CHANGES IN NUCLEAR STRUCTURE DURING BINARY FISSION IN THE CILIATE NASSULA

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SUMMARY

During binary fission intranuclear spindles are formed in the three micronuclei and in the macronucleus. The nuclear envelopes remain intact throughout nuclear division. At one stage all the nuclei are linked together by the formation of membrane bridges in continuity with the outer membranes of their envelopes. Three types of filamentous structure have been distinguished in the spindles: microtubules of diameter 210-240 Å, fine branching filaments, and filaments which have a C-shaped transverse profile and a diameter of about 240 Å, for which the term C-filament is suggested. At metaphase a polar vesicle is situated at each pole of the micronuclei but centrioles are absent. During late anaphase a long central spindle, called here a separation spindle, is situated inside each micronucleus and elongates between the separating chromosomes. The nuclear pores of both types of nuclei are partly filled with dense material. In long, late anaphase micronuclei, pores are abundant in the parts of the envelope surrounding the ends of the nuclei but are rarely found elsewhere. Large numbers of ribosome-like granules are attached to the outer surfaces of the micronuclear envelopes where they sheathe the separation spindles.

INTRODUCTION

This paper is mainly confined to a description of structural changes in the spindles and the nuclear envelopes of the dividing macronucleus and micronuclei of the ciliate Nassula during binary fission. Some of the possible functions of these changes as well as some of the possible developmental mechanisms responsible for them are discussed. Particular attention has been directed to two changes: the remarkable elongation of late anaphase micronuclei, and the interconnexion of the envelopes of all the nuclei just before completion of fission. The former change has been described in several ciliates but the latter has not been reported before. Unlike other cells, some ciliates segregate several nuclei of two distinct types in a regular and precise manner during cell division. The possibility is considered here that the two changes referred to may facilitate the distribution of equal numbers of nuclei to each daughter Nassula. In this organism a long central spindle is situated within each late anaphase micronucleus between the separating chromosomes (Raikov, 1962). Similar long micronuclear spindles have been appropriately called separation spindles in the hymenostome Paramecium by Wenrich (1926). Their formation has been described in several orders of ciliates, for example in the entodiniomorph Opisthotrichum (Dogiel, 1925) and the hypotrich Euplotes (Turner, 1930). In Paramecium each separation spindle has also
been referred to as a *middle piece* (Mittelstück) or *connecting strand* by Hertwig (1889)
and Calkins & Cull (1907), respectively.

Detailed accounts of nuclear division in *Nassula ornata* during binary fission have been given by Raikov (1962, 1966). Those aspects of the investigation recorded below which duplicate some parts of his study for the most part confirm and extend his observations. Nuclear structures which have already been described by Raikov are not described in detail here, but some are mentioned briefly where such information is necessary for a clear comprehension of previously undescribed changes in nuclear structure.

MATERIALS AND METHODS

**Culture**

The species of *Nassula* (a cyrtophorine gymnostome) which has been studied was isolated by Professor E. Fauré-Fremiet and has not yet received a specific taxonomic designation. A culture of this species (Culture no. 1650/1) is being maintained in The Culture Collection of Algae and Protozoa, Botany School, Cambridge, England.

The filamentous blue-green alga *Phormidium inundatum* was used as food for *Nassula*. The alga was grown in 250-ml conical flasks plugged at the top with non-absorbent cotton wool and each flask contained 100 ml of culture medium L+C (Bourrelly, 1948). The flasks and culture medium were sterilized by autoclaving for 5 min at 15 lb/in². After inoculation with the alga the flasks were continuously illuminated and kept in a constant temperature room at 22 °C. *Phormidium* was subcultured by pipetting algae from 2-week-old cultures into fresh culture medium. Before being supplied to *Nassula* the algal filaments were washed three times with glass-distilled water.

*Nassula* was grown at 22 °C in Petri dishes filled with glass-distilled water to which *Phormidium* was added. The bottom of each dish was lined with a thin layer of 2 % agar, because in its absence a large proportion of the organisms in some of the dishes did not feed and divide. To start new cultures starved ciliates from old cultures were transferred with a pipette to dishes containing fresh agar, water and algae. Approximately 24 h after subculturing in this way a higher proportion of *Nassula* was observed at various stages of binary fission than during any other phase in the growth of new cultures. Six days after subculturing, all the *Nassula* were starving and none was dividing if no more fresh algae had been added to the dishes.

**Electron microscopy**

The dividing *Nassula* that have been examined were obtained from 1-day-old cultures; starving *Nassula* from 6-day-old cultures were also examined to ascertain the fine structure of their interphase nuclei. Organisms were concentrated by mild centrifugation and fixed for 30 min at room temperature with a fixative containing 2.5 % glutaraldehyde (Sabatini, Bensch & Barnett, 1963) and 0.6 % sucrose (Caulfield, 1957), buffered at pH 7.8 with phosphate buffer (18 mM). Washing for 12 h (7 changes) in the same phosphate buffer with 2 % sucrose was followed by post-fixation for
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30 min in 1% osmium tetroxide with 4% sucrose and phosphate buffer (10 mM). After washing once for 5 min as described above, *Nassula* were embedded in small flat plaques of 2% agar, essentially following the instructions of de Haller, Ehret & Naef (1961). Each plaque (approximate diameter 1 cm) contained about 100 *Nassula* and was handled in the same way as a piece of tissue. Dehydration in ethanol was followed by 3 changes of propylene oxide and flat embedding in Araldite (Luft, 1961). The orientation and fission stage of individual *Nassula* selected for sectioning were ascertained by examination with a phase-contrast microscope after embedding. Blocks were trimmed accordingly and thin sections were cut with glass knives on a Huxley ultramicrotome. The sections were picked up on grids coated with celloidin and carbon films and double stained with uranyl acetate (Gibbons & Grimstone, 1960) for 90 min followed by lead citrate (Reynolds, 1963) for 2 min. They were examined with a Philips EM 200 electron microscope operated at 60 kV with a 25-μm objective aperture.

