Variations in nucleolar morphology in Eumycetozoans

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ORIGINAL

Variaciones en la morfología nucleolar en Eumycetozoos

Resumen. A pesar de que la mayoría de biólogos están familiarizados con el tipo de núcleo celular, central, más o menos esférico y conteniendo un solo nucleolo, existen muchas variaciones al respecto. Algunas de estas permutaciones pueden ser encontradas en las diferentes amebas de los eumycetozoos. Estas diferencias en la morfología nucleolar pueden tener algún significado filogenético y son muy utilizadas por los investigadores para identificar los taxones en los cuales se encuentran. Mediante una combinación de técnicas microscópicas, se ilustran los nucleolos típicos encontrados en mixomicetes, dictiostélidos y los protostélidos *Soliformovum* spp. y *Echinosteliopsis oligospora*.
Palabras clave: Dictiostélido, *Echinosteliopsis*, microscopía, mixomicetes, protostélido.

Abstract. While most biologists are familiar with nuclei that have a single, central, more or less spherical nucleolus, there are many variations on this theme. Several of these permutations are found in the various amoebae of eumycetozoans. These differences in nucleolar morphology may have some phylogenetic significance, but are clearly useful in helping researchers to identify the taxa in which they occur. Using a combination of microscopy techniques, we illustrate the typical nucleoli found in myxomycetes, most protostelids, dictyostelids, and the protostelids *Soliformovum* spp. and *Echinosteliopsis oligospora*. We emphasize the previously unpublished details of *E. oligospora*.
Key words: Dictyostelid, *Echinosteliopsis*, microscopy, myxomycete, protostelid.

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Introduction

The slime molds in the taxon Eumycetozoa Olive [10, 1] are a remarkable group. Many of their unique qualities, such as the plasmodium of myxomycetes and the cooperative multicellular aggregations of dictyostelids, are well known for their important applications throughout the biological sciences. Less widely appreciated is the array of striking differences within the eumycetozoans. Here we illustrate one such difference: an unusual degree of intraclade variation in nucleolar morphology.

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The Mycetozoa (or slime molds) is a polyphyletic group of amoebae that produce fruiting bodies consisting of a stalk and one or more spores [10, 29]. A subset of the Mycetozoa that is hypothesized to be monophyletic is the Eumycetozoa [10]. The taxon Eumycetozoa consists of three groups: protostelids (Protostelia), dicytostelids (Dicytostelia), and myxomycetes (Myxogastria).

Protostelids have a variety of life-cycles that range from simple (amoeba - fruiting body - amoeba) to more complex (amoeboflagellate - obligate amoeba - fruting body - amoeboflagellate) (See 20 for illustrations, 27 for definitions). Dictyostelids have life-cycles that include free-

living amoebae that aggregate to form multicellular fruiting bodies with stalks [15]. The myxomycete life-cycle consists of amoeboflagellates, plasmodia (obligate amoebae), and fruiting bodies with spores that germinate as amoeboflagellates [30].

The dictyostelids and myxomycetes are groups that are each clearly monophyletic based on life-cycle characters, amoebal morphology, and molecular systematics [15, 10, 21, 2, 3]. The protostelids are paraphyletic and show greater variation in life-cycle and morphology than the other groups [10, 21, 22, 29]. Spiegel [21, 22, 27] divided the protostelids into 8 groups [Table 1]. Groups that contain species with amoeboflagellates we consider to be eumycetozoans. Among the completely non-flagellated groups of protostelids, Spiegel [21] hypothesized some to be eumycetozoans and others to be non-eumycetozoans [Table 1].

Our interest is in the comparative morphology of eumycetozoan amoebae. In dictyostelids, with the possible exception of size, no major morphological differences have been recorded among the amoebae of the roughly 100 species [15, 10, 29]. The amoeboflagellate state of all the nearly 1,000 described species of myxomycetes is identical and there are only a few variations in plasmodial morphology [6, 10, 20, 21, 26]. Considerable variation is found among the amoebae of protostelids [21, 22, 23, 25, 27, 26, 19, 24].

Nucleolar morphology is a highly variable character within the *Eumycetozoa,* but it is a stable morphological feature of individual taxa (Table 1). For instance, as has already been implied, the nucleoli of all dicytostelids are thought to be identical and distinct from any other eumycetozoan nucleoli [15]. The nucleoli of myxomycetes, also indistinguishable from one another, are the typical round, central nucleoli that we often think of as the "general" eukaryotic nucleolus [7]. Protostelids, however, show a range of nucleolar variation [21, 26, 16]. Within organisms commonly referred to as protostelids there are at least three distinct types of nucleoli. First, the myxomycete-like (Group 5) and the other flagellated protostelids (Groups 1, 2, and 4) contain the single, central, spherical nucleolus identical to those found in myxomycetes [23]. Second, nucleoli of the genus *Soliformovum* (Group 3) which contains two species, *S. irregularis* and *S. expulsum*, are irregularly shaped and diffused throughout the cell [26]. Finally, the protostelid *Echinosteliopsis oligospora,* Eumycetozoa *incertae sedis,* has what appear to be multiple, peripheral nucleoli [16, 17].

