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ISOLATION AND PARTIAL CHARACTERIZATION OF DINOFLAGELLATE CHROMATIN*

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Summary

Chromatin was prepared by two different methods from isolated nuclei of *Gyrodinium cohnii* (*Cryptothecodinium cohnii*) and *Peridinium trochoideum*. These isolation procedures are different from those generally used to prepare eukaryote chromatin, because the latter do not work for dinoflagellate chromatin. The chemical composition of this chromatin is similar for both methods of preparation and both organisms. Dinoflagellate chromatin contains DNA, RNA, acid-soluble and acid-insoluble protein as does chromatin from higher plants and animals, but the amount of acid-soluble protein relative to DNA (0.02—0.08) is much lower than that of typical eukaryotes (about 1). Evidence is presented to show that proteolytic degradation is unlikely to account for the low acid-soluble protein content in dinoflagellate chromatin. Exclusion chromatography of the chromatin on large-pore gels (Bio Gel A-15m or Sephadex G-200) indicates that the bulk of the protein present in the chromatin preparations migrates with the DNA. *G. cohnii* and *P. trochoideum* chromatin show an ultraviolet absorption spectrum, which is intermediate between DNA and typical eukaryote chromatin, and this is not significantly changed by gel exclusion chromatography. Preliminary results suggest that the dinoflagellate DNA-associated proteins do not stabilize the DNA against melting. Chromatin prepared from log-phase cells has more protein and RNA than chromatin from stationary-phase cells. The chemical composition of dinoflagellate chromatin is compared with that of prokaryotes and eukaryotes.

Introduction

The chromosomes of the more advanced eukaryotes are much more com-

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plex than those of prokaryotes. Since this large difference in complexity may represent the extremes of an evolutionary continuum [1], the chromosomes of primitive eukaryotes are of special interest as these organisms may represent transition forms between the two extremes [2]. Thus it is significant that the nuclear organization of the dinoflagellates seems to be intermediate between that of prokaryotes and eukaryotes [3—7]. In fact, a third kingdom, the *Mesokaryota*, has been proposed to include the dinoflagellates [8].

One of the most striking differences between the chromosomes of dinoflagellates and those of typical eukaryotes is that their chromosomes remain condensed through the entire cell cycle. Another difference is in the relative amount of protein. Cytochemical tests for basic amino acids in protein suggest that the dinoflagellate chromosome is devoid of basic protein [3,4,9], such as histones, and furthermore, this can be interpreted to show that dinoflagellate chromosomes lack protein of any kind [3,4,10]. In contrast, a study using immunofluorescent techniques indicated that a DNA—histone complex is present in the chromosomes of dinoflagellates [11].

In order to resolve the question about the presence of histone or non-histone proteins associated with the nuclear DNA of dinoflagellates in the present study, chromatin was prepared from isolated nuclei of two dinoflagellates by two different methods. It was found that the chromatin from *Gyrodinium cohnii (Cryptothecodinium cohnii)* and *Peridinium trochoideum* contains some protein, but only a very small portion of this protein is acid-soluble, as are histones, and even the acid-insoluble protein content is less than that of typical eukaryotes. Clearly, the chromatin of these dinoflagellates is very different from that of typical eukaryotes, and different techniques are required to prepare it. A preliminary report of this work has been published [12].

Materials and Methods

Culture conditions and isolation of nuclei

As described for *G. cohnii (C. cohnii)* and *P. trochoideum* by Rizzo and Noodén [13]. These two species are quite different; for example the former is not photosynthetic whereas the latter is.

The term "log phase" is used here to designate rapidly growing cultures, which have not yet reached maximum cell density. Since a given inoculum reaches maximum cell density in a predictable period of time under our highly controlled conditions, the length of the incubation period can be used to estimate the growth phase of the cells. The term "stationary phase" is used here to designate cultures which have stopped dividing, and this can be judged from the incubation time as described above or visually. Under the set of culture conditions employed for *G. cohnii*, for example, log-phase cells were harvested 3.5 days after inoculation. Stationary phase cells were allowed to grow 6—7 days before harvesting. Difficulty in isolating the nuclei prevented a larger temporal separation of log and stationary phase cells. If the cells were harvested too soon, large amounts of "starch" prevented the isolation of clean nuclei. On the other hand, if the cells were allowed to grow for longer than 7 days, very few intact nuclei were recovered due mainly to poor cell disruption resulting from thickening of the cell walls.

