RESEARCH REVIEW

INTERNATIONAL MICROBIOLOGY (2015) 18:209-216
doi:10.2436/20.1501.01.252. ISSN (print): 1139-6709. e-ISSN: 1618-1095
www.im.microbios.org

INTERNATIONAL MICROBIOLOGY
OPEN ACCESS

Chromosomes of Protists: The crucible of evolution

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Received 15 September 2015 · Accepted 10 October 2015

Summary. As early as 1925, the great protozoologist Edouard Chatton classified microorganisms into two categories, the prokaryotic and the eukaryotic microbes, based on light microscopical observation of their nuclear organization. Now, by means of transmission electron microscopy, we know that prokaryotic microbes are characterized by the absence of nuclear envelope surrounding the bacterial chromosome, which is more or less condensed and whose chromatin is deprived of histone proteins but presents specific basic proteins. Eukaryotic microbes, the protists, have nuclei surrounded by a nuclear envelope and have chromosomes more or less condensed, with chromatin-containing histone proteins organized into nucleosomes. The extraordinary diversity of mitotic systems presented by the 36 phyla of protists (according to Margulis et al., *Handbook of Protoctista*, 1990) is in contrast to the relative homogeneity of their chromosome structure and chromatin components. Dinoflagellates are the exception to this pattern. The phylum is composed of around 2000 species, and characterized by unique features including their nucleus (dinokaryon), dinomitosis, chromosome organization and chromatin composition. Although their DNA synthesis is typically eukaryotic, dinoflagellates are the only eukaryotes in which the chromatin, organized into quasi-permanently condensed chromosomes, is in some species devoid of histones and nucleosomes. In these cases, their chromatin contains specific DNA-binding basic proteins. The permanent compaction of their chromosomes throughout the cell cycle raises the question of the modalities of their division and their transcription. Successful in vitro reconstitution of nucleosomes using dinoflagellate DNA and heterologous corn histones raises questions about dinoflagellate evolution and phylogeny. [**Int Microbiol** 18(4):209-216 (2015)]

Keywords: dinoflagellates · protist chromosomes · dinokaryon · dinomitosis · eukaryotic nucleus

Introduction

As early as 1925, Edouard Chatton (1904–1947), who had a profound knowledge of protists based on the work carried out by others over more than a century, distinguished for the first time the fundamental differences between prokaryotes and eukaryotes [36,]. In a long, accurate article devoted to *Pansporella perplexa*, an amoeboid parasite of *Daphnia*, he discussed the classification and phylogeny of Protozoa, trying to find a place for *Pansporella*. The article contains a simple table without any explanation, which is an attempt at protist classification, and differentiates between prokaryotes and eukaryotes [6]. In 1973, Roger Stannier and André Lwoff [42] resumed and simplified Chatton's fundamental distinction, well demonstrated by modern cytology. They wrote that

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This article is based on the lecture given by M.O.S-G at the Ramón Areces Foundation, Madrid, on 12 November 2012 for the International Symposium in the memory of Lynn Margulis (deceased on 22 November 2011). M.F.D. was one of the long-standing collaborators of Prof. Margulis, and her best support in her teaching duties in UMASS-Amherst.

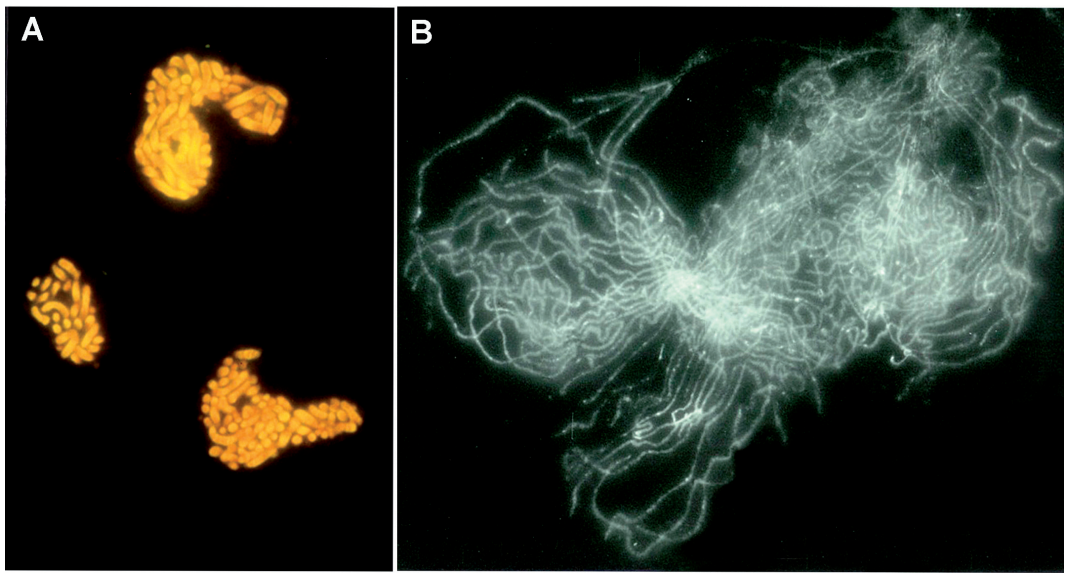


Fig. 1. Different light microscope preparations of dinoflagellate nucleus. **(A)** Semi-thin sectioned nuclei of *Prorocentrum micans* embedded in Epon. Note the chromosomal DNA contrasted with acriflavine (about 65 chromosomes per nucleus). Magnification 2000× (Preparation and image by the author). **(B)** Whole nucleus and chromosomes of *P. micans* prepared by squashing, stained with the intercalating bases fluorescent ethidium bromide, which contrasts DNA, and observed with a fluorescence light microscope. Chromosomes are totally unwounded. Magnification 3600×. From [38], with permission of Humana Press.

