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1 **Chromatin domains in space and their functional implications**

2

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17 **Abstract**

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

31

32 **Introduction**

33 In eukaryotes, the nuclear DNA is wrapped around histone octamers to form the  
34 chromatin. Chromatin is subject to extensive modifications including DNA methylation  
35 and post-translational histone modifications [1]. These modifications, also named  
36 epigenetic marks, form the epigenome. To understand the three-dimensional genome  
37 organization in relation to local epigenetic states, it is also necessary to consider the  
38 subnuclear components that include (i) nuclear bodies such as the nucleolus, nuclear  
39 speckles and Cajal bodies, as well as (ii) nuclear pores and the nuclear periphery [2,3].  
40 In mammalian cells, large chromatin regions associate at the nuclear periphery with a  
41 network composed of lamin fibers are named Lamina-associated domains (LADs) [4].  
42 Some chromatin domains also associate with the nucleolar periphery, which actually  
43 belongs to nucleolus, and are named nucleolus-associated chromatin domains (NADs)  
44 [5,6]. Besides, mammal genomes predominantly form thousands of self-organizing  
45 chromatin domains known as topologically associated domains (TADs), which are  
46 relatively insulated from one another [7]. In plants, chromatin domains comparable to  
47 animal LADs, NADs, and TADs have been found. It should be pointed out here that  
48 our knowledge of these plant chromatin domains is still preliminary, at the moment,  
49 they cannot be deemed fully equivalent to their animal analogues.

50

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

68

## 69 **Identification of plant chromatin domains from a 3D perspective**

### 70 *Functional annotation of plant long-range cis-regulatory elements*

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

83 chromatin regions from freely interacting with one another. Such spatial constraints of  
84 chromatin contacts are part of how distal cis-regulatory elements regulate expression  
85 of their target genes via establishing specific long-range physical interactions. Over  
86 the past few years, there have been increasing efforts in systematically identifying cis-  
87 regulatory elements and enhancers in various plant species, such as *Arabidopsis* [18-  
88 22], rice [22-24], tomato [22,25], maize [26], and wheat [27]. These approaches are  
89 based on searching for chromatin regions with local structural and epigenetic features  
90 similar to those in animal genomes. A challenge downstream of this approach is how  
91 to correctly annotate these potential regulatory elements by assigning them to their  
92 target gene loci.

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

115 In summary, these work demonstrate the advantage of identifying and annotating  
116 functional regulatory chromatin regions by integrating both one- and three-dimensional  
117 genomic features. In our opinion, a combinatory strategy with two steps can be

118 considered as a standard practice for functional annotation of regulatory elements in  
119 a given plant genome. The first step involves identifying chromatin regions with  
120 features of interest (e.g., epigenetic marks), and the second step involves using Hi-C-  
121 related methods that explore their chromatin-chromatin interaction network.

122

### 123 *Identifying plant LADs and NADs*

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

130 *Arabidopsis* perinuclear chromatin domains were initially identified with an artificial  
131 system, which did not reveal direct interactions between the nuclear envelope and  
132 these domains [38]. Nevertheless, these plant perinuclear chromatin domains were  
133 enriched with various repressive marks (e.g., H3K27me3 and DNA methylation),  
134 suggesting that the plant nuclear periphery was a compartment in favor of holding  
135 repressed genes [38]. Later on, it was shown that some plant-specific nuclear lamin  
136 candidate proteins, CROWDED NUCLEI (CRWN), were required to tether chromatin  
137 to the nuclear periphery in *Arabidopsis* [39,40]. By using CRWN1 as bait, chromatin  
138 domains bound by CRWN1 at the nuclear periphery (named plant LADs) were  
139 identified with chromatin immunoprecipitation [39]. Pattern analyses of plant LADs  
140 confirmed the previous conclusion that the plant nuclear periphery is a repressive  
141 environment [39]. On the other hand, the identification of NADs was achieved by  
142 isolating intact nucleoli [41,42]. In addition to ribosomal RNA loci, NADs are clearly  
143 enriched with lowly expressed protein-coding genes, as well as inactive chromatin  
144 marks and transposons [41]. Thus, plant LADs and NADs are both transcriptionally  
145 inactive; however, as they are located in different nuclear compartments, the  
146 respective silencing mechanisms might be different to a certain extent. For instance,  
147 the silencing of NAD-genes might be due to preventing RNA polymerase II from being  
148 associated with the nucleolus [43]. On the other hand, the plant lamin protein CRWN1  
149 was shown to interact with PWWP INTERACTOR OF POLYCOMBS 1 (PWO1), which  
150 associated with *Polycomb*-group proteins, suggesting the involvement of H3K27me3-  
151 mediated transcriptional repression in LADs [44]. At the moment, research of plant

