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Characterization of Giardia cell nucleus: Its implication on the nature and origin of the primitive cell nucleus

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The central problem of the origin and early evolution of the eukaryotic cell is the origin of the cell nucleus, because the cell nucleus, with its double-layered nuclear envelope, is the most prominent and important morphological mark distinguishing eukaryotic cells from prokaryotes. Eukaryotic cells may have no chloroplast, mitochondria or flagellum, but they must possess a cell nucleus.

The cell nucleus itself is a very complicated structure. If we try to make clear the origin and early evolution of the cell nucleus, we ought to investigate the origin and evolution of each morphological component of a cell nucleus. My monograph on 'The genesis of eukaryotic cells in the evolutionary history of life' (1979, Science Press, Beijing, in Chinese)[1], mainly attempted to make some effort in this regard. However, to date, the hypothetical model of a fully depicted nucleus of a primitive cell has not been constructed. It seems likely that our recent studies of the nucleus of Giardia (diplomonad) have provided a realistic basis to construct such a model.

I. The characteristics of the nucleus of *Giardia*

Kingdom Archezoa (Cavalier-Smith, 1989), contains those parasitic and free-living protists which originally possess no mitochondria and no typical Golgi apparatus, but they have a 70s type of ribosomes containing 16s and 23s rRNAs, just as prokaryotes have. Diplomonads, Microsporidia, *Entamoeba*, free-living giant polynucleate amoeba *Pelomyxa*, etc., belong to this kingdom.

According to the molecular evolution studies on small subunit rRNAs[2], large subunit rRNA and 5.8s rRNA[3] of various protists, *Giardia* is the species that earliest branched from the trunk of the eukaryotes, and it was regarded as the missing link between the prokaryotes and eukaryotes[4].

The nucleus of Giardia has very seldom been studied. Recently, we have investigated the cell nucleus of *Giardia lamblia* in more detail and found some unique and interesting characteristics which seem to be vitally important in understanding the primitive cell nucleus.

(A) The absence of nucleolus

All kinds of eukaryotes, including such as *Entamoeba* and *Pelomyxa*, were known to possess nucleolus in their cell nucleus. Microsporidia were also reported to have nucleolus[5], although the present author has some doubt about its real existence. To date the only eukaryote in which the nucleolus has not been reported is Giardia. Only in a limited number of old parasitology textbooks has the central heterochromatin in *Giardia* nucleus been mistaken for the nucleolus.

The cytochemical work done by Russian scientists showed that in the nucleus of Giardia *lamblia* no RNA-containing nucleolus-like structure was observed[6]. Our unpublished cytochemical works on the *Giardia muris*, using the methyl green pyronin method, showed that the cytoplasm was clearly stained pink, the two nuclei clearly stained very faint blue-green while the centrally located heterochromatin sphere was clearly stained dark blue-green. No structure stained pink or red was found in the two nuclei, indicating the absence of any nucleolus-like structure (Dai Jialing and Li Jingyan, unpublished). Furthermore, we recently examined the nucleus of *Giardia lamblia,* using the specific method of demonstrating nucleolus by bismuth[7], and also found no nucleolus-like structure within the nucleus, while the nucleolus of the reference species was stained dark brown.

No nucleolus was found in the nucleus of Giardia muris through electron-microscopical examination[8]. Similar work done on *Giardia lamblia* produced the same result (Dai Jialing, Shen Jianzhao). Therefore, we believe that nucleolus is absent from the Giardia nucleus.

The essential functional role of nucleolus is to produce ribosomes. In prokaryotes without nucleolus, ribosomes are also produced. The processing steps of prRNA in eukaryotic cells is functionally the same as the steps in prokaryotes, although there are several differences in details, e.g. eukaryotic prRNA contains no 5s rRNA, prokaryotes have no independent 5.8s rRNA, but only a 5.8s region in 23s rRNA. The essential difference between the ribosome production of eukaryotes and that of prokaryotes is that in eukaryotic cells the precursor particle of the large subunit of ribosome ought to detain in the nucleolus for some time to form the pars granulosa of the nucleolus, while the precursor particle of the small subunit does not[1]. This kind of specific detainment is not present in prokaryotic type of ribosome production, and it obviously does not exist in *Giardia*, because there is no nucleolus-like structure in its nucleus.