**Classification of division stages**

All studies of division by both light and electron microscopy have been confined to four stages, designated stages A–D (Fig. 1). Stage A, the earliest detectable, is identifiable by a small swelling on one side of the organism (Fig. 1A). At stage B a shallow furrow can be seen for the first time on both sides of *Nassula* (Fig. 1B). At stages C and D the widths of dividing organisms across the cleavage constriction are approximately one-half and one-eighth, respectively, of the maximum width of *Nassula* (Fig. 1C, D). Stage D is readily recognizable because at this time the cleavage constriction becomes too narrow to harbour large food vacuoles and the cytoplasm in this region appears clear and transparent. The cytoplasm of all dividing cells was otherwise densely packed with dark green, highly refractile food vacuoles, making it impossible to see the nuclei in living dividing ciliates.

**Light microscopy**

Serial longitudinal Araldite sections (thickness 1–2 μ) of *Nassula* at stages A–D were stained with methylene blue (Mullinger, 1964), and were used to correlate the different sizes, shapes and positions of the nuclei and their spindles with stages A–D in the development of the cleavage furrow. The time periods between the various stages were recorded by observing individual *Nassula* in 1-day-old cultures with a dissecting microscope while they were dividing.

**RESULTS**

**Interphase nuclei**

The species of *Nassula* which has been studied usually possesses a single spherical macronucleus and three micronuclei of approximate diameters 45 μ and 4 μ, respectively. The micronuclei are often situated close to the macronucleus and are either spherical or approximately hemispherical in shape (Fig. 2). In the latter case the flat surface of the hemisphere is closely apposed to the surface of the macronucleus.
The macro- and micronuclear envelopes appear identical in structure (Fig. 2). Each is formed of an inner and an outer membrane (each 90 Å thick), separated by a distance of about 200 Å, so that the total thickness is about 400 Å. The envelopes contain pores, which are partly filled with dense material. Sections which graze an envelope tangentially (Fig. 13) show that near the outer surface of the envelope each

For legend see facing page.
pore appears as a dense disc in which a dense peripheral region or annulus surrounds some other dense material, which in turn surrounds a small granule or rod of even greater density. The latter is at the centre of the pore and has a diameter of about 200 Å. However, close to the inner surface of the envelope each pore appears hollow, with a dense peripheral annulus which has a maximum diameter of about 700 Å and a radial thickness of about 170 Å. The small dense granule or rod is situated at the centre of the less-dense central region (Fig. 13). Sections perpendicular to the plane of the nuclear envelope do not reveal a hollow or less-dense region passing through each pore between its inner and outer surfaces; instead the pores appear to be completely occluded by dense material in both interphase and dividing nuclei (Fig. 5). However, the term nuclear pore has been retained for these structures in this account. As the nuclear membranes and the dense material inside the pores appear equally dense, it has not been possible to ascertain whether the membranes cover the inner and outer surfaces of the pores, or whether the regions occupied by the pores represent circular holes in the membranes, each of which is filled with dense material. In the macronuclear envelope the pores are closely packed in a hexagonal arrangement in which the centre-to-centre spacing is about 900 Å. Nuclear pores may be similarly arranged in the micronuclear envelopes but sections grazing these nuclei have not been obtained. The macronucleus contains dense bodies of two types, large bodies and small bodies with diameters of about 0.5 μ and 350 Å, respectively (Fig. 2). In thin sections the

Legend to Fig. 1

Fig. 1. Stages A–D in the development of the cleavage furrow are indicated in the stippled drawings (right), which show profiles of *Nassula*, after fixation and embedding, viewed from the aboral surface, with the anterior end towards the top of the figure. The macronuclei (black) have been drawn to the same scale as the profiles of the whole cell and their positions are accurately represented. The diagrams to the left of the figure show corresponding stages in the division of the macronucleus and one of the micronuclei. Each diagram shows the nuclear arrangement as ascertained from sections of *Nassula* at a cleavage stage corresponding to that shown in the drawing to its immediate right. The thin black lines show regions within the nuclei where microtubules and C-filaments have been found and also show their orientation. The length and spacing of the lines is not intended to indicate the length or density of packing of these structures, except in the macronucleus at stage B, where there is a lower concentration of microtubules and C-filaments than in any of the other situations illustrated. The outer membrane of each nuclear envelope is shown by a thick black line. The regions containing the micronuclear dense bodies are shown in black. The nuclei and their component parts are not drawn accurately to scale as the diagrams are intended only to show changes in the overall shape and spatial distribution of the dividing nuclei and some of their parts.

A. The micronucleus is at metaphase and the macronucleus has started to elongate. At this stage a small swelling is apparent on the left side of the organism.

B. During late anaphase the chromatin of each micronucleus is concentrated in two terminal knobs which are connected by a long, elongating separation spindle.

C. The macronucleus is constricting and a compact macronuclear spindle is situated in its central constriction. The terminal knobs and the macronucleus are linked to the separation spindle by membrane bridges (arrows).

D. The macronucleus and the separation spindle have divided and each new daughter macronucleus and micronucleus are still connected by membrane bridges (arrows) to one of the halves of the separation spindle.
large bodies have an irregular profile but this usually approximates to a circular or oval shape. At the centre of each large body is a dense, apparently amorphous medulla, which is surrounded by a peripheral cortex of dense granules (Fig. 3). The granules are about 130 Å in diameter and their appearance and dimensions are similar to those of ribosome-like granules attached to the outer surface of cisternae of rough-surfaced endoplasmic reticulum in the cytoplasm. The depth of the cortex varies from two to four granules, but the granules apparently do not form discrete layers surrounding the medulla.

