Chromatin Domain Architecture and Its Role in Gene Regulation

Élodie Dubois

Department of Endocrinology, Sorbonne Medical Institute, France

Abstract

Genome organization displays functional compartmentalization. Many factors, including epigenetic modifications, transcription factors, chromatin remodelers, and RNAs, shape chromatin domains and the three-dimensional genome organization. Various types of chromatin domains with distinct epigenetic and spatial features exhibit different transcriptional activities. As part of the efforts to better understand plant functional genomics, over the past a few years, spatial distribution patterns of plant chromatin domains have been brought to light. In this review, we discuss chromatin domains associated with the nuclear periphery and the nucleolus, as well as chromatin domains staying in proximity and showing physical interactions. The functional implication of these domains is discussed, with a particular focus on the transcriptional regulation and replication timing. Finally, from a biophysical point of view, we discuss potential roles of liquid-liquid phase separation in plant nuclei in the genesis and maintenance of spatial chromatin domains.

Introduction

In eukaryotes, the nuclear DNA is wrapped around histone octamers to form the chromatin. Chromatin is subject to extensive modifications including DNA methylation and post-translational histone modifications [1]. These modifications, also named epigenetic marks, form the epigenome. To understand the three-dimensional genome organization in relation to local epigenetic states, it is also necessary to consider the subnuclear components that include (*i*) nuclear bodies such as the nucleolus, nuclear speckles and Cajal bodies, as well as (*ii*) nuclear pores and the nuclear periphery [2,3]. In mammalian cells, large chromatin regions associate at the nuclear periphery with a network composed of lamin fibers are named Lamina-associated domains (LADs) [4]. Some chromatin domains also associate with the nucleolar periphery, which actually belongs to nucleolus, and are named nucleolus-associated chromatin domains (NADs) [5,6]. Besides, mammal genomes predominantly form thousands of self-organizing chromatin domains known as topologically associated domains (TADs), which are relatively insulated from one another [7]. In plants, chromatin domains comparable to animal LADs, NADs, and TADs have been found. It should be pointed out here that

our knowledge of these plant chromatin domains is still preliminary, at the moment, they cannot be deemed fully equivalent to their animal analogues.

Genome organization is also highly dynamic, and is subjected to changes according to the cell cycle progression, developmental transition like commencing photomorphogenesis or flowering, and external cues [8]. For instance, in the presence of light, as a result of progressive compaction of heterochromatin, nuclei in germinating *A. thaliana* seedlings produce chromocenters, which appear as large, bright spots upon DAPI staining [9]. How are chromatin organization patterns, with a certain degree of orderliness in space, formed? For long, affinity between different molecules was thought to be the most important force determining how they are distributed in space. A protein can diffuse through the nucleus and thanks to its affinity and specificity to other factors, this protein might be retained longer in some nuclear compartment than others [10]. Recent advances also revealed the potential role of proteins possessing intrinsically disordered regions (IDRs) in the establishment and maintenance of nuclear compartments [11,12]. In this short review, we refer to plant “chromatin domains” as chromatin regions identified with methods concerning three-dimensional (3D) chromatin organization and positioning. With a focus on the demarcation and functionality of selected plant chromatin domains, we summarize and discuss recent progress in plant three-dimensional genomics.

Identification of plant chromatin domains from a 3D perspective

Functional annotation of plant long-range cis-regulatory elements

Besides identifying functional chromatin domains via acquiring a detailed picture of epigenomic and structural features (e.g., by using ChIP-seq and ATAC-seq approaches), investigating 3D chromatin conformation provides complementary structural and functional insights into them. In particular, this information is crucial for identifying gene(s) regulated by a given candidate enhancer element and *vice versa*. In the past decade, Hi-C (Chromosome Conformation Capture coupled with High Throughput Sequencing) has become the most widely used approach to study physical chromatin contact networks in 3D [13,14]. Hi-C approaches have been applied to a variety of plant species, from which both expected and surprising chromatin organization patterns as opposed to animals have been discovered (reviewed recently in [15-17]). Similar to those in animals, chromatin compartmentalization and local chromatin insulation are present in plants, implying that they can prevent chromatin regions from freely interacting with one another. Such spatial constraints of chromatin

contacts are part of how distal cis-regulatory elements regulate the expression of their target genes via establishing specific long-range physical interactions. Over the past few years, there have been increasing efforts in systematically identifying cis-regulatory elements and enhancers in various plant species, such as *A. thaliana* [18-22], rice [22-24], tomato [22,25], maize [26], and wheat [27]. These approaches are based on searching for chromatin regions with local structural and epigenetic features similar to those in animal genomes. A challenge downstream of this approach is how to correctly annotate these potential regulatory elements by assigning them to their target gene loci.

