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Fine structure of nucleoli in the ciliate *Didinium nasutum*[*]

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Summary

The macronuclear nucleoli of vegetative non-dividing cells of *Didinium nasutum* display inverted position of the main parts: the granular component is inside and the dense fibrillar one, in form of discrete bands, is mainly at the periphery. Before binary fission, the nucleoli are degranulated and their fibrillar bands scatter throughout the macronucleus, to be segregated during division between the daughter cells, where they begin to re-form the granular parts. In young resting cysts small nucleoli consist of granular material only, larger nucleoli show a clear segregation of its fibrillar and granular elements. During conjugation, nucleoli of the old macronucleus or of its fragments become segregated into granular and fibrillar parts and the latter are largely eliminated. The nucleoli lose contact with the chromatin bodies. In the developing anlagen of the new macronuclei the first nucleoli appear as fibrous bodies and only later develop granular parts; simultaneously the number of nucleoli increases.

Key Words: macronucleus, nucleoli, cell cycle, cysts, *Didinium nasutum*

Introduction

Macronuclear nucleoli are known to be very labile structures. In all known cases, they are formed on extrachromosomal (amplified) copies of ribosomal RNA gene sequence, the so-called rDNA, which may be monomeric, as in *Glaucoma* and all hypotrichs, dimeric palindromic, as in *Tetrahymena* or oligomeric as in *Paramecium* (Blackburn, 1982; Raikov, 1982, 1989, 1995). The organization of rDNA in gymnostomes, including *Didinium*, is not known yet.

The macronuclear nucleoli can fuse, separate, segregate into granular and fibrillar parts, or become degranulated depending on various environmental factors, clonal growth phase, nutritional state, cell cycle period, encystment, conjugation and so on (Raikov, 1982, 1995). These changes may occur on the replication level (i.e., via change of the copy number of the rDNA due to differential replication of the amplified extrachromosomal rDNA) or on the transcription level (i.e., due to differences in the rate of preribosomal RNA synthesis), or else on the posttranscriptional level (i.e.,

[*] In memory of Professor Igor Borisovich Raikov (30.12.1932-27.10.1998)

due to differences in the rate of rRNA processing and maturation of ribosomal particles).

This paper aims to follow the fine structural changes of nucleoli at various stages of cell cycle of *Didmium nasutum*.

Material and methods

The laboratory strain of *Didinium nasutum* used in the present work has been cultivated at room temperature in lettuce medium and fed with *Paramecium caudatum* cultivated separately.

The following fixation schedules were used:

1) 2% osmium tetroxyde in a 0.05 M sodium cacodylate buffer (pH 7.2), 30 min on ice.

2) A freshly prepared mixture of glutaraldehyde and osmium tetroxyde with the following final concentrations: glutaraldehyde, 1%; osmium tetroxyde, 1%; phosphate buffer, pH 7.2, 0,05 M. Fix 30 min to 1 h on ice and in the darkness.

The sections were routinely stained and studied with a JEM-100C electron microscope operated at 80 kv.

Results

Vegetative cell and binnary fission

The macronucleus of normally fed interphase cells contains numerous rather conspicuous nucleoli. The nucleoli show an inverted disposition of its main components: the dense fibrillar component mainly occurs in form of discrete bands at the surface of the nucleoli and around intranucleolar chromatin bodies, and the granular component lies inside the nucleoli. The nucleolar organizers in form of compact chromatin bodies either lie inside the nucleoli, surrounded by a ring of dense fibrillar bands, or at the surface, where they also contact bands of the dense fibrillar component (Fig. 1A). The latter bodies are indistinguishable from "free'' chromatin bodies (see: Karadzhan and Raikov, 1977). Chromatin bodies, both intranucleolar and peripheral, often decondense forming fibrillar centres (NORs) as defined by Goessens (1974).

