RESEARCH HIGHLIGHT

In phase with the nucleolus

Denis L. J. Lafontaine [1]✉

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The nucleolus is a biomolecular condensate responsible for ribosome biogenesis. Using super-resolution microscopy, the team of Ling-Ling Chen has identified a novel nucleolar subcompartment, ascribing to it a function in processing the 3′ ends of primary ribosomal RNA transcripts synthesized by RNA polymerase I.

The nucleolus, a multilayered biomolecular condensate formed by liquid–liquid phase separation, is where the initial steps of ribosome biogenesis occur, along with other processes important for cell homeostasis.[1]

This membraneless organelle, prominent in the cell nucleus, was among the first observed by early microscopists. Its structure, investigated by many techniques, often reveals morphology-function links. Its role as a potent stress biosensor and biomarker highlights the importance of studying nucleolar structure–function relationships.

In most amniotes (mammals, birds, lizards, etc.), the nucleolus is described as having three main nested layers,[2] now called phases. At its core are modules comprising a fibrillar center (FC) surrounded by a dense fibrillar component (DFC) (Fig. 1). Transcription by RNA polymerase I (Pol I) occurs at the FC/DFC interface, with nascent precursor ribosomal RNAs (pre-rRNAs) radiating into the DFC. Nascent transcripts are bound co-transcriptionally by assembly factors and ribosomal proteins, forming precursor ribosomal subunits. There are multiple FC/DFC modules within a single large mass of granules, the granular component (GC). These three main subcompartments, enclosed by a compact perinucleolar chromatin (PC) shell, are obvious by transmission electron microscopy.

Established 20 years ago, the nucleolar proteome includes thousands of proteins, often in fast exchange with the surrounding nucleoplasm. Over 1000 proteins have recently been mapped by confocal microscopy to one or sometimes several nucleolar subcompartments or shown to define, at the periphery of the organelle, a nucleolar rim (notably containing the chromosome-surfactant protein MKI67).[3] The locations of the other proteins remain unclear, as does exactly where most individual steps of ribosome biogenesis take place.

Dawn of a new phase. Using super-resolution microscopy, the authors had previously obtained a hint that additional phases might exist in the nucleolus: they found individual nucleolar proteins to define subareas within the key layers. Examples include the box C+D snoRNA-guided 2′-*O*-methyltransferase fibrillarin, involved in pre-rRNA sorting at the FC/DFC interface and concomitant processing of the 5′ end of nascent transcripts,[4] and the helicase DDX21,[5] involved in regulating rRNA synthesis and modification,[6,7] with repercussions on physiology.[8]

In a candidate-based screen, the team has now mapped 200 nucleolar proteins by super-resolution microscopy in live cells.[9] Besides the three classical layers and nucleolar rim, they identified a novel layer between the DFC and the GC, coined "periphery of DFC" or PDFC.

Thus far, 12 proteins have been assigned to the PDFC: two novel, structurally related proteins (unhealthy ribosome biogenesis 1 and 2, URB1 and URB2), three known factors (Nucleolin (NCL), NIP7, and SRFBP1), and seven helicases (including DDX21). Unlike other PDFC proteins, URB1 and URB2 are rather static and contain no intrinsically disordered regions known to be important for phase separation. The presence of NCL in the PDFC is interesting, as in previous works, its location (DFC, GC, or both?) was not always clear. Now we know why: NCL localizes mainly to the interface. The PDFC is not visible by electron microscopy because it does not produce sufficiently electron-dense structures. With super-resolution microscopy, it became clearly identifiable (Fig. 1a).

Nucleolar structure often reflects nucleolus function, as best illustrated in cells treated with low doses of the Pol I inhibitor actinomycin D: they display dramatic reorganization of the nucleolar layers, which become juxtaposed instead of nested, with DFC components forming well-identifiable "caps". This phenotype, known as nucleolar segregation, has been used to prove that anamniotes (e.g., yeasts) have only two main nucleolar layers.[10] In this assay, the PDFC components appeared to underlie, and not mix with, the DFC caps (Fig. 1b). This supports the existence of the new PDFC phase.

Despite tremendous insights into the assembly mode, composition, and functions of the nucleolus,[1] we still do not know where most steps of ribosome biogenesis occur within this fascinating organelle. URB1-depleted cells showed a strong accumulation of pre-rRNAs having retained the 3′-external transcribed spacer (3′-ETS) segment, causing severely impaired production of mature large ribosomal subunit rRNA (Fig. 1a). This phenotype is quite rare and associated to date with only a few ribosome assembly factors, including the box C+D snoRNA U8.[11] U8 aids pre-rRNA folding and processing by establishing Watson–Crick base-pairing with pre-rRNAs. The authors found URB1, U8, and 3′-ETS-pre-rRNAs to colocalize at the intact PDFC (URB1 is required for PDFC integrity). They then found URB1 to interact functionally with U8: in the absence of URB1, U8 interacted less well with pre-rRNAs, and pre-rRNA structure was not properly remodeled.

