Structure of Plant Cells with Special Reference to Lower Plants

IV. Structure of *Trachelomonas* sp.¹

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Recently, the submicroscopic structure of the cell of lower plants has been studied by several workers. For instance Wolken and Palade (1953) have investigated the structure of *Euglena*, Sager and Palade (1957) studied *Chlamydomonas*, and Rouiller and Fauré-Fremiet (1958) worked on *Chromulina*.

These workers have partially clarified the fine structure of the chloroplasts and of what is called the endoplasmic reticulum. However, there are other organellae the structure of which is not well known and one of these is the nucleus.

In the present investigation, cells of *Trachelomonas* were studied and new information obtained on the structure of the nucleus, chloroplasts, and endoplasmic reticuli.

Material and methods

Trachelomonas was collected in a pond near Kyoto University. Most of the material was fixed with weak Flemming's solution or with a sublimate-alcohol mixture and then stained with Heidenhain's haematoxylin or methyl green pyronin. The structure was then studied with an optical microscope. Some of the same material was stained by Feulgen's stain. For electron microscopy some of the same material was fixed with 1% OsO₄ solution buffered at pH 7.0 and after standard treatment was embedded in methacryl resin and sectioned with an ultra-thin microtome. It was then examined with an electron microscope without removal of the resin.

Results

Nucleus. Nuclei are found at the posterior part of the cells of *Trachelomonas* (Fig. 1). A nucleus is surrounded by a nuclear membrane which consists of two thin layers (Fig. 2, m). In the nucleus, many chromonemata are seen in preparations stained with acetocarmine (Fig. 4) as well as in electron micrographs (Fig. 2). Chromonemata stained with acetocarmine

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appear to consist of two or occasionally four threads (Fig. 4. indicated by arrows). In electron micrographs, chromonemata are composed of fine fibrils of about $15\text{ m}\mu$ in thickness (Figs. 2, 3). These fine fibrils seem to be grouped into two bundles of $180\text{ m}\mu$ in thickness (Fig. 2. C'). Although all the four threads seen by microscope in the chromonemata were not shown clearly in

Fig. 1. longitudinal section of a cell. $\times 6,000$. ce, cell membrane. ch, chloroplast. co, contractile vacuole. en, envelope. er, endoplasmic reticulum. es, granules of the eyespot. f, flagellum. m, mitochondria. n, nucleus. p, pyrenoid. pa, paramylum body. r, reservoir. v, vacuole.

the electron micrographs, C'' in Fig. 3 may correspond to one of them. It may possibly be that C'' in Fig. 3 is composed of two fibrils of $15\text{ m}\mu$ in thickness. These fibrils appear in some parts to be formed from two finer fibrils which are about $6\text{ m}\mu$ thick (Fig. 3. arrow).

With a microscope a spherical nucleolus is seen in the center of the

nucleus. In electron micrographs, however, nucleoli are somewhat irregular in shape (Figs. 5-8). In these nucleoli there are electron dense regions and

Fig. 2. a part of the nucleus. $\times 45,000$. C, chromonema. C', two threads constructing a chromonema. m, double layers of a nuclear membrane. b, electron dense body. g, small granules in the nucleus.

Figs. 3-8. 3, sectional diagram of a chromonema. $\times 110,000$. C' , two threads constructing C' thread. C'' , two threads constructing C'' thread. An arrow shows a thin fibril which seems to be composed of two thinner fibrils. 4, nucleus stained with acetocarmine. Arrows show chromonemata which are composed of four threads. 5-8, nucleoli and bundles of electron dense bodies. $\times 6,000$.

electron coarse regions, the former being thread-like and the latter globular. Sometimes nucleoli appear to be divided into several parts (Fig. 6), and in some cases the parts are elongated and rod shaped (Fig. 7).

When nuclei were stained with pyronin-methyl green solution, chromonemata were stained with methyl green and nucleoli with pyronin. When the nuclei were stained by Feulgen's method, the chromonemata were strongly coloured while the nucleoli were colourless.

It must be noted that there are many electron dense bodies in the nucleus (Figs. 5-8). These bodies appear rod shaped and are about $250\text{ m}\mu$ in diameter. They are surrounded by a membrane. They appear to be collected into several groups (Figs. 5-8). These bodies could not be observed with an optical microscope in preparations stained with haematoxylin, pyronin-methyl green, acetocarmine or Feulgen's stain.

Besides chromonemata, nucleoli and electron dense bodies, there are many small granules in the nucleus (Fig. 2, g). These granules are $60\text{--}130\text{ m}\mu$ in diameter, and are frequently found near the nuclear membrane.

Chloroplast. At the periphery of the cell, plate-like chloroplasts are seen, each of which has one pyrenoid covered with paramylum bodies (Fig. 1). There are many thin lamellae in the chloroplast outside the pyrenoid. These thin lamellae are about $7\text{ m}\mu$ in thickness, and in most cases four of these lamellae are grouped into one layer of about $40\text{--}50\text{ m}\mu$ thick (Fig. 9).

The pyrenoid which appears to be a large electron dense globe in the chloroplast has canals in it (Fig. 10). In some cases, the canals of the pyrenoid could not be observed clearly so that the pyrenoid seems to be an electron dense core in the chloroplast (Fig. 11). The canals have connections with the lamellae of the chloroplast (Fig. 12). Often a lamella of the chloroplast extends into the canal of the pyrenoid (Fig. 13). Inter-canal substance of the pyrenoid is quite similar in appearance to the stroma substance of the chloroplast, but the former is more denser than the latter.