Hi-C can provide scientists information regarding chromatin domain interactions; however, Hi-C has limited sensitivity in systematically detecting chromatin loops, as it is financially costly to increase the sequencing depth of a genome-wide Hi-C map to boost the statistical power of loop calling. Nonetheless, Hi-C studies in Arabidopsis [28], rice [29], and cotton [30] show that chromatin regions involved in forming chromatin loops are enriched at gene promoters, reflecting the existence of extensive yet largely uncharted contacts between genes and their regulatory elements in plants. Compared to using Hi-C, one can better resolve spatial organization among chromatin domains with approaches that dedicate sequencing resource to genomic regions of interest. For instance, both the ChIA-PET (Chromatin Interaction Analysis by Paired-End Tag Sequencing) and HiChIP (Hi-C Chromatin Immunoprecipitation) methods aim to reveal chromatin interaction networks of regions associated with a defined chromatin mark or transcriptional regulator [31,32]. Recently, several studies using ChIA-PET unveiled chromatin interaction patterns associated with expressed genes in maize and rice [33-35]. The two maize ChIA-PET studies by Li et al. [33] and Peng et al. [34] focused on chromatin domains with H3K4me3, H3K27ac, and RNA Pol2, which were hallmarks of active promoter, enhancer, and transcribed regions, respectively. Collectively, their work identified unprecedented networks of promoter-enhancer and promoter-promoter interactions in maize, some of which were well known as contributors of important agronomic traits. Likewise, a recent ChIA-PET study of rice revealed physical interactions between many eQTLs (expression Quantitative Trait Loci) and their target genes [35].

In summary, these work demonstrate the advantage of identifying and annotating functional regulatory chromatin regions by integrating both one- and three-dimensional genomic features. In our opinion, a combinatory strategy with two steps can be considered as a standard practice for functional annotation of regulatory elements in

a given plant genome. The first step involves identifying chromatin regions with features of interest (e.g., epigenetic marks), and the second step involves using Hi-C-related methods that explore their chromatin-chromatin interaction networks

Identifying plant LADs and NADs

Another way of annotating 3D chromatin domains is based on their localization in the nucleus. In animals, active and repressed chromatin regions tend to be separated from each other, and some compartments in the nucleus, such as nuclear periphery and nucleolar periphery, are enriched with repressed chromatin [36,37]. Recently, chromatin domains preferentially localized at the nuclear and/or nucleolar periphery in *A. thaliana* have been identified (Figure 1a).

A. thaliana perinuclear chromatin domains were initially identified with an artificial system, which did not reveal direct interactions between the nuclear envelope and these domains [38]. Nevertheless, these plant perinuclear chromatin domains were enriched with various repressive marks (e.g., H3K27me3 and DNA methylation), suggesting that the plant nuclear periphery was a compartment in favor of holding repressed genes [38]. Later on, it was shown that some plant-specific nuclear lamin candidate proteins, CROWDED NUCLEI (CRWN), were required to tether chromatin to the nuclear periphery in *A. thaliana* [39,40]. By using CRWN1 as bait, chromatin domains bound by CRWN1 at the nuclear periphery (named plant LADs) were identified with chromatin immunoprecipitation [39]. Pattern analyses of plant LADs confirmed the previous findings that the plant nuclear periphery is a repressive environment [39].

The identification of NADs was achieved by isolating intact nucleoli [41,42]. In addition to ribosomal RNA loci, NADs are clearly enriched with lowly expressed protein-coding genes, as well as inactive chromatin marks and transposons [41]. Thus, plant LADs and NADs are both transcriptionally inactive; however, as they are located in different nuclear compartments, the respective silencing mechanisms might be different to a certain extent. For instance, the silencing of NAD-genes might be due to preventing RNA polymerase II from being associated with the nucleolus [43]. On the other hand, the plant lamin protein CRWN1 was shown to interact with PWWP INTERACTOR OF POLYCOMBS 1 (PWO1), which associated with *Polycomb*-group proteins, suggesting the involvement of H3K27me3-mediated transcriptional repression in LADs [44]. At the moment, research of plant LADs and NADs are still at an infant stage, as the knowledge of proteins required for forming these chromatin domains is extremely

limited. Also, it is not known how variable plant LADs and NADs demarcations are across different cell types and growth conditions. Given the highly dynamic nature of plant nuclei [45,46], we envisage that these plant chromatin domains possess a certain degree of flexibility, participating in modulating 3D genome organization and transcriptional regulations.

A comparison between *A. thaliana* LADs and NADs revealed that a tiny fraction of the genome is enriched both at the nuclear periphery and the nucleolus [47]; notably, most of these domains overlap with pericentromeric regions at chromosome 4, and to a less extent with those at chromosome 2 (Figure 1b). The occurrence of interchangeable perinuclear and nucleolar chromatin domains has been found in animals before [5,48]. A recent study of NADs identification in mouse embryonic fibroblast cells reported that a small subset of NADs were also frequently associated with the nuclear lamina [49]. These chromatin domains, shared by LADs and NADs (named “type I NADs”), appeared to be more heterochromatic; while the other type of NADs (“type II NADs”) tend to be relatively promoting gene expression and enriched with developmentally regulated genes [49]. We speculate that the chromatin domains shuffling between the nuclear periphery and the nucleolus in plants might be functionally distinct from the domains without such dual localization. For the *A. thaliana* genome, it would also be interesting to investigate whether these LAD/NAD interchangeable regions are involved in modulating dynamics of chromocenter (specifically chromosomes 2 and 4) structures during plants’ growth and development [50].

