STADLER SYMP. Vol. 8(1976) University of Missouri, Columbia
pp. 111-128

DINOFLAGELLATE GENETICS AND DNA CHARACTERIZATION

(dinoflagellates, evolution, nucleus, DNA, genetics, Mesocaryota)

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SUMMARY

Nuclear features of dinoflagellates that were used originally to support the Mesocaryota concept are reviewed. Although dinoflagellates possess some procaryotic nuclear features, the remainder of the cell is obviously eucaryotic. The fibrillar diameter of the chromatin, low level of chromosomal basic proteins, membrane attachment of the chromosomes and swirl pattern seen in sectioned chromosomes all support a procaryotic affinity. On the other hand, the repeated and highly complex DNA, S-phase of DNA synthesis, and the presence of basic proteins and extranuclear spindle support the contention that dinoflagellates are eucaryotic. The possession of both procaryotic and eucaryotic nuclear features indicate that this group evolved relatively early in biological time. A review is made of recent attempts to analyze the genetics of this algal group. Although incomplete, these studies have suggested that dinoflagellates have an unusual meiotic process.

INTRODUCTION

The dinoflagellates play a significant ecological role in the seas today (see Fig. 1). Paleontological evidence shows that they have been a dominant phytoplanktonic group since the Jurassic (SARJEANT 1974), while the detection of some primitive nuclear features by cytological and fine structural studies suggests that they are geologically older than indicated by the fossil record alone (LOEBLICH 1976). In spite of their importance, until recently, few studies have been made of this group's biology and biochemistry (LOEBLICH 1967). This can perhaps be explained by the unavailability of good experimental organisms, lack of suitable culture media and difficulties encountered in freeing them from contaminants and in growing them in the laboratory.

The dinoflagellate nucleus, termed dinokaryon by CHATTON

(1920, p. 424), has been of interest to cytologists for a number of years due to the permanent condensation of the chromosomes. Interest in this algal division has been further stimulated by the proposal that dinoflagellates may represent an intermediate between procaryotes and eucaryotes with respect to their nuclear

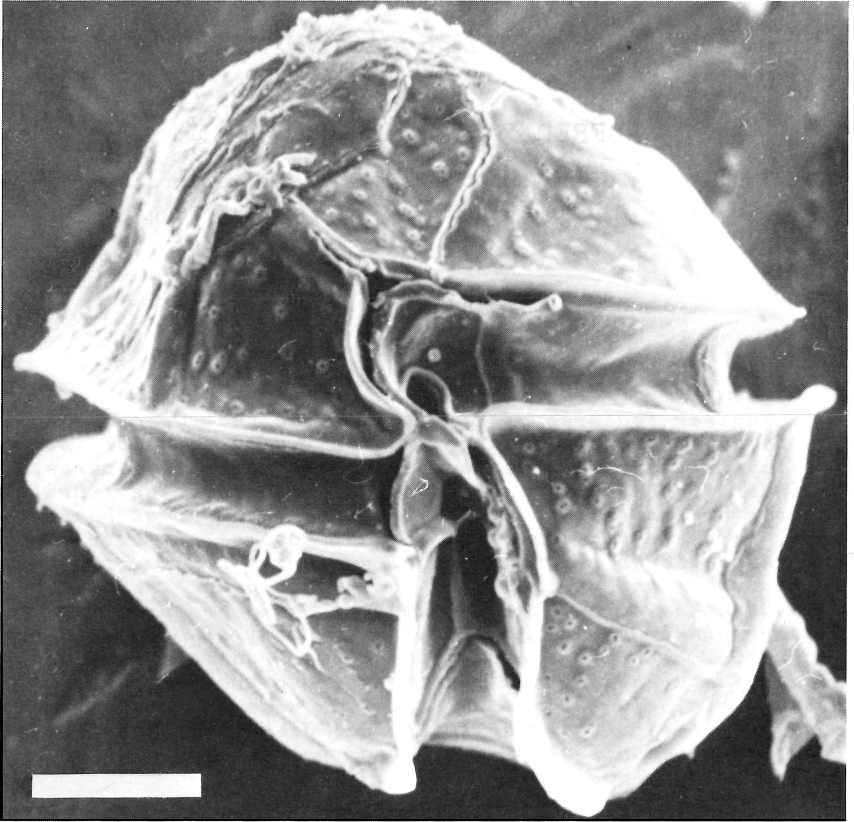

Figure 1. Scanning electron micrograph of *Gonyaulax excavata* (isolate 426), the dinoflagellate causing red tides along the New England coast. The picture shows the typical cell shape for a free-living motile dinoflagellate; however, the flagella have been lost from the transverse and longitudinal grooves. Dinoflagellates possess a complex cellular covering. In this specimen the cellulosic plates comprising one layer of the cellular covering can be seen; they are pierced by trichocyst discharge pores. Cells were cultured in medium GPM (LOEBLICH 1975) and prepared for scanning electron microscopy by the critical point drying method (LOEBLICH & LOEBLICH 1975). Scale = 5μm.

organization. DODGE (1965, 1966) proposed the term Mesocaryota for dinoflagellates. Before the last ten years practically no significant information other than chromosome counts was available concerning the group's nucleus, chromosomes, DNA or genetics.