(B) Nuclear envelope with large openings

In the nucleus of *Giardia*, the perinuclear space between the inner and outer nuclear membranes of the nuclear envelope is prominent. Here, we found a very special and interesting phenomenon: there were often large openings in the envelope (Fig 1 a, b). Even the smallest nuclear openings are much larger than the nuclear

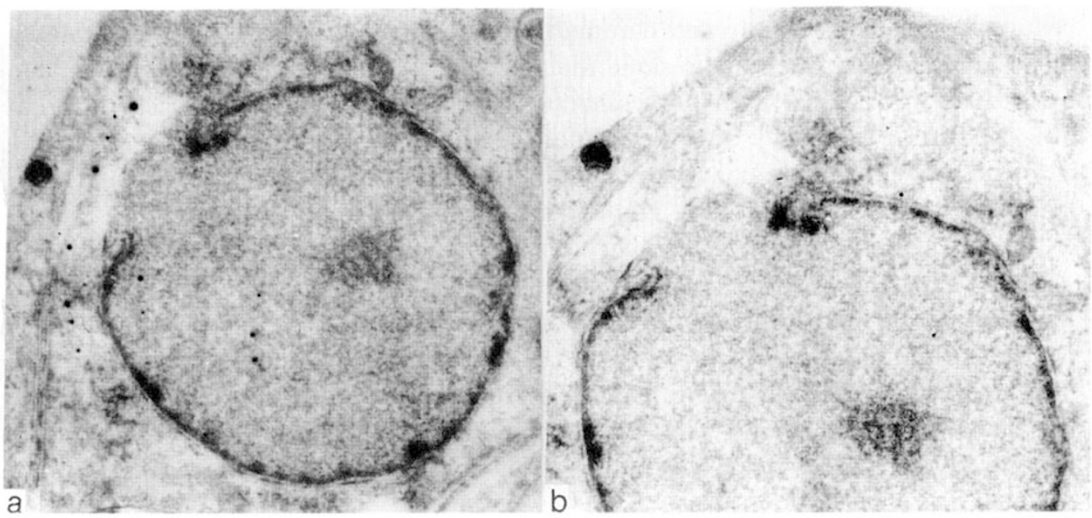

Fig 1. The nucleus of *Giaxdia lamblia*, showing a nuclear envelope opening (an electronmicrograph from Shen Jianzhao et al., unpublished). (a) 17,400 x, (b) a magnified part of (a), 28,000 x.

pores. At the margin of the opening, the two nuclear membranes are continuous with each other. This means that the nuclear envelope of *Giardia* is incomplete. It has been reported that the nuclear envelope was continuous with ER[1]. Therefore, this incomplete envelope might be regarded as a special part of the whole ER system.

According to the hypothesis that the nuclear envelope originated from the primitive ER of the closest prokaryotic ancestor of eukaryotic cells, the nuclear envelope must have been incomplete in the early stage[1]. Therefore, the existence of nuclear openings can be regarded as a very primitive characteristic.

We had been looking for this incomplete nuclear envelope for many years until we found it in *Giardia* nucleus. To the best of my knowledge, aside from the nucleus of Giardia, no such nuclear openings have been reported in any other protists, including other archezoa.

It seems rather unlikely that the opening in the nuclear envelope is the product of adaptation to parasitic life. If it were so, then nuclear openings would have been possible to find in other unicellular parasites. However, this kind of structure has never been observed. For example, in microsporidia, which are a group of intracellular parasites with very high parasitic adaptability and infectious to a wide range of hosts among different animal kingdoms, it is lacking.

(C) The presence of nuclear matrix

The nuclear matrix is a stable fibrillar network within the cell nucleus. At present, it can be demonstrated only after sequential specific extractions, when various intracellular soluble components and chromatin are removed, and only nuclear lamina, nuclear matrix and nucleolus residue remained in the nucleus. Our works and the works from Prof. Zhai Zhonghe's laboratory have shown that the nuclear matrix even existed in the nuclei of typical dinoflagellates[9, 10], although in these nuclei chromosomes are highly condensed, just as nucleoids of eubacteria, and undergo transcription only by extending DNA loops. However, whether the archezoan nucleus also possesses a nuclear matrix or not is unknown.