Finally, it should be pointed out here that apart from sequencing-based methods, high-to-super-resolution microscopic techniques, as complementary tools, serve critical roles in visualization and characterizing chromatin domains. The application of these methods in plant 3D genomics studies is increasing [51,52]; due to space limitation, we leave this undiscussed.

Functions of plant chromatin domains in 3D

In this section we discuss functional implications of the abovementioned chromatin domains.

co-expression of genes

In an earlier Hi-C work by Dong and colleagues, tomato and maize genomes were shown to form a large number of long-range chromatin loops linking interstitial active chromatin regions [53], suggesting spatial clustering of expressed genes. Later on, the

interaction networks of maize active chromatin were revealed by two research groups using the ChIA-PET method, and suggested a role for these physical interactions on gene expression [33,34]. Albeit the datasets from these two teams are difficult to compare due to the use of different growth conditions, tissues types, and antibodies (for ChIP) [33,34], three consensus patterns can be extracted. Firstly, a substantial fraction of the identified chromatin loops connects gene loci; secondly, genes forming long-range chromatin interactions tend to show higher expression levels than those without; thirdly, gene pairs linked with chromatin loops tend to show co-expression. In a recent rice ChIA-PET study, coordinated expression of active genes were also found among those connected by chromatin loops [35]. Together, these results strongly suggest that active chromatin domains in plant nuclei can form extensive physical contacts via chromatin interactions.

Earlier studies of gene expression in several plant species have pointed out that it is common to observe co-expression between neighboring genes [54-57]. An explanation of this phenomenon is that neighboring genes (especially those with overlapping divergent promoters) share some common *cis*-regulatory elements. The promoters of neighboring gene can also contact with one another via forming chromatin loops. Most of the reported plant promoter-promoter interactions are between physically linked loci in the genome [33,34]. Considering chromatin as a polymer, due to distance-dependent stochastic contacts, it is known that nearby genomic loci have much stronger contacts than do loci separated by large genomic distances [58]. This correlates well with the fact that Hi-C maps, regardless of species and cell types, always display strong contacts around their diagonal lines (indicative of interactions over short genomic distances). We speculate that stochastic contacts among loci along the chromatin fibre, as a function of genomic distance, contribute significantly to interactions between promoters and *cis*-elements. In addition, we also speculate that transcriptional regulators are involved in forming these chromatin contacts (Figure 1a) (see discussion in the next section). Together, the cooperative interactions among multiple transcribed loci form a spatial domain of “transcriptional ecosystem equilibrium” in the nucleus that fosters co-expression patterns [59]. Such physical interactions among active chromatin could be a mechanism underlying co-expression of metabolic genes residing close to each other (i.e., members belonging to a gene cluster in the linear genome) [60,61].

DNA replication timing

Spatial chromatin domain distribution is not only associated with gene transcription regulation, but also with other essential chromatin activities. As part of the cell cycle, DNA replication is a process by which genomic content is duplicated before cells enter mitosis. Interestingly, DNA replication timing across the genome is not homogeneous, rather, it displays a correlation to local histone marks and 3D chromosome structures [62]. In animals, euchromatin, which is localized in the nuclear interior, is replicated earlier than perinuclear-localized heterochromatin [62]. Similarly, studies comparing chromatin regions with different replication timing patterns in maize root tip nuclei showed that open chromatin and densely packed heterochromatin domains tend to be duplicated in early and late S phases, respectively [63,64]. The same correlation was seen in *A. thaliana* suspension cells, that repressed chromatin were enriched in late replicated loci [65,66]. Further, live imaging of *A. thaliana* replisomes revealed their dynamic distribution in early and late S phase [67]. All these observations suggest that plant DNA duplication happens in accordance with chromatin features (e.g., heterochromatin tends to be replicated late).

As mentioned above, *A. thaliana* nuclei show enrichment of repressed chromatin regions at the nuclear periphery and the nucleolus; therefore, it is expected that such chromatin compartmentalization correlate with late/early replication patterns. Indeed, for chromatin loci belonging to either LADs or NADs in *A. thaliana*, they clearly show a preference for being replicated in the last S phase (Figure 2a, b). Interestingly, further analyses on DNA replication origins with these chromatin domains reveal that the distribution of leading nascent strands over LADs and NADs are different (Figure 2c, d). Overall, among the nascent strands identified in a recent study [68], LADs overlap more with those pointing inward; while NADs overlap more with those pointing outward, suggesting that the replication of these two types of repressed chromatin domains are regulated by different mechanisms. Recent work in mammals has led to the identification of Early Replicating Control Elements (ERCEs) that play roles in regulating both DNA replication timing and 3D chromatin organization [69]. It would be interesting to study if plants also have such a mechanism that integrates DNA replication and chromatin organization. Many plant species can carry on endoreduplication, which is a process doubling the nuclear genome in the absence of mitosis [70]. As an extreme example, during tomato fruit development, the endopolyploidy level of pericarp cells can reach 512C (C is the haploid DNA content; and 512C means eight rounds of endoreduplication) [71]. So far, it is not known

whether the recurring DNA replication during endoreduplication cycle is accompanied with changes in chromatin organization and epigenetic landscape.

