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Edouard Chatton (1883–1947) and the dinoflagellate protists: concepts and models

Summary. Edouard Chatton contributed to our knowledge of single-celled protoctists, especially ciliates and dinoflagellates, free-living and/or symbiotic, in relation to the marine invertebrate animals in which they reside. More than the description of many new families, genera and species, and of their life cycles, he anticipated several major concepts of cell biology, including the fundamental difference between prokaryote and eukaryote protists, long time before the advent of electron microscopy. These concepts included: the reproductive ability of the kinetosome-centriole system; the homology of the kinetosome with the mitotic centriole of animal cells; and the different kinds of mitotic systems. Chatton trained more than thirty student collaborators, among them André Lwoff, who won the 1965 Nobel Prize in Physiology or Medicine. Later, the great cell biologist Hans Ris and I completed Chatton's light microscopy descriptions on syndinian mitosis dinoflagellate. We had at our disposal sophisticated electron microscopes as well as biochemical and molecular techniques and thus succeeded in corroborating the correct interpretation by Chatton of chromosome structure and mitotic cytology. [*Int Microbiol* 2006; 9(2):173-177]

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Introduction

The great scientist Edouard Chatton (1883–1947) studied many microorganisms of the kingdom Protoctista during his extraordinary scientific career (1904–1947) [17,21], and described them extensively in his *Notice de Titres et Travaux*, published in 1938 [6,14]. Chatton had a particular love for dinoflagellates and ciliates [17]. In 1925, thanks to his profound knowledge of protists [21], based on more than a century of previous work by others, he was able to distinguish, for the first time, the fundamental differences between unicellular eukaryotes and prokaryotes [5]. In the table of a paper devoted to *Pansporella perplexa*, an amoeboid parasite of *Daphnia*, Chatton provided “reflections about the biology and

phylogeny of protozoa”. In 1973, Stanier and Lwoff, the later being the most prestigious pupil and collaborator of Chatton, summarized and developed the important concept of differentiation of prokaryotic vs. eukaryotic protists (Ernst Haeckel, who coined the term ‘protist’ in the nineteenth century, also included bacteria in that group) [25,27,29]. Since then, this differentiation has become increasingly well-accepted by botanists and zoologists. The latest revised tentative classification of unicellular eukaryotes incorporates the results of both ultrastructural and molecular phylogenetic studies [1].

This review focuses on some unusual dinoflagellates belonging to the genera *Blastodinium* and *Syndinium*, first studied by Chatton and subsequently by other authors [28], and analyzes the conceptual contributions by Chatton and later cell biologists to these models.

Edouard Chatton and the Blastodinids; further studies

In 1905, at the Laboratoire Arago, in Banyuls, France, Chatton discovered unusual mixotrophic Blastodinids in the digestive tract of pelagic copepods. His first publication on this new order, in 1906, together with the many others that followed, constituted a major part of his doctoral thesis on parasitic dinoflagellates, published in 1920 [2]. Using the light microscope of the time, Chatton very carefully described the cytology of this binucleated (“fixed in anaphase”) trophocyte (Fig. 1), which sporadically divides to form sporocytes. These, in turn, synchronously develop and divide in each sporogenic layer. At first, Chatton thought that peridinian blastodinids became multicellular individuals. He also described large “archoplasmic spheres” that contained the Golgi apparatus, one at each pole (see Fig. 1 for details).

