



**HAL**  
open science

# Three-dimensional nuclear organization in *Arabidopsis thaliana*

Frederic Pontvianne, Stefan Grob

► **To cite this version:**

Frederic Pontvianne, Stefan Grob. Three-dimensional nuclear organization in *Arabidopsis thaliana*. *Journal of Plant Research*, 2020, 133 (4), pp.479-488. 10.1007/s10265-020-01185-0 . hal-02925853

**HAL Id: hal-02925853**

**<https://univ-perp.hal.science/hal-02925853>**

Submitted on 9 Sep 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# 1 Three-dimensional nuclear organization in *Arabidopsis thaliana*

2

3 Frédéric Pontvianne<sup>1,2\*</sup> and Stefan Grob<sup>3\*</sup>

4 <sup>1</sup>UPVD, LGDP UMR5096, Université de Perpignan, Perpignan, France

5 <sup>2</sup>CNRS, LGDP UMR5096, Université de Perpignan, Perpignan, France

6 <sup>3</sup>Institute of Plant and Microbial Biology, University of Zurich, Zurich, Switzerland

7

8 \*Correspondence to: [sgrob@botinst.uzh.ch](mailto:sgrob@botinst.uzh.ch) ; [fpontvia@univ-perp.fr](mailto:fpontvia@univ-perp.fr)

9

## 10 Abstract

11 In recent years, the study of plant three-dimensional nuclear architecture received increasing  
12 attention. Enabled by technological advances, our knowledge on nuclear architecture has  
13 greatly increased and we can now access large data sets describing its manifold aspects. The  
14 principles of nuclear organization in plants do not significantly differ from those that in  
15 animals. Plant nuclear organization comprises various scales, ranging from gene loops to  
16 topologically associating domains to nuclear compartmentalization. However, whether plant  
17 three-dimensional chromosomal features also exert similar functions as in animals is less  
18 clear. This review discusses recent advances in the fields of three-dimensional chromosome  
19 folding and nuclear compartmentalization and describes a novel silencing mechanism, which  
20 is closely linked to nuclear architecture.

21

## 22 Keywords:

23 *Arabidopsis*, Chromosome Conformation Capture, Hi-C, Nuclear Compartments

24

25

## 26 Introduction

27 The nucleus is the core-organelle of eukaryotic cells and provides manifold services. The  
28 nuclear genome is responsible for storage, amplification, release, and integration of vital  
29 information. Most cellular responses to external cues rely on nuclear responses, including the  
30 up- or down-regulation of relevant genes and non-coding RNAs. The highly complex  
31 organization of genomes allows to provide these variable and sometimes conflicting services  
32 simultaneously. Differential genome regulation can be achieved as genomes are organized on  
33 several levels: 1) the linear DNA sequence, which contains the genetic information and  
34 regulatory sequences, 2) the chromatin landscape that comprises chemical modifications of  
35 the DNA and DNA-associated proteins, 3) the three-dimensional (3D) genome folding, and 4)  
36 the chromatin's absolute 3D-position within the nucleus.

37 The genome of the model plant *Arabidopsis thaliana* ([L.](#)) Heynh. belongs to the best studied  
38 ones in plants and information for all four levels is available, however, to different extent.  
39 Whereas we have access to a nearly complete assembly of the linear DNA sequence (Lamesch  
40 et al. 2012) and in-depth epigenomic profiling of cytosine methylation and numerous histone  
41 modifications (e.g. Lister et al. 2008; Stroud et al. 2014; Zhang et al. 2006), much less is known  
42 on 3D genome folding and nuclear compartmentalization. Using cytological approaches, 3D  
43 aspects of plant genomes have been studied for a many years, revealing the existence of  
44 specific chromosome configurations in cereals (Santos and Shaw 2004), chromosome  
45 territories, and epigenetic compartmentalization into hetero- and euchromatin in mosses  
46 (Heitz 1928) and higher plants (Fransz et al. 2002). In *A. thaliana*, heterochromatin can be  
47 cytologically detected as chromocenters (Fransz et al. 2002) that likely correspond to the  
48 constitutive heterochromatin of pericentromeres. In addition to observing these basic 3D  
49 chromosome organization units, microscopy-based approaches also allowed to study the  
50 responses of the 3D genome to ectopic stimuli, such as biotic stress and light (Barneche et al.  
51 2014; Kaiserli et al. 2015; Pavet et al. 2006; Pecinka et al. 2010; Tessadori et al. 2007).  
52 However, despite steadily improving resolution of microscopy (Szabo et al. 2018), cytological  
53 approaches can still not be employed to monitor single transcriptional units, such as genes of  
54 several kilo bases (kb), a dimension, which is likely relevant for 3D organization of chromatin.  
55 In recent years, the field made a step forward and our knowledge on plant 3D nuclear  
56 architecture has significantly increased (Doğan and Liu 2018; Sotelo-Silveira et al. 2018; Stam  
57 et al. 2019). Novel technologies enabled this progress and increased resolution to study

58 nuclear architecture was achieved by employing molecular techniques, such as Chromosome  
59 Conformation Capture (3C) (Dekker et al. 2002) technologies that reveal 3D contact  
60 frequencies between chromatin fragments of typically less than 10 kilobases in size. It is worth  
61 noting that certain discrepancies between cytological observation and 3C results can arise,  
62 likely as - in contrast to microscopy - 3C technologies are usually employed on large pools of  
63 cells (Grob and Grossniklaus 2017) and, thus, reveal averaged 3D-folding principles. In recent  
64 years, 3C technologies definitely arrived in the plant field and especially the genome-wide  
65 derivative of 3C, Hi-C, has been conducted in many different plant species, providing 3D  
66 contact maps throughout the plant kingdom (Dong et al. 2017; Hu et al. 2019b, 2019c; Jibrán  
67 et al. 2018; Liu et al. 2017; Maccaferri et al. 2019; Mascher et al. 2017; Moissiard et al. 2012;  
68 Raymond et al. 2018; Shi et al. 2019; VanBuren et al. 2018; Zhang et al. 2018; Zhu et al. 2017).  
69 Interestingly, these data sets also include 3D-contact maps of non-canonical genomes, such  
70 as those of hybrids and allotetraploids (Maccaferri et al. 2019; Wang et al. 2018; Zhu et al.  
71 2017). Despite the large efforts to generate data relevant for 3D genome maps, many of them  
72 await in-depth analysis, as a majority of Hi-C experiments were performed to enable *de novo*  
73 assembly of genomes and limited effort has been invested to study 3D genome architecture  
74 in the respective species.

### 75 *Principles of plant genome folding.*

76 Eukaryotic 3D genome organization comprised several scales, ranging from large  
77 chromosomal domains of several megabases (Mb) to genomic loops, spanning less than one  
78 kb. How and whether the different scales depend on each other is still a matter of debate. At  
79 the largest scale and in accordance to cytological observation, Hi-C data can be used to  
80 describe chromosome territories, which correspond to distinct volumes occupied by single  
81 chromosomes within the nucleus (Cremer and Cremer 2001). Furthermore, Hi-C is also  
82 suitable to describe certain chromosome configurations that were previously cytologically  
83 defined: In *A. thaliana*, frequent contacts between telomeres and centromeres can be readily  
84 observed, which are hallmarks of the rosette configuration (Fransz et al. 2002; Grob et al.  
85 2014). Interestingly, other cytological features of the rosette configuration (Fransz et al. 2002),  
86 such as frequent and periodic contacts between chromosome arms and pericentromeres  
87 could not be confirmed by Hi-C (Grob and Grossniklaus 2017). The apparent discrepancy is not  
88 easily explained and does not necessarily indicate a weakness of either 3C or cytological  
89 techniques. The rosette loop anchors may not be well defined, leading to signal dilution in Hi-

90 C data, whereas these loops may still be readily observable by whole chromosome painting  
91 (Fransz et al. 2002). In the Rabl-configuration centromeres and telomeres are located to  
92 opposite poles of the nucleus and the two chromosome arms are placed in parallel to each  
93 other (Fang and Spector 2005). This Rabl-configuration specific feature, can be observed in Hi-  
94 C maps by increased contact frequencies perpendicular to the diagonal of the maize Hi-C maps  
95 (Dong et al., 2019). In non-rabl configured genomes with metacentric chromosomes, two  
96 distinct chromosome-arm-territories are detected, manifested by depleted contact  
97 frequencies between fragments located on either side of the centromere. Hence, the  
98 centromere can act as 3D boundary (Grob et al. 2013; Pecinka et al. 2004).