Here we illustrate four of the types of nucleoli found within the Eumycetozoa, including the first transmission electron micrographs published for *Echinosteliopsis oligospora.*

Materials and Methods

Cultures

The protostelids *Protostelium mycophaga* Type (ATCC PRA-154), *Soliformovum irregularis* Mex 81 (ATCC 26826) and *Echinosteliopsis oligospora* HIO4-33a-3a (ATCC PRA-125) were all cultured on weak malt yeast extract agar (wMY [21]) with *Rhodotorula mucilaginosa, Flavobacterium* sp., and *E. coli*, respectively, as their food sources. *Polysphondylium violaceum* (local isolate) was grown on wMY with *E. coli.* All were grown in the laboratory at ambient temperatures (approx. 21-25C).

Light microscopy

Agar coated slides [28] were prepared and inoculated with amoebae of each species and allowed to acclimate for approximately one hour in a Petri dish. Amoebae, cysts and spores were then observed on a Zeiss Axioskop 2 Plus under the 40x dry objective using both phase contrast and DIC techniques and photographed using Auto Montage (Syncroscopy).

Table 1. Nucleolar morphology of Eumycetozoans. Protostelid groupings modified from Spiegel (1990). Dictyostelid taxonomy as in Raper (1984).

		Nucleolar Morphology			
Group	Examples	Single, Central, Round	Diffuse	Peripheral Lobed	Multiple Peripheral
Protostelid Group 1	*Protostelium, s.s.*	X			
	Planoprotostelium	X			
Protostelid Group 2	*Ceratiomyxella*	X			
	Nematostelium	X			
	Schizoplasmodium	X			
Protostelid Group 4	*Cavostelium*	X			
	Schizoplasmodiopsis, s.s.	X			
Protostelid Group 5	*Protosporangium*	X			
	Clastostelium	X			
	Ceratiomyxa	X			
Myxomycetes	*Echinostelium*	X			
Protostelid Group 3	*Soliformovum*		X		
Dictyostelids	*Dictyostelium*			X	
	Polysphondelium			X	
	Acytostelium			X	
Eumycetozoa	*Protosteliopsis*	X			
incertae sedis	*Microglomus*	X			
	Echinosteliopsis				X
	Schizoplasmodiopsis amoeboidea		X		
Non-Eumycetozoa	*Endostelium*	X			
incertae sedis	*Protostelium arachisporum*	X			

Transmission Electron Microscopy

A 1cm square piece of agar containing a feeding front of *Echinosteliopis amoebae* was placed amoeba side down into a formvar coated fixation boat containing Karnovsky's fixative and fixed under weak vacuum. After 30 seconds, the sample was rinsed 3X in 0.05 M cacodylate buffer and post fixed in the dark for 30 min in 1% osmium tetroxide, buffered in 0.05M cacodylate buffer. During this time the agar block was floated off the sample and removed. The sample was rinsed 1X in distilled water and prestained overnight in 0.5% uranyl acetate. The uranyl acetate stained sample was dehydrated in a graded ethanol series, 1 min/change (30%, 50%, 70%, 80%, 95%, 3 changes of 100%). Samples were further dehydrated by 2 changes of propylene oxide at 20 min/change, then infiltrated with 50%-50% popylene oxide-Spurr's medium for 1 hour. *Echinosteliopsis* was infiltrated overnight in 100% Spurr in fresh desiccator. After 12 hours a thin layer (> 1 cm) of fresh 100% Spurr's medium was poured over the sample

and placed under the vacuum for several hours and then put into a 70C oven overnight. The fixation boat was cut off of the sample and amoebae were identified under the compound microscope. The block was trimmed around the amoebae, sectioned with a diamond knife, placed on copper grids, and post-stained as per standard protocol: rinsed for a few seconds in ddH_20 then stained in uranyl acetate 2% for 4 min. The section-containing-grids were rinsed again and placed in lead citrate for 2 min. then rinsed a final time in water. Grids were observed and photographed in a JOEL 100 CX transmission electron microscope.

Polysphondylium violaceum amoebae were prepared similarly except that they were fixed in suspension, pelleted by centrifugation between each step, and the pellets embedded in Spurr blocks.