152 LADs and NADs are still at an infant stage, as the knowledge of proteins required for  
153 forming these chromatin domains is extremely limited. Also, it is not known how  
154 variable plant LADs and NADs demarcations are across different cell types and growth  
155 conditions. Given the highly dynamic nature of plant nuclei [45,46], we envisage that  
156 these plant chromatin domains possess a certain degree of flexibility, participating in  
157 modulating 3D genome organization and transcriptional regulations.

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

174

## 175 **Functions of plant chromatin domains in 3D**

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

### 178 *co-expression of genes*

179 In an earlier Hi-C work by Dong and colleagues, tomato and maize genomes were  
180 shown to form a large number of long-range chromatin loops linking interstitial active  
181 chromatin regions [51], suggesting spatial clustering of expressed genes. Later on, the  
182 interaction networks of maize active chromatin were revealed by two research groups  
183 using the ChIA-PET method, and suggested a role for these physical interactions on  
184 gene expression [33,34]. Albeit the datasets from these two teams are difficult to

185 compare due to the use of different growth conditions, tissues types, and antibodies  
186 (for ChIP) [33,34], three consensus patterns can be extracted. Firstly, a substantial  
187 fraction of the identified chromatin loops connects gene loci; secondly, genes forming  
188 long-range chromatin interactions tend to show higher expression levels than those  
189 without; thirdly, gene pairs linked with chromatin loops tend to show co-expression.  
190 Based on a recent rice ChIA-PET study, coordinated expression of active genes can  
191 also be found among those connected by chromatin loops [35]. Together, these results  
192 strongly suggest that active chromatin domains in plant nuclei can form extensive  
193 physical contacts via chromatin interactions.

194 Earlier studies of gene expression in several plant species have pointed out that it is  
195 common to observe co-expression between neighboring genes [52-55]. An  
196 explanation of this phenomenon is that neighboring genes (especially those with  
197 overlapping divergent promoters) share some common *cis*-regulatory elements. The  
198 promoters of neighboring gene can also contact with one another via forming  
199 chromatin loops. Most of the reported plant promoter-promoter interactions are  
200 between physically linked loci in the genome [33,34]. Considering chromatin as a  
201 polymer, due to distance-dependent stochastic contacts, it is known that nearby  
202 genomic loci have much stronger contacts than do loci separated by large genomic  
203 distances [56]. This correlates well with the fact that Hi-C maps, regardless of species  
204 and cell types, always display strong contacts around their diagonal lines (indicative of  
205 interactions over short genomic distances). We speculate that stochastic contacts  
206 among loci along the chromatin fibre, as a function of genomic distance, contribute  
207 significantly to interactions between promoters and *cis*-elements. In addition, we also  
208 speculate that transcriptional regulators are involved in forming these chromatin  
209 contacts (Figure 1a) (see discussion in the next section). Together, the cooperative  
210 interactions among multiple transcribed loci form a spatial domain of “transcriptional  
211 ecosystem equilibrium” in the nucleus that fosters co-expression patterns [57]. Such  
212 physical interactions among active chromatin could be a mechanism underlying co-  
213 expression of metabolic genes residing close to each other (i.e., members belonging  
214 to a gene cluster annotated in the linear genome) [58,59].

215

#### 216 *DNA replication timing*

217 Spatial chromatin domain distribution is not only associated with gene transcription  
218 regulation, but also with other essential chromatin activities. As part of the cell cycle,



