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Birth of a nucleolus: the evolution of nucleolar compartments

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In eukaryotes, ribosome synthesis largely takes place in a specialized nuclear domain – the nucleolus. It has recently become apparent that this organelle is involved in the biogenesis of most cellular ribonucleoprotein particles (RNPs), as well as in cell-cycle regulation, making it central to gene expression. The field has traditionally acknowledged that each nucleolus is organized in three morphologically distinct compartments. Here, however, we discuss our view that in fact many eukaryotes have bipartite nucleoli. We propose that, during evolution, a third nucleolar compartment emerged at the transition between the anamniotes and the amniotes, following a substantial increase in size of the rDNA intergenic region. We believe that these conclusions have important implications for understanding the structure–function relationships within this key cellular organelle.

Introduction

The nucleolus is the site of rDNA transcription, pre-rRNA processing and modification and initial steps of pre-ribosome assembly (reviewed in [1–4]). Historically, the nucleolus was considered as a 'ribosome factory', literally resulting from the 'act of building a ribosome' [5,6]. The nucleolus is now known to serve essential functions in many other processes in addition to ribosome synthesis. This includes subtle regulations such as cell-cycle control, through the timely sequestration of specific *trans*-acting factors, and the biogenesis of most cellular ribonucleoprotein particles (RNPs) [7–13]. Most classes of cellular RNAs, including small nuclear snRNAs, transfer tRNAs, the signal recognition particle SRP RNA (involved in protein secretion), the telomerase RNA (the TEL RNP is required for maintenance of chromosome ends) and several mRNAs, indeed transit through the nucleolus during their life-cycle. The 'new'-nucleolus thus appears as a generic site of RNP biogenesis.

The nucleolus is compartmentalized in several morphologically distinct domains, the respective functions of which remain largely unknown. Indeed, even for such a basic activity as rRNA synthesis, there has been intense debate in the field over the past 30 years regarding the precise location of the sites of transcription. There is no consensus yet on this issue (see below). As for the numerous other reactions that take place within nucleoli, we are only starting to address where they occur. A major problem is that even the exact number of nucleolar subcompartments is not universally established. In the best-studied cases – human nucleoli – three subcompartments have been described morphologically. For certain reasons, it is widely acknowledged that all eukaryotic nucleoli have three such subcompartments. However, we believe that many researchers have erroneously supported this conclusion as they tried to fit their data into a tripartite organization in line with a preconceived notion that the human scheme is prevalent.

In this article, we carefully review the evidence for nucleolar subcompartmentalization and propose the important notion that eukaryotes do not all have three nucleolar subcompartments. In fact, a very wide variety of eukaryotes, comprising most of the lineage up to the transition to the amniotes, fall into a category of organisms possessing bipartite nucleoli. Starting from typical bi-compartmentalized nucleoli, we further propose an evolutionary scheme for the emergence of a third nucleolar compartment, thus providing for the first time important insights into the evolution of an organelle that, considering the importance and diversity of its newly discovered functions, should be a major focus of interest to many researchers.

Human nucleoli

When inspected at the ultrastructural level (Figure 1a, see panel (c) for a 'blueprint' cartoon), human nucleoli typically reveal three subcompartments: (i) one or several pale structures composed of fine fibrils of ~50Å in diameter, referred to as fibrillar centers (FCs), (ii) each surrounded by a compact layer of densely stained fibrous material (dense fibrillar component, DFC), (iii) altogether embedded within a single large granule-rich region (granular component, GC) [14]. This architecture is thought to largely reflect the vectorial maturation of the pre-ribosomes, with the transcription of the rDNA likely occurring at the interface between the FCs and the DFC [15–17], nascent transcripts reaching out into the body of the DFC and nascent pre-ribosomes progressively migrating from the DFC to the GC, as pre-rRNA processing, pre-rRNA modification and ribosome assembly occur [13,18,19]. Note that the location of the primary sites of rDNA transcription remain controversial and are still