Mitochondria. Mitochondria are rod shaped, and are found here and there in the cytoplasm (Fig. 1). A mitochondrion has many "cristae mitochondriales" which radiate from the membrane of the mitochondrion (Fig. 17). Cristae, which are about $35\text{ m}\mu$ thick are composed of a dense outer layer and a coarse inner part. The outer layer is about $9\text{ m}\mu$ thick.

Endoplasmic reticulum. There are several cylindrical bodies in the cytoplasm, about 2μ long and almost twice as wide (Fig. 1). These bodies appear to be formed from numerous round thin discs with protuberant edges. These discs are piled on top of each other to form a cylinder (Fig. 14). Each disc is about $23\text{ m}\mu$ thick, and has an outer layer, about $5\text{ m}\mu$ thick, and an inner part (Fig. 14). Similar organellae have been reported by Rouiller and Fauré-Fremiet (1958) in *Chromulina*, and Sager and Palade (1957) in *Chlamydomonas*. The former workers identified them as the Golgi apparatus and the latter as the dictyosome. In both cases and in our organism,

Figs. 9-13. 9, part of a chloroplast. $\times 45,000$. Four of thin lamellae are grouped into one layer. m, nuclear membrane. 10, pyrenoid. $\times 12,000$. pa, paramylum bodies. 11, pyrenoid having no clear structure. $\times 18,000$. 12, connection of chloroplast-layer and pyrenoid-canal. $\times 30,000$. Arrows show the entrances of the layers. 13, part of a pyrenoid. $\times 35,000$. A lamella can be seen in the canal of the pyrenoid.

Figs. 14-17. 14, endoplasmic reticulum sectioned longitudinally. $\times 63,000$. 15, endoplasmic reticulum sectioned crosswise. $\times 40,000$. 16, cluster of small vesicles in the cytoplasm. $\times 30,000$. 17, mitochondrion. $\times 40,000$. Cristae mitochondriales are observed.

the organella is formed from thin layers one on the other, and seems to be an organella homologous to the endoplasmic reticulum or the cytomembrane. Here, the term "endoplasmic reticulum" is used in a broad sense although other terms could equally well be employed.

When living cells were stained with neutral red a number of granules of about 2μ diameter in the cytoplasm were coloured red. These granules may correspond to the endoplasmic reticulums mentioned above, although definite proof has not yet been obtained on this point.

Cytoplasm. The ground substance of the cytoplasm is formed from thin fibers of less than 3μ diameter. These fibers are sinuous but not always spiral as described by Strugger (1957).

There are many small vesicles in the cytoplasm. They are distributed irregularly throughout the cytoplasm though they often form clusters (Fig. 16). These vesicles are $50-120\mu$ in diameter. They are more frequent near the endoplasmic reticulum and are similar in size and appearance to the protuberances of the endoplasmic reticulum.

Contractile vacuoles. By microscope, a contractile vacuole can be seen near the base of the reservoir. In electron micrographs however it is found that it consists of many small vacuoles (Fig. 1). These small vacuoles are of different sizes; the average size is about 0.7μ , but the largest about 2.5μ .

Discussion and conclusions

Yasuzumi (1956), Amano *et al* (1956), Shigenaga (1957), and Ris (1957) consider that a chromonema is composed of several thin threads. In the present investigation, it was shown that a single chromonema is composed of at least eight fibrils each of which may consist of two thinner fibrils.

The structure of the nucleolus appears to be quite similar to that of *Euglena* (Ueda 1958). After staining with pyronin-methyl green and Schiff's reagent the nuclei did not appear difference from that of higher plants and animals.

Electron dense bodies, which seem to be grouped in bundles in the nucleus, resemble the nucleolus in density and structure. Nucleoli are, in some cases, divided into several parts which are somewhat elongated. Therefore it is reasonable to assume that some parts of the nucleolus may divide and elongate in such a manner that after lengthwise split of the elongated nucleolar bodies they are transformed into bundles of electron dense bodies.

From the results of several investigators, the thickness of each lamella of the chloroplasts of both lower and higher plants, is $5-8\mu$. The lamellae having a thickness of this order, therefore, are assumed to be fundamental units of structure in the chloroplasts. In the chloroplast of *Trachelomonas*, lamellae of about 7μ thickness are found and in the large parts of the chloroplast four of these lamellae are grouped into one layer of $40-50\mu$ thickness. Since the canals of the pyrenoid continue to the lamellae and the inter-canal substance to the stroma substance and the inter-canal substance

is quite similar to the stroma substance, the pyrenoid is assumed morphologically to be an organelle in which, in the chloroplast, some materials of the stroma accumulate and then the lamellar structure of the chloroplast is partially or completely disintegrated. Further discussion on this point will be published in later.

Small vesicles, which are 50–120 $m\mu$ in diameter and are distributed more densely near the endoplasmic reticulum, may be derived from protuberances of the discs of the endoplasmic reticulum. That is, the protuberances of the discs may be pulled off and then distributed in the cytoplasm.

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Summary

The submicroscopic structures of the cells of *Trachelomonas* were studied.

Each chromonema in the nucleus consists of at least eight threads of about 15 $m\mu$ in thickness.

Chloroplasts are formed from many thin lamellae of about 7 $m\mu$ thickness four of which are, in most parts of the chloroplasts, grouped into one layer of 40–50 $m\mu$ thickness.

The endoplasmic reticulum is composed of many thin discs stacked one on another.

The so called contractile vacuole is composed of many small vacuoles.

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