In the micronuclei there are large numbers of dense bodies, about 700 Å in diameter and mainly concentrated around the periphery of the nuclei (Fig. 2). Structures resembling the large bodies of the macronucleus have not been found in the micronuclei. The central region of each micronucleus contains fine branching filaments, usually about 70 Å in diameter (Fig. 2). Lengths of the filaments exceeding 0.2 μ have often been observed in thin sections.

In both types of nuclei the various types of dense bodies often appear closely juxtaposed and sometimes appear to be fused together to form short beaded filaments (Fig. 2). This is especially true of the small bodies of the macronucleus and the dense bodies of the micronuclei.

The interphase nuclei of well-fed Nassula from fresh cultures have not been examined. Their structure may differ from that of the interphase nuclei of starving Nassula described above.

**Micronuclear mitosis**

The main details of shape, position and structural composition of the micronuclei were found to be the same for all ciliates examined, provided that the ciliates had been fixed at the same cleavage stage, so particular stages in mitosis are temporally correlated with stages in the development of the cleavage furrow. At stage A, the earliest stage in mitosis examined, the 3 micronuclei are all at metaphase. The diameter of the nuclei is about 6 μ and therefore greater than at interphase, when it is about 4 μ. The micronuclear envelopes remain intact throughout mitosis but sections perpendicular to them show that their thickness is irregular, in contrast to their uniform thickness in interphase nuclei. The distance between adjacent pores is also usually greater in mitotic nuclei (Figs. 2, 5). The inner and outer membranes of the envelopes may be separated by distances ranging between about 200 and 700 Å, so that the thickness of the envelopes varies from a minimum of about 400 Å near the pores, to a maximum of about 900 Å where the membranes bulge apart between the pores (Fig. 5).

Clumps of dense material, about 0.2 μ in diameter and which probably represent the chromatin, are concentrated near the equatorial plane of each nucleus during metaphase (Fig. 4). Each clump is not a single solid body but appears to be a concentration of small granules or filaments, the diameter of which varies between about 150 and 300 Å. Each micronucleus contains large numbers of microtubules which are oriented approximately at right angles to the equatorial plane. Some of them pass straight through this plane and do not terminate in contact with the clumps of dense material (Fig. 4). Short branching filaments, similar to those described in interphase
nuclei, are situated in the spaces between the microtubules throughout mitosis, and some of them appear to be attached to the microtubules (Fig. 5). As this attachment of the filaments and microtubules has been observed in sections cutting the microtubules transversely, it is unlikely that this apparent contact results from the superposition of images. The two regions of a metaphase micronucleus which are most distant from its equator will be referred to as the poles of the nucleus. A saucer-shaped vesicle, bounded by a single continuous membrane about 90 Å thick, is situated at each of the poles (Figs. 4, 5) inside the nuclear envelope. Centrioles were never found near the poles. The lumen of the polar vesicle is about 200 Å wide. The convex surface is nearest to the nuclear envelope. Incomplete series of sections passing through the poles of several metaphase micronuclei were examined and, although continuity between the nuclear membranes and the membrane of the polar vesicle could not be found, the possibility remains that each polar vesicle is an inpushing of part of the nuclear envelope. Sections revealed that in some micronuclei the microtubules converge towards at least one of the poles (Fig. 5), but sections perpendicular to the equatorial plane and passing close to both poles of a metaphase micronucleus were obtained in only one instance, in which the microtubules converged towards one pole but not, apparently, towards the other (Fig. 4). It is not known whether this example is typical, or whether the microtubules also converge towards both poles in some nuclei and not to either pole in others.

Metaphase micronuclei are not situated closely alongside the macronucleus as they are during interphase but may be separated from it by distances of at least 2 μ. The orientations of the polar axes of metaphase micronuclei are extremely variable. They may differ in the same ciliate and are sometimes at right angles to the direction of extensive elongation of the nuclei which occurs later in division.

For a period of about 55 min (at 22 °C) during anaphase, the micronuclei elongate markedly to form an attenuated dumb-bell-shaped structure with a final length of about 100 μ (Fig. 1, B, C). Dense particulate material which probably represents the chromatin is concentrated at the ends of the nuclei (Figs. 1, B and 7) in the lobe-shaped regions which will be referred to as terminal knobs, as they are in Paramecium (Sonneborn, 1947). Most of this dense material is more finely divided during anaphase than it is in interphase (compare Figs. 2 and 7). It consists mainly of granules and filaments of about 200 Å diameter, distributed fairly uniformly throughout the terminal knobs. Dense bodies of about 700 Å diameter are less numerous at this stage than during interphase. The long separation spindle which is situated between the two terminal knobs of each micronucleus is approximately circular in cross-section (Fig. 11) with a diameter of about 1 μ. It consists mainly of a compact bundle of microtubules, aligned approximately parallel to each other and to the longitudinal axis of the separation spindle. These microtubules often exhibit a curved or undulating profile in longitudinal section (Figs. 7, 8). Continuous lengths of microtubules of up to 4 μ have been observed in sections of the separation spindles and it is possible that individual microtubules extend the whole length of the spindles. The microtubules are mainly confined to the long strand connecting the terminal knobs, but at each end of the separation spindles the microtubules splay apart and some of them project for several
microns into the terminal knobs, although none of the microtubules has been observed to continue as far as the ends of the nuclei (Fig. 7). During the period of micronuclear elongation the diameter of the separation spindles remains about 1 \( \mu \) and the number of microtubules seen in transverse sections of the spindles remains fairly constant.

In both the metaphase micronuclei and the separation spindles of anaphase nuclei the microtubules are circular in cross-section and their diameters range between about 210 and 240 Å. They appear hollow, with a more dense peripheral region or wall, about 70 Å thick, which often has an irregularly beaded or granular appearance in transverse section indicating that it may be composed of closely apposed subunits (Fig. 6). In addition, filamentous structures which appear C-shaped in transverse section have been found in the separation spindles and in metaphase micronuclei. The dense C-shaped wall seen in transverse sections of these filaments has the same thickness as the wall of the microtubules and also has the same granular appearance (Fig. 6). Filamentous structures of similar appearance and dimensions have been reported in other cells (see Discussion), and the term C-filament is suggested for such structures.