Liquid-liquid phase separation (LLPS) as a prominent biophysical process implicated in arranging chromatin domains in 3D

Role of liquid-liquid phase separation in the nucleus

LLPS (liquid-liquid phase separation) drives the formation of condensate or droplets via spontaneous nucleation of certain types of molecules at high concentrations. Recent advances clearly demonstrated the implication of this biophysical process in the establishment of non-membrane organelles [12]. In the nucleus, LLPS participates in the creation of functional hubs that allow the enrichment of factors required in a specific biological process such as mRNA biosynthesis or ribosome biogenesis [72] [Wei et al., 2019, BioRxiv, DOI: 10.1101/737387]. For example, the MEDIATOR complex subunit MED1 was shown to form nuclear puncta at enhancers, concentrating RNA polymerase II to achieve desired expression levels at target loci [73]. Also, droplets generated via LLPS can potentially act as “mechano-active chromatin filters” that change how genomic loci are localized relative to each other [72].

Recent work demonstrated that LLPS could act at a chromosomal scale (e.g., separating heterochromatin from euchromatin), at a scale of a chromatin loop, and also at a scale of the nucleosome [11,74-77]. For example, plant-specific Agenet Domain Containing Protein 1 (ADCP1) has been shown to drive the phase separation of H3K9me3-marked nucleosome arrays to form condensates [78]. Such a mechanism might be employed in the rice nucleoplasm to form physical contacts among multiple heterochromatin loci, which was reported in a recent ChIA-PET study [35]. Nucleosome arrays behave like LLPS, which has been shown to be modulated by linker histone H1, internucleosomal linker DNA lengths, and histone tail [79,80].

Proteins IDRs play a crucial role in the genesis and maintenance of phase-separated bodies. IDRs are usually composed of Arginine/Glycine (R/G) rich and/or Glutamine/Asparagine (Q/N) rich domains [81,82]. One of the best-studied cases of IDRs-mediated LLPS is the nucleolus [83]. In mammal cells, the R/G-rich domains of the nucleolar proteins Nucleoplasmin and fibrillarin were both shown to be required for the formation of the nucleolus, as well as for the sub-nucleolar compartments [84]. In plants, there is no homolog of Nucleoplasmin, but FIBRILLARIN 2 (FIB2), NUCLEOLIN

1 (NUC1), and many other nucleolar proteins possess strong IDRs (Figure 3). Thus, potential LLPS driven by these nucleolar proteins might be crucial for forming functional plant nucleoli. This hypothesis is supported by the fact that NUC1 disruption leads to the nucleolus disorganization [85,86].

Arabidopsis thaliana proteins with IDRs

Although the plant science community is aware of the potential importance of LLPS in shaping chromatin domains, there are few examples described in plants so far [15,87]. We therefore attempted to search for *A. thaliana* proteins containing IDRs with R/G-rich and/or Q/N-rich stretches (Figure 3a). Amongst the 27416 proteins encoded by the *A. thaliana* genome, 1234 R/G-rich and/or Q/N-rich IDR-containing proteins were identified (Supplemental Table 1). Interestingly, there are only 4 proteins that have both types of IDR motifs (Figure 3b). The 51 proteins containing at least 4 GGRG motifs are implicated in the RNA metabolism (GO:0016070; *p* value 3.5E-3) and are found in nuclear bodies like the nucleolus, nuclear speckles, photobodies or Cajal bodies (Figure 3a) [88-91]. This list is also composed of proteins known to localize in cytoplasmic bodies like processing bodies and stress granules (e.g., DCP5 and AGO1) [92,93].

Among the 80 proteins containing a long Q/N-rich stretch (at least 40 Q/N residues), half of them are implicated in transcriptional regulation (GO: 0006355; *p* value 1.05E-14). Notably, a member of this list, FCA, is involved in LLPS and required for proper transcriptional termination [94]. Our screen also identified a strong Q/N-rich IDR in NERD, a nuclear protein implicated in the 3' end formation of another subset of mRNAs [95]. In this case, proper mRNA termination requires both NERD and FIP37-dependent N6-adenosine mRNA methylation [95]. The fact that NERD forms nuclear foci through LLPS remains to be investigated. Additionally, we observed many transcription factors (e.g., MADS-box proteins) and sub-units of the MEDIATOR complex in this list (Figure 3a). MADS-box proteins have been long known for mediating chromatin looping via forming protein complexes [96,97]. MED25, which contains an extraordinarily strong Q/N-rich stretch, has been recently shown to be required for establishing chromatin contacts between enhancers and target genes in the jasmonate signaling pathway in plants [98]. Although our IDR-containing protein list implies an existing 3D interaction network functioning in transcriptional regulation (e.g., promoter-promoter interactions and cooperative transcription), the functional implication of these Q/N-rich stretches

remains to be evidenced in plants. A systematic analysis of the nuclear localization of plant IDRs should lead to the discovery of proteins implicated in LLPS-dependent nuclear puncta formation.

