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REVIEW

The nucleolus: reviewing oldies to have new understandings

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The nucleolus is the most prominent compartment in the nucleus and known as the site for ribosome biogenesis in eucaryotes. In contrast, there is no such equivalent structure for ribosome synthesis in procaryotes. This raises two concerns that how does the nucleolus evolve and that whether the nucleolus remains playing a single role in ribosome biogenesis along the evolution. Increasing data support new nucleolus functions, including signal recognition particle assembly, small RNA modification, telomerase maturation, cell-cycle and aging control, and cell stress sensor. Multiple functions of the nucleolus possibly result from the plurifunctionality of nucleolar proteins, such as nucleolin and Nopp140. Proteomic analyses of human and *Arabidopsis* nucleolus lead a remarkable progress in understanding the evolution and new functions of nucleoli. In this review, we present a brief history of nucleolus research and new concepts and unresolved questions. Also, we introduce hepatitis D virus for studying the communication between the nucleolus and other subnuclear compartments, and *Caenorhabditis elegans* for the role of nucleolus in the development and the epistatic control of nucleologenesis.

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Introduction

The major different feature between the procaryote and the eucaryote is as their given names that the latter has a "true nucleus" with membranes to envelope whole genome, whereas the former has a "nucleoid" structure containing the genome without membranes. Inside the nucleus, the nucleolus is the most prominent structure. Owing to the difference in density between nucleolus and its surrounding nucleoplasm (0.215 g/cm^3 versus 0.106 g/cm^3), known for a long time but recently determined by the refractive indices in the nucleus of *Xenopus* oocytes [1], the nucleolus is easily observed in monolayer culture cells under a phase-contrast microscope (Figure 1A). It appears as an oval to round shape but varies in sizes and numbers in different types of the cell. Generally speaking, a larger size and higher number of nucleolus are detected in the most tumor cells than in the corresponding normal cells. Therefore, abnormal sizes and higher numbers of nucleoli are commonly used as indicators for many cancers in prognosis [2, 3].

Although the presence of nucleolus was described more than two centuries ago, the terminology of nucleolus was coined in 1839, around the time that "cell theory" was postulated. It means "small nucleus" or "nucleus of the nucleus" [4]. However, to understand the structure and function of nucleolus then took more than one century. Till 1960s, two major progresses in nucleolus research have been accumulated and summarized as follows: (i) the nucleolus is membrane-less structure and appears fibril and granule, which are recognized as a tripartite structure, including the dense fibrillar component (DFC), the fibrillar center (FC), and the granular component (GC), under an electron microscope [4-6] (Figure 1B); and (ii) it is the site for pre-rRNA transcription and processing and for ribosome subunit assembly and thus known as a "factory of ribosome" [5-8]. On the other hand, the dynamic disappearance and reappearance of nucleoli in mitotic cells were described in 1893 and 1894 [4]. However, the dynamic movement of macromolecules in and out of the nucleolus in the interphase cell has been established around 1990s

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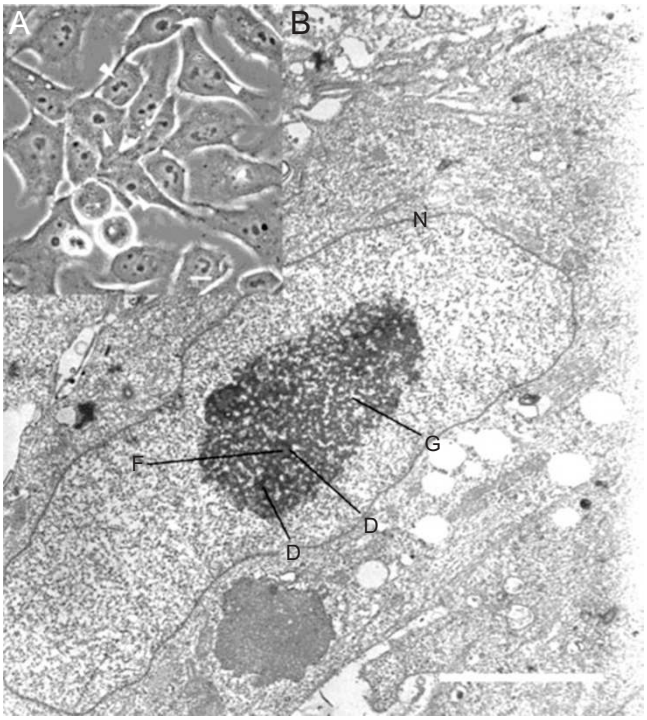