Transverse sections of the separation spindles show that there are approximately ten times as many microtubules as there are C-filaments during anaphase.

Nuclear pores are a common feature of the nuclear envelopes of anaphase micronuclei where the envelope bounds the terminal knobs (Fig. 7), but they are uncommon in the region where the envelope sheaths the separation spindle (Figs. 7, 8, 10, 11). In the latter region dense granules are attached to the outer surface of the envelope (Fig. 8), but these are not present in such large numbers where it surrounds the terminal knobs. These granules are about 130 Å in diameter and have the same dimensions and appearance as the ribosome-like granules attached to the rough-surfaced endoplasmic reticulum.

During stage C, when the nuclei are about 100 \( \mu \) long, the inner membrane of each envelope pinches off at the junctions of the separation spindle with the terminal knobs, which have become spherical in shape, so that 3 discrete compartments are formed. The 2 terminal ones contain the chromatin and the long central one contains the separation spindle. The three compartments remain within a common outer nuclear membrane, so that each terminal knob (new daughter micronucleus) is linked to the separation spindle by a narrow membrane bridge (Fig. 1, C). At the same time the separation spindles assume a bow-shape and become closely applied to the dividing macronucleus. The outer membranes of the nuclear envelopes surrounding each of the micronuclear separation spindles fuse with each other and with the membrane of the dividing macronucleus. Sequences of transverse sections of the 3 separation spindles and the dividing macronucleus show that the outer membranes are not fused along the whole length of these 4 organelles but that they are linked by numerous membrane bridges (Figs. 1, C and 11). Hence the perinuclear spaces within the envelopes surrounding the dividing macronucleus, the 6 daughter micronuclei, and the 3 separation spindles are interconnected, although these 10 structures are isolated from each other in discrete compartments bounded by an inner nuclear membrane.

Separation spindles have not been found in transverse sections which pass through the cleavage furrow of Nassula at stage D. However, at this time separation spindles
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are still found attached by membrane bridges to each of the daughter macronuclei, which indicates that the separation spindles have divided transversely (Fig. 1, D). Series of longitudinal sections sufficiently complete to assess the length of each half of the separation spindles have not been obtained, but no profiles longer than 20 μ were found. Transverse sections of the spindles revealed that the number of microtubules in the spindles was about half the total usually found during the period of anaphase elongation at stages B and C. They also showed that the diameter of the spindles was about 0.5 μ (Fig. 12) compared with 1 μ during anaphase. Membrane bridges have sometimes been found extending between each daughter micronucleus and an adjacent portion of a separation spindle in the form of anastomosing cisternae of rough-surfaced endoplasmic reticulum, which are continuous with the outer membranes of the envelopes surrounding these two structures (Fig. 12). They appear long and thin and have an undulating profile (Fig. 12). Thus it is to be expected that direct continuity between the nuclear envelopes of the micronuclei and separation spindles maintained by such membrane bridges, as revealed in a single thin section (Fig. 12), would be found infrequently even if these membrane bridges are always present at this stage. Since continuity has been demonstrated between the outer membranes of one daughter micronucleus and one separation spindle in two of the five Nassula sectioned at stage D, it seems likely that the membrane bridges uniting the daughter micronuclei and the separation spindles usually persist at this stage (Fig. 1, D). Thus at stage D, which occurs approximately 15 min before the completion of cleavage (as indicated by the complete separation of the proter and opisthe), there are two nuclear complexes on each side of the cleavage furrow. Each complex consists of one of the new daughter macronuclei (the macronucleus has completed division by stage D), 3 of the new daughter micronuclei, and 3 half-portions of the 3 separation spindles. Although the 7 components within each complex are joined by membrane bridges, no evidence has been found for any connexion or membrane bridge extending through the cleavage constriction, between the proter and opisthe, to unite the 2 nuclear complexes. At stage D the dense material in the micronuclei is still in a finely divided state compared with its condition in interphase (compare Figs. 2 and 12).

The changes outlined above usually take place within a period of 75 min at 22 °C. The completion of cleavage occurs approximately 90 min after the appearance of cleavage stage A. About 25 min elapse between metaphase when the micronuclei are approximately spherical with a diameter of about 6 μ and their elongation to form a dumb-bell of approximate length 70 μ. After a further 30 min the micronuclei are about 100 μ in length and anaphase elongation is apparently complete. During the next 20 min each separation spindle divides transversely into 2 portions. Thus the micronuclei elongate at an average rate of about 1.7 μ per min during anaphase.