Preparation of chromatin—calcium method

This is a modification of the method used by Towill and Noodén [14] for isolation of higher plant chromatin. Purified nuclei were washed twice by suspending them in 5—8 ml of buffer containing 0.14 M NaCl, 5 mM $MgCl_2$, 10 mM Tris (pH 7.6) and centrifuging at 12000 × *g* for 10 min. All operations were carried out at 0—4° C. The resulting pellet of washed nuclei was then suspended in 7 ml of buffer containing 10 mM Tris, 1 mM EDTA (pH 8.0) and sonicated for 3 s at 20000 Hz and maximum output with a Branson S-75 sonifier. The sonicate was then centrifuged at 12000 × *g* for 10 min (centrifugation at 25000 × *g* for 15 min gave the same results).

The pellet was resuspended in 3 ml of the Tris—EDTA buffer, and the previous centrifugation was repeated. The supernatants were combined, made 10 mM in $CaCl_2$ by adding solid $CaCl_2$ [15], allowed to stand 15 min, and then centrifuged at 12000 × *g* for 15 min. The supernatant was discarded. The chromatin pellet was suspended in 5 ml of 0.14 M NaCl—5 mM $MgCl_2$ —10 mM Tris (pH 7.6) and centrifuged at 12000 × *g* for 10 min. The resulting pellet, containing about 80% of the DNA present in the nuclei, constituted the purified chromatin.

Preparation of chromatin—2 M NaCl method

This method is similar to that outlined by Hnilica [16]. Purified nuclei were washed twice by suspending them in 5—8 ml of buffer containing 0.14 M NaCl, 5 mM $MgCl_2$ and 10 mM Tris (pH 7.6) and centrifuging at 12000 × *g* for 10 min. All operations were carried out at 0—4° C. The resulting pellet of washed nuclei was suspended in 10—12 ml of 2 M NaCl—10 mM Tris (pH 8.0) and stirred overnight. The preparation was then centrifuged at 12000 × *g* for 15 min, and the supernatant, which contained about 95% of the DNA present in the nuclei, was removed with a pasteur pipet. The chromatin was precipitated with 1 vol. of 40% trichloroacetic acid overnight and then centrifuged at 25000 × *g* for 15 min. The chromatin pellet was washed twice with 80% ethanol. For chemical determinations, the 2 M NaCl supernatant was divided into 2 portions at the stage immediately preceding the addition of 40% trichloroacetic acid. One portion was used for the determination of DNA and RNA and the other for protein.

Chemical determinations

DNA and RNA were extracted by the method Ogur and Rosen [17]. DNA was determined by the diphenylamine reaction of Burton [18], RNA by the orcinol test [19] and protein by the method of Lowry et al. [20]. Calf thymus or salmon sperm DNA, purified yeast RNA and bovine serum albumin were used as standards. For the determination of protein, all samples (including the standard) were dissolved by heating at 80° C for 10 min in 0.5 M or 1.0 M NaOH.

Extraction of acid-soluble proteins

Nuclei were suspended in 1—2 ml of 0.25 M HCl or 0.125 M H_2SO_4 and extracted for 20 min with intermittant shaking. These suspensions were centrifuged in a Sorvall SS-34 rotor, at 25000 × *g* for 15 min. The pellets pro-

duced were extracted again as described above. The combined supernatants were centrifuged again to remove the small amounts of particulate material that occasionally break away from the 25000 × *g* pellet upon removal of the supernatant. The acid-soluble proteins were precipitated overnight with either 1 vol. of 40% trichloroacetic acid at 4° C or 8 vol. of acetone at −15° C. In both cases, the precipitates were collected by centrifugation at 25000 × *g* for 30 min in a Sorvall SS-34 rotor. The acid precipitates were washed twice with 3—5 ml of 80% ethanol. The acetone precipitates were dried in a desiccator under a partial vacuum.

Gel filtration chromatography

The chromatin used was prepared according to the calcium method through the step immediately before the addition of solid $CaCl_2$. In order to keep the volume small, the 3 ml wash with Tris—EDTA was also omitted. A total volume of 7 ml was applied to a Biogel A-15m (molecular weight exclusion $15 \cdot 10^6$) or Sephadex G-200 (molecular weight exclusion $2 \cdot 10^5$) column pre-equilibrated with 10 mM Tris—1 mM EDTA (pH 8.0). The column dimensions were 25—30 cm × 2.4 cm, and 3—4 ml fractions were collected. The DNA content of the fractions was determined by absorption at 260 nm, and the protein content of the fractions was determined by the method of Lowry et al. [20]. Absorption spectra of the chromatin solutions were determined by checking absorbance at 1 nm intervals before and after passage through the column.