Figure 1 Structure of nucleolus under light and electron microscope. **(A)** Human cervical cancer cell line, HeLa, was attached on coverslips and then visualized under a phase contrast microscope. Nucleoli are seen as small black dots indicated by white arrowheads. **(B)** Human hepatoma cell, HepG2, was fixed and embedded in Epon, thin-sectioned, and visualized by electron microscopy. The nucleolus was heavily stained with uranyl acetate and lead citrate and appears as an oval shape in the center. N: nuclear envelope; F, fibrillar component; D: dense fibrillar center; and G: granular component. Bar=20 μm.

owing to the application of green and red fluorescence proteins (GFP and RFP) in living cells and the discovery of subnuclear compartments (speckles and paraspeckles) by various antibodies, using time-lapsing and confocal microscopy [6]. At the same period of time, new functions, of the nucleolus, in addition to the ribosome biogenesis, which are called non-traditional or non-conventional functions, were emerged [5, 9, 10]. More importantly, in the past few years, a new era of bioinformatic analyses of the nucleolus began [11, 12]. For a glance of the early accomplishment of nucleolus researches, in this review, the milestones of nucleolus researches are highlighted in Table 1. Regarding the evolution, recent findings and novel emerged concepts of the nucleolus will be reviewed and some unanswered questions will be discussed in the following sections.

Evolution of the nucleolus

How does the nucleolus evolve? Does it co-evolve with the nucleus when procaryotes evolved to eucaryotes? As eucaryotes evolve, they increase genome size and have a nuclear envelope to keep all genomic DNA inside. When a eukaryotic cell grows and divides, to allow each cell to inherit a complete genome, a new mechanism called "mitosis" evolves that differs from the binary fission of procaryotes (eubacteria and archaebacteria). From the binary fission to the eukaryotic mitosis, there is an intermediate stage, which is called the "closed mitosis" because the nucleus remains intact during cell division, found in many unicelluar protists [13]. The nucleolus also remains intact in some unicellular organisms performing closed mitosis

Table 1 The milestones of early nucleolus research [4]

Year	Events
1835	Discovery of the nucleolus (termed "Keimfleck"or "macula germinatova") in germinal vesicles.
1838	First description of the nucleolus (unnamed) in plants.
1839	Introduction of the term "nucleolus" (small nucleus or nucleus of the nucleus).
1893	Observation that the nucleoli gradually disappear during prophase.
1917	Association between nucleoli and chromosomes was reported.
1934	Demonstration that special chromosomal regions are called secondary chromosomal constrictions or nucleolus-organizing regions (NORs), a term which indicates that nucleoli originate there.
1952	First description of nucleolar ultrastructure in vertebrate cells. And first isolation of nucleoli. The presence of both RNA and DNA was demonstrated in the nucleolar preparation.
1955	Autoradiographic demonstration that radioactive precursor is incorporated into the RNA of the nucleoli.
1962	After a pulse of radioactive precursor, the label is initially found in 45S RNA and then transferred from 45S to 28S RNA.
1969	The spreading technique made it possible to visualize the transcription process. Each transcription unit looks like a "Christmas tree". These transcription units are tandemly arranged in the same polarity and are interspersed with non-transcribed (intergenic) sequences.

that might support the idea of co-evolution of nucleus and nucleolus. This observation further suggests that a same or similar mechanism was probably evolved for controlling the disintegration of nucleus and nucleolus in those cells performing the "open mitosis".