After routine sequential specific extractions, we found that the nuclear matrix was already present in the nucleus of *Giardia lamblia*, while the nuclear lamina and nucleolus residue have not been observed (Dai Jialing and Li Jingyan, submitted). The presence of a nuclear matrix in the *Giardia* nucleus proposed a possibility that this structure might already exist in the "nuclear region" of the prokaryotic ancestor of eukaryotic cells. Regarding to the absence of a nuclear lamina in the *Giardia* nucleus, it obviously needs further studies, because there is a possibility that the structure of the nuclear lamina of *Giardia* may be rather special and can not bear routine extractions. If nuclear lamina is really proved to be absent in the *Giardia* nucleus, then it must have emerged in the later stage of the evolution of cell nucleus.

(D) Nuclear division without spindle formation

The electron-microscopical observation on the nuclear division of *Giardia* has not been reported in the literature. My graduate student Shen Jianzhao made some observations on the nuclear division of *Giardia lamblia* and found that it has very special features. In the dividing nucleus: the nuclear envelope persisted; a few small chromatin spheres, which might be the cross sections of chromosomes, were observed adjacent to the inner membrane of nuclear envelope; and no spindle, or microtubule-made structure, was found. The absence of spindle was further confirmed by observations with anti-tubulin antibodies, although flagella of *Giardia* gave strong positive reactions.

Thus, the nuclear division of *Giardia* is far different from those of other archezoa: For example, the nuclear divisions of microsporidia and *Entamoeba* are just typical endomitosis with prominent intracellular spindle.

It appears extremely likely that the nuclear division of *Giardia* represents a very primitive one. Obviously, thorough investigation of its mechanism is needed, an important step for understanding the origin of mitosis.

(E) The primitiveness of the centromere/kinetochore proteins

Dr. Wu Chuanfen in our laboratory has recently initiated a study on the origin

and early evolution of centromere/ kinetochore proteins in various protists, including *Giardia lamblia*, and archaebacteria with Western blotting and other metho- ds. The results showed that not only various protists possess their own centromere/kinetochore proteins, but also the archaebacteria studied have their own analogous components. One of the most interesting finding is that the estimated molecular weight of the centromere /kinetochore proteins of *Giardia* lies just between the range of those proteins of other protists and archaebacteria[11]. A part of her works is shown in Tab 1, where ra-ACA-2 is a polyclonal antiserum against human centromere protein B (CENP-B) and mACA-2 is a monoclonal antibody against the amino terminal region of CENP-B, both being the gifts from Prof. WC Earnshow (The John Hopkins University, U.S.A.), and mAb37A5 is a monoclonal antibody against two kinetochore proteins of CHO cell obtained from Prof. R. Hancock (Laval University, Canada.)

Tab 1. Western blotting of centromere/kinetochore proteins of protists and archaebactaria

Antibodies / Antigens(KD) / Materials	mACA-2	ra-ACA-2	mAb37A5
Human Hep-2 cell	80, 120	50, 60, 80	140, 160
Protozoa			
Tetrahymena thermophila	80, 120	50, 60, 80	120
Euglena gracilis	80, 120	50, 60, 80	45 120
Oxyrrhis marina	80, 120	50, 60, 80	45 120
Cryptothecodinium cohnii	80, 120	50, 60, 80	45, 50, 120
Archezoa			
Giardia lamblia	80	60, 80	45, 50, 120
Archaebactna			
Halobacterium dachaidanensis		60, 80	40, 45, 50
Thermoplasma acidophilum		60, 80	40, 45, 50
Sulfosphaerellus thermoacidophilum		60, 80	40, 45, 50

It has been inferred that four eukaryotic nucleosomal histomes must have originated from a common ancestral molecule[12]. In the case of *Giardia*, there has been no report about its histone constituents in the literature up to now. We analyzed it and found that Giardia already possess four species of nucleosomal histones and histone H1 (Wu Gang and Li Jingyan, unpublished data) (Fig 2). However, the presence of a true nucleosome structure in the Giardia chromatin has not yet been studied. The above result would imply that if the nucleus of *Giardia* represents a very primitive nucleus, then the undifferentiated ancestral nucleosomal histone would only be found in the prokaryotic ancestor of eukaryotes, and the differentiation of four nucleosomal histones must have taken place before the formation of the most primitive cell nucleus.

