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Nuclear Architecture and Chromatin Dynamics in Interphase Nuclei of *Arabidopsis thaliana*

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Key Words

Arabidopsis thaliana · Chromosome territories · Heterochromatin · Interphase nucleus · Nuclear compartment

Abstract

The interphase cell nucleus is extraordinarily complex, ordered, and dynamic. In the last decade, remarkable progress has been made in deciphering the functional organisation of the cell nucleus, and intricate relationships between genome functions (transcription, DNA repair, or replication) and various nuclear compartments have been revealed. In this review, we describe the architecture of the *Arabidopsis thaliana* interphase cell nucleus and discuss the dynamic nature of its organisation. We underline the need for further developments in quantitative and modelling approaches to nuclear organization.

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of genome sequences, and an increasing number of studies have reported dynamic epigenomes. This progress has given rise to new challenges, namely to approach the genome in its three-dimensional nuclear framework (3D), to examine the interplay between the main functions of the genome and the architecture of the interphase cell nucleus, and to decipher the relationships between nuclear structure and function. Thus, there is a renewed interest in nuclear compartments, some of which were described more than 1 century ago, and in 3D nuclear architecture. The complexity of the interphase cell nucleus, its ordered structure, and the dynamics of this organelle at different scales are thus being investigated in both animal and plant cells. Much has been learnt about the composition and fine structure of the nucleus and the mechanism of formation and dynamics of its various functional compartments. A better understanding of the structural and functional interplay between chromatin and the other nuclear compartments is emerging. These studies have been accompanied by the development of specific 3D approaches and tools, such as 3D imaging and modeling, and methods that capture chromosome conformation. Numerous reviews have been published on diverse aspects of nuclear organisation [Delgado et al., 2010; Raj-

The linear dimension of eukaryotic genomes can be readily analysed using various high-throughput techniques. Thus, we are now able to decipher the evolution

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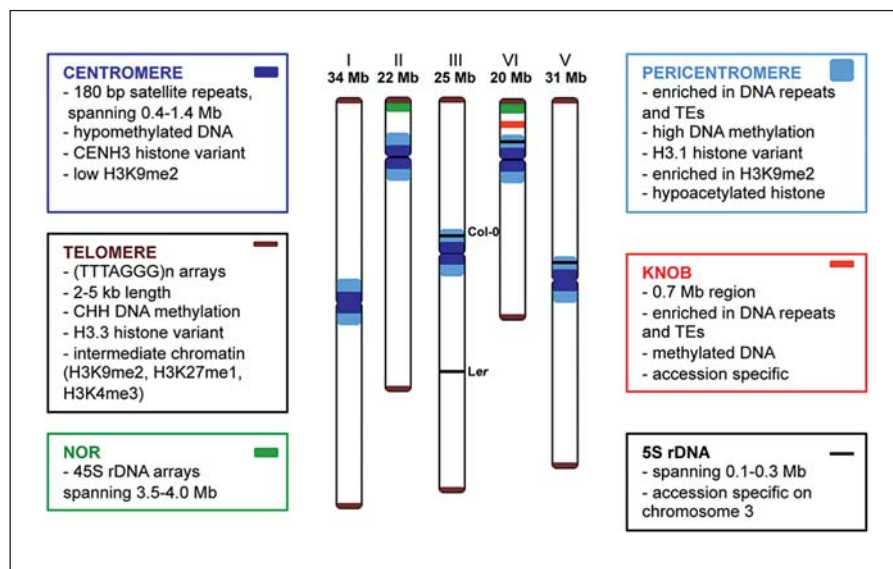
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Fig. 1. Heterochromatin compartments in *A. thaliana*. Map of the metacentric (1 and 5), submetacentric (3), and acrocentric (2 and 4) chromosomes. Polymorphic cytological markers (5S rDNA and knob) are indicated by the names of the accessions: Columbia-0 (Col-0), Landsberg *erecta* (*Ler*). TEs = Transposable elements.



apakse and Groudine, 2011; de Wit and de Laat, 2012; Taddei and Gasser, 2012; Dekker et al., 2013; Dion and Gasser, 2013; Towbin et al., 2013]. However, much remains to be learnt about chromatin dynamics in plants. In this review, we summarize our current knowledge of nuclear compartments of the interphase nucleus in the model plant *Arabidopsis thaliana* with a special emphasis on heterochromatin. Indeed, whereas little is known about euchromatin dynamics at the scale of the nucleus, heterochromatin is highly plastic, exhibits large-scale reorganizations, and participates to genome organisation. We also discuss 3D modeling and quantitative techniques for analyzing the architecture of interphase nuclei in *A. thaliana*.

Components of Plant Heterochromatin

In 1928, Emil Heitz classified chromatin into 2 types: heterochromatin and euchromatin. Whereas the former remains highly condensed throughout the cell cycle, the latter decondenses during interphase. This binary classification system, which was originally based on cytological observations in mosses, is still widely used to describe chromatin in all eukaryotes. However, it has evolved tremendously in the past 15 years, and central dogmas, such as the inertness and transcriptional inactivity of heterochromatin, have been challenged. The classification system has been expanded to include molecular and biochemical characteristics, such as symmetric or asym-

metric DNA methylation, post-translational histone modifications, nucleosome composition and arrangement, and transcriptional status, as determined by specialized polymerases. However, chromatin states at the scale of the nucleus are difficult to determine due to limitations in resolution, and only the relatively large-scale heterochromatin compartments of interphase nuclei have been analysed using cytological approaches.

The main heterochromatic regions of *A. thaliana*, which are visible by microscopy after DNA counterstaining, occur at the centromeres, pericentromeric regions, telomeres, and nucleolar organizer regions (NORs) (fig. 1, 2). These regions are referred to as constitutive heterochromatin, whereas chromatin that occasionally acquires heterochromatin characteristics and is dispersed throughout the genome is known as facultative heterochromatin. The cytological appearance of plant heterochromatin varies depending on the genome size (ranging from ~63–149,000 Mb) [Heslop-Harrison and Schwarzscher, 2011] and chromosomal organisation (ranging in dicotyledonous species from $2n = 4$, such as in *Haplopappus gracilis*, to $2n = \sim 640$ in *Sedum suaveolens*; <http://www.tropicos.org/Project/IPCEN>). Plant heterochromatin is either located in discrete and well-defined subnuclear regions that exhibit intense labeling with DNA stain, also called chromocenters (CCs) in some species, e.g. *A. thaliana* and *Oryza sativa* (rice), or it is distributed throughout the genome in less defined substructures as, for instance, in *Zea mays* (maize). The heterochromatin fraction of *A. thaliana* is estimated to account for 7.1% of

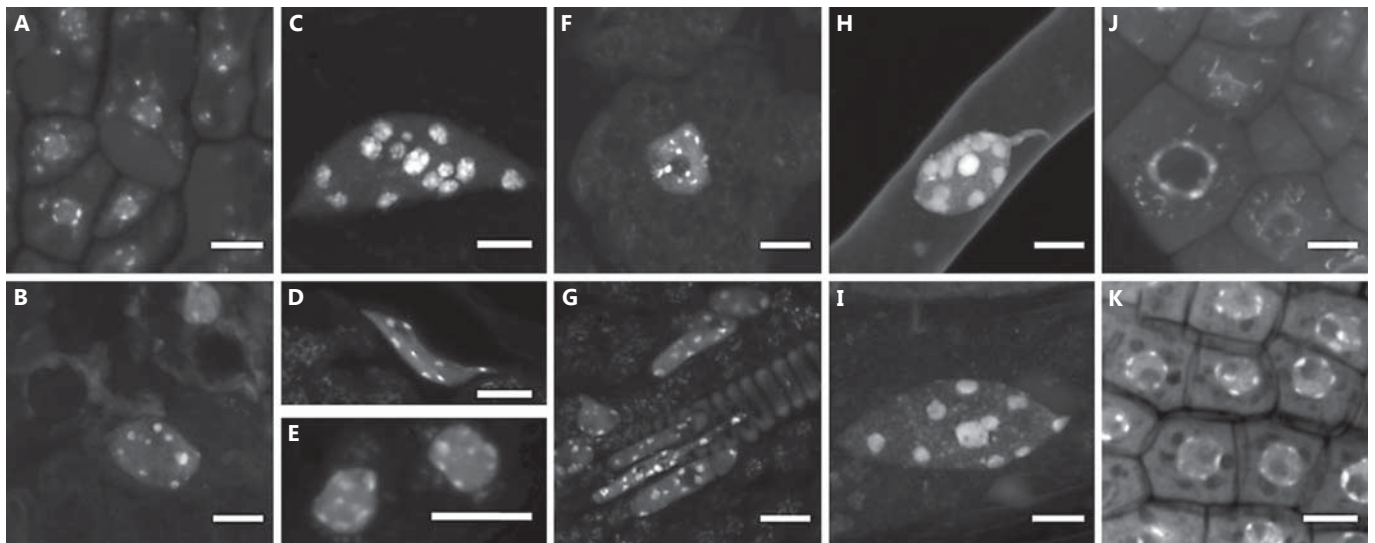


Fig. 2. Nuclear diversity in *A. thaliana*. Cell nuclei were observed in different cell types, either using cryosections (**A–G**) or whole-mount tissues (**H–K**) after DAPI staining. A selection of images is presented to highlight the diversity in shape and size of the nuclei but also in the number and size of chromocenters. Images correspond either to a single confocal section of the nucleus (**A, J, K**) or to the maximum z-projection of an image stack (**B–I**) for optimal

2D visualization. **A** Nuclei of cotyledon cells of a mature embryo. **B** Nucleus of a seed coat cell in a mature embryo. **C–G** Three-week-old seedlings. **C** Trichome nucleus. **D** Leaf epidermal cell nucleus. **E** Stomata nuclei. **F** Nucleus of a leaf mesophyll cell. **G** Nuclei of leaf vascular tissues. **H–K** Young root. **H** Nucleus of a root hair cell. **I** Nucleus of a root epidermis cell. **J** Nuclei of root meristem. **K** Nuclei of the root cap. Scale bars = 5 μ m.

the total chromosome length at pachytene ($\sim 330 \mu\text{m}$) based on a cytological approach [Fransz et al., 1998], for 10–15% of the genome based on the genome sequence [Arabidopsis Genome Initiative, 2000], and for 16% of the genome (22 Mb out of the ~ 135 Mb of the genome) based on DNA accessibility analysed by DNase I chip [Shu et al., 2012]. The relative heterochromatin fraction (RHF), defined as the area and fluorescence intensity of CCs in relation to the area and fluorescence intensity of the entire nucleus in DAPI counterstaining, is estimated to be $\sim 15\%$ [Soppe et al., 2002; Schonrock et al., 2006], with variations depending on cell type and developmental and environmental cues. Heterochromatin is rich in repetitive DNA sequences and transposable elements, has few genes, and exhibits little or no transcriptional activity. Furthermore, it exhibits distinct molecular and biochemical variations according to localization and function.

