

Ribosomal RNA genes shape chromatin domains associating with the nucleolus

Ariadna Picart-Piccolo , Nathalie Picault , and Frédéric Pontvianne 

CNRS, Laboratoire Génome et Développement des Plantes (LGDP), Perpignan, France; Université de Perpignan Via Domitia, LGDP, Perpignan, France

ABSTRACT

Genomic interactions can occur in addition to those within chromosome territories and can be organized around nuclear bodies. Several studies revealed how the nucleolus anchors higher order chromatin structures of specific chromosome regions displaying heterochromatic features. In this review, we comment on advances in this emerging field, with a particular focus on a recent study published by Quinodoz *et al.*, that developed a new method to characterize simultaneous genomic interactions in the same cell. Highlighting studies conducted in animal and plant cells, we then discuss the establishment of inactive chromatin at nucleolus organizer region (NOR)-bearing chromosomes.

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Introduction

In 1928, the cytogeneticist Heitz described two major chromatin states that differ according to their condensation level in the nucleus [1,2]. He named the condensed chromatin regions heterochromatin, while more loosely packed chromatin regions were named euchromatin. The identification of genome-wide chromatin–chromatin interactions maps confirmed this bipartite organization. Today, euchromatin and heterochromatin are generally referred to as A/B compartments [3]. This type of organization is relevant to both plant and animal cells [4]. However, this bipartite organization can be subdivided into several states according to their epigenetic signatures such as, but is not restrictive to, DNA methylation, histone modifications, histone variant distribution or nucleosome positioning [5–7]. Notably, these different states strongly participate in gene transcriptional regulation by modifying the access, or the attractiveness, of any loci to the transcriptional machineries.

At the nucleus scale, cytological analyses already revealed the existence of chromosome territories that can be distributed randomly, as in *Arabidopsis thaliana* or non-randomly, as in human cells, within interphase nuclei [8–11]. In addition, recent technical advances deeply increased our knowledge of how the genome is organized within the nucleus. For

example, the development of the chromosome conformation capture (3C) approach to a genome-wide scale revealed in various organisms and cell-types how inter and intra-chromosomal DNA contacts shape this 3D organization [12]. These studies revealed the existence of topologically associating domains or (TADs) that are self-interacting genomic regions, separated by boundaries, and confined either in A or B compartments, depending on their chromatin signatures. In plants, although the presence of TADs is not obvious in *Arabidopsis thaliana*, they were described in other plant species with larger genomes [4,13].

Recent studies pushed forward our understanding of the 3D genome organization, by taking into consideration the subnuclear compartments such as the nucleolus and the nuclear speckles. In this review, we aim to combine data of recent studies that demonstrate an important role of ribosomal RNA genes distribution and expression in global genome organization.

The nucleolus, a platform for genome organization

Ribosomal RNA (rRNA) genes are organized as tandem repeats in specific genomic regions named nucleolus organizer regions (NORs) by

Barbara McClintock [14]. The nucleolus is the site of ribosome biogenesis, starting with rRNA transcription, and is present in most eukaryote cells. Its size is dependent on the ribosome biogenesis activity, but can represent a large volume of the cell, especially in actively dividing cells [15]. In human cells, several nucleoli are usually present, while in *A. thaliana*, usually one round nucleolus is present. The nucleolus is a direct consequence of ribosome biogenesis. However, this large subnuclear compartment is also implicated in other cellular processes, especially in the maturation or sequestration of several ribonucleoproteins complexes such as the spliceosome, the proteasome, the RNA telomerase and others implicated in RNA degradation [15–20].

In 2010, two studies conducted in human cells demonstrated the direct role of the nucleolus in genome organization [21,22]. DNA co-purified with nucleoli from two types of human cells were analyzed, demonstrating that all chromosomes contain, at least, one region associating with the nucleolus. These genomic regions were named NADs for nucleolus-associated chromatin domains. In parallel, a network composed by nuclear lamina also anchors genomic regions with repressive epigenetic features at the nuclear periphery [23]. These regions are named LADs for lamina-associated domains. NADs were subsequently identified in *A. thaliana* cells [24]. In both plant and animal cells, excluding rRNA genes, NADs are composed of around 4% of the genome and display repressive chromatin features [24]. Interestingly, genes are also present in NADs and tend to be lowly expressed. Majority of NADs are presumed to be located at the nucleolus periphery, but cytological analyses demonstrated that the DNA inside the nucleolus is not only composed of rRNA genes [24]. Since RNA polymerase II is absent from inside the nucleolus, it has been proposed that the nucleolus would have a negative impact on gene expression [25,26].

