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The nucleolus

When two became three

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Though the nucleolus is considered today as a multifunctional domain, its primary function is ribosome biogenesis. We have shown at the ultrastructural level that there are primarily two types of nucleolar organization: nucleoli containing three components in amniotes and two components in all other eukaryotes. In a recent report we made the additional and surprising, finding that both types of nucleolar arrangement are found among living reptiles, viz. a bicompartmentalized nucleolus in turtles and a tricompartmentalized nucleolus in lizards, crocodiles and snakes. This latter organization occurs regardless of the species, the tissue or the developmental stages analyzed. These results are compatible with the view that the transition between bipartite and tripartite nucleoli coincided with the emergence of the amniotes within the Reptilia. They also support the previous hypothesis that turtles are primitive reptiles. The emergence in amniote vertebrates of a third nucleolar compartment might have imparted novel regulatory functions to the nucleolus, as well as perhaps, expanding the adaptability of ribosome synthesis to an ever changing environment, thus, enhancing the overall fitness of amniotic vertebrates.

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The nucleolus is a prominent and highly dynamic nuclear organelle central to gene expression where the initial steps of ribosome biogenesis take place. This is the site where rDNA genes are transcribed by RNA polymerase I into long precursor transcripts, the pre-rRNAs; three of the four rRNAs, the 18S-5.8S-25/28S rRNAs, reside in these primary transcripts. The fourth rRNA, 5S, is produced independently by RNA polymerase III and is recruited to nascent pre-ribosomes in the nucleolus. Pre-rRNA molecules undergo a complex maturation pathway, largely initiated cotranscriptionally, that comprises extensive pre-rRNA processing steps (i.e., cleavage), to release the mature rRNA sequences, as well as base and ribose modifications, folding, transient association with assembly-facilitating accessory proteins and packaging with ribosomal structural proteins (reviewed in ref. 1 and 2). This generates precursor subunits, which are released from the nucleolus, diffuse through the nucleoplasm and are eventually translocated through the nuclear pore complexes into the cytoplasm. For both the small and large ribosomal subunits, maturation is finalized there and involves a cascade of energy-dependent reactions leading to a substantial three-dimensional remodelling of the ribonucleoprotein structure and final processing of the rRNAs (reviewed in ref. 3–6).

Most of our understanding of eukaryotic ribosome biogenesis is derived from studies in *Saccharomyces cerevisiae* where no less than ca. 200 protein transacting factors have been characterized. The factors and the mechanisms involved in ribosome synthesis were long assumed to be largely conserved throughout eukaryotes; however, the picture that emerges from recent research is that the situation is likely far more complex in human cells than in budding yeast.[7,8]

