

Unusual pattern of mitosis in the free-living flagellate *Dimastigella mimosa* (Kinetoplastida)

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Summary. The three-dimensional ultrastructural organization of the mitotic apparatus of *Dimastigella mimosa* was studied by computer-aided, serial-section reconstruction. The nuclear envelope remains intact during nuclear division. During mitosis, chromosomes do not condense, whereas intranuclear microtubules are found in close association with six pairs of kinetochores. No discrete microtubule-organizing centers, except kinetochore pairs, could be found within the nucleus. The intranuclear microtubules form six separate bundles oriented at different angles to each other. Each bundle contains up to 8 tightly packed microtubules which push the daughter kinetochores apart. At late anaphase only, midzones of these bundles align along an extended interzonal spindle within the narrow isthmus between segregating progeny nuclei. The nuclear division in *D. mimosa* can be described as closed intranuclear mitosis with acentric and separate microtubular bundles and weakly condensed chromosomes.

Keywords: Kinetochore; Kinetoplastida; Intranuclear microtubules; Mitosis; Ultrastructure.

Abbreviation: MTOC microtubule-organizing center.

Introduction

Mitosis is the basic nuclear division in eukaryotic cells which leads to equal distribution of the previously replicated genetic material (chromosomes) between daughter cells. The mitotic machinery and chromosome structure in unicellular eukaryotes or protists is usually different from that of higher organisms (Heath 1980, Pickett-Heaps 1986, Raikov 1994). One of the most enigmatic types of chromosome segregation has been found in kinetoplastid flagel-

lates (Vickerman and Preston 1970). The kinetoplastids comprise the largely free-living bodonids and the exclusively parasitic trypanosomatids. These protozoan groups, together with the related euglenoids, branched off very early during the eukaryotic evolution (Schlegel 1994) and have many unique features in their cell structure, metabolism, and genome organization (Triemer and Farmer 1991, Vickerman 1994, Vanhamme and Pays 1995). The kinetoplastid chromosomes do not condense during mitosis, the number of intact chromosomal DNAs on pulsed-field electrophoresis gels far exceeds the number of kinetochore-like plaques in mitotic spindles, and the nuclear envelope remains intact throughout the entire course of nuclear division (Skarlato et al. 1987, Frolov and Karpov 1995, Solari 1995, Ersfeld and Gull 1997, Skarlato and Lom 1997).

At the fine-structural level, mitotic events have been studied in nine of ten genera of trypanosomatids, namely, *Blastocrithidia*, *Crithidia*, *Endotrypanum*, *Herpetomonas*, *Leptomonas*, *Leishmania*, *Phytomonas*, *Proteomonas*, and *Trypanosoma* (Vickerman and Preston 1970; Croft 1979; Solari 1980, 1983, 1995; Triemer et al. 1986; Ureña 1986; Skarlato et al. 1987, 1990; Frolov and Karpov 1995; Frolov et al. 1997b). In contrast, nuclear division has been described only in two (*Bodo* and *Trypanoplasma*) of about ten genera of bodonids (Skarlato 1987, Frolov et al. 1996, Skarlato and Lom 1997).

The present paper describes the three-dimensional ultrastructural organization of mitotic nuclei of the free-living bodonid *Dimastigella mimosa*. A descrip-

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tion of general morphology and ultrastructure of this species has been recently published elsewhere (Frolov et al. 1997a). The genus *Dimastigella* attracted special attention because nuclear synaptonemal complexes in kinetoplastids were first discovered in *D. trypaniformis*, Glasgow strain (Vickerman 1991, Breunig et al. 1993).

