, whereas the single-copy component is essentially undetectable (Table I). These data provide a direct demonstration that (a) single-copy and repeat sequences may occur on the same 3000-nt fragment of *Olisthodiscus* nuclear DNA and (b) given the significant reduction in the proportion of single-copy kinetic component which is observed in this experiment, single-copy DNA must be primarily located less than 3000 nt in distance from a repeat sequence.

Two experiments were designed to assay for the average proportion of double-stranded DNA found in the HAP-bound structures. First, 3000-nt tracer fragments were reassociated with excess 440-nt driver with a buffer which would allow S1 nuclease digestion of the resulting structures. After S1 nuclease treatment which removes single-stranded DNA regions (single-copy sequences) the enzymatically digested DNA was subjected to HAP fractionation. As seen in Figure 3, 45% of the tracer binds at C_0t = 100 M·s whereas almost 90% of this same DNA binds in samples which have not been S1 treated. These data indicate that approximately half of an *Olisthodiscus* 3000-nt nuclear DNA fragment is composed of repeat sequences.

In a second experiment, unlabeled 3000-nt nuclear DNA fragments were reassociated to C_0t = 100 M·s in 300 mM NaCl and 10 mM Pipes (pH 6.7), and the resulting structures (C_0t = 100 M·s duplex) were collected by preparative HAP chromatography. These structures were thermally denatured with a temperature-programmed spectrophotometer (Figure 4). The hyperchromicity of these structures was 0.148 (Figure 4c) whereas analysis of denatured unsheared native DNA resulted in the substantially higher hyperchromicity value of 0.295 (Figure 4a). The elevated hyperchromicity value of the native DNA control sample vs. that value observed in the C_0t = 100 M·s duplex indicates that a smaller proportion of double-stranded DNA is present in the latter. When hyperchromicity data were used to calculate the extent of base pairing in the C_0t = 100 M·s duplex fraction relative to that of the native DNA control sample, 47% double-strand formation (Table II) was observed. This result offers additional evidence that the C_0t = 100 M·s duplex contains both unrenatured single-copy as well as renatured repeat DNA segments.

Thermal melt profiles of (a) native DNA, (b) reassociated 440-nt DNA (C_0t = 0.01–100 M·s fraction), (c) C_0t = 100 M·s duplex, and (d) S1 nuclease resistant duplexes recovered from the enzymatic digestion of C_0t = 100 M·s duplex DNA

Table II: Duplex Content of *Olisthodiscus* Long-Fragment DNA Reassociated to C_0t = 100 M·s

duplex assay	fraction of HAP bound	T_m (°C) of bound material[c]	T_m (°C)[d]	H of bound material	duplex content
S1 nuclease resistance	0.45[a]	83.7	−5.8	0.256	0.45[e]
hyperchromicity	0.90[b]	83.8	−5.7	0.148	0.47[f]

[a] Fraction of 3000-nt DNA fragments S1 nuclease resistant at C_0t = 100 M·s (Figure 3); determined by HAP binding. [b] Optical determination from preparative HAP column. [c] The material used in the S1 assay is shown as sample d and the material for the hyperchromicity is shown as sample c in Figure 4. [d] Calculated as T_m(sample) − T_m(native unsheared). [e] Duplex content is the fraction of HAP bound at C_0t = 100 M·s. [f] Duplex content calculated from the hyperchromicity of C_0t = 100 M·s, 3000-nt HAP-bound DNA relative to the hyperchromicity of native DNA. Calculated according to the formula of Graham et al. (1974): duplex content = (0.148 − 0.030)/[0.295(1 − 0.054) − 0.030] = 0.47, where 0.148 is the hyperchromicity of HAP-bound 3000-nt fragments (C_0t = 100 M·s duplex) containing duplex and single-stranded DNA regions and 0.030 is the hyperchromicity of single-stranded *Olisthodiscus* DNA attained between 60 and 98 °C. The fraction 0.295 is the hyperchromicity of native *Olisthodiscus* DNA. The sequence mismatch term (0.054) is taken from the T_m of reassociated *Olisthodiscus* repetitive DNA (Figure 4, sample b) relative to the T_m of native DNA. Since a 1 °C lowering of T_m results from each 1% mismatch in paired sequences (Britten et al., 1974), the mismatch in *Olithodiscus* repetitive DNA is 5.4%.