99 Another organizational feature, which also occurs at a Mb-scale are A- and B-compartments,  
100 which highly correlate to the epigenetic landscape (Grob et al. 2014; Lieberman-Aiden et al.  
101 2009). Characteristic for A- and B-compartments are enriched contact frequencies among  
102 chromosomal regions with similar epigenetic features throughout the genome. Generally, A-  
103 compartments are associated with an active epigenetic state, whereas B-compartments  
104 include chromosomal regions of repressed epigenetic state (Grob et al. 2014; Lieberman-  
105 Aiden et al. 2009). However, A- and B- compartments cannot be clearly associated to  
106 biologically relevant organizational features. They are determined by the algebraic sign of the  
107 Eigenvector resulting from a principal component analysis (PCA) and the PCA highly depends  
108 on the data fed into the analysis. A- and B- compartments of entire and fully assembled  
109 chromosomes usually correspond to the euchromatin of chromosome arms and  
110 pericentromeric heterochromatin, respectively. As these compartments were identified well  
111 before the onset of 3C technologies [5], one may argue that their identification by Hi-C does  
112 not bring along a major advance in our understanding of 3D genome architecture. However,  
113 the two compartments may describe very different chromosomal region when only Hi-C data  
114 from chromosome arms are fed into the analysis, in which case previously undetected sub-  
115 compartmentalization of chromosome arms can be detected. In *A. thaliana*, these  
116 compartments are referred to as loose and closed structural domains (LSDs and CSDs) and  
117 correlate to active and partially repressed euchromatin, respectively (Grob et al. 2014). Thus,  
118 A- and B- compartments are not strictly biologically relevant terms and they do not only rely  
119 on the Hi-C data fed into the PCA but also heavily on the assembly status of the genome in  
120 question. Many genome assemblies do not include repetitive regions, which are covering large  
121 sections of pericentromeric heterochromatin (dos Santos et al. 2015), thus the resulting A-

122 and B- compartments in species with incomplete genome assemblies may rather resemble *A.*  
123 *thaliana* LSDs and CSDs of euchromatic chromosome arms.

124 Chromosome arms are typically further subdivided into topologically associating domains  
125 (TADs). A TAD comprises neighboring chromosomal regions that exhibit high 3D contact  
126 frequencies among each other but depleted contacts with other chromosomal regions in close  
127 proximity (Bonev and Cavalli 2016; Sexton et al. 2012). In a less technical view, TADs can be  
128 perceived as a chain of pearls of different sizes, being 3D structures that are clearly delineated  
129 from each other. Interestingly, unlike in most other species analyzed by Hi-C (Bonev and  
130 Cavalli 2016), TADs are not prominent features of *A. thaliana* Hi-C maps (Feng et al. 2014;  
131 Grob et al. 2014; Moissiard et al. 2012). However, the apparent absence of TADs in *A. thaliana*  
132 cannot be extrapolated to other plant species, as since the first Hi-C experiments conducted  
133 in *A. thaliana*, TADs have been detected in many other plant species (Dong et al. 2017; Hu et  
134 al. 2019b, 2019c; Jibrán et al. 2018; Liu et al. 2017; Maccaferri et al. 2019; Mascher et al. 2017;  
135 Moissiard et al. 2012; Raymond et al. 2018; Shi et al. 2019; VanBuren et al. 2018; Zhang et al.  
136 2018; Zhu et al. 2017). *A. thaliana* may even exhibit a rather unusual 3D chromosomal  
137 architecture, as many Hi-C maps from plants (including both, mono- and dicots) resemble  
138 those acquired in animals.

139 Despite these phenotypic similarities to animal 3D chromosome organization, plant TADs  
140 likely differ from animal TADs in molecular and possibly functional aspects. In animals, TAD  
141 borders are enriched in CCCTC-binding factor (CTCF) and previous experiments have shown  
142 that both the presence and orientation of CTCF binding sites are essential for TAD formation  
143 (Guo et al. 2015; Phillips and Corces 2009). In plants, CTCF homologues have not been  
144 identified to date, indicating that it is not *per se* required for 3D boundary formation. This  
145 hypothesis is in line with previous studies showing that bio-physical processes independent of  
146 specific insulator proteins may be sufficient to form 3D chromosomal structures, such as TADs  
147 (Alipour and Marko 2012; Cook and Marenduzzo 2018; Rada-Iglesias et al. 2018; Strom et al.  
148 2017). In animals, CTCF exerts its insulating function in association with cohesin (Schwarzer et  
149 al. 2017), which is thought to form a ring-like structure and thereby tethering contacting  
150 chromosomal regions (Rowley and Corces 2018; Szabo et al. 2019). According to the currently  
151 most favored model – the “loop extrusion model” (Sanborn et al. 2015) –, loop and TAD  
152 formation is a dynamic process, which requires a fine-tuned balance of both CTCF binding and  
153 cohesin activity (Rowley and Corces 2018; Szabo et al. 2019). In contrast to CTCF, cohesin

154 homologs can be found in plants (Schubert et al. 2009) and appear to play a vital role. In *A.*  
155 *thaliana*, microscopical observation revealed severe phenotypes affecting chromosomal  
156 architecture in cohesin-complex mutants (Schubert et al. 2009). To date, no Hi-C data sets  
157 describing cohesin mutants have been published in plants.

158 On a global scale, plant and animal TADs share similar characteristics, such as active  
159 transcription and enrichment of active epigenetic marks at TAD borders (Liu et al. 2017).  
160 However, they may differ on a functional level: animal TADs appear to mainly function in  
161 transcriptional control of the within located genes (Rowley and Corces 2018; Szabo et al.  
162 2019). The compartmentalization within single TADs may ensure that promoters are in contact  
163 with relevant enhancer elements and also protects these promoters from ectopic contacts  
164 with distant enhancers, which may lead to deleterious mis-expression of genes (Szabo et al.  
165 2019). Plant TADs may exert different – yet to date unknown – function (Dong et al. 2017;  
166 Stam et al. 2019), as regulatory contacts between putative enhancer and promoter elements  
167 occur across TAD boundaries (Dong et al. 2017).

168 The wide-spread occurrence of TADs and their apparent absence in *A. thaliana* has led to  
169 speculations on additional factors enabling TAD formation. One of these factors may be  
170 genome size: comparative analysis between several plant species (Dong et al. 2017; Stam et  
171 al. 2019) has shown that in species with genomes smaller than 400 Mb, prominent TADs  
172 cannot be detected. However, whether genome size really affects TAD formation can be  
173 debated. Hi-C data sets for plant species with genomes of less than 400 Mb are scarce and  
174 these species are highly related (*Arabidopsis thaliana* and *Arabidopsis lyrata* (Zhu et al. 2017)).  
175 Furthermore, *Drosophila melanogaster* has a genome size (ca. 180 Mb (dos Santos et al.  
176 2015)) comparable to *A. thaliana* and clear delineation into TADs can be observed. Features  
177 of *Drosophila* 3D genome architecture may however hint another explanation for the absence  
178 of TADs in *A. thaliana*. *Drosophila* TADs can be categorized by their epigenetic landscape and  
179 significant changes in epigenetic characteristics of chromatin coincides with borders between  
180 TADs (Sexton and Cavalli 2015). Intriguingly, the *A. thaliana* epigenetic landscape only rarely  
181 shows abrupt changes. Thus, the rather uniform distribution of epigenetic marks along *A.*  
182 *thaliana* chromosome arms may explain why TADs cannot be found in these chromosomal  
183 regions (Rowley et al. 2017; Stam et al. 2019). In support of this, TAD border-like 3D  
184 delineation can be observed in regions where the epigenetic landscape abruptly changes. The  
185 *A. thaliana knob hk4s* clearly shows TAD-like features (Grob et al. 2013, 2014) and boundaries

186 between pericentromeres and euchromatic chromosome arms are clearly detectable in *A.*  
187 *thaliana* Hi-C data sets.

188 Despite the large efforts done in recent years, we still lack functional insight into large-scale  
189 structures, such as chromosome territories, A/B compartments, and TADs, and their relevance  
190 for nuclear processes remains to be elucidated. Possibly, they are not suited to be investigated  
191 in an isolated fashion, but their occurrence should be rather put into context to the absolute  
192 3D localization of chromosomes within nuclei.