Material and methods

The strain Y of *Dimastigella mimosa* (Frolov et al. 1997a) was isolated from sewage waters of the waste-water treatment plant in Yaroslavl (Russia). The flagellates were grown at room temperature in a cerophyl medium (Breunig et al. 1993). Cells were harvested at or near the peak of mitosis, 2 h after placing in fresh cultural medium. For electron microscopy cells were fixed on ice (1.5% glutaraldehyde and 1.5% OsO₄ in 0.1 M cacodylate buffer, pH 7.2) for 30 min. Postfixation was carried out with 2% OsO₄ in 0.1 M cacodylate buffer, pH 7.2, for 1 h. After dehydration in an ethanol and propylene oxide series, the flagellates were embedded in an Epon-Araldite mixture. Serial ultrathin sections were cut with an LKB III microtome (LKB Products, Stockholm-Bromma, Sweden). Sections were stained with uranyl acetate and lead citrate and examined in a JEM 100C transmission electron microscope (JEOL Ltd., Tokyo, Japan). For 3-D reconstructions, negatives of the studied nuclei were digitized in a serial order with a Primax scanner (Primax International, Utrecht, The Netherlands). Image analyses and spatial modelling were performed with the graphic software Corel Xara 1.1 (Xara Ltd., Hemel, Hempstead, U.K.). In total, thirteen mitotic nuclei were reconstructed.

Results

Interphase

The interphase nucleus of *D. mimosa* lies in the middle portion of the flagellate and has a spherical or slightly elongated form (Figs. 1 and 2). The diameter usually varies from 2 to 2.5 µm. The nuclear envelope is perforated by pores 80–100 nm in diameter and bears numerous ribosomes on its outer surface (Fig. 2). The condensed chromatin is fragmented into a number of discrete masses which are primarily located just beneath the inner membrane of the envelope. A few condensed chromatin bodies may occur free in the karyoplasm alongside granules and fibers of various diameters. A single nucleolus is generally

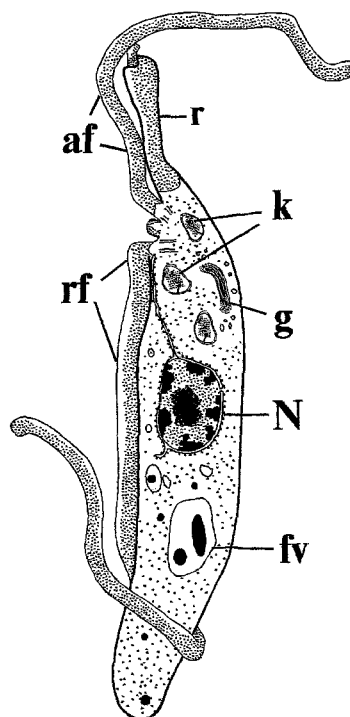


Fig. 1. Schematic drawing of *Dimastigella mimosa*. af Anterior flagellum, fv food vacuole, g Golgi apparatus, k kinetoplasts, r rostrum, rf recurrent flagellum, N nucleus

present at the center of each nondividing nucleus (Fig. 2). Both fibrillar and granular components, as well as meandering lacunae, can be found within the nucleolar domain.

Mitosis

In kinetoplastids, mitotic events are difficult to subdivide into clear prophase, metaphase, anaphase, or telophase because their chromosomes do not condense during nuclear division. Therefore, it has been suggested that the following four stages in kinetoplastid mitosis be recognised, namely, preliminary, equatorial, elongational, and reorganizational stage (Solari 1980). However, even this terminology can only partially be applied to *D. mimosa*, since no typical equa-

Figs. 2–6. Fine structure of nuclei in *Dimastigella mimosa*. mt Microtubules, ne nuclear envelope, nu nucleolus, p nuclear pore