"protists represent an heterogeneous group including on one hand the prokaryotes (bacteria and cyanophyta) and on the other hand the eukaryotes (protists, algae and fungi)." Transmission electron microscopy (TEM) made it possible to know that a unique characteristic of prokaryotes is the absence of nuclear envelope surrounding bacterial chromosome (the nucleoid), which is more or less condensed. In addition prokaryotic chromatin lacks histone proteins but contains specific HU basic proteins (histone-like proteins that were first isolated from *Escherichia coli* strain U93 and were so called factor U [28]).

Protists show an extraordinary diversity of morphology and mitotic systems throughout the 36 phyla recognized in 1990, as described by Margulis et al. in their impressive multi-authored *Handbook of Protoctista*, 1st edition [19b]. Nevertheless, their chromosome structure and chromatin components are relatively homogeneous. Dinoflagellates, however, are a distinctive group of protists that challenges that homogeneity. Their large nuclei have no nucleosomes and their chromosomes are permanently condensed. In addition, they have few histones. Due to these features, which might be primitive, and suggest that dinoflagellates could be intermediary between prokaryotes and eukaryotes, in 1965 Dodge coined the term "mesokaryotes" to call them [7], a term Raikov also used in 1982 [23].

Here we will briefly review some distinctive characteristics of the components of the dinoflagellate nucleus, and how it can be interpreted in terms of the evolution of this group, with several hypotheses suggested.

Dinoflagellates' nuclear characteristics

Dinoflagellates are a phylum of unicellular eukaryotic microorganisms among the protists, a paraphyletic group that comprises microorganisms that do not fit into the traditional kingdoms of Plants, Fungi and Animals. Protists are single celled organisms that, collectively, have developed all the known cellular functions including motility, reproduction (sexual or not), respiration, photosynthesis, secretion, nutrition, and vision—some having even an eyespot, sometimes a sophisticated photoreceptor [8]. More than 100,000 species of protists have been described and many thousands more await discovery. The number, in each phylum, might be even higher in extinct groups.

The protists, like all eukaryotes, have the nucleus surrounded by a nuclear envelope and chromosomes more or less condensed during mitosis, with chromatin that includes histone proteins and is organized into nucleosomes. In most eukaryotic cells, cyclic chromatin compaction is linked to the

stages of the cell cycle, the maximum of compaction being reached during the mitosis.

Dinoflagellates show a great ecological diversity. They can be either autotrophic, heterotrophic, mixotrophic, parasitic or symbiotic, and are widely distributed worldwide throughout the seas and freshwaters, playing major roles in trophic chains. The diversity of this group is also displayed in both their external morphology and the organization of their external thecal plates when present. In fact, thecal plates are the basis for the classification of approximately 2000 living species, 161 genera, 48 families and 17 orders described to date. Here we will review three models selected to study the structure and functioning of their chromosomes: *Prorocentrum micans* Ehr., which is an autotrophic, planktonic species, *Noctiluca scintillans* MC., a free-living species that can form extensive red tides in many parts of the world, and *Crypthecodinium cohnii* B., which is an heterotrophic marine species, with a complex cell cycling comprising both swimming cells and cysts, the latter accompanying cell division [3]. All specific techniques used to study and try to understand the dinoflagellate chromosome organization and functioning have been improved and are summarized in [38] (Fig. 1).

Some remarkable aspects of the dinoflagellate nucleus are distinctive of this group. These include a persistent nuclear membrane during all the cell cycle, including during the mitosis, permanently condensed chromosomes (except for several rare species), no longitudinal chromosome differentiation as Q-, G-, C-banding [11] and, particularly, lack of telomeric heterochromatin.

Nucleofilaments are coiled into a double helical [10,32], which explains their regular arch-shaped visualization in thin section (Fig. 2). Chromatids are coiled in an anorthospiral arrangement, and have a very regular pitch (Fig. 3A). This architecture is maintained by structural RNA [35] and by Ca^{2+} and Mg^{2+} divalent cations as demonstrated by divalent cation chelating agents ethylenediaminetetraacetic acid (EDTA) and ethylene glycol tetraacetic acid (EGTA) [14]. These observations have been later confirmed by high-resolution ion probe mass spectrometry [18].