Temperature melting studies

Purified DNA was prepared from pelleted calcium chromatin by the method of Marmur [21] as described by Stern [22] for plant tissues, except that the pronase incubation step (pronase B, Calbiochem) was performed at 37° C. The samples of DNA or chromatin were dissolved in 0.15 M NaCl—0.015 M sodium citrate, pH 7.0, and dialysed against several hundred volumes of 0.015 M NaCl—0.0015 M sodium citrate overnight in the cold with three changes. The A_{260nm} of the samples was adjusted to about 0.5 (approximately 20 μg DNA) with the dialysing buffer, and the samples were placed in cuvettes in a Beckman spectrophotometer. The temperature of the cuvette containing dialysing buffer only was monitored by a thermistor probe inserted into the cuvette. The absorbance at 260 nm was read for all cuvettes at 1° C intervals during the critical portion of the melting curve. In one experiment, 0.25 mM EDTA (pH 8.0) was used as the solvent.

Results

Preparation of chromatin

When the dinoflagellate nuclei are ruptured in the low ionic strength Tris—EDTA medium, the chromosomes are dispersed; the chromatin does not form a gel as does the chromatin from most higher eukaryotes. Because the most common procedures for chromatin isolation depend on formation of a gel at low ionic strength, it was necessary to work out other procedures to isolate the DNA and DNA-associated proteins from dinoflagellates. The first involves

breaking the 0.14 M NaCl washed nuclei and releasing the chromatin in a soluble state. Heavy debris such as nucleoli, nuclear membrane fragments, cell walls, starch grains and unbroken nuclei are then centrifuged out. The heavy debris pellet also contains 10—15% of the cells' DNA depending on the efficiency of nuclear breakage. EDTA was included to aid in nuclear disruption and has been used in the extraction of chromatin from isolated nuclei by others [23—25]. The chromatin extracted in this manner was found extremely difficult to pellet by centrifugation. For example, centrifugation at 269000 × *g* for 2 h pelleted only 23% of the DNA present and centrifugation at 122000 × *g* for 12—16 h pelleted 38—51% of the DNA present.

Since a large percentage of the chromatin could not be pelleted by centrifugation from the Tris—EDTA solutions, attempts were made to precipitate the chromatin from this solution by adding an excess of divalent cations. The procedure of Bhagavan and Atchley [26] which used 20 mM $MgCl_2$ and pH 6.5 to precipitate the deoxyribonucleoprotein from *Bacillus subtilis* enabled 80—90% of the DNA to be centrifuged out at 12000 × *g* for 10 min. Alternatively, the bulk of the DNA present could be pelleted at 12000 × *g* for 10 min if the chromatin solution was adjusted to 10 mM in $CaCl_2$ with solid $CaCl_2$ as described by Frenster et al. [15]. This latter procedure was adopted, because it kept the volume small. The chromatin pellet was washed once with 0.14 NaCl—10 mM Tris—5 mM $MgCl_2$ (pH 7.6) to remove any loosely bound protein and RNA that may have co-precipitated with the chromatin [16,27]. This chromatin will be referred to in the present study as "calcium chromatin". Relative to the DNA present in the nuclei, the yield is 75—85%.

In the second method of chromatin preparation, the chromatin is extracted from washed nuclei by stirring overnight in 2 M NaCl and then precipitated with 20% trichloroacetic acid. Washes with 80% ethanol are included to remove the trichloroacetic acid, which could otherwise interfere with ultraviolet estimates and colorimetric determinations [28]. Chromatin prepared by this method will be designated as "2 M NaCl chromatin" in the present study even though the DNA and the proteins are dissociated. The yield is 93—95% of the DNA present in the nuclei.

Chemical composition of dinoflagellate chromatin

The chemical composition of chromatin from isolated nuclei of *G. cohnii* and *P. trochoideum* is shown in Table I and II, respectively. Comparing the ratios of RNA or protein to DNA for chromatin and nuclei, it can be seen that the chromatin contains only half of the protein from the nuclei and even less, 10—30%, of the RNA.