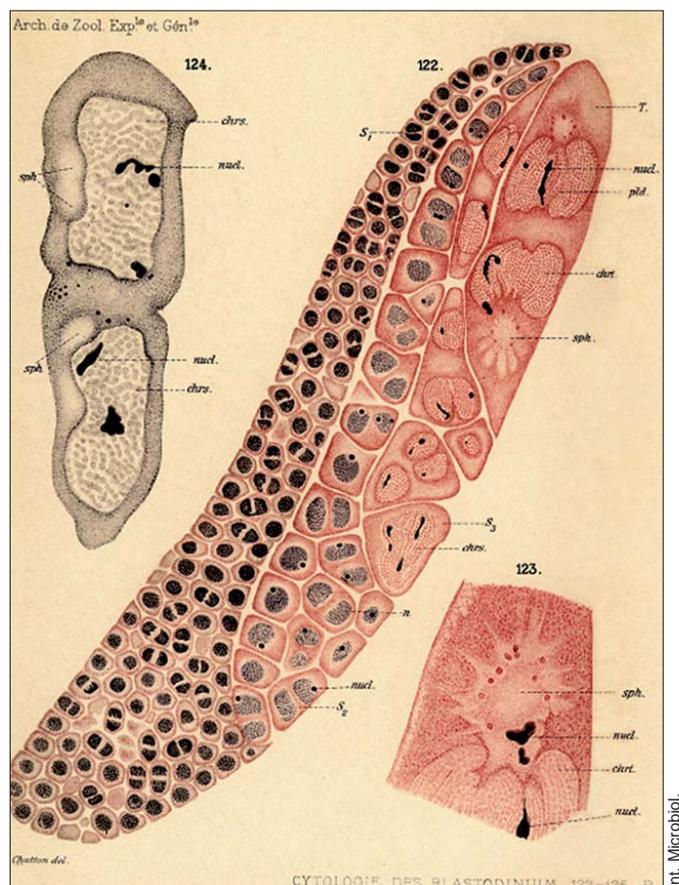


Fig. 1. Cytology of *Blastodinium* sp., Plate XI, extracted from the Edouard Chatton's doctoral thesis [2]. *Blastodinium* sp. during sporulation is depicted. The binucleated sporocytes synchronously develop and divide in each sporogenic layer as soon as their chromatin becomes increasingly condensed. The binucleated trophocyte (upper right) that divides sporadically maintains uncondensed chromatin.

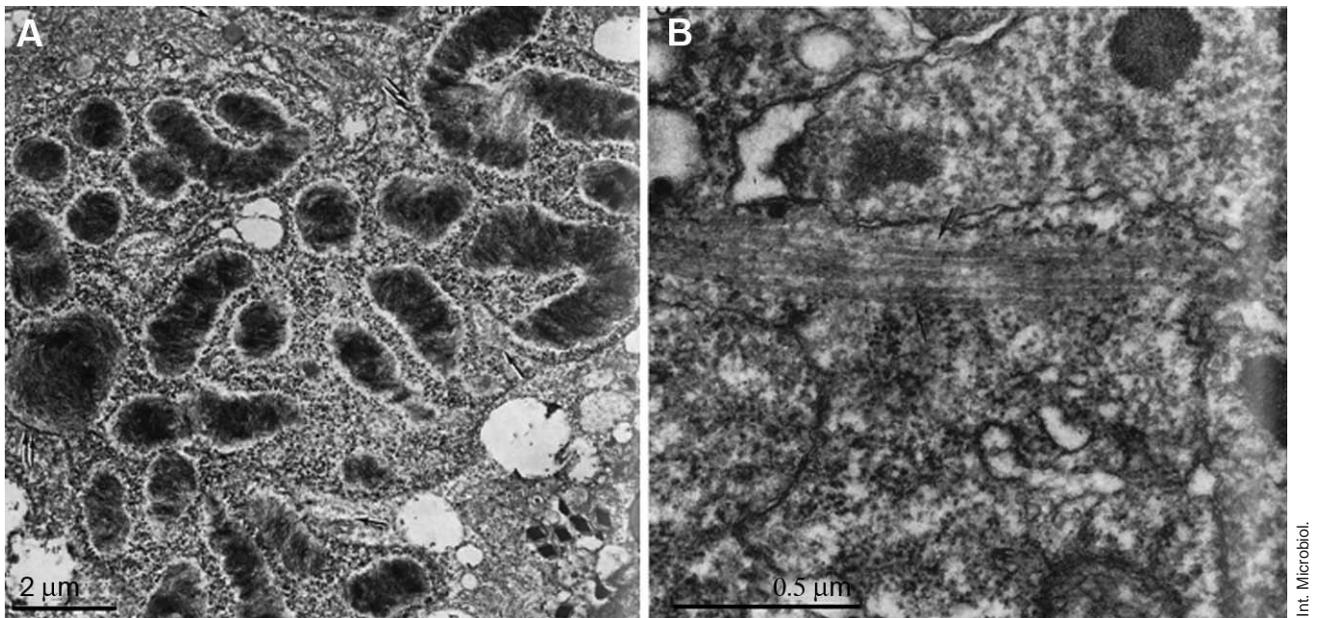
Also at the Laboratoire Arago, but about 70 years later, I continued the work of Chatton but used transmission electron microscopy (TEM) to study the ultrastructure of the same Blastodinids as part of my doctoral thesis. I confirmed Chatton's observations and described both *Blastodinium* chromosome structure and chromatin condensation during sporogenesis (Fig. 2A). In addition, I reported the presence of an extranuclear microtubular spindle (Fig. 2B) [15], characteristic of mitosis in this organism.