Then a question will be asked: "what are the bases that procaryotes have for evolving to the nucleolus of eucaryotes?" The rDNA gene might be one of the answers, because an experiment shows that a single copy of rDNA gene introduced into *Drosophila* results in the transgenic fly to have a mini-nucleolus, indicating that rDNA is an essential component for nucleolus formation. Similarly, thousands of amplified small nucleoli are present in amphibian oocytes as thousands of extra-chromosomal rDNA genes are amplified during oogenesis. In human cells, the rDNA genes in a cluster are located at the acentric region of chromosomes 13, 14, 15, 21, and 22. During the mitotic phase, the rDNA genes accompanying with proteins, such as RNA polymerase I (RNA Pol I), are recognized by silver stain and known as Ag-NOR (nucleolus organizer region). At the end of telophase, transcription of rRNA by RNA Pol I initiates the nucleologenesis. If the function of RNA Pol I is inhibited by actinomycin D, no nucleolus would be generated. When RNA Pol I is inhibited in the interphase cells, the FC, DFC, and GC segregate into distinct regions within the nucleolus. It indicates that the interphase nucleolus is also maintained by transcription and ribosome assembly and the concept that the nucleolus is formed by "the act of building a ribosome" is thus postulated [7]. Therefore, those enzymes and factors involving in pre-rRNA transcription and processing and those for ribosome assembly are the second important components for the nucleolus evolution.

Similar to the *Drosophila* experiment described above, a yeast mutant strain on losing RNA Pol I activity forms a mini-nucleolus when a plasmid containing a copy of rRNA gene driven by RNA Pol II is introduced. However, it never appears like the crescent-shaped nucleolus as the wild-type does, even when multiple copies of rDNA are introduced. The ribosome synthesis is normal in the above yeast mutants and raises two questions: i) is a normal nucleolus required for ribosome biogenesis and ii) why a long tandemly repeated rDNA (around 50 copies) is required for forming a normal nucleolus? Although there is no direct evidence, the presence of nucleolus may facilitate the ribosome biogenesis or accommodate for non-traditional functions (see below). A longer length of rDNA genes may provide a better scaffold for those nucleolar proteins' binding. Recently, Thiry and Lafontaine [14] proposed that the length of intergenic space of rDNA may result in the evolution of two-compartmentation to three-compartmentation nucleolus. The authors analyzed the length of rDNA transcript units versus the length of intergenic spacer regions across evolution and found that organisms that appear as bipartite structure of nucleolus usually have a smaller or similar intergenic spacer as the transcript unit, while those that appear as tripartite nucleolus have always much longer intergenic spacers than the transcript unit. The increasing size of intergenic spacer might allow it looping out longer to induce a new compartment formation, in which FC and DFC are separated. However, the tandem copy number of rDNA seems not to play a role in the new compartment formation.

Regarding the origin of nucleolar proteins, Staub *et al*. [15] proposed a beautiful model based on the data of nucleolus proteome from HeLa cells and bioinformatic data of eubacteria, archaebacteria, yeast, *Caenorhabditis elegans*, *Drosophila*, mouse, and human to define unique protein domains for the nucleolus. They found 115 known domains and 91 novel domains. Among them, 59 domains are found in all kingdoms: 25 are shared by both archaebacteria and eucaryotes, 13 are shared by eubacteria and eucaryotes, and the rest are unique to eucaryotes. Then they concluded that i) the core proteins of the eucaryotic nucleolus stem from an archaebacterial ancestor, ii) eubacterial nucleolar protein domains were added lately in nucleolus evolution, and iii) a large fraction of nucleolar protein domains evolved in eucaryotes. They also excluded the possibility that the nucleolus evolution was through the process of endosymbiosis. Since the size of eukaryotes is much larger than that of procaryotes, for avoiding a lower efficiency of ribosome biogenesis, a dense subnuclear organelle without membrane evolved in the early eucaryotes.

The hypothesis proposed by Thiry and Lafontaine and the model proposed by Staub and co-workers as described above have pointed out that a late and continued evolution occurred to the nucleolus across species. However, several questions remain unanswered i) What is the evolutionary selection force to localize rDNA genes at the acentric region of chromosomes in the most of organisms? ii) How does the mechanism evolve in the higher eukaryotic organisms to control their number, size, and shape of the nucleolus in various types of cell? iii) Is it possible that more functional domains of the nucleolus, in addition to GC, FC, and DFC, will be discovered when better tools are applicable? Nevertheless, comparative studies of rDNA genomic structure and nucleolar proteome among various species will give more insight into the relationship of structure and function of nucleolus.