Results

The four variations of nucleoli seen thus far in eumycetozoans are illustrated with light microscopy in Figure 1. The single, central, homogeneous spherical to subspherical type of nucleolus is represented by *P. mycophaga* (Figure 1a). A diffuse, lobed, central nucleolus is typical of the genus *Soliformovum* (Figure 1b). Dictyostelids, represented here by *P. violaceum,* all have a peripheral, reticulate nucleolus that appears as thin straps with enlarged thickenings (Figure 1c). The protostelid *E. oligospora* has one to several nuclei per amoeba and each has numerous peripheral nucleolar bodies and perhaps a small central nucleolus as well (Figure 1d). With through focus examination, the peripheral nucleolar bodies appear to be distinct and not joined into a reticulum.

The ultrastructure of the peripheral nucleoli of *E. oligospora* and the dictyostelids, represented by *P. violaceum*, are distinct from each other (Figure 2). Many electron dense, peripheral bodies were observed in the nuclei of *Echinosteliopsis oligospora* under transmission electron microscopy (Figure 2a,b) These dark nuclear constituents contain a core region of greater electron density and an outer region of less electron density. Sections of the same nucleus indicate that the electron dense core regions are spherical and disjunct from other such regions. The surrounding less electron dense regions are irregular in shape and may or may not be interconnected. The less electron dense portions of the nucleoli are not closely appressed to the nuclear envelope and tend to have half-moon shaped pits filled with nucleoplasm in the areas immediately opposite nuclear pores (Figure 2b). In addition, at least one nucleolar body appears to be located near the center of the nucleus. Conversely, the nucleoli of *P. violaceum* (Figure 2c) are essentially uniformly electron dense. They are closely appressed to the inside of the nuclear envelope, and there is no indication of a portion of the nucleolus in the center of the nucleus.

Discussion

Single, Central Nucleolus

The typical single, central, round eukaryotic nucleus displayed in the myxomycetes and protostelids (Groups 1, 2, 4, and 5) is not static. In these organisms, as in other "typical" eukaryotes, the nucleolus degenerates when the nucleus divides mitotically [8, 12, 9, 11, 13]. After telophase, multiple nucleoli appear in the reorganizing nucleus of each daughter cell depending on the species [12, 9]. These eventually fuse into a single centrally located nucleolus, but are reported to persist for some time before fusion [9]. This observation contributed to Olive's 1967 hypothesis that the dictyostelids arose from a nonflagellated protostelid ancestor [9]. The recognition that there are a number of distinct types of fragmented nucleoli in the eumycetozoans suggests that this simple hypothesis may be incorrect.

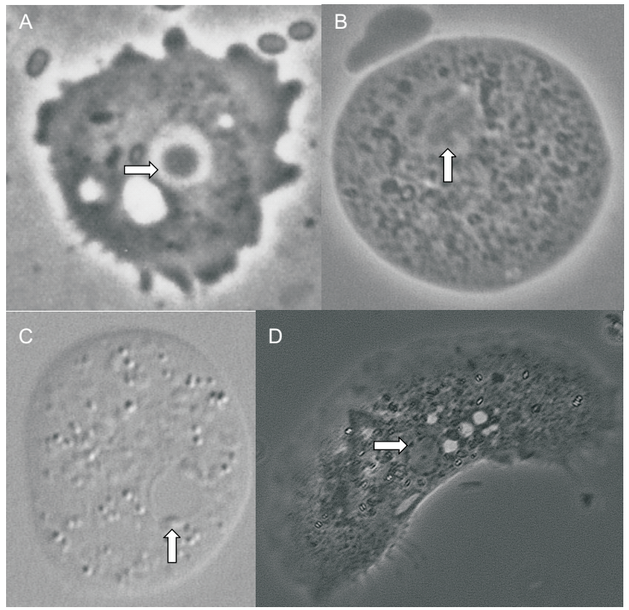

Figure 1. Mycetozoan nucleolar morphologies. Nucleoli indicated by white arrows a) phase contrast *Protostelium mycophaga* amoeba with single, central nucleolus b) phase contrast *Soliformovum irregularis* amoeba with diffuse nucleolus c) differential interference contrast of *Polysphondelium violaceum* amoeba with peripheral lobed nucleolus d) phase contrast *Echinosteliopsis oligospora* amoeba showing multiple peripheral nucleoli.

Multiple Peripheral Nucleoli

The prominent dark objects within the *Echinosteliopsis* nuclei are assumed to be nucleoli because previous literature has reported that they have the staining properties of RNA [16, 17]. If these are indeed nucleoli, then they appear to be distinct from the large central nucleoli of Groups 1, 2, 4, and 5 protostelids [10, 21] and the myxogastrids [7], the peripheral lobed nucleoli of the dictyostelids [15], and the diffuse, central, lobed nucleoli of *Soliformovum* [26]. We know of no other organism with a nucleolar morphology that is identical to that found in *Echinosteliopsis oligospora.* The odd nucleolar morphology of *Echinosteliopsis oligospora* does not match that of any eumycetozoan. Further phylogenetic analysis is needed before we can understand the evolution of this nucleolar character.