The relative amounts of DNA, RNA, acid-insoluble and acid-soluble protein depend on the growth phase of the cells at the time of harvest (Table I). With both methods, chromatin prepared from log-phase cells has considerably more RNA, acid-insoluble and acid-soluble protein relative to DNA than chromatin prepared from stationary phase cells. Table I also shows that in almost all instances the protein and especially the RNA and acid-soluble protein contents are higher for calcium chromatin than for the equivalent 2 M NaCl chromatin.

Table II shows the chemical composition of calcium chromatin from log-

TABLE I

CHEMICAL COMPOSITION OF NUCLEI AND CHROMATIN FROM *G. COHNII*

Preparation	Method	RNA/DNA ratio (w/w)	Acid-insoluble protein/DNA ratio (w/w)	Acid-soluble protein/DNA ratio (w/w)	Total protein/DNA ratio (w/w)
Nuclei, log phase		0.32	0.99	0.13	1.12
Chromatin, log phase	Calcium	0.094 ± 0.004*	0.485 ± 0.019	0.075 ± 0.005	0.561 ± 0.019
Chromatin, log phase	2 M NaCl	0.041 ± 0.001	0.451 ± 0.025	0.028 ± 0.001	0.487 ± 0.025
Chromatin, stationary phase	Calcium	0.049 ± 0.003	0.241 ± 0.039	0.059 ± 0.009	0.300 ± 0.034
Chromatin, stationary phase	2 M NaCl	0.029 ± 0.002	0.268 ± 0.051	0.019 ± 0.002	0.287 ± 0.053

* Standard error.

phase cells and 2 M NaCl chromatin from stationary phase of a very different dinoflagellate, *P. trochoideum*. Comparing Tables I and II, one can see that the chemical composition of chromatin from the two dinoflagellates is quite similar.

Since histones (acid soluble) may be degraded by proteases, dinoflagellate nuclei and thence chromatin were prepared in the presence of known inhibitors of proteolysis [29]. The relative amount of acid-soluble protein does not change significantly when chromatin was prepared in the presence of proteolytic enzyme inhibitors phenylmethylsulfonylfluoride or sodium bisulfite (Table III).

Gel filtration chromatography of dinoflagellate chromatin

Fig. 1 shows the behavior of *G. cohnii* chromatin (prepared by the calcium method but applied to the column just before precipitation with $CaCl_2$) in a Bio Gel A-15 m column (molecular weight exclusion $15 \cdot 10^6$). As can be seen from the figure, the bulk of the protein present in the chromatin preparation migrates with the DNA. Since the DNA runs in the exclusion volume with little or no tailing, the DNA has a high molecular weight, above $15 \cdot 10^6$. Chromatography of *G. cohnii* chromatin was also performed using Sephadex G-200 instead of Bio Gel A-15m, and the elution profile was very similar to

TABLE II

CHEMICAL COMPOSITION OF NUCLEI AND CHROMATIN FROM *P. TROCHOIDEUM*

Preparation	Method	RNA/DNA ratio (w/w)	Acid-insoluble protein/DNA ratio (w/w)	Acid-soluble protein/DNA ratio (w/w)	Total protein/DNA ratio (w/w)
Nuclei, log phase		0.22	1.22	0.08	1.30
Chromatin, log phase	Calcium	0.060 ± 0.004*	0.502 ± 0.008	0.056 ± 0.008	0.558 ± 0.009
Chromatin, stationary phase	2 M NaCl	0.029 ± 0.004	0.209 ± 0.028	0.022 ± 0.002	0.230 ± 0.027

* Standard error.

TABLE III

EFFECT OF INHIBITORS OF PROTEOLYTIC ENZYMES ON THE CHEMICAL COMPOSITION OF *G. COHNII* CHROMATIN

In these tests, the sodium bisulfite was included in all solutions used for the preparation of nuclei and chromatin except the 2.2 M sucrose solution. This omission was necessary, because a precipitate formed when bisulfite and Triton X-100 were both present in the 2.2 M sucrose. Phenylmethylsulfonylfluoride (PMSF) was included in the isolation medium and all the solutions used during the preparation of chromatin.