Among the important aspects pointed out by Nemeth *et al.* [22], is the potential redundancy between NADs and LADs. Additional studies confirmed this observation and demonstrated that a portion of LADs, identified as facultative LADs, can reshuffle stochastically either at the nuclear or the nucleolar periphery after mitosis [27,28]. Conversely, LADs remaining at the nuclear

periphery in the daughter cells are now identified as constitutive LADs [29]. In plant cells, no homolog of nuclear lamina proteins have been identified so far, but putative homologs like nuclear matrix constituent protein (NMCP), nuclear envelope-associated proteins (NEAPs) or CROWDED NUCLEI (CRWN) proteins, all localized at the nuclear periphery, are suspected to play a similar role [30–33]. Chromatin domains associating with proteins located at the nuclear periphery have not been identified so far, but Chang Liu's laboratory was able to identify the genomic regions associated with the nuclear periphery using a derived chromatin immunoprecipitation (ChIP) protocol. The nucleoporin NUP1, which is one of the subunits of the nuclear pore complex, was used as a bait [34]. As for NADs, chromosome regions identified in this study (hereafter referred to as NUP1-enriched domains) are enriched in repressive chromatin signatures, transposable elements and contain genes that tend to be lowly expressed [34]. Since at least a portion of NADs overlap with LADs in human cells, we analyzed the potential connection between NADs and NUP1-enriched domains in *A. thaliana* (Figure 1). At the chromosome scale, NADs correspond to genomic regions flanking telomeres, as well as the short arm of chromosome 4, where active rRNA genes are located [35,36]. In contrast, NUP1-enriched domains mainly correspond to peri-centromeric regions, and large portions of chromosome arms (Figure 1(a)) [24,34]. The partial overlap observed on the short arm of chromosome 4 could potentially link the nucleolus and the nuclear periphery. Interestingly, the heterochromatic knob, corresponding to the conspicuous gap located between the 3,5 and 4,5 MB region, only associate with the nucleolus.

Therefore, the overlap between NADs and NUP1-enriched domains appears minor, and genes usually either associate with the nuclear periphery or with the nucleolar periphery (Figure 1(b)).

Influence of RNA genes in nucleolus-associated chromatin domains and inactive hubs

The Hi-C approach allows the detection of direct or indirect chromatin interactions in a cell population [12]. However, Hi-C is not compatible to detect

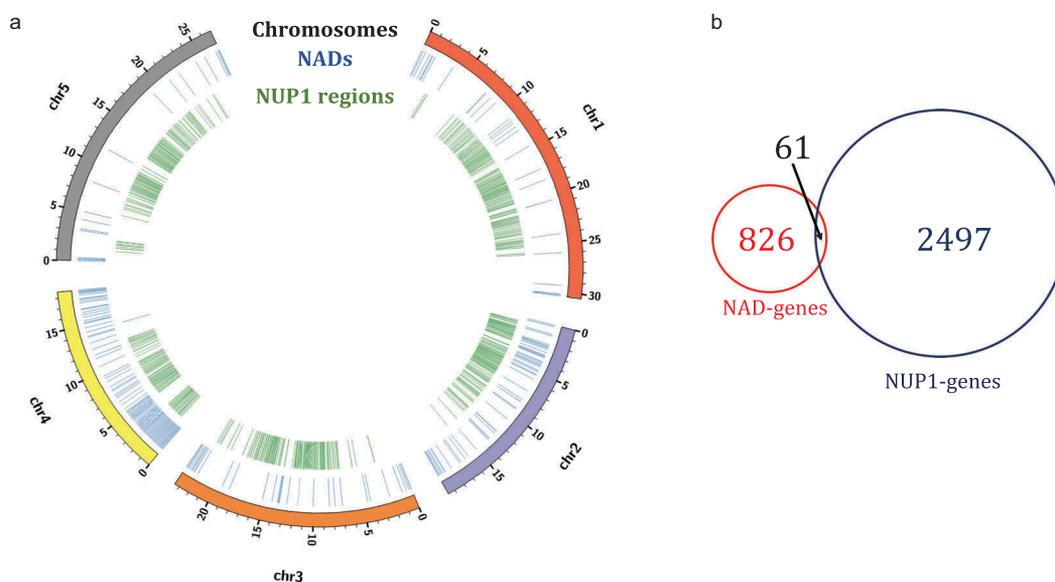


Figure 1. Overlap between NADs and NUP1-regions in *Arabidopsis thaliana*. (a) Genome-wide view of NAD-genes and the regions enriched at the nuclear periphery (NUP1-enriched regions) in the *A. thaliana* genome produced by Circos [49]. Considering the concentric circles from the outside inward, the outermost (and first) circle indicates the five chromosomes of *A. thaliana*. The next two circles are showing the location of NADs-genes (blue) and NUP-enriched regions (green) throughout the genome. (b) Venn diagram of NAD-genes and NUP1-enriched regions corresponding to protein-coding genes in TAIR10. Data from [24,34] were used to generate this figure.