We recently learned that processing at the 5′ end of the pre-rRNA occurs at the FC/DFC interface concomitantly with sorting.[4] With the view that 3′-ETS processing occurs at the PDFC, the field

[1]RNA Molecular Biology, Fonds de la Recherche Scientifique (F.R.S./FNRS), Université libre de Bruxelles (ULB), Biopark Campus, B-6041 Gosselies, Belgium.
✉email: denis.lafontaine@ulb.be

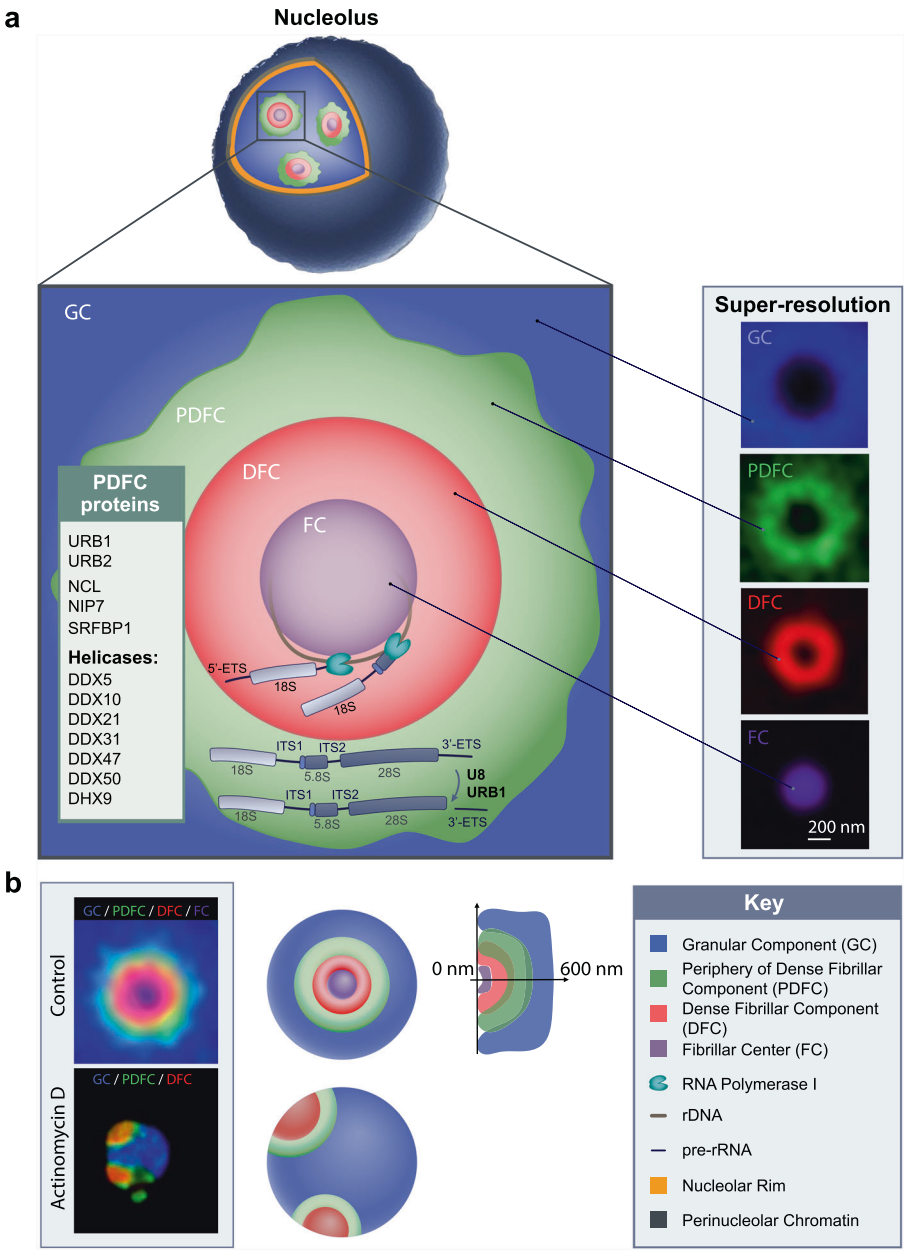

Fig. 1 PDFC and 3′-ETS processing. a Four main layers of the nucleolus. Picture insets: super-resolution (structured illumination) microscopy imaging of part of a human cell nucleolus. Spatial functional specialization of the PDFC in 3′-ETS processing involving URB1 and U8. The mature rRNAs (18S, 5.8S, and 28S) flanked by external (ETS) and internal (ITS) transcribed spacers in pre-rRNAs. **b** Nucleolar segregation upon actinomycin D treatment: the PDFC (in green) underlying the DFC "caps" (in red) proves the existence of a distinct phase (FC not shown). For comparison, a control cell nucleolus displays four nested layers.

has moved a step further toward demonstrating functional compartmentalization of the nucleolus.

Note: numerous surveillance mechanisms ensure the faithful production of mature ribosomes. Aberrantly processed RNAs are typically recognized and targeted for degradation, notably by the RNA exosome, a multi-subunit complex with both endoRNase and 3′-5′ exoRNase activities. Interestingly, so are the 3′-ETS-extended precursors accumulating in the absence of URB1.

There exist associations between disease and either an excess (cancer) or an insufficiency (ribosomopathies) of functional ribosomes. Ribosomopathies, caused by mutations in ribosomal proteins or assembly factors, are highly tissue-specific syndromes (the blood, brain, and bones being prime targets) and are often deeply rooted in embryonic development.

U8 deficiency has been associated with a debilitating brain disease: Labrune syndrome or Leukoencephalopathy with Calcification and Cysts (LCC).[12] URB1 fish morphants showed abnormal early-stage embryogenesis, including cranio-facial morphogenesis defects, and some of the phenotypes observed in a fish model of LCC. Embryonic development was also delayed in URB1-deficient mice.

There remain many open questions: How many more phases does the nucleolus possess? Does interfacial energy contribute to ribosome biogenesis? Will specific functions map to other nucleolar subphases? Why are so many helicases (seven) in the PDFC? Is URB1 impairment associated with a disease whose symptoms overlap with those of LCC? What are the enzymatic activities involved in 3′-ETS removal?

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ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Denis L. J. Lafontaine.