Macronuclear division

During binary fission the macronucleus first elongates parallel to the longitudinal axis of the dividing Nassula, then constricts in the middle and finally divides into 2 discrete nuclei (Fig. 1). As with micronuclei, particular stages in macronuclear division
are temporally correlated with stages in the development of the cleavage furrow. Most of the elongation occurs before the central constriction in the nucleus becomes apparent. Throughout division the nuclear membranes remain intact, but the thickness of the envelope is more irregular and the distance between adjacent nuclear pores is often greater than in interphase nuclei (compare Figs. 2 and 10). The interphase nucleus is spherical, but during stages A and B the macronucleus starts to elongate. During stage B microtubules are found in the mid-region of the nucleus, with their longitudinal axes roughly aligned with the longitudinal axis of the elongating macronucleus, as indicated in Fig. 1B. Longitudinal sections of macronuclei at this stage reveal continuous longitudinal profiles of individual microtubules with a length of up to 2 \mu, often with a curved or undulating profile. At this stage the microtubules are not closely packed together in parallel array as are the microtubules in the micronuclear separation spindles, and, although some are grouped together in compact bundles of 5–20 microtubules, others occur singly, separated by distances of up to 0.2 \mu from the nearest microtubule. Some organisms examined at stage C possessed long sausage-shaped macronuclei about 80 \mu in length which were only slightly constricted in the middle and in which the microtubules were arranged as described for stage B. In other *Nassula* the macronucleus was slightly longer, ranging between 90 and 100 \mu long in different individuals, and narrowed in the middle to a transverse diameter of between 5 and 1 \mu where a long central constriction extended between the lobe-shaped ends of the nucleus (Fig. 1, C). Large numbers of microtubules and C-filaments are situated within this constriction, but very few were found at either end of the nucleus. Within the constriction the majority of the microtubules and C-filaments are concentrated together in a closely packed parallel array to form a macronuclear spindle, which is circular in cross-section and about 2 \mu in diameter (Fig. 9). Whether the microtubules of the macronuclear spindle are produced by concentration of the less closely packed microtubules and C-filaments found in the nucleus during stage B, or whether they are formed *in situ* as a closely packed bundle in the spindle has not been ascertained. Most of the other microtubules and C-filaments form an irregular layer just inside the nuclear envelope (Fig. 11), but a few of them are distributed throughout the remainder of the nucleoplasm, either singly or in small bundles. At this time, cisternae of rough-surfaced endoplasmic reticulum project from, and are continuous with, the outer membrane of the macronuclear envelope (Fig. 9). These cisternae were not found in such large numbers during interphase. In addition, during this period of macronuclear constriction the macronuclear envelope is joined to the envelopes of the micronuclear separation spindles by membrane bridges (Figs. 1, C and 11) as described in the previous section. The central constriction continues to narrow during stage C and simultaneously the number of microtubules and C-filaments located in the central constriction decreases (compare Figs. 9 and 10). It has not been determined whether this decrease is due to their breakdown or to their translation to another part of the macronucleus. However, the diameter of the micronuclear separation spindles does not change appreciably during this period (Figs. 9, 10). By stage D 2 spherical daughter macronuclei were found, one on either side of the cleavage furrow. No trace of the central constriction or any other connexion between the daughter nuclei could be
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found, and very few microtubules or C-filaments were present inside the nuclei. Nuclear pores are a common component of the macronuclear envelope, even where it surrounds the macronuclear spindle in the region of the central constriction, and the outer membrane of the envelope is not crowded with dense granules (Fig. 10). This contrasts with the structure of the micronuclear envelopes in the region where they surround the separation spindles (Fig. 10). The dimensions of the microtubules and C-filaments of the macronucleus are apparently identical with those already described in the micronuclei, but in the macronucleus the outer surface of the walls of these structures seems to be covered with an additional layer of amorphous dense material. Also there is usually a higher ratio of C-filaments to microtubules in the macronuclear spindle (approximately 3:10) than in the micronuclear separation spindles (approximately 1:10). Cross-sections of the macronuclear spindles show that fine branching filaments similar to those described in the micronuclear spindles are situated between the microtubules and C-filaments and sometimes seem to interconnect them.

The changes in the structure of the macronucleus described above occur within a period of about 75 min at 22 °C. Macronuclear constriction commences about 55 min after the start of macronuclear elongation. About 20 min after this and approximately 15 min before the completion of cell cleavage, the macronucleus has divided to produce 2 daughter nuclei.

**Discussion**

*Interphase nuclei*

Large and small bodies with dimensions comparable to those described in the macronucleus of *Nassula* have been reported in the macronucleus of *Paramecium* by Dippell & Sonneborn (1957) and by Jurand, Beale & Young (1962). The large bodies described above are structurally identical with the RNA-rich macronuclear nucleoli of *Nassula ornata*, which also consist of a dense medulla surrounded by a cortex of ribosome-like particles (Raikov, 1962, 1966). Seshachar (1964) has described a similar granular component forming part of the large bodies in the macronucleus of the heterotrich *Blepharisma*. Dippell & Sonneborn (1957) showed that the large bodies of *Paramecium* are RNA-positive and identified them as nucleoli. The fine structure of the cortex and medulla of the large bodies of *Nassula* (Fig. 3) closely resembles that of the nucleonema and the pars amorpha, respectively, of the nucleoli in spermatogonia of opossum testis (Fawcett, 1966). In both instances the amorphous component is surrounded by a granular component. Hence numerous RNA-positive bodies closely resembling the nucleoli described in cells of some other organisms have been found in ciliate macronuclei, but there are no definite reports of such bodies in micronuclei. This is significant, as it is generally accepted that nucleoli participate as part of a mechanism involved in gene expression, and in those ciliates in which it has been studied only the macronuclear genes are expressed phenotypically (Sonneborn, 1947).

The small bodies of the macronucleus correspond in size and appearance to the Feulgen-positive chromatins strands in the macronucleus of *N. ornata* (Raikov, 1962, 1966). The dense bodies and branching filaments of the micronuclei correspond to
the Feulgen-positive chromatin net and the achromatic protein bodies, respectively, as described in the micronuclei of *N. ornata*. The finely divided state of the contents of the interphase micronuclei of *Nassula* contrasts with the single spherical mass of dense material in the micronuclei of *Tetrahymena, Paramecium* and *Blepharisma* as described by Roth & Minick (1961), Jurand *et al.* (1962) and Seshachar (1964), respectively.