At the same time, Kubai and Ris ultrastructurally described “dinomitosis” in another species of dinoflagellate, *Gyrodinium* (*Crypthecodinium*) *cohnii* [11]. None of the numerous sections at the level of the “archoplasmic spheres” ever showed the presence of centrioles, except in *Syndinium* (see below). These observations eventually led me to study the particular architecture of dinoflagellate chromosomes by using ultrathin sections of specifically fixed *Prorocentrum micans* cells (Fig. 3A) [20] and the whole-mount technique (Fig. 3B) [18]. Together with other authors, I also demonstrated the absence of longitudinal differentiation of dinoflagellate chromosomes [8], their fibrillar organization [7], their division [19], and the maintenance of their architecture by divalent cations [9,10] and structural RNAs [22]. This work has been summarized in a review [23].

Edouard Chatton and syndinian mitosis

In the years 1920 and 1921, Chatton described the genus *Syndinium*, symbionts of marine copepods [2] and radiolarians [4]. On the basis of the morphology of free-living swimmers and their released spores, Chatton considered *Syndinium* to be a specialized dinoflagellate. Based on the apparent simplicity of *Syndinium* division and the fact that it has only five chromosomes, Chatton considered it as a model example for dinoflagellate mitosis [3], which he thus named ‘syndinian mitosis’. Ris and Kubai investigated a *Syndinium* sp. parasite of the radiolarians *Collozoum* and *Sphaerouzoum* [13], and I studied *Syndinium turbo* Chatton, a parasite of the pelagic copepod *Corycaeus venustus* Dana [16]. Further ultrastructural studies showed *Syndinium* to undergo a very peculiar peridinian mitosis, characterized by a closed permanent nuclear membrane outside of which lies an extranuclear mitotic spindle with its centrioles and kinetochores attached to the inner surface of the nuclear membrane. Other dinoflagellates have no centrioles.

Typical dinoflagellate chromosomes lack basic proteins, such as histones, but have other basic nuclear protein associated within their DNA, as shown in biochemical studies



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Fig. 2. (A) Transmission electron microscope view of an ultrathin section through a dividing sporocytic nucleus of *Blastodinium contortum* Chatton. Note the presence of an invaginated nuclear envelope that forms cytoplasmic channels, near which condensed chromosomes are attached. From MO Soyer (Gobillard), *Chromosoma*, 1971, 33, p. 93. (Diameter of a chromosome, ca. 0.8 µm). (B) Longitudinal ultrathin section through the microtubular mitotic spindle of *Blastodinium*. The spindle passes through the nucleus in cytoplasmic channels formed by the invaginated nuclear envelope. (Bar = 0.5 µm).

[9,23,28]. Ris and Kubai stained *Syndinium* chromosomes with alkaline fast green and obtained clear and strong staining, demonstrating that *Syndinia* differ from typical dinoflagellate chromosomes in that they do not have histone family proteins associated with their DNA [13]. Further biochemical and molecular studies could be important to analyze and characterize such basic nuclear proteins.

