in metabolism of each of these components and others with geminal-dichloro groupings, at least in the variety of systems used in examining the metabolism of toxaphene component **1**. Preliminary findings (*13*) with **3** in the iron(II) hematin system indicate that the dichloromethyl substituent is less susceptible to attack than other substituents such as the ring geminal-dichloro group.

Relatively few major polychlorobornane components of toxaphene have proper configurations for high toxicity. This structural specificity and the available knowledge concerning the potency of polychlorobornane metabolites suggest that metabolic reductive dechlorination in the geminal-dichloro substituent and dehydrochlorination are likely to detoxify many toxaphene components or initiate a series of metabolic events leading to their detoxification. The toxicity of **1** to mice and houseflies is increased by a factor of 5 to 8 by piperonyl butoxide (*10*), indicating the importance of cytochrome P-450–mediated detoxification mechanisms (*12*), either reductive (*9, 13*) or oxidative (*9*). Piperonyl butoxide also increases the toxicity of several other polychlorobornanes and polychlorobornenes (Table 1). A combination of reductive dechlorination at geminal-dichloro groups, dehydrochlorination, and oxidation of carbon substituents probably contributes to the extensive dechlorination noted for toxaphene components in rats (*8*). These findings help explain the low persistence of toxaphene as compared with that of many other chlorinated hydrocarbons in mammals (*23*). They also suggest possible pathways for environmental degradation of toxaphene residues.

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Histone Occurrence in Chromatin from *Peridinium balticum*, a Binucleate Dinoflagellate

Abstract. Peridinium balticum *is one of two dinoflagellates known to have dissimilar nuclei together in the same cell. One nucleus (dinokaryotic) has permanently condensed chromosomes, while the other (eukaryotic) does not have morphologically distinct chromosomes. Acid extracts of chromatin prepared from a mixture of dinokaryotic and eukaryotic nuclei and purified eukaryotic nuclei give four bands that co-migrate with four of the five histones from calf thymus when analyzed in urea-containing polyacrylamide gels.*

Although it has been shown that the chromosomes of dinoflagellate algae do not contain histones, all of the studies on histone occurrence in these organisms were done on uninucleate dinoflagellates. We now report on the occurrence of histones in a binucleate dinoflagellate and provide evidence that one of the two dissimilar nuclei present in *Peridinium balticum* contains four acid-soluble proteins which comigrate with histones H1, H2a, H2b, and H4 from calf thymus in urea-containing polyacrylamide gels. Dinoflagellates are eukaryotic algae that have several bacterial traits with respect to the organization of their nuclei and chromosomes. The occurrence of prokaryotic traits in a eukaryotic nucleus has led many investigators to suggest that the nuclear organization of dinoflagellates is intermediate between that of prokaryotes and eukaryotes (*1–5*). Some of the unusual features of dinoflagellate nuclear organization are the absence of a centromere and conventional mitotic spindle (*6*), and the presence of extremely high amounts of DNA (*3, 7, 8*). The DNA is organized into numerous, identical appearing chromosomes (*4, 9*) that are attached to the nuclear envelope (*2, 6*). The nuclear envelope does not disintegrate during division (*6*), and the chromosomes remain condensed throughout the entire division cycle (*2, 6, 9*). Although little is known about the genetics of dinoflagellates, recent work suggests that the vegetative cells of dinoflagellates are haploid (*10*), and genetic recombination occurs in at least some species (*11*). One of the most interesting aspects of dinoflagellate chromosomes is the absence of histones. Although typical eukaryotic histones are absent, chromatin from isolated nuclei of *Crypthecodinium cohnii* (*12, 13*) and *Peridinium trochoideum* (*13*) log phase cells contains a histone-like protein that has a mobility in acid-urea polyacrylamide gels slightly lower than that of histone H4. The protein from *Crypthecodinium cohnii* has been partially characterized and does not appear to be analogous to any of the five vertebrate histones (*13*).

In addition to the peculiar features of the dinoflagellate nucleus, an even more intriguing condition exists in the binucleate dinoflagellates *Glenodinium foliaceum* (*14*) and *Peridinium balticum* (*15*). These organisms have two dissimilar nuclei within the confines of a single cell. One of these nuclei (dinokaryotic) is similar in ultrastructure to that of uninucleate dinoflagellates and contains permanently condensed chromosomes. The chromatin of the second nucleus (eukaryotic) is not organized into chromosomes at any stage in the cell cycle. It was recently suggested (*16*) that *Peridinium balticum* obtained its eukaryotic nucleus after the invasion of a chrysophyte-like endosymbiont. Al-