**Spindle structure, function and development**

Microtubules are present in the spindles of both types of nuclei in *Nassula*, as they are in *Diplodinium* (Roth & Shigenaka, 1964), *N. ornata* (Raikov, 1966) and certain peritrichs (Carasso & Favard, 1965). Hence microtubules seem to be a common component of the intranuclear spindles of both the micro- and macronuclei of ciliates. Their occurrence as part of the spindles in cells of a diversity of other organisms has been reported in, for example, the blastomeres of the sea-urchin *Strongylocentrotus* (Harris, 1962), the amoeba *Pelomyxa* (Roth & Daniels, 1962) and in wheat meristem cells (Pickett-Heaps & Northcote, 1966). Raikov (1966) reports that the microtubules in the micronuclei of *N. ornata* have a greater diameter (about 250 Å) than those in the macronucleus (150–180 Å), but the diameter of the microtubules in both types of nucleus in the species of *Nassula* described above all fall within a range of 210 – 240 Å. In the macronuclear spindle of the peritrich *Campanella* (Carasso & Favard, 1965), the microtubules are grouped together to form sinuous bundles, an arrangement identical with that of some of the microtubules in the macronucleus of *Nassula* during stage B.

The granular appearance of the wall of the intranuclear microtubules of *Nassula* (Fig. 6) is similar to that described for the wall of certain cytoplasmic microtubules (Ledbetter & Porter, 1964; Grimstone & Cleveland, 1965; Bassot & Martoja, 1965; Philips, 1966), and also similar to that described for the wall of intranuclear microtubules (which did not, however, seem to form part of a spindle) in certain nuclei of the stick insect *Carausius* (Smith & Smith, 1965). The C-filaments described in the intranuclear spindles of *Nassula* may represent a fixation artifact produced by rupture of the wall of microtubules. It is relevant that the wall of the C-filaments has the same thickness and granular appearance as the wall of the microtubules, and that if the two ends of the C-shaped profile of these filaments were pushed together until they made contact then they would have approximately the same diameter and appearance as microtubules. As the C-filaments cannot be distinguished from microtubules in longitudinal section, no estimate of their length has been obtained, and it has not been possible to determine whether the two structures recognized in transverse section as C-filaments and as microtubules ever form parts of a single continuous filament. Cameron (1965) has described similar C-shaped profiles of the wall of developing accessory fibres of *Tenebrio* sperm, and in *Nassula* the C-filaments might represent a stage in the development of the microtubules. Filaments with a C-shaped profile very similar to that of the C-filaments of *Nassula* are also spatially associated with microtubules and an elongating nucleus in the spermatids of the dragonfly *Aeschna* (Kessel, 1966), where they are closely applied to the outside of the nucleus only during the period of its elongation. Kessel suggests that the C-shaped profiles might be produced...
by a break in the wall of the microtubules. However, in the flagella of *Trichonympha*, Gibbons & Grimstone (1960) noted that the outer fibres cannot consist of two subfibres (or microtubules) simply juxtaposed together, because the central partition of each outer fibre has the same thickness as the outer wall. This observation is emphasized by a report that cross-sections through the tips of the sperm-tails of certain insects reveal nine microtubular profiles and nine C-shaped profiles in each sperm-tail, which indicates that the A and B subfibres are separate in this region and that the transverse profile of one type of subfibre is C-shaped (Philips, 1966). Thus in this instance there is evidence for the presence of C-filaments in close association with microtubules in a situation where the presence of the C-filaments is not accounted for by simple rupture of the wall of microtubules. Hence other examples of such associations, as described in *Nassula* and *Aeschna*, should not be dismissed as fixation artifacts without more careful investigation.

In the mitotic micronuclei of the peritrichs *Episty lis* and *Vorticella* and the entodiniomorph *Diplodinium*, Carasso & Favard (1965) and Roth & Shigenaka (1964), respectively, noted that centrioles were apparently absent and that non-convergent microtubules were present in the spindle. Study of *Nassula* also failed to reveal the presence of centrioles but showed that sometimes some of the intranuclear microtubules converge towards and terminate in the vicinity of a polar vesicle. Similar spatial relationships between intranuclear microtubules and vesicular structures or differentiated portions of the nuclear envelope have been described in two other unicellular organisms; in the microsporidian-like protozoon *Metchnikovella* (Vivier, 1965) and in the yeast *Saccharomyces* (Robinow & Marak, 1966). In both, typical centrioles have not been found in the vicinity of the dividing nucleus and the nuclear membranes remain intact during nuclear division. In these three organisms, apart from the proximity of the vesicles and modified portions of the nuclear envelopes to the ends of the microtubules at the time of the appearance of the spindles, no other evidence is available to indicate that these structures influence the development of the spindles.

In many organisms each mitotic apparatus nearly fills the cell containing it and the separation of sister chromosomes and their movement towards opposite poles of a mitotic apparatus also displaces the two sets of chromosomes to opposite ends of the cell, so that they are situated on either side of the cleavage furrow or cell plate when the cell divides. In *Nassula*, and in most ciliates, the mitotic apparatus of each micronucleus during metaphase and early anaphase is small compared with the size of the ciliate, so that the separation of the chromosomes and their concentration at either end of the nuclei in the terminal knobs, which takes place during early anaphase, does not position them at opposite ends of the cell. The extensive elongation of the micronuclei in *Nassula* during late anaphase and the development of long separation spindles presumably represent a special phase of mitosis concerned with the translation of the terminal knobs to opposite ends of the dividing ciliate. Mazia (1961) distinguishes two aspects of chromosomal movement during anaphase; namely, movement which reduces the distance between the chromosomes and the poles, and movement in which the increasing distance between sister chromosomes can be accounted for solely by elongation of the whole spindle where the poles also move apart. In terms of the distance
moved by the chromosomes, the latter type of movement predominates in *Nassula*. No examination has been made of changes in micronuclear fine-structure during early anaphase but some form of polar migration of chromosomes presumably occurs, as they are concentrated in an equatorial plate during metaphase but distributed throughout the terminal knobs during late anaphase. This polar movement may be mediated by a different mechanism from that which displaces the terminal knobs towards opposite ends of the cell. For instance, movement of the chromosomes may be effected by the combined action of pulling by chromosomal fibres and pushing by a central spindle, as suggested for the flagellate *Barbulanympha* (Cleveland, 1954).