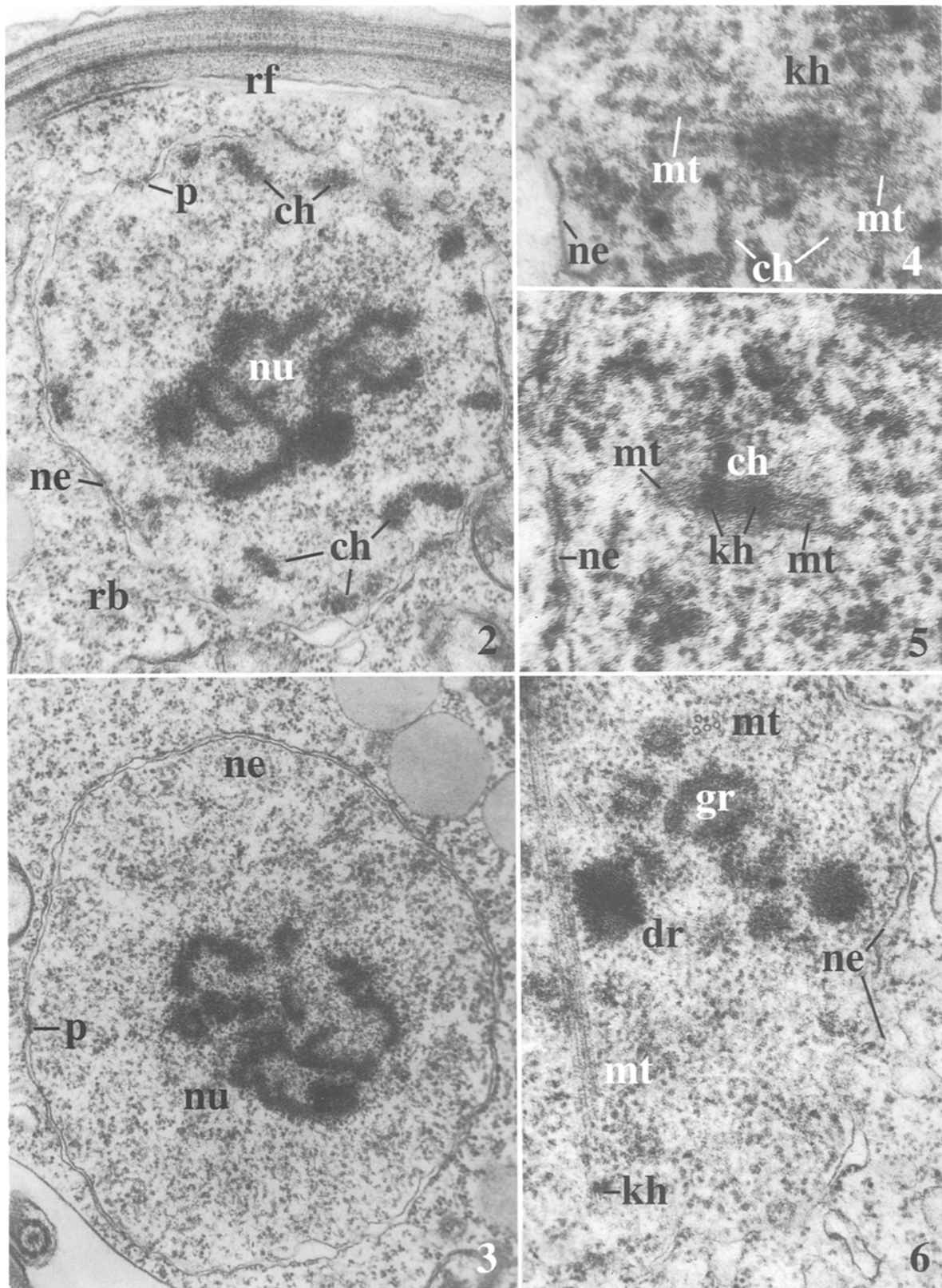
Fig. 2. The interphase nucleus. ch Condensed chromatin, rb ribosomes. × 39000