When the macronucleus prepares to divide, it contracts from a horseshoe shape to a more or less rounded one and then begins stretching in a rod. During the stretching phase, numerous bundles of longitudinal microtubules assemble in the macronucleus. The nucleoli become largely degranulated and the dense fibrillar bands become free and scatter throughout the macronucleus during the contraction phase; their contacts with chromatin bodies are no more evident (Fig. 1B). The general disposition of the nucleolar remnants is reminiscent of a swirling movement described during pre-

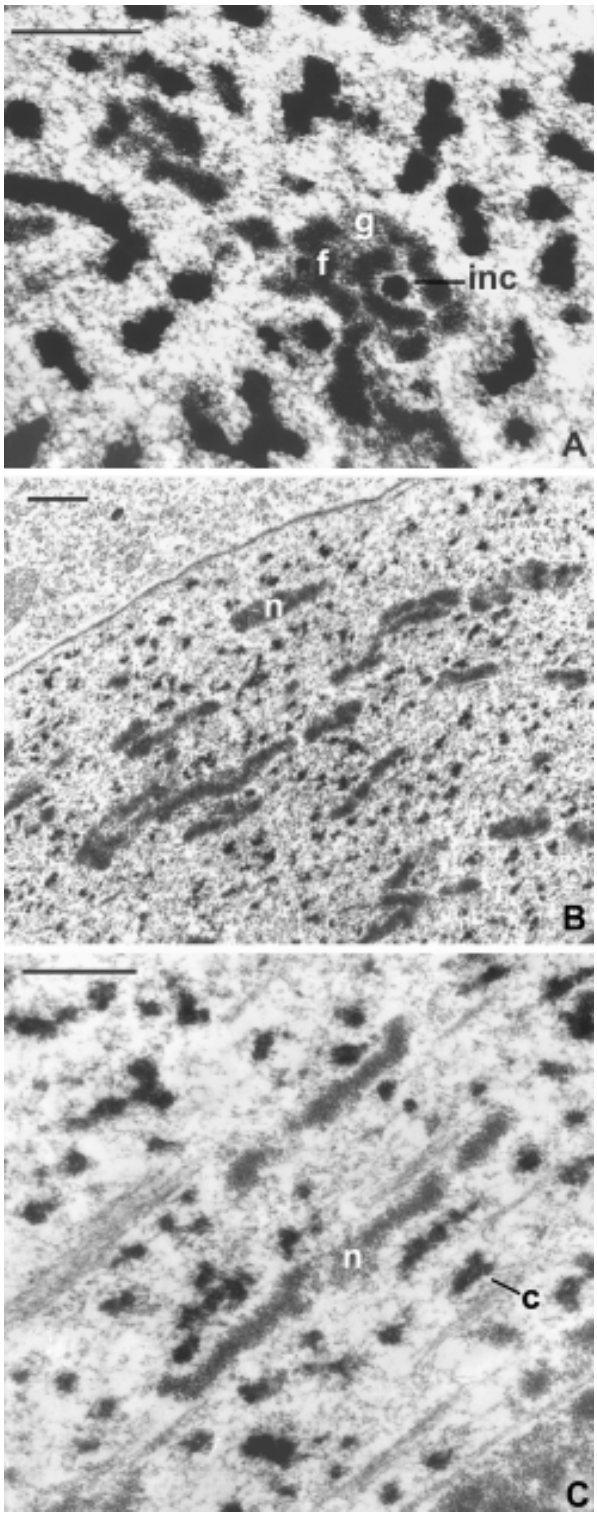

Fig. 1. Nucleoli in the interphase (A) and in a dividing macronucleus (B, C). Abbreviations: c- chromatin bodies, f - fibrillar component of the nucleolus, g - granular component of the nucleolus, inc - intranucleolar chromatin body, n - nucleoli. Scale bars: A, C - 0,5 μm, B - 1 μm.

