Chapter

The Mitosis of *Entamoeba histolytica* Trophozoites

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Abstract

The mechanisms of mitosis in higher eukaryotic organisms are very well studied; however, regarding protozoa, there are still many questions in need of an answer. Because of the complexity with which it carries out this process, many forms of mitosis exist, such as open orthomitosis, semi-open orthomitosis, semi-open pleuromitosis, closed intranuclear pleuromitosis, closed intranuclear orthomitosis, and closed extranuclear pleuromitosis. The fascinating aspect about the mitosis of *Entamoeba histolytica* trophozoites is that it falls out of the context of this classification, but not entirely. The *Entamoeba histolytica* trophozoites first carry out karyokinesis and then cytokinesis. The mitosis of this parasite is comprised of the following phases: prophase, metaphase, early and late anaphase, early and late telophase, and karyokinesis. The difference lies in the mechanism by which it carries out the distribution of the genetic material because it forms three mitotic spindles: two radial spindles that practically surround every group of chromosomes and one that we call inter microtubule-organizing centers (IMTOCs). The latter transports each group of chromosomes at each of the nucleus poles. Based on these observations, we propose that *Entamoeba histolytica* trophozoites carry out a type of mitosis we have called modified intranuclear pleuromitosis open.

Keywords: *Entamoeba histolytica*, mitosis, chromatin, mitotic spindle

1. Introduction

Mitosis in the cells of living beings guarantees the cells' multiplication during the processes of tissue replacement and repair. However, in some protozoa it carries out the purpose of maintaining the species, such as in the case of *Entamoeba histolytica* trophozoites. Differences in the intracellular structures of the mitotic apparatus of human somatic cells [1] (**Figure 1a**) and of *E. histolytica* trophozoites [2–6] (**Figure 1b**) generate the need to briefly review what the cell cycle of the former is like. This will serve as a basis for explaining the equivalence of the intracellular structures of mitosis between the cells of these two species of organisms so distant in evolution.

In order to understand how mitosis occurs in *E. histolytica* and the structures involved, in this chapter we first present a brief review of the cell cycle and mitosis in higher eukaryotes, schematized in **Figures 2** and **3**, which will serve as basis

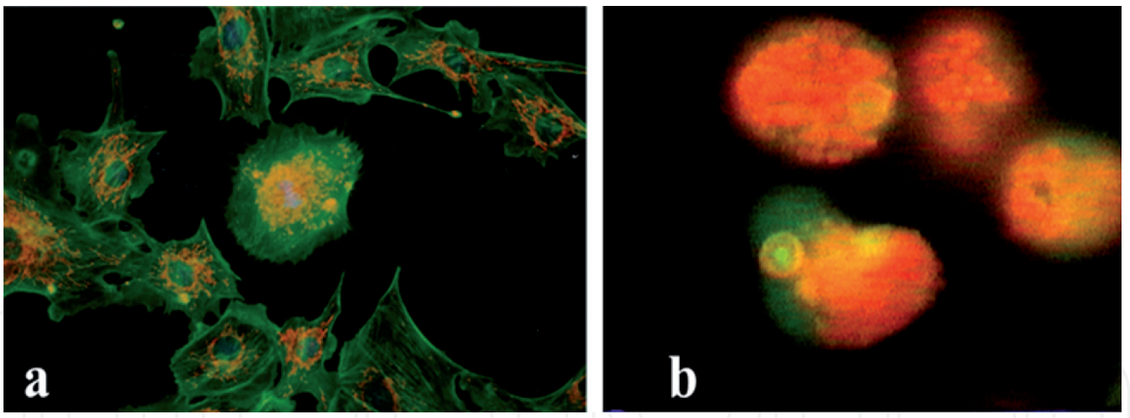

Figure 1.
Higher eukaryotic cells (a) and trophozoites of Entamoeba histolytica *(b).*

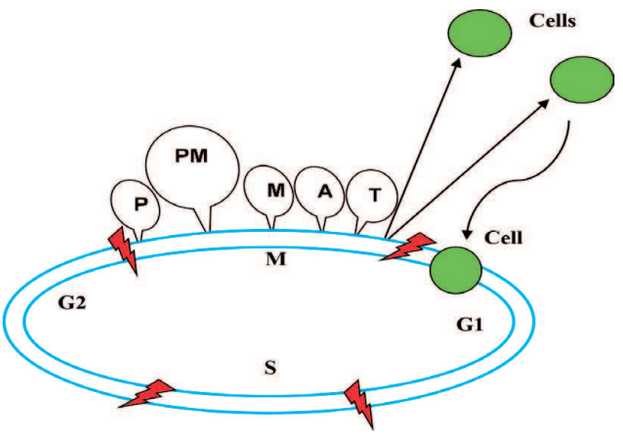

Figure 2.
Schematic diagram representing the cell cycle phases of higher eukaryotic cells. G1, growth 1; S, synthesis; G2, growth 2; and M, mitosis. Prophase (P), prometaphase (PM), metaphase (M), anaphase (a), and telophase (T) occur during mitosis.

used to compare with the mitosis of lower eukaryotes. Afterward, we explain the cell cycle in protozoa and the different mitotic models that occur in them based on different authors, observations masterfully compiled by Raikov IB. In addition, we relate information about the cell cycle of *E. histolytica* and explicitly the mitosis of this protozoan. Regarding the mitosis of *E. histolytica*, we considered scientific evidence published by other authors and our own observations obtained through phase-contrast techniques, video microscopy, acridine orange vital stain, and immunofluorescence, all of which allow us to propose a mechanism on how the mitotic process occurs in this protozoan parasite found in humans.

2. The cell cycle of higher eukaryotes

The cell cycle phases have been divided into interface and M phase (mitosis). During the interface, the cell performs functions of the tissue in which it differentiated into (phenotype) in order to stay alive (G1 phase), duplicate its genetic material (S phase), and prepare for mitosis (G2 phase). During the G1 phase, the cell maintains its biochemical integrity, expresses its phenotype, and synthesizes elements necessary for the duplication of genetic material. In the S phase, the cell carefully duplicates its genetic material so that each chromosome is doubled. During the G2 phase, the cell prepares for the M phase. Sometimes, some cells that are in the G1 phase enter a state of latency or rest, known as the G0 phase [1].

