DIFFERENTIATION OF Giardia intestinalis: FROM LIGHT TO ELETRON MICROSCOPY

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Giardia intestinalis is a parasite protist considered a living fossil because it is placed in the earliest divergence and deepest branch in eukaryote evolution. This parasite grows in vitro as trophozoites and under some conditions differentiates into cysts, characterized by presence of a cyst wall (CW). Proteins and carbohydrates compose the CW of the parasite and are transported by the Encystation Secretory Vesicles (ESVs) and Encystation Carbohydrate-contain Vesicles (ECVs), respectively [1]. In addition to these encystation specific organelles, Giardia presents mitosomes, which are mitochondria related organelles that are modulated during the differentiation process. These cytoplasmic organelles are commonly analyzed by confocal and conventional electron microscopy, however, due its limited resolution and depth, some information is always missed. In this study, we took advantage of the SR-SIM and PALM methodology, electron tomography and Dual-beam (FIB) microscopy to reach new insights of these cytoplasmic structures during G. intestinalis encystation. Immunofluorescence assays were performed using anti-cyst wall protein 1 (CWP1) and DBA fluorescent-conjugated lectin, as well mitosomal anti-IscU protein, with 21h-encysted cells. The samples were observed with the super resolution microscopy Zeiss ELYRA SP.1 (SR-SIM and PALM). To compare the results obtained using confocal and super-resolution microscopy, the samples were analyzed with both methodologies. In addition, the cells were processed to routine electron microscopy and analyzed by Electron Tomography and Dual-beam microscopy. During the parasite differentiation the ESVs are easily identified. These vesicles present a continuous labeling pattern along the membrane by confocal microscopy. However, when the same experiment was analyzed by SR-SIM, revealed a non-continuous staining in the organelle membrane and fluorescence dots are noted throughout the cytoplasm. The FIB microscopy revealed ESVs surrounded by endoplasmic reticulum and a very close proximity to the nuclei at early stages of encystation. The ECVs are smaller than ESVs, distributed throughout the cytoplasm, preferentially close to the ESVs. A diffuse labeling was seen within the cell with confocal microscopy. By SR-SIM the same labeling was observed clearly, without fluorescence spreading, allowing the exact identification of these organelles. A non-encysted Giardia presents peripheral and central mitosomes. By confocal microscopy the central mitosome appears as a unique tinny structure between the two nuclei. A different perspective was obtained by SR-SIM, and different labeled spots formed the central mitosome but not a unique tinny ovoid organelle. In the cyst form, a disorganization of mitosomes was observed. Thus, the use of advanced light and electron microscopy added new insights of Giardia organelles.