Centromeres are the primary constrictions along mitotic/meiotic chromosomes. The relative location of the centromere differs for each type of chromosome (fig. 1) [for a detailed review, see Ma et al., 2007]. The centromere directs the assembly of the proteinaceous kinetochore which interacts with spindle microtubules and facilitates the segregation of sister chromatids during mitosis [Del-

gado et al., 2010]. Centromeres of *A. thaliana* are composed of arrays of a 178-bp satellite repeat, ranging from 0.4 to 1.4 Mb in different chromosomes [Fransz et al., 1998; Copenhaver et al., 1999; Heslop-Harrison et al., 2003; Ma et al., 2007; Zhang et al., 2008]. Substantial variation in the copy number of the centromeric repeat was reported in different ecotypes [Hall et al., 2006]. The DNA sequences of centromeric satellite repeats differ markedly even among closely related species [Heslop-Harrison et al., 1999; Lysák, 2009; Heslop-Harrison and Schwarzacher, 2011]. Also, the centromeric chromatin region shows low levels of DNA methylation and of the H3K9me2 epigenetic mark [Zhang et al., 2008]. Independent of the DNA sequence, the location of the centromere is epigenetically specified by the presence of a histone H3 variant, CENH3 (also named HTR12 in *A. thaliana*). Despite an essential role in mitosis and meiosis, CENH3 is rapidly evolving and participates in the formation of centromeric nucleosomes with unique properties, thereby allowing the centromere to fulfill essential roles in kinetochore formation and genome partitioning [Lermontova et al., 2011; Tachiwana and Kurumizaka, 2011; Tachiwana et al., 2011]. In *A. thaliana*, the rapidly evolving N-terminal domain of CENH3 is specifically required for

centromeric loading in meiotic nuclei, suggesting that different CENH3 loading mechanisms exist in mitosis and meiosis [Ravi and Chan, 2010; Lermontova et al., 2006, 2011; Ravi et al., 2011]. These data have key functional implications for genome maintenance. A recent study reported that KINETOCHORE NULL2 (KNL2) participates in loading CENH3 at centromeres [Lermontova et al., 2013]. KNL2 contains a SANT-associated (SANTA) domain which is present in chromatin remodeling proteins and is associated with centromeres during all phases of the mitotic cell cycle, except from metaphase to mid-anaphase. KNL2 inactivation affects both mitotic and meiotic division without abolishing CENH3 assembly at centromeres [Lermontova et al., 2013]. In the *A. thaliana* cell nucleus, centromeres can be visualized by fluorescent in situ hybridization (FISH) by using a centromeric satellite repeat probe [Fransz et al., 1998], immunocytochemistry by using an antibody against HTR12 [Talbert et al., 2002] or MSI12, which colocalizes with HTR12 [Sato et al., 2005], or by live cell imaging using fluorescently tagged HTR12 [Fang and Spector, 2005]. These approaches have facilitated studies of the dynamics of the centromeric sub-compartment (see below).

The flanking pericentromeric heterochromatin is enriched in repetitive DNA sequences and transposable elements (TEs) [Copenhaver et al., 1999]. It is also characterized by a high level of 5-cytosine DNA methylation, with some strand-specific bias [Luo and Preuss, 2003], and an increased methylation of lysine 9 of histone H3 (H3K9me) and of the histone H3.1 variant [Stroud et al., 2012]. Recently, pericentromeric heterochromatin was shown to be the least accessible chromatin to DNase I, and blocks of accessible chromatin are progressively more abundant with increasing distance from the centromere [Shu et al., 2012]. Thus, there is not a sharp boundary between pericentromeric heterochromatin and euchromatin but rather a gradual transition to chromatin with an increased protein-coding gene density and a decreased TE density [Shu et al., 2012]. The assembly of H3K9me2-marked heterochromatin requires the transcription of repetitive sequences by specific RNA polymerases and RNA-directed DNA methylation [Verdel et al., 2009; Beisel and Paro, 2011; Zhang and Zhu, 2011; Castel and Martienssen, 2013].

Telomeres are protective nucleoprotein structures at the extremities of linear chromosomes that stabilize chromosome termini and prevent chromosome fusion and degradation by exonucleases [Lamb et al., 2007; Zellinger and Riha, 2007; Watson and Riha, 2010]. They consist of relatively short tandem repeat arrays (2–5 kb in *A. tha-*

liana) of a conserved short motif (TTTAGGG in most plant species) and associated telomere proteins. Interestingly, non-functional telomere-like repeats have been identified in chromosomal regions [named interstitial telomeric repeats (ITRs)], some of which are in close proximity to centromeres [Uchida et al., 2002], whereas other short interstitial telomere motifs (named *telo* box) were preferentially observed in the 5'-flanking region of genes [Regad et al., 1994; Gaspin et al., 2010]. The existence of these interstitial telomeric repeats strongly challenges the structural and functional analysis of terminal telomeric repeats but also provides interesting perspectives on regulatory mechanisms. The length of telomeres, which is related to life span, is under genetic control and varies among species. Although telomeres were originally thought to consist of heterochromatin, a recent molecular analysis of epigenetic marks in *A. thaliana* telomeres revealed that telomeric chromatin has some unexpected and unique features that are characteristic of intermediate heterochromatin [Vrbsky et al., 2010] or even euchromatin [Vaquero-Sedas and Vega-Palas, 2013]. Indeed, *Arabidopsis* telomeres are enriched in H3K9me2 and H3K27me1 heterochromatic marks but still retain the euchromatic H3K4me3 mark [Vrbsky et al., 2010; Vaquero-Sedas et al., 2012]. Furthermore, the *A. thaliana* telomeres are also relatively enriched in the H3.3 histone variant (which is usually associated with transcriptionally active regions) in comparison to centromeres that are enriched in H3.1 in comparison to telomeres [Vaquero-Sedas and Vega-Palas, 2013]. Interestingly, subtelomeres and ITRs are heterochromatic [Vaquero-Sedas et al., 2012]. The telomeric enrichment in the histone H3.3 variant is probably related to the unique juxtapositioning of telomeres and transcriptionally active genes in *A. thaliana*. Furthermore, as in other eukaryotes, *A. thaliana* telomeres are transcribed into non-coding telomeric repeat-containing RNAs (named TERRA) [Vrbsky et al., 2010]. Telomeric repeats are methylated at asymmetric cytosine sites via an RNA-dependent DNA methylation pathway, and small RNAs are derived from TERRAs produced from telomeres and from ITRs located near centromeres [Vrbsky et al., 2010]. Telomeric methylation was shown to be dependent on the activity of DNA methyltransferase MET1 and the remodeling factor DDM1 [Ogrocka et al., 2013].

Interstitial blocks of heterochromatin, called knobs, occur on the chromosome arms of maize and related species [Poggio et al., 2005; Albert et al., 2010]. Knobs vary in size and composition but are highly enriched in DNA repeats and TEs. Some knobs are visible throughout the

cell cycle and are used as cytological markers [Ghaffari et al., 2013]. Knobs are associated with low gene density and low transcriptional and recombinational activity. Some *A. thaliana* accessions, such as Wassilewskija and Columbia-0 (Col-0), bear a ~0.7-Mb knob on the short arm of chromosome 4 (hk4S) which does not colocalize with the CC of chromosome 4 (CC4) and is not visible during interphase (fig. 1) [Fransz et al., 1998, 2000; Koornneef et al., 2003]. The knob hk4S is enriched in centromeric and pericentromeric repeats and has few expressed genes. It was proposed to originate from an inversion event that moved DNA sequences from the pericentromeric outer domain to a distal euchromatin region of the 4S chromosome arm [Fransz et al., 2000]. Maize knobs do not have such a pericentromeric origin.

The nucleolar organizer region [McClintock, 1934] consists of tandem arrays of 45S rRNA-encoding DNA (rDNA) and is another major functional genomic region with heterochromatic characteristics. *A. thaliana* contains 2 NORs of similar size (each spanning 3.5–4.0 Mb of tandem repeat arrays), located at the subtelomeric regions of the acrocentric chromosomes 2 and 4 [Copenhaver and Pikaard, 1996] (fig. 1). The 5S rDNA loci are also organized in tandem arrays (of ~1,000 copies) which span 0.1–0.3 Mb and are located at pericentromeric regions of chromosomes 3, 4, and 5 in the Col-0 accession [Campbell et al., 1992; Murata et al., 1997]. The presence, location, and size of the 5S rDNA cluster on chromosome 3 are accession specific (fig. 1) with some possible intra-accession polymorphisms such as in the Cape Verde Islands (Cvi) accession [Fransz et al., 1998; Sanchez-Moran et al., 2002; Lopez et al., 2012]. It was shown that in the Col-0 accession, only the 5S rDNA clusters, located on chromosomes 4 and 5, contribute to the 5S RNA pool [Cloix et al., 2002].

***Arabidopsis* Model of Chromosome Organisation Centered on Heterochromatin**

In *A. thaliana*, CCs correspond to the coalescence of centromeric and pericentromeric regions of a chromosome and the NOR (if the chromosome bears a NOR). These heterochromatic structures function as genome organizer centers. Indeed, euchromatic chromosomal regions form loops that are 0.2–2 Mb long and are anchored to CCs [Fransz et al., 2002]. This organisation contributes to the overall structure of chromosome territories as described in the chromocenter-loop model [Fransz et al., 2002], also named the rosette-like model [van Driel and

Fransz, 2004]. Furthermore, it was shown that highly repetitive elements and TEs located on euchromatic chromosomal arms colocalize with CCs and remain associated with CCs despite extensive demethylation of the genome [Soppe et al., 2002]. This suggests that TEs both anchor the euchromatin loops and organize the pericentromeric regions [Soppe et al., 2002].

Variations in the number, size, and shape of centromeric foci and CCs as well as the cell type-specific organisation of heterochromatin have been reported in a number of studies. The nuclei of most cells (e.g. parenchyma cells, epidermal guard cells, and root cells) exhibit a ‘classical CC’ pattern, with 4–10 (mean ~8) conspicuous CCs (fig. 2) [Fransz et al., 2002]. The heterochromatin index (HX), defined as the percentage of nuclei showing the classical CC pattern, was thus calculated in numerous studies to quantify heterochromatin distribution [Fransz et al., 2003]. However, nuclei with uniform DAPI fluorescent nucleoplasm have been reported in some cells such as the diploid interphase tapetal cells of premeiotic anthers [Weiss and Maluszynska, 2001; Talbert et al., 2002]. In the root tip, centromeric foci exist in a variety of shapes, from dots of 0.5 µm in diameter to discontinuous strings (1.0–2.0 µm in length) of smaller bead-like dots, suggesting that centromeres have a range of compaction ratios [Talbert et al., 2002]. Given that the root tip is actively dividing, this range in centromeric foci shape might be, at least partially, cell cycle dependent. Interestingly, nuclei of the triploid endosperm tissue also have a peculiar heterochromatin organisation, with small CCs and additional heterochromatic foci interspersed in euchromatin which is likely linked to parental dosage [Baroux et al., 2007].