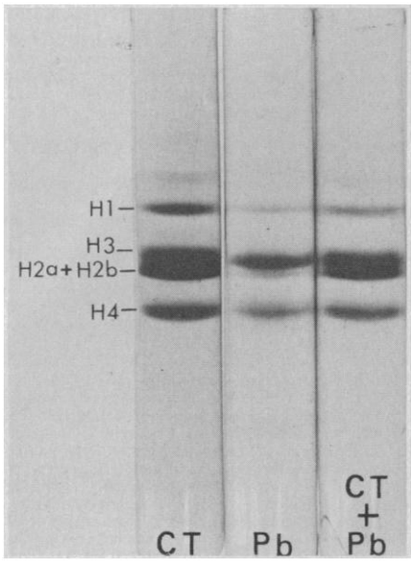

Fig. 1. Comparison of *Peridinium balticum* acid-soluble proteins with calf thymus histones by polyacrylamide gel electrophoresis in the presence of 6.25*M* urea. Electrophoresis was carried out for 3 hours at 2 ma per gel. Gels (7.5 cm) were stained for 2 hours with 0.5 percent (weight to volume) naphthol blue black (Sigma) in 20 percent (by volume) ethanol and 7.5 percent (by volume) acetic acid, and destained by diffusion in 20 percent ethanol and 7.5 percent acetic acid. *CT*, calf thymus whole histone (50 μg). *Pb*, *Peridinium balticum* acid-extract of chromatin prepared from dinokaryotic plus eukaryotic nuclei. Approximately 20 μg of protein was applied to the gel. *CT + PB*, coelectrophoresis of calf thymus (20 μg) and *Peridinium balticum* (20 μg).

though this second nucleus has been termed eukaryotic, it has several characteristics indicating that it is not a typical eukaryotic nucleus (*17*), including the absence of a spindle apparatus.

Cell disruption and isolation of dinokaryotic plus eukaryotic nuclei and the purification of eukaryotic nuclei were achieved by modifying methods described for the isolation of nuclei from *Peridinium trochoideum* (*8*). Cultures of *Peridinium balticum* in the log phase of growth (*18*) were covered with aluminum foil for 24 hours to reduce the number and size of accumulated starch grains, which otherwise tended to collide with and rupture nuclei during subsequent sonication; the dark treatment also reduced the amount of sonication necessary to achieve satisfactory cell breakage. The cells from 2 liters of culture (5×10^4 cell/ml) were collected by centrifugation and disrupted by sonication in 60 ml of a slightly modified Honda medium (*19*). To inhibit serine protease activity, 0.6 ml of a stock solution of $10^{-2}M$ phenylmethysulfonylfluoride (PMSF) in ethanol was added to the isolation medium immediately before use (the final PMSF concentration was 0.1 m*M*). Nuclei were prepared by differential centrifugation through solutions of sucrose or of sucrose plus dextran (*8*). The purity of all nuclear preparations was checked by light microscopy; the cells were stained with methyl green-pyronin B, which stains nuclei blue and cytoplasm pink (*20*). The nuclei appeared free of cytoplasmic contamination, although some cell wall fragments and starch grains were present.

Chromatin was prepared from isolated nuclei by the calcium precipitation method (*21*), except that 0.1 m*M* PMSF was included in the nuclear isolation medium and the 0.14*M* NaCl used for washing the nuclei unless indicated otherwise. The chromatin thus prepared had a ratio of RNA to DNA of 0.04. DNA and RNA were separated (*22*); DNA was estimated by the diphenylamine reaction (*23*), RNA was estimated by the orcinol reaction (*24*), and protein was estimated by the Folin procedure (*25*) with the use of calf thymus DNA, yeast RNA, and calf thymus histone as standards.

When acid-soluble proteins (*26*) from chromatin of dinokaryotic plus eukaryotic nuclei are subjected to electrophoresis in polyacrylamide gels containing 6.25*M* urea (*27*), four major bands are detected; these bands have the same mobilities as the four major bands from calf thymus histone, the most commonly used electrophoretic standard for eukaryotic histones (Fig. 1). Coelectrophoresis of the dinoflagellate and calf thymus samples provides further evidence of their identical mobilities (Fig. 1). If nuclei are isolated in the absence of PMSF a faint, high mobility band, presumably a degradation product, is present.

Although the most sharply defined resolution of histones H1, H3, and H4 is obtained in gels containing 6.25*M* urea, the maximum number of major bands from cell nuclei of vertebrates is obtained with gels containing 2.5*M* urea (*27*). It was therefore of interest to compare the banding patterns of dinoflagellate and calf thymus samples in gels containing 2.5*M* urea. Only four bands are detectable in the dinoflagellate sample (Fig. 2). Specifically, there is no band present in the position where calf thymus histone H3 migrates. It is interesting that the same result was obtained by Cohen and Stein (*28*) when they compared acid extracts of *Phycomyces blakesleeanus* chromatin with that of HeLa S_3 cells. If dinoflagellate chromatin is washed with 5 percent perchloric acid, which selectively removes H1 (*29*), a band is no longer

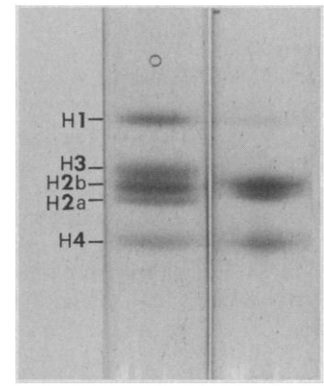

Fig. 2. Polyacrylamide gel electrophoresis in the presence of 2.5*M* urea. Left, calf thymus histone. Right, *Peridinium balticum* acid extract of chromatin prepared from eukaryotic nuclei. Approximately 40 μg of protein was applied to each gel.

present in the position where calf thymus H1 migrates. Preliminary results with sensitivity to ferric chloride destaining (*30*), in which plant and animal histones H1 and H2B are selectively destained, also suggests that this band is probably H1. However, the absolute identification of the dinoflagellate proteins in terms of the five vertebrate histones must await further analysis such as determination of molecular weights and amino acid analysis, as well as chromatographic and solubility behavior (*31*).