Chromosomal fibers composed of circular chromatids [10,12] are compacted into a hierarchy of six organizational levels helically coiled (Fig. 3) as schematized in Fig. 3E, after TEM observations of isolated, squashed and shadowed chromosomes (Fig. 3A–D), level 6 being the chromosome itself. This organization allows a DNA content 5 to 10 times higher than in other eukaryotic nuclei to be compacted into chromosomes in the absence of DNA-binding histone proteins [13] and consequently of nucleosomes. For example,

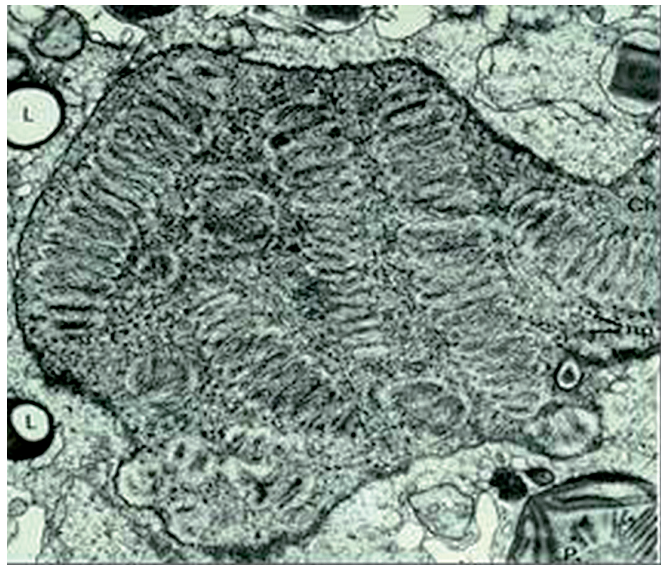


Fig. 2. Micrograph of a prophase nucleus of *Prorocentrum micans* showing the well protected organization of the compact chromosomes which chromatin fibrils give an arch shaped aspect in ultrathin section. Magnification 18,000×. From [31].

in *Prorocentrum micans*, which has 65 chromosomes, the DNA amount per cell is 7.0×10^{10} nucleotide pairs for a nuclear volume of 3,450 μm^3 and a chromosome volume of 20 μm^3. In *Crypthecodinium cohnii*, which has 95–100 chromosomes, the DNA amount per cell is 1.4×10^{10} nucleotide pairs for a nuclear volume of 690 μm^3 and a chromosome volume of 2.6 μm^3 [10]. These measurements demonstrate the extraordinary compaction of DNA.

Another distinctive feature of dinoflagellate chromosomes is the absence of diffuse chromatin during the interphase, except for some genera including *Noctiluca*. For example, in *N. scintillans* [30] chromatin of the vegetative nucleus is uncondensed. During mitosis dinoflagellates lack a "metaphase" plate, kinetochores and centrioles (except for some rare species as *Syndinium* sp., which undergo a very peculiar peridinian mitosis [37], and see cover. Dinoflagellates undergo longitudinal chromosome fissure (Fig. 4B), and segregation of daughter chromatids (Y- and V-shaped; Fig. 4A,C) [33] attached to the nuclear envelope (Fig. 4D). For a review see [39].

The presence of chromosomes in a permanently condensed state throughout the cell cycle raises the question of how such structures can transcribe and can be replicated. Fibrillar loops protruding from chromosomes have been described and evidenced by treating the cells with the proteolytic enzyme pronase, which removes the bulk of non fibrillar chromosome material [34]. Both right-handed double helix

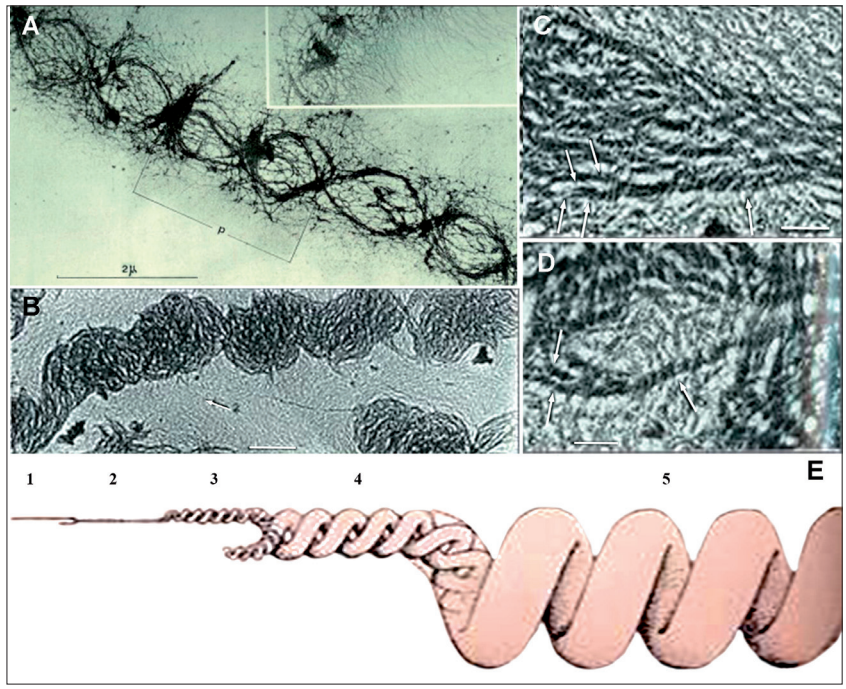


Fig. 3. Different preparations of whole mounted dinoflagellate chromosomes. **(A)** Chromosome squashed on water and observed in transmission electron microscopy (TEM) after staining with uranyle acetate shows a figure-eight conformation of the chromatid bundles. Bar, 2 µm. Reproduced from [32]. **(B)** Details of TEM observations of chromosome fibers after squashing next rotary shadowing with platinum. Bar, 1 µm (Magnification 16,000×). **(C)** and **(D)** Details of TEM showing the organizational chromosome levels 3, 4, 5 (arrows) of the chromatin bundles. (Magnification 56,000×). Reproduced from [14]. **(E)** Schematic representation of the hierarchy of five organization levels of the supercoiled chromosomal fibers, level 6 being the whole chromosome itself. Reproduced from [16].