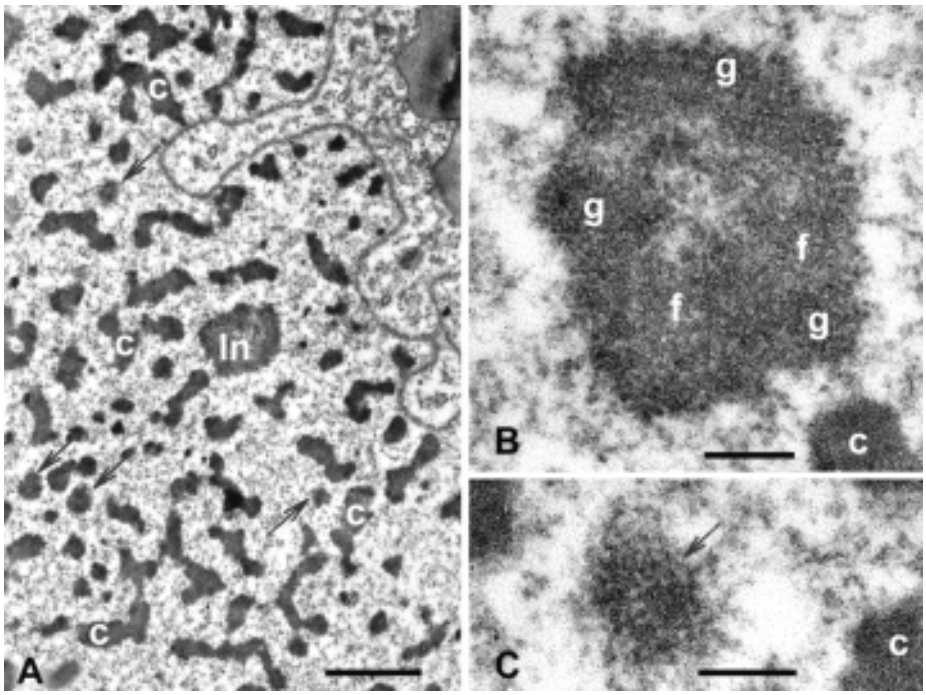

Fig. 2. Nucleoli in a young resting cyst. A - a general view of the macronucleus of a young cyst. The small granular nucleoli are designated by arrows. B - a relatively large nucleolus shown in A, at higher magnification. C - a small granular nucleolus (arrow) at higher magnification. Abbreviations: ln - a relatively large nucleolus (~ 800 nm in size) with segregated fibrillar and granular components, other abbreviations are the same as in Fig. 1. Scale bars: A - 1 μm, B, C - 0,2 μm.

divisional contraction of the macronucleus in several ciliates (Raikov, 1982).

During the stretching phase and the actual division (pinching in two), the granular nucleolar component begins to re-appear, but it is not yet organized into regular nucleoli (Fig. 1C). The nucleolar material is caught between microtubule bundles, the dense fibrillar bands are mostly isolated and have the same longitudinal orientation as microtubules (Fig. 1C). However, the contact between chromatin bodies and fibrillar bands is re-established. All this argues for that the biosynthetic activity of the nucleoli is resumed.

Cysts

Cells of *Didinium nasutum* can differentiate into resting cysts under unfavorable condition, such as starvation, temperature stress and so on. We have investigated the young resting cysts, less than 16 h old, which formed spontaneously in a somewhat inhibited culture.

Number of nucleoli as well as the size of single nucleoli in resting cysts reduce drastically (Fig. 2A). The size of each nucleolus remains within the limits of 150 to 900 nm. The structure of nucleoli also changes considerably. Larger nucleoli show a clear segregation of fibrillar and granular component, but the segregation of the nucleolar component in young resting cysts obviously proceeds differently from that observed in the fragments of old macronucleus during conjugation. The fibrillar component in this case is immersed in a granular mass of the nucleolus (Fig. 2B). The small nucleoli consist of granular material only (Fig. 2C). Nucleolar organizers, both inter- and perinucleolar, are not observed.

Old macronucleus during conjugation

During the first meiotic division of the micronucleus (2-3 h after the start of conjugation), the old macronucleus fragments into 20-30 pieces (Karadzhan and Raikov, 1979). Each piece usually contains several nucleoli. During the second meiotic division the peripheral position of the bands of the dense fibrillar nucleolar component becomes clearer than in vegetative cells; intranucleolar chromatin bodies disappear and the connection of the main chromatin mass with the nucleoli is lost (Fig. 3A). This can be considered as the onset of nucleolar segregation into the fibrillar and the granular components.

Somewhat later, usually still during the 2nd meiotic division, the peripheral fibrillar bands contract and

coalesce into compact blocks (Fig. 3B). The rest of the nucleoli is granular. Nucleolar organizers, both intra- and perinucleolar, are absent.

During the first or the second synkaryon division the chromatin of the macronuclear fragments condenses into a common spongy mass and at the same time the envelope of the fragments begins «to peel off» establishing direct contact of the macronuclear contents with the cytoplasm (Karadzhan and Raikov, 1979). The nucleoli remain segregated and devoid of NORs. They are located in holes in the chromatin mass and are always surrounded by a rim of structureless nucleoplasm (Fig. 3C). Possibly the nucleoli give off a part of their granules to the cytoplasm. About the same time or a little later each macronuclear fragment, together with some adjacent cytoplasm, becomes enclosed in a single-membraned vacuole. These vacuoles are likely to be autophagic. Later, during the early development of the macronuclear anlagen, when the fragments of old macronucleus actually degene-rate, the nucleoli become completely degranulated and their fibrillar blocks are hardly distinguishable inside the fused mass of pycnotic chromatin.