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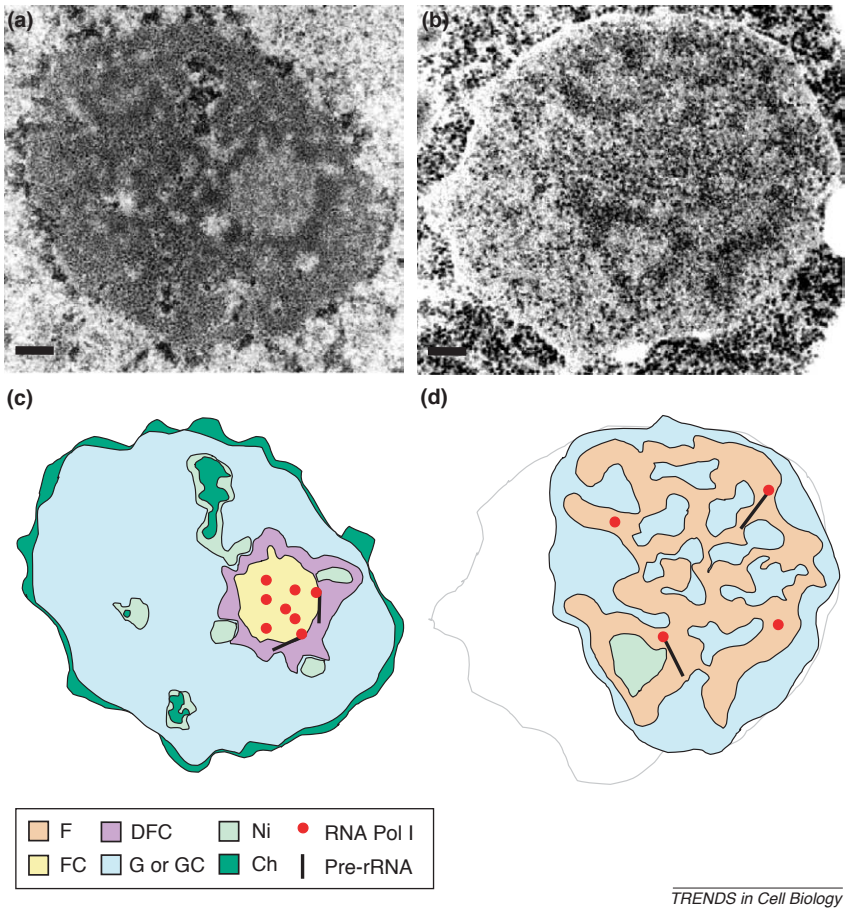

Figure 1. Nucleolar organization in human and *Saccharomyces cerevisiae* budding yeast cells. Electron microscope analysis on cultured human **(a)** and yeast **(b)** cells. **(a)** Human nucleolus (Hep-2 cells: larynx epidermoid carcinoma), **(b)** yeast nucleus and nucleolus. Bars, 0.25 μm. From these micrographs, it can be estimated that the size of a typical human nucleolus roughly corresponds to that of the entire yeast nucleus. **(c,d)** 'Blueprint' cartoons of panels (a) and (b), respectively. Key: F, fibrillar component; FC, fibrillar center; DFC, dense fibrillar component; G or GC, granular component; Ni, nucleolar interstices; Ch, condensed chromatin. Note that condensed chromatin is often readily detected within nucleolar interstices. The RNA polymerase I (RNA Pol I) and the nascent pre-rRNAs (Pre-rRNA) are represented for the benefit of our discussion and are not visible in panels (a) and (b). In panel (d), the yeast nuclear envelope is outlined in light grey.

formally considered to be either in the FCs, the DFCs, the FC–DFC interface or a combination of these locations (discussed in [15,20]).

Human nucleoli are in addition generally surrounded by a shell of condensed chromatin (Ch) that occasionally penetrates deeply into the organelle reaching the FCs (Figure 1a and c); condensed perinucleolar chromatin and FCs are thus contiguous. In sections, these invaginations are often visualized as 'nucleolar interstices' (Ni) (Figure 1a and c, discussed in [21]).

Besides their respective morphological properties, each nucleolar subcompartment is characterized by a distinct biochemical composition (Table 1 and Supplemental data S1). FCs contain DNA, including rDNA in a transcription-competent structure, some nascent pre-rRNAs (especially in its cortical area) and most importantly transcription factors, such as the RNA polymerase I (RNA Pol I), the upstream binding factor (UBF) and the DNA topoisomerase I. The resulting structure is sensitive to silver and detected in a specific cytochemical reaction, known as AgNOR, largely used in cancer prognosis [22,23]. The DFC, largely acknowledged as the site of early pre-rRNA processing and modification reactions, contains nascent pre-rRNAs and antigens such as the *Fibrillarin* (a core component of the box C+D small nucleolar snoRNPs involved in RNA modification [24]). The DFC also stains AgNOR positive. The GC, which accounts for up to 75% of the nucleolar mass in actively dividing cells, comprises