(B-DNA) and Z-DNA conformations in chromosomes of *Prorocentrum micans* (Fig. 5) were detected and located by immunoelectromicroscopy [40]. This was in agreement with the proposed model of a chromosome that, to allow transcription to occur, must allow local untwisting of supercoiling of these loops, where active chromatin is located [29].

The usual conformation of DNA is a right-handed double helix (B-DNA). DNA with stretches of alternating purine-pyrimidine (G–C or A–T) can also form a left-handed helix (Z-DNA). In these species of dinoflagellates, the absence of histones, the stabilization of DNA supercoiling by divalent cations, the presence of rare bases, and the high G–C content [15] are factors known to facilitate local B to Z transitions of DNA [45]. The dinoflagellate chromosome has been a suitable model to study this dynamic phenomenon because it does not contain the nucleosomal system that would modulate local supercoiling necessary for transcription (Fig. 6) [9].

At the molecular level, dinoflagellate DNA is peculiar in terms of density and thermal denaturation due to the presence of an unusual base, 5-hydroxymethyluracil (HOMedU), which replaces 16–28% of the thymines [15,24]. The occurrence of this pyrimidine base replacing thymine was described for the first time in a bacteriophage [17]. The presence of an unusual nucleotide containing the base HOMedU has been also detected in the heterotrophic, free-living *Noctiluca miliaris (scintillans)* DNA by in vitro labeling using *Escherichia coli* DNA polymerase I. Another characteristic of dinoflagellate DNA is its high G–C content [25] as well as a high proportion (55–60%) of repeated, interspersed DNA [2].

Low amounts of basic nuclear proteins (12,000–13,000 daltons) have been detected in several dinoflagellate species while the general absence of histones (basic nuclear proteins of eukaryotes) and nucleosomes has been confirmed. For a transcriptome-level analysis that suggests the presence of nucleosomes, see [20]. In fact, the amino acid composition of those basic proteins greatly differs from that of histones [13,26,27]. By in vitro reconstitution [16], it has been possible to form nucleosomes in the presence of foreign histones and

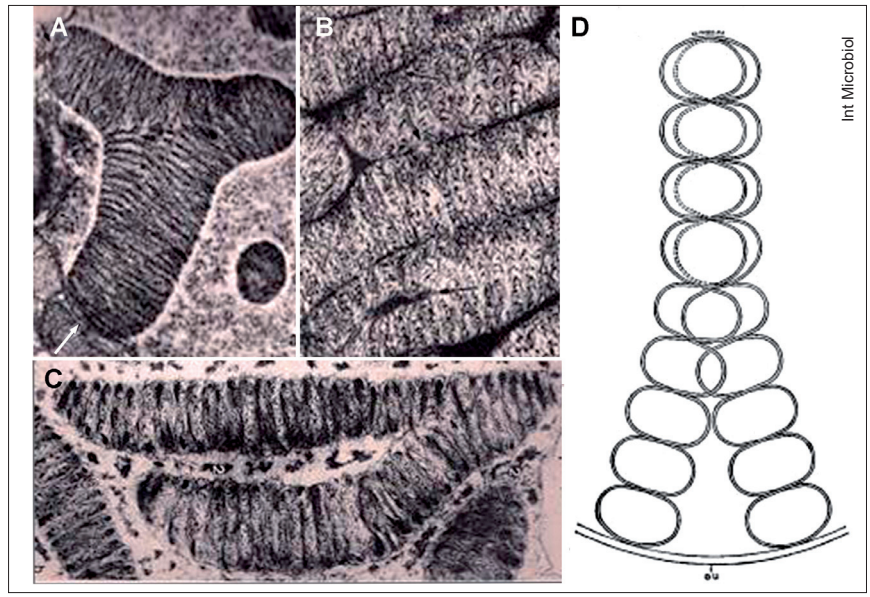


Fig. 4. Stages of dividing dinoflagellate chromosomes. **(A)** Ultrathin sectioned dividing *Blastodinium* sp. (Magnification 32,000×) and **(B)** *Prorocentrum micans* chromosomes: tips of this Y-shaped chromosome are attached to the nuclear envelope (arrow). (Magnification 21,900×). Reproduced from [34]. **(C)** Beginning of the longitudinal fissure of *Noctiluca scintillans* chromosomes. (Magnification 32,000×). Reproduced from [30]. **(D)** Model of the nuclear membrane-mediated dividing dinoflagellate circular chromatids. Reproduced from [34].

purified dinoflagellate DNA (Fig. 7), which confirms that the high amount of HOMedU in their DNA is not a hindrance to in vitro formation of nucleosomes by heterologous histones [16].