As Ris, Kubai, and myself have shown by TEM observations, the nuclear characteristics of *Syndinium* appear completely different from those of typical dinoflagellates. The attachment of chromosomes to the mitotic spindle—even if it is extranuclear and passes through the nucleus in nuclear channels—and the visible kinetochores show that “syndinian” mitosis is closer to the orthodox mitosis of typical

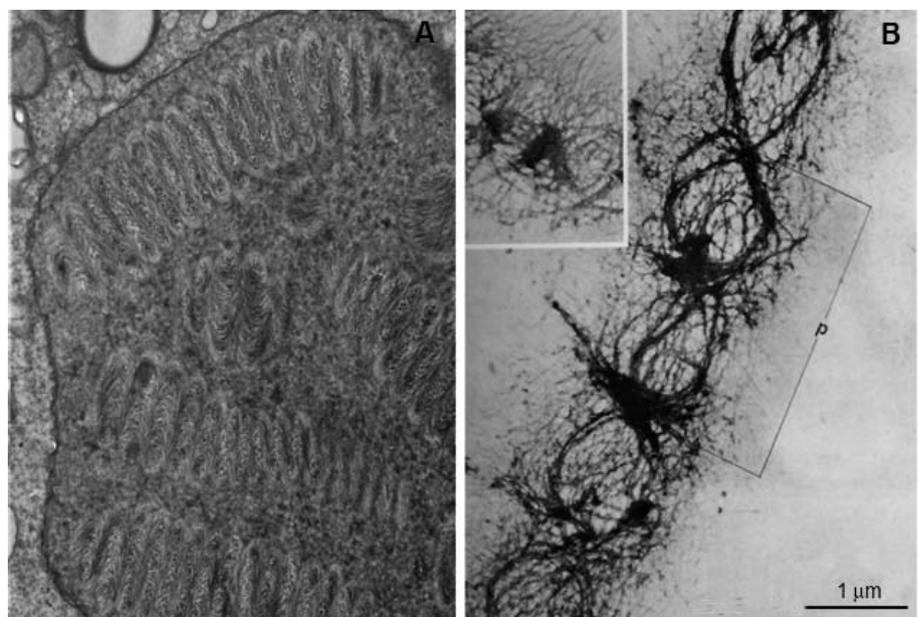


Fig. 3. (A) Ultrathin section of a *Prorocentrum micans* dinoflagellate nucleus in prophase. Observe the regular organization of the chromosome architecture, which is maintained by divalent cations and structural RNA. Specific Karnovsky-Soyer (Gobillard) fixation [19]. (B) Whole-mounted dinoflagellate chromosome, spread on water and observed by TEM after contrast with uranyl acetate. Note the regular supercoiling of the twisted nucleofilaments in an orthospiral arrangement with regular pitch. From Soyer (Gobillard) and Haapala [18], and Haapala and Soyer (Gobillard) [7].

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eukaryotic cells [13]. However, the presence of differentiated structures at the spindle poles, such as centrioles, or the persistence or disappearance of the nuclear envelope are clearly secondary variations rather than essential aspects of this type of mitotic mechanism [13].

Centrosomes in dinoflagellates

Centrosomes (archoplasmic spheres of Chatton) have been shown to be present in all dinoflagellate cells. As shown by Kubai and Ris [11], after ultrathin serial sections through whole cells of *Gyrodinium* (*Crypthecodinium*) *cohnii*, and by Soyer (Gobillard) [15] after sectioning whole *Blastodinium* trophocytes and sporocytes, or *Prorocentrum micans* cells [19], no centrioles have been observed inside the “archoplasmic spheres” located at the poles of the dividing cells. The archoplasmic spheres do contain sizeable Golgi apparatus. These results were later confirmed by incorporation of anti-tubulin antibodies and immunodetection of tubulin antigens in *Crypthecodinium cohnii* dividing cells as well as by confocal laser fluorescence scanning microscopy of semi-thin sections and by TEM [24,26].