Table 1. Distribution of various constituents in bi- and tripartite nucleoli as inspected at the ultrastructural level[a]

Constituents	Bipartite nucleoli	Tripartite nucleoli
DNA, including rDNA	F	FC
AgNOR proteins	F	FC+DFC
RNA polymerase I	F	FC
UBF	F	FC+DFC
DNA topoisomerase I	n.d.	FC
Fibrillarin	F	DFC
Nucleolin[b]	F+G	DFC+GC
Ribosomal protein S1	G	GC
Ribocharin[c]	G	GC
rRNA	F+G	FC[d]+DFC+GC

[a]A fully referenced version of this table is available in the supplemental data S4 and at www.ulb.ac.be/sciences/rna.
[b]Nucleolin, an abundant and phylogenetically conserved nucleolar antigen that has been involved in most steps of ribosome synthesis.
[c]Ribocharin, a pre-60S ribosome synthesis factor of ill-defined function.
[d]rRNA molecules have been detected preferentially in the cortex of the fibrillar centers. n.d., not determined.

particles resembling ribosomes and, presumably, corresponding to pre-ribosomes at an advanced stage of maturation. The GC is not sensitive to AgNOR staining.

Nucleolar interstices are morphologically fairly similar to the nucleoplasm (Figure 1a) and contain DNA, including rDNA, but strikingly lack transcription-associated factors and the silver-sensitive AgNOR proteins (Table 1 and Supplemental data S1).

Yeast nucleoli

In the budding yeast *Saccharomyces cerevisiae*, a primitive eukaryote, the nucleolus is consistently confined to one territory occupying up to about one-third of the nuclear volume as a crescent- shaped zone of fibrillo-granular material juxtaposed to the nuclear envelope (Figure 1b and d). Despite controversial reports [25–28], close re-inspection of the published literature strongly convinced us that, in yeast, only two compartments can be unambiguously identified, that is – (i) a network of fibrillar strands (F) embedded within (ii) granules (G) (Figure 1b and d). In addition, 'electron-lucid zones', partially surrounded by fibrillar strands, are conspicuously observed (labeled as 'Ni' in Figure 1d). While the yeast 'electron-lucid zones' contain rDNA [26] and are somehow morphologically reminiscent of the mammalian FCs, these structures lack RNA Pol I and associated transcription factors, as well as nascent pre-rRNAs [26]. For this reason, we believe that these structures in fact correspond to the nucleolar interstices detected in vertebrates. We therefore propose that the yeast *Saccharomyces cerevisiae* has only two nucleolar subcompartments.

Nucleoli through evolution

The inspection of several dozen organisms across the eukaryotic lineage, including insects, amphibians and plants, led us to the conclusion that bipartite nucleoli are not unique to yeast (Supplemental data S2). In fact, the vast majority of eukaryotes have only two nucleolar subcompartments; these include the plants, all invertebrates and the anamniote vertebrates (see Figure 2, and Supplemental data S3 for a complete list of organisms experimentally tested). Indeed, in our opinion, homogeneous nucleolar structures of low electron density, even if they consist of fine fibrils, should only be referred to as FCs in the presence of RNA Pol I and associated factors. According to this definition, FCs are only detected in the amniote clade. We therefore propose that the transition from two to three subnucleolar compartments is a recent evolutionary acquisition.

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Furthermore, on compiling information examining the length of the rDNA transcription units versus the length of the intergenic regions (Figure 3 and Supplemental data S4), a striking correlation appeared between the transition from two to three nucleolar compartments and a tremendous increase in the size of the rDNA intergenic region. Indeed, in species containing bipartite nucleoli, the size of the rDNA transcription unit was larger or of similar length to that of the intergenic spacer. In species containing tripartite nucleoli, the intergenic region was always much larger than the transcription units. Note that the

