

The nucleolus of *Giardia lamblia*

Abstract

The nucleolus is the major site of ribosome biogenesis in eukaryotes. Until recently, *G. Lamblia* was considered the only eukaryote lacking nucleoli. Recently, light and electron microscopy cytochemical techniques have been used to demonstrate the presence of nucleoli in the interphase nucleus of *G. Lamblia*. Here we review the work made during the last few years on the nucleolus of *G. Lamblia* in interphase and cell division. This mini review indicates that nucleoli are present in *G. Lamblia* interphase cell nuclei. Also, the persistence of nucleoli during cell division was documented more recently. Therefore, microscopical anatomy of *G. Lamblia* should include the presence of authentic morphological nucleoli.

Keywords: *G. Lamblia*, *G. Duodenalis*, nucleolar, golgi apparatus, endoplasmic reticulum, hybridization, nuclei, mitosomes, flagella, ribonucleoprotein

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Abbreviations: AgNOR, silver staining for nucleolar organizer region; NOR, nucleolar organizer region; Rna, ribosomal rna; TEM, transmission electron microscopy

Introduction

G. Lamblia, also known as *G. Intestinalis* or *G. DSuodenalis*, is a unicellular parasite infecting human large intestine and causing severe diarrhea.¹⁻⁶ According to B. Ford, it was first observed by Antony van Leeuwenhoek back in 1681,⁷ as very small organisms, with a large body and a flattish belly with little paws and stir movements. *G. Lamblia* was later described taxonomically.⁸

G. Lamblia also belongs to early divergent lineages of eukaryotes as revealed by protein or nucleic acids sequence analysis,⁹⁻¹¹ although reduced processes also may have occurred.¹² Studies on the cell biology of *G. Lamblia* have described several organelles as nuclei, mitosomes, flagella, several elements of cytoskeleton, but definite proof for the presence of Golgi apparatus or endoplasmic reticulum have not been documented.¹⁻⁶ For a long time *G. Lamblia* was known as the only eukaryote lacking nucleoli. Since the detailed analysis searching for nucleoli failed to show the presence of nucleoli in *Giardia* even by using immunocytochemistry and *in situ* hybridization combined with the use of confocal microscopy,¹³ it was asked whether classical cytochemical staining as silver staining for nucleolar organizer would produce better contrast than fluorescent techniques.

The first evidence for the presence of nucleoli in *G. Duodenalis*, *G. Intestinalis* or *G. Lamblia* was obtained after using silver staining for nucleolar organizer regions (AgNOR) for light microscopy. Nucleolar material was observed as small fiber-granular areas of impregnation around 0.3µm large (Figure 1).¹⁴ Then, transmission electron microscopy (TEM) confirmed this result. In fact, standard technique for TEM produced results indicating an intranuclear electron dense material at the periphery of nuclei (Figure 2), that in addition was positive to silver staining for NOR, for RNA using the EDTA method for RNA, silver staining for NOR at the EM level followed by immunoelectron microscopy for fibrillarin and high resolution *in situ* hybridization for rRNA. Analysis of expression of transfected rRNA-pseudouridine synthase (CBF5) and confocal microscopy confirmed these results. Later on, additional light and

electron microscopy techniques also revealed the presence of nucleoli (Figure 3).¹⁵ Microscopical anatomy of *G. Lamblia* therefore, includes the presence of nucleoli.^{4-6,16}

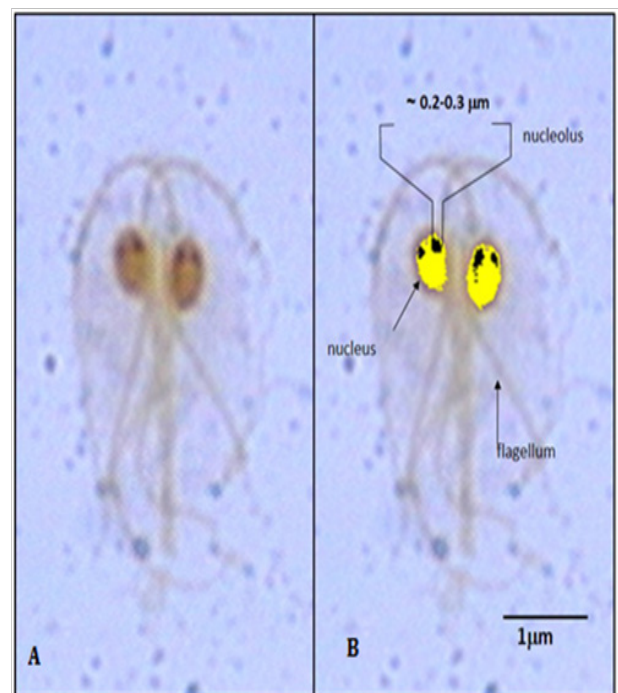


Figure 1

- A) Bright field light microscopy after silver staining for nucleolar organizer region (AgNOR) staining in *G. lamblia*.
- B) Same image as A processed to enhance the silver stained material. Nucleolus is present as peripheral structures within both cell nuclei.