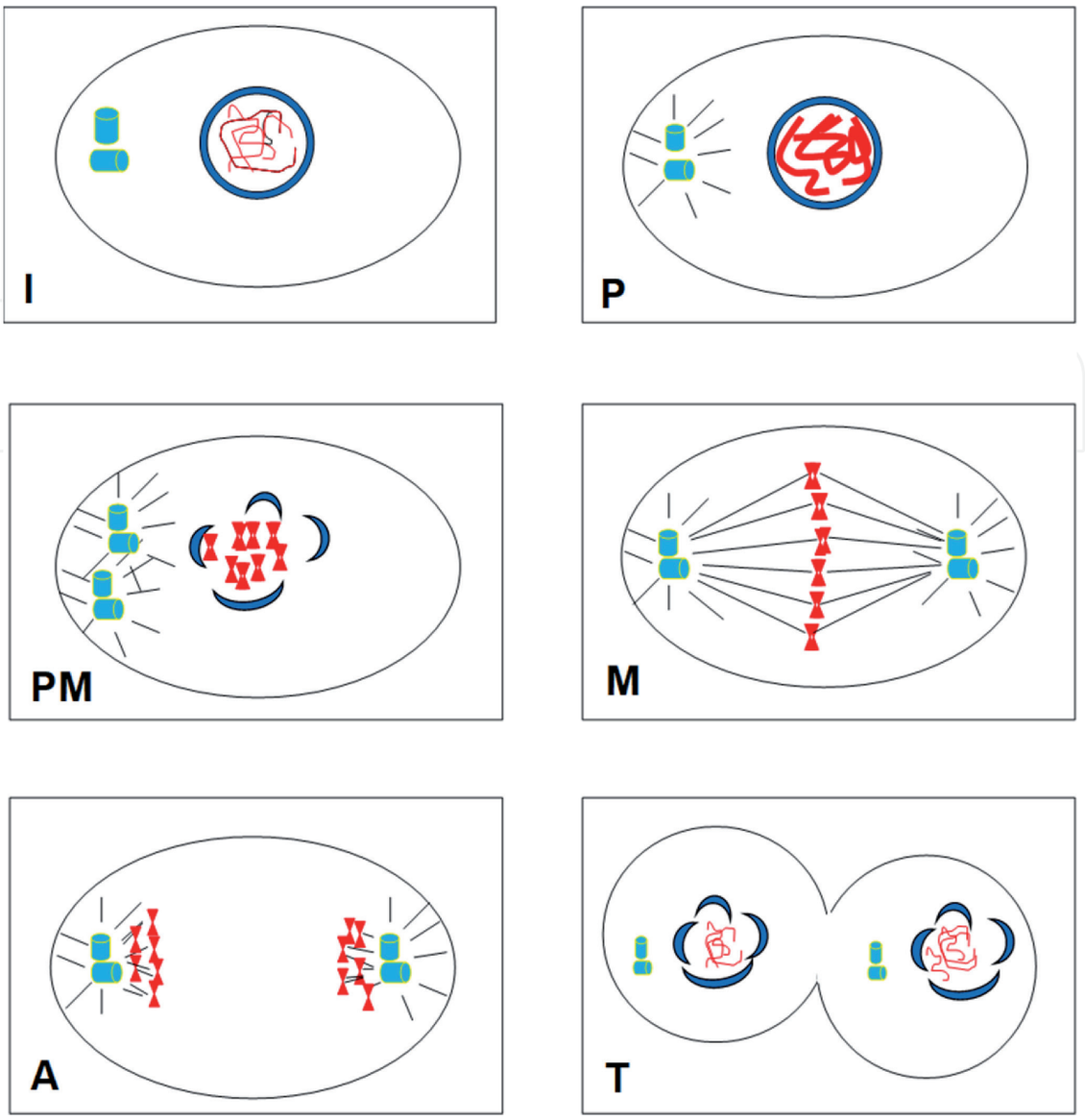

Figure 3.
Schematic diagram depicting the phases of mitosis in a higher eukaryotic cell. (I) Interface, (P) prophase, (PM) prometaphase, (M) metaphase, (A) anaphase, and (T) telophase.

The G1 phase covers the end of the M phase up until the beginning of the S phase. The S phase follows at the end of the G1 phase and ends at the beginning of the G2 phase. The G2 phase starts at the end of the S phase and finishes at the beginning of mitosis (**Figure 2**).

The duration of the interface is longer than the M phase, which lasts only 1 h. However, in the embryonic cells of higher eukaryotic organisms, the S phase is reduced; consequently these cells' cycle is short [1]. The cell cycle of embryonic cells explains the accelerated cell multiplication and the rapid growth of the embryo. The cell cycle of fruit fly embryos lasts only 8 h in contrast to the cell cycle of mammals which lasts 24 h [1].

The duration of the cell cycle varies but the process is similar in all cases. It involves the preparation of the cell in order to give rise to a new organism, as it occurs in unicellular organisms, or to form two identical cells during embryonic development or cell regeneration. Although the two newly formed cells of multicellular organisms are identical, during their cell cycle, each one can modify its phenotype to specialize in a specific function, as it occurs during the advanced stages of the embryonic development of higher eukaryotic organisms [1].

Before a cell initiates mitosis, it needs to duplicate its genetic material and prepare optimal cytoplasmic conditions that will allow it to form two identical cells. Mitosis of higher eukaryotic cells begins with prophase (P), during which the chromatin gradually condenses until the duplicated chromosomes are visible, each with its two sister chromatids joined by the centromere. The microtubules of the cytoskeleton are disassembled, and the formation of the mitotic spindle begins in between the centrosomes that move away from each other. In this phase, the nucleolus is disorganized and not visible during the entire mitosis. Prometaphase (PM) begins abruptly with the disorganization of the nuclear envelope that remains in the form of small vesicles around the mitotic spindle during mitosis. Several protein complexes called kinetochores mature and assemble in the centromere of each chromatid. The fibers of the mitotic spindle that are attached to these structures are called microtubules of the kinetochore; the fibers that do not bind to the kinetochore are known as polar microtubules, and the fibers that are outside the spindle are called astral microtubules. When changing to metaphase (M), the microtubules of the kinetochore align the condensed chromosomes into an equatorial plate. The other end of the kinetochore microtubules attaches to the centrosome of each pole opposite the spindle. Anaphase (A) begins exactly at the moment where the kinetochore pair separates, aided by the microtubules of the mitotic spindle, and is directed toward the opposite poles of the nucleus. The polarization of the chromosomes produces shortening of the kinetochore microtubules, whereas the polar microtubules become longer. During telophase (T) the daughter chromosomes reach the poles of the nucleus, and the kinetochore microtubules disappear. The polar microtubules have lengthened further, and the nuclear envelope begins to organize around the daughter chromosomes. In this phase the nucleolus reappears [1]. During cytokinesis, the cytoplasm divides, and the cell membrane is strangulated in the middle portion of the cell by myosin rings causing the cell to separate, forming two daughter cells (**Figure 3**).

