ULTRASTRUCTURAL AND BIOCHEMICAL NUCLEAR ASPECTS OF EUKARYOTE CLASSIFICATION: INDEPENDENT EVOLUTION OF THE DINOFLAGELLATES AS A SISTER GROUP OF THE ACTUAL EUKARYOTES?

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Abstract. Structural and functional features of the dinoflagellate nucleus are examined and compared to those commonly found in Prokaryotes and in Eukaryotes. It appears that dinoflagellate protists, while showing several ancestral characters also found in Prokaryotes are above the prokaryote level in terms of their organization but below that of the other Eukaryotes. Some characters are typical of dinoflagellate nuclei alone, and no correspondence is found in either bacterial nucleoïd or typical eukaryote nuclei. This supports Loeblich's (1976) proposal that dinoflagellate evolution may have been independent of that of the Eukaryotes. This concept can now be refined using an argumentation plan *sensu* Hennig (Hennig and Schlee, 1978) and appears to be in accordance with the Mesokaryote model introduced by Dodge (1965).

1. Introduction

Dinoflagellate protists are a large, highly diversified group of planktonic algae which comprise a variety of morphologies and life styles, and different levels of complexity. Paleontological investigations have shown that Dinoflagellates form an important phytoplankton group since the Jurassic (Sarjeant, 1974), but some primitive aspects, in particular of their nuclear structure and composition, suggest a considerably earlier origin. This is especially clear in the *Prorocentrales*, which are considered to be the most primitive living order within the Dinoflagellates (Loeblich, 1976; Taylor, 1980).

As early as 1920, Chatton considered the dinoflagellate nucleus as unique among cell nuclei, and proposed the term 'dinokaryon'. Chatton (1937) also made the distinction between 'prokaryotic' and 'eukaryotic protists'; Dinoflagellates are characterized by a well-defined nucleus and by this sign belong to the latter category. The first detailed ultrastructural observations of the dinokaryon were published by Grassé and Dragesco (1957), and by Grell and Wohlfarth-Bottermann (1957); these revealed the characteristic arch-shape aspect of their chromosomes as observed only in thin sections. Cytochemical data from Ris (1962) and Dodge (1964) pointed out another peculiar feature of these permanently condensed chromosomes: the absence of cytochemically detectable basic proteins (histones), except for the parasitic species *Syndinium* sp. (Ris and Kubai, 1974). Histones are common constituents of eukaryotic chromatin and form the protein part of the nucleosomes (for a review see Kornberg, 1977).

It is generally accepted that the dinoflagellate nucleus is above prokaryote level in terms of organization, but below the level of the other Eukaryotes. Questions

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remaining at present thus concern the position of this group among the 'lower Eukaryotes'. Taylor (1978) considered Dinoflagellates as the most primitive eukaryotic cells, but viewing all their cellular features has not so far resulted in a clear picture of their position.

Our approach to this problem will be to concentrate on the nuclear features as a guideline for further discussions, essentially in the way Dodge and Loeblich have outlined the problem. The concept of 'Mesokaryotes' based essentially on their available ultrastructural and cytochemical data (Dodge, 1965) has been refined by Loeblich (1976) on biochemical grounds. Loeblich suggested that Dinoflagellates "are a geologically old group, one that perhaps diverged from the higher eukaryotic lineage before evolution of eukaryotic chromatin but after the evolution of repeated DNA". This phylogenetic model has been called in question by Cavalier-Smith (1981) with the argument that the lack of histones (and nucleosomes) in dinoflagellate chromatin could be explained by deletion of the clustered histone genes typical of all other Eukaryotes. This hypothesis is put forward quite categorically, but as far as we can see, without objective support.

The aim of our contribution is to add a few new aspects to the Mesokaryote concept by deliberately focussing our attention on the typical nuclear features of Dinoflagellates as far as they can be now defined in macromolecular, fine structural and functional terms. We then compare these features with typical nuclear conditions of both Prokaryotes and Eukaryotes. From this basically typological approach, we attempt to proceed towards a phylogenetically consistent picture by sorting the nuclear features according to aspects of plesiomorphy (common ancestral features) and apomorphy (uniquely derived features) following the views of Hennig and Schlee, 1978. This attempt is made in full awareness of the problems that will have to be solved later by a detailed analysis of structural and functional correlations between nuclear and extranuclear features. But we think that the analysis has to set out from the central apparatus of the cell, i.e. the nucleus as the carrier of genetic information.