219 DNA replication is a process by which genomic content is duplicated before a cell  
220 enters mitosis. Interestingly, DNA replication timing across the genome is not  
221 homogeneous, rather, it displays a correlation to local histone marks and 3D  
222 chromosome structures [60]. In animals, euchromatin, which is localized in the nuclear  
223 interior, is replicated earlier than perinuclear localized heterochromatin [60]. Similarly,  
224 studies comparing chromatin regions with different replication timing patterns in maize  
225 root tip nuclei showed that open chromatin and densely packed heterochromatin  
226 domains tend to be duplicated in early and late S phases, respectively [61,62]. The  
227 same correlation was seen in *Arabidopsis* suspension cells, that repressed chromatin  
228 were enriched in late replicated loci [63,64]. Further, live imaging of *Arabidopsis*  
229 replisomes revealed their dynamic distribution in early and late S phase [65]. All these  
230 observations suggest that plant DNA duplication happens in accordance with different  
231 chromatin features (e.g., heterochromatin tends to be replicated late). As mentioned  
232 above, *Arabidopsis* nuclei show enrichment of repressed chromatin regions at the  
233 nuclear periphery and the nucleolus; therefore, it is expected that such chromatin  
234 compartmentalization correlate with late/early replication patterns. Indeed, for  
235 chromatin loci belonging to either LADs or NADs in *Arabidopsis*, they clearly show a  
236 preference for being replicated in the last S phase (Figure 2a, b). Interestingly, further  
237 analyses on DNA replication origins with these chromatin domains reveal that the  
238 distribution of leading nascent strands over LADs and NADs are different (Figure 2c,  
239 d). Overall, among the nascent strands identified in a recent study [66], LADs overlap  
240 more with those pointing inward; while NADs overlap more with those pointing  
241 outward, suggesting that the replication of these two types of repressed chromatin  
242 domains are regulated by different mechanisms. Recent work in mammals has led to  
243 the identification of Early Replicating Control Elements (ERCEs) that play roles in  
244 regulating both DNA replication timing and 3D chromatin organization [67]. Certainly,  
245 it would be interesting to study if plants also have such a mechanism that integrates  
246 DNA replication and chromatin organization. Many plant species can carry on  
247 endoreduplication, which is a process doubling the nuclear genome in the absence of  
248 mitosis [68]. As an extreme example, during tomato fruit development, the  
249 endopolyploidy level of pericarp cells can reach 512C (C is the haploid DNA content;  
250 and 512C means nine rounds of endoreduplication) [69]. So far, it is not known whether  
251 the recurring DNA replication during endoreduplication cycle is accompanied with  
252 changes in chromatin organization and epigenetic landscape.

253

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

256 *Role of liquid-liquid phase separation in the nucleus*

257 Nuclear sub-compartments are membrane-less organelles or condensates and are  
258 characterized by liquid-phase properties. In that case, they are liquid-phase  
259 compartments and remain separated from each other through liquid-liquid phase  
260 separation (LLPS). LLPS form nuclear condensate or droplets, and are generated by  
261 spontaneous nucleation of a given molecules resent at a high concentration. These  
262 phenomenon participate in the creation of functional hubs that allow the enrichment of  
263 factors required in a specific biological process such as mRNA biosynthesis or  
264 ribosome biogenesis. Recent advances clearly demonstrated the implication of LLPS  
265 in the establishment of non-membrane organelles in the nucleus [12]. Proteins with  
266 intrinsically disordered regions (IDRs) play a crucial role in the genesis and  
267 maintenance of phase-separated bodies. Recent work demonstrated that LLPS could  
268 act at the scale of large chromatin domains (i.e TAD or NAD), at the scale of a  
269 chromatin loop to participate in transcriptional regulation and also at the scale of the  
270 nucleosome [11,70,71]. For example, plant-specific Agenet Domain Containing  
271 Protein 1 (ADCP1) has recently been shown to drive the phase separation of  
272 H3K9me3-marked nucleosome arrays to form condensates [72]. Such a mechanism  
273 might be employed in the rice nucleoplasm to create physical contacts between  
274 multiple heterochromatin loci, which was revealed by a ChIA-PET study [35]. For  
275 example, nucleosome arrays behave like LLPS, with histone tail and linker histone H1  
276 playing a substantial role in their level of compaction [73]. Interestingly, histone tail  
277 acetylation seems to be able to regulate LLPS of nucleosome arrays [74]. Another  
278 example is with the clustering of RNA polymerase II, which is due to LLPS mediated  
279 by the presence of IDRs in its C-terminal domain [75]. Furthermore, droplets generated  
280 via LLPS can potentially act as mechano-active chromatin filters. Most IDR-containing  
281 proteins indeed exclude chromatin, which explain why nuclear bodies usually display  
282 a low chromatin density. This mechanism facilitate chromatin factors to target genomic  
283 loci by changing their concentration in a given compartment [76] (Wei et al. 2019). For  
284 example, the MEDIATOR complex subunit MED1 was shown to form nuclear puncta  
285 at enhancers, concentrating RNA polymerase II to achieve desired expression levels  
286 at target loci [77].