In plants, endoreduplication cycles occur in differentiated cells, leading to ≥4C cell nuclei. A positive correlation between CC association and ploidy levels was reported for a number of plant species [Ceccarelli et al., 1998]. In *A. thaliana*, endoreduplicated sister centromere associations have also been reported using live cell imaging [Fang and Spector, 2005]. These associations are cell type-dependent, being for instance more frequent in root epidermal cells than in leaf epidermal cells [Fang and Spector, 2005]. Similar results were observed in fixed cell nuclei with an alignment of the majority of the sister centromeres up to 16C [Schubert et al., 2006]; however, surprisingly, a dispersed pattern was reported in 32C nuclei [Schubert et al., 2006].

Distribution of Heterochromatin in the Nuclear Space

In some species, chromosomes exhibit a polarized orientation with all centromeres clustered at one pole of the interphase nucleus and all telomeres at the other. This peculiar interphase nuclear organisation, originally observed in salamander cell nuclei, was named the Rabl configuration [Rabl, 1885]. It has been described in *Allium cepa* (onion), *Hordeum vulgare* (barley), *Triticum aestivum* (wheat), *Secale cereale* (rye), and *Avena sativa* (oats) [Stack and Clark, 1974; Schwarzacher et al., 1989; Dong and Jiang, 1998; Santos and Shaw, 2004; Roberts et al., 2009]. The Rabl configuration is possibly a remnant of the preceding mitotic event. Indeed, during mitosis, the chromosomes condense, separate, and move to opposite poles, with the centromeres being pulled by kinetochore microtubules and leading the way and the telomeres lagging behind.

The Rabl configuration is not present in *A. thaliana* interphase nuclei. Rather, the centromeres are located at CCs which preferentially occupy peripheral positions, and the telomeres are preferentially associated with the nucleolus [Armstrong et al., 2001; Fransz et al., 2002; Schubert et al., 2012]. Interestingly, it was observed that plants with large genomes, e.g. *A. cepa*, ~149,000 Mb, tend to exhibit the Rabl pattern, whereas those with smaller genomes, e.g. *A. thaliana*, ~135 Mb, tend to exhibit a non-Rabl pattern. These data suggest a correlation between the Rabl configuration and genome size; however, the non-Rabl configuration was also reported in *Sorghum bicolor* (sorghum) and maize [Dong and Jiang, 1998], 2 species with quite large genomes. Thus, other determinants of the Rabl configuration may exist. Interestingly, the non-Rabl configuration appears to be tissue-specific in diploid rice; whereas the Rabl configuration is present in root xylem vessels, it is absent in other root tissues [Prieto et al., 2004]. Endoreduplication may occur in the large nuclei of vascular tissues and induce these changes in chromatin distribution, in agreement with the previously described correlation, or the large nucleolus of xylem cells might interfere with the redistribution of centromeres and telomeres.

The preferential locations of telomeres at the nucleolus and the dispersed peripheral distribution of centromeres were also observed during meiotic interphase in *A. thaliana* [Armstrong et al., 2001]. In meiotic prophase of most species (e.g. *A. thaliana* and maize), the ends of chromosomes cluster together on the inner surface of the nuclear envelope and form a structure called the 'bouquet' [Franklin and Cande, 1999; Cowan et al., 2001;

Tiang et al., 2012]. Thus, in maize the Rabl configuration is observed prior to the last premeiotic cell division and is lost during the following interphase [Bass et al., 1997] and a bouquet is formed in meiotic prophase. These observations demonstrate that the distribution of chromosomes in the nuclear volume is tightly regulated.

Many studies using fixed nuclei have reported that *A. thaliana* centromeres tend to preferentially localize to the nuclear periphery. This centromere distribution was confirmed by measuring the distances between centromeres and the nuclear envelope in 3D images of various diploid living cells from transgenic *A. thaliana* plants expressing HTR12-GFP [Fang and Spector, 2005]. However, this pattern might be more complex. Live-cell imaging also revealed that centromeres cluster transiently at opposite poles at the end of mitosis in root meristematic cells [Fang and Spector, 2005] and in root tip cells [Lindhout et al., 2007]. Lastly, in *A. thaliana* and *A. lyrata* interphase nuclei, CCs from NOR-bearing chromosomes 2 and 4 are more frequently located in close proximity to the nucleolus [Fransz et al., 2002; Berr et al., 2006; Schubert et al., 2012]. Using spatial statistics, a recent study showed that the 3D intra-nuclear distribution of CCs in leaf cell nuclei was not completely random and that this distribution was more regular than a completely random one [Andrey et al., 2010]. This finding was observed in both round and elongated nuclei of plant cells which differ in differentiation stage and ploidy level. This repulsive trend was evidenced based on the global analysis of the CC population. Therefore, it is not incompatible with some frequent associations of specific CCs, such as CC2s and CC4s. Observation of CCs in close proximity (CC clusters) has also been reported by de Nooijer et al. [2009]. However, in this study the frequency and the intensity of the phenomenon remain elusive as no quantification was provided. Therefore, the spatial distribution of CCs seems to obey to a global apparent repulsive tendency with some attractive trends for specific CCs. It remains to be determined whether this regular distribution of CCs can be fully explained by peripheral positioning or if additional constraints have to be invoked to explain the apparent mutual repulsion between CCs. For example, the existence of euchromatin loops anchored at CCs, as proposed by the rosette model of chromosome organisation [Fransz et al., 2002], could prevent CCs from coming into close proximity. Specific proteins may also be involved as recently demonstrated by the clustering of centromeres in CAP-D protein mutants [Schubert et al., 2013].

Dynamics of the Heterochromatin Compartment during Development

The plant life cycle is characterized by major developmental phase transitions and the reiterative production of plant phytomers but also by diverse adaptations to environmental changes. These events require transcriptional reprogramming events that modulate the expression of specific sets of genes. Recent studies showed that these transcriptional reprogramming events are accompanied by reorganization of heterochromatin compartments, illustrating that the nucleus is highly plastic [Baroux et al., 2011; Schubert and Shaw, 2011; van Zanten et al., 2012a]. Whether this reorganization participates in or is a consequence of gene regulation remains to be elucidated.

The female spore mother cell (or megaspore mother cell, MMC) differentiates from somatic cells within ovules and ultimately gives rise to female gametes. Large-scale chromatin reprogramming occurs during the specification of the MMC, and this probably contributes to the acquisition of the gametophyte fate [Baroux et al., 2011]. During this nuclear reorganization, the nucleolus and nucleus expand, the RHF and CC undergo a reduction in number, and the heterochromatin decondenses [She et al., 2013]. MMC chromatin reprogramming may be divided into 2 distinct phases: an early and rapid phase during which the composition of the nucleosome changes, followed by a late phase during which histone modifications undergo important changes [She et al., 2013].

In *A. thaliana*, embryonic development is completed about 10 days after pollination (DAP). After a phase of seed maturation, which involves the accumulation of sufficient reserves and desiccation (from 10–20 DAP), the seed undergoes a period of dormancy. Seed maturation is accompanied by 2 independent processes, nuclear shrinkage and chromatin compaction, which occur between 8 and 12 DAP and precede the major dehydration event of the maturing seed [Mansfield and Briarty, 1992; van Zanten et al., 2011, 2012b]. The RHF in embryonic cotyledon nuclei increases sharply during the maturation phase, while the 45S rDNA loci and the centromeric and pericentromeric repeats remain localized to the CCs. Interestingly, the nuclear volume is independent of both the moisture content and dormancy status of the seed but is developmentally controlled. ABSCISIC ACID INSENSITIVE3 (ABI3), a key transcription factor in seed maturation, participates in nuclear shrinkage which is thought to be a general adaptive response to desiccation tolerance [van Zanten et al., 2011]. During the early

events of seed germination (48–72 h after imbibition), the nuclear volume increases again, and this increase requires the activity of LITTLE NUCLEI1 (LINC1) and LINC2, 2 lamin-like analogues [van Zanten et al., 2011, 2012b; Ciska et al., 2013]. Furthermore, chromatin reorganization accompanies this event. Whereas the 45S rDNA loci remain localized to CCs during germination, the centromeric and pericentromeric repeats are more dispersed at the onset of germination [van Zanten et al., 2012b]. These CCs are smaller than those present in mature seeds. The classical conspicuous CC pattern reappears later during seedling growth.

During floral transition, which corresponds to the short developmental switch from the vegetative to the reproductive phase, a transient reduction in both RHF and HX was observed in 3 accessions [Landsberg *erecta* (*Ler*), Col-0, Cvi] which was accompanied by the decompaction of pericentromeric regions and 5S rDNA chromatin, followed by their subsequent relocation to CCs 3 days after bolting [Tessadori et al., 2007a].

Dynamics of the Heterochromatin Compartment in Response to Environmental Cues

Two recent studies reported a correlation between heterochromatin organisation and ambient light intensity; specifically, the RHF and HX increase with a rise in light intensity [Tessadori et al., 2009; van Zanten et al., 2010, 2012a]. In the first study, Tessadori et al. [2009] analysed the HX in 21 *A. thaliana* accessions originating from different geographical habitats and identified a significant correlation between geographical latitude, which determines the photon flux density (light intensity) of the region, and the HX. Interestingly, the HX was found to plateau (at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ for Col-0 and at $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ for *Ler*, a widely-used Central-European accession). The lowest HX was observed in the sub-tropical Cvi-0 accession which has smaller and fewer CCs than *Ler*. Furthermore, the Cvi-0 accession exhibited dispersed 5S rDNA and pericentromeric repeats, and the centromeric and 45S rDNA sequences remained in the reduced CCs. This chromatin arrangement is reminiscent of the one observed during floral transition. The second study showed that chromatin compaction progressively decreases after a reduction in light intensity from 200 to $15 \mu\text{mol m}^{-2} \text{s}^{-1}$. This heterochromatic event is reversible with return to normal light conditions, and the intensity of the response varies in different accessions (with Col-0 being more sensitive than *Ler*) [van Zanten et al., 2010]. Therefore, chro-

matin plasticity seems to contribute to the plant's adaptation to environmental light conditions.

