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Research brief

Entamoeba histolytica: Ultrastructure of the chromosomes and the mitotic spindle

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Abstract

We have analyzed by transmission electron microscopy the mitotic process of *Entamoeba histolytica* trophozoites in an asynchronous population of axenically cultured parasites. Our observations showed that nuclear microtubules, initially located at random in the karyosome during prophase, formed in subsequent stages a mitotic spindle closely related to the nuclear membrane at the polar regions of dividing nuclei. In late prophase and in anaphase, chromosomes appeared as dense bodies 0.1–0.5 μm. At least 15 chromosomes appeared in favorable planes of section, arranged as an incomplete elliptical circle, in close contact with microtubules. There was no morphological evidence of structures resembling the kinetochores of higher eukaryotes. When cut in cross-section, the mitotic spindle was made of 28–35 microtubular rosette assemblies. The latter probably correspond to a similar number of chromosomes, as has been shown by others with pulse-field electrophoresis and fluorescence microscopy of trophozoite spreads. In turn, each microtubular rosette was constituted by 7–12 parallel microtubules. In later stages of the metaphase, two sets of chromosomes were disposed forming a pair of elliptical circles. An additional finding in the dividing nuclei of *E. histolytica* trophozoites was the presence of compact conglomerates of numerous particles 50 nm in diameter, of similar electron density, shape, and size, probably corresponding to RNA episomes.

Index Descriptors and Abbreviations: Protozoa; Ameba; Mitosis; Microtubules

The ultrastructure of the nuclear division of the human intestinal parasite *Entamoeba histolytica* is still a subject of debate. There is general agreement concerning the following facts: (a) mitosis occurs without dissolution of the nuclear membrane, (b) microtubules are prominent in the nucleus once trophozoites enter into mitosis, but are absent during the interphase both in the nucleus and in the cytoplasm, and (c) chromosomes are ill defined and their number has been difficult to estimate either by fluorescence microscopy or by electron microscopy.

In the first and most detailed ultrastructural study of the mitosis of an *Entamoeba*, Gicquaud (1979) reported in *E. histolytica*-Laredo that in the course of the prophase the karyosome, the centrally located region of the nucleus where chromatin is concentrated, acts initially as a microtubule-organizing center (MTOC), from where abundant microtubules radiate toward 10–15 small condensations, considered as chromosomes. Metaphase images were reported as rare. According to this study, the MTOC disappears early in anaphase, when chromosomes move toward the nuclear poles. A second set of microtubules, this time disposed in parallel array, form a thick bundle that progressively distorts the nuclei of *E. histolytica*-Laredo, giving an ovoid outline to the nucleus, until two daughter cells are formed (Gicquaud, 1979).

Later, essentially the same sequence of ultrastructural events described above for *E. histolytica*-Laredo amebas was found in electron microscopy studies of the nuclear division of *E. histolytica*, except for the fact that in the latter ameba the karyosome was reported to be present at all stages of the mitotic process (Orozco et al., 1988; Solis and Barrios, 1991).

With specific DNA-binding dyes and fluorescence microscopy, the number of chromosomes in *E. histolytica* was

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reported initially as five (Solis and Adams, 1997) or six (Gómez-Conde et al., 1998). More recently, the use of spreads of fixed trophozoites revealed 30–50 chromosomes, in accordance with results obtained by pulse-field gel electrophoresis (Willhoeft and Tannich, 2000). Due to limitations in spreading and the fragility of amebic chromosomes, their exact number could not be determined, although they were visualized as long, linear and thin structures, with a visible centromeric region (Willhoeft and Tannich, 2000).

We report here novel observations concerning the ultrastructure of the chromosomes and the mitotic spindle during the nuclear division process of *E. histolytica*.

Entamoeba histolytica trophozoites of the strain HM1-IMSS were cultured at 36 °C in TYI-S-33 medium added with 10% bovine serum and vitamins mixture, in borosilicate glass culture tubes. To stimulated cell division, parasites in the log phase of growth (72 h) were subcultured with fresh medium for 60, 90, and 120 min (Gómez-Conde et al., 1998).