THE MESOCARYOTA

DODGE's proposal (1965, 1966) to place the dinoflagellates in a new kingdom intermediate in nuclear organization between the procaryotes and eucaryotes was based on: a) lack of histochemically detectable histones (DODGE 1964); b) 3 to 6 nm diameter of the chromatin (BOULIGAND et al. 1968b, DODGE 1965, GIESBRECHT 1962, HALLER et al. 1964); c) membrane-attached chromosomes (BOULIGAND et al. 1968a, KUBAI & RIS 1969, SOYER 1969); d) absence of a mitotic spindle (KUBAI & RIS 1969, LEADBEATER & DODGE 1967); e) continuous DNA synthesis (DODGE 1965, 1966); f) the ultrastructural pattern of the chromatin fibrils arranged in arched swirls similar to that found in bacterial nucleoids (GIESBRECHT 1962); g) permanent condensation of the chromosomes throughout the cell cycle (LOEBLICH 1971). I shall discuss subsequent information that has accumulated on the features originally used to support the Mesocaryota concept.

Histones

Using the histochemical stain Fast Green, no one has been able to detect basic proteins or histones in free-living dinoflagellates (DODGE 1966). However, in the dinoflagellate *Syndinium* sp., a parasite of radiolarians, RIS and KUBAI (1974) noted that the chromosomes stain intensely with Fast Green, indicating that basic proteins are present. By immunofluorescence a DNA-histone complex was detected in five free-living dinoflagellate species (STEWART & BECK 1967). RIZZO and NOODÉN (1974b) found acid-soluble proteins in log phase cells that were apparently lacking in stationary phase cells of the free-living species, *Crypthecodinium cohnii* and *Scrippsiella trochoidea*. One major acid-soluble protein of these two species was similar in electrophoretic mobility on polyacrylamide gels to corn histone IV but was 45% larger and contained fewer basic amino acids. The decrease in the amount of DNA/cell as the population shifts from exponential to stationary phase of growth (see discussion of cell cycle) may be related to this decrease in basic protein associated with the DNA. Thus both free-living as well as parasitic dinoflagellates have basic proteins, although these proteins differ qualitatively and quantitatively from the histones of higher eucaryotes.

Chromosome Attachment and Extranuclear Spindle

The chromosomes of all dinoflagellates are attached to the persistent nuclear membrane; however, recent studies of mitotically dividing nuclei demonstrate the existence of two major patterns of cell division (KUBAI & RIS 1969, RIS & KUBAI 1974). Free-living species have many membrane-lined cytoplasmic tunnels piercing the dividing nucleus (LOEBLICH & HEDBERG 1976). These

tunnels contain elongating extranuclear microtubules, some of which end on the nuclear membrane at the point of membrane attachment of the chromosomes (CACHON & CACHON-ENJUMET 1974, OAKLEY & DODGE 1974). No centrioles are associated with the mitotic apparatus of free-living species and the chromosomes are rod-shaped, varying from 12 to 400 per nucleus (LOEBLICH 1976). The other major pattern of cell division is seen in the intracellular, parasitic dinoflagellates, e.g. *Syndinium* sp., which have centrioles and only one microtubule-containing membrane-lined tunnel piercing the dividing nucleus. An end of some of the microtubules attaches to regions of the nuclear membrane, such as the nuclear pores, in which the chromosomal kinetochores are recessed. The other end of these microtubules fastens to the centrioles located at both ends of the tunnel. Separation of the chromosomes is accomplished through the elongation of microtubules other than those connecting the centrioles and kinetochores (RIS & KUBAI 1974). The chromosome number of the intracellular parasites, *Syndinium* and relatives, is low (4-10), and the chromosomes are V-shaped with the apex of the V attached to the nuclear membrane.

The earlier belief that dinoflagellates differ from higher eucaryotes in their lack of a spindle is giving way to interpretation of the extranuclear microtubules as an extranuclear spindle (OAKLEY & DODGE 1974). The presence of the microtubule-chromosome attachment sites and the progressive elongation of some microtubules during mitosis suggest a direct role for these elements in chromosomal separation during mitosis and thus that they represent an extranuclear mitotic spindle.

Cell Cycle

A procaryotic pattern of DNA synthesis (continuous DNA synthesis) was originally given as a feature of the Mesocaryota. Various attempts have been made to determine whether dinoflagellates have an eucaryotic pattern (a discrete period in the cycle during which DNA is synthesized) or a prokaryotic pattern of DNA synthesis (continuous synthesis). These studies are fragmentary, and perhaps some of the variations can be attributed to the differences in organisms, in manner of growth and methods of analysis. From a radioautographic study of the incorporation of labelled nucleotides in *Prorocentrum micans*, DODGE (1965, 1966) suggested that DNA synthesis in dinoflagellates was continuous. Recent work on several species conflicts with his proposal and indicates that a discrete DNA-synthesis phase (S-phase) is present.

