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When two became three: Shaping the nucleolus with *Treacle*

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The nucleolus is a multiphase biomolecular condensate responsible for the initial steps of ribosome biogenesis. Jaberi-Lashkari et al.[1] report that *Treacle*, a protein associated with a craniofacial distortion disease, played an evolutionary role in the spatial specialization of the nucleolus.

The nucleolus is a multilayer biomolecular condensate formed by liquid-liquid phase separation where the initial steps of ribosome biogenesis and other reactions important for gene expression regulation take place.[2]

In humans and other amniotes (e.g. birds and lizards), the nucleolus comprises three main nested layers (now also called phases).[3] At the core of the organelle lies a fibrillar center (FC) surrounded by a dense fibrillar component (DFC) (Figure 1A). The FC-DFC interface is where ribosome production starts, with synthesis by RNA polymerase I (Pol I) of a long polycistronic precursor encoding three of the four mature ribosomal RNAs (rRNAs). The FC and DFC form functional modules present in one or often several copies per nucleolus, immersed in the granular component (GC), a large mass of granules consisting of maturing precursor ribosomes (Figure 1A). FC, DFC, and GC are sufficiently electron-dense to be visible by electron microscopy (EM). Other layers (not visible by EM and not discussed here for simplicity) have been described more recently, including the periphery of DFC, involved in 3′-end maturation of primary transcripts,[4] and a nucleolar rim of unknown function.

In fact, most eukaryotes display only two main subnucleolar compartments: a fibrillar and a granular zone. Acquisition of a third compartment, i.e., the transition from a bipartite to a tripartite structure, is suggested to have occurred in the *Reptilia* (Figure 1B).[3,5] It is also proposed to have coincided with a tremendous lengthening of the sequence separating rDNA repeats in the genome[3] or of an upstream spacer destined to be processed in the primary rRNA transcript.[6] Heretofore, what drove this transition molecularly has remained unclear. Jaberi-Lashkari et al. have now made a case for the involvement of a single self-assembling nucleolar phosphoprotein protein, *Treacle* (TCOF1), in this important evolutionary step.[1]

The authors suspected that *Treacle* might play a scaffolding role in the FC, where it normally resides. Previously, they had found expression of a *Treacle* variant to cause formation of large condensates outside the nucleolus and that those could recruit FC components (Pol I and other critical rRNA synthesis factors) but not DFC proteins (fibrillarin).[7] Using EM, they now show that depleting human cells of *Treacle* leads to complete FC ablation, while leaving the DFC and GC apparently intact.

Remarkably, they also show that it's possible to modulate FC size simply by changing the *Treacle* expression level, which may have therapeutic applications.

The nucleolus is an RNA- and protein-rich structure where scores of multivalent, weak, transient interactions take place between components. This collective behavior is understood to promote formation of the layers by phase separation.[2] In a multi-component system, increasing the concentration of a single component leads to increased condensate size, but only up to a point because the concentrations of the partners remain the same. In contrast, the authors show that increasing the cellular concentration of *Treacle* causes the condensate size to increase without reaching such a limit. They conclude it is a single-component self-assembling protein. They then mapped the segment of *Treacle* important for self-assembly to a central domain containing a repeat of serine/glutamate-rich low-complexity regions.

As *Treacle* can self-assemble into foci with FC identity (recruiting FC components and excluding others, thus preventing their mixing), it seems plausible that it might have contributed during evolution to acquisition of a novel nucleolar compartment, especially since *Treacle* emerged broadly at the anamniote-amniote transition.

To provide molecular grounds for a putative involvement of *Treacle* in FC acquisition, the authors performed a very elegant experiment: they successfully converted a two-compartment nucleolus to a three-compartment nucleolus, simply by expressing *Treacle*.

Practically, they used as model the fish *Danio rerio*, whose cells display bipartite nucleoli (Figure 1C). They microinjected single-cell-stage embryos with mRNAs encoding a fluorescent version of *Treacle* and derived primary cells from a growing embryo. In these cells they found exogenously expressed *Treacle* to form a condensate nested within naturally expressed fibrillarin (a typical DFC marker), but not when it lacked its central domain important for self-assembly. *Treacle* expression alone was sufficient to reorganize the fish nucleolus. As in human cells, the size of the FC depended on the level of *Treacle* expression. By co-injecting mRNAs encoding *Treacle* and fish MPP10 (used as a GC marker) fused to different fluorescent protein variants, they confirmed that the *Treacle* body was nested in fibrillarin, which in turn was nested in MPP10. Furthermore,