3. The cell cycle in protozoa

For their survival, unicellular organisms also need to duplicate their DNA, divide, and, thus, give origin to a new organism [7]. Like in the cell cycle of higher eukaryotic organisms, in the protozoa interphase (I) and mitosis (M) also occur. The phases (G1), (S), and (G2) are also present in interphase. The phases of mitosis are the same as those in higher eukaryotic cells [7]. The duration of the M phase, as well as each of its phases, depends on the culture conditions. However, the beginning of the S phase and its duration and culmination are evidently regulated by the genetic material [7]. For example, the cell cycle of the *Entamoeba histolytica* clone L-6 lasts from 15 to 18 h: G1 lasts 1 h; the S phase lasts 6 h; and the G2 phase lasts 3 h [8]. The cell cycle of protozoa is very similar to that of prokaryotic organisms because both have states of inactivity in situations of environmental stress, for example, the cystic form in some protozoa and the formation of spores in bacteria, which suggests that they have alternating functional states during the G1 phase [9].

The presence of cyclin-dependent protein kinases related to human cdc2 in some parasites such as *Trypanosoma brucei* (tbcrk1–3) and *Paramecium tetraurelia* suggests their participation in cell cycle regulation of lower eukaryotic organisms [10, 11]. Variation in cytoplasmic calcium concentrations has also been found to be involved in cell cycle regulation in trypanosomes [10]. In particular, it regulates the expression of procyclin mRNA during the differentiation of elongated forms into short ovoid forms [12]. In *Plasmodium falciparum*, the Pfcrk-1 gene has been identified; it encodes a cdc2-related protein kinase that is regulated during the development of the parasite [13].

The cell cycle can be interrupted experimentally with drugs that inhibit the activity of proteasomes such as lactacystin which maintains the procyclic forms of *T. brucei* in the G2 + M phases [14] or with drugs that induce morphological changes and rapid and effective inhibition of DNA synthesis such as sinefungin, which blocks the beginning of the S phase of *Leishmania donovani* promastigotes, stopping them in the G1 phase [15], or with drugs that stabilize the microtubules of the mitotic spindle of *L. donovani* such as taxol that interferes with the progression of G2/M [15].

Mitosis is the main type of nuclear division of protozoa. The fundamental characteristic of mitosis is that the two copies of chromosomes or chromatids are equally distributed between the two daughter nuclei. Consequently, each daughter nucleus receives a complete series of chromosomes. The details of mitotic mechanisms vary widely, particularly in lower eukaryotes including protozoa. In protozoa the level of development of the spindle and centrioles (or structures that are functionally similar to them, such as the microtubule-organizing centers (MTOC)) and the behavior of the nucleolus during mitosis vary widely. In spite of the variants, the two chromatids of each replicated chromosome migrate toward the daughter nucleus while conserving the fundamental characteristic of mitosis [7].

The mitosis observed in protozoa has been classified according to the site in which the formation of the mitotic spindle occurs, the appearance of a complete spindle or formation of two half spindles, and the disintegration, or not, of the nuclear envelope. If the mitotic spindle forms inside the nucleus, it is an intranuclear mitosis, whereas if the spindle forms outside the nucleus, it is said to be an extranuclear mitosis. If a complete spindle is formed, it is an orthomitosis; on the other hand, if two half spindles are formed, it is called pleuromitosis [7]. Finally, if the nuclear envelope remains intact, it is a closed mitosis; if it partially disintegrates, it is said to be a semi-open mitosis, but if it disintegrates completely, it is called a eumitosis, which is equivalent to the mitosis of the higher eukaryotic cells [7]. The combination of these events, during the reproduction of the protozoa, allows the mitosis to be classified into six types: (1) open orthomitosis, (2) semi-open orthomitosis, (3) semi-open pleuromitosis, (4) intranuclear pleuromitosis, (5) intranuclear orthomitosis, and (6) extranuclear pleuromitosis [7] (**Table 1**).

Open orthomitosis has all the characteristics of prototypic mitosis of eukaryotic organisms: the nuclear envelope disorganizes, the nucleolus disappears, and a bipolar, axial, and symmetric spindle with chromosomal fibers joined to the kinetochore forms. Also, the MTOC is located in the cytoplasm where the formation of an equatorial chromosome plate occurs. This type of division occurs in *Phytomastigophora*, *Sarcodina*, *Labyrinthomorpha*, *Gregarines*, *and Dinoflagellates* [16].

Semi-open orthomitosis is peculiar in the way in which the filaments of the mitotic spindle pass through the nuclear membrane via fenestrations located at the poles of the nucleus. The spindle is symmetric and bipolar and contains continuous chromosomal fibers. Chromatin is poorly condensed and formation of the equatorial plate varies. This form of division is found in green flagellates [7].

In **semi-open pleuromitosis**, two identical half spindles are formed with radial and chromosomal fibers that pierce the nuclear membrane before the centrioles occupy the poles of the nucleus. The microtubules penetrate through fenestrations formed in the nuclear envelope. In this type of division, there is no formation of an equatorial plate. It is common in *Gregarines*, *Coccidia*, *Toxoplasmids*, *and Sarcosporidia* [7].

Closed intranuclear pleuromitosis displays two symmetrical half spindles inside the nucleus, each one originating in a MTOC. The chromosomes are less condensed, and there is no formation of the equatorial plate. It is observed in *Microsporidia*, *Kinetoplastid*, *Oximonadida*, *Foraminifera*, *Radiolarians*, and some green flagellates [7].

Types of mitosis	Spindle	Nuclear envelope	MTOC	Nucleolus	Chromatin
Open orthomitosis	Bipolar, axial, and symmetrical	Disorganized and not observable	Yes	Disorganized and not observable	Condensed
Semi-open orthomitosis	Bipolar and symmetric	Perforated at the core poles	Yes	¿?	Less condensed
Semi-open Pleuromitosis	Two identical hemi-spindles	Perforated near spindle formation	Yes	¿?	Less condensed
Closed intranuclear pleuromitosis	Two intranuclear symmetrical hemi-spindles	Whole	Yes	Yes	Less condensed
Closed intranuclear orthomitosis	Axial, bipolar, symmetrical, and intranuclear	Whole	Yes	¿?	Condensed
Closed extranuclear pleuromitosis	Two extranuclear mitotic hemi-spindles	Whole	Yes	Remains	Condensed

¿?: Absent.

Table 1.
Types of mitosis in protozoa. Microtubule-organizing center.

Closed intranuclear orthomytosis is characterized by the formation of a symmetric, axial, bipolar, and intranuclear spindle. The chromosomes form an equatorial plate which occurs in *Rhizopoda*, *Gregarines*, *Euglenids*, *and the micronucleus of the ciliates* [7].

Closed extranuclear pleuromitosis presents an intact nuclear envelope with the formation of two extranuclear mitotic half spindles, adjacent to the nucleus. The kinetochores are found in the nuclear envelope, which allow interaction with spindle fibers and the distribution of chromosomes during nuclear strangulation. It has been observed to occur in *Trichomonadida and Hypermastigida* [7].

