Fine Structure of the Macronucleus of the Gymnostome Ciliate, *Chlamyodon pedarius*

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As emphasized in previous publications (Kaneda 1960 a, b) *Chlamyodon pedarius* provides unusual opportunities for studying the composition and the behavior of the components during division of the macronucleus in a single celled organism. The reason is that the macronucleus exhibits several features of an interesting and uncommon nature, such as the different structures in the anterior and posterior halves, the distinct appearance of an endosome (caryosome) in the center of the anterior half at interphase, the advent of "reorganization band", chromonemata, polar caps and central substance at the division stage. And the anterior half shows light color with Heidenhain's haematoxylin and faint reaction in Feulgen's and methyl-green tests, but the posterior half and the endosome dark color and strongly positive reaction.

The aims of the present study are to gain a more detailed knowledge of the ultra-structure of the macronucleus by electron microscopy and to relate the biochemical results to the underlying fine structure.

A preliminary account of this work was presented at the 32nd Meeting of the Genetics Society of Japan held in Kyushu University, in November 1960.

Material and Methods

The organisms used in this study were collected from a blackish water ditch in Hiroshima City. They were cultured with the previously reported method (Kaneda 1960).

For thin sectioning and electron microscopy, first the organisms were removed with capillary pipette from the culture dish to the spill tube. They are concentrated in the bottom of the tube by a light centrifugation, after which the supernatant fluid was decanted quickly and replaced with a solution of the fixing reagent—One per cent OsO_4 buffered with 0.028 M acetate-veronal to a pH 7.8–8.3, and added NaCl to 1/4 M. In order to minimize the extraction of the specimens by the fixative, the fixation was shortened in many cases to 30–60 minutes and carried out at about 2°C. The fixed specimens were thereafter washed rapidly in one change of veronal-acetate buffer solution (diluted 1: 1 with distilled water). Then the specimens were rapidly dehydrated with graded ethanol, and embedded in a mixture of butyl and methyl methacrylate (8: 2) containing 0.2 per cent benzoyl peroxide. The centrifugation was repeated

before each change of fluid. Polymerization was carried out at 55°C for 12 hours. Sections about 50 m μ thick were cut with a glass knife from the resulting plastic blocks. They were mounted on copper mesh, the grids of which were previously covered with a thin supporting film of formvar or collodion, and in the case of the latter still fixed with vapor of carbon. The sections were examined without removing the plastic, with Akashi TRS-50 type electron microscope.

Observations

The macronucleus of this ciliate at interphase is of an ovoidal form in the young stage but it becomes ellipsoidal form of about 29 μ in length and about 13 μ in width as it approaches the division stage. The division of the macronucleus takes place daily between about 11 a. m. and about 4 p. m. under normal conditions.

In the light microscopy, the macronucleus at interphase comprises two different structures: The anterior half is a vesicular structure with an endosome in its center and the posterior half is a massive structure. The nuclear membrane is so very thin as to be almost beyond the resolving power of the light microscope.

Electron micrographs of longitudinal sections through the macronucleus at interphase show evidently that the macronucleus is divided clearly into two halves of different structures, the anterior and posterior parts (Fig. 1). However, they reveal that the fine structure of a young macronucleus is different from that of the macronucleus in the interphase just before the division.

The anterior half of the young macronucleus is filled with a fairly homogeneous matrix of very lower density, containing in its center an endosome of round and more dense structure than the matrix material, and distributing several dense nucleoli of various shapes in its periphery. The matrix consists of diffuse filamentous material, in which are seen dispersively more electron-scattering strands and threads than the other material. The strand is measured about 100 m μ in width, as their longitudinal appearance and in diameter of their cross-sectional views (Fig. 1 arrows). The strands consist of one or two very dense threads and a less dense matrix. The thread is much helically coiled structure, and in the strands the two threads intertwine in each other. Each width of the threads ranges in different places between 25 m μ and 30 m μ . The difference may be due to degree of the extension of the threads.

The endosome is composed of compact filamentous material and consists of a very dense cortex and a slightly light core (Fig. 1 e). The density of the cortex is similar to that of the dense particles of the nucleoli distributing in the periphery of the macronucleus. The filamentous material constituting the inner core is much coiled and shows a similar density to that of the threads dispersing in the matrix. The endosome has not a defined retaining membrane and out of it extends clumps of the filamentous material resembling the dense threads described above. The fact may possibly support the observation reported previously by the writer (1960) that reorganization of the endosome after nuclear division was a conglobation of chromatin materials distributed within the macronucleus.