### 193 *Putting back the chromatin domains in the nuclear context*

194 Taking into account the context of a TAD is important to understand its biology. TADs can  
195 indeed associate or be in close proximity with different nuclear compartments such as the  
196 nucleolus, nuclear speckles, Cajal bodies, nuclear pore complex, or the nuclear periphery. The  
197 nuclear periphery occupies a large volume in the nucleus. As a result, a large part of the  
198 genome is associated with the nuclear periphery. At least in *A. thaliana*, repeated sequences  
199 present in the centromeric and the pericentromeric regions, as well as ribosomal RNA genes  
200 repeats, are organized as chromocenters (CCs) (Fransz et al. 2002). Analyses of their relative  
201 distribution revealed their preferential association with the nuclear periphery, displaying a  
202 repulsive-like spatial distribution (Andrey et al. 2010). In metazoans, large portions of the  
203 genome are maintained at the nuclear periphery by a network of fibers composed largely of  
204 nuclear lamins. These domains were called LADs, for lamina-associated chromatin domains  
205 (Guelen et al. 2008; Pickersgill et al. 2006; Vertii et al. 2019). LAD regions correspond to around  
206 40% of the genome and display heterochromatic characteristics and are relatively depleted of  
207 genes, which tend to be poorly transcribed. In plant cells, although no clear homologs of  
208 lamina have been found so far, plant-specific nuclear proteins CROWDED NUCLEI (CRWN) are  
209 thought to be lamina orthologs (Dittmer et al. 2007; Doğan and Liu 2018; Sakamoto and Takagi  
210 2013). Plant mutated in *crwn* genes show an alteration of the CC nuclear distribution (Poulet  
211 et al. 2017). A first identification of LADs-like regions in plants has been performed using the  
212 NUCLEOPORIN 1 (NUP1) protein, located in the inner part of the nuclear pore complex (Bi et  
213 al. 2017). More recently, plant LADs (pLADs) have been identified using CRWN1 protein as a  
214 bait, by chromatin immunoprecipitation (Hu et al. 2019a). Pattern analyses of pLADs  
215 confirmed the enrichment of genomic domains displaying heterochromatic features (Bi et al.  
216 2017; Hu et al. 2019a). How genes are silenced at the nuclear periphery remains an open  
217 question, but the fact that CRWN1 interacts with the protein PWWP INTERACTOR OF

218 POLYCOMB 1 (PWO1), a partner of the polycomb-group proteins (PcGs), suggest the  
219 involvement of PcGs in pLAD-gene silencing (Mikulski et al. 2019).

220 The largest nuclear compartment is the nucleolus, the site of ribosome biogenesis. Due to its  
221 high content in proteins, mainly implicated in the ribosome biogenesis pathway (Montacié et  
222 al. 2017; Sáez-Vásquez and Delseny 2019; Weis et al. 2015), the nucleolus is a very dense  
223 nuclear compartment, thought to contain only low amounts of the genomic sequences.  
224 However, in human cells, identification of nucleolus-associated chromatin domains (NADs)  
225 demonstrated that all 23 chromosomes possess at least a portion at the nucleolar periphery  
226 (van Köningsbruggen et al. 2010; Németh et al. 2010). Like LADs, NADs are mainly composed  
227 by large genomic regions displaying heterochromatic features. Interestingly, there is some  
228 overlap between LADs and NADs in human cells (Kind et al. 2013). A recent report was able  
229 separate two classes of NADs. “Type I NADs” are more heterochromatic and share domains  
230 with LADs. In contrast, “type II NADs” tend to be more prone to gene expression and enriched  
231 with developmentally regulated genes (Vertii et al. 2019). Microscopic observations suggest  
232 that interchangeable NADs and LADs are reshuffled after mitosis (Kind et al. 2013).

233 Using a technique enabling to isolate pure nucleoli directly from plant tissues, NADs were  
234 identified in *A. thaliana* (Pontvianne et al. 2016a, 2016b). As in mammalian cells, NADs in *A.*  
235 *thaliana* are enriched in genomic regions displaying repressive chromatin marks. Short arm of  
236 the chromosome 4 is by far the most enriched in *A. thaliana* (Pontvianne et al. 2016b). This  
237 chromosomal region has the particularity of containing actively transcribed rRNA genes,  
238 whose expression requires their association with the nucleolus, their site of transcription by  
239 the RNA polymerase I (Pontvianne et al. 2013). Additional rRNA genes are present on  
240 chromosome 2, but kept silent by epigenetic repressive marks and are located outside the  
241 nucleolus area (Chandrasekhara et al. 2016; Pontvianne et al. 2013). However, in a mutant  
242 that is affected in rRNA genes transcriptional regulation and in which rRNA genes from both  
243 chromosomes 2 and 4 are expressed, NADs composition is enriched by large genomic domains  
244 from the short arm of chromosome 2 (Picart and Pontvianne 2017; Pontvianne et al. 2016b).  
245 This suggests that NADs composition is largely dependent on rRNA genes organization and  
246 expression, a hypothesis that was recently confirmed by studies in human cells (Picart-Piccolo  
247 et al. 2019; Quinodoz et al. 2018).

248 In *A. thaliana*, telomeres cluster at the nucleolar periphery (Armstrong et al. 2001; Franz et  
249 al. 2002). NADs identification confirmed the presence of 200 to 300 kb of subtelomeric regions

250 of each chromosome end in the nucleolus (Pontvianne et al. 2016b). The role of telomeric  
251 sequences in the nucleolus of *A. thaliana* is not known, but a loss of their nucleolar localization  
252 correlates with a decrease in their length (Pontvianne et al. 2016b).

253 In addition, NADs are also composed of several hundred genes that tend to be silent  
254 (Pontvianne et al. 2016b). Because the RNA polymerase II is absent from the nucleolar interior,  
255 one hypothesis is that the nucleolus could be a sequestering area to keep genes silent  
256 (Pontvianne et al. 2016b). Some of them are developmentally regulated genes and future  
257 studies will certainly reveal whether their association is lost when there are expressed.

258 A comparison of NADs and pLADs from *A. thaliana*, unlike in animal cells, did not revealed a  
259 significant overlap (Picart-Piccolo et al. 2019). However, regions of the short arm of the  
260 chromosome 4 have also been identified as pLADs. One possible explanation is the link  
261 between the nucleolus and the nuclear periphery. Indeed, the CC of the chromosome 4  
262 associates with both the nucleolus and the nuclear periphery, creating a physical and indirect  
263 link between the nucleolus and the nuclear periphery.

#### 264 *Chromosomal loops*

265 Chromosomal loops, leading to contacts of distant genomic regions at their base are  
266 recognized to play an important role in gene regulation. Interestingly, the first plant loop  
267 described has been associated to one of the most exotic modes of gene regulation, namely  
268 paramutation (Hollick 2016; Louwers et al. 2009). The maize *b1* gene has two epialleles, *B-I*  
269 and *B'* (Arteaga-Vazquez et al. 2010; Louwers et al. 2009; McEachern and Lloyd 2012). They  
270 share an identical DNA sequence but exhibit differential expression, leading to a clearly  
271 distinguishable pigmentation phenotype of husks (leaves surrounding the corncob). Green  
272 husks can be observed when the lowly expressed *B'* epiallele is present, whereas plants  
273 carrying the highly expressed *B-I* show purple husks. If the two are crossed, the transcriptional  
274 state of the paramutagenic *B'* allele is transmitted to *B-I*, leading to its downregulation and  
275 green husks throughout a segregating F2 generation (Louwers et al. 2009). This difference in  
276 expression levels may be mainly due to their differential 3D folding properties. In *B'*, a single  
277 loop between its transcription start site (*TSS*) and a repeat region 117 kb upstream has been  
278 detected, whereas *B-I* additionally has loops anchoring between the *TSS* and regions lying in-  
279 between the repeats and the *b1* gene. However, an association of differential 3D folding and  
280 transcriptional state seems to be tissue specific, thus other – yet unknown – factors may be  
281 involved in epiallele formation in other tissues (Louwers et al. 2009).