The structures similar to the centrioles of kingdom Protista, currently known as MTOC, have received different names such as centrosphere, rhizoplast, spindle polar body, kinetosome, atractophores, or organelle associated with the nucleus [17]. Even though they present great diversity in their arrangement, they adequately participate in the spatial organization and behavior of microtubules during the cell cycle. In *E. histolytica*, the presence of a MTOC has been observed in the center of the nucleus and in one of the poles during mitosis [3, 4, 6].

Apparently there is just one MTOC in this protozoan. However, the binding of recombinant anti-tubulin γ antibodies, from the amoeba, at the MTOC site, suggests that this structure is doubled and polarized, respectively, during anaphase and telophase [18].

The chromatin of the protozoa during interphase is in a decondensed and condensed form. In some protozoa, chromatin is dispersed, while in others it is condensed forming peripheral groups, reticular fibers, individual chromosomes, chromocenters, karyosome, or a dense mass that occupies the whole nucleus [7].

The level of compaction of chromatin mainly varies from one species to another. (i) Finely dispersed chromatin has been found in protozoa that have very large nuclei as seen in *Gregarines and Coccidia* (ii) Granular chromatin is very rare and has only been observed in organisms such as *Trichomonadida*.

(iii) Dispersed chromatin is characteristic of protozoa such as *Amoeba proteus* and *Chaos illinoisensis*. (iv) The compacted chromatin is located at the periphery of the nucleus in the form of a continuous plate or as individual chromocenters. Its structure can be finely granular as it occurs in the kinetoplastids, Foraminifera gametes, and life cycle dispersive states of *Sporozoa* (sporozoites, merozoites, and endozoites). Chromatin that is located in the periphery can be decondensed during the transformation from sporozoites or merozoites to active growth states such as trophozoites or gametocytes [7]. The structure of the chromatin in the form of filaments is located in the central portion of the nucleus of Foraminifera, Mixoteca, and Alogramia gametes. At the ultrastructural level, they form blocks and chains that give the appearance of a reticle. The filamentous structure becomes decondensed during the encystment of *A. schizopyrenidae and Naegleria fowleri*. (vi) Highly condensed chromatin is observed in *euglenids*. The level of compaction varies according to the conditions of the medium and exposure to light. The Euglena chromosomes maintained in darkness are very compact, while exposed to light are very dispersed. (vii) Permanently condensed chromatin is found in chromosomes that are linked by the kinetochore to the internal face of the nuclear envelope of organisms such as *Trichonympha*, *Barbulanympha*, *and Spirotrichonympha*. (viii) Especially compacted chromatin is found in holomastigotes where they acquire a clearly defined spiral structure [7].

Due to the fact that some protozoa have chromatin organized in a similar way to that of prokaryotes, it has led to classify this phylum in (a) mesocarion and (b) eukaryotes. Both types have a well-defined nuclear envelope, but the former possess chromatin arranged in fibers aggregated in a similar way to bacteria [7].

(a) Mesocarion protozoa include organisms of the *dinoflagellates'* order, and (b) eukaryotes include organisms of the Plasmodroma and Ciliophora subphylum. Dinoflagellates are protozoa with well-formed nucleus, of which its chromatin remains compacted during all the phases of the cell cycle. The chromosomes of the dinoflagellates have a shape similar to coarse threads or fibrillar rods. In some species, chromosomes are decondensed with a structure similar to the nuclei of prokaryotic organisms (noctiluca and parasitic dinoflagellate forms) and appear like strands of DNA lacking histones [19].

The number of chromosomes of protozoa has been determined by applying electrophoresis in a pulse field gradient (PFG) and obtaining karyotypes through cellular explosion. By means of the PFG, the DNA size of the chromosome in two species of fish microsporidia has been established. The molecular karyotype of *Glugea atherinae* shows 16 bands of DNA from 240 to 27,000 kb, and *Spraguea lophii* (the smallest nuclear genome of eukaryotic organisms) has 12 bands of 230 to 980 kb [20]. It has also been found that *G. duodenalis* presents 4 to 6 chromosomal bands between 1 and 4 Mb [21] and *Leishmania* has 20 to 28 chromosomal bands from 250 to 2600 [22]. In *Entamoeba histolytica*, 6 to 9 chromosomal bands have been identified of approximately 2000, 1140, 800, 575, 490, 400, 340, and a doublet of 280 Kb [23] and *Plasmodium falciparum* has 7 chromosomal bands ranging from 750 to 2000 kb [24]. In *T. vaginalis* six chromosomal bands of 5700, 4700, 3500, 1200, 1100, and 75 kbp have been identified [25].

In general, the chromatin of the flagellates is compacted, although there are variations of condensation sometimes forming dense rods that touch the nuclear envelope [26]. In functional terms, decondensed chromatin is considered transcriptionally active [27]. Its arrangement is fibrillar with the formation of aggregates of granular appearance as it occurs in organisms of the *Cryptomonadida* order. In some cases, it forms a fibrous sheet as in *E. invadens* [28]. In *A. proteus* it is identified as dispersed chains of 80–90 nm [29]. In *Leishmania*, it has been proposed that

chromatin comprises long diploid holochromosomes that contain all functional structures, such as telomeres, centromeres, and replication origins [30].

In the ciliated protozoa, the genetic material is stored in the macro- and micronucleus. The DNA contained in the macronucleus is transcriptionally active unlike the content in the micronucleus which is inactive [31]. Both nuclei contain DNA and RNA [32]. The chromatin of the macronucleus of the ciliated protozoa is commonly organized into numerous discrete masses called "small bodies" [33] although it has also been observed in the form of spongy masses [34], reticular formations, long chains of chromatin thin plexuses [7], discretely elongated bodies [35], compact spheres, spherical masses, and clear halo granules [28]. The macronucleus of these protozoa contains a structure made visible by light microscope called the replication band, which is a specific site of DNA replication that migrates from the macronucleus and advances along the edge of this band generating the rearrangement of the chromatin in two zones: the proximal and the distal. The proximal reorganizes the chromatin for the synthesis of DNA, and the distal carries it out [36]. Apparently in these organisms, chromosomal fragmentation and the elimination of internal sequences as a route of DNA processing occur [37].

4. Cell cycle of the trophozoites of *Entamoeba histolytica*

One of the main problems in studying the cell cycle and the mitosis of the trophozoites of *Entamoeba histolytica* has been how difficult it is obtaining synchronized cultures. Even when good synchronization with hydroxyurea and nucleotide starvation is obtained, the viability of the trophozoites is low. The synchronization of the cultures of trophozoites of *Entamoeba histolytica* of clone L6 with high doses of colchicine and tritiated thymidine labeling allowed to identify that the phases G1, S, and G2 last 5, 6, and 3 h, respectively [38].