Inhibitor	Preparation	RNA/DNA ratio (w/w)	Acid-insoluble protein/DNA ratio (w/w)	Acid-soluble protein/DNA ratio (w/w)	Total protein/DNA ratio (w/w)
None	Chromatin log phase (calcium method)	0.094	0.485	0.075	0.561
$NaHSO_3$ (5 mM)	Chromatin log phase (calcium method)	0.124	0.492	0.095	0.587
None	Chromatin stationary phase (2 M NaCl method)	0.029	0.269	0.019	0.287
PMSF (25 μM)	Chromatin stationary phase (2 M NaCl method)	0.025	0.241	0.014	0.255

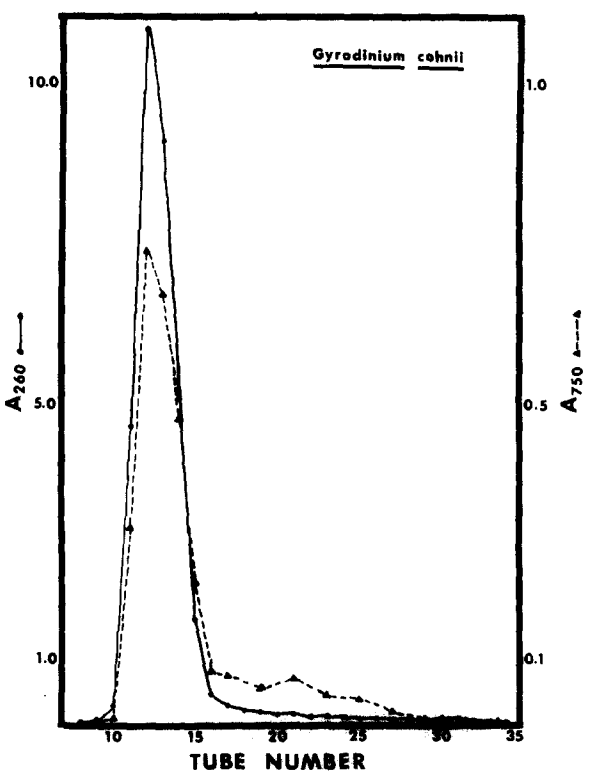

Fig. 1. Chromatography of *G. cohnii* chromatin on a Bio Gel A-15m column. The sample was eluted with 10 mM Tris—1 mM EDTA (pH 8.0). DNA was estimated by absorption at 260 nm, protein by the Lowry method.

that shown in Fig. 1 as was the elution profile of *P. trochoideum* chromatin in Bio Gel A-15m column.

The protein and DNA peaks in Fig. 1 were cut out and weighed to give an estimate of the protein/DNA ratio based on the Lowry method for protein determination and absorption at 260 nm for DNA. The ratios obtained by this method were 0.78 for *G. cohnii* and 0.67 for *P. trochoideum*, which are a bit higher than the ratios obtained by direct chemical determinations (see Tables I and II).

The absorption spectra of the chromatin peak in 10 mM Tris—1 mM EDTA (pH 8.0), before and after chromatography are shown in Fig. 2. Passage through the column alters the shape of the spectrum only slightly; the major change being less absorption in the trough region (230—240 nm). This is most likely due to a difference in turbidity, but it could also be due to removal of some low molecular weight compounds. However, the absorption spectra for *G. cohnii* and *P. trochoideum* both before and after chromatography are similar, but not identical, to typical chromatin preparations [30], showing little absorption at 320 nm with a peak at about 260 nm, a trough at about 240 nm, and a ratio of $A_{280\ nm}/A_{260\ nm}$ of about 0.6. The ratio $A_{230\ nm}/A_{260\ nm}$ is about 0.6, which is a bit lower than typical chromatin (0.7—1.0) and may reflect the lower protein content of dinoglagellate chromatin. Thus dinoflagellate chromatin is intermediate between DNA and typical eukaryote chromatin in its ultraviolet absorption.

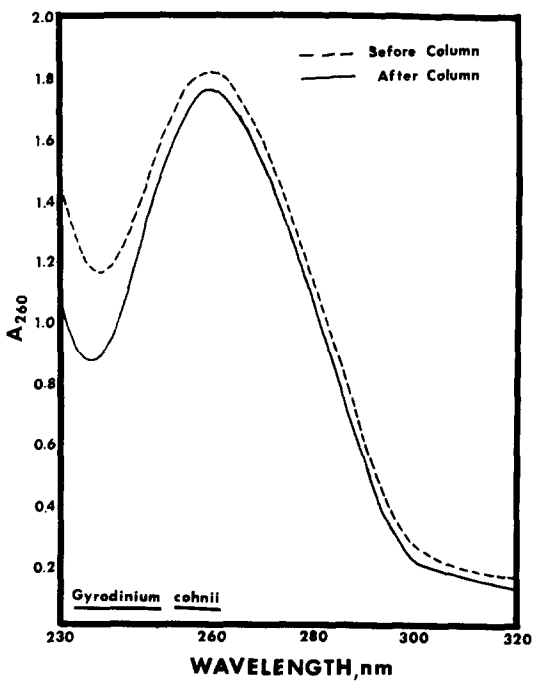

Fig. 2. Ultraviolet absorption spectra of *G. cohnii* chromatin before and after Bio Gel A-15m column chromatography.

