

EXTRA VIEW

## Plant nucleolar DNA: Green light shed on the role of Nucleolin in genome organization

Claire Picart<sup>a,b</sup> and Frédéric Pontvianne <sup>a,b</sup>

<sup>a</sup>CNRS, Laboratoire Génome et Développement des Plantes, UMR5096, F-66860, Perpignan, France; <sup>b</sup>Université de Perpignan Via Domitia, Laboratoire Génome et Développement des Plantes, UMR5096, F-66860, Perpignan, France

### ABSTRACT

The nucleolus forms as a consequence of ribosome biogenesis, but it is also implicated in other cell functions. The identification of nucleolus-associated chromatin domains (NADs) in animal and plant cells revealed the presence of DNA sequences other than rRNA genes in and around the nucleolus. NADs display repressive chromatin signatures and harbour repetitive DNA, but also tRNA genes and RNA polymerase II-transcribed genes. Furthermore, the identification of NADs revealed a specific function of the nucleolus and the protein Nucleolin 1 (NUC1) in telomere biology. Here, we discuss the significance of these data with regard to nucleolar structure and to the role of the nucleolus and NUC1 in global genome organization and stability.

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### Introduction

In eukaryotic cells, a precise organization of chromatin is essential for proper gene regulation and genome integrity. Epigenetic changes such as DNA methylation and post-translational histone modifications participate in the establishment of different states of chromatin condensation.<sup>1</sup> According to a classic textbook definition, heterochromatin is highly condensed and transcriptionally repressed, being mainly composed of repetitive DNA elements and transposons. Euchromatin is in a relaxed state permissive for transcription and is enriched in genes. However, deeper classifications of chromatin organization delineate several subcategories of chromatin.<sup>2</sup> One major challenge in cell biology today is to accurately situate the epigenome in its nuclear context. Chromatin organization within the nucleus is governed by several aspects of cell organization. Among these are intra and inter-chromosomal interactions, nuclear periphery association and localization to specialized nuclear bodies. It is clear now that the nuclear distribution of heterochromatin is regulated by several factors. In mammals, the nuclear lamina was shown to anchor a large portion of

heterochromatin at the nuclear periphery. Although the positioning of heterochromatin at the nuclear periphery is not solely dependent on the nuclear lamina, this process is crucial for genome integrity and its misregulation can provoke diseases in humans that include muscle dystrophies, neurological disorders or progeria syndromes.<sup>3</sup>

Heterochromatin was also shown to organize in and around the nucleolus, as demonstrated by the characterization of nucleolus-associated chromatin domains (NADs) in human cells.<sup>4,5</sup> Several megabases of genomic DNA encompassing at least one genomic region from each of the 23 chromosomes physically associate with the nucleolus, demonstrating that NAD identity is not determined by genetic linkage to rRNA (rRNA) gene arrays located on Chr, 13, 14, 15, 21 and 22.

In plant cells, we recently reported the identification of NADs from *Arabidopsis thaliana* leaf cells<sup>6</sup>: DNA purified from isolated nucleoli was analyzed by high-throughput sequencing.<sup>7</sup> In *A. thaliana*, like in human cells, NADs display heterochromatic signatures and encompass genomic domains from all 5 *A. thaliana* chromosomes. Moreover, NAD identification in a

**CONTACT** Frédéric Pontvianne  [fpontvia@univ-perp.fr](mailto:fpontvia@univ-perp.fr)  Bat T, Université de Perpignan, 58 Avenue Paul Alduy, F-66860, Perpignan, France

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*nucleolin 1* (*nuc1*) mutant, where the nucleolus structure is affected,<sup>8</sup> demonstrated several important aspects of NAD biology. Indeed, our recent study demonstrated the importance of rRNA gene expression for NAD composition, and the role of NUC1 protein in nucleolar heterochromatin organization and telomere maintenance. Here, we discuss the emerging role of the nucleolus in the nuclear organization of the epigenome and the impact of its alteration in the *nuc1* mutant.

