Chromosomal Proteins in the Dinoflagellate Alga Gyrodinium cohnii

Abstract. *Chromatin has been prepared from nuclei isolated from the dinoflagellate alga* Gyrodinium cohnii. *This chromatin contains RNA, acid-insoluble proteins, and acid-soluble proteins; the respective ratios to amount of DNA are about 0.09, 0.48, 0.08 (by weight). Not only is the amount of acid-soluble protein associated with the DNA much less than it is in the typical eukaryote, but polyacrylamide gel electrophoresis in urea at* p*H 3.2 produces a banding pattern different from that of typical histones. There is one predominant band that migrates about as fast as does histone IV from corn. These findings are of interest, because the nuclear organization in the dinoflagellates appears to be intermediate between the prokaryotes and the eukaryotes.*

The chromosomes of dinoflagellate algae possess some characteristics which may prove helpful in analyzing the chromosome structure and the regulation of gene action in eukaryotes. It has been reported that the chromosomes remain condensed throughout the mitotic cycle (*1*, *2*), and also that they (according to cytochemical tests) lack histones or protein of any kind (*1*, *3*, *4*). In contrast, a study with immunofluorescent techniques indicates that a complex of histone-DNA is present in the chromosomes of dinoflagellates (*5*).

Since many believe that histones are responsible for the repression of gene activity (*6*, *7*) and possibly even for the maintenance of chromosome condensation (*8*), the question of whether or not histone or any protein is associated with DNA in dinoflagellate algae is significant. Furthermore, the nuclear organization of the dinoflagellates is considered to be intermediate between that of prokaryotes and eukaryotes (*3*, *9–11*), and a third kingdom, the Mesokaryota, has been proposed to include these organisms (*10*). Thus, the study of the dinoflagellate nucleus and chromatin might shed some light on the evolution of eukaryotes.

We report here, based on direct chemical determinations, that two dinoflagellate chromatins do indeed contain protein; however most of this protein is not soluble in acid as are histones. The small amount of protein that is soluble in acid does not give the characteristic histone band patterns on polyacrylamide gel electrophoresis.

An axenic culture of *Gyrodinium cohnii* (Schiller) (*12*) was grown in a modified AXM medium (*11*) at 23°C, and the cells were collected by centrifugation.

Purified nuclei were used as the starting material for the isolation of chromatin. Cells in mid log phase of growth were broken by light sonication in a modified Honda medium at 4°C (*13*), and nuclei were obtained by differential centrifugation through a discontinuous sucrose gradient containing 0.1 percent Triton X-100 (Rohm and Haas). A typical preparation contained about 2×10^8 nuclei, each with 6.9 pg of DNA. The purity of these nuclear and chromatin preparations was determined by staining with methyl green–pyronin B (*14*), which produces blue chromosomes and reddish cytoplasms and nucleoli.

Chromatin was prepared by modification of Frenster's method (*15*). Briefly, isolated nuclei were washed twice with a solution of 0.14*M* NaCl, 5 m*M* $MgCl_2$ and 10 m*M* tris(hydroxymethyl)aminomethane (tris) (*p*H 7.6) (*16*), and suspended in a solution of 10 m*M* tris and 1 m*M* ethylenediaminetetraacetic acid (EDTA) (*p*H 8.0) with a homogenizer (Potter-Elvehjem). This suspension was sonicated for 3 seconds at 20,000 cycle/sec on a Sonifier (Branson S-75). This mixture was then centrifuged at 12,000*g* for 10 minutes, and the pellet was washed with a solution of 10 m*M* tris and 1 m*M* EDTA (*p*H 8.0). The combined supernatants were adjusted to 10 m*M* in $CaCl_2$, allowed to stand for 15 minutes, and then centrifuged at 12,000*g* for 15 minutes. The pellet (about 80 percent of the DNA present in the nuclei) was washed with a solution of 0.14*M* NaCl, 5 m*M* $MgCl_2$, and 10 m*M* tris (*p*H 7.6) to remove any loosely bound protein [nuclear sap proteins or ribosomes (*16*)] and constituted the purified chromatin.

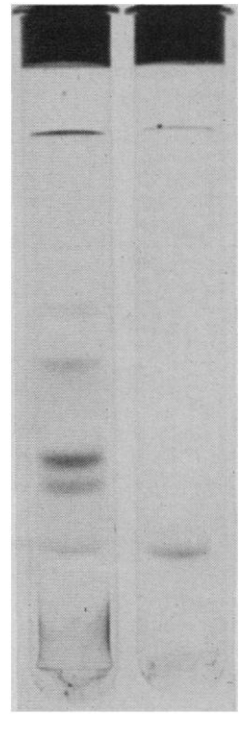

Fig. 1. Electrophoresis in polyacrylamide gels of chromosomal proteins, soluble in 0.25*N* H_2SO_4, from *Gyrodinium cohnii* and from corn root. These gels were stained overnight in 0.5 percent Buffalo Black (Allied Chemical), and the stain was removed by diffusion in a solution of 7.5 percent acetic acid and 20 percent ethanol. (Left) Soluble protein from corn root chromatin, ~ 25 μg; (right) soluble protein from *G. cohnii* chromatin, ~ 10 μg.