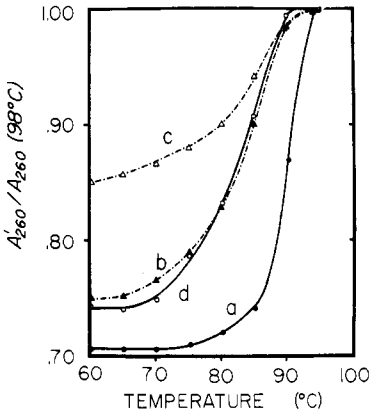

FIGURE 4: Thermal melts of reassociated *Olisthodiscus* DNA. Denaturations were performed in a Gilford 2400 recording spectrophotometer equipped with a thermoprogrammer accessory. Samples b–d were eluted from preparative HAP columns at 60 °C in 500 mM sodium phosphate (pH 6.8) and diluted to 120 mM sodium phosphate. Sample a was dialyzed into 120 mM sodium phosphate. Hyperchromicity (H) values were calculated from the formula $H = (A_{260}$ at 98 °C − A_{260} at 60 °C)/A_{260} at 98 °C. T_m values were taken from the points at which the samples had reached 50% of their final respective hyperchromicity values and are given in degrees Celsius. (a) Native DNA, unsheared, H = 0.295, T_m = 89.5 (●). (b) Reassociated 440-nt nuclear DNA, C_0t = 0.01–100 M·s fraction, H = 0.265, T_m = 82.6 (▲). (c) Reassociated 3000-nt DNA, HAP bound at C_0t = 100 M·s (C_0t = 100 M·s duplex), H = 0.148, T_m = 83.2 (△). (d) C_0t = 100 M·s duplex, S1 nuclease resistant, H = 0.256, T_m = 83.1 (○). The T_m values corrected for fragment length (Britten et al., 1974) are (b) 84.1 (440 ntp), (c) 83.8 (1115 ntp), and (d) 83.7 (1115 ntp). The fragment lengths for samples c and d were taken from the weight-average length of S1 nuclease resistant repetitive DNA (Figure 5).

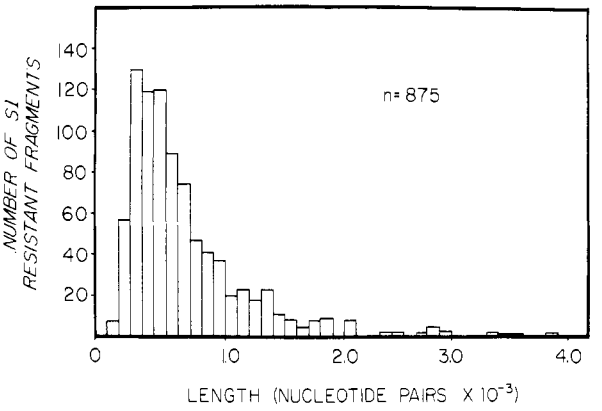

FIGURE 5: Length distribution of *Olisthodiscus* C_0t = 100 M·s duplexes resistant to S1 nuclease. Nuclear DNA fragments 3000 nt in length were reassociated to C_0t = 100 M·s in 300 mM NaCl and 10 mM Pipes (pH 6.7) and HAP fractionated prior to spreading for electron microscopy. The duplex lengths were determined relative to the average contour length of double-stranded SV40 DNA, length = 5224 ntp (Fiers et al., 1978). The weight-average length of all *Olisthodiscus* fragments measured was 1115 ntp.