Molecular evolutionary study on the four nucleosomal histones and histone-related proteins from methanogenic archaebacteria was done by the Present-Day Ancestor method with five species of HU proteins from eubacteria as references, (Fig 3, Li Jingyan's unpublished work). HMt, HMf and HMv in the figure represent histone-related proteins separated from *Methanobacterium*, *Methanothermus* and Methanococcus respectively[13-15]. A similar tree was obtained by Neighboring Joint Method. These results confirmed the above described prediction. The reconstructed evolutionary tree showed that the differentiation of nucleosomal histones took place at an early time and was much earlier than the differentiation between HMt protein of *Methanobacterium* and HMf protein of *Methanothermus*, but later than the differentiation between HMv protein of Methanococcus and the ancestral protein of HMt and HMf proteins.

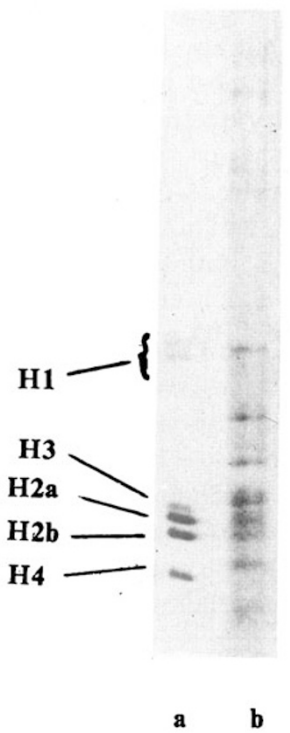

Fig 2. Electrophoresis of basic proteins of *Giardia lamblia* and calf thymus histones on sodium dodecyl sulfate gel, stained with coomassie brilliant blue R-250.
a. calf thymus histones.
b. *Giardia lamblia* basic proteins.

The results described and analyzed above, showed that the nucleus of *Giardia* is very primitive. This is particularly evident when one considers the presence of its nuclear envelope opening, peculiarities of nuclear division and the absence of nucleolus which are all remarkably different from the nuclei of other archezoa and all other eukaryotes. Because of the profound significance of these differences, we believe the kingdom of Archezoa would be divided into two kingdoms: Proarchezoa (incIuding diplomonads) and Archezoa (including all other archezoa). Both kingdoms belong to the Superkingdom Archezoa (Cavalier-Smith, 1989), which, according to him, had only one kingdom under this superkingdom[16].

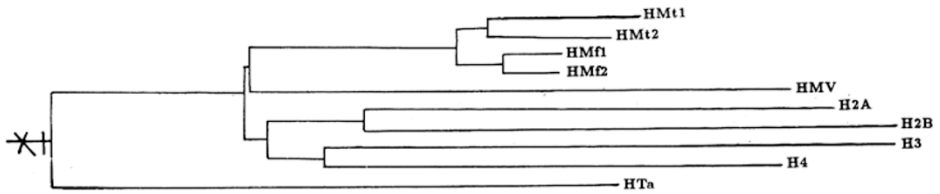

Fig 3. The evolutionary tree of histone-related proteins and nucleosomal histones. HMf, HMt and HMv are histone-related proteins isolated from methanogenous *Archaebacteria Methanothermus, Methanobacterium* and *Methanococcus* respectively. HTQ is the DNA-binding basic protein of *Archaebacterium thermoplasma*. H2A, H2B, H3 and H4 are nucleosomal histones. The amino acid sequences we used here are the consensus sequences of eukaryotic nucleosomal histones (according to Wells and McBride, Nucleic Acid Res. 1989; **17**:311 -46.)

II. The implication of the *Giardia* nucleus on the possible nature. and origin of primitive cell nucleus

(A) The nature of the closest prokaryotic ancestor of eukaryotic cell

If we regard the *Giardia* cell as a present-existing model of primitive eukaryotic cells, we can deduce the nature of the closest prokaryotic ancestor of eukaryotic cells from the nature of the *Giardia* cell and that of present-existing archaebacteria as follows:

It must have been a large anaerobic organism with a size similar to protozoan cells. It might have had flagella already, or at least the precursors of flagella, i.e. an anaerobic ectosymbiotic spirochaete according to Lynn Margulis's hypothesis[17]. It might possess 70s type of ribosomes with rDNA just as other prokaryotes, but without nucleolus.