Alternatively, the heterochromatin response to low light can be viewed as an abiotic stress response. Upon exposure to another abiotic stress, namely prolonged heat stress, the transcription of centromeric and pericentromeric repeats is reactivated, and these regions exhibit a dispersed pattern in FISH [Pecinka et al., 2010]. Interestingly, throughout recovery, transcription of centromeric and pericentromeric repeats was progressively silenced, whereas decondensation persisted for up to 1 week. Therefore, this is another example showing that chromatin condensation status and gene expression can be uncoupled. Furthermore, such alterations did not occur in meristematic cells or in cells from leaves produced after a period of heat stress. It was proposed that the specific meristematic chromatin response indicates the existence of a safeguard mechanism that minimizes genome damage in the germline [Pecinka et al., 2010]. Interestingly, heterochromatin decompaction was not observed after freezing or UV-C treatments [Pecinka et al., 2010]. Therefore, decondensation of the heterochromatin compartments is either not a general stress response or each type of stress is associated with chromatin reorganization in a specific compartment or with a distinctive timing and amplitude pattern. It will be interesting to decipher the signaling mechanisms that induce large-scale chromatin reorganization in differentiated cells and prevent such reorganization in rapidly dividing cells. Reorganization of heterochromatin was also observed in response to biotic stress [Pavet et al., 2006]. A drastic reduction in RHF and CC number (with most nuclei having only 2 small CCs) and loosening of CCs were observed within 1 day of infection with the bacterial pathogen *Pseudomonas syringae*.

A drastic decondensation involving pericentromeric regions, 5S rDNA, centromeric repeats, and 45S rDNA was described during the isolation of *A. thaliana* protoplasts [Tessadori et al., 2007b]. Despite general NOR decondensation, a fraction remains partially condensed, participating in small CCs close to the nucleolus. The protoplast chromatin reorganization is accompanied by the acquisition of totipotency and major transcriptional reprogramming that affects, for example, chromatin-associated genes and genes encoding histone variants [Chupeau et al., 2013]. It remains to be determined whether the reorganization of protoplast chromatin results from a stress response due to enzymatic digestion and osmotic and light changes and/or is necessary for acquisition of totipotency and major transcriptional reprogramming [Chupeau et al., 2013].

Mechanisms Involved in the Spatial Heterochromatin Distribution

Two main patterns of heterochromatin distribution emerge from the previous examples: the first pattern involves the partial decondensation of CCs at the 5S and pericentromeric regions, and the second affects all heterochromatic compartments of the CCs. A detailed study of the progressive and sequential reformation of CCs during protoplast culture provided complementary information about the highly ordered structure of CCs [Tessadori et al., 2007b]. During sequential CC recompaction, the NOR regions (3.5–4 Mb) reorganize first, followed by the centromeric (0.4–1.4 Mb), 5S rDNA (0.1–0.3 Mb), and dispersed pericentromeric repeats, including transposons, suggesting that the recompaction timing and the size of the repeat arrays are correlated [Tessadori et al., 2007b]. Thus, the 5S and pericentromeric sequences might participate in one core domain of a CC, which is first mobilized in chromatin decondensation events, and the centromeric repeats and 45S rDNA in another CC core domain, with a more central location and/or different properties. Establishing whether this latter core domain decondenses independently of the other core domain would provide insights into the structure of CCs. The number of anchoring sites might also be proportional to the size of the arrays and may thus contribute to the kinetics and formation of sub-compartments of the CCs.

Finally, the underlying biochemical properties of heterochromatin, such as DNA methylation, epigenetic marks, or histone composition, are also expected to contribute to this sort of 'CC breathing'. Heterochromatin dynamics have been considered as being either dependent or independent of epigenetic changes, suggesting that several mechanisms with possible self-reinforcing feedbacks exist. For instance, by using molecular approaches, the 5S rDNA arrays were shown to be hypomethylated when they loop out of CCs during seed germination [Mathieu et al., 2003], and demethylation of the centromeric and pericentromeric repeats was shown to accompany biotic-induced chromatin decondensation [Pavet et al., 2006]. However, no change in DNA methylation was observed at centromeric repeats during floral transition [Tessadori et al., 2007a], in protoplasts [Tessadori et al., 2007b], and in response to heat stress [Mittelsten Scheid et al., 2002; Pecinka et al., 2010]. Despite the large-scale reorganization, there is no change in H3K9me2 and H3K4me3 contents in protoplasts as determined by immunoblot analysis of total histones. In heat-stressed cell nuclei, a reduction in nucleosome occupancy

with a small reduction in H3K9me2 was observed [Mittelsten Scheid et al., 2002; Pecinka et al., 2010]. From these data, it is tempting to speculate that the epigenetic-dependent pathway might contribute to the formation of a putative 5S-pericentromeric core domain and the independent pathway to that of the other putative core domain. However, it is important to note that most studies used methods with low sensitivity at the global nuclear scale to detect epigenetic changes and did not consider all of the chromatin marks and their combinations [Baubec et al., 2010]. Furthermore, all sub-compartments were not simultaneously analysed. Therefore, specific epigenetic variations may not have been identified yet. Alternatively, ‘CC breathing’ may be seen as a continuous process with various amplitude and timing patterns. Finally, another key missing element is a better understanding of the higher-order structures of chromatin. The existence of 30-nm chromatin fibers is still a matter of debate, and an alternative chromatin model that involves interdigitation of nucleosomal arrays, which is more compatible with rapid conformational changes providing access to DNA, is currently proposed [Fussner et al., 2011; Luger et al., 2012] and might also impact on ‘CC breathing’.

A few mutations that have a marked impact on the formation and/or spatial distribution of conspicuous heterochromatin sub-compartments have been described (table 1). Three main classes of genetic determinants involved in heterochromatin dynamics can tentatively be distinguished based on their functions (table 1). The first class (class I) corresponds to genes involved in the formation of heterochromatin and the maintenance of silencing in *A. thaliana* (i.e. *MET1*, *CMT3*, *NRPD2*, and *NRPE1*) [Mittelsten Scheid et al., 2002; Soppe et al., 2002; Onodera et al., 2005; Vaillant et al., 2008; Douet et al., 2009]. It is important to note that mutations that affect silencing do not necessarily alter nuclear heterochromatin organisation. For instance, the nuclear shape and CC structure of the *morpheus*’ *molecule1* mutant (*mom1*), which is affected in an epigenetic regulator, are normal [Probst et al., 2003]. The second class (class II) includes genes encoding chromatin-associated proteins, such as the ATP-dependent SWI2/SNF2-like chromatin remodeling DDM1 factor [Soppe et al., 2002; Probst et al., 2003] which was shown to have specific functions in heterochromatin remodeling [Zemach et al., 2013], the histone-modifying enzyme HDA6 [Probst et al., 2004], and the chromatin assembly subunits FAS1 and FAS2 [Schonrock et al., 2006]. The first 2 classes of genetic determinants may participate in the epigenetic-dependent pathway which is involved in heterochromatin dynamics. The third emerging

class (class III) contains genes that encode diverse nuclear structural proteins, such as the 2 lamin-like proteins LINC1 and LINC2 [Dittmer et al., 2007] that might be involved in an epigenetic-independent pathway. Based on the identification of nuclear lamina-associated chromatin domains in *Drosophila* and humans [Pickersgill et al., 2006; Guelen et al., 2008], we expect that mutations that affect the plant nuclear envelope [Graumann et al., 2010; Boruc et al., 2012] might have an impact on plant chromatin organisation. Furthermore, some subunits of the cohesion and condensin complexes, such as the SYN4 and CAP-D3 proteins, also play important roles in sister chromatid organisation and centromere distribution and thus affect the interphase chromatin architecture [Schubert et al., 2009, 2013].

Different signaling pathways involved in heterochromatin reorganization are expected to exist that might converge on key regulators of heterochromatin reorganization, such as the factors described in table 1. Indeed, development and environmental processes affect the spatial organisation of heterochromatin. These regulatory components might be specific to or shared by different signaling pathways. As described above, light intensity is an important environmental signal that controls chromatin compaction. The light-signaling pathway is mediated by well-described photoreceptors, some of which are localized to the nucleus. Among them, CRYPTOCHROME 2 (CRY2) and PHYTOCHROME B (PHYB) are involved in chromatin reorganization, whereas others, such as phototropins and CRYPTOCHROME1, are not [Tessadori et al., 2007a; van Zanten et al., 2010]. These data suggest that CRY2 and PHYB also participate in the spatial organisation of heterochromatin. Thus, many elements that affect the timing and molecular events of heterochromatin dynamics remain to be identified, and the impact of heterochromatic reorganization on the 3D organisation of the genome remains to be established.

Chromosome Territories

In plant and animal cell nuclei, each chromosome occupies a discrete portion of the nuclear space, named the chromosome territory (CT) [Boveri, 1909; Cremer and Cremer, 2010; Tiang et al., 2012]. Deciphering the rules and mechanisms that govern the spatial nuclear distribution and internal organisation of chromosomes is a highly dynamic and challenging research field, with the aim to better understand genome functions [Bickmore and van Steensel, 2013; de Graaf and van Steensel, 2013]. The

Table 1. Mutations affecting the spatial organisation of heterochromatic compartments in *A. thaliana*

Class	Mutant	Protein function	Nuclear phenotype	Reference
I	<i>methyltransferase 1 (met1)</i>	DNA methyltransferase	small CCs (chromocenter fraction reduced by ~25–30%), pericentromeric sequences away from CCs, reduced heterochromatic 5S rDNA fraction, DNA methylation reduced by ~70%, decreased H3K9 methylation, transcriptional reactivation of silent genes	Soppe et al. [2002]; Vaillant et al. [2008]
	<i>chromomethylase 3 (cmt3)</i>	DNA methyltransferase	reduced heterochromatic 5S rDNA fraction, decreased symmetrical methylation at 5S rDNA	Vaillant et al. [2008]
	<i>nuclear RNA polymerase D2A and D2B (nrpd2a nrpd2b double mutant)</i>	Second largest subunit of Polymerase IV	numerous but small CCs, decondensation of 5S rDNA with less colocalization with CCs, increased number of NOR signals due to dissociation, H3K9me2 signals are dispersed and colocalize with the numerous small DAPI foci	Onodera et al. [2005]; Douet et al. [2008]
	<i>Nuclear RNA polymerase E1 (nrpe1)</i>	Largest subunit of Polymerase V	decondensed 5S rDNA at chromosome 4 but not for 5S rDNA at chromosomes 3 and 5, decondensed NOR4	Douet et al. [2009]
	<i>repressor of silencing1 (ros1)</i>	DNA glycosylase/demethylase	reduction of the transient decondensation of 5S rDNA loci at 3 days post-germination	Douet et al. [2008]
II	<i>decrease in DNA methylation 1 (ddm1)</i>	SWI2/SNF2 chromatin remodeling factor	small and decondensed CCs with looping out of pericentromeric sequences, smaller heterochromatic 5S rDNA fraction, reduction of DNA methylation by ~70% and of H3K9 methylation, transcriptional reactivation of silent loci	Soppe et al. [2002]; Mathieu et al. [2003]; Probst et al. [2003]
	<i>histone deacetylase 6 (hda6)</i>	RPD3-like histone deacetylase – involved light response of chromatin	reduction of RHF and HX, decondensation of rDNA loci with enrichment of H4ac4 and H3K4me at rDNA loci, transcriptional reactivation of the <i>TSI</i> pericentromeric repeats	Probst et al. [2004]; Tessadori et al. [2009]
	<i>fasciata 1, fasciata 2 (fas1, fas2)</i>	Subunits of Chromatin Assembly Factor 1 (CAF-1)	reduced total heterochromatin fraction, maintenance of transcriptional silencing at heterochromatic loci	Schonrock et al. [2006]
III	<i>little nuclei 1 and 2 (linc1 linc2 double mutant)</i>	Lamin-like analogs – related to Nuclear Matrix Constituent Protein1 (NMCP1)	reduced nuclear size, altered nuclear morphology, reduction of CC number (4.6 ± 0.1), higher DNA packaging ratio, altered polyploidy	Dittmer et al. [2007]; Sakamoto and Takagi [2013]
	<i>syn4</i>	alpha-kleisin subunit of the cohesin complex	decreased sister chromatin alignments along chromosome arms in 4C differentiated leaf nuclei and impaired sister centromere cohesion	Schubert et al. [2009]
	<i>cap-D3</i>	HEAT-repeat containing condensin CAP-D subunit	alterations of centromeric and pericentromeric heterochromatin association, decreased sister chromatid cohesion in 4C nuclei	Schubert et al. [2013]