### **NAD composition in *A. thaliana***

In *A. thaliana* leaf cells, NADs are mainly composed of sequences displaying 2 types of epigenetic features: regions enriched in dimethylated lysine 9 of Histone 3 (H3K9me2) or regions displaying trimethylated lysine 27 of Histone 3 (H3K27me3). H3K9me2 enriched regions correspond to genomic domains mainly composed of repeated elements such as transposons, while H3K27me3 enriched regions are mainly composed of silent genes or pseudogenes. In total, NADs represent around 4% of the genome, 3% of the genes and 11% of transposable elements in *A. thaliana*.<sup>6</sup>

This organization may differ depending on the cell type or stress condition analyzed, but we observed that 45S rRNA gene regulation is one important determinant of NAD composition. 45S rRNA genes are arranged in large tandem arrays called Nucleolus Organizer Regions (NORs). A change in NOR transcriptional activity modifies its subnuclear localization, as demonstrated in mutant backgrounds that show perturbations in rRNA gene transcription, such as *nuc1* or *histone deacetylase 6* (*hda6*).<sup>9-11</sup> As a consequence, changes in NOR transcription affect the global organization of chromosomes in the nucleus. In wild-type *A. thaliana* (ecotype Col-0), only the short arm of chromosome 4 (Chr4S) associates with the nucleolus, due to *NOR4*-derived rRNA gene expression. However, in the *nuc1* mutant where *NOR2* rRNA genes also become transcriptionally active, the short arm of chromosome 2 (Chr2S) now associates with the nucleolus. This observation demonstrates the importance of rRNA gene regulation in the global organization of nuclear DNA.

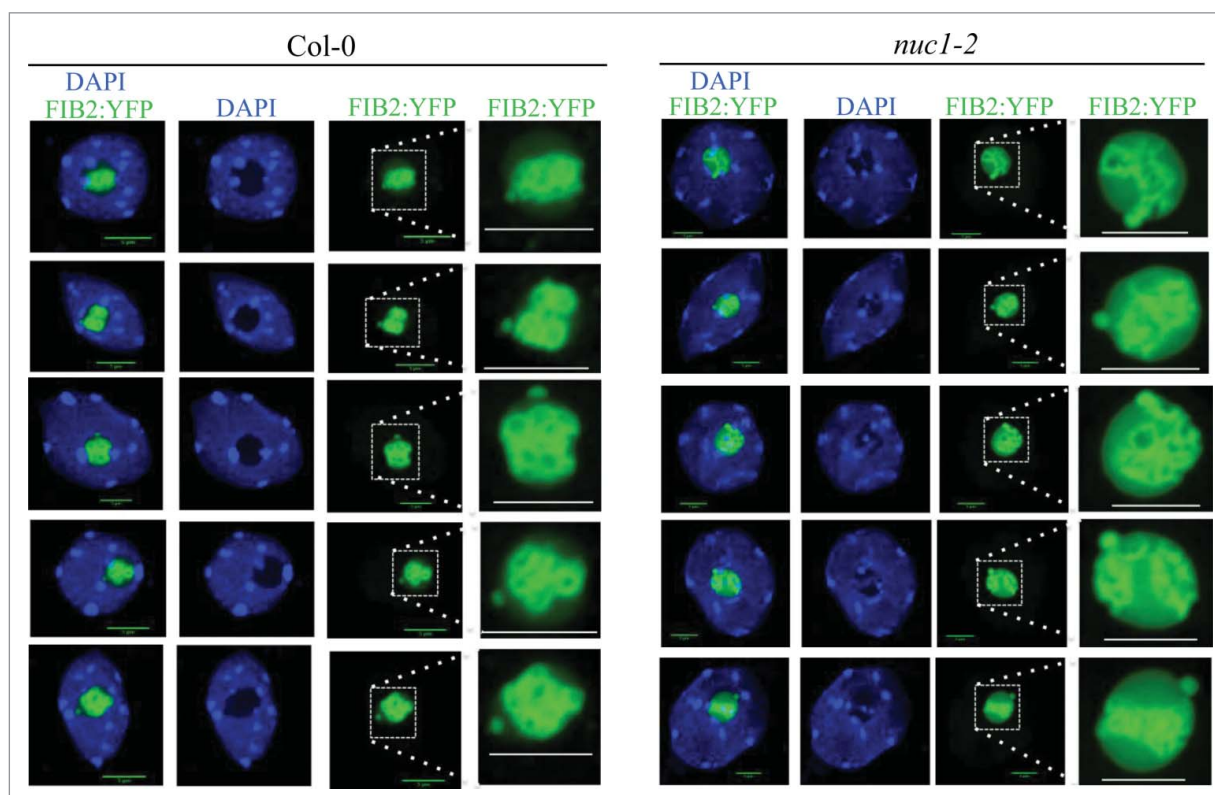
### **Heterochromatin and the nucleolus**