Fig. 1. A low power survey picture showing a longitudinal section of the entire young macronucleus. Notice the different structures in the anterior and posterior parts, the endosome (e) situated at center of the anterior half part, the nucleoli (n) distributed on the periphery, and the "reorganization band" (B) (enlarged in Fig. 2) crossing the middle portion of the posterior half part.

The nucleoli are situated beneath the nuclear membrane with a short distance (Fig. 1 n). The structure is composed of filamentous material, dense particles of various size and less dense matrix. The large ones of dense particles contain clear vacuole of least density respectively. The nucleoli have not any retaining membrane. The nucleolus at anterior end and easily visible with the light microscope appears in largeness of about $0.9\ \mu$ in thickness and about $8.4\ \mu$ in width.

The posterior half of the young macronucleus is a dense structure, and is composed of a coarse reticulum, of ill-defined dense bodies in the meshes and of little

matrix material of very low density. And there are seen usually one or two large nucleoli at the posterior margin. The reticulum superimposes on it dense material resembling the dense body. It is clear from their number, size, shape and distribution that the dense bodies represent sections through masses of the nucleolar material of the light microscope image. It is characteristic of the nucleoli in this part that each of them is surrounded by a clear zone of least density.

A remarkable feature is a band crossing the middle portion so as to divide the posterior half of the macronucleus into the anterior and posterior portions (Fig. 1 and 2 B). This band is about one micron in width, and consists of two different structures, the less dense and very dense parts. The less dense part, the density of which is slightly superior to that of the matrix material, occupies its anterior main portion of about 0.7μ wide and comprises diffusing filamentous material of about $25\text{ m}\mu$ wide. The filamentous materials are similar to those found in the matrix of the anterior half part in configuration. The very dense part occupies the posterior border of about one third width of this band, and is composed of very dense strands or particles. Some of the particles contain clear vesicle (Fig. 2 thick arrow). The strand has a thickness of about $75\text{ m}\mu$ and contains two twisted dense threads, respectively about $25\text{ m}\mu$ in thickness (fine arrow). When both components in the portions of anterior and posterior to the band are compared the anterior part shows more compact structure, and consists

Fig. 2. An enlargement of the posterior half part of the young macronucleus to show fine structure of the "reorganization band" (B) and the difference of the structure in the portions of the anterior and posterior to the band. Notice the strands (fine arrows) composed of the dense threads, the dense particles (thick arrow) and the nucleoli (n) surrounded by a clear zone respectively.

of more dense reticulum and less achromatic spaces than those of the posterior portion. The reticulum in the anterior portion has so little superimposed material that the component strands about $100\text{ m}\mu$ wide are plainly detectable. The individual strand contains two very dense and intertwined threads of each about $25\text{ m}\mu$ thick (arrow). The nucleoli in the anterior portion show small homogeneous structure but the ones at the posterior margin manifest the same complicated structure as described about

Fig. 3. Semidiagrammatic interpretation of a longitudinal section through a very early prophase macronucleus. For purposes of clarity, the longitudinal stripes in the anterior half part and the reticulum structure made of many cords (chromonemata) in the posterior half part of the macronucleus are somewhat exaggerated. E, endosome; N, nucleolus.

a. A low power electron micrograph showing the vanishing nucleus (arrow) and the corrugated nuclear membrane in the anterior end portion of the macronucleus.

b. A low power electron micrograph showing the middle portion of the anterior half part of the macronucleus. Notice the longitudinal stripes (a part of which is enlarged in Fig. 4). e, endosome.

c. A low power electron micrograph showing the reticulum structure made of many chromonemata in the posterior half part of the macronucleus. n, nucleolus.

the nucleoli in the anterior half part. These facts correspond fairly with the feature of the "reorganization band" observed previously with the light microscope (Kaneda 1960 a).

Micrographs of longitudinal sections through the elongated interphase macronucleus reveal more fine complicated structures than those of the young macronucleus. The matrix material of the anterior half changes into very fine filaments and many fine granules, which are about $10\text{ m}\mu$ to $15\text{ m}\mu$ in diameter. And the nucleoli show very loose and vanishing structure of aggregate of granular texture, especially the nucleolus at anterior end has almost vanished and only remains some broken particles beneath the corrugated membrane (Fig. 3 a).