4.1 Mitosis in the trophozoites of *Entamoeba histolytica*

Studies on the organization of the nucleic acids of live trophozoites *of Entamoeba histolytica* with acridine orange stain [5] and those fixed with paraformaldehyde and observed by phase-contrast microscopy (**Figure 4**) show six mitotic phases: prophase, metaphase, early anaphase, late anaphase, early telophase, and late telophase (**Figure 4**).

Unlike the mitosis of the higher eukaryotic cells, the nuclear envelope of the *Entamoeba histolytica* trophozoites remains present during all phases. Another important observation is that the intranuclear RNA located near the nuclear envelope (**Figure 5a** and **b**) remains present during all the phases of mitosis and is fragmented and distributed between the two daughter nuclei [5].

The permanence of the nuclear envelope of *Entamoeba histolytica* trophozoites throughout the whole process of mitosis suggested that the nucleus is first divided (karyokinesis) and then the cell (cytokinesis), resulting in two daughter cells. The chromatin (DNA and RNA) of *Entamoeba histolytica* trophozoites apparently does not show changes of condensation during the phases of mitosis. It is observed as large and small spherical structures (**Figure 5a**). Nuclei during interphase show large chromosomes in the center of the nucleus and the RNA near to the inner face of the nuclear envelope.

During prophase, the oval nucleus shows 5 to 6 chromosomes and 16 to 18 small chromosomes. The chromosomes are observed to be arranged around the microtubule-organizing center (**Figures 4b**, **5c** and **d**) in the center of the nucleus, and the RNA is located around the periphery in a ring shape [5].

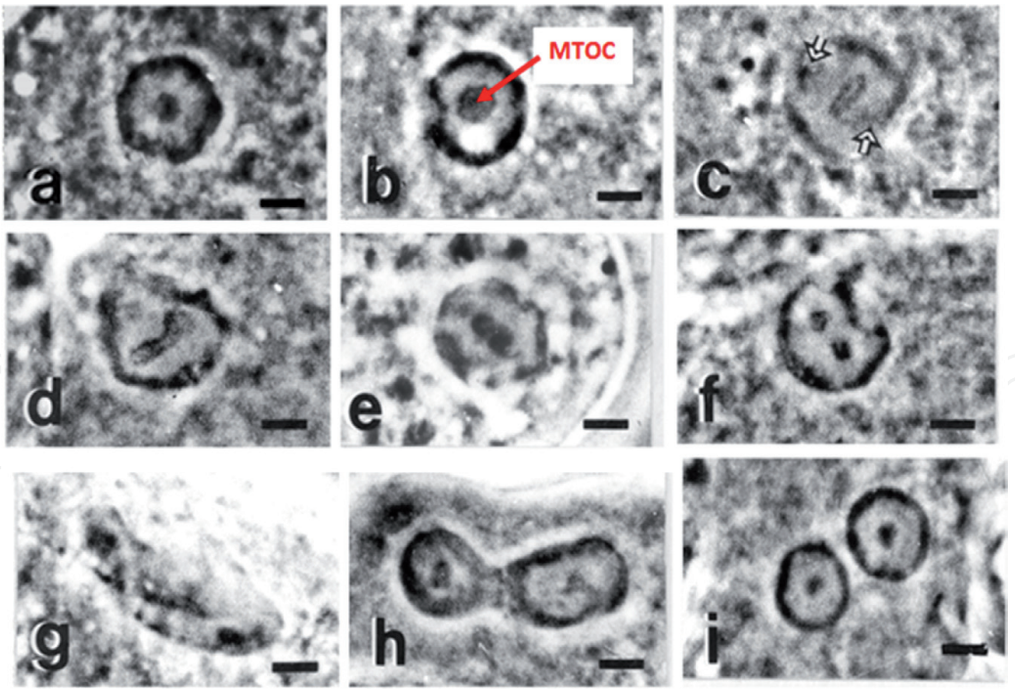

Figure 4.
Phases of mitosis in the nuclei of Entamoeba histolytica. *Trophozoite (a) interface, (b) prophase, (c) metaphase, (d) early anaphase, (e) delayed anaphase, (f) early telophase, (g) late telophase, (h) early karyokinesis, and (i) late cytokinesis. MTOC, microtubule-organizing center.*

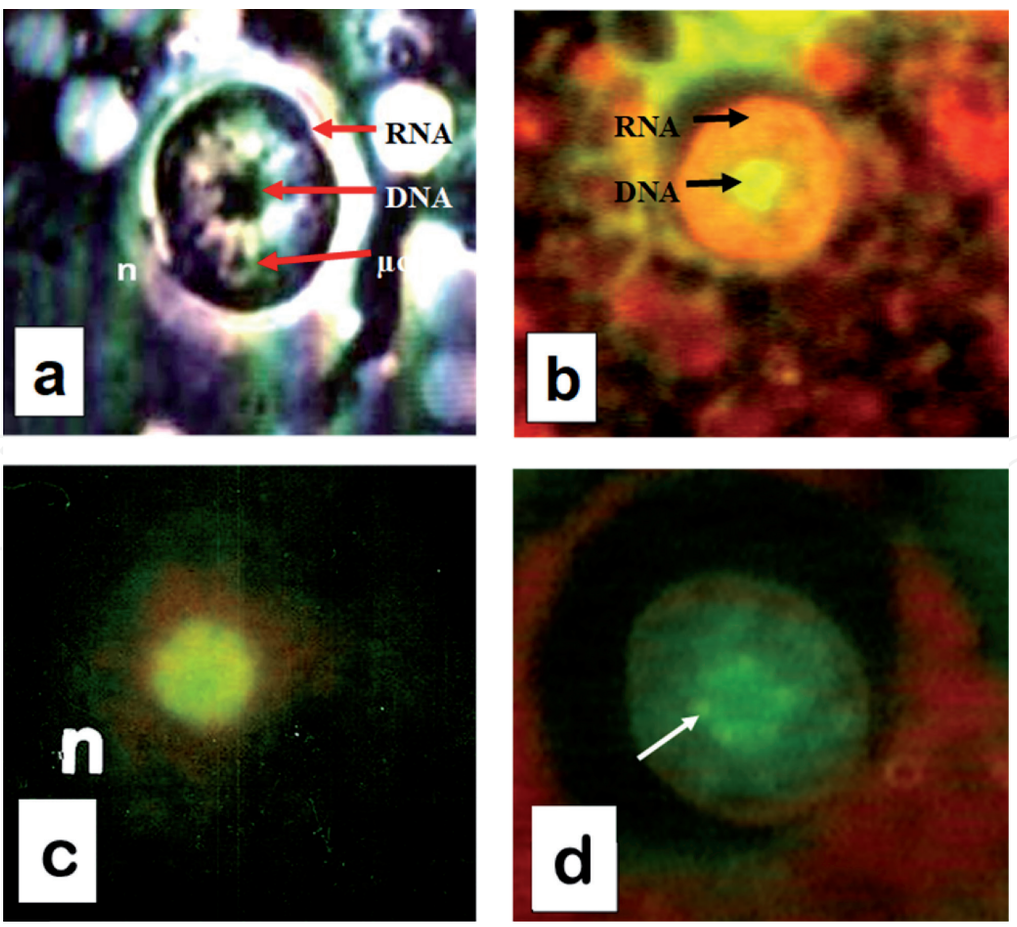

Figure 5.
Interphase and prophase nuclei of Entamoeba histolytica *trophozoites. (a) Phase-contrast microscopy, (b) stained with orange acridine and observed in the fluorescence microscope. (c) Tubulin β in the microtubule-organizing center (green color). (d) Vital stain with orange acridine which are seen in spherical chromosomes (arrow) of green color arranged in a hexagon. μc, microchromosomes.*