We tentatively suggest that there is a monophyletic group of unicellular organisms comprising most of the actually free-living Dinoflagellates, and that it forms a sister group to the 'higher' Eukaryotes. We feel that this hypothesis, which is basically identical to the mesokaryote model, is of greater promise for further clarifications than evasive arguments about overall similarities.

2. Eukaryotic Features Observed in Dinoflagellate Nuclei

Several nuclear features of the Dinoflagellates are very similar to those of the other Eukaryotes, as suggested by numerous ultrastructural studies published over the last forty years. Dinoflagellates have a membrane-bounded nucleus (Grell and Schwalbach, 1965), with highly organized chromosomes (Figure 1). They also have one or several nucleolar structures (Figure 1b) generally in close contact with chromosomes (Soyer and Haapala, 1974). Although a mitotic spindle does not exist, long extranuclear microtubules could act as an extra-nuclear spindle; this is at least conceivable

for a few species (Oakley and Dodge, 1979). In other species, however, a more primitive mechanism seems to exist (see below). More recent molecular data again indicate some eukaryotic features in the dinokaryon. Firstly, a distinct S-phase of DNA synthesis has been demonstrated in several partially synchronized species (Franker, 1971; Franker *et al.*, 1974; Galleron and Durrand, 1979). Secondly, renaturation studies published by several authors (Britten and Kohne, 1968; Allen *et al.*, 1975; Steele, 1980; Hinnebusch *et al.*, 1980) demonstrate the presence of a large amount of repeated DNA (50 to 60%) interspersed in a manner similar to the other Eukaryotes, with a highly complex non-repetitive DNA. Finally, Steele (1980) using recombinant DNA techniques, reported that in *Crypthecodinium cohnii* ribosomal DNA (rDNA) exists as a complex gene family organized in tandemly repeated 15 Kb units, as in other eukaryotic rDNA.

One could also take ribosomal RNA into consideration for which molecular weight, sedimentation coefficient (S), base methylation and sequence homologies suggest that dinoflagellate rRNA represents an early form of eukaryotic rRNA (Rae, 1970; Gressel *et al.*, 1975; Werner-Schlenzka *et al.*, 1978; Hinnebusch *et al.*, 1981). Also, Reddy *et al.* (1983) demonstrated the presence of six capped small nuclear RNAs in *C. cohnii* similar in several respects to the U_1 to U_6 Sn RNAs of higher Eukaryotes.

3. Prokaryotic Features Observed in Dinoflagellate Nuclei

Along with the eukaryotic features, dinoflagellate nuclei display several more primitive traits, in particular the arch-shaped fibrillar appearance (Figure 1a, b) of the thin sectioned chromosomes which are reminiscent of the bacterial nucleoïd (Giesbrecht, 1965; Gourret, 1978). For some species it has been shown that chromosome attachment on the nuclear membrane is concomitant with the chromatid segregation. In functional terms, this could be compared with the corresponding mechanism in bacteria where the dividing nucleoïd is attached to the plasmic membrane.

Moreover, we have recently been able to show that the stabilization of the chromosomal fabric is maintained by divalent cations (Herzog and Soyer, 1983) and by structural RNA molecules (in prep.). Involvement of the latter was demonstrated also in the stabilization of the supercoiling of DNA loops in bacteria (Pettijohn *et al.*, 1973).