chromosome conformation capture method (3C) and derived methods, such as high-throughput chromosome conformation capture (HiC), have been developed in animal systems to provide maps of interaction frequencies between genomic regions at the locus, chromosome, or whole genome scales [de Wit and de Laat, 2012]. Three-dimensional conformations of the genomes have thus been computationally reconstructed from these data [Lieberman-Aiden et al., 2009; Dekker et al., 2013]. Based

on HiC results, the fractal globule model of CT organisation was proposed. According to this model, the chromatin fiber is packed into 1-Mb domains in meta-stable conformational states [Mirny, 2011; Dekker et al., 2013]. In plants, the 3C method has only recently been used [Hovel et al., 2012], and no equivalent or alternative model is available for plant CT organisation yet.

Plant CTs were observed in species both with [Abranches et al., 1998] and without [Lysak et al., 2001, 2003;

Pecinka et al., 2004] Rabl configuration by FISH studies. Using a wheat line containing an extra pair of rye chromosomes, it was shown that the rye CTs were roughly parallel and elongated nuclear domains extending between the centromeric and telomeric nuclear poles in a Rabl configuration [Abranches et al., 1998]. More recently, this organization was shown to be present in meristematic but not in differentiated nuclei [Schubert et al., 2011]. In small genome species with non-Rabl configuration, such as *Arabidopsis*, CTs have a different spatial distribution. The association frequencies between *A. thaliana* chromosome pairs were computed based on simultaneous painting of all chromosome pairs [Pecinka et al., 2004]. Based on a computer model of CT formation determined by polymer decondensation, it was shown that these frequencies were not significantly different from those expected under randomness, except for NOR-bearing chromosomes which are frequently associated with each other [Pecinka et al., 2004]. Likewise, no differences were found between random expectations and observed associations of homologous genomic regions of ~100 kb [Pecinka et al., 2004]. Similar results were found in related species [Berr et al., 2006] and in differentiated cells [Berr and Schubert, 2007]. Hence, the current view of CT organisation in *A. thaliana* and related species is globally random and contrasts with results from animal studies [Schubert et al., 2012]. It has, for example, been suggested that CTs in animals obey a size-dependent radial distribution, with smaller chromosomes being located toward the nuclear center. Other studies reported a gene density-dependent spatial distribution, with gene-rich chromosomes being located towards the center [van Driel and Fransz, 2004]. The absence of such patterns in *A. thaliana* may be due to its relatively uniform chromosome size and gene density and its low number of chromosomes (fig. 1).

Similarly, few arguments favor any cell type- and tissue-specific organisation of CTs in *Arabidopsis* and other plants. Chromosome painting in *A. thaliana* revealed that CTs were larger in endosperm nuclei than in nuclei of other cell types, corroborating the notion that less chromatin compaction occurs in this tissue [Baroux et al., 2007]. The tissue-specific spatial distribution of CTs has been reported in mouse, and CT distribution patterns were found to be maintained throughout mitosis [Parada and Misteli, 2002]. By contrast, the spatial distribution of CTs is not conserved between mother and daughter cells in *Arabidopsis*, and only a transient mirror-image distribution has been reported between daughter cells [Berr and Schubert, 2007]. The absence of maintenance of CT organisation during mitosis and within tissues is proba-

bly also related to the small genome and chromosome sizes of *Arabidopsis*.

No specific role of CTs in genome expression has been reported in plants until now [Tiang et al., 2012]. In animals, transcription sites are largely localized to the CT boundaries, and genes have been reported to relocalize to sites outside the CT upon activation [Geyer et al., 2011; Davidson et al., 2013]. By contrast, the relative localization of the flowering gene *FWA* to its CT did not seem to differ with its transcriptional status [Pecinka et al., 2004]. In wheat, transcription sites were not preferentially localized to the periphery of CTs [Abranches et al., 1998]. Only a few studies have investigated gene-to-CT positioning in relation to transcriptional regulation, and these studies were performed using coarse spatial descriptions. Further large-scale and systematic quantitative analyses are required to better document the role, if any, of chromosome organisation in the regulation of gene expression in plant nuclei.

Chromatin Dynamics at the Locus Scale

Numerous studies in yeast and animals have measured the chromatin motion of genomic regions to investigate the interplay between gene positioning and transcriptional status. In plants, the first evidence of chromatin dynamics and remodeling at the scale of the locus was provided by a 3D FISH experiment that revealed 2 chromatin conformations, one open and one closed, in the non-hair and hair cell nuclei of the *A. thaliana* root epidermis, respectively, in the genomic region of the *GLA-BRA2* (*GL2*) transcription factor locus [Costa and Shaw, 2006]. *GL2* is expressed in non-hair cells; however, mutant analysis revealed that neither *GL2* expression nor the belonging to a specific cell lineage is required for the open chromatin conformation in the *GL2* genomic region. The chromatin conformation in the *GL2* region depends on positional information in root cells and is reset at mitosis.

Currently, our knowledge is limited due to the complexity of the nuclear system and technical problems in visualizing, recording, and analyzing small portions of the genome in wild-type environments. The chromatin beacon or chromatin-tagging method is a powerful technique for analyzing the dynamics of tagged genomic regions in living organisms and for quantifying chromatin structural parameters at these genomic locations [Amini et al., 2011]. In this technique, exogenous concatameric arrays (~2–10 kb) of a DNA sequence, which is specifically recognized by a selected DNA-binding domain, are

inserted into the genome, and the location of the arrays (termed beacon arrays) is molecularly characterized. The expression *in trans* of a chimeric protein resulting from the fusion between the selected DNA-binding domain and a fluorescent reporter protein allows visual tracking of chromatin movement at the tagged loci in the nuclei of live cells. A 2-component labeling system based on the recognition of tandem *lac* operator binding sites (*lacO*) by the *lac* repressor (LacI) was successfully used in yeast, *Drosophila*, mammals, and plants [Robinett et al., 1996; Marshall et al., 1997; Kato and Lam, 2001; Vazquez et al., 2001]. A bacterial tetracycline operator/repressor (*tetO*/TetR-YFP) system [Matzke et al., 2003] and a combination of the *lacO*/LacI-dsRed and *tetO*/TetR-YFP systems [Matzke et al., 2005] have been developed to analyze the 3D distribution of chromosomal sites in plants. By introducing a gene into the cassette bearing the *lacO* repeat array, it was possible to study chromatin movement in relation to transcriptional activity of the reporter gene at the location of the insertion [Rosin et al., 2008; Rosa et al., 2013].

The approaches described above were used to study the relationship between the physical and functional properties of chromatin. In animals, it was shown that chromatin undergoes random diffusive motion in interphase nuclei, and that this Brownian motion is constrained in a way that a given genomic segment moves within a limited nuclear sub-region. Complex motion patterns have been recorded for genomic regions which exhibit short- and long-range motion with different velocities. The long-range diffusive motion fluctuates over a 36-fold distance range during the cell cycle [Vazquez et al., 2001]. These findings support a highly organized nuclear space and the existence of mechanisms that control chromatin motility according to cell type and throughout the cell cycle. Furthermore, these findings suggest that chromatin motion participates in nuclear processes that require chromosome mobility and chromosome reorganization in the nuclear space. For instance, it was recently shown that the induction of double-strand breaks in the yeast *Saccharomyces cerevisiae* dramatically increases the mobility of the broken loci but also of the homologous unbroken loci without changing the speed at which chromosomal regions move. The increased mobility allows a wider exploration of the nuclear volume which facilitates the pairing of homologous regions [Mine-Hattab and Rothstein, 2012, 2013].

Using the chromatin beacon technique, a few studies have demonstrated that chromatin in the *A. thaliana* nucleus also exhibits constrained diffusive motion and that

the amplitude and kinetics (diffusion coefficient) of the motion depend on the ploidy level and cell type [Kato and Lam, 2003; Rosin et al., 2008]. The constrained area increases with the ploidy level: it was 6.6 times larger, on average, in elongated polyploid epidermal pavement cell nuclei than in smaller disk-shaped diploid guard cell nuclei, whereas the diffusion coefficient was lower (<2.3 times) in polyploid cells than in diploid ones. The confinement radius in the *A. thaliana* nucleus ranges from 0.1–0.4 μm , depending on the ploidy level and activity of the locus [Kato and Lam, 2003; Rosin et al., 2008]. In *A. thaliana*, the chromatin compaction ratio, as determined by measuring the *in vivo* distance between 2 fluorescence-tagged transgene inserts on the same chromosome [Matzke et al., 2005], is approximately 670-fold. For instance, 2 transgenes, located 2.4 Mb apart (which corresponds to a DNA sequence of 0.8 mm) on chromosome 2, had an observed physical separation of 2.1 μm (380-fold), whereas another pair of transgenes, situated 25 Mb apart on chromosome 1, exhibited a physical separation of 8.7 μm (960-fold). Furthermore, the frequencies of homologous pairing and association with heterochromatin of transgenic repeats differed with the construct, the chromosomal insertion position, the cell type, and the number and repetitiveness of inserts [Pecinka et al., 2005].

The first evidence that gene position and transcriptional activity are linked in the plant cell nucleus emerged with the study of the nuclear organisation of transgenic *FLC* alleles tagged with the *lacO* repeat array (*FLC-lacO*) in response to vernalization [Rosa et al., 2013]. During cold treatment and after ethanol induction of the LacI-YFP-NLS fusion protein, a cluster of *FLC-lacO* alleles was observed with 1–2 foci in endoreduplicated root cells and only 1 in meristematic diploid root cells, whereas in non-vernalized plants, 6 or more foci were observed. The *FLC-lacO* clustering was quantitatively dependent on the cold treatment and dependent on Polycomb proteins of the PHD-PRC2 complex which are required for *FLC* silencing before and during the cold treatment. Therefore, physical repositioning of loci associated with transcriptional changes occurs in plants and can be mediated by Polycomb-mediated epigenetic mechanisms in both plants and animals, suggesting that the key mechanisms are conserved.