Cryofixation and cryofracture techniques enabled us to complement the description of the ultrastructure of these centrosome regions that are devoid of a classical centriole [24]. Moreover, cytochemical, biochemical, and immunocytochemical approaches together with molecular sequencing studies allowed us to detect centrosome-associated proteins and to monitor their behavior during the cell cycle: p80, a nuclear and cytoplasmic protein recently characterized, myosin II antigens, β - and γ -tubulins, CTR 210 antigens, p72 (an HSP 70 protein), α -actin, and p56^{cdc13}, a homologue of *Schizosaccharomyces pombe* cyclin B [24].

Conclusions

These older studies together with more recent ones have confirmed that to develop taxonomy in so diverse a kingdom as protocista, light, confocal laser scanning microscopy, TEM, SEM, as well as biochemical and molecular phylogenetic analyses must be taken into account. Further studies on dinoflagellates, of typical and/or atypical families, have demonstrated that Chatton, using the microscopes of his time, was already correct in suggesting a new classification of eukaryotic cells based on the presence or absence of the centrosome and its relationship to kinetosomes. On “course boards” for students, Chatton depicted cell evolution by centrosome-centriole-mitotic spindle morphology. Indeed, in the

dinoflagellate model and according to Chatton, most dinoflagellates are classified as *mastigosomées* and they show the presence of an extranuclear microtubular paradesmose linked to kinetosomes whereas *Syndinium*, which has canonical centrosomes, belongs to the *cinetosomées* group [12].

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Edouard Chatton (1883-1947) y los protistas dinoflagelados: conceptos y modelos

Resumen. Edouard Chatton contribuyó a nuestro conocimiento de los protoctistas unicelulares, especialmente ciliados y dinoflagelados, simbioses o de vida libre, en relación con los animales invertebrados marinos en los que residen. Más que la descripción de muchas nuevas familias, géneros y especies y de la descripción de su ciclo vital, Chatton anticipó algunos conceptos principales de la biología celular como la diferencia fundamental entre los protistas eucariotas y los procariotas mucho antes del uso del microscopio electrónico; la capacidad reproductora del sistema del cinetosoma-centriolo; la homología del cinetosoma con el centriolo mitótico de las células animales; y las diversas clases de sistemas mitóticos. A lo largo de su vida formó a más de treinta estudiantes colaboradores, entre ellos André Lwoff, que en 1965 ganó el premio Nobel de Fisiología o Medicina. Posteriormente, el gran biólogo Hans Ris y yo misma completamos las descripciones microscópicas de Chatton sobre mitosis del dinoflagelado *Syndinium*. Tuvimos a nuestra disposición técnicas sofisticadas de microscopía electrónica, bioquímicas y moleculares que nos permitieron corroborar la interpretación correcta de Chatton de la estructura cromosómica y de la citología mitótica. [*Int Microbiol* 2006; 9(3):173-177]

Palabras clave: microorganismos eucarióticos · conceptos de biología celular · dinoflagelados

Edouard Chatton (1883-1947) e os protistas dinoflagelados: conceitos e modelos

Resumo. Edouard Chatton contribuiu para nosso conhecimento dos protistas unicelulares, especialmente ciliados e dinoflagelados, simbioses e/ou de vida livre, em relação aos animais invertebrados marinhos nos quais residem. Mais que a descrição de inúmeras novas famílias, gêneros e espécies e de seu ciclo vital, Chatton antecipou alguns dos grandes conceitos da biologia celular: A diferença fundamental entre protistas eucariotas e procariotas, muito antes do uso do microscópio eletrônico, a capacidade reproductora do sistema do cinetossomo-centríolo; a homologia do cinetossomo com o centríolo mitótico das células animais, e as diversas classes de sistemas mitóticos. Ao longo de sua vida formou mais de trinta estudantes colaboradores, entre eles André Lwoff, que em 1965 ganhou o prêmio Nobel de Fisiologia ou Medicina. Mais adiante, o grande biólogo Hans Ris e eu mesma completamos as descrições microscópicas de Chatton sobre a mitose do dinoflagelado *Syndinium*. Tivemos a nossa disposição sofisticadas técnicas de microscopia eletrônica, bioquímicas e moleculares que nos permitiram complementar a interpretação correta de Chatton da estrutura cromossômica e da citologia mitótica. [*Int Microbiol* 2006; 9(3):173-177]

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