FRANKER (1971), using natural populations of a *Zooxanthella* sp. living symbiotically within the sea anemone *Anthopleura elegantissima*, fractionated the population by absorbing division "cysts" to plastic surfaces. Unfortunately, with this method only one synchronous division occurs. Using these cells, ^{14}C thymidine was incorporated into a fraction precipitatable by cold 1M trichloroacetic acid over a relatively long period of the cell cycle (40 hr of the 70 hr cell cycle). Neglecting the time spent in mitosis, FRANKER's data suggests that the phases of the cell cycle are: G_1 =10 hr, S =40 hr, and G_2 =20 hr. How-

ever, the incorporation pattern may have been influenced by the thick wall of the "encysted" cells, diminishing permeability of the labelled substrate and/or perhaps to the effects of several antibiotics included in the culture medium. Notably, the period of thymidine incorporation coincides with the vegetative phase (thin-walled phase) of the cell cycle where one would expect the substrates to be incorporated more readily.

Two more recent studies on synchronously dividing dinoflagellates indicate a somewhat different pattern, namely a cycle with a longer G_1- than G_2-phase. Using light-dark (12 hr: 12 hr, L:D) synchronized cultures of the free-living *Cachonina niei* where cell division occurs at the end of the dark period, the average amount of DNA/cell was calculated using the 3,5-diaminobenzoic acid spectrofluorometric method. In synchronized cultures of *C. niei* DNA synthesis begins at the end of the light period and is completed within the first seven hours of the dark period. Neglecting the time spent in mitosis, a cell cycle of 24 hours could be resolved into: G_1 =12 hr, S =7 hr and G_2 =5 hr (LOEBLICH 1976).

Measurement of ^{32}P incorporation into alkali insoluble material of *Crypthecodinium cohnii* populations synchronized by absorption to plastic indicates that DNA synthesis occurs late in the cell cycle (FRANKER et al. 1974). Again, neglecting time spent in mitosis, the 9 hour cell cycle could be represented by: G_1 =4 hr, S =4 hr and G_2 =1 hr. The morphological similarities, namely alternation of motile and nonmotile cell types of both *C. niei* and the *Zooxanthella* sp., imply that the cell cycle phases might be similar.

The data for three species (*Crypthecodinium cohnii, Gyrodinium resplendens* and *Gymnodinium* sp.) demonstrating that log phase cells possess twice the DNA content of stationary phase cells could be explained by a relatively long G_2-phase and consequently short G_1- and S-phases (ALLEN et al. 1975). Measurements of the average amounts of DNA per cell for *C. cohnii* and *G. resplendens* (Figures 2 & 3) indicate that there is a gradual decrease in the amount of DNA per cell as the population enters stationary phase. Microspectrofluorometric measurements (data not shown) of the amount of DNA in single cells of *G. resplendens* using Ciba's 2,5-bis-[4'-amino-phenyl-(1')]-1,3,4 oxdiazole (RUCH 1966), sampled at three times during the log phase and once in stationary phase, resulted in a distribution of values centered around one number, with the mean slightly higher than the median. From the timing of DNA synthesis in light-dark synchronized dinoflagellates one might expect a bimodal distribution of amounts of DNA/cell, the two peaks representing cells in the G_1- and G_2-phases.

Microspectrophotometric measurements of the amount of Feulgen bound dye in the nuclei of log phase and stationary phase cells of *Gymnodinium* sp. (Figure 4) showed a single major peak of DNA per cell in each growth phase. The measurements also demonstrated that log-phase cells contain roughly twice the amount of DNA per cell as compared to stationary phase cells. Interestingly, the decrease in DNA per cell is not paralleled by a decrease

in the chromosome number in *Scrippsiella trochoidea* and *S. faeroense*. Chromosome counts of these species indicate that the number of chromosomes is the same in both log and stationary phase cells (FINE & LOEBLICH 1976).