The RNA and DNA were prepared by the Ogur-Rosen method (*17*). The amount of DNA was determined by the diphenylamine test (*18*), that of RNA by the orcinol test and ultraviolet absorption (*19*, *20*), and that of protein by the Lowry method (*20*). Because the orcinol test gave variable results with these nuclei, the amount of RNA was determined by absorption at 260 nm, and probably represents a slight overestimate (*19*). The chromatin was extracted twice for 20 minutes with intermittent shaking in 0.25*N* HCl or 0.25*N* H_2SO_4 (no difference noted between the two acids) to obtain the acid-soluble proteins. These acid extracts were then precipitated overnight at 4°C with eight volumes of acetone and centrifuged. The pellet was dissolved in 10*M* urea and electrophoresed by the method of Panyim and Chalkley in 6.25*M* urea (*21*).

The ratio of the amount of protein to the amount of DNA and especially the ratio of the amount of RNA to that of DNA in the chromatin from *G. cohnii* are much lower than those of the nuclei. Thus the chromatin fraction, even though it represents a high percentage of the nuclear DNA (80 percent), carries with it only a small proportion of the nuclear RNA and total protein, but a very high percentage of the acid-soluble protein which precipitates with the DNA (Table 1). Hence, there is little or no loss of the acid-soluble protein due to proteolysis, or washing during the preparation of chromatin from the nuclei.

Another feature of this chromatin is that the ratio of the amount of protein to the amount of DNA is lower than usual for eukaryote chromatin [see (*6*) for a comparison]. On the other hand,

the ratio of the amount of protein to that of DNA in dinoflagellate chromatin is higher than that in prokaryotes; for example, in blue-green algae the ratio is 0.09 (*22*), and in a bacterium, it is 0.25 (*23*). Although higher ratios of amounts of protein to amounts of DNA have been reported for bacteria, most of these preparations were probably greatly contaminated with ribosomes or other material (*7*). It appears, then, that the amount of protein associated with the DNA of dinoflagellates is greater than that with the DNA of prokaryotes, yet definitely less than that with the DNA of eukaryotes. The greatest difference between dinoflagellate chromatin and that of typical eukaryotes is the ratio of the amount of acid-soluble protein to the amount of DNA, which is about 0.08 for *G. cohnii* and about 1 (due mainly to the histones) for typical eukaryotes (*6*).

Figure 1 shows the electrophoretic profiles of acid-soluble proteins from corn-root chromatin and from *G. cohnii* chromatin in polyacrylamide gels with urea at *p*H 3.2. The acid-soluble proteins from the chromatin of corn, a typical eukaryote (prepared by essentially the same method used with dinoflagellates), contains the major histone bands, while the acid-soluble protein from dinoflagellates migrates as one major band with a mobility about the same as histone IV from corn. Although the mobility of the major band of the acid-soluble protein from dinoflagellates is very similar to the histone IV band of corn, the two bands do not stain the same color with Buffalo Black. If the gels are loaded with three times as much protein, a second prominent band becomes apparent directly behind the single band, and several minor bands of lesser mobility are also evident. As judged by the amount of stain in these bands, the major band is about 80 percent of the total acid-soluble protein. The acid solubility and the electrophoretic mobility of the major components at *p*H 3.2 indicate a possibility that the protein (or proteins) comprising the dominant band are small basic proteins like histones; nevertheless, it would be highly premature to conclude that these bands do or do not represent histones.

With chromatin prepared by this and other methods, there is always a question of the inclusion of proteins and RNA which do not belong. We believe that the washes with 0.14*M* NaCl remove the bulk of these (*16*). This is supported by our observation that the bulk of the protein present in the chromatin preparation (including the major acid-soluble protein) just before precipitation with $CaCl_2$ migrates with the DNA in large pore chromatographic exclusion gels such as Sephadex G-200 and Bio-Gel A-15 (molecular weight exclusion, 15×10^6). On the other hand, the larger debris present after disruption of the nuclei, such as unbroken nuclei, nuclear membranes plus a few cell wall fragments and "starch" grains, is centrifuged out before adding the $CaCl_2$. The preparation of chromatin by extracting washed nuclei with 2*M* NaCl (*16*) provides chromatin similar to that from the $CaCl_2$ method but with slightly less RNA, and acid-soluble and acid-insoluble protein. This indicates that our determinations of the composition of *G. cohnii* chromatin are accurate. Furthermore, the chemical composition and the electrophoretic pattern of the acid-soluble protein (or proteins) of chromatin from another dinoflagellate, *Peridinium trochoideum*, are similar to that of *G. cohnii*; thus our findings may be valid for dinoflagellates in general.

Table 1. Chemical composition of nuclei and chromatin from *Gyrodinium cohnii*. The chromatin was prepared by the $CaCl_2$ method. Results are expressed as means ± standard errors; the numbers in parentheses indicate the number of determinations.