Melting of DNA and chromatin

Preliminary experiments were done to see if the DNA-associated proteins of dinoflagellates could stabilize the DNA against melting (increase the T_m). The T_m values in 0.015 M NaCl—0.0015 M sodium citrate were approximately 67, 64 and 66° C for *G. cohnii* DNA, whole chromatin (prepared by the calcium method) and acid-extracted chromatin, respectively, and the melting profiles were indistinguishable. Using $2.5 \cdot 10^{-4}$ M EDTA as the solvent, *G. cohnii* DNA and chromatin had a T_m of about 47° C.

Discussion

DNA-associated proteins in dinoflagellates

Although the earlier cytochemical studies have suggested that the dinoflagellate chromosome is devoid of protein [3,4,10] the present study has shown that dinoflagellate chromatin contains a small amount of acid-soluble protein and a considerable amount of acid-insoluble protein. From the probable role that chromosomal proteins play in the regulation of gene activity and the maintenance of chromosome structure in other eukaryotes [1,2] the presence of DNA-associated proteins would be expected. Indeed, their absence would be surprising. Moreover, Franker [31] has recently reported DNA-binding proteins in *C. cohnii (G. cohnii)*. Why then, have cytochemical tests failed to detect the presence of protein in dinoflagellate chromosomes? It is possible that the amount of protein is too low and the staining levels are too close to the background to be detected by these methods.

Calcium chromatin versus 2 M NaCl chromatin

Since dinoflagellate chromatin does not form a gel which is readily centrifuged from low ionic strength solutions, the standard procedures [23,30] for chromatin isolation do not work, and other techniques had to be developed. The two different methods of chromatin isolation used in the present study produced chromatins with similar but not identical compositions. In general, chromatin prepared by the calcium method contained a higher proportion of RNA and acid-soluble protein. This suggests ribosomal contamination; however, this is considered unlikely, because the nuclei were washed with 0.14 M NaCl to remove nuclear sap proteins and nuclear ribosomes [16,27]. Furthermore, if any free ribosomes were present, they would be extracted with 2 M NaCl and thus should also be present in the 2 M NaCl chromatin.

Since the nuclear membranes and nucleoli are insoluble in 1—2 M NaCl [27,32—36], the nuclear proteins which are not removed with 0.14 M NaCl but are solubilized by 2 M NaCl are probably not nucleolar or nuclear membrane proteins. Although there is a difference in the acid-soluble protein/DNA ratios of the chromatins prepared by the two methods, electrophoresis in urea—acrylamide gels at pH 3.2 shows banding patterns which are quite similar [37]. This similarity would not be expected if the quantitative differences in the acid-soluble proteins stemmed from nuclear membrane or nucleolar protein contamination in the calcium chromatin. Thus we are at present unable to account for the quantitative differences in acid-soluble protein between the calcium and the 2 M NaCl chromatin. Other explanations such as interference

with the Lowry determination [38] or the RNA measurement [28,39] cannot be ruled out especially in the case of acid-soluble protein which is present in very small amounts and therefore more affected by interference. Even though there are small quantitative differences in the RNA and acid-soluble protein content, the two chromatin preparations are very similar in their total protein content and the protein gel electrophoretic patterns [37] which suggests that the two methods produce very similar chromatin preparations.

Since the acid-soluble protein (and therefore histone) content is very low and chromatin aggregation is known to be dependent on histones [40], the absence (or paucity) of histones explains why the dinoflagellate chromatin does not aggregate in low ionic strength solutions and why the standard procedures for chromatin isolation did not work.

How representative is the isolated dinoflagellate chromatin?