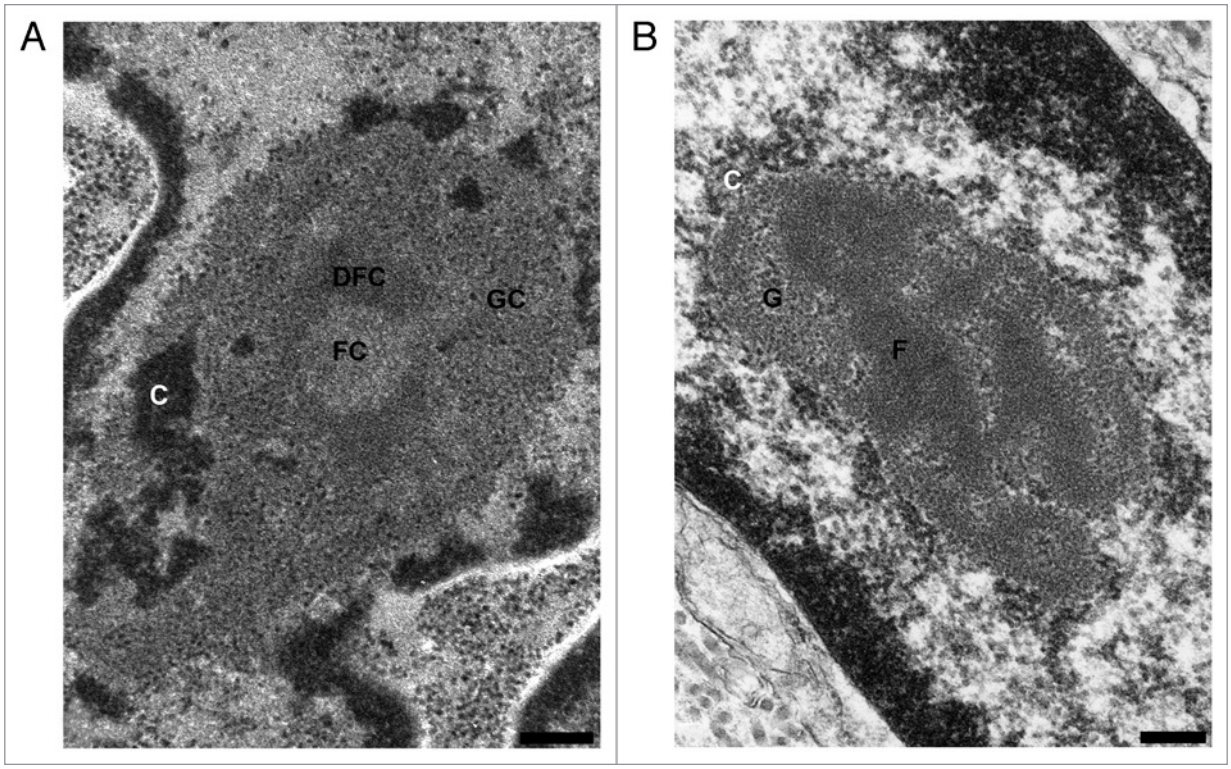

Figure 1. Tripartite and bipartite organization of the nucleolus. In the Mac-T bovine mammary epithelial cell line nucleolus (A), three main nucleolar compartments are observed: the fibrillar center (FC), the dense fibrillar component (DFC) and the granular component (GC). In the follicular cell nucleolus from the newt *Pleurodeles waltlii* ovary (B), only two main nucleolar compartments are obvious: a fibrillar zone (F) surrounded by a granular zone (G). In both types of nucleolus, condensed chromatin clumps (C) are found around and inside the nucleolar body. Bars = 0.25 μm.

When observed by electron microscopy mainly two types of nucleolar organization can be distinguished, with the organelle containing either two or three major components (reviewed in ref. 9). In mammalian cells (**Fig. 1A**), three morphologically distinct nucleolar subcompartments have typically been described: the fibrillar centers (FCs), the dense fibrillar component (DFC) and the granular component (GC). The FCs are structures with a low electron density composed of fine fibrils of ~0.1 to 1 μm in diameter. They are partly surrounded by the DFC that is formed by densely stained fibrous material. Nucleoli often contain several functional FC/DFC modules, which are embedded within a single GC that consists of granules of ~15–20 nm in diameter. It was established that transcriptionally active rDNA resides in the FCs, especially at its perimeter, and that nascent transcripts extend into the intimately associated DFC.[10-14] Initial pre-rRNA cleavage and the early steps of pre-rRNA modification and assembly occur in the DFC; this is where the many snoRNPs that act as antisense guides in ribose-2'-O-methylation and pseudouridylation of the pre-rRNAs largely accumulate. Further assembly steps occur in the GC.