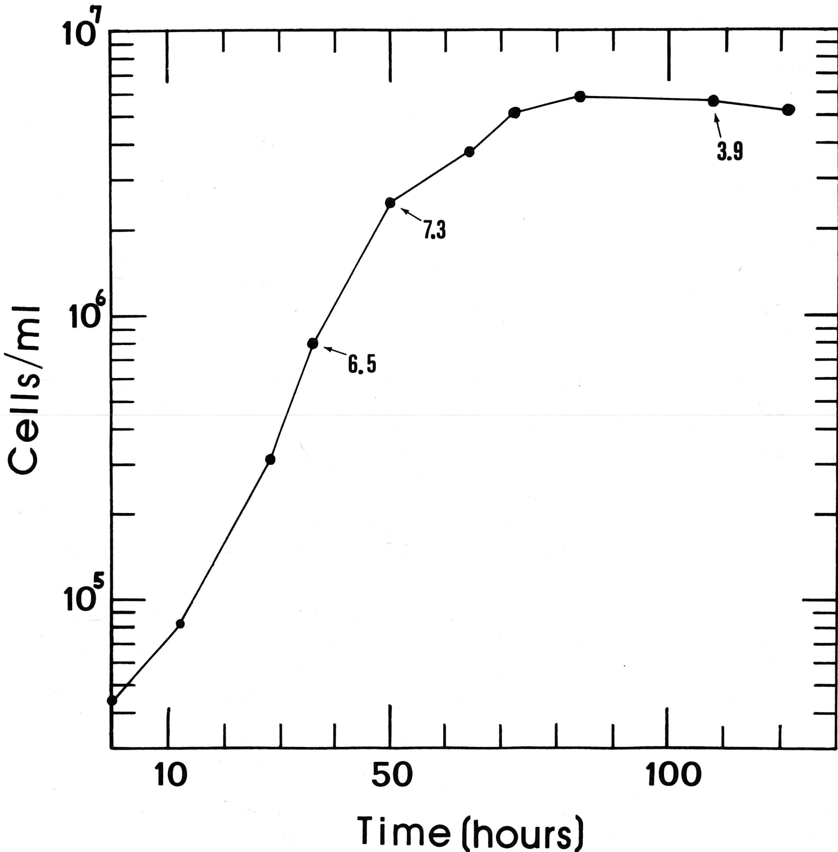

Figure 2. Growth curve of *Crypthecodinium cohnii*, a non-photosynthetic, marine dinoflagellate, showing a progressive decrease in DNA per cell as the population enters the stationary phase of growth. The values indicated at the three points on the growth curve are amounts of DNA per cell in pico grams (pg). Each value given is the mean of nine measurements (triplicate determination of each of three different sample sizes). DNA content was determined by the 3,5 diaminobenzoic acid fluorometric method as given in ALLEN et al 1975). The values (pg/cell are 6.5 (range 5.9 - 7.2), 7.3 (range 5.7 - 9.2) and 3.9 (3.1 - 4.6). Cells were cultured in medium MLH (TUTTLE & LOEBLICH 1975) at 27C° in darkness.

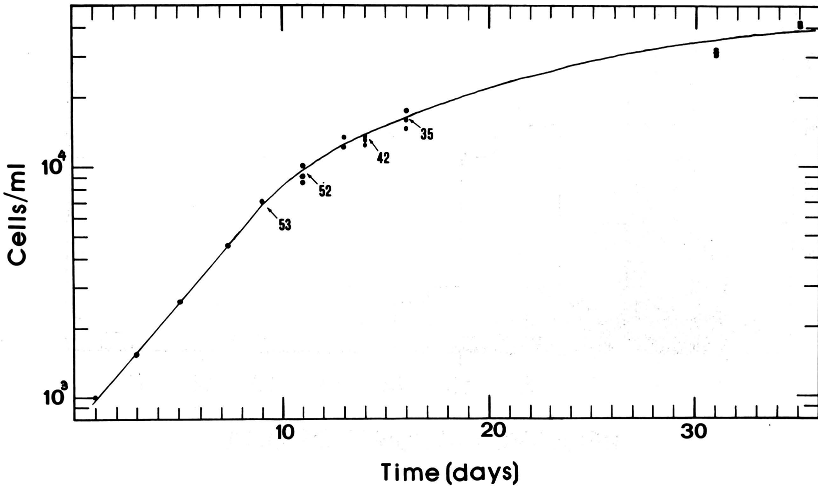

Figure 3. Growth curve of *Gyrodinium resplendens*, a photosynthetic marine dinoflagellate, showing a progressive decrease in DNA per cell as the population enters the stationary phase of growth. The values indicated at the four points on the growth curve are amounts of DNA in picograms per cell (pg). Each value given equals the mean of six measurements, triplicate determinations of each of two different sample sizes. DNA content was determined by the 3,5-diaminobenzoic acid fluorometric method given in ALLEN et al. (1975). The means and the ranges of the measurements in pg/cell are 53 (39-66), 52 (44-62), 42 (39-50) and 35(31-39). Cells were cultured in medium GPM with an equimolar substitution of disodium glycerophosphate for K_2HPO_4 at 20C° in 240 ft-candles.

It appears that the cell cycle in synchronized populations may differ from that of exponentially growing cultures with respect to the relative lengths of the G_1- and G_2- phases. An explanation for this difference might be that light inhibits mitosis, delaying it, and, consequently, that the percentage of the cell cycle occupied by the G_2-phase increases in cells grown in continuous light.

We can conclude that in all the dinoflagellates examined the data is not consistent with the hypothesis of continuous DNA synthesis.