There are several limitations of the chromatin beacon technique: (i) the beacon array is not targeted precisely, because site-directed mutagenesis can not be performed in most plants; (ii) an artificial transgenic environment that differs from the original one may be created due to the interruption and/or rearrangement of the genomic

region where the beacon is inserted, and the 3D organisation of the chromatin fiber resulting from the insertion may be modified; and (iii) the high number of tandem repeats can induce repeat-induced silencing and generate modifications in the epigenetic chromatin of the beacon array and/or in the adjacent genomic chromatin. Indeed, a high copy number of repeats alters the local chromatin arrangement, resulting in more frequent somatic pairing of *lacO*-harboring regions and more frequent association with heterochromatic CCs [Pecinka et al., 2005]. These changes depend on the size and number of repeat units per locus [Jovtchev et al., 2008]. To minimize recombination events, replication instability, and the repeat-induced silencing usually associated with tandem repeats, Rosa et al. [2013] used a low number of concatameric *lacO* binding sites of the *lacO* DNA sequence and inserted ~10-bp random sequences between *lacO* DNA sequences [Jovtchev et al., 2008; Rosa et al., 2013]. There is an urgent need to develop alternative techniques to label specific genomic regions so that the relationship between the physical and functional properties of chromatin can be analysed more thoroughly. Zinc finger and transcription activator-like effector proteins have been customized to introduce targeted genome modifications (i.e. mutations, insertions, and substitutions) [Chen and Gao, 2013; Gaj et al., 2013]. These proteins have also been adapted to visualize heterochromatic repeat sequences, both in plants [Lindhout et al., 2007] and animals [Miyinari et al., 2013]. Novel optimization and adaptation techniques for single-copy gene detection would be tremendously useful. Furthermore, the development of programmable RNA-guided DNA labeling systems derived from RNA-guided nuclease technology [Belhaj et al., 2013; Gaj et al., 2013; Puchta and Fauser, 2013] might give rise to alternative useful techniques. A recent study using rolling-circle amplification of gene-specific padlock probes coupled with FISH reported an example of gene repositioning being associated with transcriptional activation [Feng et al., 2014].

Dynamics of Nuclear Protein Compartments

Chromatin occupies only a fraction of the interphase nuclear space (i.e. ~5% for DNA and ~20% for the 10-nm chromatin fiber) [Fussner et al., 2011]. Thus, interchromatin and perichromatin nuclear domains correspond to large functional spaces, harboring numerous and diverse nuclear bodies (NBs). NBs are membraneless subdomains that were originally described as being morpho-

logically distinct from their surrounding neighborhood when observed by transmission electron microscopy (TEM) [Shaw and Brown, 2004; Dundr, 2012]. The definition was extended to subnuclear compartments observed by immunocytochemistry or fluorescence microscopy but without prior morphological evidence of their ultrastructure (i.e. Polycomb bodies or transcription factories).

NBs are nuclear microenvironments that function in specific biochemical processes, such as splicing (nuclear speckles and Cajal bodies), transcription (transcription factories), or gene silencing (Polycomb bodies). Most are highly conserved in animals and plants, but some, such as photobodies, are plant specific. Photobodies are discrete accumulations of photoreceptors with key regulatory functions in plant growth and development in response to light signals. They contain red and far-red sensing phytochromes (A to E) or cryptochrome blue light receptors (CRY2 and possibly CRY1) and other associated proteins. Light induces rapid conformational changes of the photoreceptors and directly controls the assembly of photobodies. Although they have been proposed to have functions in storage, transcriptional regulation, or protein degradation, these possibilities remain to be confirmed [Chen, 2008; Chen and Chory, 2011; Van Buskirk et al., 2012]. As previously described, light signaling can induce heterochromatin reorganization. It remains unclear whether photobodies interact with chromatin and participate in such reorganization. Based on fluorescence imaging studies, other NBs with specific functions in abscisic acid or auxin signaling pathways [Ng et al., 2004; Tao et al., 2005] or in the control of the circadian clock [Strayer et al., 2000] have been reported. Plant nuclear dicing bodies, which are located close to nucleoli, are sites of plant-specific miRNA processes [Fang and Spector, 2007; Liu et al., 2012].

Most NBs are highly dynamic in terms of composition, number, shape, and size, and these dynamics are modulated by the cell differentiation state, metabolic state, and transcriptional activity. Whereas animal NBs are fairly well characterized, the components, structures, and assembly mechanisms of plant NBs remain elusive, with the exception of the largest NB, the nucleolus. It was proposed that NBs generally result from a local dynamic equilibrium between assembly and disassembly activities of protein complexes. Two distinct assembly models for NBs have been proposed. The first model is based on an orderly manner of assembly that involves central scaffolding factors, whereas the second model depends on random aggregation [Matera et al., 2009]. Due to the spa-

tial and temporal dynamics of NBs in the cell nucleus and their intricate functions in genomic activities (i.e. transcription, replication, and DNA repair), it is difficult to decipher the relationship between NB functions and genome organisation. How the spatial dynamics of NBs relate to chromatin dynamics is largely unknown. Indeed, the spatial distribution of NBs relative to CTs, chromatin domains, and other nuclear compartments is poorly documented.

The largest NB, the nucleolus, was first described in 1835 by Rudolf Wagner and is a well-studied multifunctional compartment in both animals and plants. Mainly known for its functions in rDNA transcription, rRNA processing, and pre-ribosome assembly, the nucleolus is also involved in the processing and assembly of ribonucleoproteins, transcriptional gene silencing, and mRNA surveillance and export [Brown and Shaw, 2008; Dundr, 2012; Morimoto and Boerkoel, 2013]. The nucleolus is a complex NB that contains different subdomains with their own dynamics (e.g. the fibrillar center, dense fibrillar and granular components, and peripheral heterochromatic shell) [Nemeth and Langst, 2011]. Interestingly, the NOR plays a crucial role in its assembly [McClintock, 1934]. In humans, a model was recently proposed for a role of distal (telomeric side) NOR-flanking regions as anchoring sites for active NORs in the perinucleolar heterochromatic region, whereas inactive NORs are relocated from the nucleolar periphery [Floutsakou et al., 2013]. The nucleolus has emerged as a genome organisation center that is involved in nuclear architecture [Nemeth et al., 2010; van Koningsbruggen et al., 2010]. Indeed, genomic regions associated with the nucleolus (nucleolar-associated domains, NADs) were identified and found to account for ~4% of the genome. NADs are characterized by a high density of AT-rich sequence elements and satellite repeats, a low gene density, and a significant enrichment in transcriptionally repressed genes belonging to different gene families. It will be interesting to identify the *cis* and *trans* determinants of NAD formation and the mechanism underlying the dynamic maintenance of NADs in different physiological conditions. The plant nucleolus was also intensively studied [Brown and Shaw, 2008], and a large set of *A. thaliana* nucleolar proteins (217) was identified within the first extensive proteomic analysis of a plant NB [Pendle et al., 2005]. In *A. thaliana*, the nucleolar periphery seems to be an important functional interface with other NBs (D-bodies and Cajal bodies) and also with heterochromatic compartments (NORs, telomeres). It is thus very likely that NADs exist in plants.

In *Drosophila* and mammals, local enrichments in Polycomb group (PcG) proteins have been described as Polycomb bodies (PcG bodies) [Pirrotta and Li, 2012]. PcG proteins are key chromatin factors that maintain gene repression via several mechanisms, such as histone post-translational modifications, chromatin compaction, or formation of higher-order chromatin structures [Schuettengruber and Cavalli, 2009; Bantignies and Cavalli, 2011; Simon and Kingston, 2013]. PcG proteins participate in the formation of multiprotein complexes, such as Polycomb Repressive Complex1 (PRC1) and PRC2 which are the best-studied PRC complexes in eukaryotes [Schuettengruber and Cavalli, 2009]. Animal PcG bodies can be visualized in the cell nucleus by labeling subunits of PRC1, such as Polycomb and Polyhomeotic [Pirrotta and Li, 2012]. PcG bodies participate in gene silencing by creating a specific microenvironment in which remote PcG-silenced targets colocalize as a result of long-distance interactions between silent targets and chromatin-fiber folding [Bantignies et al., 2011; Cheutin and Cavalli, 2012; Delest et al., 2012]. Some RNAi components, such as Dicer-2, PIWI, and Argonaute1, which also form specific nuclear bodies, can colocalize with PcG bodies and participate in PcG repression [Grimaud et al., 2006]. Furthermore, PcG bodies are dynamic, with proteins being rapidly exchanged between the nucleoplasm and PcG bodies, and vary both in number and size with cell type; their number increases as cells differentiate. Time-lapse experiments also revealed a fast regime of PcG motion within volumes smaller than CTs as well as a slow regime of long-range motion that depended on coordinated large-scale chromatin movements which might be associated with displacements of whole or large parts of chromosomes and led to occasional association and dissociation events of PcG bodies [Cheutin and Cavalli, 2012]. However, whether PcG bodies are true nuclear entities or 3D chromosomal domains enriched in PcG proteins is still a matter of debate [Smigova et al., 2011; Pirrotta and Li, 2012]. Thus, the mechanism by which PcG bodies form, which may involve PRC1 components with polymerization properties [Isono et al., 2013], as well as the role of PcG bodies in transcriptional regulation remain to be clarified. In *A. thaliana*, LHP1, the plant functional homolog of Polycomb, represses gene expression [Turck et al., 2007; Zhang et al., 2007]. LHP1 is located in discrete nuclear foci, and its distribution changes during cell differentiation [Gaudin et al., 2001; Libault et al., 2005]. In young proliferating cells, a uniform LHP1 distribution pattern was reported. As root hair cells mature progressively from the root apex to the

crown area, the number of foci in the root hair nuclei increases. The distribution of LHP1 in the nucleus was also shown to be dynamic throughout the cell cycle [Libault et al., 2005]. Therefore, LHP1 foci are reminiscent of Polycomb bodies in animals. LHP1 was shown to repress *FLC* expression. However, LHP1 does not seem to be required for the vernalization-induced clustering of *FLC-lacO* alleles, whereas PRC2 subunits participate in such processes [Rosa et al., 2013]. EMBRYONIC FLOWER1 (EMF1), a putative PRC1 subunit, has been shown to participate in chromatin compaction [Beh et al., 2012]. Whether EMF1 or others PcG proteins participate in the formation of plant PcG bodies and function in the spatial organisation of the genome as in animals requires further investigation.