One approach to establishing the structure/function relationship within the nucleolus is to take a close look at our ancestors and to deduce how this organelle evolved. In a previous review we discussed the somewhat overlooked fact that the vast majority of eukaryotes have only two subnucleolar compartments.[9] Indeed, tripartite nucleoli appear as the exception rather than the rule, since they are only found in amniote vertebrates, whereas bipartite nucleoli are present in all the other eukaryotes, including arthropods, fishes and amphibians.[15-19] For example, in amphibian cells (**Fig. 1B**), only two nucleolar compartments are unambiguously identified: a single large contiguous fibrillar zone (F), surrounded by a granular zone (G). These findings suggested to us that the emergence of a third nucleolar compartment coincided with the transition between the anamniote and the amniote vertebrates. This hypothesis needed to be examined by a thorough investigation of the ultrastructural features of the nucleoli of selected species in the class Reptilia that phyletically map at this transition. In amniote vertebrates, a tripartite nucleolar organization has been described repeatedly in many species of mammals and birds but has so far only been reported in two species of lizard species among the reptiles.[20,21] Besides lizards, the Reptilia also comprises turtles, snakes and crocodiles. The fine structure of the nucleolus in these other subgroups of living reptiles remained however, completely unknown.

Recently, we have examined the ultrastructural organization of the nucleolus in various tissues among four subgroups of the Reptilia, including three species of turtles, three lizards, three snakes and two crocodiles, with each species within a subgroup being members of different genera.[22]