Nucleofilament structure also displays prokaryotic characters as demonstrated by chromatin spreading and biochemical investigations. Chromatin is composed of smooth filaments, 3–6 nm in diameter, which do not present the typical eukaryotic 'beads-on-a-string' appearance (Hamkalo and Rattner, 1977; Rizzo and Burghart, 1980, 1982; Herzog and Soyer, 1981). Absence of nucleosomes was confirmed (Bodansky *et al.*, 1979; Rizzo, 1981; Herzog and Soyer, 1981) by demonstrating the lack of histones (basic nuclear proteins of Eukaryotes) and the absence in nuclease-treated nuclei of the DNA fragment repeats which in true Eukaryotes are protected by the nucleosome structure. On the other hand, low amounts of basic nuclear proteins (12 000 and 13 000 daltons) were detected in several dinoflagellate species. In the case of *Crypthecodinium cohnii*, Rizzo and Nooden (1974) indicated that their composition in amino acids was different from that of histones. The possible relationship with HU

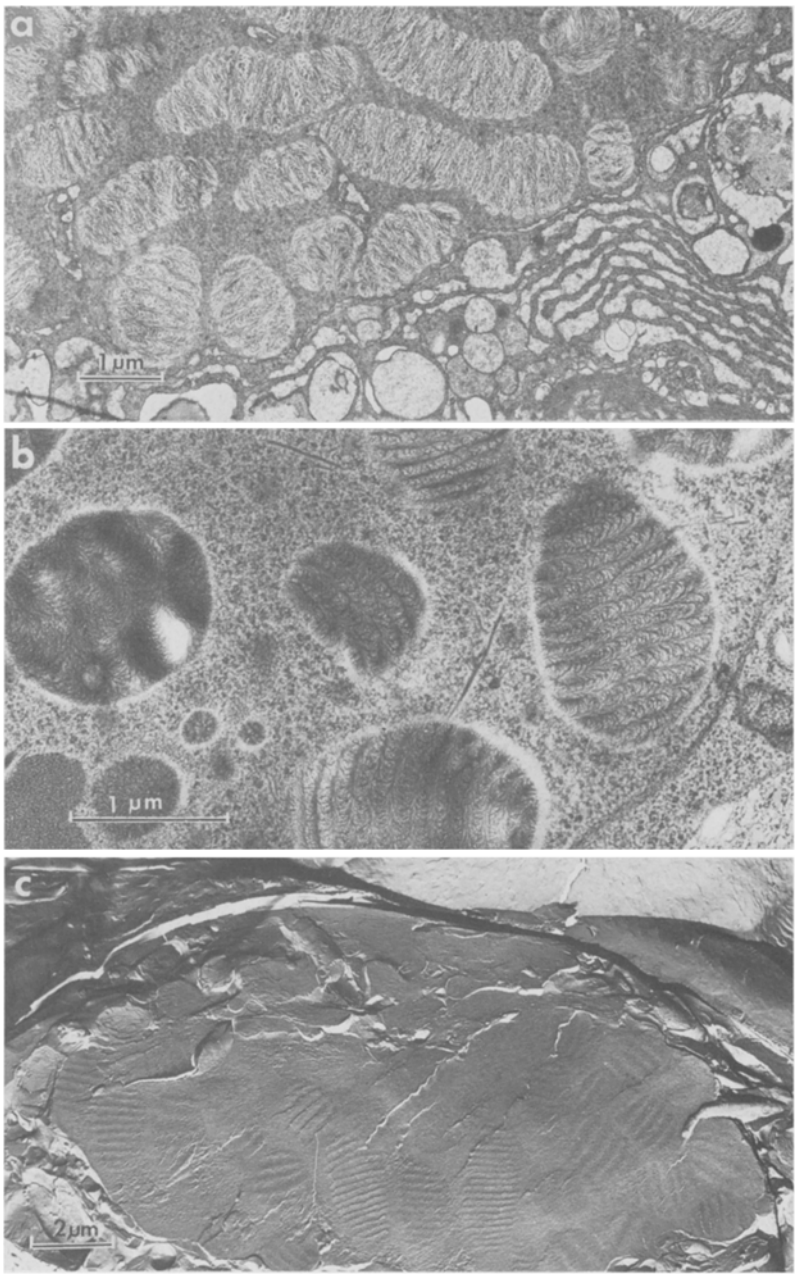

Fig. 1. The typical arch-shape appearance of *Prorocentrum micans* chromosomes when thin sectioned (a) after chemical fixation (Soyer, 1977) or (b) after cryofixation (Escaig *et al.*, 1977). In (c), a freeze-fractured nucleus in which chromosomes show transverse striation with a periodicity of about 150 to 200 nm. (a) 11 000 ×; (b) 21 000 ×; (c) 5 400 ×.