Taxonomic position and phylogeny of dinoflagellates

Dinoflagellates occupy a special place among protists, and many questions remain about their phylogeny. The absence of nucleosomes and histones in several species, and the permanently condensed and highly ordered supercoiled chromosomes bound to nuclear envelope during segregation, led Dodge to coin the "mesokaryote" concept [7]. He suggested the fact that dinoflagellates have prokaryotic traits conserved along with typical eukaryotic features. Later studies showed that dinoflagellates have also characters of true eukaryotes including distinct cell cycle phases and typical genomic organization. Different phylogenetic studies based on ribosomal gene sequences have shown that dinoflagellates emerged late in evolution and have a common ancestor with Apicomplexa and Ciliates, which group together into Alveolata [1,5,44]. In 1981, Cavalier-Smith suggested that dinoflagellates should be true eukaryotes that could have lost their histones and consequently their nucleosomes,

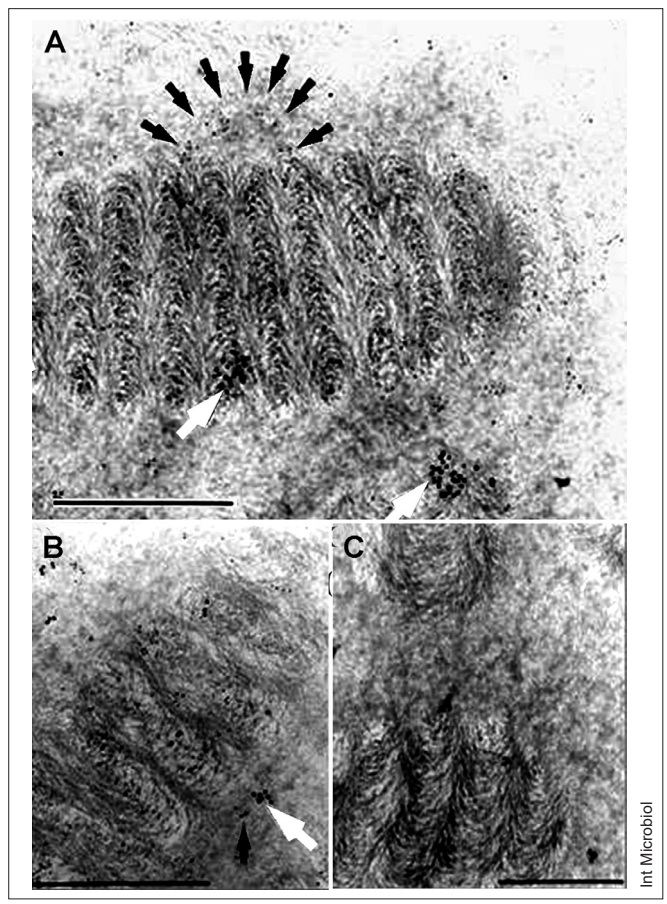


Fig. 5. Nucleus of *Prorocentrum micans* double-immunolabelled with antibodies against B- and Z-DNA coupled with 5 nm gold particles (B-DNA, black arrows) or 7 nm gold particles (Z-DNA, white arrows). **(A)** B-DNA is visible in the chromosome and the nucleoplasm where an extrachromosomal loop is visible. (Bar 0.5 μm). **(B)** Clusters of Z-DNA (left-handed) are located in the periphery of the chromosome. (Bar 0.1 μm). **(C)** Negative control. (Bar 0.5 μm). Reproduced from [7] by copyright permission of The Rockefeller University Press.

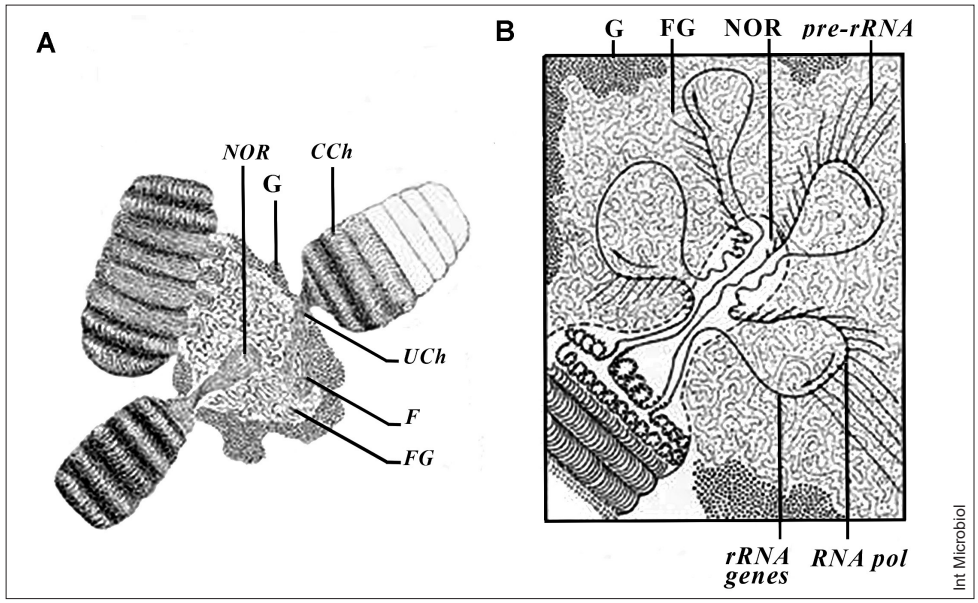
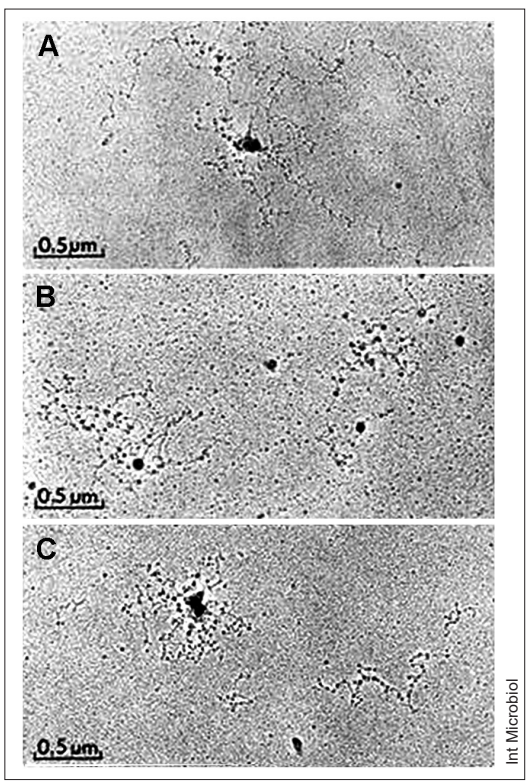