To improve the contrast between the different nucleolar components, we

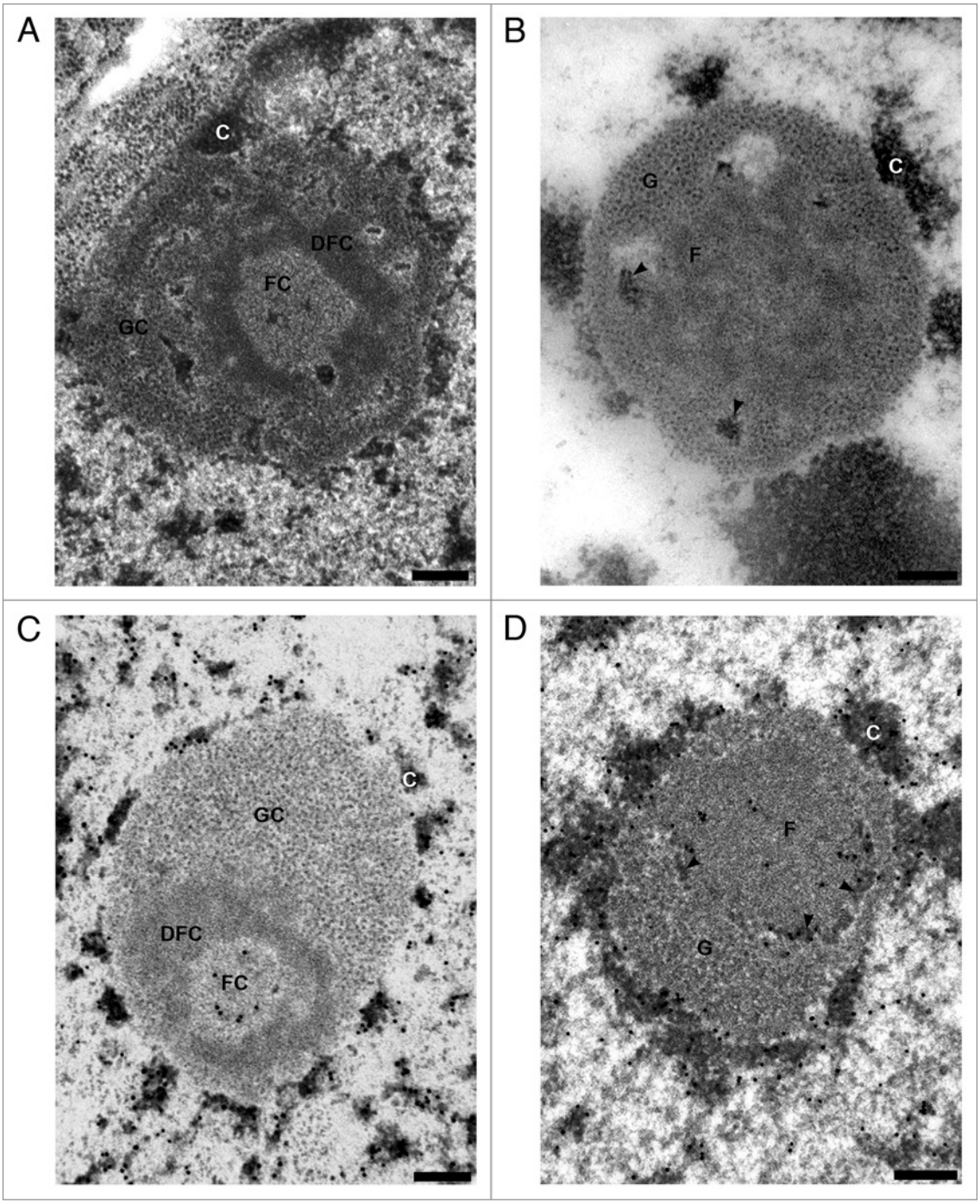

Figure 2. Nucleolar organization in reptiles. Lizard (A and C) and turtle (B and D) epithelial cells are characterized by a tripartite (FC, DFC and GC) versus a bipartite (F and G) nucleolar organization, respectively. Besides an intense labelling over the condensed chromatin (C) associated with the nucleolus, DNA is detected in the FC of lizard nucleolus (C) and in the F of turtle nucleolus (D), as revealed with the immunogold TdT labeling procedure. Arrowheads point to a concentric ring of intranucleolar chromatin. (A and C) stomach epithelial cells from the lizard Japalura sp. (B) Stomach cells from the aquatic turtle *Pseudemys scripta* elegans. (D) tracheal cells from the aquatic turtle *Trachemys scripta scripta*. Bars = 0.25 μm.

applied an acetylation method.[23] This technique is based on a glutaraldehyde fixation step followed by acetylation in pyridine, a procedure which we usually apply on the tissue blocks prior to embedding. Under these experimental conditions, the three fundamental nucleolar components (FC, DFC and GC) were readily detected in lizards, snakes and crocodiles, regardless of the species and the tissues studied (**Fig. 2A**), as has been classically described in avian and mammalian cell nucleoli.[16,24,25] In the crocodile *Crocodilus niloticus*, we also found that nucleolar compartmentalization was independent of the developmental stage. Specifically, in the epithelial cells of the stomach at the embryonic and adult stages, we observed that the

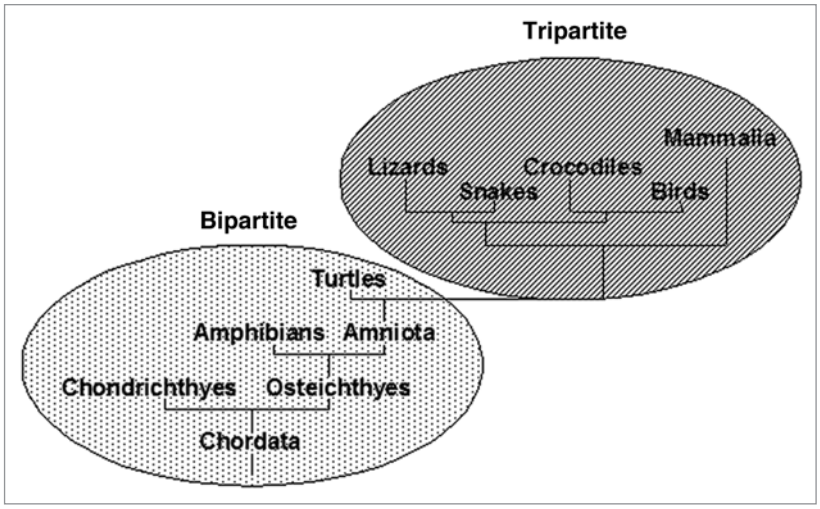

Figure 3. Nucleolar compartmentalization across phyla. Simplified chordate tree (http://tolweb.org/tree/). Chordata containing bicompartmentalized nucleoli are represented in stippled area, chordates possessing tricompartmentalized nucleoli are represented in the hatched area (encompassing all the amniotes except for the turtles).

nucleolus presents a tripartite organization. By contrast, in the turtle nucleolus only two main nucleolar compartments were conspicuously detected regardless of the species or the tissues analyzed: a fibrillar zone (F), and a granular zone (G) (**Fig. 2B**). The fibrillar zone was always located in the central part of the nucleolar body, with the granular zone being preferentially found at the periphery. The presence of frequent nucleolar interstices, often in contact with the fibrillar constituent of the nucleolus and presenting patches of heterochromatin, was found in both types of nucleoli (**Fig. 2A and B**).