282 In *A. thaliana*, several genomic loops associated with gene regulation have been described  
283 since the introduction of 3C technologies in plants. Loops have been described for prominent  
284 genes, such as the *FLOWERING LOCUS C (FLC)* (Crevillén et al. 2013; Jegu et al. 2014). FLC is a  
285 key regulator of flowering, which is suppressed when FLC is expressed. FLC transcription is  
286 gradually inhibited during vernalization by a Polycomb complex, leading to flowering initiation  
287 (Alexandre and Hennig, 2008). When FLC is transcribed, a gene loop anchored 5' and 3' of FLC  
288 can be observed and this loop is abolished after vernalization (Crevillén et al. 2013). This  
289 abolishment may be induced by binding of BAF60, a subunit of the SWI/SNF remodeling  
290 complex (Jegu et al. 2014) and may lead to the transcription of an FLC-antisense transcript,  
291 which is involved in FLC silencing (Swiezewski et al. 2009). Similar to FLC, an involvement of  
292 BAF60 in loop abolishment has also been reported in other genes (Jégu et al. 2015, 2017),  
293 however, whether BAF60 generally is involved in loop abolishment and subsequent gene  
294 silencing is questionable as chromatin binding profiles of BAF60 could generally be associated  
295 with active transcription (Jégu et al. 2017). FLC is not only regulated by a gene loop but also is  
296 involved in loop formation of its targets. Binding of FLC to the 3' regulatory region of *TARGET*  
297 *OF FLC AND SVP1 (TFS1)* was reported to enable the formation of a gene loop anchoring at  
298 this 3' region and the *TFS1* gene body and promote *TFS1* silencing (Richter et al. 2019).  
299 For *WUSCHEL*, another prominent *A. thaliana* gene, an association of gene looping with its  
300 transcriptional control has been reported (Guo et al. 2018). In case of *WUSCHEL*, the gene  
301 loop formed between its *TSS* and 3' flanking region is associated with *WUSCHEL* repression.  
302 Guo and colleagues studied the contribution of the loop anchor sequences and reported that  
303 neither the exact sequence nor distance between anchors is necessary for loop formation  
304 (Guo et al. 2018).  
305 Gene loops are wide-spread features of *A. thaliana* 3D genome folding and additional loops  
306 have been identified by both 3C (Ariel et al. 2014; Cao et al. 2014; Liu et al. 2013; Wang et al.  
307 2019) and Hi-C experiments (Liu et al. 2016). Generally, gene loops are associated with  
308 elevated transcription levels, not only in *A. thaliana* (Liu et al. 2016) but also in rice (Dong et  
309 al. 2018) and maize (Li et al. 2019) and, thus a positive correlation between gene looping and  
310 transcriptional activity may be a general feature in flowering plants.  
311 Using chromatin interaction analysis by paired-end tag sequencing (ChIA-PET) for the first time  
312 in plants, two independent studies described numerous loops in maize (Li et al. 2019; Peng et  
313 al. 2019). These loops can be established between loci of similar epigenetic and transcriptional

314 state and, interestingly, between expression quantitative trait loci (eQTL) and their target  
315 genes (Peng et al. 2019). Additionally, long-range (up to 1 Mb) enhancer-promoter contacts  
316 were described that are associated with tissue-specific gene expression (Li et al. 2019). This  
317 may indicate that long-range contacts are rather part of a more complex and specialized  
318 regulatory machinery as opposed to short-range gene loops, which might be a hallmark of  
319 general gene regulation.

320 Despite the large number of known chromatin loops described in plants, their functional  
321 relevance remains largely elusive. Studying the exact biological role of chromatin loops is  
322 extremely difficult. As of today, our knowledge on factors mediating chromatin loops is scarce  
323 and one of the few ways available to analyze loops is to manipulate their anchor sequences.  
324 However, by doing so, there is always the risk that binding sites of important regulators may  
325 have been compromised. Thus, it is challenging to discriminate effects of loop abolishment  
326 and a potential lack of regulatory proteins.

327 The existence of thousands of gene loops in *A. thaliana* may compensate for the apparent  
328 absence of TADs. Gene loops may serve to insulate gene regulatory regions, similar as it is  
329 anticipated for animal TADs. In *A. thaliana*, transcriptional and epigenetic states of  
330 neighboring genes can widely differ, hence, making it more favorable to insulate single genes  
331 including their regulatory regions instead of insulating clusters of genes with similar  
332 transcriptional state as observed in animals (Szabo et al. 2019).

### 333 *KNOT-linked silencing – a 3D silencing phenomenon*

334 One of the most prominent features of *A. thaliana* 3D genome architecture is the *KNOT*, a 3D  
335 genomic structure formed by tight long-range *cis* and *trans* contacts among ten *KNOT engaged*  
336 *elements (KEEs)* (aka Interactive Heterochromatic Islands (IHIs))(Feng et al. 2014; Grob et al.  
337 2014), which are distributed over all five *A. thaliana* chromosomes. High-frequency contacts  
338 among *KEEs* can be readily detected in Hi-C maps as they clearly stand out from contact  
339 frequencies observed between regions surrounding *KEEs*. *KEEs* are enriched in transposable  
340 elements (TEs) and associated small RNAs (sRNAs) (Grob et al., 2014) and exhibit a moderately  
341 heterochromatic constitution (Feng et al. 2014). However, their occurrence appears to be  
342 independent of a larger epigenetic context, as *KEEs* can be found in euchromatic regions of  
343 chromosome arms as well as constitutive heterochromatin of pericentromeres. TEs found  
344 within *KEEs* are significantly enriched in fragments of *VANDAL6* DNA transposons and  
345 potentially full-length *ATLANTYS3* retrotransposons (Dong et al. 2018; Grob and Grossniklaus

2017; Grob et al. 2014). Furthermore, the *KNOT* is likely conserved, both in structure and sequences, as high-frequency contacts between TE islands can also be detected in rice (Dong et al. 2018).

Factors involved in the *KEE-KEE* contact and *KNOT* formation have not been identified to date, despite the various mutants that have been screened by Hi-C in *A. thaliana* (Feng et al. 2014; Grob et al. 2014). Interestingly, the *KNOT* is not abolished in various mutant affecting epigenetic pathways:

Histone H3 lysine 9 di-methylation (H3K9me<sub>2</sub>) is an important repressive epigenetic mark and enriched in heterochromatin and is impaired in *su(var)3-9* homolog 4 (*suvh4*) *suvh5* *suvh6* triple mutants. However, despite slight enrichment of H3K9me<sub>2</sub> in *KEEs*, *KNOT* formation is not impaired in the *suvh4* *suvh5* *suvh6* triple mutants (Feng et al. 2014). Similarly, DNA methylation mutants *decreased in DNA methylation 1* (*ddm1*), *methyltransferase 1* (*met1*), and *chromomethylase 3* (*cmt3*) do not lead to *KNOT* abolishment. Moreover, in *ddm1* and *met1* mutants, ten additional *KEEs* can be found, which completely overlap between the two mutants. Interestingly, three additional *KEEs* can also be observed in H3K9me<sub>2</sub> mutants and two of them overlap with those found in the *met1* and *ddm1* mutants (Feng et al. 2014). Additional *KEEs* do not appear to emerge from random positions, as ectopic *KEEs* are also enriched in *VANDAL6* TE fragments. Mutations in other suspects, such as the Polycomb gene *curly leaf* (*clf*) and *swinger* (*swn*) and chromatin remodelers *microchidia 6* (*morc6*) and *morpheus molecule 1* (*mom1*) do also not negatively affect the *KNOT* (Feng et al. 2014).

Despite the scarce knowledge on how the *KNOT* is formed, more is known about its biological function. DNA transposons have been shown to preferentially insert into *KEEs* (Grob et al. 2014), leading to the speculation that *KEEs* could be involved in TE biology. This hypothesis has been supported by the finding of a novel silencing phenomenon, termed *KNOT-linked silencing* (KLS) (Grob and Grossniklaus 2019): The insertion site of T-DNA transgenes can form ectopic contacts with single *KEEs* or the entire *KNOT* and these ectopic contact correlate to the transgene's expression state. Transgenes that form ectopic contacts with the entire *KNOT* are fully silenced, whereas transgenes exhibiting ectopic contacts to a single *KEE* only are stably expressed, similar to transgenes that do not form ectopic *KEE* contacts at all. RNA-dependent DNA methylation (RdDM) and sRNA-induced post-transcriptional gene silencing are not involved in KLS, leading to speculations whether KLS represents a completely novel silencing mechanism. The differential pattern observed in KLS by active transgenic lines that

378 show only ectopic contacts to single *KEEs* and repressed lines that exhibit full *KNOT* association  
379 is of special interest: the study of KLS offers the rare opportunity to investigate both transgene  
380 detection (single *KEE* contacts) and subsequent transgene silencing (full *KNOT* association).