Perspectives

Recent advances have greatly helped scientists better understand the mechanisms by which chromatin domains are brought together in 3D. It is noteworthy that the list of chromatin organization regulators is expanding rapidly. Recent work from Xiang-Dong Fu and colleagues revealed the presence of a large, unexpected subset of RNA-binding proteins at numerous chromatin sites [99]. As many RNA-interacting proteins are implicated in LLPS, it is reasonable to speculate that some of them may regulate chromatin looping and compartmentalization. This study also gives us a hint that the interactions between RNA-binding proteins and chromatin in plants might have been unsuspected.

As discussed earlier, there is a correlation between chromatin domains and their local epigenetic signatures. Understanding the transcriptional activity of a given gene requires investigating not only its regulation in the context of linear sequence (e.g., epigenetic modification, transcription factor bindings, etc.), but also the regulation in the context of 3D chromatin (e.g., other genomic regions, RNAs, and proteins at its close proximity). Considering growing evidence in animal models [77], LLPS processes are likely to play an equally important role in the organization of plant chromatin and its partitioning into functional, spatially separated domains.

Conflict of interest statement

The authors declare no conflict of interest.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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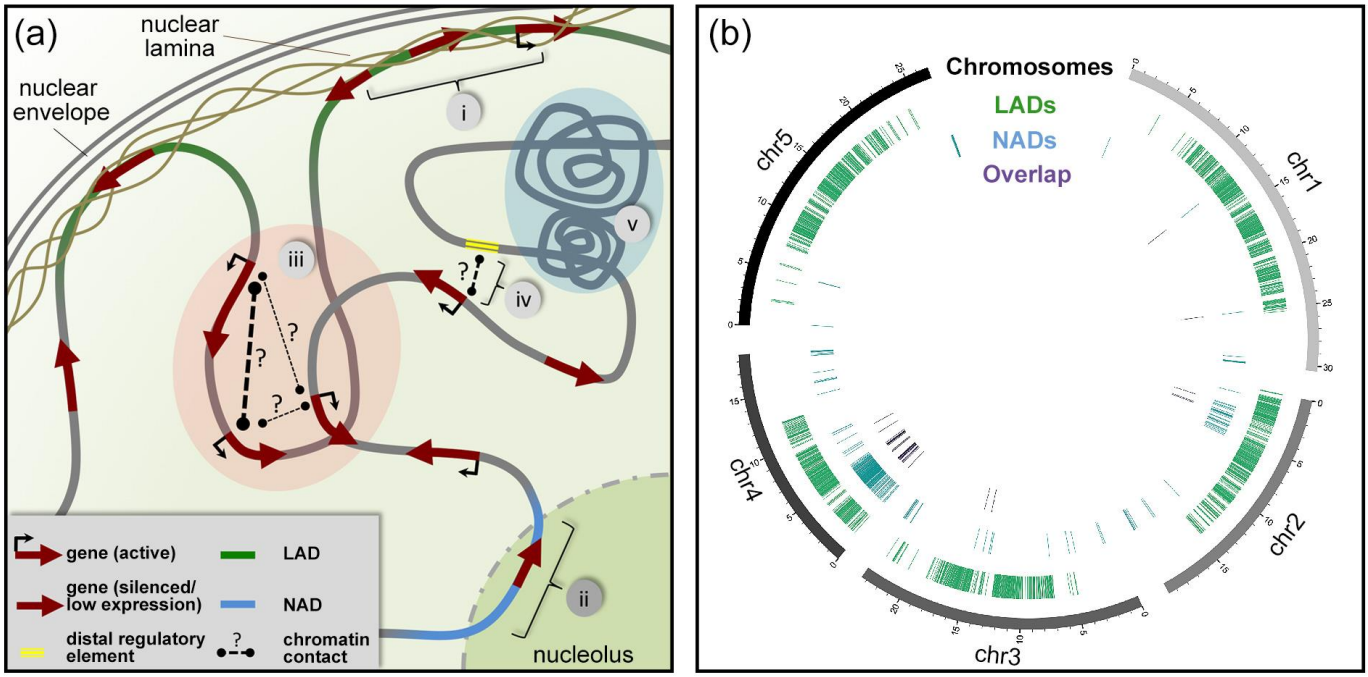

Figure 1. Spatial distribution of chromatin regions.