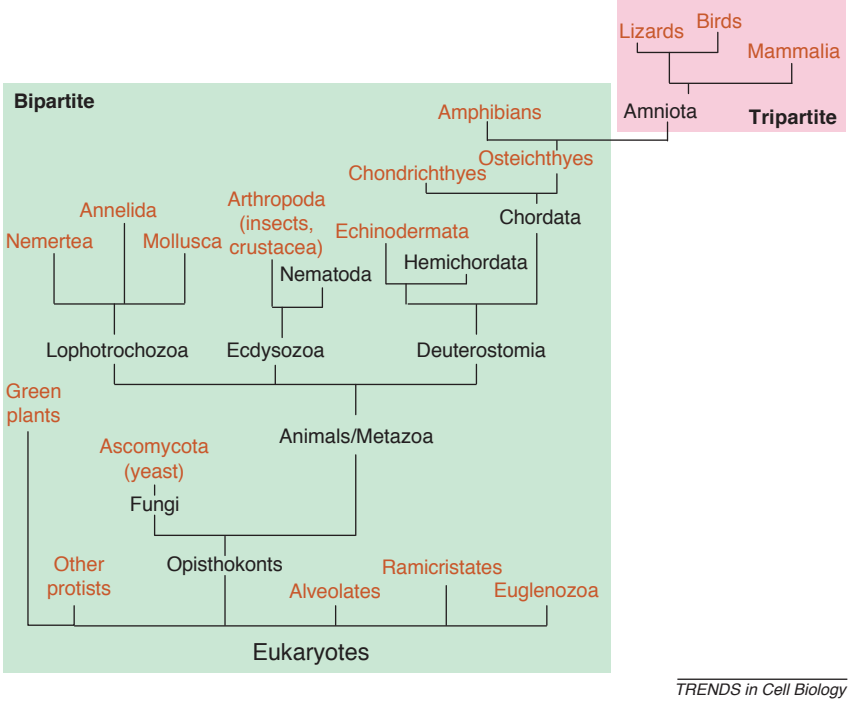

Figure 2. Nucleolar compartmentalization across evolution. Simplified eukaryotic tree of life (inspired by 'The tree of Life Web Project', http://tolweb.org/tree/). Eukaryotes containing tri-compartmentalized nucleoli (amniotes and upwards in the evolutionary tree) shaded in pink; eukaryotes possessing bipartite nucleoli shaded in light green. Phyla inspected experimentally (see Supplemental data S3) labelled in red.