It has been known for decades that the nucleolus is surrounded by a layer of highly condensed late-replicating heterochromatin, often called the nucleolar shell.<sup>12</sup>

Whether this localization was an indirect consequence of the transcriptional activity of DNA recruited to the nucleolar shell or whether nucleolar proteins were directly implicated in nucleolar heterochromatin tethering remained an open question for several years. However, studies performed in *Drosophila* demonstrated a direct role for the respective protein homologs of Nucleolin (known as Modulo in *Drosophila*) and Nucleoplasmin.<sup>13</sup> These two nucleolar proteins form a stable platform together with the CCCTC-binding factor to anchor centromeres at the periphery of the nucleolus in *Drosophila*.<sup>13</sup> In our recent study in *A. thaliana*, analyses of NADs in the *nuc1* mutant revealed a perturbed association of centromeres with the nucleolus: centromere association with the nucleolus was slightly increased in the *nuc1* mutant. In addition, heterochromatin can be detected in a larger proportion of nucleoli in the *nuc1* mutant as compared to wild-type nucleoli. Previous analyses of subnucleolar compartments in *nuc1* by transmission electron microscopy revealed defects in their organization. All the 3 nucleolar compartments normally observed in eukaryotic cells—the granular component (GC), dense fibrillar component (DFC) and fibrillar center (FC)—are disrupted in *nuc1*.<sup>8</sup> We know that rRNA genes transcription and processing is affected in these plants,<sup>10,14</sup> but we wanted to see whether intra-nucleolar heterochromatin could be responsible for this disorganization. Fibrillarin is a nucleolar protein implicated in the snoRNA-guided methylation of rRNA precursors in the DFC.<sup>15,16</sup> Therefore, Fibrillarin mainly localizes to the DFC, forming ring-like structures in the nucleolus (Fig. 1A). However, in the *nuc1* mutant, *A. thaliana* homolog Fibrillarin 2 (FIB2) subnucleolar localization varies dramatically; instead of forming ring-like structures, it surrounds the intra nucleolar heterochromatin signals (Fig. 1B). These data suggest that the intra-nucleolar chromatin accumulating in *nuc1* nucleoli affect the typical organization of nucleolar factors implicated in ribosome biogenesis. Additional analyses should reveal whether or not this observation is also true for other factors required for proper pre-rRNA processing.

### **Role of NUC1 in the phase separation of sub-nucleolar domains**

The anchoring of centromeres and heterochromatin at the nucleolar periphery probably helps keep heterochromatin away from the nucleoplasm, where most RNA Polymerase II transcription of genes occurs. Conversely,



**Figure 1.** Chromatin and Fibrillarin distribution in WT and *nuc1* nuclei Confocal images of 5 nuclei from WT (A) or *nuc1* mutant (B) expressing the Fibrillarin fused to YFP (green). DNA is labeled by DAPI (blue) and scale bars represent 5  $\mu$ m.