More recently, it was reported further confirmation of the presence of nucleoli in *G. Lamblia* in interphase and also the persistence of this organelle through cell division,¹⁷ therefore indicating that the process of nucleolar biogenesis does not include the formation of the so called pre nucleolar bodies, as in other eukaryotes.¹⁸

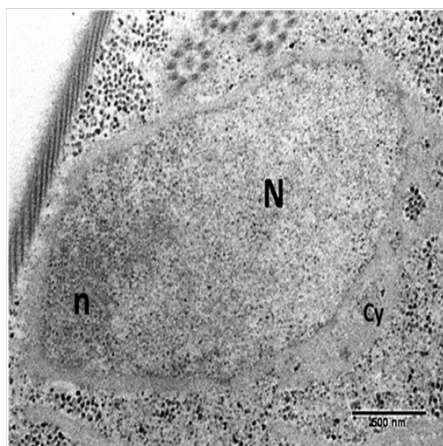


Figure 2 Transmission electron microscopy of *G. lamblia* nucleolus. Nucleolus (n) is present at the periphery within the cell nucleus (N). Cy, cytoplasm; n, nucleolus; N, cell nucleus

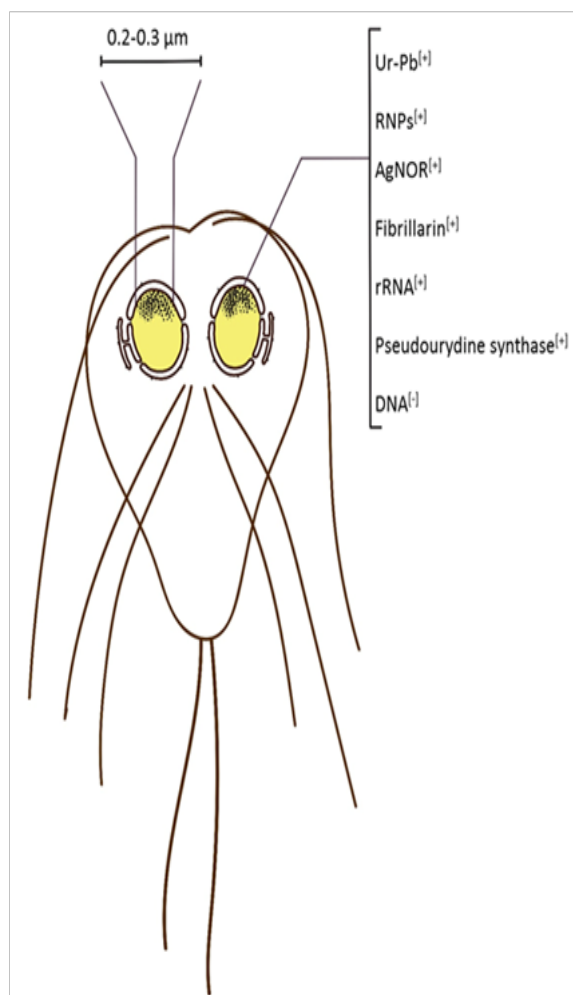


Figure 3 Drawing of *G. lamblia* showing the nucleolus in both nuclei of the cell summarizing the different nucleolar markers tested. Ur-Pb [+], positive in samples contrasted with uranyl acetate for standard transmission electron microscopy; RNPs [+], positive for the EDTA regressive staining for ribonucleoproteins; AgNOR [+], positive for silver staining for nucleolar organizer region; Fibrillarin [+], positive for immunoelectron microscopy for nucleolar protein fibrillarin; rRNA [+], positive for high resolution *in situ* hybridization for rRNA; pseudouridine synthase [+], transfection for expression of this protein; DNA [-], negative for immunoelectron microscopy for DNA.

Discussion

G. Lamblia displays the smallest nucleoli so far described in nature. It is about 0.2-0.3 μm large, which is also about the resolving power for standard light microscopy. The reason of why it was not observed before, may have to do with its size and the use of fluorescent probes that, even they are so specific, however they do not display the highest contrast. On the other hand, silver staining for nucleolar organizer offers the highest contrast when analyzing by bright field light microscopy. The first observations suggested that a fine study had to be made with TEM. The best fixation techniques and further cytochemical, immunocyto chemical and *in situ* hybridization techniques at the ultrastructure level confirmed that nucleolar material was present in *G. Lamblia*. A fibrous and granular component characterizes an intranuclear region. Further analysis with transfection of the gene for the rRNA-pseudouridine synthase or fibrillarin also confirmed the presence of a nucleolar domain within the nuclei of *G. Lamblia*.¹⁴

The results mentioned for the presence of this nuclear organelle in such early divergent eukaryote organism,¹⁴ suggest that nucleoli may be considered a *sine qua non* structure for eukaryotes, further suggesting that the origin of nucleoli predates the origin of nuclei. In addition, the persistence of nucleolar material during cell division,¹⁷ may also indicate that nucleoli are active through cell division, an aspect that have to be explored. In fact, the role of active nucleolus or nucleolar material during cell division may confer some advantages in such environments as stomach and intestine. Recent results also have been observed in other unicellular parasites as *Trypanosoma cruzi*.¹⁹

Conclusion

Nucleoli are present in both interphase nuclei of the unicellular parasite *G. Lamblia*. We found in current literature that *G. Lamblia* nucleolus was about 0.2 μm in size, is present as peripheral fibro-granular ribonucleoprotein material, is positive to several nucleolar markers and it is negative for DNA presence. Morphological and cytochemical evidence indicate that nucleoli are persistent during the cell cycle.

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Conflict of interest

Author declares that there is no conflict of interest.

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