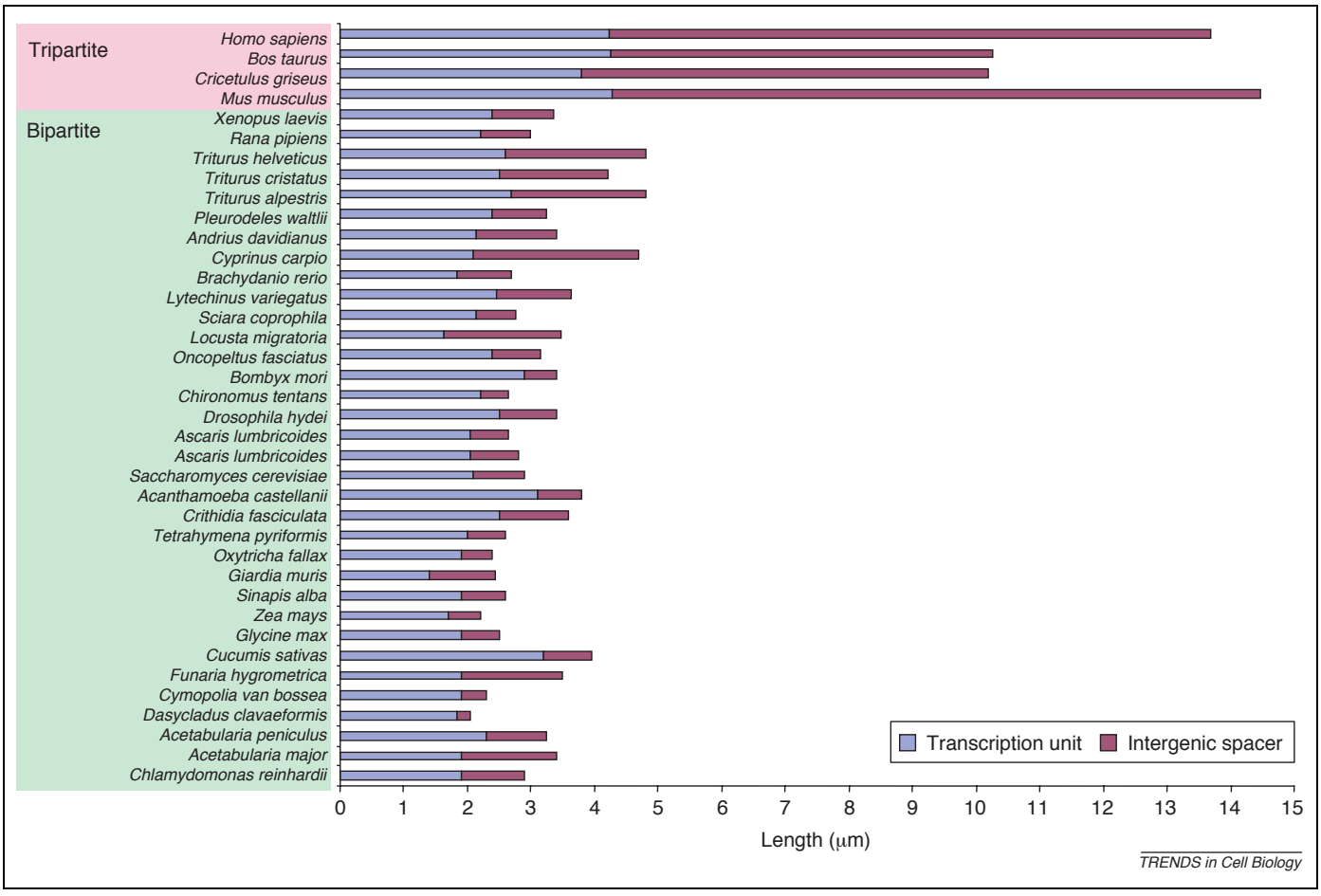

Figure 3. Length of rDNA transcription units versus intergenic spacer regions across evolution. Chart diagram representing the length of the rDNA transcription units (in blue) versus the length of the intergenic rDNA spacers (in purple) in different species. Color-code for bi- and tri-compartmentalized nucleoli as in Figure 2. See supplemental data S4 for hard data and references.

number of rDNA units per genome varies greatly across evolution and could not be correlated in such a way with the presence of bi- or tripartite nucleoli (Supplemental data S4).

Model for the function of bi-compartmentalized nucleoli

In tripartite nucleoli, and despite considerable research effort, it is not yet clear whether rDNA transcription occurs in FCs, in DFCs or in a combination of these locations (see Introduction). For bipartite nucleoli, the situation is not quite as complex, we think, as there is only one fibrillar component in which we propose that rRNA synthesis occurs (see cartoon, Figure 1d). Furthermore, since the F compartment of bi-compartmentalized nucleoli features both characteristics of mammalian FCs and DFCs (see Table 1 and Supplemental data S1), we suggest that it carries at least some of their core functions in ribosome synthesis and that, during evolution, a primordial fibrillar compartment specialized into FCs and DFCs. We further propose that the physical segregation of the functions initially carried out by a single compartment into two distinct locales somehow relates to an increase in size in the rDNA intergenic regions.

What are the function(s) of FCs?

In humans, both FCs and nucleolar interstices contain rDNA; however, these contiguous compartments differ substantially by the exclusive presence in FCs of transcription-associated factors [29]. It seems evident to us that the transition between a transcriptionally inactive to a transcription-competent form of chromatin is acquired at the interface between nucleolar interstices and FCs. To put it simply, what makes an FC and a nucleolar interstice different is that in FCs 'everything is ready to go' – regardless of whether transcription *per se* occurs within the FC core or at its cortical surface. We speculate that the reported increase in size in the rDNA intergenic region in amniotes allowed for a physical separation between these two forms of rDNA chromatin. One possibility is that the increase in size in the rDNA spacers allowed the specific exclusion of one form of chromatin by 'looping it out' from a defined nucleolar location into a new compartment. The acquisition of a third nucleolar subcompartment, containing a pool of RNA Pol-I-related factors, offers the potential for rapid responses to environmental stimuli (stresses, nutrient availability etc.), as well as for improved quality control and fine-tuning, for instance through the selective sequestration of specific *trans*-acting factors.

For the aficionados… nucleolar delicatessen

In higher eukaryotes, nucleoli undergo a cycle of disassembly–reassembly during mitosis ([30]; reviewed in [12,29,31]). Nucleoli disappear during prophase and

reform at the end of telophase around specific chromosomal regions termed nucleolar organizer regions (NORs), which map to the rDNA loci. Chromosome-associated NORs and interphase FCs are often considered as equivalent structures. However, within the NORs, silver-positive and silver-negative regions can be discriminated (Table 2 and Supplemental data S1); the interphase FCs in fact strictly correspond to the silver-positive portion of the NORs. These contain the non-condensed rDNA and remain associated throughout mitosis with the inactive RNA Pol I transcription machinery. Yeast strains that express their rDNA from non-chromosomal locations do not show the nucleolar 'electron-lucid zones' [25], suggesting that these structures correspond to genuine FCs. We believe that this indicates that the electron-lucid zones are equivalent to the mammalian heterochromatic, silver-negative region of the NOR.