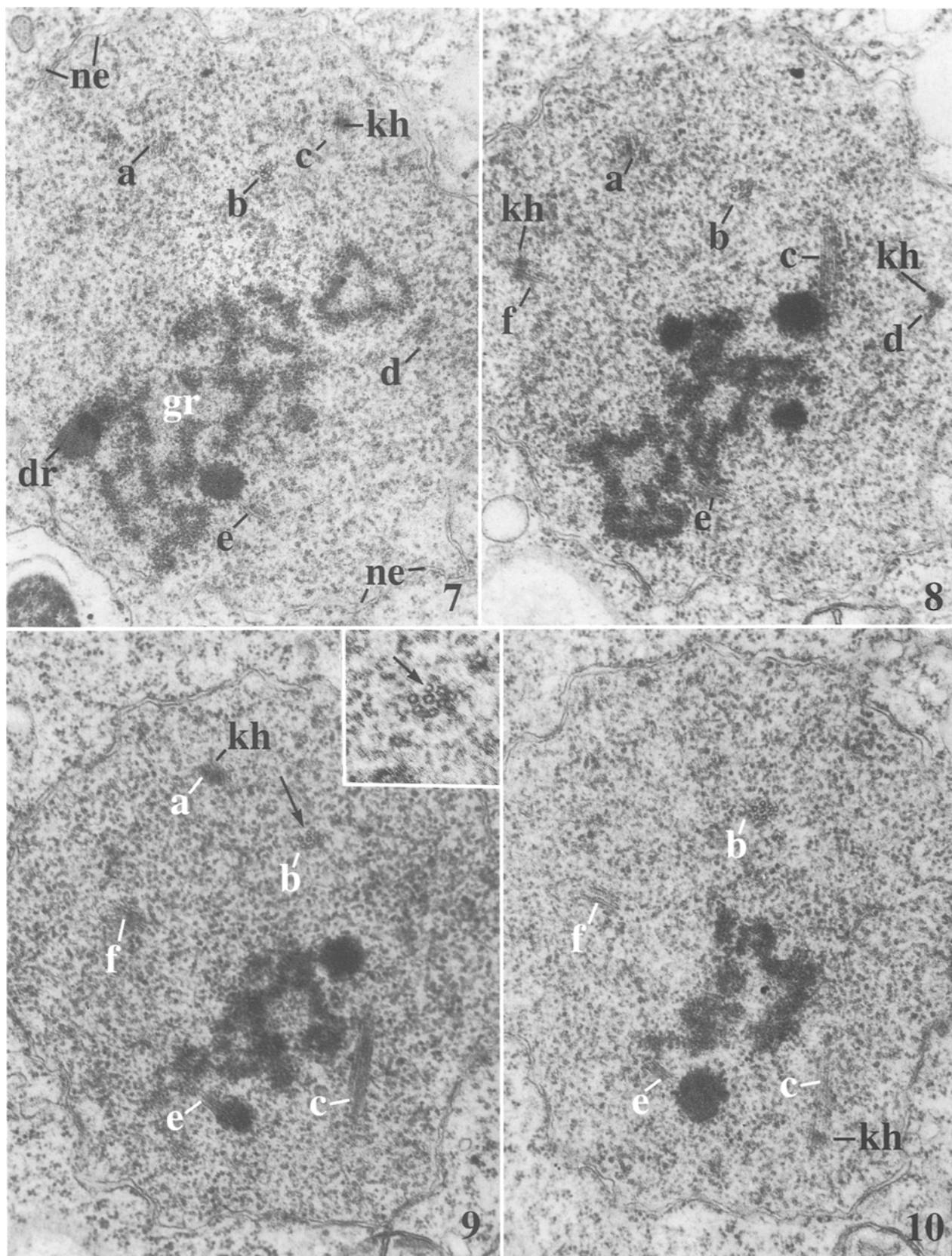
Fig. 3. The nucleus at the preliminary stage of mitosis. × 35000

Fig. 4. A pair of joint kinetochore-like plaques (kh) shows layer structure. ch Chromatin filaments. × 125000

Fig. 5. Kinetochore pair splits into sister kinetochores (kh). ch Chromatin filaments. × 79000

Fig. 6. Section through the nucleus at the six-independent-bundle stage. dr Dense fibrillar rod, gr granular ribbon, kh kinetochore. × 44000





Figs. 7-10. Serial sections (nr. 8, 10, 12, and 13) through a dividing nucleus of *Dimastigella mimosa* at the six-independent-bundle stage (a-f). *dr* Dense fibrillar rod, *gr* granular ribbon, *kh* kinetochore, *ne* nuclear envelope. $\times 35000$. **Inset 8** microtubules of the bundle *b* (arrow) at higher magnification, $\times 68000$