287 IDRs are usually composed of Arginine/Glycine (R/G) rich and/or  
288 Glutamine/Asparagine (Q/N) rich domains [78,79]. One of the best-studied cases of

289 IDRs-mediated LLPS is the nucleolus [80]. In mammal cells, the R/G-rich domains of  
290 the nucleolar proteins nucleoplasmin and fibrillarlin were both shown to be required for  
291 the formation of the nucleolus, as well as for the sub-nucleolar compartments [81]. In  
292 plants, there is no homolog of nucleoplasmin, but FIBRILLARIN 2 (FIB2), NUCLEOLIN  
293 1 (NUC1), and many other nucleolar proteins possess strong IDRs (Figure 3). Thus,  
294 potential LLPS driven by these nucleolar proteins might be crucial for forming  
295 functional plant nucleoli. This hypothesis is supported by the fact that NUC1 disruption  
296 leads to the nucleolus disorganization [82,83].

297

### 298 *Arabidopsis thaliana* proteins with IDRs

299 Although the plant science community is aware of the potential importance of LLPS in  
300 shaping chromatin domains, there are few examples described in plants so far [15,84].  
301 We therefore attempted to search for *A. thaliana* proteins containing IDRs with R/G-  
302 rich and/or Q/N-rich stretches (Figure 3a). Amongst the 27416 proteins encoded by  
303 the *A. thaliana* genome, 1234 R/G-rich and/or Q/N-rich IDR-containing proteins were  
304 identified (Supplemental Table 1). Interestingly, there are only 4 proteins that have  
305 both types of IDR motifs (Figure 3b). The 51 proteins containing at least 4 GGRG  
306 motifs are implicated in the RNA metabolism (GO:0016070; *p* value 3.5E-3) and are  
307 found in nuclear bodies like the nucleolus, nuclear speckles, photobodies or Cajal  
308 bodies (Figure 3a) (Love et al. 2017; Zhu and Brangwynne 2015; Montacie et al. 2017;  
309 Li et al. 2019). This list is also composed of proteins known to localize in cytoplasmic  
310 bodies like processing bodies and stress granules (e.g., DCP5 and AGO1).

311 Among the 80 proteins containing a long Q/N-rich stretch (at least 40 Q/N residues),  
312 half of them are implicated in transcriptional regulation (GO: 0006355; *p* value 1.05E-  
313 14). Notably, a member of this list, FCA, is involved in LLPS and required for proper  
314 transcriptional termination [85]. Our screen also identified a strong Q/N-rich IDR in  
315 NERD, a nuclear protein implicated in the 3' end formation of another subset of mRNAs  
316 [86]. In this case, proper mRNA termination requires both NERD and FIP37-dependent  
317 N6-adenosine mRNA methylation [86]. The fact that NERD forms nuclear foci through  
318 LLPS remains to be investigated. Additionally, we observed many transcription factors  
319 (e.g., MADS-box proteins) and mediators in this list (Figure 3a). MADS-box proteins  
320 have been long known for mediating chromatin looping via forming protein complexes  
321 [87,88]. MED25, which contains an extraordinarily strong Q/N-rich stretch, has been  
322 recently shown to be required for establishing chromatin contacts between enhancers

323 and target genes in the jasmonate signaling pathway in plants [89]. Although our IDR-  
324 containing protein list implies an existing 3D interaction network functioning in  
325 transcriptional regulation (e.g., promoter-promoter interactions and cooperative  
326 transcription), the functional implication of these Q/N-rich stretches remains to be  
327 evidenced in plants. A systematic analysis of the nuclear localization of plant IDRs  
328 should lead to the discovery of proteins implicated in LLPS-dependent nuclear puncta  
329 formation.

330

### 331 ***Perspectives***

332 Recent advances have greatly helped scientists better understand the mechanisms by  
333 which chromatin domains are brought together in 3D. It is noteworthy that the list of  
334 chromatin organization regulators is expanding rapidly. Recent work from Xiang-Dong  
335 Fu and colleagues revealed the presence of a large, unexpected subset of RNA-  
336 binding proteins at numerous chromatin sites [90]. As many RNA-interacting proteins  
337 are implicated in LLPS, it is reasonable to speculate that some of them may regulate  
338 chromatin looping and compartmentalization. This study also gives us a hint that the  
339 interactions between RNA-binding proteins and chromatin in plants might have been  
340 unsuspected.