were analyzed to determine the degree of base pairing fidelity in the reassociated structures. The experimental DNA samples melted at T_m values (Figure 4b–d) which were lowered by 5.4, 5.8, and 5.7 °C, respectively. These values were corrected (Britten et al., 1974) for the influence of fragment length on thermal stability. Since a 1.0 °C depression of T_m results from each 1% sequence mismatch in the reassociation of DNA (Britten et al., 1974), the mismatch of the reassociated repeat DNA sequences is approximately the same for all sequences studied. These data demonstrate that the DNA sequences which reassociate at low C_0t values and which are recovered by the various experimental methods listed above are of comparable composition and therefore provide a good representation of the repeat sequences present within the *Olisthodiscus* genome.

Length Determination of Reassociated Repetitive Sequences. For further elucidation of the sequence organization of the *Olisthodiscus* genome, both the length of the repetitive sequences and the pattern of distribution of these sequences were analyzed. Size measurements were made by using the electron microscopic technique on the S1 nuclease resistant duplexes formed when 3000-nt fragments were reassociated to C_0t = 100 M·s (Figure 5). The blunt-ended duplexes produced by enzymatic removal of single-stranded regions of the DNA were strongly skewed toward a smaller size class. That is, 87% of the fragments counted were shorter than 1200 ntp with a mean length of 605 ntp (modal length 350 ntp). This fraction represents 68% by weight of the duplexed DNA. Although repeat sequences of a length longer than 1200 ntp represent a large proportion by weight of the total DNA, repeats of this size class were less abundant in number. Repeat lengths as long as 4000 ntp were observed in our analysis. However, repeat sequences longer than this probably exist in the *Olisthodiscus* genome but are not recovered due to the fragment size chosen for the experiment. If an average 3000-nt segment of *Olisthodiscus* DNA is composed of 45% repetitive DNA (Table II), then this DNA segment would have the capacity to contain 2–4 short repetitive sequences if the variable size of this repetitive DNA class is considered. However, it must be noted that although in low number, repeated segments do exist which are long enough to occupy a major portion of a 3000 base pair DNA fragment.

Discussion

Reassociation kinetics analysis of isolated single-copy DNA has demonstrated that 1.66 × 10^{-12} g of DNA/nucleus rep-

resents a haploid genomic complement in an *Olisthodiscus* cell. This information places *Olisthodiscus* among the few unicellular algae, most of which are chlorophytes (Bayen & Dalmon, 1975; Rawson et al., 1979; Howell & Walker, 1976; Siu et al., 1974; Roberts et al., 1974) for which genome size is reliably known. It is interesting to note that a cellular DNA content such as that observed in *Olisthodiscus* is not unique among the chromophytic algae. Although low DNA amounts (Holm-Hansen, 1969; Cattolico & Gibbs, 1975) of 0.1 pg/cell (*Monochrysis*) to 0.2 pg/cell (*Ochromonas*) are seen in the Chrysophyceae, diatoms may contain (Holm-Hansen, 1969) a DNA complement of 0.6 pg/cell (*Skeletonema*) to 25 pg/cell (*Ditylum*). The largest nuclear DNA content seen among chromophytes (Holm-Hansen, 1969; Allen et al., 1975) is that observed in the dinoflagellates, where a value of 200 pg/cell (*Gonyaulaux*) to 650 pg/cell (*Gymnodinium*) is not uncommon. If, in many cases, these high DNA values represent a haploid genomic complement, as indicated by our *Olisthodiscus* studies and as suggested (Roberts et al., 1974) by nitrosoguanidine mutation frequency studies in *Gymnodinium*, then this observation presents an intriguing paradox. Although the algal cell is often morphologically and functionally plastic, why should so much DNA exist in a unicellular system if only a small proportion of the genome is expressed during growth and/or differentiation? For example, an analysis (Howell & Walker, 1977) of *Chlamydomonas* (0.1 pg of DNA/haploid cell) over different phases of the cell cycle and experiments (Curtis & Rawson, 1979) with greening *Euglena* (3 pg of DNA/diploid cell) demonstrate that RNA transcripts represent a utilization of less than 12% of the total nuclear genome. Similar results have been obtained for higher plant (Kamalay & Goldberg, 1980), invertebrate (Galau et al., 1974), and vertebrate (Rosbash et al., 1974) systems even when structural gene regulation at the posttranscriptional level (Davidson & Britten, 1979) is taken into account. If this observation is universal, then algae, much like higher eukaryotes, seem to possess (Thompson & Murray, 1980) DNA (secondary DNA) whose genetic utility is presently unknown. How the variable structural and biochemical capacity of the algal cell relates to genome size and expression awaits further experimentation.