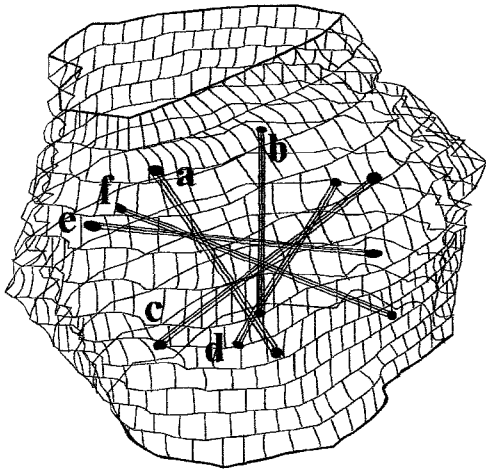


Fig. 11. 3-D reconstruction of the intranuclear-containing portion of *Dimastigella mimos* from 27 serial sections, fifteen of which (including those in Figs. 7–10) cross the nucleus. Outer membrane of the nuclear envelope and nucleolar fragments are not shown. Adjacent section contents are connected by vertical stripes. *a–f* Six independent bundles of microtubules

torial stage (characteristic metaphase of kinetoplastids) has been found in this species.

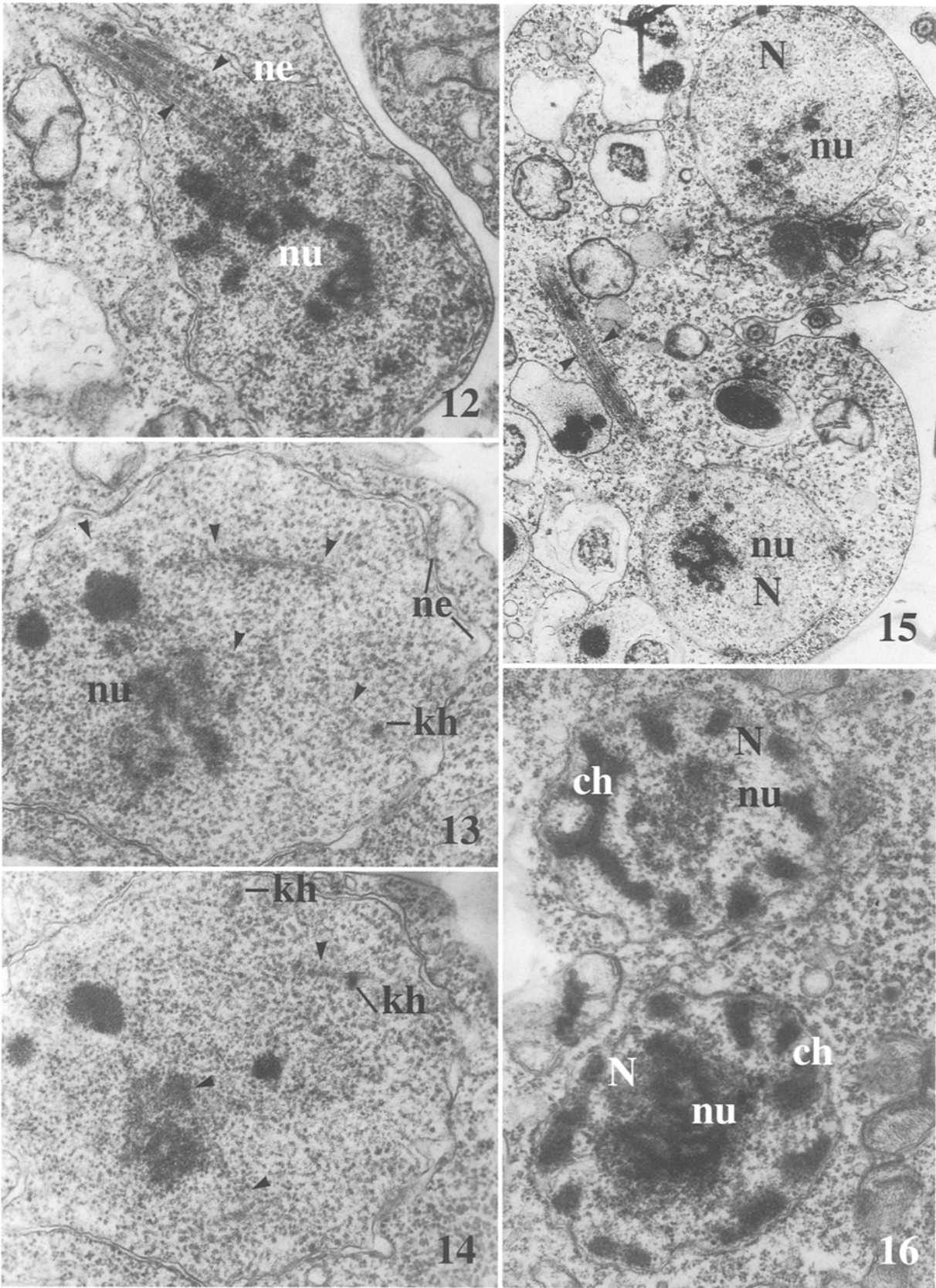
At the preliminary stage of mitosis, the nucleus increases up to 3.5 μm in diameter maintaining a nearly spherical shape (Fig. 3). The peripheral chromatin masses of *D. mimos* decondense and the nucleolar domain becomes looser (Fig. 3). The karyoplasm appears more homogeneous and electron-translucent, compared with the interphase nucleus. The nuclear envelope, with its rare pore complexes, does not break down during mitosis. It should be mentioned that the preliminary stage is presumably prolonged, because the majority of dividing nuclei in cultured *D. mimos* were found at this stage.