Mitotic apparatus	Human cells [1]	*E. histolytica* trophozoites
Spindle:		
Number.	One	Three
Types	Bipolar, axial, and Symmetric	Two radial and one inter MTOCs
Location	Centrosome-kinetochore) Cytoplasmic	Two radial are intranuclear, The inter MTOCs is intranuclear and cytoplasmic [2] Bipolar spindles [39]
Origin of spindle microtubules	Centrosome	Duplicated MTOC
Tubulins:		
γ	Centrosome	MTOC
α and β	Cytoplasmic	Intranuclear and Cytoplasmic
Nuclear envelope	Disorganized during Mitosis	Remains during mitosis
Nucleolus	Disorganized during mitosis and not observable	No nucleolar structure has been demonstrated
RNA	Disorganized and not Observable	It remains condensed [5]
Chromatin	Condensed in the metaphasic chromosomes	It remains condensed throughout mitosis
DNA	Condensed and Decondensed	It remain condensed
Chromosomes:		
Number	46	Indeterminate:
	44 autosomes	24–32 [23]
	2 sex chromosomes	6 [4]
		5 [40]
		6 [5]
		30–50 [41]
		24–32 [42]

Table 2.
Differences in the structures of the mitotic apparatus between human somatic cells and Entamoeba histolytica trophozoites.

In metaphase, the round nucleus increases in size, and the chromosomes move further away from the center of the nucleus. The side view of the duplicated chromosomes shows two parallel rows of round bodies. The RNA ring breaks and forms two to three oval portions located in opposite poles (**Figure 4c**).

Early and late anaphase is characterized by the separation of the chromosomes into two apparent equal parts, each with six chromosomes. In this phase, the nucleus is observed to be elongated with an unchanged RNA (**Figure 4d** and **e**).

In early and late telophase, the nucleus size is 21 μm. Each group of six chromosomes, with the respective small chromosomes, is located in the opposite poles of the nucleus arranged in a ring shape, while the RNA forms small condensations evenly distributed between the two daughter nuclei (**Figure 4f, g**, and **h**).

During karyokinesis, the daughter nuclei separate, each with their own DNA and RNA, and in the end they are observed to be joined by a cytoplasmic filament [5] (**Figure 4h** and **i**).

As mentioned at the beginning of this chapter, there are variations in the types and in the location of the mitotic spindles in different protozoa. By transmission electron microscopy [3] and immunofluorescence studies [39, 2], the mitotic spindle of *Entamoeba histolytica* trophozoites is shown to be intranuclear. In this chapter we not only show the presence of three intranuclear mitotic spindles in this parasite, but we propose a model that explains how the spindle microtubule fibers distribute the chromosomes into the two daughter nuclei.