Development of the new macronucleus

The nucleoli appear in the anlagen of the new macronucleus for the first time at the «homogeneous» (decondensed) stage of development of the anlagen that occurs about 3 hours after separation of the conjugants (Karadzhan and Raikov, 1979). They are not very numerous (10-20 per anlage) and extremely dense. Almost all of them are ring-shaped, with a clearer centre and denser periphery (Fig. 3D). The centre is often structured: it displays lighter and darker spots, whereas the periphery is similar to the material of the dense fibrillar bands of the nucleoli of vegetative macronuclei (Fig. 3D). The former possibly corresponds to the NOR region, and the latter to the fibrillar part of the nucleolus; no nucleolar granules exist at this stage. These nucleolar primordia are usually surrounded with clear halos (shrinkage artefact?) and only incidentally connect with the general chromatin network with various threads (Fig. 3D). There are no bodies of condensed chromatin in this network yet. Later, 4 to 6 hours after separation of the conjugants, the nucleoli enlarge and acquire a more typical structure. At the same time small bodies of condensed chromatin begin to form throughout the anlage; they grow and become more and more abundant until they reach the morphology typical of the vegetative macronucleus. The clear halos around the nucleoli diminish, and the nucleoli come into contact with some chromatin bodies which depress the nucleolar surface (Fig. 3E). These would enter the nucleoli and become the intranucleolar chromatin bodies. Simultaneously granular material begins to accumulate inside each nucleolus; additional nucleoli are possibly also formed during this time, as their number grows. The fibrous bands always remain at the nucleolar surface (Fig. 3E). Sometimes such nucleoli fuse forming a large compound one.

Discussion

Vegetative macronucleus

In vegetative non-dividing macronuclei the nucleolar morphology mainly depends on the nutritional state of the culture and/or on the phase of its development. The results mainly concern *Tetrahymena* where nucleoli fuse into larger aggregates during a nutritional shift-down or simply in the stationary phase (Raikov, 1982). This occurs also under the action of certain drugs and other unfavorable conditions such as elevated temperature. Fused nucleoli are partly degranulated and show a strong inhibition of RNA synthesis. Segregation of the nucleoli into fibrillar and granular parts under the action of certain drugs is also frequently observed (Caratero et al., 1983; Nilsson, 1985). After a nutritional shift-up (transfer to fresh medium or refeeding) there is a burst of rDNA replication not associated with cell division (apparently, more copies of extrachromosomal rDNA are produced): at the same time, rRNA synthesis is resumed and the nucleoli return to normal morphology. Our study of *Didinium* concerns only normally fed cells.

Macronuclear nucleoli usually display a fibrillar core and a granular cortex (Raikov, 1982, 1995). The nucleolus-organizing region is then inside the fibrillar core. However, in *Didinium* the position of the main components is inverted: they have a granular core and a dense fibrillar cortex, and the NOR regions are mainly at the periphery. A similar inverted position of components occurs in *Dileptus* (Vinnikova, 1974) and *Nyctotherus* (Vinnikova and Golikova, 1979). Moreover, the macronuclear nucleoli of *Didinium* appear to be composite (a product of fusion of several unit nucleoli) since they have several NOR regions.

Cell division

It is well known that nucleoli diminish in size, are fragmented, or even disappear (at least, become invisible with the light microscope) during division of the macronucleus in many ciliates. Often the nucleoli are degranulated (Raikov, 1982). In *Didinium*, the composite