In the very thin portions of the section, it is observable that the filamentous material of the matrix has clumped to show longitudinal stripes running parallel to the antero-posterior axis of the macronucleus (Fig. 3b). The individual stripes are evenly spaced around $120\text{ m}\mu$, and their width is generally equal at the same level from the posterior ends and about $200\text{ m}\mu$, but at more anterior ends about $100\text{ m}\mu$. The width of the stripes and spaces between the stripes change from place to place, but this may be due to slight changes in the angle of sectioning or compression during sectioning. In the stripe within the endosome, two dense helical threads are apparently found (Fig. 4). The thickness of each thread is about $25\text{ m}\mu$ to $30\text{ m}\mu$. The individual thread may be composed of two more fine filaments of about $10\text{ m}\mu$. The same threads are detectable within the other stripes. These facts, together with vanishing of the nucleoli, seem probably to show that the macronucleus sectioned was entering to the prophase of nuclear division. The filamentous material of the matrix also may be helical structure but they appear in less dense and smaller in diameter than the threads described above.

Fig. 4. A neighbor portion of the endosome such as that indicated at square in Fig. 3b, showing the clumps of the filamentous material and the dense helical threads (arrow).

The posterior half part of the elongated interphase macronucleus shows almost the same structure as the constitution of the anterior portion to the "reorganization band" in the young macronucleus. However, the network is more typical structure than the latter one, and the dense nucleoli are composed of much fine granules and

filaments (Fig. 3 c). The network is made of many cords of about $100\text{ m}\mu$ wide and ill-defined length. The cords show respectively a rough helical form. It is seen obviously that at least two dense helices are contained in the cord (Fig. 5 a). The thickness of the individual helix is of the order of $15\text{ m}\mu$ to $20\text{ m}\mu$. Moreover, under high magnification the section shows parallel dense walls which bound a less dense core and they seem to be a longitudinal section of the filaments making up the helices. The section also shows in other places some rings of very electron-scattering material surrounding a less dense core and they seem to be cross-sectional views of the filaments (Fig. 5 b). The thickness of the wall is about $50\text{ \AA}$, and the core is about $80\text{ \AA}$ in diameter. However, the dimension of the fundamental components can not be determined precisely.

Fig. 5 a. An enlargement of the portion indicated at oblong in Fig. 3 c, showing the dense helices (arrows) in the chromonemata.

b. An enlargement of the portion enclosed with upper square in Fig. 3 c, showing sections of the dense filaments. Notice that each dense filament consists of very dense walls bounding a less dense core in longitudinal section (bottom arrow) and appears as a ring surrounding a less dense core in cross-section (upper left arrow). Upper right arrow indicates the dense filaments.

c and d. Diagrammatic representation of the chromonematic filaments in sections. c, longitudinal appearance. d, cross-sectional view. CN, chromonema; T, thread; F, filament.

Sections normal to the surface of the macronucleus reveal two component membranes, each about $50\text{ \AA}$ thick, separated by a space of about $200\text{ \AA}$ wide. The total thickness of the whole structure is, thereafter, about $300\text{ \AA}$ (Fig. 6 a). Constituent

differences between the inner and outer membranes as reported by Årzelius (1955) in the nuclear membrane of sea urchin oocytes and by Pappas (1956) in the nuclear envelope of *Amoeba proteus* have not been detected. At frequent intervals the two membranes approach and join each other, thereby delimiting pores (arrows). The width of the individual pores is usually about 400 Å. Tangential sections of the envelope, on the other hand, reveal many annuli, their diameter is approximately 600 Å (Fig. 6 b). They are made up of dense rims, about 100 Å wide, surrounding

Fig. 6 a. Electron micrograph of the section normal to the nuclear envelope showing the two component membranes and the pores (arrows). N and c indicate the nuclear side and the cytoplasmic side, respectively.

b. Electron micrograph of tangential section of the nuclear envelope showing annuli (arrows). They consist of rims surrounding less dense cores. N and c indicate the nuclear side and the cytoplasmic side, respectively.

a central less dense area of about 400 Å in diameter. The density of the rims is slightly greater than that of the material between the annuli. The pores can be interpreted as perpendicular section views of the annuli. They agree in frequency and dimensions. However, in the sections normal to the envelope, the portions beneath which the collapsing nucleoli are distributed show that the component membranes are remarkably separated and make themselves conspicuous pores of about 600 Å or more wide.