(a) A sketch illustrating spatial patterns of plant chromatin in the nucleus and their association with gene expression. Note that plants do not encode lamin proteins. The term “plant nuclear lamina” refers to filamentous protein structures that underlie the inner nuclear membrane [100]. Plant nuclear lamina very likely consists of plant-specific Nuclear Matrix Constituent Proteins (NMCP, also known as CRWN in *A. thaliana*) [101]. In general, chromatin regions located at the nuclear periphery (i) and at the nucleolus (ii) tend to be inactive. Recent studies have revealed a large number of chromatin contacts linking actively expressed gene with one another (iii), as well as with distal regions having potential roles in transcriptional regulation (iv). These chromatin contacts are established by factors yet unknown (question marks), which we speculate to be combinatorial activities of stochastic chromatin movements, specific bridging interactions of proteins and RNAs, and liquid-liquid phase separation. The contacts among expressed genes foster the formation of sub-compartments and coordinated transcription. Besides, multiple H3K9me-marked loci can interact with each other in the nucleoplasm (v), which is likely driven by liquid-liquid phase separation mediated by plant-specific ADCP1 proteins [78]. LAD, lamina-associate domains; NAD, nucleolus-associated domains. **(b)** Location of LADs and NADs loci across the *A. thaliana* genome. This circos plot is generated based on domain coordinates described in [39] and [41].

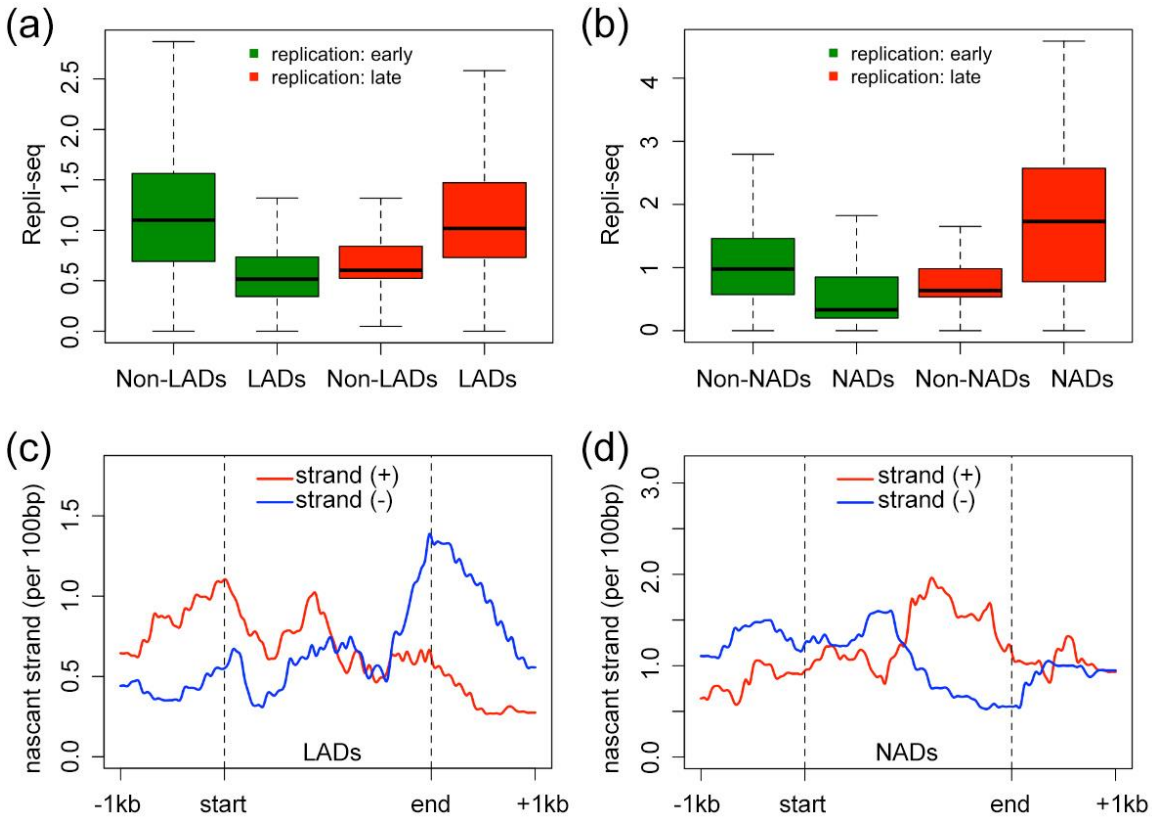

Figure 2. Association between DNA replication timing and chromatin localization in *A. thaliana*.