simultaneous chromatin interactions and cell-to-cell variability. Single-cell Hi-C has recently been developed to counteract this problem, but sequencing depth remains an issue, leading to very low genome coverage [37]. In a recent study, Quinodoz and colleagues enable the detection of simultaneous interactions *via* the development of a new technique named SPRITE for split-pool recognition of interactions by tag extension [1]. SPRITE allows the detection of multiple chromatin interactions mediated by an individual complex within a nucleus, revealing the context of the interactions. Briefly, unique complex, from which all RNA, DNA and protein components are covalently crosslinked, are sorted in individual wells. Therefore, all DNA reads from one individual complex are identified as a SPRITE cluster [1]. In other words, a chromatin loop can be associated with its TAD and its compartment. Using the SPRITE technique, Quinodoz *et al.*, linked some A/B compartments with nuclear bodies. They identified active hubs, corresponding to A compartments, in association with nuclear speckles. In parallel, inactive hubs, corresponding to B compartments, were shown to associate with the nucleolus [1]. This corroborates previous

observations made in both animal and plant cells [25,38], regarding the important role of the nucleolus in organizing inactive chromatin regions at the nucleolar periphery.

Importantly, inactive hubs are present on rRNA-genes bearing chromosomes [1]. In other words, inactive hubs correspond to genomic regions flanking rRNA genes. This raises the question of the role of rRNA gene expression and location on chromosomes in defining the identity of inactive hubs at the nucleolar periphery. In *A. thaliana*, NADs are mainly composed of genomic regions corresponding to the NOR-bearing chromosomes 2 and 4, as well as genomic regions from the subtelomeric regions [24]. In wild-type leaves, only NOR4-derived rRNA genes associate with the nucleolus and are actively transcribed, while NOR2-derived rRNA genes are silent and excluded from the nucleolus [35,36,39–42]. Interestingly, NOR2-derived rRNA genes expression provokes a complete reorganization of the short arm of chromosome 2 that associates with the nucleolus [24]. Collectively, these data suggest that rRNA genes expression or silencing impact the global heterochromatin nuclear distribution. To test the

potential presence of inactive hubs in *A. thaliana*, we analyzed the global gene expression of nucleolus-associated genomic regions. As in mammalian cells, the short arms of NOR-bearing chromosomes tend to contain low-expressed genes compared to the entire genome (Figure 2). However, we do not see such correlation for genes of the subtelomeric regions that also associate with the nucleolus in a wild-type (Col-0) *A. thaliana* line [43].

Conclusions and perspectives

Overall, recent studies revealed the importance of nuclear bodies in the distribution of the epigenome. In these cases, nuclear body specific proteins play crucial roles in these regulations. For example, the nucleolar protein Nucleolin was demonstrated to regulate (i) telomere nucleolar clustering in *A. thaliana* [24], (ii) the two-cell embryo master regulator gene *DUX* in human embryonic stem cells [44] or (iii) centromere nucleolar clustering in drosophila [45]. A missing aspect is an overlap between inactive hubs identified using the SPRITE method and NADs identified in human cells [1,19,20]. We indeed expect NADs and inactive hubs to overlap. To further demonstrate how rRNA genes distribution in the genome defines NADs or inactive hubs, it will be important to identify these regions in cells with the variable NOR chromosomal location, or in mutant showing reduction or increase numbers of rRNA genes. Previous observations in yeast and in drosophila demonstrated that reducing the number of rRNA genes alter heterochromatin organization and genome stability [46–48]. In the light of these new

data, we expect to observe changes in global genome organization in cells with a reduced number of rRNA genes.

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ORCID

Ariadna Picart-Piccolo  <http://orcid.org/0000-0001-6380-7969>

Nathalie Picault  <http://orcid.org/0000-0003-2770-9688>

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

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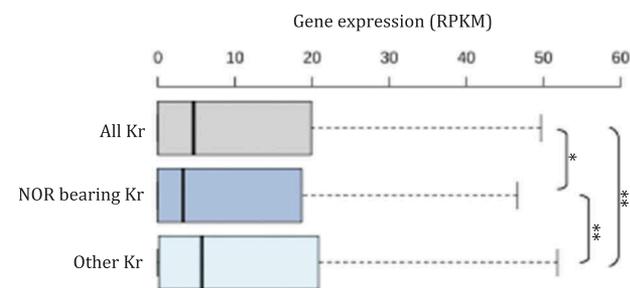


Figure 2. Gene expression of nucleolus-associated genomic regions in *A. thaliana*. Box plot representing the global expression of genes located in the short arm of NOR-bearing chromosomes 2 and 4 (NOR-bearing Kr) compare to other chromosomes 1, 3 and 5 (other Kr 1, 3 & 5) or all five chromosomes (all Kr) in *A. thaliana*. A p value < 0.05 *; p value < 0.01 : ** (Wilcoxon rank sum test).

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