Discussion

The description given here of the fine structure of the macronucleus at interphase corresponds fairly well with the accounts previously reported by the writer (1960 a, b), who studied the fixed and living material with the light microscope. However, the following facts observed in the ultra-thin sections through the macronucleus are far beyond the limit of resolution of the light microscope and strikingly remark-

able subjects for the cytological study of the macronucleus.

The first is the structure of the nucleolus. Hertl (1957) has recently published an admirable report about the structure and function of the nucleolus (nucleolar apparatus) which studied in the diencephalon of white mouse with the light microscope, in which he concluded that "Der Nucleolus ist eine chemische tätige Kernorganelle. Er ist schwamm- oder knäuelartig aufgebaut, enthält basische und andere Eiweisskörper, ferner RNS". Regarding the structure of the nucleolus in the macronucleus of *Chlamydomonas pedarius*, it is concluded that in the young macronuclear stage the nucleolus is composed of filamentous material, dense particles of various sizes and less dense matrix, but those of the interphase macronucleus approaching the division are of aggregate of granular texture, and that the structure of the nucleolus is variable at the stages of the macronuclear cycle and in the places of the macronucleus, even at the interphase. Further discussion about the structure and function of the nucleolus will be furnished in another publication.

The second is the longitudinal stripes of the order of $100\text{ m}\mu$ to $200\text{ m}\mu$ in width. The stripes are observed in the macronucleus approaching the division. They run parallel to the long axis of the macronucleus. It is thought that the stripes begin to be formed before the occurrence of the changes visible with the light microscope in the very early prophase macronucleus, and that the formation continues during the prophase, contributing to elongation of the macronucleus. Because it has been ascertained in the living and fixed materials with the light microscope that the macronucleus elongates from the onset of prophase until it becomes about four thirds in length of the interphase macronucleus. The elongation is accompanied with extension of many chromonemata from the posterior half part toward the anterior half (Kaneda 1960 a, b).

The third is the highly electron-scattering threads and the electrondense helices. The threads are found in the matrix of the anterior half, within the endosome and the strands which distribute on the posterior border of the "reorganization band" and in the anterior portion to the band of the young macronucleus, and within the longitudinal stripes of the very early prophase macronucleus. The helices are found in the cords constituting the network of the posterior half part of the interphase macronucleus. Since the respective threads of about $25\text{ m}\mu$ to $30\text{ m}\mu$ wide may be composed of two finer filaments of about $10\text{ m}\mu$ thick and this filament appears to be identical with the filament making up the helix of about $15\text{ m}\mu$ to $20\text{ m}\mu$ in thickness, it is considered that the individual thread may possibly appear as a result of duplication of the filament. The fluctuation of diametrical size of the threads and helices may be explained with the account of Amano *et al.* (1956), namely it is large in highly contracted coiling, while small in loose coiling. In general, the fiber appears to be more contracted and intimately associated with the other one in the young macronucleus than the interphase macronucleus approaching the division.

On the other hand, seeing that where the strands and threads are found, likewise the cords constituting the network of the posterior half of the elongated macronucleus

show intensively positive reaction by Feulgen's and methyl-green tests (unpublished), it is inferred that the threads and the helices found within the cord respond morphologically to DNA containing elements in the macronucleus. Under high magnification the component filaments of the threads and helices consist of electron-dense material of about 50 Å thick bounding less dense core of approximately 80 Å. The structure corresponds fairly with that of 200 Å thick fibril observed by Ris (1956) in meiotic prophase chromosomes of anthers of *Lilium longiflorum* and *Tradescantia paludosa*, in oocytes of *Triturus viridescens* and testis of the rat, and in somatic interphase nuclei of lily anthers and onion root tips. Ris (1956) has described that the appearance of the fibrils strikingly resembles the structure of the tobacco mosaic virus described by Hart (1955) and Schramm *et al.* (1955) superficially at least, and he has suggested that in analogy to the structure of the tobacco mosaic virus the chromosomal microfibril may have an inner core of DNA surrounded by a shell of protein. On the other hand, the filament described in this paper seems to correspond to the nucleoprotein extracted from calf thymus nuclei by Liquier-Milward (1953), though the thickness of it is of the order of 10 m μ to 20 m μ which is much greater than that of DNA alone. According to Kahler and Lloyd (1953), sodium desoxyribonucleate prepared from calf thymus shows a spiral fiber structure of 15 ± 5 Å diameter. Moreover, James and Mazia (1953) have reported that high polymer desoxyribonucleic acid isolated from calf thymus or from chicken erythrocytes was of approximately 22 Å in thickness. From these facts, it may be concluded that the thread and helix are fundamental components of the chromonema of this ciliate macronucleus. Consequently, the following conclusions are drawn: the strands are fragments of the chromonemata in the young macronucleus; the longitudinal stripes are preliminary form of the chromonemata in the very early prophase macronucleus; the cords constituting the network of the posterior half represent the chromonemata in the interphase or very early prophase macronucleus.