Since the chromosomes of *Giardia* were reported to be closely related to each other[18], they can be considered as the differentiating nucleoids. The closest ancestor of eukaryotic cells must have several nucleoids, and, like *Giardia* nucleus, already have had a nuclear matrix. These nucleoids, while in the process of differentiation and association with the nuclear matrix structure, would form "a nuclear region", which will be surrounded by a primitive ER system.

The centromere/kinetochore proteins of *Giardia* are similar to the homologous proteins in various archaebacteria. However, the localization of these proteins in Giardia chromosomes has not been studied, and that of the homologous proteins of nucleoid in archaebacteria is also not known. Therefore, it is presently difficult to formulate acutely corresponding precursor molecules in the closest ancestor of eukaryotic cells. Nevertheless, according to our molecular evolutionary analysis of nucleosomal histones and histone-related proteins, .the closest ancestor of the eukaryotic cells must have already had four nucleosomal histones and even a possible true

nucleosomes structure.

(B) The origin of the closest ancestor of eukaryotic cells

Because histone-related proteins were only found in one branch of methanogenous archaebacteria, the closest prolmryotic ancestor of eukaryotic cells must have originated from this branch. Due to the fundamental fact that there is a prominent distinction between the size of eukaryotic cells and that of present-existing archaebacteria, the distant ancestor of the eukaryotic ceils must have evolved along the way to increasingly enlarge its size. There were a series of benefits in the struggle for existence to evolve in this manner. However, the enlargement of individuals would decrease the multiplication rate of the unicellular organisms, which will be very harmful for the maintenance of prokaryotic species in the struggle for existence. Therefore, only very few prokaryotes could evolve successfully this way.

During such evolution, the invagination of plasma membrane and the formation of an endomembrane system were inevitable. In fact a primitive ER was already formed in a large prokaryote Thiovulum majus[19]. Another inevitable thing which accompanied the enlargement of individual size was the increase in the number of genome copies; and, on this ground, the number of nucleoid might have also increased, although in Thiovulum majus only one single very long nucleoid was formed. As mentioned in the previous section, the close association of nuclear matrix with several nucleoids of prokaryotie ancestor of eukaryotic cells to form a 'nuclear region' might be intimately related to the development of the nuclear envelope and eventually, to the formation of a typical cell nucleus in further evolution. In this connection, it is interesting to note that *Thiovulum* majus, with a single long nucleoid, possibly being not concentrated in a limited region, did not evolve to form a nuclear envelope-like structure.

(C) The evolutionary formation of the nuclear envelope

Nuclear envelope is hypothesized to be originated from the primitive ER around the 'nuclear region' of the closest prokaryotic ancestor of eukaryotic cells[1]. The basis of such hypothesis are as follows: (1) Primitive ER-like endomembrane system has already been observed in huge bacteria, e.g., *Thiovulum majus*. (2) Nuclear envelope is often continuous with ER in various protists, and this continuity is also observed in higher eukaryotes: Therefore, the nuclear envelope can be regarded as a specific part of the ER system, playing a special functional role. (3) The nuclear envelope of the two daughter nuclei following mitosis originated from ER or ER-like components. (4) The repair of the injured part of the nuclear envelope was found to be dependent on ER to provide the membranous component[1]. The primitive ER around "nuclear region" was assumed to take an active role in providing necessary materials to this region by active transport and forming a convenient microenvironment for the activities of chromatin. However, in order to keep this

specific microenvironment in a dynamic state, a large amount of energy ought to be consumed.

Since the "nuclear region" was fine and dense in texture, the primitive ER could not penetrate into it. On this ground, during evolution, part of the primitive ER, which was directly adjacent to the "nuclear region" became enlarged and spread along the margin of this region to strengthen the supply. Gradually, an incomplete envelope around the "nuclear region" would have been formed, which could hinder and block the diffusion and loss of necessary materials, and, therefore, decrease the energy consumption in transport. Through a long period of natural selection, a complete nuclear envelope with nuclear pore complexes would have formed which could have decreased the energy consumption to its least extent, and at the same time could have provided a more stable microenviroment for the genome.