Fig. 6. Schematic representation based on TEM observations of nucleolar chromosomes of *Prorocentrum micans* showing the unwinding of nucleofilaments located in either telomeric or lateral regions. **(A)** Several chromosomes are contributing to the formation of a new nucleolus. CCh condensed chromosome; UCh unwound chromosome region; NOR nucleolar organizing region; F fibrillar region; FG fibrillogranular region; G granular (preribosomal) region. Reproduced from [40] by copyright permission of the Company of Biologists Ltd. **(B)** Predicted molecular organization of the dinoflagellate transcriptionally active nucleolus deduced from TEM observation after in situ hybridization with a ribosomal biotinylated probe. The rDNA transcription is initiated at the periphery of the NOR and carried on in the proximal part of the fibrillo-granular (FG) compartment to generate the rRNA transcripts, whereas the distal FG region is devoted to rRNA processing and packaging of preribosomes of the granular G region. Reproduced from [9] by copyright permission from Elsevier Science.



leading to their peculiar condensed DNA structure [4]. A study of the parasitic dinoflagellate *Amoebophrya* suggested that dinoflagellates' condensed chromosomes may be a relict trait of their primordially parasitic ancestor [21]. Geological analyses based on the examination of fossilized thecae have shown that the first unambiguous dinoflagellate fossils occurred in the Triassic and belong to Gymnodiniales. But biogeochemical analysis of early Cambrian sediments (520 million years ago) detected specific dinosterols [22]. Those sediment, however, are more recent than the period during which the first photosynthetic eukaryotes appeared, around 750 million years ago. This ambiguity could be resolved by a better knowledge of the very old Proterozoic fossils acritarchs, which would confirm whether dinoflagellates evolved earlier than other protists.

The similarities of bacterial (circular) and dinoflagellate chromosomes in both chemical composition and structure

Fig. 7. In vitro reconstitution of nucleosomes using a mixture of purified corn histone (without Histone H1) and sonicated DNA. **(A)** and **(B)** From the dinoflagellate *Prorocentrum micans (P.m.)*. **(C)** From calf thymus (Sigma). Histone to DNA ratio were respectively: 1:1, 2:1 and 2:1. This indicates that the presence of the unusual base hydroxymethyluracil (HOMedU) in dinoflagellate DNA does not impede accurate DNA-histone interactions. Reproduced from [16], with permission of Springer Science.

Fig. 8. Participants in the 5th Meeting of the International Society for Evolutionary Protistology (ISEP), held in the famed Laboratoire Arago on the Mediterranean Sea, at Banyuls-sur-Mer in Catalunya in June 4-6, 1983. The meeting was directed by Marie-Odile Soyer-Gobillard and hosted some 70 people representing a dozen nations (Belgium, Canada, Denmark, England, France, Germany, the Netherlands, Poland, Scotland, Spain, and the USA). Lynn Margulis is the first woman at the right of the picture. Reproduced from [19a].

imply common principles in the replication, segregation and functioning. The circular chromatid model described by Haapala and Soyer in 1973 [10] explained the segregation of two identical bundles of chromatids. The origin of the circular chromatid, present also in the bacterial chromosome, remains unexplained. One hypothesis is that the concatemeric structure—i.e., copies of the entire genome linked end to end—found in T7 and lambda phages could be an ancestor of the chromosome because it can produce a single circular chromosome [43]. Nevertheless, as there are still too few molecular data to resolve dinoflagellates phylogeny, morphological and cell biological analyses will continue to be crucial tools in studying this group.

