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, microscopic 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

**Introduction**

*G. Lamblia*, also known as *G. Intestinalis* or *G. DSuodenalis*, is a unicellular parasite infecting human large intestine and causing severe diarrhea. According to B. Ford, it was first observed by Antony van Leeuwenhoek back in 1681, as very small organisms, with a large body and a flattish belly with little paws and stir movements. *G. Lamblia* was later described taxonomically. *G. Lamblia* also belongs to early divergent lineages of eukaryotes as revealed by protein or nucleic acids sequence analysis, although reduced processes also may have occurred. 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. 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, it was asked whether classical cytochemical staining as silver staining for nucleolus 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). 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).

Microscopical anatomy of *G. Lamblia* therefore, includes the presence of nucleoli.
Discussion

*G. Lamblia* displays the smallest nucleoli so far described in nature. It is about 0.2-0.3µm 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, immunocytochemical 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 RNA-pseudourydine 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 0.2µm in size, is present as peripheral fibrogranular 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.

References

1. Gillin FD, Reiner DS. Cell biology of the primitive eukaryote *Giardia lamblia*. *Annu Rev Microbiol*. 1996;50:679‒705.
2. Upcroft J, Upcroft P. My favorite cell: *Giardia*. *Bioessays*. 1998;20(3):256‒263.
3. Adam RD. Biology of *Giardia lamblia*. *Clin Microbiol Rev*. 2001;14(3):447‒475.
4. Ankarklev J, Jerlström-Hultqvist J, Ringqvist E, et al. Behind the smile: cell biology and disease mechanism of *Giardia* species. *Nat Rev Microbiol*. 2010;8(6):413‒422.
5. Carranza PG, Luján HD. New insights regarding the biology of *Giardia lamblia*. *Microbes Infect*. 2010;12(1):71‒80.
6. Luján HD, Svärd S. *Giardia*, a model organism. Wien, Austria: Springer-Verlag; 2011.
The nucleolus of Giardia lamblia

7. Ford B. The discovery of Giardia. Microscope. 2005;53:147–153.

8. Kudo RR. Protozoologia. CECSA, Mexico City, Mexico; 1966. p. 350–355.

9. Sogin ML, Gunderson JH, Elwood HJ, et al. Phylogenetic meaning of the kingdom concept: an unusual ribosomal RNA from Giardia lamblia. Science. 1989;243(4887):75–77.

10. Van Keulen H, Gutell RR, Gates MA, et al. Unique phylogenetic position of Diplomonadida based on the complete small subunit ribosomal RNA sequence of Giardia ardeae, G. muris, G. duodenalis and Hexamita sp. FASEB J. 1993;7(1):223–231.

11. Hashimoto T, Nakamura Y, Nakamura F, et al. Protein phylogeny gives a robust estimation for early divergences of eukaryotes: phylogenetic place of a mitochondria-lacking protozoan, Giardia lamblia. Mol Biol Evol. 1994;11(1):65–71.

12. Morrison HG, McArthur AG, Gillin FD, et al. Genomic minimalism in the early diverging intestinal parasite Giardia lamblia. Science. 2007;317(5846):1921–1926.

13. Narcisi EM, Glover CVC, Fechheimer M. Fibrillarin, a conserved pre-ribosomal RNA protein of Giardia. J Eukaryot Microbiol. 1998;45(1):105–111.

14. Jiménez-García LF, Zavala G, Chávez-Munguía B, et al. Identification of nucleoli in the early branching protist Giardia duodenalis. Int J Parasitol. 2008;38(11):1297–1304.

15. Tian XF, Yang ZH, Shen H, et al. Identification of the nucleoli of Giardia lamblia with TEM and CFM. Parasitol Res. 2010;106(4):789–793.

16. Benchimol M, de Souza W. The ultrastructure of Giardia during growth and differentiation. In: Luján H, Svärd S, editors. Giardia. Wien, Austria: Springer-Verlag; 2011. p. 141–160.

17. Lara-Martínez R, Segura-Valdez ML, de la Mora-de la Mora I, et al. Morphological studies of nucleologenesis in Giardia lamblia. Anat Rec. 2016;299(5):549–556.

18. Hernandez-Verdun D. Assembly and disassembly of the nucleolus during the cell cycle. Nucleus. 2011;2(3):189–194.

19. Nepomuceno-Mejía, Lara-Martínez, Hernández R, et al. Nucleologenesis in Trypanosoma cruzi. Micrsc Microanal. 2016;22(3):621–629.