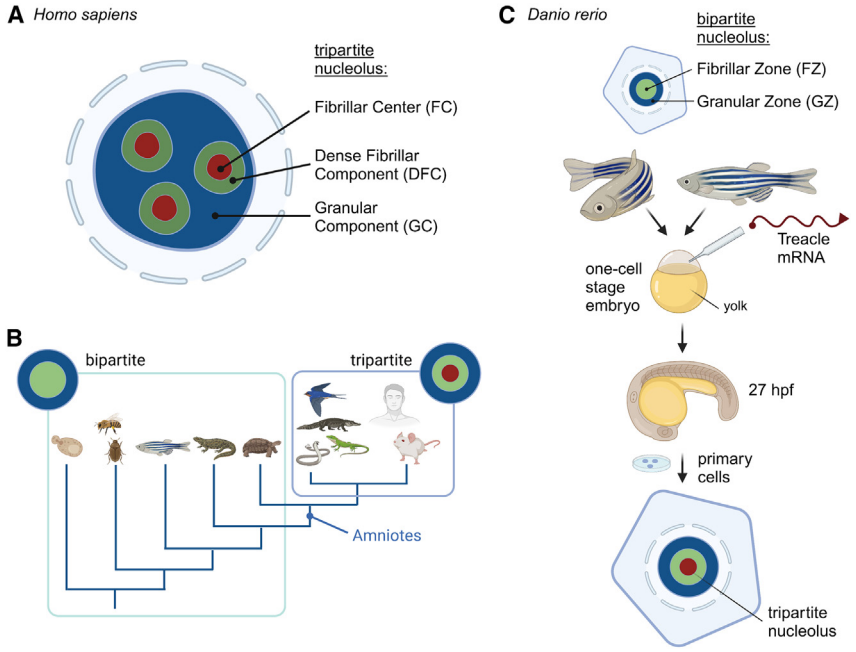

Figure 1. Model of the involvement of *Treacle* in the conversion of bipartite to tripartite nucleoli at the anamniote-amniote transition

(A) Typical tripartite nucleoli (e.g., human cells) with EM-visible FC, DFC, and GC layers.

(B) Anamniote-to-amniote transition coinciding with FC acquisition. Schematics of bipartite and tripartite nucleoli are shown.

(C) Converting a fish bipartite nucleolus to a tripartite one by expressing *Treacle*.

hpf, hours postfertilization.

upon analyzing where rRNA was synthesized in their hybrid nucleoli by means of metabolic labeling with a traceable nucleotide, they observed nascent transcripts forming a ring at the periphery of the artificially induced FC, precisely where it would be expected in tripartite nucleoli.

In a nutshell, expression of *Treacle* alone in a fish cell with bipartite nucleoli was sufficient to reorganize the nucleolus, inducing formation of a nested FC-like body, and to give rise to a core-shell architecture similar to that of tripartite nucleoli.

What might be the benefit of having well-defined fibrillar centers? Both regulatory aspects and an increased adaptive capacity have been considered. One hypothesis is that FCs are repositories of factors important for rRNA synthesis and "poised for action," enabling cellular ribosome production to respond promptly to environmental cues and cell needs.[3] Another is that an additional compartment offers more options for specific sequestration of factors, for process fine-tuning.[3]

Treacle is a disease protein important for optimal rRNA synthesis[8] and its mutation can lead to Treacher Collins Syndrome (TCS, also known as mandibulofacial dysostosis), a disease associated with severe facial abnormalities caused by impaired neural crest cell (NCC) maturation during embryogenesis.[9] NCC loss results from excessive p53-dependent apoptosis due to nucleolar stress activation.

In light of this work, it will be interesting to investigate whether *Treacle* phosphorylation contributes to condensation, scrutinize nucleolar morphology in TCS patients for possible FC alterations, and expand bottom-up *in vitro* nucleolar reconstitution (a DFC-GC core has been beautifully constructed[10]—it would be nice to add an FC). As there are diseases linked to both insufficient (e.g., TCS) and excessive (cancer) production of functional ribosomes, it could be of therapeutic value to modulate ribosome biogenesis by changing *Treacle* expression, altering FC size, and perhaps altering the presentation of factors important for rRNA synthesis at the FC/DFC interface.

DECLARATION OF INTERESTS

The author declares no competing interests.

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