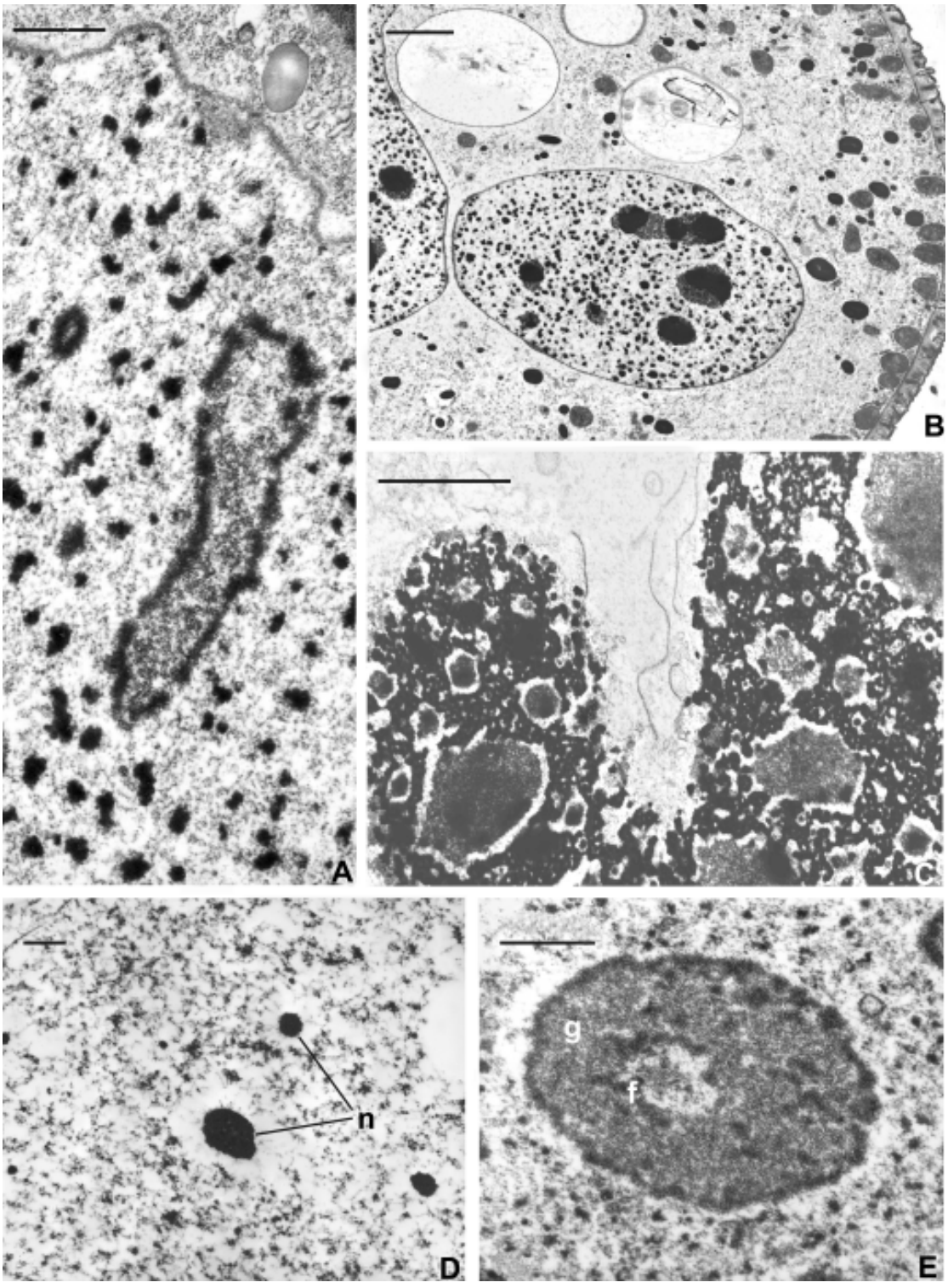

Fig. 3. Nucleoli in the old macronuclear fragments and in an anlage of a new macronucleus following conjugation. A, B - segregation of nucleolar material into granular and fibrillar components. C - a fragment of an old macronucleus, showing spongy chromatin mass, segregated nucleoli and ruptures of the nuclear envelope. D - first nucleoli with a clearer center and denser periphery in the anlage of a new macronucleus. E- a nucleolus in the anlage of a new macronucleus, 24 h after separation of the conjugants. Abbreviations are the same as in Fig. 1. Scale bars: A, B, D - 1 μm, C - 5 μm, E - 0,5 μm.

nucleoli are not only degranulated but fall into unit fibrillar bands already during preparation to division, and during division itself the nucleolar granules begin to re-form. All this indicates a temporary decrease of the biosynthetic activity of the nucleoli just prior to cell division.