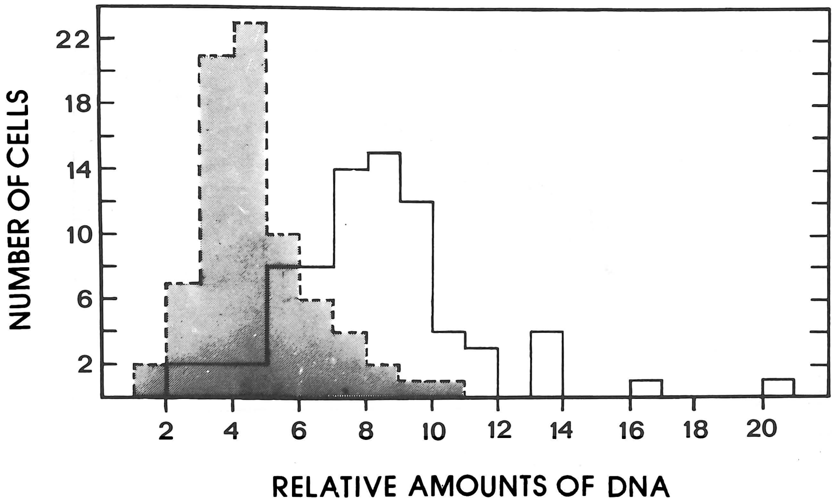

Figure 4. Measurements of amounts of DNA in individual cells from logarithmically growing populations (open regions enclosed in solid line) and from the subsequent stationary phase (shaded region enclosed in dashed line) of the freshwater dinoflagellate *Gymnodinium* sp. (isolate 160). Measurement of nuclear Feulgen dye-DNA complex by the one wavelength method (at 456 nm) in arbitrary units on 151 cells according to procedure of LEUCHTENBERGER (1958). The mean (and range) in arbitrary units are for log phase cells: 9(3-21) and for stationary phase cells 5(2-11). Cells grown in medium FWVM (unpublished) at 13C° in 250 ft-candles.

Fine-Structural Aspects of Dinoflagellate Chromosomes

GIESBRECHT (1962) has presented a comparison at the fine-structural level of the fibrillar arrangement in the dinoflagellate chromosome and the bacterial nucleoid. In thin sections these two have surprisingly similar arch-like whorls of fibrils (Figure 5). Perhaps the reason for this similarity is the low level of chromosomal proteins present in the dinoflagellate chromatin which allows it to fold into a state similar to that assumed by the bacterial chromosome.

DINOFLAGELLATE CHROMOSOME MODELS

Five models have been proposed for the structure of the dinoflagellate chromosomes, based on electron-microscopical observations. Three models for the chromosome assume a high de-

gree of polyteny (GRASSÉ et al. 1965, HAAPALA & SOYER 1973, SOYER & HAAPALA 1974). Although attempts have been made to prove polyteny by measurement of chromosomal-sized DNA pieces in electron micrographs, no unequivocal evidence has been published, nor has any statistical treatment of measurements been made (HAAPALA & SOYER 1974).

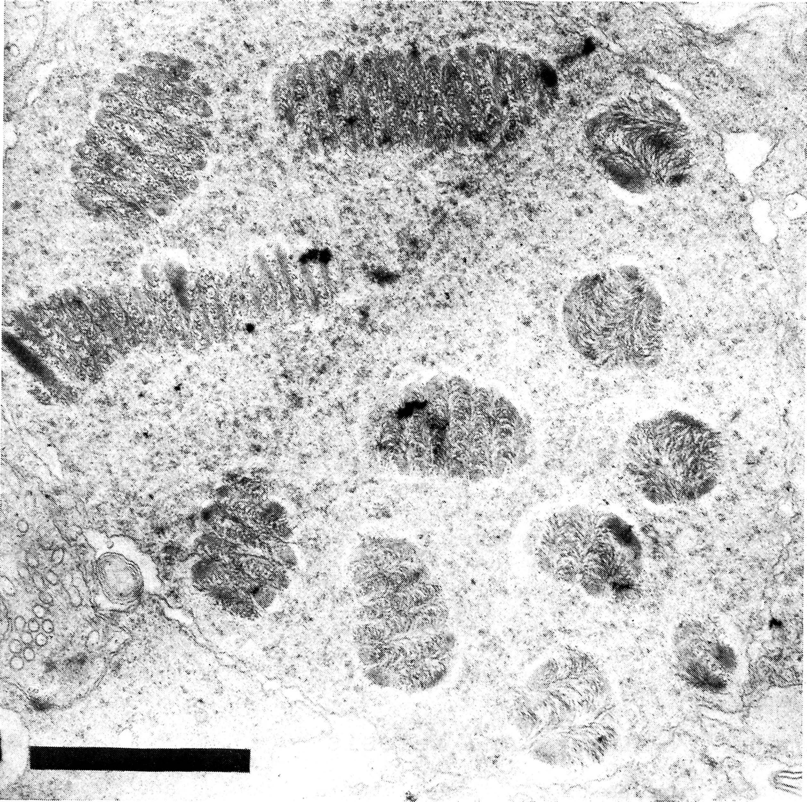

Figure 5. A thin section through the nucleus of the dinoflagellate *Prorocentrum mariaelebouriae* (isolate 403). Chromosomes display a variety of fibrillar patterns depending on their orientation. Cells cultured in medium ASP_7 PROVASOLI (1964) at 20C° in 200 ft-candles and fixed according to method B of LOEBLICH (1976). Scale =1 µm.