As with chromatin prepared from any organism, there is always a question about the inclusion of components that were not present in vivo or the removal of components that were present in vivo. In order to minimize cytoplasmic contamination, chromatin was prepared from isolated nuclei rather than directly from whole cells. In addition, chromosomal material is also less likely to be lost from the intact nucleus. Thus the addition or removal of material is largely limited to the steps going from nuclei to chromatin. The fact that the composition of dinoflagellate chromatin is quite similar when prepared by two different methods indicates that no gross changes occurred during preparation from the nuclei.

Experiments with inhibitors of proteolytic enzymes indicate that the low protein/DNA ratio, in particular the low acid-soluble protein/DNA ratio, is not due to proteolysis such as the degradation of histones from rat liver [29]. In addition, the acid-soluble protein content of the nuclei is also low, and these are isolated quickly enough that the effect of proteolytic enzymes should be minimal. The fact that the purified nuclei were used as starting material greatly lessens the chance of contamination due to cytoplasmic matter, and the centrifugation following nuclear rupture should remove the larger particles. Furthermore, the low RNA/DNA ratios also indicate that ribosomal contamination is at most very small. On the other hand, the movement of almost all of these proteins with the DNA on large-pore exclusion gels suggests that they are associated with the DNA. Finally, the low protein contents reported here are consistent with the histochemical observations which show that dinoflagellate chromosomes have a low protein content relative to typical eukaryote chromosomes [9].

The values given for *P. trochoideum* chromatin compare quite well with the corresponding chromatin from *G. cohnii* suggesting that data are representative for all dinoflagellate chromatin.

Chromatin from stationary and log-phase cultures

Since the transcriptional activity can be expected to differ in dividing and nondividing cells, it is of interest to compare the chromatin from these cells. The more active cells (dividing) contain not only more RNA but also more protein, both acid soluble and acid insoluble. This is in agreement with other

TABLE IV

CHEMICAL COMPOSITION OF CHROMATIN FROM VARIOUS ORGANISMS

Source of chromatin	RNA/DNA ratio (w/w)	Acid-soluble protein/DNA ratio (w/w)	Acid-insoluble protein/DNA ratio (w/w)	Reference
Rat liver	0.03	1.10	0.67	44
Sea urchin (blastula)	0.08	1.02	0.60	45
Sea urchin (gastrula)	0.08	1.04	1.15	45
Pea bud	0.05	1.10	0.41	46
S. cerevisiae	0.11	1.17	0.50	47
N. crassa	0.14	0.24	0.60	48
M. gypseum	0.05	0.03	1.20	49
E. coli		0.24		50
A. cylindrica	0.05	0.02	0.07	51
*G. cohnii**	0.04	0.03	0.45	Present study
*G. cohnii***	0.03	0.02	0.27	Present study
P. trochoideum	0.03	0.02	0.21	Present study

* 2 M NaCl chromatin from log-phase cells
** 2 M NaCl chromatin from stationary-phase cells

observations that an increase in the level of nonhistone proteins is correlated with an increase in genetic activity [23,41—43]. There is also a marked increase in the amount of nonhistone protein in chromatin from the gastrula stage as compared to the blastula stage of sea urchin embryos (Table IV), which correlates with increased template activity.

Dinoflagellate chromatin compared with other organisms

Table IV shows the differences between the chemical composition of dinoflagellate chromatin and that of several eukaryotes. Although the acid-insoluble protein content is lower than typical eukaryotes, it is similar. The most striking difference is the amount of acid-soluble protein relative to DNA. This value is approximately one for higher plants and animals, and most of the acid-soluble protein is histone. In dinoflagellates, however, the acid-soluble protein/DNA ratio is only 0.02—0.08, 12—50 times lower than that of higher plants and animals. In fact, the dinoflagellates studied here have a protein content resembling that of the prokaryotes *Escherichia coli* [50] and *Anabaena cylindrica* [51]. Although there are several studies on bacterial chromatin, the example given in Table IV was chosen, because these investigators used more rigorous methods of preparing their chromatin than those used in other studies. Like the dinoflagellates, the chromatin from other eukaryotes, *Microsporum gypseum* [49] and *Allomyces arbuscula* [52] is reported to have a very low acid-soluble protein/DNA ratio. By contrast, another fungus, the yeast *Saccharomyces cervisiae* [47] has a ratio of about 1, like typical eukaryotes, while *Neurospora crassa* seems to be intermediate between these extremes. The data presented here clearly show that the acid-soluble protein content (and therefore also the histone content) of dinoflagellate chromatin is much lower than that of the typical eukaryotes; however, some other eukaryotes particularly the fungi may resemble the dinoflagellates.

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