Plurifunctionality of the nucleolus

If a late and continued evolution happened to the nucleolus, the nucleolus of current eucaryotes would not play a

single role of "ribosome factory". Indeed, an increasing data showed that many proteins unrelated to ribosome assembly, including viral proteins encoded by DNA and RNA viruses [16], were detected in the nucleolus. Thus, many non-traditional functions of the nucleolus have been proposed [5-7, 9]. These include signal recognition particle assembly, small RNA modification, RNA editing, telomerase maturation, nuclear export, cell cycle control, and stress sensor [17-22]. The nucleolar proteins unrelated to ribosome assembly mostly contain an RNA-binding motif or have a chaperone function. Because they are derived from the ancestor ribosomal proteins, this explains why they function in RNA-containing particles (RNP). However, these RNPs exert their function either in the speckles of nucleoplasm (splicesome) or in the ER (signalosome), indicating that these non-ribosomal-related proteins can shuttle between the nucleolus and nucleoplasm as the ribosomal proteins. Similarly, an RNA-editing enzyme (ADAR) resides in the nucleolus and executes function in the SC-35 speckles [23]. Many nucleolar proteins are also present in the other subnuclear locations but without known function, for example, the newly found PSP-1, which is more abundant in the paraspeckles than in the nucleolus when cells actively transcribe rRNA. When the rRNA transcription is turned off, PSP-1 accumulates in the nucleolus [24]. Many other nucleolar proteins that can move in and out of the nucleolus for controlling the stability of tumor suppressor, p53, are presented below.

The p53 protein has been known as a "guardian of the genome" because of its important role in coordinating cellular responses to stress [25, 26]. Its activity is regulated by changing the balance between its synthesis and degradation. Under the normal conditions, cells maintain a low level of p53 by continuous synthesis and quick degradation. Under the stress conditions, cells have a higher amount of p53 by inhibition of MDM2, which is an E3 ubiquitin to tag on the p53 in the nucleus and to lead the ubiquitinated p53 degradation in the proteasome. The activity of MDM2 is inhibited by a nucleolar protein, ARF, which may be through simply binding to MDM2, sequestering the MDM2 in the nucleolus or preventing the p53-MDM2 export. Two other abundant nucleolar proteins, B23 and nucleolin, are also found to bind p53 directly; when cells are under stress, nucleoli are disrupted and nucleolin and B23 move from the nucleolus