Ratio to DNA of:	Nuclei	Chromatin
RNA	0.30 ± 0.008 (4)	0.091 ± 0.006 (6)
Acid-insoluble protein	1.09 ± 0.222 (3)	0.485 ± 0.031 (5)
Acid-soluble protein	0.09 ± 0.027 (3)	0.075 ± 0.013 (5)
Total protein	1.18 (3)	0.560 (5)

Although we confirm the earlier cytochemical observations that histones (acid-soluble protein) are not major chromosomal constituents in dinoflagellates, we find that chromatin prepared by two different methods does contain a very small amount of acid-soluble protein and a considerable amount of acid-insoluble protein. The observation that protein of any kind is present in the dinoflagellate chromosome is contrary to nearly all of the earlier (cytochemical) observations. Results from such studies have been taken as evidence that dinoflagellate chromosomes do not contain either proteins that are bases or protein of any kind (*1, 3, 4*). The limitations and difficulties involved in using cytochemical methods for the detection of proteins in situ (*5, 9, 24*); it is possible that the concentration of protein in dinoflagellate chromosomes is too close to the background concentration to be detected by staining. Since the acid-soluble chromosomal proteins are present in only very small quantities in dinoflagellates, it seems unlikely that they play the major role in gene repression that histones appear to have in typical eukaryotes.

PETER J. RIZZO
LARRY D. NOODÉN
Botany Department, University of Michigan, Ann Arbor 48104

References and Notes

1. J. D. Dodge, in *The Chromosomes of the Algae*, M. B. E. Godward, Ed. (Arnold, London, 1966), p. 96.
2. Y. Bouligand, M. O. Soyer, S. Puiseux-Dao, *Chromosoma* **24**, 251 (1968); M. O. Soyer, *ibid.* **33**, 70 (1971).
3. J. D. Dodge, *Arch. Mikrobiol.* **48**, 66 (1964).
4. D. F. Kubai and H. Ris, *J. Cell Biol.* **40**, 508 (1969).
5. J. M. Stewart and J. S. Beck, *J. Protozool.* **14**, 225 (1967).
6. J. Bonner, M. E. Dahmus, D. Fambrough, R. C. Huang, K. Marushige, D. Y. H. Tuan, *Science* **159**, 47 (1968).
7. S. C. R. Elgin, S. C. Froehner, J. E. Smart, J. Bonner, *Advan. Cell Mol. Biol.* **1**, 1 (1971).
8. J. E. Hearst and N. Botchan, *Annu. Rev. Biochem.* **39**, 151 (1971).
9. G. de Haller, E. Kellenberger, C. Rouiller, *J. Microsc. Paris* **3**, 627 (1964); D. F. Kubai and H. Ris, *Annu. Rev. Genet.* **4**, 263 (1970); P. M. M. Rae, *J. Cell Biol.* **46**, 106 (1970);
10. R. G. Zingmark, *Amer. J. Bot.* **57**, 586 (1970).
11. J. D. Dodge, *Excerpta Med. Int. Congr. Conf. Protozool. 2nd* **91**, 339 (1965).
12. L. Provasoli and K. Gold, *Arch. Mikrobiol.* **42**, 196 (1962).
13. This culture was provided by Dr. G. G. Holz, Jr., State University of New York, Syracuse.
14. S. I. Honda, T. Hongladarom, G. G. Laties, *J. Exp. Bot.* **17**, 460 (1966).
15. W. A. Jensen, *Botanical Histochemistry* (Freeman, San Francisco, 1962), p. 250.
16. J. H. Frenster, V. G. Allfrey, A. E. Mirsky, *Proc. Nat. Acad. Sci. U.S.* **50**, 1026 (1963).
17. L. Hnilica, *Progr. Nucl. Acid Res. Mol. Biol.* **7**, 25 (1967).
18. M. deDeken-Grenson and R. H. deDeken, *Biochim. Biophys. Acta* **31**, 195 (1959).
19. K. Burton, *Biochem. J.* **62**, 315 (1956).
20. Z. Dische, in *The Nucleic Acids*, E. Chargaff and N. Davidson, Eds. (Academic Press, New York, 1955), vol. 1, p. 301.
21. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
22. S. Panyim and R. Chalkley, *Arch. Biochem. Biophys.* **130**, 337 (1969).
23. F. Makino and J. Tsuzuki, *Nature* **231**, 446 (1971).
24. M. Kadoya, H. Mitsui, Y. Takagi, *Biochim. Biophys. Acta* **91**, 36 (1964).
25. D. P. Bloch and H. Y. C. Hew, *J. Biophys. Biochem. Cytol.* **8**, 69 (1960); H. Swift, in *The Nucleohistones*, J. Bonner and P. O. P. Ts'o, Eds. (Holden-Day, San Francisco, 1964), p. 169; N. R. Ringertz and A. Zetterberg, *Exp. Cell Res.* **42**, 243 (1966).

We thank L. E. Towill for the corn root histone and for helpful suggestions. Supported in part by NIH predoctoral fellowship (5 F01 GM43747-02) to P.J.R.

20 January 1972; revised 20 March 1972