### 381 *Conclusions*

382 The study of nuclear architecture gained increasing attention in recent years. With the  
383 establishment of novel technologies, we are today not only able to characterize contact  
384 frequencies among genomic regions, but we can also locate these regions in relation to  
385 important nuclear compartments. As Hi-C became a favorite tool to support genome  
386 assemblies, Hi-C data sets for various plant species are available to date. However, many of  
387 them seem to be byproducts of assemblies and not all of them suffice in quality for in-depth  
388 analysis. Thus, *A. thaliana* still represents the best-studied model concerning nuclear  
389 architecture in plants. Further studies in other species, including the characterization of LADs  
390 and NADs could shed more light on plant nuclear architecture, especially since nuclear  
391 architecture in *A. thaliana* may be rather exceptional among plants.

392

### 393 *Acknowledgments*

394 FP is supported by CNRS, the ANR JCJC NucleoReg [ANR-15-CE12-0013-01] and the French  
395 Laboratory of Excellence project TULIP (ANR-10-LABX-41 and ANR-11-IDEX-0002-02). SG is  
396 supported by the University of Zurich.

397

398

399

### 400 *References*

401 Alexandre, C.M., and Hennig, L. (2008). FLC or not FLC: the other side of vernalization. *J. Exp.*  
402 *Bot.* *59*, 1127–1135.

403 Alipour, E., and Marko, J.F. (2012). Self-organization of domain structures by DNA-loop-  
404 extruding enzymes. *Nucleic Acids Res.* *40*, 11202–11212.

405 Andrey, P., Kiêu, K., Kress, C., Lehmann, G., Tirichine, L., Liu, Z., Biot, E., Adenot, P.G., Hue-  
406 Beauvais, C., Houba-Hérin, N., et al. (2010). Statistical analysis of 3D images detects regular  
407 spatial distributions of centromeres and chromocenters in animal and plant nuclei. *PLoS*  
408 *Comput. Biol.* *6*, 27.

409 Ariel, F., Jegu, T., Latrasse, D., Romero-Barrios, N., Christ, A., Benhamed, M., and Crespi, M.  
410 (2014). Noncoding transcription by alternative RNA polymerases dynamically regulates an  
411 auxin-driven chromatin loop. *Mol. Cell* *55*, 383–396.

412 Armstrong, S.J., Franklin, F.C., and Jones, G.H. (2001). Nucleolus-associated telomere  
413 clustering and pairing precede meiotic chromosome synapsis in *Arabidopsis thaliana*. *J. Cell*  
414 *Sci.* *114*, 4207–4217.

415 Arteaga-Vazquez, M., Sidorenko, L., Rabanal, F.A., Shrivistava, R., Nobuta, K., Green, P.J.,  
416 Meyers, B.C., and Chandler, V.L. (2010). RNA-mediated *trans*-communication can establish  
417 paramutation at the *b1* locus in maize. *Proc. Natl. Acad. Sci.* *107*, 12986–12991.

418 Barneche, F., Malapeira, J., and Paloma, M. (2014). The impact of chromatin dynamics on  
419 plant light responses and circadian clock function. *J. Exp. Bot.* *65*, 2895–2913.

420 Bi, X., Cheng, Y., Hu, B., Ma, X., Wu, R., and Wang, J. (2017). Nonrandom domain  
421 organization of the *Arabidopsis* genome at the nuclear periphery. *Genome Res.* *27*, 1162–  
422 1173.

423 Bonev, B., and Cavalli, G. (2016). Organization and function of the 3D genome. *Nat. Rev.*  
424 *Genet.* *17*, 772.

425 Cao, S., Kumimoto, R.W., Gnesutta, N., Calogero, A.M., Mantovani, R., and Holt, B.F. (2014).  
426 A distal CCAAT/NUCLEAR FACTOR Y complex promotes chromatin looping at the *FLOWERING*  
427 *LOCUS T* promoter and regulates the timing of flowering in *Arabidopsis*. *Plant Cell* *26*, 1009–  
428 1017.

429 Chandrasekhara, C., Mohannath, G., Blevins, T., Pontvianne, F., and Pikaard, C.S. (2016).  
430 Chromosome-specific NOR inactivation explains selective rRNA gene silencing and dosage  
431 control in *Arabidopsis*. *Genes Dev.* *30*, 177–190.

432 Cook, P.R., and Marenduzzo, D. (2018). Transcription-driven genome organization: a model  
433 for chromosome structure and the regulation of gene expression tested through simulations.  
434 *Nucleic Acids Res.* *46*, 9895–9906.

435 Cremer, T., and Cremer, C. (2001). Chromosome territories, nuclear architecture and gene  
436 regulation in mammalian cells. *Nat. Rev. Genet.* *2*, 292–301.

437 Crevillén, P., Sonmez, C., Wu, Z., and Dean, C. (2013). A gene loop containing the floral  
438 repressor FLC is disrupted in the early phase of vernalization. *EMBO J.* *32*, 140–148.

439 Dekker, J., Rippe, K., Dekker, M., and Kleckner, N. (2002). Capturing chromosome  
440 conformation. *Science.* *295*, 1306–1311.

441 Dittmer, T.A., Stacey, N.J., Sugimoto-Shirasu, K., and Richards, E.J. (2007). *LITTLE NUCLEI*  
442 genes affecting nuclear morphology in *Arabidopsis thaliana*. *Plant Cell* *19*, 2793–2803.

443 Doğan, E.S., and Liu, C. (2018). Three-dimensional chromatin packing and positioning of  
444 plant genomes. *Nat. Plants* *4*, 521–529.

445 Dong, P., Tu, X., Chu, P., Peitao, L., Zhu, N., Grierson, D., Du, B., Li, P., and Zhong, S. (2017).  
446 3D chromatin architecture of large plant genomes determined by local A/B compartments.  
447 *Mol. Plant* *10*, 1497–1509.

448 Dong, P., Tu, X., Li, H., Zhang, J., Grierson, D., Li, P., and Zhong, S. (2019). Tissue-specific Hi-C  
449 analyses of rice, foxtail millet and maize suggest non-canonical function of plant chromatin  
450 domains. *J. Integr. Plant Biol.*

451 Dong, Q., Li, N., Li, X., Yuan, Z., Xie, D., Wang, X., Li, J., Yu, Y., Wang, J., Ding, B., et al. (2018).  
452 Genome-wide Hi-C analysis reveals extensive hierarchical chromatin interactions in rice.  
453 *Plant J.* *94*, 1141–1156.

454 Fang, Y., and Spector, D.L. (2005). Centromere positioning and dynamics in living *Arabidopsis*  
455 plants. *Mol. Biol. Cell* *16*, 5710–5718.

456 Feng, S., Cokus, S.J., Schubert, V., Zhai, J., Pellegrini, M., and Jacobsen, S.E. (2014). Genome-  
457 wide Hi-C analyses in wild-type and mutants reveal high-resolution chromatin interactions in  
458 *Arabidopsis*. *Mol. Cell* *55*, 694–707.

459 Fransz, P., De Jong, J.H., Lysak, M., Castiglione, M.R., and Schubert, I. (2002). Interphase  
460 chromosomes in *Arabidopsis* are organized as well defined chromocenters from which  
461 euchromatin loops emanate. *Proc. Natl. Acad. Sci. U. S. A.* *99*, 14584–14589.

462 Grob, S. (2019). Three-dimensional chromosome organization in flowering plants. *Brief.*  
463 *Funct. Genomics.* elz024.

464 Grob, S., and Grossniklaus, U. (2017). Chromosome conformation capture-based studies  
465 reveal novel features of plant nuclear architecture. *Curr. Opin. Plant Biol.* *36*, 149–157.

466 Grob, S., and Grossniklaus, U. (2019). Invasive DNA elements modify the nuclear architecture  
467 of their insertion site by *KNOT*-linked silencing in *Arabidopsis thaliana*. *Genome Biol.* *20*,  
468 120.

469 Grob, S., Schmid, M.W., Luedtke, N.W., Wicker, T., and Grossniklaus, U. (2013).  
470 Characterization of chromosomal architecture in *Arabidopsis* by chromosome conformation  
471 capture. *Genome Biol.* *14*, R129.

472 Grob, S., Schmid, M.W., and Grossniklaus, U. (2014). Hi-C analysis in *Arabidopsis* identifies  
473 the *KNOT*, a structure with similarities to the flamenco locus of *Drosophila*. *Mol. Cell* *55*,  
474 678–693.