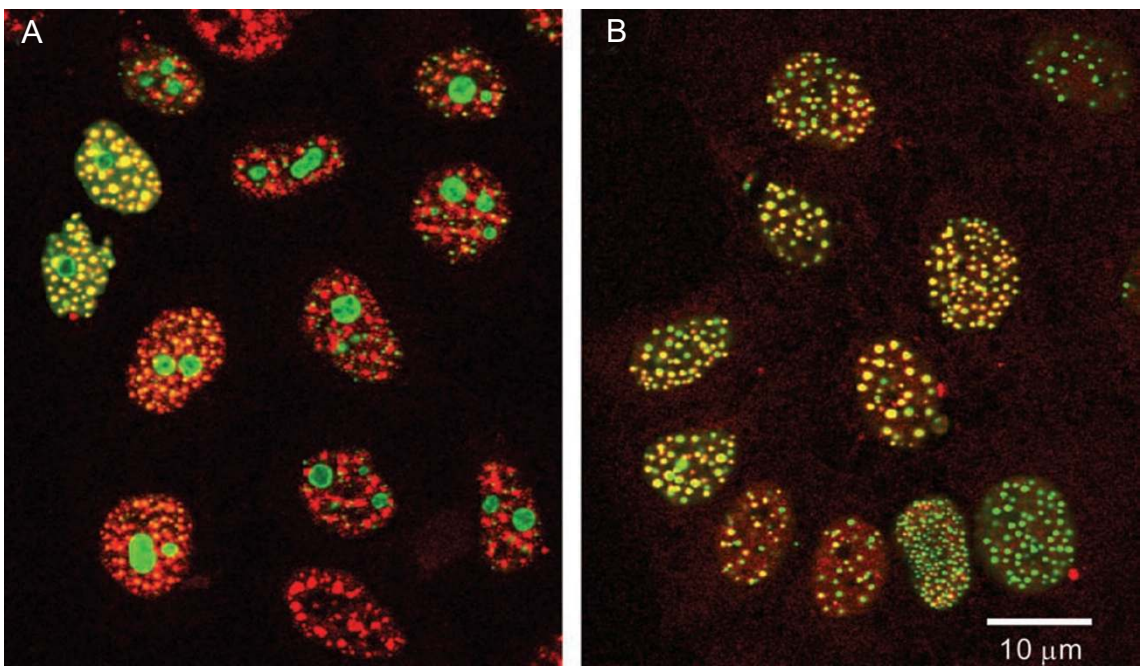

Figure 2 Different nuclear localizations of HDV antigen fused with GFP. HeLa cells were transiently transfected with a plasmid expressing green fluorescent protein fused with HDV large antigen (GFP-LD) and stained with antibody against SC-35 detected by a secondary antibody conjugated with rhodamine. **(A)** In the absence of DRB treatment, the most GFP-LD is present in the nucleolus (green oval shape) and few in speckles. **(B)** After 2-h DRB treatment, all GFP-LD move to speckles and co-localize with SC-35 appearing small yellow dot. Results suggest that the phosphorylated GFP-LD prefers to localize at the nucleolus. Bar: 10 μm.

to the nucleoplasm to bind p53. Furthermore, interaction between B23 and MDM2 also prevents the ubiquitination of p53. These conclude a new function of the nucleolus as a stress sensor of the cell. Another newly identified nucleolar protein, nucleostemin, is also found to regulate p53 activity, which adds an extra role of nucleoli in the control of stem and cancer cell proliferation [27].

The non-conventional function of the nucleolus can also be elaborated by many viral proteins and nucleic acids that interact with nucleolar proteins. More than 12 RNA viruses and five DNA viruses are found to localize their proteins in the nucleolus and subnuclear structures associated with the nucleolus, such as Cajal (coiled) bodies, PML ND-10, and nuclear speckles [16]. Among these viruses, nucleolin has been shown to interact with the poliovirus 3′ non-coding region as well as to stimulate IRES-mediated translation [28, 29]. These activities are suggested to promote poliovirus replication. In addition, nucleolin and B23 have also been shown to interact with hepatitis D virus (HDV) antigens and modulate HDV replication [30, 31]. HDV is an RNA virus with a genome in 1.7 kb in a single-stranded circular form. To complete its life cycle, HDV requires host RNA polymerase and RNA-editing enzyme and its own ribozyme [32]. The HDV antigens are known to associate with these activities and present in the nucleolus and speckles [33, 34] (Figure 2), but their location related to function is largely unknown. The association of HDV RNA metabolism with the nucleolus provides a good model for exploring possible new functions of the nucleolus.

Proteomic analyses of the nucleolus

For exploring new functions of the nucleolus today, the proteomic analysis of nucleoli is the most powerful approach. Recently, a proteomic analysis of the *Arabidopsis* nucleolus suggested that additional functions in mRNA export or surveillance in plants [35]. Owing to the invention of high-throughput technology for mass spectrometric (MS) analyses of large amounts of peptides and the application of computer search engines that access huge amount of genomic data, the first nucleolome from a human HeLa cell line was reported in 2002 [11, 36]. The authors identified 271 proteins, in which more than 30% of proteins are novel or uncharacterized. This report has a high impact on the field of nucleolus research and leads to a model explaining the evolution of nucleolus [15, 37]. Before 2002, nearly 120 proteins had been reported to localize in the nucleolus through the methods of biochemical analyses and cellular localization. Now, coupling methods of liquid chromatography and tandem mass spectrometry allow scientists to identify more nucleolar proteins and lead to the coverage of nucleolar protein that is extended to almost 700 in human [38].

The nucleolar proteome reveals that the seven most abundant motifs in the nucleolar proteins are as follows: i) RNA recognition motif, ii) DEAD/DEAH box helicase, iii) helicase conserved C-terminal domain, iv) WD domain, v) intermediate filament proteins, vi) myosin tail, and vii) elongation factor Tu GTP-binding domain [12]. Although the function of those nucleolar proteins bearing the known motif can be predicted, many proteins lacking the obvious motifs require a systematic way to analyze their interaction partners (interactome), either along a pathway or within a complex. However, one should bear in mind that many nucleolar proteins have multiple functions. Here are two examples, nucleolin and Nopp140.