The "penny-stack"chromosomal model (BOULIGAND et al. 1968b) leaves open the question as to whether the chromosomes are polytene or not. The GIESBRECHT (1965) chromosome model is uninemic and incorporates a hypothetical protein backbone. The modific-

ation of the "penny-stack" model to incorporate one to a few pieces of DNA per chromosome remains an attractive possibility. Several models have been proposed from electron-microscopical data for the pattern of chromatin folding in dinoflagellates; however, none suggest a relationship between the chromatin level present and the folding pattern. One way in which DNA can condense without the aid of basic proteins is to fold in the presence of neutral or anionic polymers. LERMAN (1974) made several physical studies on the pattern of condensation or folding of purified DNA in the presence of neutral polymers such as polyethylene oxide. By various physical methods the condensed DNA, termed ψ-DNA, was shown to have a fibrillar arrangement similar to that of the "penny-stack" dinoflagellate chromosome model (BOULIGAND et al. 1968b). In this model the fibrils are aligned parallel to each other within the plane of each disc, but each successive disc is rotated slightly in relation to those above and below. Lerman has proposed that the mechanism of condensation of dinoflagellate chromosomes may indeed be the same as that for ψ-DNA. The low level of dinoflagellate chromosomal proteins may play an integral role in the chromatin fibrillar arrangement and, consequently, in the structure of the dinoflagellate chromosome.

GENETICS

The possible unique phylogenetic position of dinoflagellates and their bizarre nuclear features has stimulated an interest in their genetics. Until the very careful studies by von Stosch, the occurrence of sexuality in this group was based on incomplete observations and the position of meiosis was unknown. Von STOSCH (1972, 1973) has offered the best cytological evidence that the vegetative cells of dinoflagellates are haploid, that they reproduce sexually, that meiosis directly follows excystation of the zygote, and that some species have mating types (heterothally) and some do not (homothally). His work concerning dinoflagellate life cycles and that of others was recently summarized (LOEBLICH 1976).

Gametes, Syngamy and Meiosis

The gametes of dinoflagellates are motile and remain so during fusion. They may be equal (isogamy) or unequal (anisogamy) in size but generally are smaller than vegetative cells. After fusion the zygote may remain motile (planozygote) or become nonmotile and secrete a cell wall. In certain species this wall is known to differ in composition and morphology from the cell wall of vegetative cells. Meiosis in some species occurs while the zygote is motile. In others both meiotic divisions may occur in the nonmotile cell-wall-covered zygote (hypnozygote). Alternatively, the first meiotic division may occur in the hypnozygote with two motile cells escaping and each transforming into nonmotile, cell-wall-covered cells. It is within these cell-wall-covered cells that the second meiotic division then occurs. The period extending from gamete fusion to the end of meiosis may be hours long, *e.g.*, *C. cohnii* (TUTTLE & LOEBLICH 1975) or else last several weeks, *e.g.* *Ceratium cornutum* (von

STOSCH 1972). There are several morphological features by which one can distinguish dinoflagellate planozygotes from vegetative motile cells: 1) paired longitudinal flagella, one being derived from each gamete (vegetative cells have only one), 2) a relatively large nucleus in comparison to the nucleus of the vegetative cell, 3) the occurrence of "nuclear cyclosis", a rotation of the chromosomal mass which occurs only during meiotic prophase of the first division, lasting several hours, and which is thought to facilitate chromosomal pairing.

Problems in Dinoflagellate Genetics

One of the most disappointing features of this algal group is the inability to culture them on solid substrates, thus hampering mutant isolation and separation of meiotic tetrads. To date I have been able to grow only three species on agar-solidified media: *Crypthecodinium cohnii* (generation time 8-9 hours), *Gloeodinium montanum* (generation time in days), *Symbiodinium* sp. (isolate 395A; generation time 30 hours). The ability to grow on solid substrates and the relatively short generation time of *C. cohnii* has made it the organism of choice for genetic studies. The absence of restrictive mating types (homothallism) in this species and the inability to differentiate gametes from vegetative cells (thus making feasible matings of single cells), however, makes genetic analysis of this organism difficult. Although a rarity, the report of hypnozygotes in clones of *Ceratium cornutum*, a heterothallic species, is attributed to a breakdown in self-sterility barriers. This phenomenon presents yet another problem for genetic analysis.