Cysts

It is well known that during encystment morphology of nucleoli undergoes drastic changes (Raikov, 1982; Gutierrez et al., 1991, 1998). In some ciliates nucleoli in resting cysts can fuse (Frenkel, 1992; Palacios et al., 1995) or disappear (Foissner and Foissner, 1987; Adl and Berger, 1997). A segregation of fibrillar and granular components, and subsequent disappearance of the latter was observed in cysts of *Tillina magna* (Frenkel, 1989, 1992). In cysts of Hypotrichida macronuclei become small, compact and homogeneous (Grimes, 1973; Walker et al., 1980; Jareno, 1985). For these species an appearance of bundles of microtubules at the early stages of encystment is typical. Nucleoli are bound to these microtubules.

In *Colpoda* and some other ciliates, the amount of preribosomal granules in the young cysts increases in comparison with precyst stage (Chessa et al., 1983). The granular component is conspicuous in nucleoli of *Colpoda steini* (Frenkel, 1987), *Dileptus visscheri* (Kink, 1973). The presence of granular component in the nucleoli and clustering of preribosomal granules into 100-200 nm RNP particles was observed in resting cysts of *Bursaria truncatella* (Popenko et al., 1998) and *B. ovata* (Samoshkin and Sergejeva, 1995). The bundles of microtubules in macronuclei of these species were not observed.

More or less similar changes of nucleoli occur also in young resting cysts of *Didinium nasutum*. Nucleoli decrease in size. Small nucleoli consist of granular material only. Intranucleolar and peripheral chromatin bodies are absent. All these structural changes correlate well with transition of the cell to the resting stage.

The presence of aggregates of preribosomal granules in cysts can be explained by accumulation of rRNP for subsequent processes of cyst formation and/or excystment in the future (Chessa et al., 1983; Frenkel, 1987). It is possible, however, that structural changes observed in early cysts can be explained by a stronger preribosomal RNA suppression in comparison with that of rRNA synthesis (Eckert and Franke, 1975).

Old macronucleus at conjugation

The first changes that occur in nucleoli in the fragments of the old macronucleus of *Didinium* is their progressive segregation and subsequent compactization of the dense fubrillar component. Segregation of nucleolar components is known to occur in cells of various organisms when biosynthesis is lowered (see review: Bush and Smetana, 1970). It also occurs during encystment of some protozoans, e.g., *Arcella* (Raikov et al., 1989), and in the old macronucleus during conjugation in some ciliates, e.g., *Blepharisma* (Kovaleva et al., 1998). After that the nucleoli in *Didinium* become largely degranu-lated, also like in *Blepharisma*, possibly as a result of migration of nucleolar granules to the cytoplasm through gaps in the nuclear envelope of the macronuclear frag-ments (Karadzhan and Raikov, 1979).

Final degeneration of the old macronucleus or its fragments usually involves destruction of its envelope and enclosure in autophagic vacuoles. This occurs, e.g., in *Stentor* (Skarlato, 1978), *Dileptus* (Vinnikova, 1974), and *Tetrahymena* (Weiske-Benner and Eckert, 1987). Simultaneously, the RNA synthesis, which has been active before this moment, is arrested.

Anlagen of the new macronucleus

In the developing anlagen of the new macronucleus development of nucleoli proceeds in some-what different ways in various ciliates (see reviews: Raikov, 1982, 1995). In *D. nasutum* the first nucleoli appear in the form of fibrous bodies about 3-4 hours after separa-tion of the conjugant. In *Paramecium aurelia*, the first nucleoli appear in the anlagen earlier, about 30 min after differentiation of the latter (Jurand et al., 1964; Berger, 1973). In most hypotrichs the nucleoli are formed much later, only after destruction of the polytene chromosomes (Ammermann, 1971; Murti, 1973; Skarlato, 1978; etc.). Late formation of the nucleoli in macronuclear anlagen was observed also in *Stentor* (Skarlato, 1978), *Blepharisma* (Kovaleva et. al., 1998) and some other ciliates.

Conclusion

Results presented in this paper show that the structure of nucleoli undergoes drastic changes at various stages of life cycle in *D. nasutum*. Fibro-granular nucleoli, containing intra- and perichromatin bodies in the macronucleus at interphase, can degranulate during binary fission, segregate in resting cysts and in fragments of old macronucleus, appear in autophagic vacuoles and re-form in the macronuclear anlagen after conjugation. The structure of nucleoli may be an indicator of the level of biosynthetic activity of the cell, indicating changes in the rRNA metabolism.

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