The *Olisthodiscus* genome is composed of 45% repeat DNA sequences. This amount of middle repetitive DNA is also seen in similar proportion in two other algae (Rawson et al., 1979; Hinnebusch et al., 1980) with high nuclear DNA content, namely, *Euglena gracilis* and *Crypthicodinium cohnii*, but is minimal (Bayen & Dalmon, 1975; Prima et al., 1974; Howell & Walker, 1976; Siu et al., 1974) in *Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, and *Polytoma obtusum*, whose small genomes have virtually no (except for rRNA cistrons) repeat sequences. Since these seven organisms represent the entire literature of DNA sequence organization studies of algal cells, it is premature to initiate an evolutionary (Eden & Hendrick, 1978) or genomic size (Hutchinson et al., 1980; Thompson & Murray, 1980) comparison as possible factors effecting repeated DNA distribution, for these organisms represent four different phyla and have almost an 80-fold difference in haploid DNA content.

The *Olisthodiscus* genome displays two patterns by which single-copy and repeat sequences are interspersed. Of the total repetitive DNA which is observed, 87% by fragment length (68% by weight) are DNA sequences shorter than 1200 base pairs with a modal length of 350 nucleotide pairs. The remaining repeated sequences occur in lengths as high as 4000 nucleotide pairs. Whether these long repeats in *Olisthodiscus* represent tandemly repeated multigene families or clusters of short repetitive sequences is presently unknown. Although the *Olisthodiscus* DNA preparations are entirely free of chloroplast DNA, given the buoyant density and copy number of mitochondrial DNA in some systems (Bendich & Ward, 1980), these long repeat sequences could represent contamination by this organelle. However, *Eco*RI digestion of the nuclear DNA fraction of *Olisthodiscus* gives no distinct restriction fragments which would be expected if mitochondrial DNA were in high concentration, and melt profiles of this repeat fraction show no obvious component which would represent a large, homogeneous organellar DNA contaminant. Moreover, in algal cells, mitochondrial DNA usually represents (Manning et al., 1971; Ryan et al., 1978) a minor portion of the total cellular DNA.

Except for the very slightly larger repeat length, the short repetitive pattern seen in *Olisthodiscus* is not unique. Repetitive sequences, 300 nucleotides in length, flanked by single-copy DNA is a characteristic sequence organization pattern found in a large proportion (50–80%) of the DNA complement in a variety of animal (Davidson et al., 1973), insect (Crain et al., 1976), and plant (Walbot & Goldberg, 1979; Flavell, 1980) systems. In many cases, as seen in *Olisthodiscus*, these varied systems may also contain (Walbot & Goldberg, 1979; Flavell, 1980) long (~1500–15000 nucleotide pairs) stretches of repeat DNA. It is important to note that many sequence organization patterns (long period, short period, and mixed) occur in eukaryotic cells. The fact that these patterns exist in such a diverse array of organisms suggests that interspersion of repeated and single-copy DNA sequences occurred as an early evolutionary event. We interpret our findings with respect to the "primitive" taxonomic position of *Olisthodiscus* to substantiate this hypothesis. The significance of the initiation of this event in terms of gene expression and/or organism fitness potential remains a contemporary research question.

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