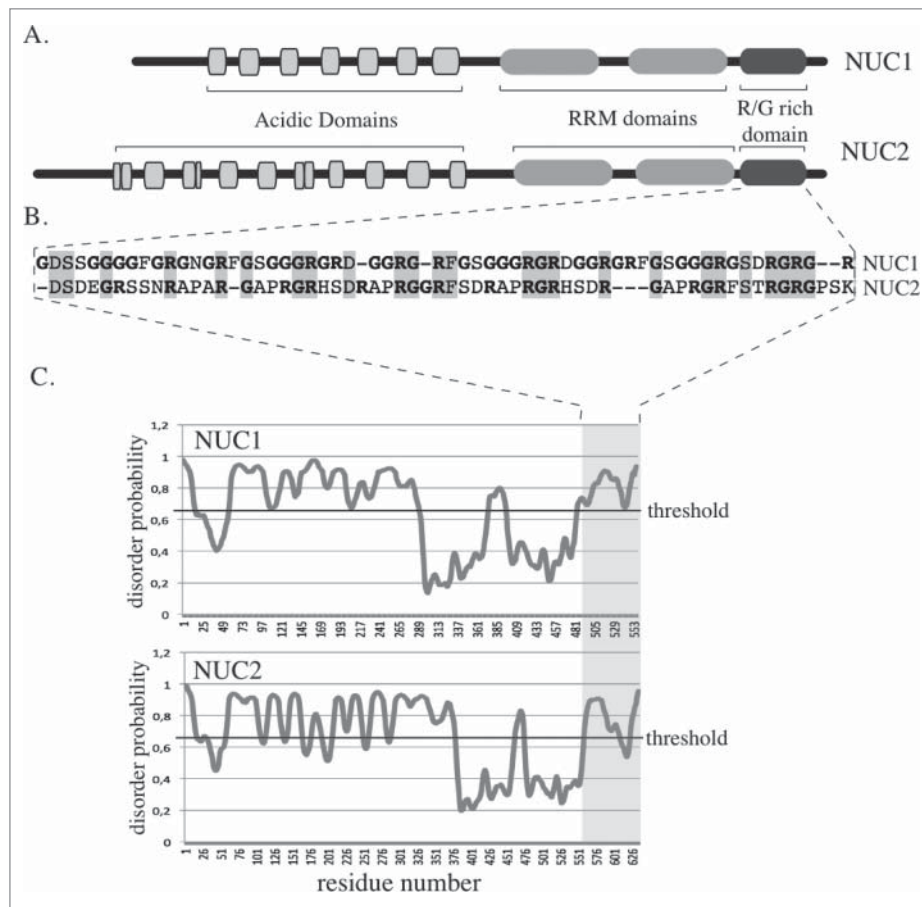
this heterochromatin tethering would also protect the nucleolus from being invaded by heterochromatin. In human cells, recent work demonstrated how 2 nucleolar proteins, Fibrillarin and Nucleophosmin, can phase separate into droplets similar to subnucleolar compartments *in vitro* and *in vivo*.<sup>17</sup> This is partially due to the physical properties of the Arginine/Glycine (R/G) rich disordered domain of Fibrillarin. The combination of the R/G-rich domain and at least one RNA Recognition Motif (RRM) in the Fibrillarin protein is required for proper subnucleolar compartment formation and maintenance.<sup>17</sup>