First described in 1903 by Ramón y Cajal, Cajal bodies (CBs) are subnuclear structures that appear to contain many coiled threads (previously named coiled bodies) in TEM images. They are present in most animal species and in several plant species [Shaw and Brown, 2004; Dundr, 2012; Machyna et al., 2013]. CBs are mainly involved in the maturation of RNA species and the assembly and trafficking of transcription-related ribonucleoprotein particles involved in splicing, ribosome biogenesis, or telomere maintenance. In *A. thaliana*, CBs have specific functions in siRNA and miRNA biogenesis [Pontes and Pikaard, 2008]. CBs are composed of a wide range of proteins (some of which also occur in the nucleolus and histone locus bodies) and various types of small RNAs that are related to their specific and various functions [Fong et al., 2013; Machyna et al., 2013]. The coilin protein, which is considered as a signature of CBs, has functions in structural scaffolding and is a key regulator of CB formation and activity [Collier et al., 2006; Machyna et al., 2013]. The CBs are either free in the nucleoplasm and/or physically associated with specific regions of chromatin, such as the nucleolus, in line with the original description of CBs as nucleolar accessory bodies [Shaw and Brown, 2004]. The *A. thaliana* nucleus usually contains a single spherical CB of ~1 µm in diameter which is often closely associated with the nucleolus of different cell types. *A. thaliana* CBs can be visualized by TEM or by using marker proteins such as AtCoilin [Collier et al., 2006] or the spliceosomal protein U2B'' [Beven et al., 1995; Boudonck et al., 1999]. Mutants with multiple CBs have been described [Collier et al., 2006], suggesting the existence of plant factors involved in the maintenance of CB cohesion. As in animals [Platani et al., 2000], plant CBs are highly motile and travel within the nucleoplasm and nucleolus [Boudonck et al., 1999]. Their numbers fluctuate with the

cell cycle, with coalescence occurring mainly at the nucleolar periphery [Boudonck et al., 1999].

Nuclear speckles (NSs), which are enriched in splicing regulators such as Ser/Arg-rich RNA-binding proteins and 3'-end processing factors, correspond to another type of NB that is also related to RNA splicing. These NBs are considered to act as storage and assembly environments for splicing regulators which are translocated to transcription sites for RNA maturation [Reddy et al., 2012; Spector and Lamond, 2011]. The NS components shuttle rapidly from the nucleoplasm and nucleolus to the NS which are motile within a constrained region and vary in size, shape, and number, as do other NBs.

Transcription factories (TFAs) are discrete local subnuclear regions enriched in RNA polymerase and ribonucleoproteins and are in close contact with chromatin [Davidson et al., 2013]. Nascent transcripts are synthesized at the surface of most TFAs, but some are not active. Genomic loci were found to relocate to TFAs in animals in a manner that was dependent on the transcriptional status of the loci, and it was proposed that TFAs co-regulate gene sets, with several transcription units being associated with a single TFA and some being remotely located. As for most NBs, TFAs differ in number, size, and distribution, depending on the cell type, the metabolic and transcriptional activity of the cell, and the cell cycle phase. Recent imaging of RNA production at the single cell level demonstrated that transcription is a discontinuous process, with bursts of transcriptional activity occurring [Chubb and Liverpool, 2010]. Furthermore, there is recent evidence that Polymerase II clusters form transiently, and due to the resulting molecular crowding occurring at these sites, the clusters might participate in the assembly of the transcriptional machinery during the rate-limiting transcription steps [Cisse et al., 2013]. In plants, parameters describing the dynamics of TFAs in the nucleus remain to be determined. A recent study using super-resolution microscopy highlighted distinct reticulate structures within euchromatin in *A. thaliana* and rye interphase nuclei that correspond to active and inactive RNA polymerases, rather than discrete factories as in animals [Schubert, this issue].

Modeling Approaches to Uncover Plant Nuclear Architecture

An increasing number of studies use multidisciplinary approaches based on quantitative image analysis and biophysical modeling to identify the organizational princi-

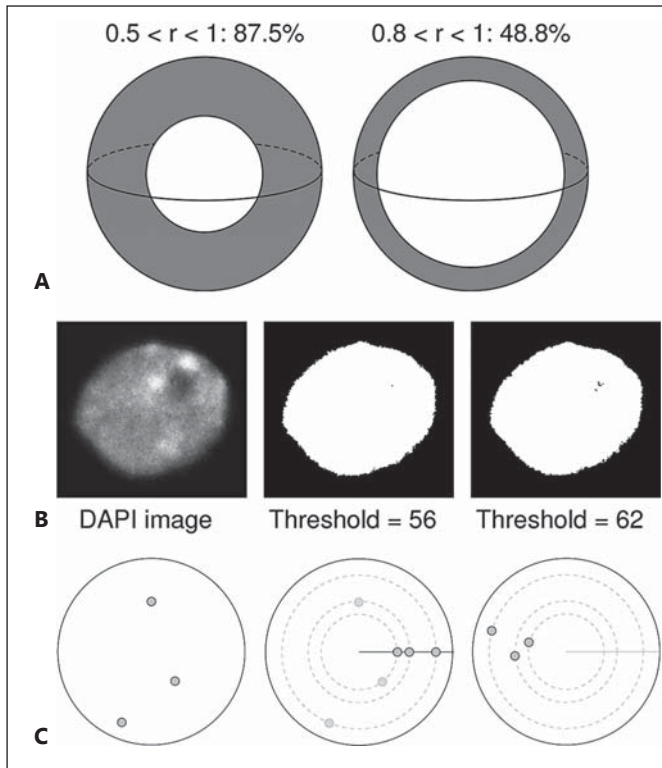


Fig. 3. Some important issues in quantitative image analysis of nuclear patterns. **A** In a 3D sphere, the probability of a point being located by pure chance closer to the periphery than to the center is quite large. **B** Image segmentation errors may have a large impact on the delineated nuclear volumes. The left image shows a 2D section from a 3D image stack that has been segmented at 2 thresholds (middle and right images). On 2D sections, the differences of the surfaces after thresholding are small (<2%). However, the volume differences are larger (8%). **C** In radial analysis, based on distances from the object to the periphery or the center, any pattern (e.g. left pattern) is virtually considered as a 1D pattern (middle). This results in a loss of spatial information and possible misinterpretation. Although different, patterns (for instance the left and right patterns) may yield the same 1D projection (middle) and thus become indistinguishable after analysis.

ples of the nucleus and underlying mechanisms. However, these new research tools need to be refined before they can adequately address these objectives.

Since nuclear patterns are dynamic and do not fully obey deterministic rules, statistical analysis over large samples of nuclei is required to better describe and analyze these patterns. However, the high variability in nuclear size and shape makes integration and comparison of data from different nuclei difficult. In some species, such as *S. cerevisiae*, nuclear landmarks can be used to superimpose different nuclei and to generate statistical repre-

sentations of intra-nuclear distributions [Berger et al., 2008]. Since nuclear landmarks are absent in plant nuclei or have not yet been identified, alternative quantitative strategies for integrating data are required. The need for quantitative approaches also results from the remarkable properties of the spatial distributions in 3D volumes. For example, the outer shell of a half radius width represents 90% of a sphere volume, and this proportion remains as high as 50% when reducing the shell width to one-fifth of the sphere radius (fig. 3A). Since nuclei frequently have a spherical topology, peripheral positioning of nuclear compartments is thus likely to occur under random distribution. Therefore, demonstrating a specific peripheral positioning requires a quantitative analysis.

Before nuclear images can be subjected to quantitative analysis, objects of interest (e.g. the nucleus, CTs, genes) must be localized in 3D. This process, called image segmentation, is generally achieved by setting an intensity threshold that separates object voxels from the others. For example, nuclear contours are frequently identified using DAPI counterstaining. The difficulty in achieving this task automatically is generally underestimated. For example, the popular Otsu's thresholding method [Otsu, 1979] is sensitive to intensity distribution features [Xu et al., 2011; Xue and Zhang, 2012]. Setting an accurate threshold is all the more critical because errors are magnified in 3 dimensions. This point can be illustrated by segmenting a 3D image at 2 slightly different thresholds: the 2D section areas displayed in figure 3B appear to be almost identical, differing by <2%; by contrast, in 3D, the difference between nuclear volumes is 8%. When there is uncertainty about the intensity threshold, it is recommended that the potential impact on quantitative measurements be evaluated using, for example, sensitivity analysis. Also, threshold-insensitive measures could be used or measurements over a range of threshold values could be integrated [Eils et al., 1996]. Alternative methods for intensity thresholding can also use a priori information about the number or size of the expected objects. It may be important to include additional image processing steps before or after the segmentation steps, such as deconvolution, noise reduction, or shape regularization, using mathematical morphological operators [Ronneberger et al., 2008]. Some of these steps may be simplified when better images become available due to technological advances in microscopy. However, the need for accurate segmentations will remain.

Two broad approaches are used to analyze nuclear positioning data which typically consist of sets of points and sometimes also of the associated object sizes. In the first

approach, object positions are analysed individually, thus focusing on the absolute positioning in the nucleus. The prominent paradigm is radial distance analysis in which object location is defined based on its distance from the nuclear envelope or nuclear centroid or on its relative position along the radius passing through it. The radial distance measurement is generally binned into a finite number of classes corresponding to concentric shells. To test for any preferential location towards/away from the envelope, the resulting histogram is compared to the expected distribution under randomness. Continuous variants have been proposed [Ballester et al., 2008] that avoid the loss of statistical power inherent in class binning. In plants, distance analysis was used to demonstrate the preferential localization of *A. thaliana* centromeres at the nuclear periphery of diploid cells [Fang and Spector, 2005]. The popularity of radial distance analysis in nuclear organisation studies [Shiels et al., 2007] is probably due to the simple spherical shape of cultured animal cell nuclei. The diversity of plant nuclear shapes (fig. 2) [Chytilova et al., 1999], however, challenges the general relevance of this approach in plant studies. Further, radial distance analysis entails a projection of 3D data onto a single dimension, thus resulting in a significant loss of spatial information (fig. 3C).