475 Guelen, L., Pagie, L., Brasset, E., Meuleman, W., Faza, M.B., Talhout, W., Eussen, B.H., de  
476 Klein, A., Wessels, L., De Laat, W., et al. (2008). Domain organization of human  
477 chromosomes revealed by mapping of nuclear lamina interactions. *Nature* *453*, 948–951.

478 Guo, L., Cao, X., Liu, Y., Li, J., Li, Y., Li, D., Zhang, K., Gao, C., Dong, A., and Liu, X. (2018). A  
479 chromatin loop represses *WUSCHEL* expression in *Arabidopsis*. *Plant J.* *94*, 1083–1097.

480 Guo, Y., Xu, Q., Canzio, D., Krainer, A.R., Maniatis, T., Guo, Y., Xu, Q., Canzio, D., Shou, J., Li,  
481 J., et al. (2015). CRISPR inversion of CTCF sites alters genome topology and  
482 enhancer/promoter function. *Cell* *162*, 900–910.

483 Heitz, E. (1928). Das Heterochromatin der Moose. *Jahrbücher Für Wissenschaftliche Bot.* *69*,  
484 762–818.

485 Hollick, J.B. (2016). Paramutation and related phenomena in diverse species. *Nat. Rev.*  
486 *Genet.* *18*, 5–23.

487 Hu, B., Wang, N., Bi, X., Karaaslan, E.S., Weber, A., Zhu, W., Berendzen, K.W., and Liu, C.  
488 (2019a). Plant lamin-like proteins mediate chromatin tethering at the nuclear periphery.  
489 *Genome Biol.* *20*.

490 Hu, L., Xu, Z., Wang, M., Fan, R., Yuan, D., Wu, B., Wu, H., Qin, X., Yan, L., Tan, L., et al.  
491 (2019b). The chromosome-scale reference genome of black pepper provides insight into  
492 piperine biosynthesis. *Nat. Commun.* *10*, 4702.

493 Hu, Y., Chen, J., Fang, L., Zhang, Z., Ma, W., Niu, Y., Ju, L., Deng, J., Zhao, T., Lian, J., et al.  
494 (2019c). *Gossypium barbadense* and *Gossypium hirsutum* genomes provide insights into the  
495 origin and evolution of allotetraploid cotton. *Nat. Genet.* *51*, 739–748.

496 Jegu, T., Latrasse, D., Delarue, M., Hirt, H., Domenichini, S., Ariel, F., Crespi, M., Bergounioux,  
497 C., Raynaud, C., and Benhamed, M. (2014). The BAF60 subunit of the SWI/SNF chromatin-  
498 remodeling complex directly controls the formation of a gene loop at *FLOWERING LOCUS C*  
499 in *Arabidopsis*. *Plant Cell* *26*, 538–551.

500 Jégu, T., Domenichini, S., Blein, T., Ariel, F., Christ, A., Kim, S., Crespi, M., Boutet-Mercey, S.,  
501 Mouille, G., Bourge, M., et al. (2015). A SWI/SNF chromatin remodelling protein controls  
502 cytokinin production through the regulation of chromatin architecture. *PLoS One* *10*,  
503 e0138276.

504 Jégu, T., Veluchamy, A., Ramirez-prado, J.S., Rizzi-paillet, C., Perez, M., Lhomme, A., Latrasse,  
505 D., Coleno, E., Vicaire, S., Legras, S., et al. (2017). The *Arabidopsis* SWI/SNF protein BAF60  
506 mediates seedling growth control by modulating DNA accessibility. *Genome Biol.* *18*, 1–16.

507 Jibrán, R., Dzierzon, H., Bassil, N., Bushakra, J.M., Edger, P.P., Sullivan, S., Finn, C.E., Dossett,  
508 M., Vining, K.J., Vanburen, R., et al. (2018). Chromosome-scale scaffolding of the black  
509 raspberry (*Rubus occidentalis* L.) genome based on chromatin interaction data. *Hortic. Res.*  
510 *5*.

511 Kaiserli, E., Pa, K., Donnell, L.O., Nusinow, D.A., Kay, S.A., Chory, J., Donnell, L.O., Batalov, O.,  
512 Pedmale, U. V., Nusinow, D.A., et al. (2015). Integration of light and photoperiodic signaling  
513 in transcriptional nuclear foci. *Dev. Cell* 311–321.

514 Kind, J., Pagie, L., Ortabozkoyun, H., Boyle, S., de Vries, S.S., Janssen, H., Amendola, M.,  
515 Nolen, L.D., Bickmore, W.A., and van Steensel, B. (2013). Single-cell dynamics of genome-  
516 nuclear lamina interactions. *Cell* *153*, 178–192.

517 van Köningsbruggen, S., Gierliński, M., Schofield, P., Martin, D., Barton, G.J., Ariyurek, Y., den  
518 Dunnen, J.T., and Lamond, A.I. (2010). High-resolution whole-genome sequencing reveals  
519 that specific chromatin domains from most human chromosomes associate with nucleoli.  
520 *Mol. Biol. Cell* *21*, 3735–3748.

521 Lamesch, P., Berardini, T.Z., Li, D., Swarbreck, D., Wilks, C., Sasidharan, R., Muller, R., Dreher,  
522 K., Alexander, D.L., Garcia-hernandez, M., et al. (2012). The *Arabidopsis* Information  
523 Resource (TAIR): improved gene annotation and new tools. *Nucleic Acids Res.* *40*, 1202–  
524 1210.

525 Li, E., Liu, H., Huang, L., Zhang, X., Dong, X., Song, W., Zhao, H., and Lai, J. (2019). Long-range  
526 interactions between proximal and distal regulatory regions in maize. *Nat. Commun.* *10*,  
527 2633.

528 Lieberman-Aiden, E., Van Berkum, N.L., Williams, L., Imakaev, M., Ragooczy, T., Telling, A.,  
529 Amit, I., Lajoie, B.R., Sabo, P.J., Dorschner, M.O., et al. (2009). Comprehensive mapping of  
530 long-range interactions reveals folding principles of the human genome. *Science.* *326*, 289–  
531 293.

532 Lister, R., O'Malley, R.C., Tonti-Filippini, J., Gregory, B.D., Berry, C.C., Millar, A.H., and Ecker,  
533 J.R. (2008). Highly integrated single-base resolution maps of the epigenome in *Arabidopsis*.  
534 *Cell* *133*, 523–536.

535 Liu, C., Teo, Z.W.N., Bi, Y., Song, S., Xi, W., Yang, X., Yin, Z., and Yu, H. (2013). A conserved  
536 genetic pathway determines inflorescence architecture in *Arabidopsis* and rice. *Dev. Cell* *24*,  
537 612–622.

538 Liu, C., Wang, C., Wang, G., Becker, C., Zaidem, M., and Weigel, D. (2016). Genome-wide

539 analysis of chromatin packing in *Arabidopsis thaliana* at single-gene resolution. *Genome Res.*  
540 *26*, 1057–1068.

541 Liu, C., Cheng, Y.-J., Wang, J.-W., and Weigel, D. (2017). Prominent topologically associated  
542 domains differentiate global chromatin packing in rice from *Arabidopsis*. *Nat. Plants* *3*, 742–  
543 748.

544 Louwers, M., Bader, R., Haring, M., Van Driel, R., De Laat, W., and Stam, M. (2009). Tissue-  
545 and expression level-specific chromatin looping at maize b1 epialleles. *Plant Cell* *21*, 832–  
546 842.

547 Maccaferri, M., Harris, N.S., Twardziok, S.O., Pasam, R.K., Gundlach, H., Spannagl, M.,  
548 Ormanbekova, D., Lux, T., Prade, V.M., Milner, S.G., et al. (2019). Durum wheat genome  
549 highlights past domestication signatures and future improvement targets. *Nat. Genet.* *51*,  
550 885–895.

551 Mascher, M., Gundlach, H., Himmelbach, A., Beier, S., Twardziok, S.O., Wicker, T., Radchuk,  
552 V., Dockter, C., Hedley, P.E., Russell, J., et al. (2017). A chromosome conformation capture  
553 ordered sequence of the barley genome. *Nature* *544*, 427–433.

554 McEachern, L.A., and Lloyd, V.K. (2012). The maize b1 paramutation control region causes  
555 epigenetic silencing in *Drosophila melanogaster*. *Mol. Genet. Genomics* *287*, 591–606.

556 Mikulski, P., Hohenstatt, M.L., Farrona, S., Smaczniak, C., Stahl, Y., Kalyanikrishna, Kaufmann,  
557 K., Angenent, G., and Schubert, D. (2019). The chromatin-associated protein PWO1 interacts  
558 with plant nuclear lamin-like components to regulate nuclear size. *Plant Cell* *31*, 1141 LP –  
559 1154.