341 As discussed earlier, there is a correlation between chromatin domains and their local  
342 epigenetic signatures. In addition, to assess the transcriptional regulation of a given  
343 gene, it is essential to identify all the direct and indirect interacting-factors of the gene,  
344 i.e., the other genomic regions, RNA, and proteins [57]. Moreover, the supra-molecular  
345 arrangements created by LLPS also seem to play a role in protein regulation via their  
346 retention as demonstrated for the DNA methyltransferase DNMT1, retained in the  
347 nucleolus during acidosis in human cells (Audas et al 2012). With the growing evidence  
348 in animal models (Zhu and Brangwynne, 2015), LLPS processes are likely to play an  
349 equally important role in the organization of plant chromatin and its partitioning into  
350 functional, spatially separated domains. In animal cells, high-to-super resolution  
351 techniques have led to a better understanding of the 3D chromatin domain analyses  
352 (Shin Y et al, 2018; Wei et al, 2019; (Szabo et al. 2018). Although important progress  
353 have been made, plant cells specificity makes the use of these techniques more  
354 challenging (Dumur et al 2019).

355

356 **Conflict of interest statement**

357 The authors declare no conflict of interest.

358

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367

368 **References and recommended reading**

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

371 •□of special interest

372 ••□of outstanding interest

373

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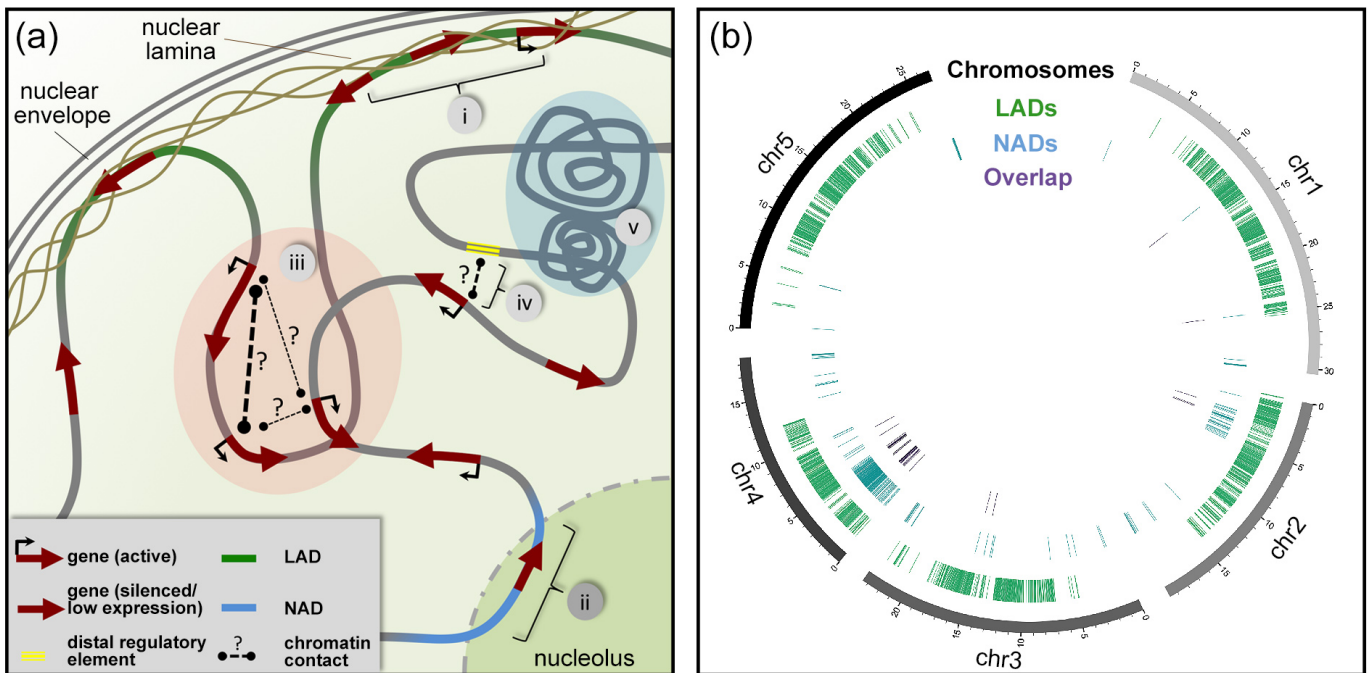