Fungi are notable in that they show a 'closed' mitosis, in which both the nuclear membrane and the nucleolus remain intact during the process of cell division, suggesting that these organisms might be less dependent on chromosome-associated NORs and related FCs. Although this model is appealing, the relationship between NORs and FCs is not quite that simple as many organisms characterized by an 'opened' mitosis and bi-compartmentalized nucleoli have NORs that are virtually indistinguishable from those observed in higher eukaryotes (both by morphological and compositional criteria [29]).

How does the nucleolus hold together?

Like most functional nuclear domains, the nucleolus is not bound by a membrane. It is also one of the most highly dynamic cellular organelles: number, size and shape of nucleoli vary greatly depending on cell type, cell-cycle stage and culture conditions [30,32,33]. Consistently, both scarce components and abundant nucleolar constituents, such as fibrillarin, are known to exchange continuously and rapidly with the surrounding nucleoplasm [34]. Despite recent proteomic analysis [35,36], it is not clear at present whether the nucleolus relies on structural components for its maintenance. Recent evidence from yeast, however, suggests that the nucleolus at least partially obeys the rules of 'self-organization' – that is, a system that is built on the transient and functional interactions of its constituents [27,37,38]. The deletion of a single nucleolar methyltransferase, which transiently interacts with its substrates to effect a base modification, was indeed sufficient to bring about the dissolution of the whole structure [27].

Table 2. Distribution of various constituents in the two portions of the nucleolar organizer region (NOR)[a]

Constituents	Pale area of the NOR	Dark area of the NOR
AgNOR-proteins	Yes	No
UBF	Yes	No
Phosphoprotein 135[b]	Yes	No
DNA	Decondensed	Condensed

[a]a fully referenced version of this table is available in the supplemental data S4 and at www.ulb.ac.be/sciences/rna.

[b]Phosphoprotein 135, a typical argyrophilic protein that remains associated with the NOR throughout the cell cycle.

Concluding remarks: many eukaryotes have bipartite nucleoli

Why is it so important to learn more about nucleolar structure? Notably, the nucleolus is not only the site of a key cellular activity – ribosome synthesis – but crucially is also involved in processes as diverse as cell-cycle regulation and the biogenesis of most cellular RNPs.

Historically, morphological analyses have allowed the classical description of three nucleolar subcompartments in human nucleoli – a tripartite organization that was thought to be conserved across evolution. Here, we have challenged this view. We believe that many eukaryotes have in fact only two nucleolar subcompartments and that they have been misclassified as a direct consequence of the incorrect assignment of one compartment, the FC. We believe that the time is ripe to define accurately and unambiguously the various nucleolar subcompartments. This is particularly true for the FC, for which we have here provided a functional definition based on the strict presence of the rDNA transcription machinery.

Furthermore, we believe it to be important to try to establish how the various subnucleolar compartments relate to each other, in essence how they emerged during evolution, to gain insight into their respective, and largely unknown, functions. We propose that, during evolution, a primordial fibrillar compartment diverged into two specialized domains, the FCs and the DFCs. We suggest that the late acquisition of a third nucleolar compartment most likely occurred at the transition between the anamniotes and the amniotes, a transition that coincided with a substantial increase in size of the rDNA intergenic regions. The emergence of a third nucleolar compartment might correspond to an increased need for fine-regulation in higher eukaryotes.

Looking to the future – the field is now facing the challenging task of delineating the largely unexplored structure–function relationships within the nucleolus. Particular attention needs to be paid to its newly assigned and exciting non-ribosomal functions. As most of our current knowledge on ribosome synthesis is based on yeast work, we hope that this article will have important implications for the field and will revive interest in parallel studies in higher eukaryotes. In humans, this will be aided by the recent proteomic analyses that have characterized the nucleolus as a whole and provided us with a comprehensive list of nucleolar components. These now need to be characterized functionally and systematically localized at the ultrastructural level.

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Supplementary data

Supplementary data associated with this article can be found at doi:10.1016/j.tcb.2005.02.007 and at www.ulb.ac.be/sciences/rna.

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