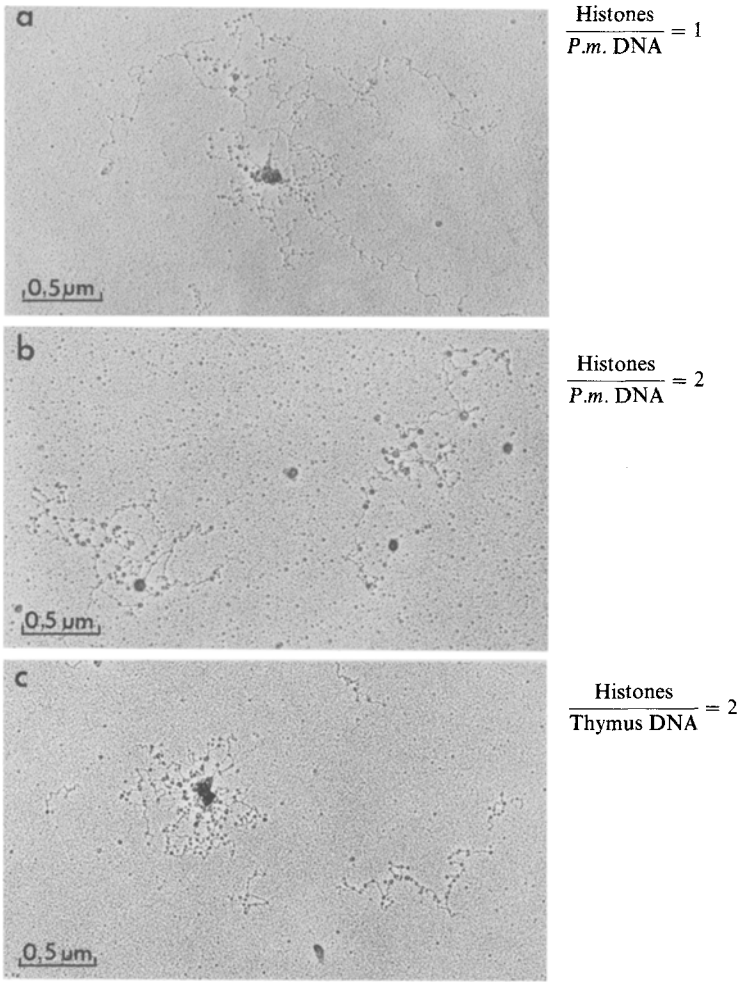

Fig. 2. *In vitro* reconstitution of nucleosomes in heterologous conditions using a mixture of purified corn histones, without histone H1 (a gift from Dr C. Gigot), and sonicated DNA from (a – b) the Dinoflagellate *Prorocentrum micans* or (c) from calf thymus (Sigma). Reconstitution conditions were as in Germond *et al.* (1976), and histone to DNA ratios were respectively (a) 1 : 1; (b) 2 : 1; (c) 2 : 1. This indicates that the presence of high amounts of the unusual base hydroxymethyluracil in dinoflagellate DNA does not impede accurate DNA-histone interactions.

bacterial basic protein (Rouviere-Yaniv and Gros, 1975; Haselkorn and Rouviere-Yaniv, 1976) has not yet been investigated. Nevertheless, we have shown, by means of *in vitro* reconstitution experiments, that purified dinoflagellate DNA was able to form nucleosomes in the presence of foreign histones (Figure 2). In addition to the evidence provided by several workers using both prokaryotic and eukaryotic DNA (Wilhelm *et al.*, 1979; Noll *et al.*, 1980) that histones, and not DNA as such, are responsible for

nucleosome formation, it is now clear that high amounts of an abnormal base (hydroxymethyluracil, see below) in the DNA are not an impediment to *in vitro* construction of nucleosomes by heterologous histones.