To further characterize the two types of nucleoli in reptiles, we applied different cytochemical and immunocytological techniques on the tricompartmentalized nucleolus of the lizard *Japalura splendida* and on the bicompartmentalized nucleolus of the aquatic turtle *Trachemys scripta scripta*. Firstly, we used the silver staining which primarily labels the DFC, and to a lesser extent the FC in the tricompartmentalized nucleoli of mammalian cells.[23,26] In the lizard nucleolus, the silver-staining pattern was similar to that observed in the mammalian nucleolus. In the turtle nucleolus, only the fibrillar zone was silver-stained.[22] Next, to identify the precise localization of DNA within the reptilian nucleolus, we applied the immunogold labeling terminal transferase (TdT) method.[29] In the mammalian nucleolus, DNA was preferentially found over the condensed chromatin associated with nucleolus as well as over the FC.[16,17] In the lizard nucleolus (**Fig. 2C**), in addition to the presence of intense labeling over intranucleolar and perinucleolar condensed chromatin, DNA was clearly detected over the FCs. By contrast, the DFC and the GC appeared completely devoid of gold particles. In the bipartite nucleolus of turtles (**Fig. 2D**), labeling was present in the fibrillar zone, in addition to the condensed chromatin associated with the nucleolus. In turtle preparations, we consistently observed intranucleolar concentric rings of condensed chromatin; these were also labeled with the TdT method (**Fig. 2D**). Whether these rings of intranucleolar heterochromatin contribute to a layered foundation in these nucleoli is an open question.

In conclusion, both types of nucleolar organization, bi- and tripartite, are present among living reptiles since we observed a bicompartmentalized nucleolus in all species of turtle studied and a tricompartmentalized nucleolus in lizards, crocodiles and snakes. Our findings are consistent, regardless of the species within a sub-group of the Reptilia, the particular tissue, or the developmental stages analyzed. These data are compatible with the idea that the transition between bipartite and tripartite nucleoli coincided with the emergence of the amniotes within the Reptilia (**Fig. 3**). This morphological evidence also supports the longstanding hypothesis that turtles are primitive reptiles, a matter of intense debate in the field of evolutionary biology.[30,31] We indeed showed that, within the living reptiles, only turtles present a bicompartmentalized nucleolus, the type of nucleolus typical of invertebrates and anamniote vertebrates.[9] This finding is also in agreement with recent genetic and organogenesis data.[32,33]

Finally, these data are also consistent with our earlier proposal that during evolution, the fibrillar constituent of bipartite nucleoli diverged into separate domains, leading to the formation of two morphologically and functionally distinct components: the FC and the DFC, and, further support the idea that FCs appeared as specialized areas of tricompartmentalized nucleoli where rRNA genes are concentrated.[9] The emergence in amniote vertebrates of a third nucleolar compartment, the FC, might impart novel regulatory functions to nucleolar processes; for example, possibly contributing to the differential sequestration of trans-acting factors. The FCs, which in essence are a repository of RNA polymerase I complexes and ancillary factors, might afford cells an opportunity to promptly adapt ribosome synthesis rates to a changing environment: modern eukaryotes are indeed exposed to an immensely more complex range of environmental challenges (growth factors, insulin, etc.,) than their ancient relatives. Further research is now needed to determine what additional repertoire of functions is afforded by having FCs. These additional functions could include an expanded dynamic range of rRNA transcription regulatory circuits, or may be even novel FC functions unrelated to ribosome synthesis altogether.

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