The micronuclear separation spindles which have been described in anaphase micronuclei correspond to the *connecting strands* (*Verbindungsstrang*) described in *N. ornata* (Raikov, 1966). Throughout the elongation of anaphase micronuclei which occurs between cleavage stages B and C the diameter of the separation spindles remains fairly constant. Also, as ascertained by examination of transverse sections of the spindles, the number of microtubules and C-filaments within each spindle does not change markedly. As the separation spindles are increasing in length but not decreasing in cross-sectional area during this period, their volumes are increasing, and as the number of microtubules and C-filaments per unit length of the spindles stays the same, either the existing microtubules and C-filaments become longer or new ones are formed. Micronuclear elongation might be a consequence of such growth, especially if individual microtubules and C-filaments span the length of the spindles from end to end, so that their linear growth actively pushes the terminal knobs apart. There are also examples of cytoplasmic situations in which microtubules are concentrated in particular intracellular regions where local elongation of part of the cell is taking place, as for example in the hair outgrowth of trichogen cells of the hemipteran *Oncopeltus* (Lawrence, 1966) and in the extending axopods of the heliozoan *Actinosphaerium* (Tilney, 1965), and it is relevant that de Thé (1964) has drawn attention to the apparent structural identity of the microtubules found in mitotic spindles and those found in the cytoplasm of non-dividing cells. Marked elongation of spindles is not confined to ciliate micronuclei but also occurs in several flagellates. On the basis of direct observations of living *Barbulanympha*, Cleveland (1953) noted an increase in the length of astral rays in the elongating central spindle which apparently pushes the daughter nuclei apart. In the case of *Gigantomonas* (Cleveland, 1966) undertaking multiple fission, in which nuclei are sometimes eliminated from the cell, the possibility that the nuclei are moved apart by some agent other than the spindle and that the spindle extends passively between the nuclei, is difficult to reconcile with the report that in some fixed preparations of this flagellate just one end of a spindle and the nucleus fastened to this end of it protruded completely out of the cell.

Transverse sections of dividing macronuclei sufficient to estimate the number and length of the microtubules and C-filaments in the nucleus at different stages in its elongation have not been obtained, but it is possible that as suggested in the case of the micronuclei the growth of microtubules and C-filaments pushes the ends of the macronucleus apart. Raikov (1966) makes the same inference and refers to the macronuclear spindle as a *pushing body* (*Stemmkorper*).
During stage D, after the separation spindles have elongated and divided transversely their diameter and the number of microtubules they contain both decrease and there is also some evidence which suggests that the spindles are shortening at this time. This indicates that during stage D the separation spindles are decreasing in volume and that the microtubules and C-filaments are being autolysed, although no stages in the breakdown of these structures have been observed.

**Growth and differentiation of nuclear envelopes**

During binary fission the surface area of both types of nuclei increases as they elongate, constrict and divide. As the nuclear envelopes apparently remain intact during nuclear division they must also increase in area at this time by the incorporation of new portions of the membranes into the structure of the existing envelopes and possibly also by the formation of new nuclear pores. It seems unlikely that this increase in area is accommodated by simple stretching or two-dimensional expansion within the plane of the nuclear membranes without addition of new material, as the thickness of the membranes of dividing nuclei is the same as that of interphase nuclei. As no evidence for breakage of the membranes has been found expansion probably takes place by the interpolation of new material into the existing structure of the continuous membranes. Sufficient data are not available to show whether new pores are formed during division, or whether their total number remains constant. The inner and outer nuclear membranes are probably linked to each other by the pores, even if they are not fused together at the edges of the pores. The bulging apart of the membranes between the pores in envelopes of dividing nuclei may reflect temporary local inequalities in the rates of expansion of the inner and outer membranes. During anaphase the nuclear envelope of each micronucleus is structurally differentiated into 3 distinct regions, the 2 regions surrounding the terminal knobs where nuclear pores are a common component of the envelopes and the central portion of the envelope surrounding the separation spindle where pores are rarely found and numerous ribosome-like granules are attached to the outer membrane. The infrequent occurrence of pores in the central region may be an indication that the interpolation of new membrane-forming material during anaphase is confined mainly to this central portion. It also indicates that the formation of new pores, if it occurs at all, takes place at a slow rate compared with expansion of the membranes.

In this ciliate and in many other cells, the outer membrane of a nuclear envelope is often continuous with membranes bounding cisternae of rough-surfaced endoplasmic reticulum. The occurrence of ribosome-like particles on the outer membrane of the micronuclear envelopes could represent a special instance in which a portion of rough-surfaced endoplasmic reticulum has developed in continuity with (or fused to) the outer membrane of the micronuclear envelope, and where, instead of the ribosome-bearing membrane projecting outwards from the envelope into the cytoplasm, its development (or fusion) is apparently accompanied by the production of an equivalent quantity of the inner nuclear membrane, so that the two membranes are closely apposed and form part of the envelope surrounding the nucleus. Studies on zymogen formation in guinea-pig pancreas indicate that proteins synthesized by ribosomes
attached to membranes of the endoplasmic reticulum are sometimes initially concentrated within the spaces enclosed by these membranes (Palade, 1956). The modified ribosome-bearing region of each micronuclear envelope appears at the same time as the separation spindle is growing by an increase in its length and volume, and an increase is taking place in either the length or number of microtubules and C-filaments present in the spindle, which suggests that this region of each micronuclear envelope is specialized to concentrate spindle-forming proteins inside the nucleus. Alternatively, the ribosome-like granules might be involved in synthesis of membrane-forming proteins which are incorporated into the expanding envelope. Large numbers of ribosome-like granules are not directly attached to the outer membrane of the macronucleus during spindle formation. However, numerous cisternae of rough-surfaced endoplasmic reticulum project from this membrane at this time but are found less frequently during interphase. They might perform for the macronucleus one of the roles suggested above for the ribosome-bearing region of each micronuclear envelope.