As mitosis progresses, both dense plaques and intranuclear microtubules are assembled in the karyoplasm (Figs. 4–6). A typical plaque is about 115 nm wide and 160 nm long and displays a heptalaminar structure with bilateral symmetry (layers of 40–15–10–30–10–15–40 nm are shown in Fig. 4). Once the dense plaques appear, their opposite faces become associated with microtubular bundles. Each bundle contains 2–3 microtubules of about 150 to 200 nm in length (Fig. 4). The microtubule-free sides of plaques contact chromatin filaments dispersed in the karyoplasm. Serial-section analysis has revealed six plaques in the peripheral region of the nucleus. Interestingly enough, the equatorial alignment of these plaques has never been observed in the central nuclear domain of *D. mimos*.

Later in mitosis, dense plaques split into two hemiplaques (Fig. 5), which are generally considered to be individual sister kinetochores of the “invisible” kinetoplastid chromosomes (for arguments, see Solari et al. 1985, Skarlato et al. 1987, Solari 1995, Skarlato and Lom 1997). Once the sister kinetochores pinch apart, their laminated structure becomes less prominent (Fig. 5). Additionally, interkinetochore microtubules nucleate shortly after kinetochore separation (Fig. 6). Members of a kinetochore pair seem to be pushed away by the growing interkinetochore microtubules. Finally, six individual bundles occur with kinetochores near their ends (Figs. 7–11). Each bundle only divides one pair of kinetochores. Figures 7–10 show 4 of 27 serial sections through the nucleus-containing portion of the dividing flagellate, with its 3-D reconstruction shown in Fig. 11. The bundles are oriented at different angles to each other (Figs. 7–11). In cross-sections of the bundle midzones (Fig. 9, bundle b), up to 8 microtubules can be counted, but only 2–3 are in contact with the kinetochore structure (Figs. 7–10, bundle c). The total number of interkinetochore microtubules per nucleus may be about 48. By the end of this stage, the kinetochore-to-kinetochore distance reaches 3 μm (Figs. 6–10). Interestingly, the kinetochore face, opposite the interkinetochore bundle, also nucleates 2–3 short (150–200 nm) microtubules which remain free at their ends throughout mitosis. No discrete microtubule-organizing centers (MTOCs), except the kinetochores, could be observed within the nucleus of *D. mimos*.

At the six-independent-bundle stage, the middle region of the nucleus of *D. mimos* is filled with dispersed nucleolar elements, i.e., granular ribbons and dense fibrillar rods (Figs. 6 and 7–10). The nucleolar domain is pervaded with bundle microtubules.