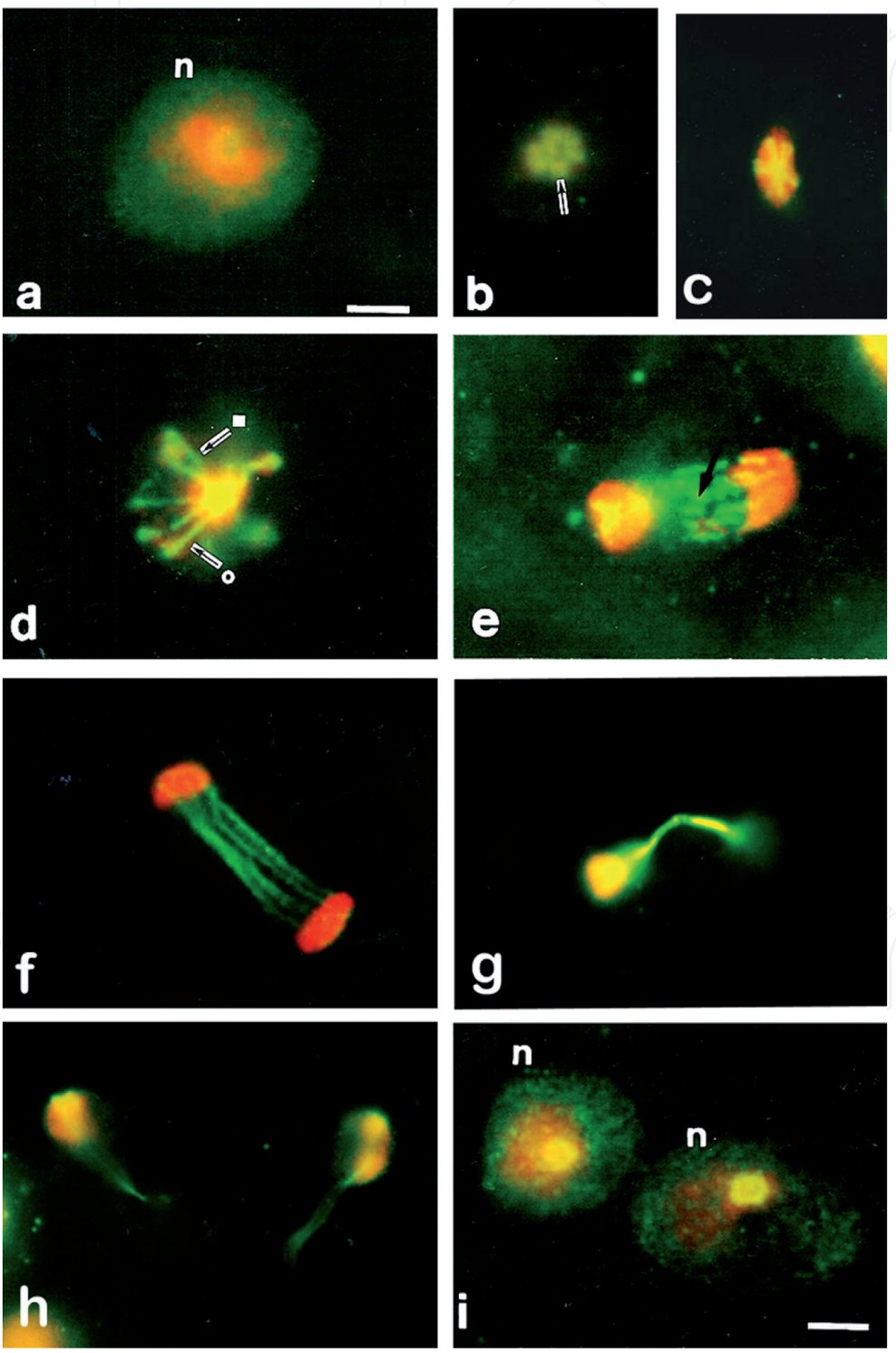

Figure 6.
Structural organization of the mitotic spindles of the nucleus of Entamoeba histolytica *trophozoites during the mitosis phases. (a) Interphase, (b and c) prophase, (d) metaphase, (e) anaphase, (f–g) telophase, and (h–i) karyokinesis. Nucleus treated with RNAse, incubated with anti-β-tubulin antibodies, and contrasted with propidium iodide.*

The MTOC of the protozoa is equivalent to the centrosome of the higher eukaryotic organisms (**Table 2**).

During prophase, the formation of many radially arranged microtubule fibers is observed in the MTOC located in the center of the nucleus [3] (**Figures 6b** and **c**, **7b** and **c**). In metaphase, the MTOC is duplicated, and radial microtubule fibers directed toward the nuclear envelope (radial spindles) emerge from each one. MTOC fibers also arise from transverse microtubules that go from one MTOC to the other (spindle inter MTOC). It is possible to appreciate in the nuclei of the *Entamoeba histolytica* trophozoites during mitosis three mitotic spindles: two radial and one inter MTOC from their MTOC (**Figure 8a**). Apparently, each radial spindle guides the free end of its microtubule fibers toward the nucleus poles, surrounding each group of chromosomes in a mesh (**Figure 8b**). The spindle that forms between the two MTOCs serves to transfer the groups of chromosomes trapped by the radial spindles toward the opposite poles of the nucleus. During anaphase, each group of

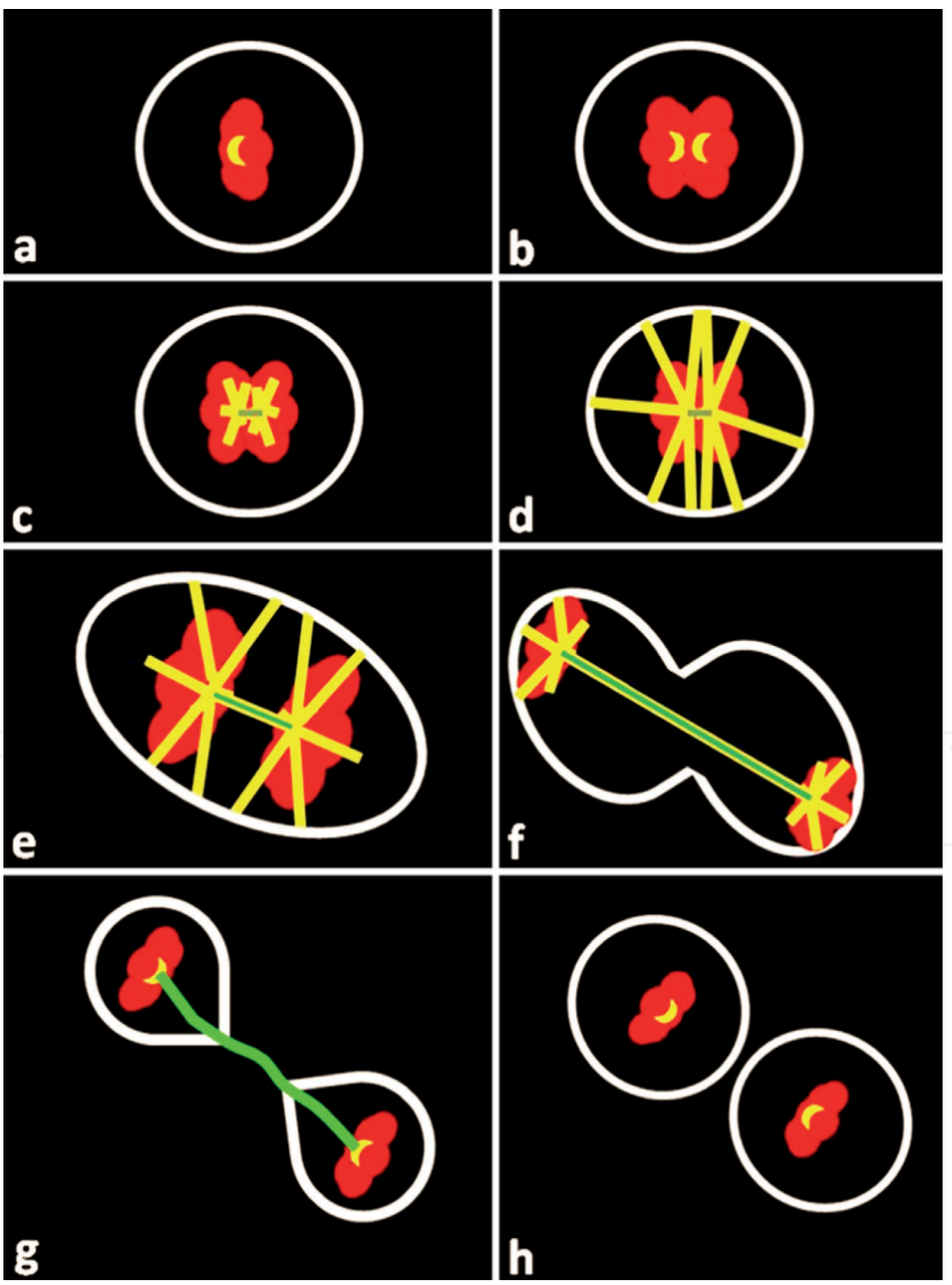

Figure 7.
Diagram of the organization of the mitotic spindles in the nucleus of Entamoeba histolytica *trophozoites. (a) Interphase, (b and c) prophase, (d) metaphase, (e) anaphase, (f) telophase, and (g–h) karyokinesis.*