Genetics of *Crypthecodinium Cohnii*

Using the mutagen N-methyl-N-nitrosoguanidine we have obtained mutation frequencies for carotene-deficient and adenine-requiring mutants of *C. cohnii* that are consistent with the vegetative cell being haploid (ALLEN et al. 1975, ROBERTS et al. 1974). Using these carotenoid-deficient mutants, we have detected genetic recombination in the homothallic *C. cohnii* (TUTTLE & LOEBLICH 1974a,b, 1975). Subsequently, tetrad analysis has been performed on crosses of several motility mutants of this species (HIMES & BEAM 1975). The tetrads resulting from crosses of impaired motility mutants of *C. cohnii* were incomplete (lacking both parental ditypes and nonparental ditypes). Himes and Beam favor a one-division meiosis rather than centromere linkage or the absence of crossing over as the explanation for the presence of only incomplete tetrads. We have also been unable to find tetratypes in crosses of carotenoid mutants. Before accepting any hypothesis further genetic analyses must be performed. There is a possibility that crossing over occurs, as meiotic chromosome pairing has been detected in several dinoflagellates. It has not been detected cytologically, however. The persistence of the nuclear membrane, the absence of an intranuclear spindle, and the presence of microtubules inserted into regions similar to nuclear pores in which the kinetochore-like regions of chromosomes are situated leads one to the conclusion that if the term

centromere is applicable to dinoflagellate chromosomes, centromeres would be terminal. However, the concept of a centromere is probably not applicable to dinoflagellates, as chromosomal separation is thought to be initiated at the point of attachment to the nuclear membrane.

Caution must be exercised in interpreting the single division of "zygotic cyst" as evidence for a one-division "meiosis". In the cases of the dinoflagellates *Woloszynskia apiculata* and *Helgolandinium subglobosum* the two products of the first meiotic division become flagellated, escape and finally settle to form another "cyst" within which the second meiotic division occurs (von STOSCH 1972, 1973). In these species one structure containing all four meiotic products is never observed. Zygotes of *C. cohnii* with four products would be normal, while those with eight could be attributed to a mitotic division following normal meiosis. Zygotic "cysts" of *C. cohnii* containing only two cells may have resulted from the degeneration of one or two of the products of meiosis, or this species may resemble *W. apiculata* and *H. subglobosum* in having the two meiotic divisions occur in separate cysts. The belief by Himes and Beam that *C. cohnii* perhaps has "genome redundancy" and/or polytenic chromosomal arrangement is not supported by their genetic data or by a physico-chemical characterization of the DNA of this organism (see the section of DNA characterization). A careful cytological study of meiosis might reveal instances of crossing over. Also, it would be helpful if we had several other classes of mutants with which to test the hypothesis of one division meiosis.

DNA CHARACTERIZATION

Among the algae the dinoflagellates are unique in having large amounts of DNA per cell (LOEBLICH 1976). Measurements ranging from 3.2 to 200 pg per cell have been made, with larger-sized species tending to have a larger amount of DNA. The gradual decrease in DNA per cell to a level of approximately 50% of the log phase value as the population enters stationary phase of growth can be explained by a gradual lengthening of the G_1-phase in relation to the G_2-phase. An alternative explanation could be that an increased percentage of cells remain in the G_1-phase after their last division. A remote but possible interpretation is that some portion of the DNA of each cell is degraded as the population enters stationary phase.

Bases

Thermal denaturation curves and buoyant density determinations have revealed that for at least two dinoflagellates there is a significant discrepancy in mole % G +C determinations (see Table 1). Chromatography of the nucleotides of *C. cohnii* DNA reveals the presence of a large amount of 5-hydroxymethyluracil, enough to account for a 37% substitution of thymidylate. This high degree of base substitution can explain the discrepancy in G + C estimations by the two methods. Such a large percentage base substitution is unknown for a eucaryotic organism; however, bacteriophages are known to possess extensive base substitutions.

Table 1. Thermal denaturation temperatures and buoyant densities of dinoflagellate DNA.

Source of DNA	T_m	mole % G+C from T_m	buoyant density (CsCl)[h]	mole % G+C from buoyant density[g]	discrepancy in mole % G+C estimation	mole % G+C from chromatography	source
Crypthecodinium cohnii							
whole cell	68.5[a]	35.6	1.715[c]	56.1	20.5	41.3	i
whole cell	86[b]	41.1[p]	--	--	--	--	j
whole cell	84.8[b]	38.2	1.7185[d]	59.7	21.5	--	k
isolated nuclei	67[a]	32.0	--	--	--	--	l
chromatin extracted from isolated nuclei	64[a]	24.6	--	--	--	--	l
acid extracted chromatin from isolated nuclei	66[a]	29.5	--	--	--	--	l
Zooxanthella sp.							
whole cell	--	--	1.695[e]	35.7	--	--	m
isolated nuclei	--	--	1.695[e]	35.7	0.7	35	m
isolated plastids	--	--	1.688[f]	28.6	0.6	29.2	m
Gymnodinium sp. isolate 160							
whole cell	88.3[b]	46.8	1.7225[f]	63.8	17.0	--	n

[a] T_m determined in 0.1 SSC and mole % G+C calculated using equation (3) of MANDEL & MARMUR 1968.