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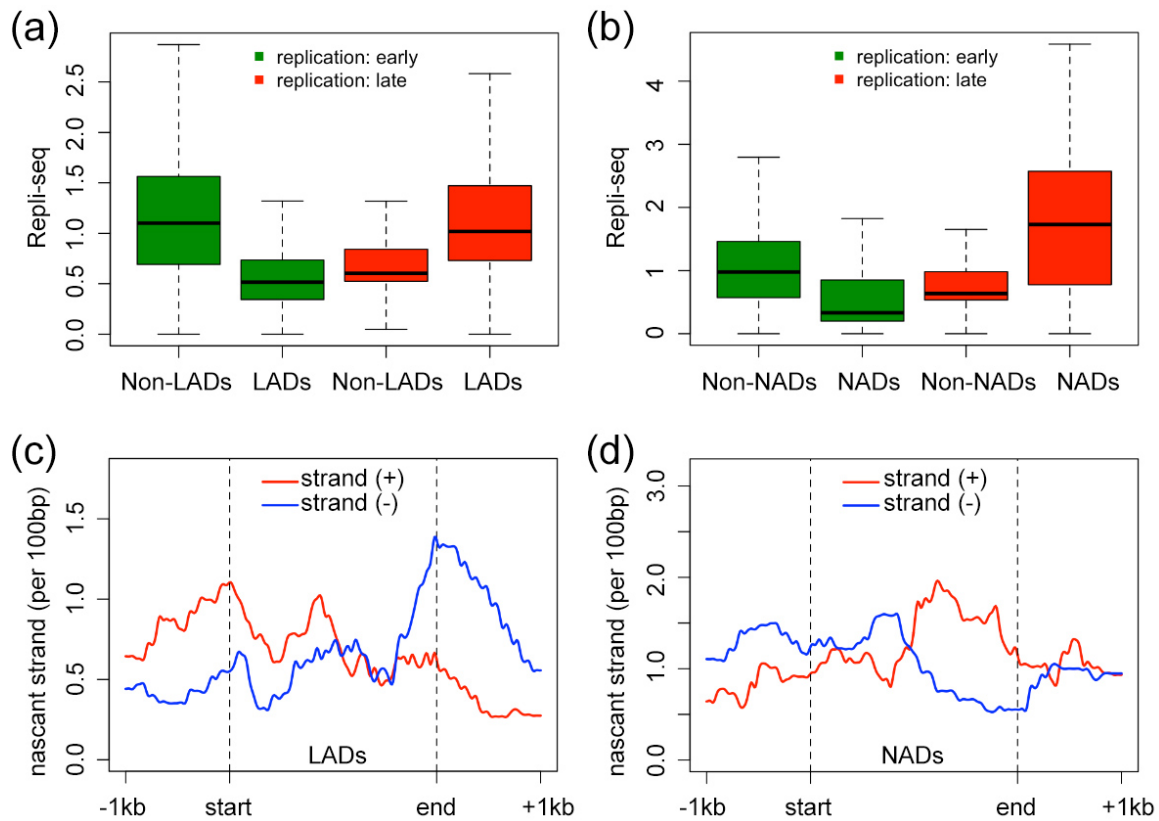
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695 **Figure 1. Spatial distribution of chromatin regions.**