4. Distinctive Features of Dinoflagellate Nuclei

Some of the nuclear aspects of Dinoflagellates are quite distinctive of this group. The nuclear membrane is persistent throughout the whole mitotic cycle. Chromosomes are permanently condensed and no longitudinal differentiation (e.g. Q-, G- or C-bands which occurs in eukaryotic metaphasic chromosomes after specific 'banding technique') was observed (Haapala and Soyer, 1974) in *Prorocentrum micans*. These chromosomes are characterized by a peculiar organization of the chromosomal fibers (Oakley and Dodge, 1979: Livolant and Bouligand, 1980; Herzog and Soyer, 1983), which are tightly coiled into a double helical bundle. We recently provided evidence that *P. micans* chromosomal DNA is compacted in a hierarchy of six organizational levels, based on the mode of helical coiling (Herzog and Soyer, 1983). This organization (Figure 3) allows the large amount of DNA (which in Dinoflagellates is 5 to 10 times higher than in eukaryote nuclei) to be compacted into chromosomes, in the absence of histones.

Another distinctive feature of the nuclei is the division mechanism (dinomitosis), which is still under investigation, but is known to be basically different from the eukaryote mitosis. It is mainly characterized by the absence of (a) a diffuse chromatin interphase (except for some genera like *Noctiluca*, *Blastodinium*) (Soyer, 1971, 1972), (b) metaphase chromosomes and metaphasic plate, (c) a *kinetochore*, with the exception of *Syndinium* and perhaps *Amphidinium* (Oakley and Dodge, 1974), *Oodinium* (Cachon and Cachon, 1979) and *Glenodinium* (Dodge, 1971), (d) centrioles, except for

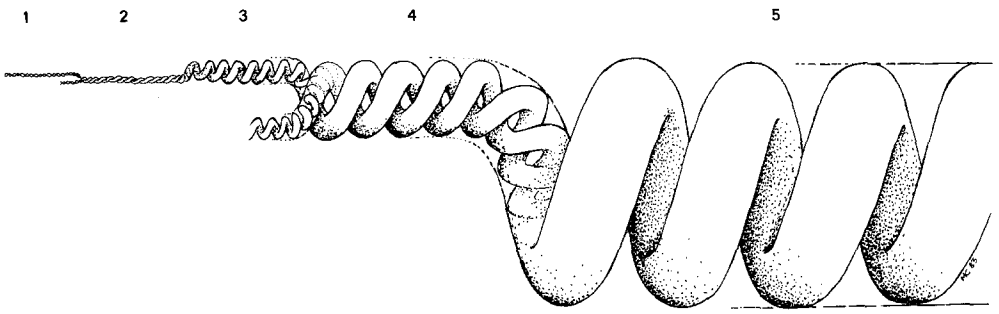

Fig. 3. Tentative representation of the helical compaction of dinoflagellate chromosomal DNA in a hierarchy of 6 organization levels. DNA molecules (level 1) form a double helix (level 2) 10 nm thick which is twisted into a single helix 18 nm in diameter (level 3). The fourth level is represented by a double-helically twisted state (25–31 nm thick) of two 18 nm filaments, which in turn are supertwisted into a left-handed single helix (level 5) with a diameter of 43–56 nm. These chromosomal fibers are finally united to form the double helical bundle of the chromosome (not shown here) with a diameter of about 1250 nm.

Syndinium (Ris and Kubai, 1974). Other features are the longitudinal division of the chromosomes which appear Y- and V-shaped, and the presence, as described in *P. micans* (Soyer, 1981 and Figure 1b), of intranuclear microcables, which might be involved in the chromosomal movement. These microcables could act in a specific mechanism of dinomitosis probably restricted to the most primitive genera.

At the molecular level, Dinoflagellates also display a peculiar trait. The abnormal behaviour of their DNA in terms of density and thermal denaturation, was shown (Rae, 1976; Herzog *et al.*, 1982) to be due to the presence of an unusual base, the 5-hydroxymethyluracil, which replaces 12–68% of the thymines in the investigated species. Figure 4 demonstrates the presence of this abnormal base in the highly complex heterotrophic species *Noctiluca miliaris*, as shown by *in vitro* labelling using *E. coli* DNA polymerase I. This base is specific to Dinoflagellates and has otherwise been found only in some bacteriophages infecting *Bacillus subtilis* (Kallen *et al.*, 1962).