(a and b) Comparisons of *A. thaliana* DNA replication activities (measured with Repli-seq by Concia *et al.* [65]) in early and late S phase stages in LADs (a) and NADs (b)**.** **(c and d)** Distribution of leading nascent DNA strands in ORIs (DNA replication origin) across LADs (c) and NADs (d). Note that the dataset describing nascent DNA strand is from a study by Sequeira-Mendes *et al.* [68], in which a size cutoff (0.3 to 2 kb) was used so that the recovered nascent strands were primarily leading strands in ORIs. This information, in turn, can be used to infer whether ORIs occur evenly across a given genomic region. For instance, the curves in (c) imply that around LAD boundary regions, ORIs fire more often outside LADs than inside. Plant materials used for generating these datasets are partly comparable: Repli-seq, 7-day-old seedlings; nascent DNA strands, 4-day-old and 10-day-old seedlings; LADs, 10-day-old seedlings; and NADs, 3-week-old seedlings. Datasets and scripts for reproducing plots in panels (a-d) are available from figshare repository at https://figshare.com/articles/Plant_chromatin_localization_and_DNA_replication_timing/8953235

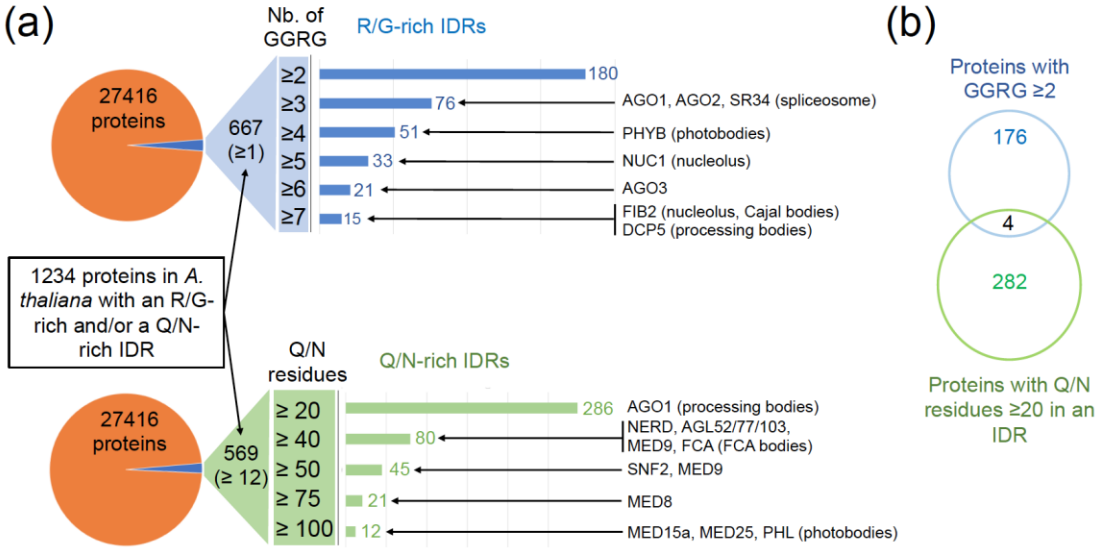

Figure 3. Identification of *A. thaliana* proteins containing an R/G-rich and/or a Q/N-rich IDR.

(a) Among the 27416 referenced proteins in the *A. thaliana* genome (TAIR10), 1234 possess at least a GGRG motif or a stretch of Q/N (at least 12 Q or N in 30 continuous amino acids). The numbers of proteins with more GGRG motifs or stronger Q/N stretch are presented. The key proteins are listed, and their respective subnuclear compartment is specified in brackets. **(b)** Venn diagram demonstrating the lack of overlap between proteins containing at least two R/G-rich and a strong Q/N-rich IDR. Protein sequences were downloaded from TAIR10 https://www.arabidopsis.org/. The identification of IDR-containing proteins was based on text mining with following criteria: R/G motifs were called if they exactly matched text string “GGRG”; Q/N motifs were called when at least 12 Q/N residues were found in a window of 30 amino acids. Overlapping Q/N motifs were further merged. For each IDR-containing protein, the number of R/G motifs and/or the number of Q/N residues in IDR can be found in Supplemental Table 1.

Reference highlight

- of special interest

•• of outstanding interest

•• Li E, Liu H, Huang L, Zhang X, Dong X, Song W, Zhao H, Lai J: **Long-range interactions between proximal and distal regulatory regions in maize**. *Nat Commun* 2019, **10**:2633.

The authors used the ChIA-PET method to investigate how maize chromatin regions marked with H3K4me3 and H3K27ac interacted with each other. A large number of chromatin loops identified in this study unveiled extensive promoter-promoter and promoter-enhancer contacts.

•• Peng Y, Xiong D, Zhao L, Ouyang W, Wang S, Sun J, Zhang Q, Guan P, Xie L, Li W, et al.: **Chromatin interaction maps reveal genetic regulation for quantitative traits in maize**. *Nat Commun* 2019, **10**:2632.

In this ChIA-PET study, the authors used antibodies against H3K4me3 and RNA polymerase to identify and analyze the maize chromatin interaction network of active chromatin regions. The authors show that expressed genes with promoters linked by chromatin loops tend to have co-expression patterns.