In *A. thaliana* there is no obvious homolog of Nucleophosmin, but NUC1, one of the most abundant nucleolar proteins, also combines 2 RRM domains with a disordered R/G rich domain in its C-terminal region (Fig. 2). Knock-out mutations of *nuc1* provoke nucleolar disorganisation and, as mentioned before, heterochromatin entering the nucleolus (Fig. 1). The *A. thaliana* genome encodes a second Nucleolin homolog, NUC2, which is not expressed in the leaf cell. However, in a *nuc1* mutant background, NUC2 becomes expressed and appears to partially complement NUC1 deficiency because the *nuc1 nuc2* double mutant is lethal.<sup>8,18</sup> Why NUC2 complementation of NUC1 is

only partial remains an open question, but sequence comparison reveals the presence of less (R/G) residues in the C-terminal part of NUC2 (Fig. 2). 45 R/G residues out of 60 are found in NUC1, while only 26 out of 59 are present in NUC2, affecting its disordered domain as analyzed using the Protein Disorder prediction System PrDOS<sup>19</sup> (Fig. 2). We propose that the subnucleolar disorganisation observed in *nuc1* mutants is due to the reduction of this R/G domain in the remaining Nucleolin homolog, NUC2, preventing it from establishing proper subnucleolar compartments. Whether the intranucleolar localization of heterochromatin is a cause or a consequence of subnucleolar disorganisation observed in *nuc1* mutants is unknown, but a systematic analysis of mutants with disrupted heterochromatin organization or altered nucleolus function might help answering this question.

### ***NUC1 is required for global genome organization and stability***

In *A. thaliana*, telomeric and subtelomeric regions cluster at the nucleolus.<sup>20-22</sup> NAD identification in *A. thaliana* confirmed the association of telomeres with



**Figure 2.** NUC1 and NUC2 proteins organization A. Schematic representation of NUC1 (AT1G48920) and NUC2 (AT3G18610) proteins from *A. thaliana*. B. Amino acid sequence alignment of the R/G rich domains of NUC1 and NUC2. C. Disorder profile plots of NUC1 and NUC2 amino acid sequences, obtained with the prediction software PrDOS. The black lines correspond to the threshold for a 2% prediction false rate.

the nucleolus in wild-type plants, but also revealed decreased telomere association with the nucleolus in the *nuc1* mutant.<sup>6</sup> Further investigation demonstrated that NUC1 co-immunoprecipitates with a telomerase. In addition, telomeres are shortened in the *nuc1* mutant, including telomeres not adjacent to NORs.<sup>6</sup> These observations indicate that NUC1 itself may play a direct role in telomere biology. Human Nucleolin interacts with a double-stranded telomeric probe *in vitro*.<sup>23</sup> But defects in telomere length could also be an indirect consequence of decreased telomere clustering at the nucleolus. The nucleolus may offer an environment that facilitates telomere protection and/or maintenance. In fact, the nucleolus has been described in animal and plant cells as a site where factors implicated in telomerase elongation are at least transiently detected (reviewed in<sup>24,25</sup>).

In *Drosophila* and yeast, the number of rRNA genes influences heterochromatin formation, global gene expression and genome stability.<sup>26-28</sup> However,

shorter telomeres are a sign of genome instability in *nuc1*. Further analyses of the *nuc1* mutant should reveal if telomeric regions defects is solely due to the absence of NUC1, or if it is also an indirect effect of changes in the global genome organization.

### Concluding remarks

Our recent work demonstrated the importance of the nucleolus in global genome organization, as well as its role with the NUC1 protein in ensuring genome stability.<sup>6</sup> This study extends previous investigations of NADs in human cells<sup>4,5</sup> and addresses novel aspects of genome organization in Arabidopsis. However, several new questions have been raised by our own studies and those of other researchers. Is the nucleolar association of NAD-genes required for their transcriptional regulation? Does the pool of NAD loci depend on cell type? Are NADs affected by stress conditions? The identification of NADs in different cell types and



growth conditions together with transcriptome analyses should allow us to determine whether the pool of NADs is dynamic, and to explore its potential role in gene regulation. Finally, our data suggest a direct link between ribosome biogenesis and genome organization within the nucleolus and further analyses should help clarify how these 2 fundamental aspects of the nucleus biology are interconnected.

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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### Notes on contributors

CP and FP performed the experiments. FP designed the experiments, analyzed the data, and wrote the manuscript.

### ORCID

Frédéric Pontvianne  <http://orcid.org/0000-0002-2913-4104>

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