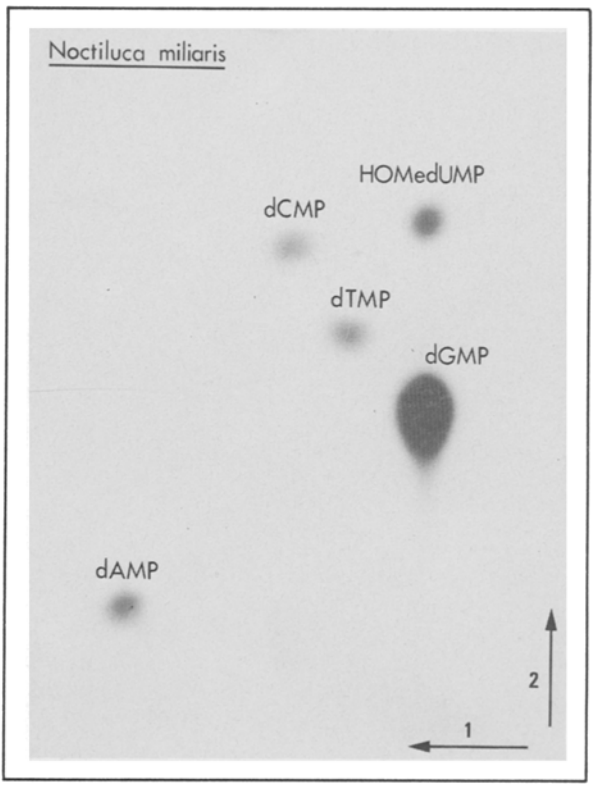

Fig. 4. The presence of the unusual nucleotide containing the base 5-hydroxymethyluracil, in *Noctiluca miliaris* DNA. Purified DNA was labelled *in vitro* by a method derived from the Nick-translation reaction, using *E. coli* DNA polymerase I, and radioactive (α ^{32}P) – d ATP. 3′ nucleotides were obtained by digestion of the labelled DNA by micrococcal endonuclease and phosphodiesterase II, and separated by two-dimensional chromatography. This unusual base has been found in all the so far investigated dinoflagellate species.

5. Discussion and Conclusion

The various features have been listed in Table I for comparison of dinoflagellate nuclei with both prokaryotic and eukaryotic conditions. Characters or character states were grouped using functional or structural criteria: nuclei, chromosomes, chromatin

TABLE I

Synoptic presentation of nuclear features listed for Prokaryotes, Dinoflagellates and higher Eukaryotes. Features that appear 'unique' for one group are encircled; many can be grouped according to their interrelationship. For some of these (e.g. permanence of nuclear membrane) it is impossible to decide whether they are common ancestral or uniquely derived characters (cf. Figure 5).

Character-states	Prokaryotes	Dinoflagellates	Eukaryotes
Nuclei			
Well defined nucleus	−	+	+
Nuclear membrane	−	+	+
– permanence of the membrane		+	−(a)
Chromosomes			
Permanently condensed chromosomes	−	+(b)	−
– C-, G- or Q-banding	−	−	⊞
– arch-shaped aspect	+	+	−
– helical compaction (6 levels)	−	⊕	−
– chromosome stabilizing RNA	+	+	−
Chromatin organization			
Nucleosomes	−	−	⊞
– histones	−	−	⊞
– HU-proteins (DNA-binding)	⊕	−	−
– basic non histone proteins (12–13 kd)	−	⊕	−
DNA:			
– thymine replacement by HOMeU	−(c)	⊕	−
– high amount of repetitive DNA	−	+	+
– tandemly repeated ribosomal DNA	−	+	+
Nuclear division			
– S-phase DNA synthesis	−	+	+
– Mitosis	−	−	⊞
– Dinomitosis	−	⊕	−
– Interphase		−	⊞
– Metaphase		−	⊞
– Kinetochores		−(d)	⊞
– Centrioles		−(e)	⊞
– Mitotic spindle		−(f)	⊞
– Membrane attachment of the dividing chromosomes	+	+	−
– Intranuclear microcables		⊕	−
– Nucleoli	−	+	+

(a) Except for some protozoa. (b) Except for *Noctiluca* and some parasitic species. (c) Except for bacteriophages SP 8. (d) Kinetochore-like structures in *Amphidinium* and some parasitic species. (e) Except for the parasitic *Syndinium*. (f) Extra-nuclear spindle in some species?