560 Moissiard, G., Cokus, S.J., Cary, J., Feng, S., Billi, A.C., Stroud, H., Husmann, D., Zhan, Y.,  
561 Lajoie, B.R., McCord, R.P., et al. (2012). MORC family ATPases required for heterochromatin  
562 condensation and gene silencing. *Science*. *336*, 1448–1451.

563 Montacié, C., Durut, N., Opsomer, A., Palm, D., Comella, P., Picart, C., Carpentier, M.-C.,  
564 Pontvianne, F., Carapito, C., Schleiff, E., et al. (2017). Nucleolar proteome analysis and  
565 proteasomal activity assays reveal a link between nucleolus and 26S proteasome in *A.*  
566 *thaliana*. *Front. Plant Sci.* *8*, 1815.

567 Németh, A., Conesa, A., Santoyo-Lopez, J., Medina, I., Montaner, D., Péterfia, B., Solovei, I.,  
568 Cremer, T., Dopazo, J., and Längst, G. (2010). Initial genomics of the human nucleolus. *PLOS*  
569 *Genet.* *6*, 1–11.

570 Pavet, V., Quintero, C., Cecchini, N.M., Rosa, A.L., and Alvarez, M.E. (2006). *Arabidopsis*  
571 displays centromeric dna hypomethylation and cytological alterations of heterochromatin  
572 upon attack by *Pseudomonas syringae*. *Mol. Plant-Microbe Interact.* *19*, 577–587.

573 Pecinka, A., Schubert, V., Meister, A., Kreth, G., Klatte, M., Lysak, M.A., Fuchs, J. rg, and  
574 Schubert, I. (2004). Chromosome territory arrangement and homologous pairing in nuclei of  
575 *Arabidopsis thaliana* are predominantly random except for NOR-bearing chromosomes.  
576 *Chromosoma* *113*, 258–269.

577 Pecinka, A., Dinh, H.Q., Baubec, T., Rosa, M., Lettner, N., and Scheid, O.M. (2010). Epigenetic  
578 regulation of repetitive elements is attenuated by prolonged heat stress in *Arabidopsis*. *Plant*  
579 *Cell* *22*, 3118–3129.

580 Peng, Y., Xiong, D., Zhao, L., Ouyang, W., Wang, S., Sun, J., Zhang, Q., Guan, P., Xie, L., Li, W.,  
581 et al. (2019). Chromatin interaction maps reveal genetic regulation for quantitative traits in

582 maize. *Nat. Commun.* *10*, 2632.

583 Phillips, J.E., and Corces, V.G. (2009). CTCF : Master weaver of the genome. *Cell* *137*, 1194–  
584 1211.

585 Picart-Piccolo, A., Picault, N., and Pontvianne, F. (2019). Ribosomal RNA genes shape  
586 chromatin domains associating with the nucleolus. *Nucleus* *10*, 67–72.

587 Picart, C., and Pontvianne, F. (2017). Plant nucleolar DNA: Green light shed on the role of  
588 Nucleolin in genome organization. *Nucleus* *8*, 11–16.

589 Pickersgill, H., Kalverda, B., de Wit, E., Talhout, W., Fornerod, M., and van Steensel, B.  
590 (2006). Characterization of the *Drosophila melanogaster* genome at the nuclear lamina. *Nat.*  
591 *Genet.* *38*, 1005–1014.

592 Pontvianne, F., Blevins, T., Hassel, C., Pontes, O.M.F., Muchova, V., Tucker, S., Mokros, P.,  
593 Muchova, V., Fajkus, J., and Pikaard, C.S. (2013). Subnuclear partitioning of rRNA genes  
594 between the nucleolus and nucleoplasm reflects alternative epiallelic states. *Genes Dev.* *27*,  
595 1545–1550.

596 Pontvianne, F., Boyer-Clavel, M., and Sáez-Vásquez, J. (2016a). Fluorescence-activated  
597 nucleolus sorting in *Arabidopsis*. In: Németh A (ed) *The nucleolus: methods and protocols*.  
598 Springer New York, New York, pp 203–211.

599 Pontvianne, F., Carpentier, M.C., Durut, N., Pavlišťová, V., Jaške, K., Schořová, Š., Parrinello,  
600 H., Rohmer, M., Pikaard, C.S., Fojtová, M., et al. (2016b). Identification of nucleolus-  
601 associated chromatin domains reveals a role for the nucleolus in 3D organization of the  
602 *A. thaliana* genome. *Cell Rep.* *16*, 1574–1587.

603 Poulet, A., Duc, C., Voisin, M., Desset, S., Tutois, S., Vanrobays, E., Benoit, M., Evans, D.E.,  
604 Probst, A. V, and Tatout, C. (2017). The LINC complex contributes to heterochromatin  
605 organisation and transcriptional gene silencing in plants. *J. Cell Sci.* *130*, 590 LP – 601.

606 Quinodoz, S.A., Ollikainen, N., Tabak, B., Palla, A., Schmidt, J.M., Detmar, E., Lai, M.M.,  
607 Shishkin, A.A., Bhat, P., Takei, Y., et al. (2018). Higher-Order Inter-chromosomal hubs shape  
608 3D genome organization in the nucleus. *Cell* *174*, 744-757.e24.

609 Rada-Iglesias, A., Grosveld, F.G., and Papantonis, A. (2018). Forces driving the three-  
610 dimensional folding of eukaryotic genomes. *Mol. Syst. Biol.* *14*, e8214.

611 Raymond, O., Gouzy, J., Just, J., Badouin, H., Verdenaud, M., Lemainque, A., Vergne, P.,  
612 Moja, S., Choisne, N., Pont, C., et al. (2018). The *Rosa* genome provides new insights into the  
613 domestication of modern roses. *Nat. Genet.* *50*, 772–777.

614 Richter, R., Kinoshita, A., Vincent, C., Martinez-Gallegos, R., Gao, H., van Driel, A.D., Hyun, Y.,  
615 Mateos, J.L., and Coupland, G. (2019). Floral regulators FLC and SOC1 directly regulate  
616 expression of the B3-type transcription factor *TARGET OF FLC AND SVP 1* at the *Arabidopsis*  
617 shoot apex via antagonistic chromatin modifications. *PLoS Genet.* *15*, e1008065.

618 Rowley, M.J., and Corces, V.G. (2018). Organizational principles of 3D genome architecture.  
619 *Nat. Rev. Genet.* *19*, 789–800.

620 Rowley, M.J., Nichols, M.H., Lyu, X., Ando-Kuri, M., Riviera, S.M., Hermetz, K., Wang, P.,  
621 Ruan, Y., and Corces, V.G. (2017). Evolutionarily conserved principles predict 3D chromatin  
622 organization. *Mol. Cell* *67*, 837-852.e7.

623 Sáez-Vásquez, J., and Delseny, M. (2019). Ribosome biogenesis in plants: from functional 45S

624 ribosomal DNA organization to ribosome assembly factors. *Plant Cell* 31, 1945 LP – 1967.

625 Sakamoto, Y., and Takagi, S. (2013). LITTLE NUCLEI 1 and 4 regulate nuclear morphology in  
626 *Arabidopsis thaliana*. *Plant Cell Physiol.* 54, 622–633.

627 Sanborn, A.L., Rao, S.S.P., Huang, S., Durand, N.C., Huntley, M.H., Jewett, A.I., Bochkow, I.D.,  
628 Chinnappan, D., Cutkosky, A., Jian, L., et al. (2015). Chromatin extrusion explains key  
629 features of loop and domain formation in wild-type and engineered genomes. *Proc. Natl.*  
630 *Acad. Sci. U. S. A.* 112, E6456–E6465.

631 Santos, A.P., and Shaw, P. (2004). Interphase chromosomes and the Rab1 configuration: does  
632 genome size matter? *J. Microsc.* 214, 201–206.

633 dos Santos, G., Schroeder, A.J., Goodman, J.L., Strelets, V.B., Crosby, M.A., Thurmond, J.,  
634 Emmert, D.B., Gelbart, W.M., and Consortium, F. (2015). FlyBase: introduction of the  
635 *Drosophila melanogaster* Release 6 reference genome assembly and large-scale migration of  
636 genome annotations. *Nucleic Acids Res.* 43, D690–D697.