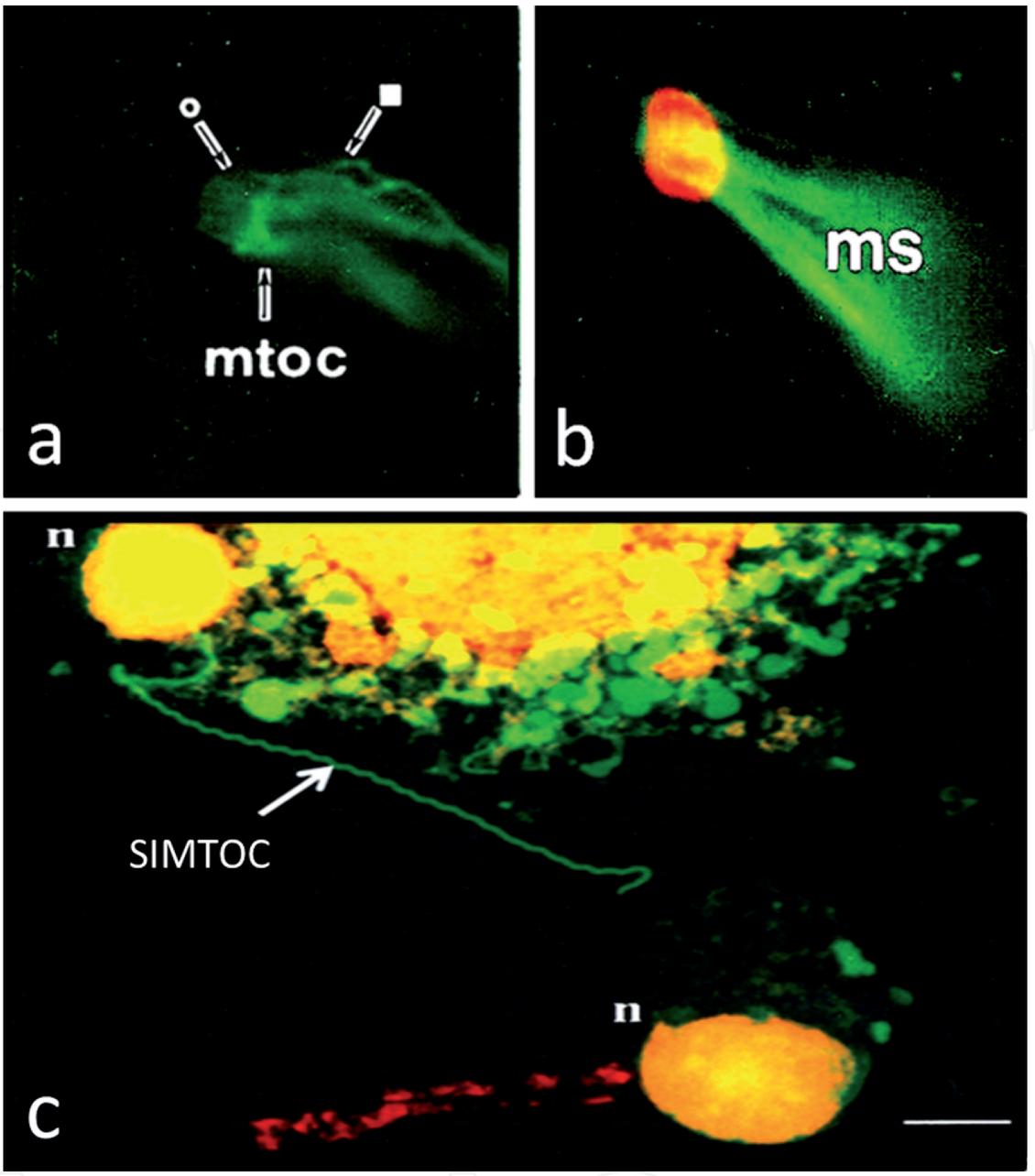

Figure 8.
Nuclear pole of Entamoeba histolytica *trophozoites during telophase and spindle inter microtubule-organizing center (SIMTOC). (a) Nucleus treated with RNAse and incubated with anti-β-tubulin antibodies. (b) Nucleus treated with RNAse, incubated with anti-β-tubulin antibodies, and contrasted with propidium iodide. O, radial spindle at one of the poles. o, part of the spindle inter microtubule-organizing center. (c)* Entamoeba histolytica *trophozoites stained with orange acridine, hypotonized with KCL 0.075 M, and observed with fluorescence microscope during karyokinesis. Spindle inter microtubule-organizing center in cord form.*

chromosomes is surrounded by fibers of the radial spindles, and the MTOC spindle increases in length, leading the chromosomes toward the opposite poles of the nucleus (**Figures 6e** and **7e**). In telophase each group of chromosomes is located at opposite poles of the nucleus. Each group of chromosomes surrounded by radial spindles has the appearance of a round pumpkin with linear marks. Karyokinesis begins with the separation of the nuclei that are still joined by microtubule fibers of the spindle inter MTOCs (**Figures 6h–i, 7g–h, 8c**). Apparently the spindle remains even when the nuclei have been divided; it is still unknown if it perforates the nuclear envelope or if this cord remains surrounded by it. An interesting observation occurs when the *Entamoeba histolytica* trophozoites are vitally stained with acridine orange and hypotonized with 0.075 M KCL. Under these conditions it is possible to observe microtubular structures during mitosis (**Figure 8c**).

After karyokinesis, cytokinesis begins; the trophozoite with two nuclei begins to divide by narrowing the cytoplasm in its middle part [5]. This process is relatively slow and occurs gradually through stretching with intervals of rest. The cytoplasm of the middle part of the trophozoite thins until it forms a very thin filament which then breaks and results in two trophozoites of *Entamoeba histolytica* with a nucleus in each of them. It is still unknown whether cytoplasmic DNA [38] duplicates during mitosis of trophozoites or if it is only genetic material with other functions. The description and observations realized in the different phases of mitosis in *E. histolytica* trophozoites using the phase-contrast microscopy technique are consistent with those described by acridine orange vital staining and transmission electron microscopy [3–6]. Peripheral RNA chromatin and central DNA chromatin behaved in a similar manner to the acridine orange staining described [5]. The data described and observed with the anti-β-tubulin antibodies of *E. histolytica* regarding the radial microtubule bundles correlate with the observations described with transmission electron microscopy of nuclei in prophase and prometaphase [3, 4, 6]. However, there is a discrepancy about the number of spindles that are present and observed between the two nuclei in formation [2, 3, 6). The description of an inter-MTOC spindle independent of radial spindles was based on its observation with immunofluorescence (**Figure 8a**). An observation that brings forth new ways of studying the nuclear division in *E. histolytica* trophozoites is the presence of a green fluorescent internuclear cord obtained with the acridine orange staining and KCL-hypotonization.

5. Conclusions

The identification of β-tubulin bundles that surround the DNA nuclei in telophase suggests a mechanism of entrapment similar to a "hand-closure movement" that allows, along with the inter-MTOC spindle, the distribution of genetic material between the two newly formed nuclei.

The mitosis of *Entamoeba histolytica* trophozoites is a type of intranuclear pleuromitosis, closed or open? modified, since three spindles are formed: two radial and one inter MTOCs. In addition, these are found within the nucleus. It remains to be discovered if the spindle that apparently is outside the nucleus has a nuclear envelope or not.

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