After the existence of mitochondria within the cells, the nuclear envelope might have taken an additional role to protect the intranuclear DNA by excluding oxygen radical and H_2O_2 arising from respiration. However, this important function would not exist in the primitive eukaryotic cells and their closest ancestor at the every beginning, because they had no mitochondria and respiration yet, and even the nuclear envelope, if formed, was still incomplete as we have observed in the *Giardia* nucleus. Therefore, free radical impediment would not be a promoting factor leading to the evolutionary formation of the nuclear envelope.

In summary, the characteristic features of the nucleus of Giardia lamblia described in the present paper would suggest that this protozoan parasite may represent, at the moment, the most primitive eukaryote among the present-existing organisms. Since G. *lamblia* can be propagated *in vitro*, it will become an exceptional model system to study the various aspects of the origin of cell nucleus. Thus, among many problems which need further investigation, the origin of nucleolus and that of mitosis will be the problems which would claim precedence over all others. Besides, the construction of a *Giardia* gene bank is tremendously critical for clarifying the early phylogeny of eukaryotes.

REFERENCES

[1] Li JY. "The genesis of eukaryotic cells in the evolutionary history of life-The origin of eukaryotic cells." Science Press, Beijing 1979 (in Chinese).

[2] Sogin ML et al. Phylogenetic meaning of the kingdom concept: An unusual rRNA from *Giardia lamblia*. Science 1989; **243**:75-7.

[3] Li JY. The PDARIC method for constructing molecular evolutionary tree from sequence data. Zool Res 1992; **13**:387-96.

[4] Kabnick KS and Peattie DA. *Giardia*: a missing link between prokaryotes and eukaryotes. American Scientist 1991; **79**:34-43.

[5] Canning EU. *Nosema helminthorum* (Microspora): a taxonomic enigma. J Protozool 1984; **31**:525-31.

[6] Soloviev MM. Cytochemical study of Lamblia duodenalis. Medizin Parasitol i Parasit bolezni(Moscow) 1963; 675-8. (in Russian)

[7] Locke M. Bismuth staining for light and electron microscopy. Tissue and Cell 1977; 9:347-71.
[8]Friend DS. The fine structure of Giardia muris. J Cell Biol 1966; **29**:317-32.
[9] Cai ST et al. Identification of nuclear matrix and chromosome scaffold in dinoflagellate Crypthecodinium cohnii. Cell Res 1992; **2**:165-82.
[10] Fang ZL, Wu CF and Li JY. The study on the nuclear matrix and its reality on *Amphidinium carterae*. Zool Res 1993; **14**:80-5.
[11] Wn CF. The studies on the origin and early evolution of centromere/kinetochore proteins. Doctoral Thesis of Beijing Normal University 1994.
[12] Reek GR, Swanson E and Teller DC. The evolution of histones. J Mol Evol. 1978; **10**:302-17.
[13] Sandman K et al. HMF, a DNA-binding protein from the hyperthermophilic archaeon *Methanothermus fervidus*, is most closely related to histones. Proc Natl Acad Sci USA 1990; 87:5788-91.
[14] Tabassum R, Sandman KM, Reeve JN. HMt, a histone-related protein from *Methanobacterium* thermoautotrophicum H. J Bacteriol 1992; **147**:7890-5.
[15] Aglia-Amiri Km Klein A. Nucleotid sequence of a gene encoding a histone-like protein in the archaeon *Methanococcus voltae*. Nuc Acid Res 1993; **21**:1491.
[16] Cavalier-Smith T. Eukaryotic evolution. International Botanical Congress, 1989: P. 203-23.
[17] Margulis L. "Origin of Eukaryotic Cells" Yale University Press, 1970.
[18] Adam RD, Nash TE and Wellems TE. The *Giardia lamblia* trophozoite contains sets of closely related chromosomes. Nuc Acid Res 1988; **16**:4555-67.
[19] Faure-Freund E et Roiller C. Etude au microscope electronique d'une bacteri fulfureuse Thiovlum majus Hinze. Exp Cell Res 1958; **14**: 29-46.

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