Nucleolin

Nucleolin (also known as C23), having around 700 aa residues across vertebrates, is encoded by a gene localized on human chromosome 12 and represents about 10% of the total nucleolar proteins. The amino acid sequence of nucleolin comprises three domains, which exert different functions: i) the N-terminal domain controlling rRNA transcription, ii) the central globular domain controlling pre-rRNA processing, and iii) the C-terminal domain controlling nucleolar localization [39]. Apart from the role of helping ribosome assembly, nucleolin promotes HDV and poliovirus replication as described above. Nucleolin also expressed on the cell surface allow Coxsackie B virus and HIV binding [40, 41]. Furthermore, nucleolin binds to topoisomerase and the growth factor midkine to localize them at the nucleolus. It also acts as a transcriptional repressor [42] and a post-transcriptional regulator to stabilize amyloid precursor protein mRNA [43] and to manifest a helicase activity capable of unwinding RNA-RNA and DNA-DNA duplexes [44]. Nucleolin is known to be modified by phosphorylation, methylation, and ADP-ribosylation, which may render it targeting to various compartments to exert different functions.

Nopp140

Nopp140 with approximately molecular weights of 140 000 was first identified as a nuclear localization signal-binding protein [45] and functioning as a chaperone for shuttling between the nucleolus and cytoplasm in rat [46]. Later, the ortholog of Nopp140 was identified in human, *Xenopus*, *Drosophila*, and worms [47-50]. They vary in the length of amino acids but share a similar organization (Figure 3). It contains the N-terminal-conserved domain, C-terminal-conserved domain, and a unique central region consisting of several (10-25; see legend of Figure 3) interspersing repeats of acidic and basic amino acid clusters. Unlike the most nucleolar proteins, Nopp140 does not have RNA-

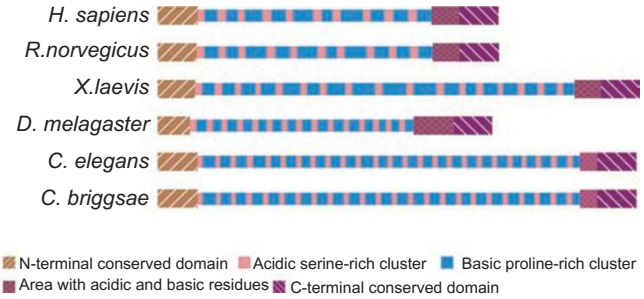

Figure 3 Schematic representation of putative domains in Nopp140s. Nopp140s from six species are aligned based on their relative lengths of amino acid. Although the lengths of Nopp140 are different among all species, they appear with similar features. The putative of domains are located at the N-terminal, central, and C-terminal regions and illustrated in different color boxes as below. The length difference results from the central region, which contains various repeats (10-25) of basic proline-rich cluster and acidic serine-rich cluster (acidic and basic domain are indicated with pink and blue color, respectively). The numbers of acid and basic domain among different species are 10 in human Nopp140 (hNopp140) and rat Nopp140 (rNopp140) (the top two lines), 17 in frog Nopp140 (xNopp160) (the third line), 15 in fruit fly Nopp140 (DmNopp140) (the fourth line), and 25 in two worm Nopp140s (CeNopp140 and CbNopp140) (the bottom two lines).

binding motif/glycine/arginine-rich stretches. The acidic regions contain exclusively aspartic acid, glutamic acid, and serine, in which the serine residues are phosphorylated by casein kinase type II [51, 52]. The interspersed basic regions are rich in lysine, alanine, and proline. A highly evolutionarily conserved C-terminus follows the central acidic and basic domain [51].

Multiple functions of Nopp140 have been reported. Since Nopp140 localizes to nucleolar DFCs [46], it suggests that Nopp140 involves in the regulation of rRNA transcription. This function is further supported by that hNopp140 interacts with the largest subunit of RNA Pol I (RPA194) [53]. Nopp140 also acts as a transcriptional regulator by interacting with C/EBP-β and TFIIB to activate the alpha-1-acid glycoprotein gene (*agp*) in mammalian liver [54]. Cells show altered nucleoli with crescent-shaped structures when an exogenous Nopp140 N-terminus is expressed and show an enlarged nucleolus when a full-length Nopp140 is overexpressed, suggesting that Nopp140 plays a structural role in maintaining the morphology of nucleoli [53]. The associated proteins of Nopp140 have also been identified, including NAP57 (Nopp140-associating protein) [55], p80-coilin [56], fibrillarin, and NAP65 [57]. NAP57 (yeast homolog termed Cbf5p) is a component of boxH/ACA snoRNPs that involves in pseudouridylation of pre-RNA processing. When the mutation occurs on the human NAP57, dyskerin, it leads to dyskeratosis congenita, a rare X-linked (Xq28) recessive disease [58]. However, when mutations are introduced to the *Drosophila* NAP57, flies appear with a reduced body size, abnormal eggs, and reduced fertility. These phenotypes imply that Nopp140 may play a role in pseudouridylation process, which is crucial in organism growth. In addition, hNopp140 has been demonstrated as a binding target of doxorubicin, a wildly used anti-cancer drug [59].