Acnowledgements. We thank Prof. R. Guerrero, University of Barcelona and University of Massachusetts-Amherst, for his stimulus to write this paper, based (see note in p. 209) on the lecture given by M.O.S-G at the Ramón Areces Foundation, Madrid, on 12 Nov. 2012 for the International Symposium in the memory of Lynn Margulis, under the direction of Prof. Guerrero. Lynn Margulis (née Lynn Petra Alexander, Chicago, IL,1938–Amherst, MA, 2011), was an outstanding biologist whose ideas were of special significance for biology and evolution in the second half of the 20th century. Besides her pioneering "Serial Endosymbiotic Theory" to explain the origin of the eukaryotic cell, her contribution to the understanding of the complex world of protists was of special importance to recognize this huge group of organisms as the basis of eukaryotic evolution, and as the origin of fungi, plants and animals. Lynn Margulis helped one of us (M.O.S-G.) to organize the 5th Meeting of the International Society for Evolutionary Protistology (ISEP), held in Banyuls-sur-Mer in June 4-6, 1983 (Fig. 8). The contributions of the meeting were published less than one year later by D. Reidel Pub. [19a] (*Origins of Life* **13**, 1984), and were also the basis for the impressive *Handbook of Protoctista*, 1st ed., by Jones and Barlett Pub., in 1990 [19b].

Competing interests. None declared.

References

1. Adl SM, et al. (2012) The revised classification of Eukaryotes. J Eukaryot Microbiol 59:429-493 doi:10.1111/j.1550-7408.2012.00644.x
2. Allen JR, Roberts TM, Loeblich AR III, Klotz JC (1975) Characterization of the DNA from the Dinoflagellate *Crypthecodinium cohnii* and implication for the nuclear organization. Cell 6:161-169 doi:10.1016/0092-8674(75)90006-9
3. Bhaud Y, Salmon JM, Soyer-Gobillard MO (1991) The complex cycle of the dinoflagellate *Crypthecodinium cohnii* as studied in vivo and by cytofluorimetry. J Cell Sci 100:675-682
4. Cavalier-Smith T (1981) The origin and early evolution of the eukaryotic cell. In: Carlile, Collins and Moseley (eds) Molecular and cellular aspects of the microbial evolution. Cambridge Univ. Press, Cambridge, UK, pp 33-84
5. Cavalier-Smith T (1993) Kingdom Protozoa and its 18 phyla. Microbial Revue 57:953-994
6. Chatton E (1925) *Pansporella perplexa*: Réflexions sur la biologie et la phylogénie des protozoaires. Ann Sci Nat Zool 8:5-84