[b] T_m determined in phosphate buffer (0.18 M Na+) and mole % G+C calculated using equation (2) of SCHILDKRAUT & LIFSON 1965.

[c] density relative to a marker of denatured DNA of *Pseudomonas aeruginosa* (1.737 gm cm^{-3}).

[d] density relative to a marker of *Micrococcus lysodeikticus* DNA (1.731 gm cm^{-3}).

[e] density relative to a marker of *Tetrahymena pyriformis* (HSM strain) DNA (1.685 gm cm^{-3}).

[f] density relative to a marker of *E. coli* DNA (1.710 gm cm^{-3}).

[g] mole fraction G+C calculated using equation (4) of SCHILDKRAUT et al. 1962 and expressed as mole % G+C.

[h] all buoyant densities are relative to that of *E. coli* at 1.710 gm cm^{-3}.

[i] RAE 1973; [j] FRANKER et al. 1974; [k] ALLEN et al. 1975 and unpublished data; [l] RIZZO & NOODÉN 1974a; [m] FRANKER 1970; [n] LOEBLICH et al. 1976 and unpublished data.

[p] originally erroneously calculated by FRANKER et al. 1974 to be 37.8.

Prior to the discovery of 5-hydroxymethyluracil in *C. cohnii*, only one other dinoflagellate, a *Zooxanthella* sp., had been analyzed for base composition. Other than the report of less than 1% 5-methyl cytosine, the bases present in this organism were normal. Clearly, the DNA of this species requires reinvestigation. A survey of the DNA of other dinoflagellates would be desirable.

DNA Renaturation Kinetics of *Crypthecodinium Cohnii*

Renaturation kinetic studies of the only dinoflagellate analyzed in detail by ALLEN et al. (1975) indicate that the DNA sequence organization is eucaryotic in its possession of roughly equal amounts of two kinetic classes. *C. cohnii* contains a repeated class representing 55-60% of the DNA and a highly complex class comprising 40-45% of the DNA. From renaturation kinetic analyses the highly complex DNA is calculated to be unique or, at most, repeated two or three times. The repeated fraction is of high complexity for a eucaryotic organism. If the repeated DNA represents only one sequence repeated many times, it would be present in approximately 4000 copies.

Renaturation of isolated repeated DNA (the fraction renatured to C_ot 20) occurs over a broad range of C_ot values, indicative of the presence of many classes of repeats. Thermal denaturation curves of the isolated, renatured, repeated DNA have a lower T_m, suggesting repeats that are approximately 10% mismatched rather than being perfect copies of each other.

Several facts suggest that the repeated DNA is interspersed with unique DNA, as is typical in higher eucaryotes. The lower hyperchromicity of thermal denaturation curves of isolated, renatured, repeated DNA suggests that the pieces (500-600 base pairs in length) of sheared DNA contain unrenatured, single-strand ends. This would be expected for the interspersion of repeated DNA with highly complex DNA. An increase in the length of the fragments being renatured to C_ot 20 from 500-600 base pairs to 3000 base pairs results in an increase in the per cent renatured. Such would be the case if the repeats were interspersed with the highly complex DNA. In preliminary experiments with ^{3}H-adenine and an adenine-requiring mutant of *C. cohnii* radioactive DNA was prepared. When this DNA (length 3000 base pairs) after renaturing to C_ot 20 was exposed to S1 nuclease, the repeated fraction was found to comprise roughly 30-35% of the total. Sizing of the double stranded DNA remaining after S1 nuclease treatment indicated that the repeats were 400-450 base pairs in length. Thus if these repeats were evenly dispersed, they would be interspersed with unique DNA 1000 base pairs in length.

Attempts to measure the molecular weight of dinoflagellate DNA using the viscoelastic technique have not been successful. ROBERTS et al. (1976) offered several suggestions for difficulties encountered in measuring dinoflagellate DNA molecular weights. The difficulties of measuring dinoflagellate DNA by this method have been attributed to several possible factors: the occurrence

of a nuclease that is extremely resistant to denaturation in dinoflagellates, the presence of single stranded regions of their DNA and/or the presence of several pieces of DNA per chromosome.

ACKNOWLEDGMENTS

This investigation was supported by research grants from the National Science Foundation (BMS 74- 22151) and from the National Institute of General Medical Sciences, U.S. Public Health Service (GM 19519 and GM 06637). I wish to thank Alan Brown, Vivian E. Clark, Margo G. Haygood and Lorraine A. Johnsrud for technical assistance. The many stimulating discussions with James R. Allen, Karen E. Fine, Lynn C. Klotz, Laurel A. Loeblich and Thomas M. Roberts were greatly appreciated.

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