• Hu B, Wang N, Bi X, Karaaslan ES, Weber AL, Zhu W, Berendzen KW, Liu C: **Plant lamin-like proteins mediate chromatin tethering at the nuclear periphery**. *Genome Biol* 2019, **20**:87.

In this paper, the authors show that plant-specific protein CRWN1 is required to anchor repressed chormatin domains to the nuclear pheriphery in Arabidopsis. CRWN1 directly binds to inaccessible chromatin regions. This paper describes genome-wide interactions between chromatin and nuclear lamina in plants.

• Pontvianne F, Carpentier MC, Durut N, Pavlistova V, Jaske K, Schorova S, Parrinello H, Rohmer M, Pikaard CS, Fojtova M, et al.: **Identification of Nucleolus-Associated Chromatin Domains Reveals a Role for the Nucleolus in 3D Organization of the A. thaliana Genome**. *Cell Rep* 2016, **16**:1574-1587.

This paper reports the identification and characterization of Nucleolus-associated domains in Arabidopsis. The authors show that these nucleolar chromatin domains are enriched with silenced chromatin. Besides, the authors also show that the nucleolar protein NUC1 is required to maintain these chromatin regions in the nucleolus.

• Mikulski P, Hohenstatt ML, Farrona S, Smaczniak C, Stahl Y, Kalyanikrishna, Kaufmann K, Angenent G, Schubert D: **The Chromatin-Associated Protein PWO1 Interacts with Plant Nuclear Lamin-like Components to Regulate Nuclear Size**. *Plant Cell* 2019, **31**:1141-1154.

The authors show that the Arabidopsis lamin protein CRWN1 interacts with Polycomb-group proteins. This finding provides us mechanistic insights into how chromatin repression happens at the nuclear pheriphery in plants.

• Vertii A, Ou J, Yu J, Yan A, Pages H, Liu H, Zhu LJ, Kaufman PD: **Two contrasting classes of nucleolus-associated domains in mouse fibroblast heterochromatin**. *Genome Res* 2019.

In this paper, the authors report the identification of two types of nuceolar chromatin domains in mouse embryonic fibroblasts via differentiaing their different spatial localization patterns. Detailed pattern analyses reveal that these two types of domains also differ in their epigenetic profiles and are enriched with genes belonging to different funtional categories.

• Sima J, Chakraborty A, Dileep V, Michalski M, Klein KN, Holcomb NP, Turner JL, Paulsen MT, Rivera-Mulia JC, Trevilla-Garcia C, et al.: **Identifying cis Elements for Spatiotemporal Control of Mammalian DNA Replication**. *Cell* 2019, **176**:816-830 e818.

The authors discovered a long-sought cis-regulatory element which regulated DNA replication timing in mammlian nuclei. Functional studies of this type of elements reveal that it is also involved in modulating chromatin compartmentalization and localizatoin.

• Brangwynne CP, Mitchison TJ, Hyman AA: **Active liquid-like behavior of nucleoli determines their size and shape in Xenopus laevis oocytes**. *Proc Natl Acad Sci U S A* 2011, **108**(11):4334–4339.

The authors demonstrate that the nucleolus displays droplet-like behaviors. They also reveal that the nucleolar viscosity is ATP-dependent.

•• Fang X, Wang L, Ishikawa R, Li Y, Fiedler M, Liu F, Calder G, Rowan B, Weigel D, Li P, et al.: **Arabidopsis FLL2 promotes liquid–liquid phase separation of polyadenylation complexes.** *Nature* 2019, **569 :**65–269.

This paper described for the first time the identification of a coiled-coil protein, FLL2, in the formation of FCA nuclear bodies, a nuclear compartment that liquid-liquid

phase separate. The authors demonstrate that the FCA liquid-like body is required to compartmentalize the 3'-end processing machinery and facilitate polyadenylation at specific sites.

• Pontier D, Picart C, El Baidouri M, Roudier F, Xu T, Lahmy S, Llauro C, Azevedo J, Laudié M, Attina A,et al.: **The m(6)A pathway protects the transcriptome integrity by restricting RNA chimera formation in plants**. *Life Sci Alliance* 2019 **2**(3). doi:10.26508/lsa.201900393.

The authors show that the 3' end RNA processing of a subset of mRNA is controlled by an N6-adenosine (m^6A)-dependent pathway involving NERD, FIP37 and CPSF30L proteins. They also show that a defect in this pathway induces mRNA chimera formation.

•• Falk M, Feodorova Y, Naumova N, Imakaev M, Lajoie BR, Leonhardt H, Joffe B, Dekker J, Fudenberg G, Solovei I, et al.: Heterochromatin drives compartmentalization of inverted and conventional nuclei. Nature 2019, 570:395-399.

The authors show that by only considering 1) interactions between heterochromatin regions and 2) interactions between heterochromatin and lamina, they can model global genome organization patterns. This includes a special pattern seen in "inverted nuclei" (in rod cells of nocturnal mammals).