Multiple Peripheral *vs*. Peripheral Lobed Nucleoli

There are many striking differences between dictyostelid nucleoli and the nucleoli of *Echinosteliopsis.* For instance, the dictyostelid nucleoli are closely appressed to the nuclear envelope, where *Echinosteliopsis* nucleoli generally maintain a spherical electron dense core with some nucleoplasm remaining between the outer nucleolus and the nuclear envelope. Secondly, one might recall that the dictyostelid nucleus typically contains one nucleolus with many lobes which wrap part way around the nucleus much like a person's fingers wrap around a tennis ball, as has been demonstrated with serial sectioning [5]. Conversely the nucleoli of *Echinosteliopsis* seem to contain individual spheres. Finally, dictyostelid nucleoli also tend to lack distinct granular and fibrillar regions within the nucleolar lobes [5, 15], while these are evident in each *Echinosteliopis* nucleolus. Peripheral lobed nucleoli are found in all dictyostelids examined so far [15, 10].

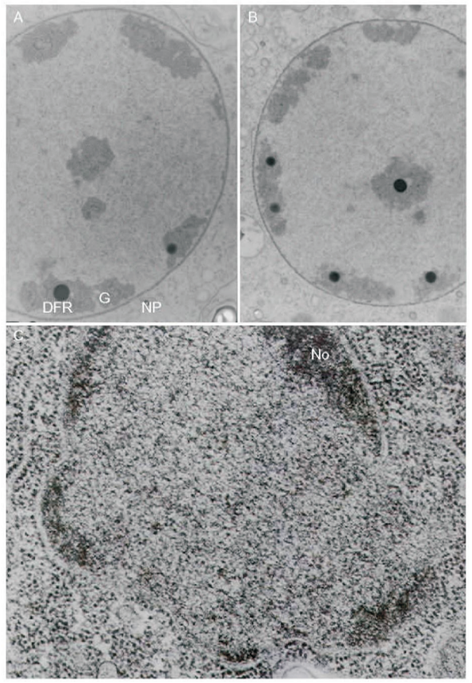

Figure 2. Transmission electron micrographs of different sections through *Echinostelipsis oligospora* round nucleus with section through a) smaller diameter section b) larger diameter section. Note discrete dense fibrillar region, diffuse granular component, and pits in granular component around nuclear pores. Dense Fibrillar Region (DFR). Granular (G). Nuclear Pore (NP). Compare with section through Dictyostelid nucleus c) *Polysphondelium violaceum* peripheral lobed nucleoli. Nuceleolar lone (NL).

Central, Diffuse Nucleoli

The central, diffuse *Soliformovum* (Group 3) nucleoli are quite different from the nucleoli of other eumycetozoan amoebae [26]. These nucleoli either have granular and fibrillar regions that are diffuse/interspersed with one another or indistinguishable [26]. A nucleolar morphology similar to *Soliformovum* is possibly found in the feeding amoebae of *Schizoplasmodiopsis amoeboidea*, a protostelid that is dissimilar to other members of the genus *Schizoplasmodiopsis* [4]. An open nucleolar arrangement like this has been hypothesized to be important for cells that are rapidly producing ribosomal precursors [18]. The *Soliformovum* type of nucleolus shows no obvious similarity with that of *E. oligospora* or the dictyostelids.

Taxonomic Implications

Intra-clade nucleolar variation does occur in other groups of Eukaryotes, such as the Hartmannellidae and certain other families within Amoebozoa, and is a character frequently used for taxonomic purposes [14]. It has been suggested that the number and size of nucleoli are strongly correlated with the number and lengths of the chromosomal secondary constrictions (nucleolar organizing regions) [18]. However, many evolutionary and developmental questions about nucleolar morphology remain unanswered.

Nucleolar morphology may be a useful character in taxonomy particularly with respect to eumycetozoans. Here we have illustrated four nucleolar morphologies present in various eumycetozoan taxa. Of the three major groups of eumycetozoans, the species described as protostelids display the greatest diversity of morphological and life-cycle variations. These morphological variations include three different types of nucleoli within the approximately 36 described species, as compared to one nucleolar morphology for all ca. 100 species of dictyostelids, and one nucleolar morphology for all ca. 1000 species of myxomycetes. Whether a wide array of nucleolar morphologies indicates long evolutionary divergence times, or represents a case of simply controlled morphological variation is still an open question. While nucleolar morphology is a useful character for identification, the biochemical and evolutionary importance of varying nucleolar morphology remains almost completely unknown. To discover the pattern of evolution of nucleolar morphology in the group, a robust phylogeny must be developed.

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