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Assembly of the nucleolus: in need of revision

Maria Carmo-Fonseca

Our current view of the nucleolus has been shaped by the concept that the organization of this prominent compartment within the nucleus is primarily dictated by its function, the making of ribosome subunits. Whether ribosome biogenesis is framed by a dedicated nucleolar scaffold has remained unclear. In this issue of *The EMBO Journal*, Caudron-Herger and colleagues present evidence for a nucleolar skeleton composed of non-coding RNA enriched in *Alu* repeat elements.

See also: **M Caudron-Herger *et al*** (November 2015)

Production of ribosomes is vital for life, and eukaryotic cells evolved a specialized compartment, the nucleolus, where ribosomal RNA genes are transcribed, pre-ribosomal RNA is processed, and pre-ribosomes are assembled (Pederson, 2010). Mammalian cells typically contain a few nucleoli that disappear during mitosis and form *de novo* in early G1 around ribosomal genes (rDNA). In humans, long stretches of rDNA arrays located on five distinct chromosomes coalesce to form between one and three nucleoli (Savino *et al*, 2001). The rDNA transcription units coupled with binding sites for the HMG-box protein UBF are sufficient to direct formation of a nucleolus (Grob *et al*, 2013). However, what drives the clustering of rDNA transcription units into only one or a few nucleoli per nucleus remains elusive. The new study by Caudron-Herger and colleagues identifies non-coding RNAs enriched in *Alu* repeat elements as potentially involved in this process.

There are over 1 million copies of *Alu* elements in the human genome, many of which localize within introns of protein-coding genes (Deininger, 2011). According to Caudron-Herger *et al* (2015), stable short intron-derived RNAs containing *Alu* repeat elements accumulate in the nucleolus and are essential for its integrity. In the absence of so-called *alu*RNAs, nucleoli dispersed into smaller domains and rDNA transcription was severely reduced. Nascent intronic *Alu* RNAs were previously shown to escape degradation after pre-mRNA splicing, being processed and packaged into metabolically stable small ribonucleoprotein particles (Jády *et al*, 2012). However, these previously described RNA species were exclusively detected in the nucleoplasm (Jády *et al*, 2012).

Caudron-Herger *et al* (2015) also carried out RNA tethering experiments using a *lac*O array consisting of multiple copies of the

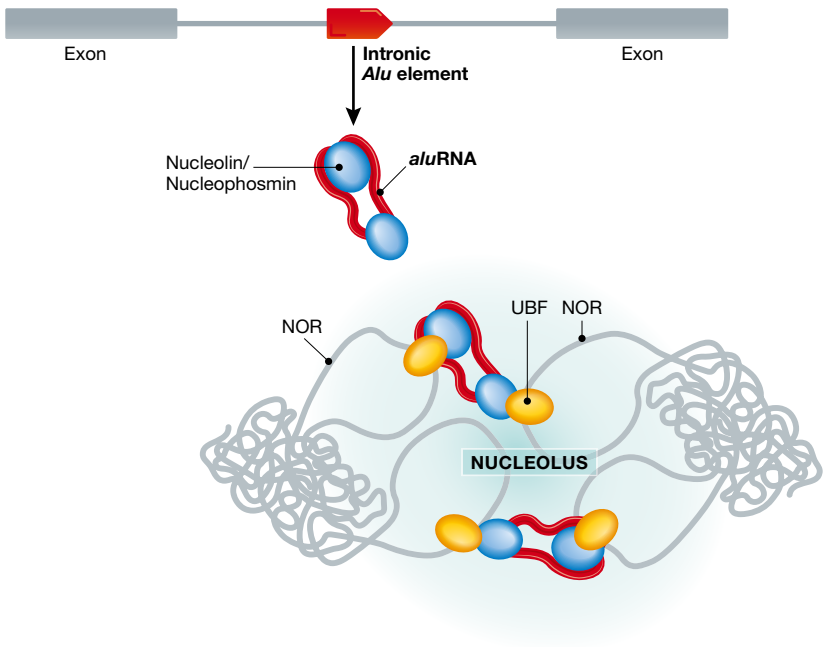

Figure 1. Scheme illustrating the *alu*RNA-scaffold model.
Splicing of pre-mRNAs containing intronic *Alu* elements generates short *alu*RNAs that associate with nucleolar proteins nucleolin and nucleophosmin. These *alu*RNAs, which are capable of attracting genomic loci to the nucleolus, create a scaffold that may contribute to clustering of nucleolar organizing regions (NORs) from different chromosomes via interactions with UBF.

Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal. E-mail: carmo.fonseca@medicina.ulisboa.pt
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binding sequence for the *lac* repressor protein, LacI (Shevtsov & Dundr, 2011). A*lu*RNA was tagged with MS2 stem loops and immobilized at the *lac*O array through binding to MS2 protein fused to LacI. This resulted in recruitment of the *lac*O genomic locus to the nucleolus. Nucleolin and nucleophosmin, two abundant structural proteins of the nucleolus, were further shown to interact *in vivo* with *alu*RNAs. Based on these observations, the following model was proposed: (i) pre-mRNA splicing of protein-coding genes containing intronic *Alu* repeat elements generates small RNA species termed *alu*RNAs; (ii) these RNAs act as a scaffold that recruits nucleolin and nucleophosmin; and (iii) interactions between nucleolin, nucleophosmin, and UBF result in coalescence of rDNA arrays that would otherwise disperse throughout the nucleus (Fig 1).

A remarkable feature of this model is that it explains the long-debated conundrum that specific inhibition of RNA polymerase II (Pol II) causes disintegration of nucleoli, although very little Pol II activity is detected in this nuclear compartment. Inevitably, however, this new model raises a number of open questions. For example, *Alu* elements are primate specific, and although targeted disruption of *Alu*-related B1-containing RNAs causes nucleolar dispersion in mouse cells (Caudron-Herger *et al*, 2015), it is intriguing how distinct RNA species co-evolved a common nucleolar scaffolding role. It is also puzzling that a subset of intron-encoded *Alu* RNAs generate nucleoplasmic box H/ACA RNPs (Jády *et al*, 2012), while others are essential for nucleolar organization. Additional mechanistic issues concern specificity of the interaction between nucleolar *alu*RNAs and the very abundant nuclear proteins nucleolin and nucleophosmin. A final remaining enigma is how nucleolar *alu*RNAs are functionally coupled to Pol I activity. Clearly, the *alu*RNA-scaffold model paves the way for further research that will likely expand our understanding of how ribosome biogenesis is controlled by nucleolar biology.

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