7. Dodge JD (1965) Chromosome structure in the dinoflagellates and the problem of the mesocaryotic cell. In: Progress in Protozoology (Abstr. II Internat. Conf. Protozool.):264-265 Excerpta Medica, London, UK
8. Gehring WJ (2005) New perspectives on eye development and the evolution of eyes and photoreceptors. J Hered 96:171-184 doi:10.1093/jhered/esi027
9. Géraud ML, Herzog M, Soyer-Gobillard MO (1991) Nucleolar organization of rRNA coding sequences in *Prorocentrum micans* Ehr. (Dinomastigote, Kingdom Protoctist) by in situ hybridization. BioSystems 26:61-74 doi:10.1016/0303-2647(91)90038-M
10. Haapala OK, Soyer MO (1973) Structure of Dinoflagellate chromosomes. Nature New Biol 244:195-197 doi:10.1038/newbio2495a0
11. Haapala OK, Soyer MO (1974) Absence of longitudinal differentiation of dinoflagellate (*Prorocentrum micans*) chromosomes. Hereditas 78:1-145 doi:10.1111/j.1601-5223.1974.tb01436.x
12. Haapala OK, Soyer MO (1974) Size of circular chromatids and DNA amount in the dinoflagellates *Gyrodinium* (*Crypthecodinium*) *cohnii* and *Prorocentrum micans*. Hereditas 76:83-90
13. Herzog M, Soyer MO (1981) Distinctive features of dinoflagellate chromatin. Absence of nucleosomes in a primitive species *Prorocentrum micans* Ehr. Eur J Cell Biol 23:295-302
15. Herzog M, Soyer MO (1983) The native structure of dinoflagellate chromosomes and their stabilization by Ca^{2+} and Mg^{2+} cations. Eur J Cell Biol 30:3
15b. Herzog M, Soyer MO, de Marcillac GD (1982) A high level of thymine replacement by 5-hydroxymethyluracil in nuclear DNA of the primitive dinoflagellate *Prorocentrum micans* E. Eur J Cell Biol 27:151-155
16. Herzog M, Von Boletzky S, Soyer-Gobillard MO (1984) Ultrastructural and biochemical nuclear aspects of eukaryote classification: independent evolution of the dinoflagellates as a sister group of the actual eukaryotes? Origins of Life 13:205-215 In: Margulis L, Soyer-Gobillard MO, Corliss J (eds) Evolutionary Protistology: The organism as cell. Reidel D, Boston, MA
17. Kallen RG, Simon M, Marmur J (1962) The occurrence of a new pyrimidine base replacing thymine in a bacteriophage DNA: 5-hydroxymethyluracil. J Mol Biol 5:248-250
18. Levi-Setti R, Gavrilov KL, Rizzo PJ (2008) Divalent cation distribution in dinoflagellate chromosomes imaged by high-resolution ion probe mass spectrometry. Eur J Cell Biol 87:963-976 doi:10.1016/j.ejcb.2008.06.002
19a Margulis L, Soyer-Gobillard MO, Corliss JO (eds) (1984) Evolutionary Protistology. The Organism as Cell. D. Reidel, Dordrecht, Holland, xii + 188 pp
19b Margulis L, Corliss JO, Melkonian M, Chapman DJ (eds) (1990) Handbook of Protoctista, 1st ed. Jones and Bartlett, Burlington, MA, USA, 914 pp
20. Marinov GK, Lynch M (2016) Diversity and divergence of dinoflagellate histone proteins. G3 Genes Genomics Genetics 6:397-422 doi:10.1534/g3.115.023275
21. Miller JJ, Delwiche CF, Coats DW (2012) Ultrastructure of *Amoebophrya* sp. and its changes during the course of infection. Protist 163:720-745 doi:10.1016/j.protis.2011.11.007
22. Moldowan JM, Talyzina N (1998) Biogeochemical evidence for dinoflagellate ancestors in the early Cambrian. Science 281:1168-1170 doi:10.1126/science.281.5380.1168
23. Raikov IB (1982) Nuclear apparatus of Mesokaryotic Protozoa. In: The Protozoan Nucleus. Morphology and Evolution. Cell Biology Monographs 9:1-174 Springer Verlag, New York, USA, 474 pp
24. Ray PMM (1973) 5-Hydroxymethyluracil in the DNA of a dinoflagellate. Proc Natl Acad Sci USA 70:1114-1125
25. Ray PMM, Steele RE (1978) Modified bases in the DNAs of unicellular eukaryotes: an examination of distributions and possible roles, with emphasis on hydroxymethyluracil in dinoflagellates. BioSystems 10:37-53
26. Rizzo PJ, Nooden LD (1974) Partial characterization of Dinoflagellate chromosomal proteins. Biochem Biophys Acta 349:5-427
27. Rizzo PJ, Burghard RC (1982) Histone-like protein and chromatin structure in the wall-less Dinoflagellate *Gymnodinium nelsoni*. BioSystems 15:27-34
28. Rouvière-Yaniv J, Gros F (1975) Characterization of a novel low-molecular-weight DNA-binding protein from *Escherichia coli*. Proc Natl Acad Sci USA 72:3428-3432
29. Sigee DC (1983) Structural DNA in genetically active DNA in dinoflagellate chromosomes. BioSystems 16:203-210
30. Soyer MO (1972) Les ultrastructures nucléaires de la *Noctiluque* (Dinoflagellé libre) au cours de la sporogenèse. Chromosoma 39:9-4
31. Soyer MO (1977) Une modification de la technique de Karnovsky pour la preservation optimale des structures nucléaires chez les dinoflagellés. Biol Cellulaire 30:297-300
32. Soyer MO, Haapala OK (1973) Filaments extra-chromosomiques: variations et relations avec l'enveloppe nucléaire pendant la division chez les dinoflagellés. Journal de Microscopie 18:267-270
33. Soyer MO, Haapala OK (1974) Division and function of dinoflagellate chromosomes. Journal de Microscopie 19:137-146
34. Soyer MO, Haapala OK (1974) Structural changes of dinoflagellate chromosomes by pronase and ribonuclease. Chromosoma 47:179-192
35. Soyer MO, Herzog M (1985) The native structure of dinoflagellate chromosomes. Involvement of structural RNA. Eur J Cell Biol 36:334-342
36. Soyer-Gobillard MO (2002) Scientific research at the Laboratoire Arago (Banyuls, France) in the twentieth Century: Edouard Chatton, the "master", and André Lwoff, the "pupil". Int Microbiol 5:37-42
37. Soyer-Gobillard MO (2006) Edouard Chatton (1883–1947) and the dinoflagellate protists: concepts and models. Int Microbiol 9:173-177
38. Soyer-Gobillard MO (2007) Methods for studying the nuclei and chromosomes of Dinoflagellates. In: The Nucleus Vol 1: Nuclei and subnuclear components. Ronald Hancock (ed). Humana Press, NY, USA, pp 93-108
39. Soyer-Gobillard MO (2011) Dinoflagellates. In: Schaechter M (ed) Eukaryotic microbes. Elsevier, Amsterdam, Netherlands, pp 263-277
40. Soyer-Gobillard MO, Géraud ML (1992) Nucleolus behaviour during the cell cycle of a primitive dinoflagellate eukaryote, *Prorocentrum micans* Ehr., seen by light microscopy and electron microscopy. J Cell Sci 102:475-485
41. Soyer-Gobillard MO, Geraud ML, Coulaud D, Barray M, Théveny B, Révet B, Delain E (1990) Location of B- and Z-DNA in the chromosomes of a primitive eukaryote dinoflagellate. J Cell Biol 111:293-304 doi:10.1083/jcb.111.2.293
42. Stanier R, Lwoff A (1973) Le concept de microbe de Pasteur à nos jours. La Nouvelle Presse Médicale 2:1191-1198
43. Thomas Jr CA (1971) The genetic organization of chromosomes. Annu Rev Genet 5:237-256
44. Van de Peer Y, Van der Auweva G, De Wachter R (1996) The evolution of stramenopiles and alveolates as derived by substitution rate calibration of small ribosomal subunit RNA. J Mol Evol 42:201-210
45. Zarling DA, Arndt-Jovin DJ, Robert-Nicoud M, McIntosh LP, Thomae R, Jovin TM (1984) Immunoglobulin recognition of synthetic and natural left-handed Z-DNA conformations and sequences. J Mol Biol 176:369-415 doi:10.1016/0022-2836(84)90495-9