Caenorhabditis elegans as a model for nucleolus study

It is very difficult to explain why mutations occurring on certain nucleolar proteins only affect the partial organs or tissues of animals, for example, Treacher Collins Syndrome (TCS) affecting craniofacial development in human. TCS results from loss-of-function of the TCOF1 gene products, treacle, a nucleolar protein sharing with several similarities of Nopp140. The TCS phenotype is also observed in *Tcof1* heterozygous mice, suggesting that the correct dosage of treacle is essential for survival of cephalic neural crest cells, which contribute significantly to formation of branchial arches [60]. This case illustrates that the nucleolus

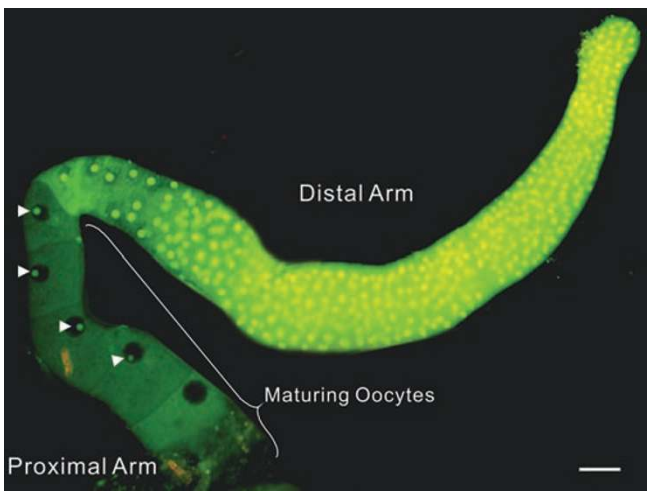

Figure 4 Fluorescence micrograph of nucleoli in germ cells of *C. elegans*. The gonads of *ok542* homozygous adults were dissected and stained with SYTO-14 to visualize RNA. One arm of gonad is shown and its distal arm on right, which comprises mitotic cells and early meiotic cells, and its proximal arm on left, which comprises maturing oocytes. The nucleolus of cells in distal arm is stained as a round-shape dot in the proximal arm. The maturing oocytes show a bigger size of cell and nucleus but a decreasing size of nucleolus. Arrowheads indicate the nucleolus in the maturing oocytes. Bar: 10 μm.