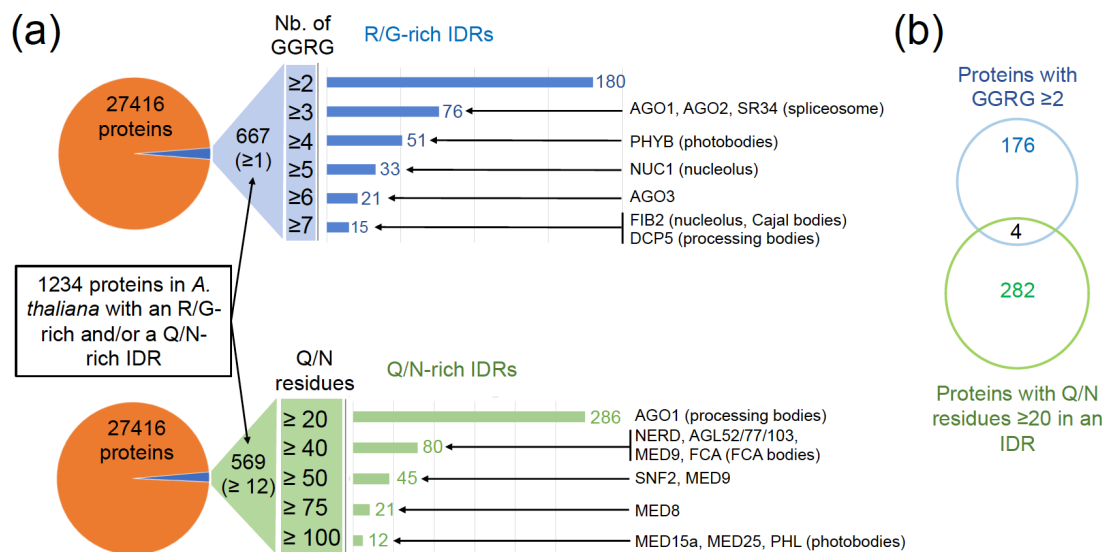
696 **(a)** A sketch illustrating spatial patterns of plant chromatin in the nucleus and their  
 697 association with gene expression. Note that plants do not encode lamin proteins. The  
 698 term “plant nuclear lamina” refers to filamentous protein structure that underlies the  
 699 inner nuclear membrane [91]. Plant nuclear lamina very likely consists of plant-specific  
 700 Nuclear Matrix Constituent Proteins (NMCP, also known as CRWN in *Arabidopsis*)  
 701 [92]. In general, chromatin regions located at the nuclear periphery (i) and at the  
 702 nucleolus (ii) tend to be inactive. Recent studies have revealed a large number of  
 703 chromatin contacts linking actively expressed gene with one another (iii), as well as  
 704 with distal regions having potential roles in transcriptional regulation (iv). The question  
 705 marks besides these chromatin contacts depict the current situation that little is known  
 706 about how the interactions are established. These chromatin contacts are established  
 707 by factor yet unknown (question marks), which we speculate to be combinatorial  
 708 activities of stochastic chromatin movements, specific bridging interactions of proteins  
 709 and RNAs, and liquid-liquid phase separation. The contacts among expressed genes  
 710 foster the formation of sub-compartments and coordinated transcription. Besides,  
 711 multiple H3K9me-marked loci can form puncta in the nucleoplasm (v), which is likely  
 712 driven by liquid-liquid phase separation mediated by plant-specific ADCP1 proteins  
 713 [72]. LAD, lamina-associate domains; NAD, nucleolus-associated domains. **(b)**  
 714 Location of LADs and NADs loci across the *Arabidopsis* genome. This circos plot is  
 715 generated based on domain coordinates described in [39] and [41].



716

717 **Figure 2. Association between DNA replication timing and chromatin localization**  
 718 **in *Arabidopsis*.**

719 **(a and b)** Comparisons of *Arabidopsis* DNA replication activities (measured with Repli-  
 720 seq by Concia *et al.* [63]) in early and late S phase stages in LADs (a) and NADs (b).  
 721 **(c and d)** Distribution of leading nascent DNA strands in ORIs (DNA replication origin)  
 722 across LADs (c) and NADs (d). Note that the dataset describing nascent DNA strand  
 723 is from a study by Sequeira-Mendes *et al.* [66], in which a size cutoff (0.3 to 2 kb) was  
 724 used so that the recovered nascent strands were primarily leading strands in ORIs.  
 725 This information, in turn, can be used to infer whether ORIs occur across a given  
 726 genomic region evenly. For instance, the curves in (c) imply that around LAD boundary  
 727 regions, ORIs fire more often outside LADs than inside. Plant materials used for  
 728 generating these datasets are partly comparable: Repli-seq, 7-day-old seedlings;  
 729 nascent DNA strands, 4-day-old and 10-day-old seedlings; LADs, 10-day-old  
 730 seedlings; and NADs, 3-week-old seedlings. Datasets and scripts for reproducing plots  
 731 in panels (a-d) are available from figshare repository with DOI:  
 732 10.6084/m9.figshare.8953235. Before publication, these datasets and scripts are  
 733 accessible with this private link: <https://figshare.com/s/e9ec0a926a1840e2455b>



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

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