organization, DNA and nuclear division. This representation clearly shows the close relationship of Dinoflagellates with Eukaryotes. Nevertheless, their peculiar features described above, suggest that Dinoflagellates have diverged early from the eukaryotic lineage, as proposed by Loeblich (1976), before the eukaryotic chromatin was organized in nucleosomal structures, but after the appearance of repeated DNA sequences and, presumably, of the permanent nuclear membrane. This latter assumption is supported by the example of some lower Eukaryotes, as most of the Sporozoa, Foraminifera, Zoomastigina and Euglenophyta (for a review, see Margulis, 1981), which show a permanently intact nuclear membrane, as do Dinoflagellates, but otherwise display regular eukaryotic features.

A correlation may be seen in Eukaryotes between presence of histones and lack of arch-shape of the chromosomes. The appearance of the nucleosome structure in the higher Eukaryotes may have implied the disappearance of the structure resulting in the arch-shaped aspect of thin-sectioned chromosomes. The different structural and biochemical characters may be used to establish the cladogram presented in Figure 5, as an 'argumentation plan' *sensu* Hennig. Dinoflagellates and Eukaryotes have a common ancestor recognizable by the nuclear structure. The peculiar characters of Dinoflagellates strongly suggest that this group is monophyletic and has evolved independent of the true Eukaryotes; it thus may be considered as their sister group. This concept is in agreement with Loeblich's (1976) proposal and does not appear in any way opposed to the mesokaryotic hypothesis introduced by Dodge (1965). If future

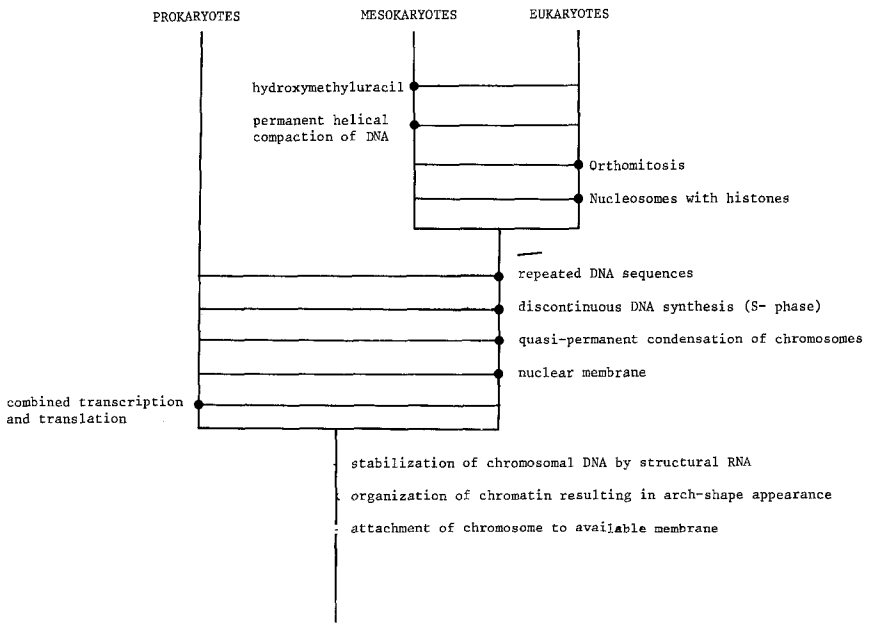

Fig. 5. A tentative cladogram based only on nuclear features supposed to present an apomorphic state (uniquely derived, or group-distinctive state) for the level considered (the corresponding plesiomorphic state on the 'sister' branch is not listed here, e.g. absence of nucleosomes).

work supports this conclusion then the current taxonomic practice of grouping Dinoflagellates with other unicellular Eukaryotes creates an unacceptable paraphyletic group.

In terms of phylogenetic systematics, the former Eukaryotes *sensu lato* would thus be subdivided into two monophyletic (sister) groups, the Mesokaryotes (or Dinokaryotes) and the Eukaryotes *sensu stricto*. However, as classification generally (and justly) lags behind the establishment of highly probable phylogenetic relationships, this nomenclature is not intended for immediate use in systematics. Rather it is proposed as a guideline for discussions focussing on the problem of dinoflagellate evolution.

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