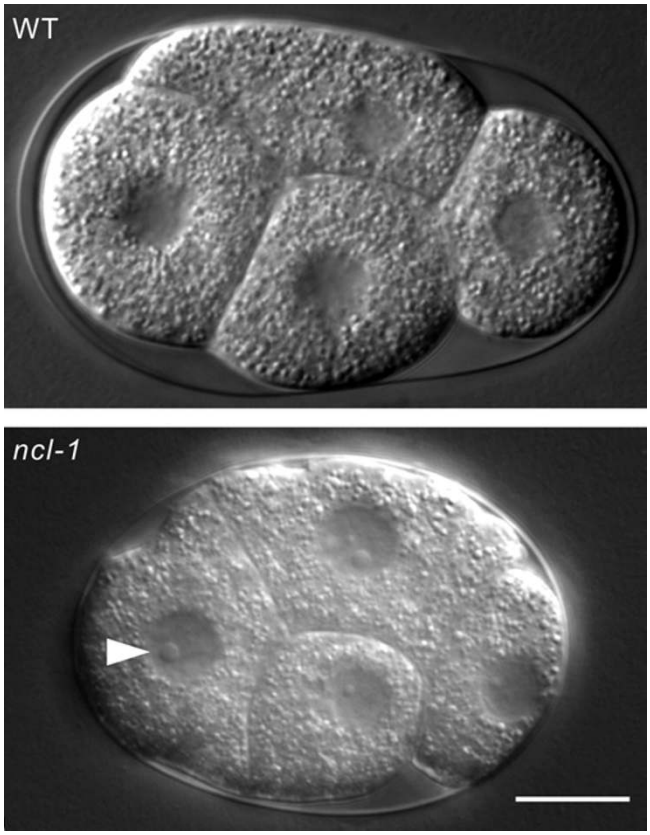

Figure 5 The four-cell stage of *C. elegans* embryos. Live wild-type (WT) and *ncl-1* embryo were loaded on slide having a thin layer of agar pad and observed using differential interference contrast (DIC) microscopy and photographed. No nucleoli can be seen in WT embryos (upper panel). One or two nucleoli can be seen in all nuclei of *ncl-1* (lower panel). The nucleolus is indicated with an arrowhead. Bar: 10 μm.

participates in a certain role in embryonic development. Therefore, to explore nucleoli involving in animal development, *C. elegans* is recommended for its short life cycle, transparent body, easy handling, and completion of genome analyses [61, 62].

In the mature hermaphrodite of worms, there are 959 somatic cells. Among them, 302 cells are neurons, which have the smallest nucleolus among all cell types. Whereas, the nucleoli of vulva and pharyngeal cells are bigger. Moreover, the germ cells at the distal arm of gonads have the largest nucleolus, which occupies the 80% to 90% volume of the nucleus (Figure 4). The oogenesis occurring in the U-shaped worm gonads provides an ideal model for studying nucleologenesis because cells are well arranged from the mitotic zone, transition zone to meiotic zone. Instead of producing thousands of small nucleoli in *Xenopus* oocytes, a single large nucleolus in *C. elegans* oocytes is responsible for synthesizing ribosome in a large amount, which are then used in embryogenesis. Genetic approach has been well established in worms that can be applied to reveal mechanisms of controlling various sizes of nucleoli in adult worm cells.

Furthermore, an *ncl-1* mutant strain of worm exhibits an early appearance of nucleoli in embryos and larger sizes of nucleoli in adult cells than in the respective cells of wild-type worms [63] (Figure 5). This feature provides a good model for studying resumption of rRNA transcription in early embryos. Collection of embryos from *ncl-1* mutant allows obtaining enough nucleoli for proteomic analyses. It has been demonstrated that NCL-1 is primarily located in the cytoplasm but controls the synthesis of ribosome. Worms with *ncl-1* mutation have a higher production rate of rRNA, presumably yielding a higher amount of ribosome than the wild type, and appear with a larger size of body [63]. This is another example illustrating that the nucleolus participates in controlling the body size of animal. The RNAi feeding method [64] is well established in worms and that can be employed to quickly identify epistatic genes in the *ncl-1* pathway. In all, the advent of worm can contribute to the nucleolus research, which is not easily applicable in other organism systems.

Conclusion remarks

It is a general knowledge in biology that the ribosome plays an essential role of protein translation in life and the nucleolus is the site for ribosome biogenesis in eucaryotes. Although it has been more than 150 years since the nucleolus was described, the major understanding of structure and function of the nucleolus was established just in the last 50 years. A remarkable progress of knowing the evolution of nucleolus has been accumulated in only few years. For a half century, the yeast scientists have contributed to the canonical foundation of ribosome biosynthesis. To explore more non-traditional functions of the nucleolus requires parallel studies in higher eucaryotes. The proteome of human and *Arabidopsis* nucleolus has shed the light on the evolution and new function of nucleoli. *C. elegans* model is recommended for proteomic study to fill the evolution gap as well as for investigating the roles of nucleolus in development and the mechanism of regulating sizes of nucleolus. More importantly, microarray techniques will be used for studying dynamic proteome and interactome. Combination of using the high-throughput techniques and advanced bioinformatic tools for analyzing nucleolome of various model organisms is a trend of future study in the nucleolus. New understandings in the structure and func-

tion of the nucleolus will then be applied in cancer and nucleolus-associated diseases in the next decade. We do believe that the more we study, the better we will understand the nucleolus.

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