637 Schubert, V., Weißleder, A., Ali, H., Fuchs, J., Lermontova, I., Meister, A., and Schubert, I.  
638 (2009). Cohesin gene defects may impair sister chromatid alignment and genome stability in  
639 *Arabidopsis thaliana*. *Chromosoma* 118, 591–605.

640 Schwarzer, W., Abdennur, N., Goloborodko, A., Pekowska, A., Fudenberg, G., Loe-mie, Y.,  
641 Fonseca, N.A., Huber, W., Haering, C.H., Mirny, L., et al. (2017). Two independent modes of  
642 chromatin organization revealed by cohesin removal. *Nature* 551, 51–56.

643 Sexton, T., and Cavalli, G. (2015). The role of chromosome domains in shaping the functional  
644 genome. *Cell* 160, 1049–1059.

645 Sexton, T., Yaffe, E., Kenigsberg, E., Bantignies, F., Leblanc, B., Hoichman, M., Parrinello, H.,  
646 Tanay, A., and Cavalli, G. (2012). Three-dimensional folding and functional organization  
647 principles of the *Drosophila* genome. *Cell* 148, 458–472.

648 Shi, J., Ma, X., Zhang, J., Zhou, Y., Liu, M., Huang, L., Sun, S., Zhang, X., Gao, X., Zhan, W., et  
649 al. (2019) Chromosome conformation capture resolved near complete genome assembly of  
650 broomcorn millet. *Nat. Commun.* 10, 464.

651 Sotelo-Silveira, M., Chávez Montes, R.A., Sotelo-Silveira, J.R., Marsch-Martínez, N., and de  
652 Folter, S. (2018). Entering the next dimension: plant genomes in 3D. *Trends Plant Sci.* 23,  
653 598–612.

654 Stam, M., Tark-dame, M., and Fransz, P. (2019). 3D genome organization : a role for phase  
655 separation and loop extrusion ? *Curr. Opin. Plant Biol.* 48, 36–46.

656 Strom, A.R., Emelyanov, A. V, Mir, M., Fyodorov, D. V, Darzacq, X., and Karpen, G.H. (2017).  
657 Phase separation drives heterochromatin domain formation. *Nature* 547, 241–245.

658 Stroud, H., Do, T., Du, J., Zhong, X., Feng, S., Johnson, L., Patel, D.J., and Jacobsen, S.E.  
659 (2014). Non-CG methylation patterns shape the epigenetic landscape in *Arabidopsis*. *Nat.*  
660 *Struct. Mol. Biol.* 21, 64–72.

661 Swiezewski, S., Liu, F., Magusin, A., and Dean, C. (2009). Cold-induced silencing by long  
662 antisense transcripts of an *Arabidopsis* Polycomb target. *Nature* 462, 799–802.

663 Szabo, Q., Jost, D., Chang, J., Cattoni, D.I., Papadopoulos, G.L., Bonev, B., Sexton, T., Gurgo,  
664 J., Jacquier, C., Nollmann, M., et al. (2018). TADs are 3D structural units of higher-order  
665 chromosome organization in *Drosophila*. *Sci. Adv.* 4, eaar8082.

666 Szabo, Q., Bantignies, F., and Cavalli, G. (2019). Principles of genome folding into  
667 topologically associating domains. *Sci. Adv.* 5, eaaw1668.

668 Tessadori, F., Schulkes, R.K., Driel, R. Van, and Fransz, P. (2007). Light-regulated large-scale  
669 reorganization of chromatin during the floral transition in *Arabidopsis*. *Plant J.* 2, 848–857.

670 VanBuren, R., Wai, C.M., Pardo, J., Giarola, V., Ambrosini, S., Song, X., and Bartels, D. (2018).  
671 Desiccation tolerance evolved through gene duplication and network rewiring in *Lindernia*.  
672 *Plant Cell* 30, 2943–2958.

673 Vertii, A., Ou, J., Yu, J., Yan, A., Pagès, H., Liu, H., and Zhu, L.J. (2019). Two contrasting classes  
674 of nucleolus-associated domains in mouse fibroblast heterochromatin. *Genome Res.* 29,  
675 1235–1249.

676 Wang, H., Li, S., Li, Y., Xu, Y., Wang, Y., Zhang, R., Sun, W., Chen, Q., Wang, X., Li, C., et al.  
677 (2019). MED25 connects enhancer–promoter looping and MYC2-dependent activation of  
678 jasmonate signalling. *Nat. Plants* 5, 616–625.

679 Wang, M., Wang, P., Lin, M., Ye, Z., Li, G., Tu, L., Shen, C., Li, J., Yang, Q., and Zhang, X.  
680 (2018). Evolutionary dynamics of 3D genome architecture following polyploidization in  
681 cotton. *Nat. Plants* 4, 90–97.

682 Weis, B.L., Kovacevic, J., Missbach, S., and Schleiff, E. (2015). Plant-specific features of  
683 ribosome biogenesis. *Trends Plant Sci.* 20, 729–740.

684 Zhang, L., Cai, X., Wu, J., Liu, M., Grob, S., Cheng, F., Liang, J., Cai, C., Liu, Z., Liu, B., et al.  
685 (2018). Improved *Brassica rapa* reference genome by single-molecule sequencing and  
686 chromosome conformation capture technologies. *Hortic. Res.* 5.

687 Zhang, X., Yazaki, J., Sundaresan, A., Cokus, S., Chan, S.W.-L., Chen, H., Henderson, I.R.,  
688 Shinn, P., Pellegrini, M., Jacobsen, S.E., et al. (2006). Genome-wide high-resolution mapping  
689 and functional analysis of DNA methylation in *Arabidopsis*. *Cell* 126, 1189–1201.

690 Zhu, W., Hu, B., Becker, C., Süheyla, E., and Berendzen, K.W. (2017). Altered chromatin  
691 compaction and histone methylation drive non-additive gene expression in an interspecific  
692 *Arabidopsis* hybrid. *Genome Biol.* 18, 157.

693

694

695

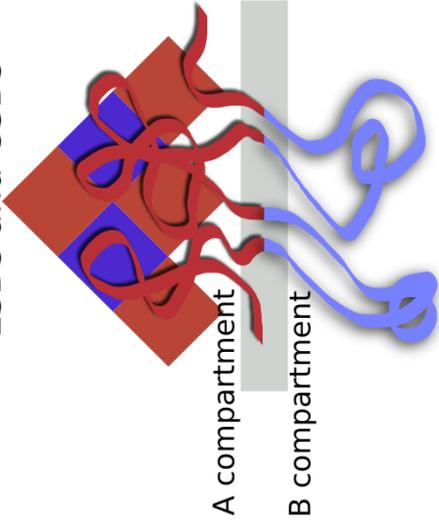
696 [Figure Legends](#)

697

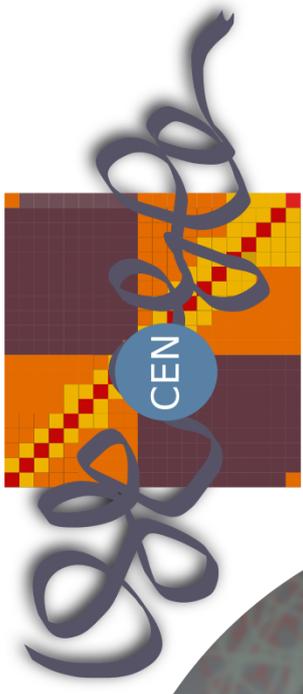
698 Fig. 1 The 3D genome is organized at multiple scales. For each scale, an illustration is given  
699 accompanied with typical features of Hi-C maps (adapted from Grob (2019)). LSD: Loose  
700 Structural Domain; CSD: Closed Structural Domain; CEN: Centromere; TAD: Topologically  
701 Associating Domain.

702

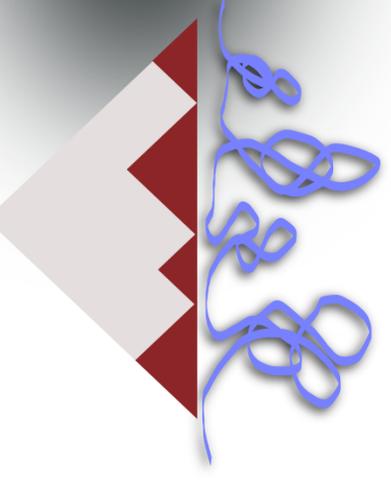
A/B compartments  
LSDs and CSDs



chromosome and chromosome arm  
territories



TADs



chromatin loops