During the elongational stage of mitosis, the nucleus extends considerably and finally becomes dumbbell-shaped (Fig. 12). What dictates the position of the two nuclear poles remains a mystery. Moreover, *D. mimos* lacks anaphase A. The length of interkinetochore microtubules increases as the nucleus progresses through mitosis (anaphase B). At late elongational stage, these microtubules reach 5.7 μm in length. Only at this stage, midzones of all six bundles of *D. mimos*, which were originally oriented in an apparently random fashion, begin to align in an extended interzonal spindle within the narrow isthmus, between end-pieces of the constricting dumbbell-shaped nucleus (Fig. 12). However, outside the isthmus the interzonal spindle spreads again into six sep-



arate bundles with kinetochore plaques near their free ends (Figs. 13 and 14).

At the end of mitosis (reorganizational stage), the daughter nuclei separate (Fig. 15).

The nuclear envelope simply constricts in two points leaving the isthmus to degenerate in the cytoplasm. Once nuclear division is completed, heterochromatin bodies begin to re-appear near the inner membrane of the nuclear envelope (Fig. 16). A typical nucleolus assembles as a spherical body in the central region of the karyoplasm. Thus, the daughter nuclei assume their original interphase appearance (Fig. 16). Finally, cytokinesis produces two new flagellates.

Discussion

Evidence accumulated over the past years has demonstrated that dividing nuclei in bodonids and trypanosomatids share some major ultrastructural features (Skarlato 1987, Frolov and Karpov 1995, Solari 1995, Frolov et al. 1996, Skarlato and Lom 1997, Somova et al. 1997). In many respects, mitosis in *D. mimosa* is also typical of other kinetoplastids. The following common characteristics of dividing nuclei in these flagellates can be noted:

1. the basal bodies do not take part in nuclear division;
2. the nuclear envelope remains intact throughout mitosis;
3. the peripheral heterochromatin of the interphase nucleus spreads in the karyoplasm at early stages of mitosis;
4. chromosomes never condense at any stage of nuclear division;
5. a few (six in *D. mimosa*) dense plaques, kinetochore pairs, associate with both intranuclear microtubules and chromatin filaments;
6. these kinetochore pairs split into individual kinetochores during mitosis.

However, the original distribution of kinetochore

pairs and intranuclear microtubules in the karyoplasm of *D. mimosa* differs significantly from those of other kinetoplastids.

In many kinetoplastid species, the unsplit kinetochore pairs congregate near the center of the nucleus, or even line up in a more or less well-defined kinetochore plate at the equatorial stage of mitosis (Solari 1980, 1983; Skarlato et al. 1987; Frolov et al. 1996; Skarlato and Lom 1997). The equatorial alignment of the kinetochore pairs has never been observed in *D. mimosa*. In this species, unsplit pairs can only be found at the nuclear periphery. Moreover, the central spindle and nuclear poles of *D. mimosa* are formed long after the separation of the sister kinetochores, at a stage which can be compared with the late elongational stage of other kinetoplastids. How equal distribution of the genetic material, which is attached to the kinetochore plaques, occurs under these circumstances remains obscure.

Recently, it has been shown that direct counts of kinetochores in the mitotic nuclei may not reflect the true numbers of chromosomes in trypanosomatid species (Skarlato et al. 1990, Skarlato and Lom 1997). Pulsed-field electrophoresis analyses have revealed considerably more intact DNA molecules in gels compared with the amount of kinetochore pairs on ultrathin sections. In *D. mimosa*, six kinetochore pairs have been found in dividing nuclei. In other bodonids, the number of kinetochore pairs was determined as 8 in *Bodo* sp. (Solari 1995), 10 in *B. curvifilus* (Frolov et al. 1996), approximately 6 in *B. designis*, *B. saltans*, *Parabodo nitrophilus* (Somova et al. 1997), and approximately 7 in *Trypanoplasma borreli* (Skarlato and Lom 1997). In bodonids, pulsed-field gel electrophoresis analysis is currently at the cradle stage (Maslov et al. 1993, Somova et al. 1997). However, preliminary results strongly indicate that the discrepancy between the numbers of kinetochore pairs and chromosomal DNA is less marked in bodonids than trypanosomatids.