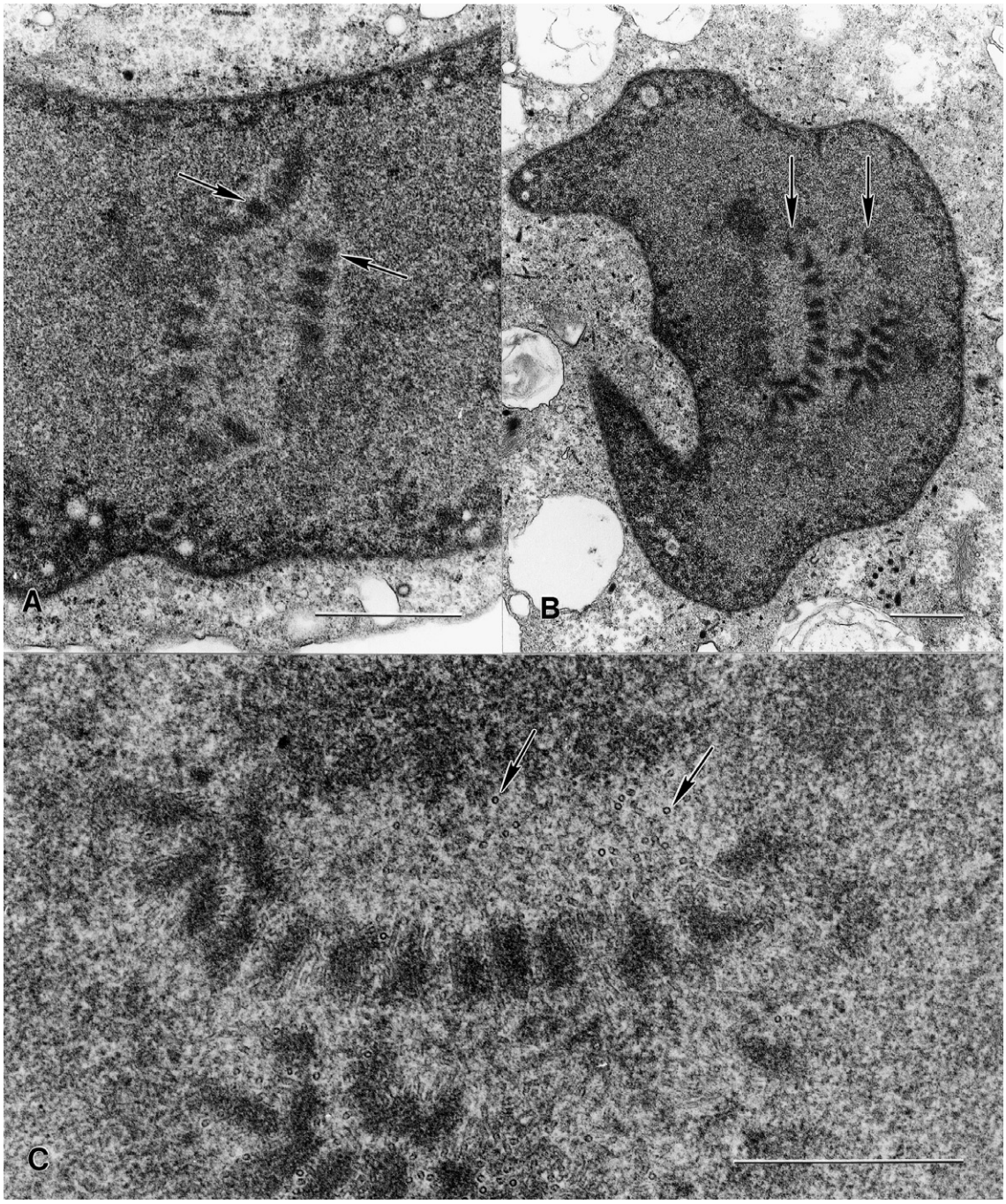

Fig. 1. (A) Fine structural appearance of a nucleus of *E. histolytica* in late prophase. At least 15 chromosomes are arranged as an irregular circle (arrows). At this level of the nucleus, the microtubules do no present a regular disposition. Bar = 1 μm. (B) A nucleus in telophase shows chromosomes disposed forming two semicircles (arrows). Bar = 1 μm. (C) Higher magnification of an *E. histolytica* nucleus in prophase demonstrates a number of microtubules (arrows) disposed perpendicular to the plane of the chromosomes, but no structure resembling kinetochores are apparent. Bar = 1 μm.