The filaments making up the thread and the helix seem to correspond with the microfibrils which have been reported by many authors (Yasuzumi *et al.* 1951; Ris 1952; Lafontaine and Ris 1955; Amano *et al.* 1956; Gay 1956; Moses 1956; Dales 1960) in various metazoa, and by Kaufmann and Deepesh (1956) in *Tradescantia reflexa*. In protozoa the helices described by Pappas (1956) and Mercer (1959) in the nucleus of *Amoeba proteus* and the fiber constituting the honeycomb or the parallelly aligned structure in the macronucleus of *Tokophrya infusionum* reported by Rudzinska and Porter (1955) seem to be identical with the helices or the threads found in the macronucleus of *Chlamydodon pedarius* from their resemblance in electron-density and diametrical size, though the formers were not found in the chromonematic structure. Then, the present writer would like to accept actually Ris's description (1956) that the microfibrils seem to be the universal and basic component of chromosomes in both animal and plant cells.

The last is the structure of the envelope enclosing the macronucleus. The structure is double membranes of about 50 Å thick respectively and numerous penetrating

pores of about 400 Å diameter in normal areas. This structure is very similar to those found in the other protozoan and metazoan cell nuclei (Bahr and Bearmann 1954; Watson 1955; Grimstone 1959). Watson (1955) has rendered a proposition that pores of the nuclear envelope are a fundamental feature of all resting cells. His proposition is actually accepted in this study of the macronucleus of *Chlamydomonas pedarius*. As the consequence, it could be most easily suspected that compounds of relatively large molecular weight pass through the intact nuclear envelope. It suggests an active nucleo-cytoplasmic correlation.

Summary

The fine structure of the macronucleus of the gymnostome ciliate *Chlamydomonas pedarius* at young stage and interphase approaching the division has been investigated by means of the electron microscopy.

The macronucleus comprises two different structures: the anterior half is less dense structure which consists of the diffuse filamentous matrix material, and contains one endosome composed of much dense filamentous material in its center; on the contrary the posterior half is a very dense structure, and consists of a reticulum made of very dense strands or cords, very dense nucleolar materials and little less dense matrix material. However, the fine structure changes in the stages of the young macronucleus and of the interphase approaching the division.

The nucleoli are distributed beneath the envelope with a short distance. The structure is composed of filamentous material, dense particles of various size, and less dense matrix in the young macronucleus, but in the interphase macronucleus approaching the division it shows very loose and vanishing structure of aggregate of granular texture. They do not have any retaining membrane always.

The longitudinal stripes of about 100 m μ to 200 m μ in width are found in the macronucleus approaching the division. They run parallel to the long axis of the macronucleus, seeming to contribute the elongation of the macronucleus, and are inferred to be preliminary form of the chromonemata in the very early prophase macronucleus.

The highly electron-scattering threads of the order of 25 m μ to 30 m μ in thickness and the electron-dense helices of about 15 m μ to 20 m μ thick are found in the corresponding places to the DNA containing elements of the macronucleus, and the filament making up them seems to correspond to a nucleoprotein complex. It is possible, consequently, to draw the following conclusions: The individual thread appears as a result of duplication of the filament; the strands in the young macronucleus are fragment of the chromonemata; the cords constituting the network of the posterior half represent the chromonemata in the interphase or very early prophase macronucleus.

The structure of an envelope enclosing the macronucleus is composed of double membranes of about 50 Å thick respectively, separated by a space of about 200 Å wide, and numerous penetrating pores of about 400 Å diameter in normal area.

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