Figs. 12–16. Late mitosis and daughter nuclei in *Dimastigella mimosa*

Fig. 12. Half a dividing nucleus, showing tightly packed array of spindle microtubules (arrowheads) within the persistent envelope (ne) of the nuclear isthmus. nu Nucleolar fragments. $\times 22000$

Figs. 13 and 14. Two (nr. 2, 4) of 17 serial sections through the dividing nucleus at the dumbbell stage. In bulb portions of migrating daughter nuclei the central spindle branches into six separate bundles (arrowheads) with kinetochores (kh) near their ends. ne Nuclear envelope, nu nucleolus. $\times 35000$

Fig. 15. Remnants of the nuclear isthmus with spindle microtubules (arrowheads) between the daughter nuclei (N). nu Nucleolus. $\times 18000$

Fig. 16. Daughter nuclei (N) of *D. mimosa*. ch Condensed chromatin, nu nucleolus. $\times 22000$

Of special interest is the nature of MTOCs in *D. mimosa*. As in other kinetoplastids, basal bodies do not play an identifiable role in nuclear division. In addition, spindle pole bodies (bundle pole bodies in *D. mimosa*) were not observed in this species. Moreover, neither nuclear membranes nor other morphologically distinct structures occur in association with the bundle extremities. In other kinetoplastids, the spindle microtubules may be in contact with the polar nuclear membrane, at least during the equatorial stage of mitosis (Solari 1980, Triemer et al. 1986, Skarlato et al. 1987; however, see Solari 1983). In three species, *Trypanosoma raiae* (Vickerman and Preston 1970), *T. equiperdum* (Paulin 1975), and *Trypanoplasma borreli* (Skarlato and Lom 1997), the spindle microtubules are thought to be nucleated by specialized MTOCs, which are flattened tightly against the nuclear envelope in the polar region of the mitotic nucleus. In *D. mimosa* we found that the first microtubules appeared associated with the unsplit kinetochore pairs. Later, the interkinetochore microtubules appear after separation of the sister kinetochores. It is noteworthy that both faces of individual kinetochores seem to be able to nucleate the bundle microtubules. However, the interkinetochore microtubules can reach 5.7 μm by the late elongational stage, while only short microtubule fragments (150–200 nm) are found to occur with the outer kinetochore faces. Thus, it is tempting to speculate that the dense plaques are MTOCs which may play the major nucleating role in the formation of intranuclear microtubules for the mitotic machinery in *D. mimosa*.

In *D. mimosa*, the intranuclear microtubules are assembled as six separate bundles which are oriented at different angles to each other. There are some other kinetoplastid examples, such as the bodonid *Trypanoplasma borreli* (Skarlato and Lom 1997) and the trypanosomatids *Trypanosoma cruzi* (Solari 1980) and *T. danilewskyi* (Skarlato et al. 1987), in which the central spindles are subdivided into a number of bundles. However, in these cases the bundle microtubules converge at the nuclear poles and seem to touch the nuclear envelope, at least during the equatorial stage of mitosis. In *D. mimosa*, direct contact between intranuclear microtubules and the nuclear envelope has never been detected.

Heretofore, both separate and unparallel spindles have only been reported for giant multinucleate cells of *T. borreli* (Skarlato 1987, Skarlato and Lom 1997). In the latter, the separate mitotic spindles lie at different angles to each other, inside the single outer mem-

brane of the nuclear envelope. In contrast to *D. mimosa*, however, the continuous inner nuclear membranes of *T. borreli* prevent the adjacent nuclei from directly touching their nucleoplasm (Skarlato and Lom 1997).

Thus, the nuclear division in *D. mimosa* is described as a closed intranuclear mitosis with acentric and separate microtubular bundles and weakly condensed chromosomes. Free-living bodonid flagellates lack a typical anaphase A. Anaphase B, however, occurs in *D. mimosa* as the central-spindle elongation is obvious in late mitosis.

In conclusion, it is worth repeating that the presence of synaptonemal complexes has been claimed for the nucleus of *Dimastigella trypaniformis*, strain Glasgow (Vickerman 1991, Breunig et al. 1993). However, these complexes have not been found in our present preparations (strain Y), or in strain U (Breunig et al. 1993).

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