Membrane bridges

The membrane bridges connect the macronucleus and the micronuclei during stages C and D throughout the final stages of macronuclear constriction and division of the micronuclear separation spindles. A possible function of the membrane bridges may be to enhance the probability of each daughter macronucleus being accompanied by 3 daughter micronuclei after nuclear division. The membrane bridges may help to bind the long, thin anaphase micronuclei alongside the long macronucleus before either type of nucleus divides, so that the terminal knobs of each of the 3 micronuclei are associated with opposite ends of the macronucleus. The length of the macronucleus just before it divides is usually greater than half the length of Nassula and as the cleavage furrow constricts transversely the ends of the macronucleus must always be situated on opposite sides of the cleavage furrow. When macronuclear division is complete the minimum width of Nassula measured across the cleavage constriction is less than the diameter of each daughter macronucleus. Hence one daughter is confined to the proter and the other to the opisthe. By contrast, the diameters of the daughter micronuclei are less than that of the cleavage constriction during stage D. As the separation spindles have divided they can no longer be expected to restrain the movement of sister micronuclei relative to one another and hold them on either side of the cleavage furrow. Thus if the 6 daughter micronuclei were otherwise free to move through the cytoplasm they might be unequally distributed on either side of the furrow when the proter and opisthe finally separate. Cyclosis of food vacuoles and the movement of small vacuoles across the cleavage constriction has been observed during stage D in uncompressed Nassula dividing in culture dishes. Therefore cytoplasmic streaming sometimes takes place at this time. Thus the attachment of daughter micronuclei to each daughter macronucleus during stage D and until the end of cleavage could reduce the likelihood of an unequal distribution of micronuclei to the daughter cells. The size relations of the nuclei and the cleavage constriction of N. ornata are similar to those described above and it is notable that in this instance the daughter micronuclei are also clustered closely alongside the dividing macronucleus (Raikov, 1962).
Nuclear division in Nassula

The envelopes of the macro- and micronucleus of the trichostome *Colpoda* are also sometimes united by a common outer membrane but this has only been described in non-dividing organisms (Rudzinska, Jackson & Tuffrau, 1966). Raikov (1966) has described the invagination and growth of the inner membrane of each micronuclear envelope to form a new envelope which replaces the old one during late anaphase and at the beginning of interphase in *N. ornata*. This has not been observed in the species of *Nassula* studied here and it is apparently a quite different phenomenon from the membrane bridge formation described in this paper.

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REFERENCES


Fig. 2. Interphase, showing two of the micronuclei and part of a macronucleus. The macronucleus contains large (l) and small (b) dense bodies and the micronuclei also contain dense bodies (d). Fine, branching filaments fill the central region (asterisk) of each micronucleus.

Fig. 3. Interphase. One of the large bodies of the macronucleus showing the structure of its amorphous medulla and granular cortex. ×100,000.
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(Facing p. 498)
Fig. 4. Metaphase, stage A. A micronucleus sectioned medially close to both poles and perpendicular to the equatorial plane, where clumps of dense material are concentrated. The nucleus contains a large number of microtubules, and a polar vesicle at each pole (arrows).
Fig. 5. Metaphase, stage A. The polar region of a micronucleus showing the close apposition of the polar vesicle (v) to the nuclear envelope which is composed of an inner (i) and an outer (o) membrane. Two nuclear pores (p) are also shown. The microtubules (t) converge towards the polar vesicle and short, branching filaments (arrow) are attached to them.

Fig. 6. Late anaphase, stage C. Cross-section of some of the microtubules and one of the C-filaments (c) in a separation spindle. The walls of some of the microtubules have a granular appearance (arrow). × 450,000.
Fig. 7. Late anaphase, stage B. Median longitudinal section through one end of a micronucleus. Most of the microtubules (t) are concentrated in the separation spindle (s) and the dense bodies (d) are confined to the terminal knob.

Fig. 8. Late anaphase, stage C. Median longitudinal section of part of a separation spindle showing the parallel arrangement of the microtubules. The envelope surrounding the spindle is composed of two membranes. Dense granules (arrows) are attached to the outer membrane.
Fig. 9. Stage C. Three separation spindles (s) and the central constriction of the macronucleus (m) in cross-section showing the macronuclear spindle (s) and cisternae of rough-surfaced endoplasmic reticulum (arrow) which project from the outer membrane of the macronuclear envelope.

Fig. 10. Stage C. Cross-section of a separation spindle (s) and the central constriction of a macronucleus (m), in which constriction is more advanced than in Fig. 9. Pores (p) form part of the macronuclear envelope but they are an uncommon feature of the envelope surrounding the separation spindle. C-filaments (c) are present in both separation and macronuclear spindles.
Fig. 11. Stage C. The 3 separation spindles (s) and part of the central constriction of the macronucleus (m) in cross-section showing membrane bridges (arrows) uniting the outer membranes of the envelopes of two of the separation spindles and the macronucleus. Nuclear pores are absent from those portions of the envelopes included in this section which surround the separation spindles. Some of the macronuclear microtubules (t) are concentrated immediately inside the nuclear envelope.
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Fig. 12. Stage D. One of the separation spindles (s) is linked to one of the daughter micronuclei (n) by a long membrane bridge (arrows) which forms part of an anastomosing cisterna of rough-surfaced endoplasmic reticulum.

Fig. 13. Interphase. Part of the macronuclear envelope sectioned at a shallow angle to the plane of the envelope. On the right of the figure the outer portions of three nuclear pores are contained in the section and the pores appear as dense discs with a central granule of even greater density. To the left the inner portions of two pores are shown and an annulus surrounding a less-dense region with a dense granule (arrow) at the centre can be distinguished for each pore. × 120000.