Although purified eukaryotic nuclei can be prepared from *Peridinium balticum*, the yield is much lower than that of nuclear preparations containing both kinds of nuclei. Hence, initial electrophoretic analyses were done with acid extracts of chromatin prepared from a mixture of dinokaryotic and eukaryotic nuclei. However, two lines of evidence show that the eukaryotic nucleus is the source of the basic proteins described in our study. First, cytochemical staining with alkaline fast green, a specific stain for basic proteins (*32*), gave a negative test for the dinokaryotic nucleus and a positive test for the eukaryotic nucleus of *Peridinium balticum* with whole cells or isolated nuclei. The negative test for the dinokaryotic nucleus is in agreement with previous results with this stain (*1*, *9*) and suggests that this nucleus is similar to that of uninucleate dinoflagellates in its content of basic proteins. Second, when acid extracts of chromatin prepared from eukaryotic nuclei free of dinokaryotic nuclei are analyzed electrophoretically, the same gel pattern is observed.

Studies on the occurrence of histones in lower eukaryotes are useful for determining the evolutionary history of this highly conserved group of proteins.

However, owing to difficulties in isolating purified nuclei in high yields, and problems with proteolytic degradation, progress in this area has been slow and many conflicting reports exist (*33*). In our report, the low ratio of DNA to RNA of the chromatin indicates that ribosomal contamination is insignificant, and the gels show no indication of proteolytic activity if PMSF is included in the isolation medium.

Our results show that the eukaryotic nucleus of the binucleate dinoflagellate *Peridinium balticum* contains basic proteins with the same gel mobilities as four of the five histones from calf thymus. The question then arises as to whether these proteins should be called histones. Caution should be used in this regard because of the possibility that low-molecular-weight basic proteins arising from ribosomal or other cytoplasmic contamination can migrate in the same region of a gel as histones (*33, 34*). Furthermore, there are histone-like nonhistone chromosomal proteins which have gel mobilities nearly identical to those of known histones (*13, 35*). Thus the co-migration of a particular low-molecular-weight basic protein with a known histone could be coincidental. However, it is unlikely that the co-migration of all four of the bands detected in our study with four calf thymus histones is purely coincidental. It is suggested, therefore, that the proteins detected in our study are histones, although definite proof will require characterization of the individual bands. If these proteins are indeed histones, the apparent absence of H3 requires explanation. If just one histone is absent, one would not expect it to be a histone as highly conserved as H3 (*36*). It should be kept in mind that H3 could be present but may migrate in an anomalous manner. For example, the existence of histones in *Euglena* has recently been confirmed (*37*), and H1 was found to migrate in an anomalous manner. It is possible that H3 from *Peridinium balticum* has a slightly higher mobility than H3 from calf thymus and merges with the band of next highest mobility.

It is suggested that mitotic chromosome morphology is a result of further compaction of the nucleosomes, the DNA-histone complexes which constitute the basic structure of interphase chromatin (*38*). Thus one would expect histones to be present in organisms possessing morphologically distinct chromosomes, but absent in organisms whose DNA does not condense into chromosomes, and this is usually the case. A unique situation may therefore exist in binucleate dinoflagellates where we find a nucleus containing condensed chromosomes but no histones, and a nucleus containing histones but lacking condensed chromosomes.

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Chemical Evidence for Separating the Psilotaceae from the Filicales

Abstract. *The distribution of flavonoid compounds in* Psilotum, Tmesipteris, *and primitive filicalean ferns shows that it is unlikely the two groups are closely related. The Psilotaceae synthesize only amentoflavone and related biflavonyls which are totally absent from ferns. The primitive ferns contain the full complement of flavonols and proanthocyanidins, as found in all other filicalean families.*

The two living psilophyte genera, *Psilotum* and *Tmesipteris*, have been regarded as relics of ancient Devonian Rhyniophytes. As such, they are considered to be living fossils although there is no direct evidence regarding the origin and early evolution of the Psilotaceae and no *Psilotum*-like remains have been identified with certainty from intervening geological periods (*1*). Bierhorst has suggested, on the grounds of the similarity of the gametophytes and other morphological and anatomical features, that the primitive fern family Stromatopteridaceae and the Psilotaceae are very closely related and that the Psilotaceae should be transferred to the order Filicales and hence be regarded as primitive ferns (*2*). This proposal has, however, met with some degree of opposition (*3*), but the problem has not, so far, been resolved. This report presents information on the distribution of flavonoids in the two groups which strongly suggests that they are unrelated.

An analysis of the distribution of sec-