The second approach considers all object positions from a single nucleus together, thus evaluating their mutual positioning. In its simplest form, such an analysis relies on object association frequencies, with an association being defined by the inter-object distance below some fixed thresholds. Comparing measured frequencies with expected frequencies under random object distributions reveals associations or exclusions. This approach revealed a random association between homologous genomic regions of ~100 kb in *A. thaliana*, thus demonstrating the absence of somatic homologous pairing in this species [Pecinka et al., 2004]. Similar results were later obtained in other species [Berr et al., 2006; Schubert et al., 2007]. Spatial statistics offer more elaborate tools for analyzing point patterns based on summary statistics such as cumulative distance functions [Diggle, 2003]. Standard spatial descriptors are the distance from any object to its nearest neighbor or the distance between any position and the nearest object. As above, measured distribution functions are compared to expected functions under the hypotheses of reference models, which are generally based on complete spatial randomness. Spatial statistics have historically been developed for ecology, forestry, and epidemiology studies, where data are positions recorded within a single sampling window. This contrasts with nuclear data

which result from exhaustive position recordings over finite domains with replication over samples of nuclei. Initial attempts to apply spatial statistical tools to nuclear studies ignored these difficulties by considering nuclear data as classical spatial data sampled from virtually infinite point processes [Noordmans et al., 1998; Beil et al., 2005; Buser et al., 2007]. Adapting methods rather than data was recently proposed as an alternative with the introduction of spatial statistical tools specifically designed to analyze nuclear organisation data [Andrey et al., 2010].

Because of the lack of statistical power in per-individual analyses, uncovering spatial effects of small amplitude requires integrating data extracted from large populations of nuclei. Early studies incorporated no size or shape normalization and relied, for example, on average cumulative distance functions [Noordmans et al., 1998; Beil et al., 2005]. In other studies, inter-nucleus size normalization was achieved by dividing measured distances by nuclear size [Fang and Spector, 2005] or maximal observed distance [McManus et al., 2006]. Shape normalization was recently proposed for testing spatial models of object distributions, based on the comparison of nuclear patterns to model outcomes conditioned by nuclear morphology. Comparisons were either performed on an individual basis [Russell et al., 2009] or at the group level [Andrey et al., 2010]. The latter was made possible by introducing a normalized measure of model/data discrepancy for each nucleus. Results reported on simulated data later demonstrated the superior statistical power of this method as compared with other strategies for data integration over samples of nuclei [Weston et al., 2012]. This methodology is particularly relevant for nuclear organisation studies in plants, as plant nuclei have diverse shapes that deviate significantly from simple spherical ones [Chytilova et al., 1999]. Since this methodology is generic, it is also likely that it will be generalized in the future to evaluate more elaborate spatial models and to support inter-group comparisons.

Several studies in both animals and plants have used polymer modeling to analyze the mechanisms underlying the spatial organisation of the genome. The chromatin fiber is generally modeled as a linear chain of monomers, and an energy function is defined to incorporate physical forces and constraints, such as attraction, repulsion, and volume exclusion, between adjacent or distant monomers. Starting from initial configurations, computer simulations iteratively update monomer positions to progressively reach lower energy configurations. In the Spherical Chromatin Domain (SCD) model, which has been used both in animal and plant studies, chromo-

somes are modeled as strings of 1-Mb spherical domains connected by elastic springs and obeying short-range exclusion/long-range attraction potentials. Chromosome territories are obtained by relaxing initially condensed linear configurations representing metaphase chromosomes [Kreth et al., 2004]. By comparing observed association frequencies between homologous chromosomes with those predicted by the SCD model, it was shown that homologue associations in *A. thaliana* and the closely related species *A. lyrata* are globally not significantly different from random, except for chromosome pairs 2 and 4, which bear NORs [Pecinka et al., 2004; Berr et al., 2006]. Using a coarse-grain molecular dynamics model closely related to the SCD model, the contribution of non-specific physical interactions in the positioning of CCs and nucleoli in *Arabidopsis* leaf nuclei was examined [de Nooijer et al., 2009]. In that study, chromosomes were represented by heteropolymers that could form loops, and large monomers were used to model CCs. Based on a comparison of the different models of polymer structure, the authors concluded that non-specific interactions between polymers with CC-anchored loops were sufficient to explain the experimentally observed positioning of CCs and nucleoli, as well as the segregation of chromosomes into territories, thus supporting the previously proposed rosette model [Fransz et al., 2002]. One difficulty in interpreting the results of this model is that equilibrium configurations were obtained through chromosome condensation and reduction of the nuclear volume. It is also unclear how a completely non-specific determinism of heterochromatin positioning in *A. thaliana* nuclei can be reconciled with the heterochromatin reorganizations described previously. An important future challenge will be to shift from simulations in theoretical, simple nuclear shapes (spheres or ellipsoids) to actual shapes determined from 3D images. In parallel, the ability to associate heterogeneous properties with monomers along chromosomes will be essential for further expanding the potential of these models in investigations of the principles underlying nuclear organisation and of genetic determinants.

Conclusion

In the past decade, tremendous progress has been made in deciphering chromatin properties at the genomic scale in eukaryotes, and the molecular and biochemical diversity of chromatin has been highlighted. Despite its repetitiveness, which for a long time largely excluded it from molecular studies, heterochromatin has started to reveal

its secrets and is no longer considered as a monotonous and silent compartment. Furthermore, besides key functions in chromosome segregation and genome stability and maintenance, a new function in genome organisation has emerged for heterochromatin, highlighting the interdependence of euchromatin and heterochromatin. The development of 3C and related methods, which have been used to unravel the 3D conformation of the genome and, in turn, have raised questions about the relationship between 3D genome organisation and its main functions in the interphase nucleus (i.e. transcription, replication, and DNA repair), has revolutionized chromatin biology. Combining these molecular approaches and classical cytological studies with modeling approaches promises to answer these questions while taking nucleus-to-nucleus variability and the stochasticity of nuclear processes into account.

In *A. thaliana*, most cytological studies have been performed on 2D nuclei. However, the number of 3D approaches is rapidly increasing, and this increase calls for a change in perspective and tools. The principles that govern plant heterochromatin organisation are slowly emerging. It will be interesting to determine whether these principles, which were established in a diploid model plant, extend to plants with complex and large genomes containing large heterochromatin fractions. The repertoire of known plant nuclear compartments has expanded tremendously. However, the mechanisms underlying their assembly and functions are still poorly understood, as is their functional interplay, particularly with the 3D conformation of the plant genome.

The complex and intricate interplay between nuclear components, nuclear architecture, and genome functions remains to be further investigated. We predict that much will be learnt about the interphase plant nucleus and its dynamics in the coming years and that this knowledge will depend on intensive interdisciplinary dialogues on subjects ranging from biophysics and modeling to imaging and cytology.

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References

- Abranches R, Beven AF, Aragon-Alcaide L, Shaw PJ: Transcription sites are not correlated with chromosome territories in wheat nuclei. *J Cell Biol* 143:5–12 (1998).
- Albert PS, Gao Z, Danilova TV, Birchler JA: Diversity of chromosomal karyotypes in maize and its relatives. *Cytogenet Genome Res* 129: 6–16 (2010).
- Amini A, Luo C, Lam E: Chromatin beacons: global sampling of chromatin physical properties using chromatin charting lines. *Methods Mol Biol* 701:301–314 (2011).
- Andrey P, Kieu K, Kress C, Lehmann G, Tirichine L, et al: Statistical analysis of 3D images detects regular spatial distributions of centromeres and chromocenters in animal and plant nuclei. *PLoS Comput Biol* 6:e1000853 (2010).
- Arabidopsis Genome Initiative: Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408:796–815 (2000).
- Armstrong SJ, Franklin FC, Jones GH: Nucleolus-associated telomere clustering and pairing precede meiotic chromosome synapsis in *Arabidopsis thaliana*. *J Cell Sci* 114:4207–4217 (2001).
- Ballester M, Kress C, Hue-Beauvais C, Kieu K, Lehmann G, et al: The nuclear localization of WAP and CSN genes is modified by lactogenic hormones in HC11 cells. *J Cell Biochem* 105:262–270 (2008).
- Bantignies F, Cavalli G: Polycomb group proteins: repression in 3D. *Trends Genet* 27:454–464 (2011).
- Bantignies F, Roue V, Comet I, Leblanc B, Schuettengruber B, et al: Polycomb-dependent regulatory contacts between distant *Hox* loci in *Drosophila*. *Cell* 144:214–226 (2011).
- Baroux C, Pecinka A, Fuchs J, Schubert I, Grossniklaus U: The triploid endosperm genome of *Arabidopsis* adopts a peculiar, parental-dosage-dependent chromatin organization. *Plant Cell* 19:1782–1794 (2007).
- Baroux C, Raissig MT, Grossniklaus U: Epigenetic regulation and reprogramming during gamete formation in plants. *Curr Opin Genet Dev* 21:124–133 (2011).
- Bass HW, Marshall WF, Sedat JW, Agard DA, Cande WZ: Telomeres cluster de novo before the initiation of synapsis: a three-dimensional spatial analysis of telomere positions before and during meiotic prophase. *J Cell Biol* 137: 5–18 (1997).
- Baubec T, Dinh HQ, Pecinka A, Rakic B, Rozhon W, et al: Cooperation of multiple chromatin modifications can generate unanticipated stability of epigenetic states in *Arabidopsis*. *Plant Cell* 22:34–47 (2010).
- Beh LY, Colwell LJ, Francis NJ: A core subunit of Polycomb repressive complex 1 is broadly conserved in function but not primary sequence. *Proc Natl Acad Sci USA* 109:E1063–E1071 (2012).
- Beil M, Fleischer F, Paschke S, Schmidt V: Statistical analysis of the three-dimensional structure of centromeric heterochromatin in interphase nuclei. *J Microsc* 217:60–68 (2005).
- Beisel C, Paro R: Silencing chromatin: comparing modes and mechanisms. *Nat Rev Genet* 12: 123–135 (2011).
- Belhaj K, Chaparro-Garcia A, Kamoun S, Nekrasov V: Plant genome editing made easy: targeted mutagenesis in model and crop plants using the CRISPR/Cas system. *Plant Methods* 9:39 (2013).
- Berger AB, Cabal GG, Fabre E, Duong T, Buc H, et al: High-resolution statistical mapping reveals gene territories in live yeast. *Nat Methods* 5:1031–1037 (2008).
- Berr A, Schubert I: Interphase chromosome arrangement in *Arabidopsis thaliana* is similar in differentiated and meristematic tissues and shows a transient mirror symmetry after nuclear division. *Genetics* 176:853–863 (2007).
- Berr A, Pecinka A, Meister A, Kreth G, Fuchs J, et al: Chromosome arrangement and nuclear architecture but not centromeric sequences are conserved between *Arabidopsis thaliana* and *Arabidopsis lyrata*. *Plant J* 48:771–783 (2006).
- Beven AF, Simpson GG, Brown JW, Shaw PJ: The organization of spliceosomal components in the nuclei of higher plants. *J Cell Sci* 108:509–518 (1995).
- Bickmore WA, van Steensel B: Genome architecture: domain organization of interphase chromosomes. *Cell* 152:1270–1284 (2013).
- Boruc J, Zhou X, Meier I: Dynamics of the plant nuclear envelope and nuclear pore. *Plant Physiol* 158:78–86 (2012).
- Boudonck K, Dolan L, Shaw PJ: The movement of coiled bodies visualized in living plant cells by the green fluorescent protein. *Mol Biol Cell* 10:2297–2307 (1999).
- Boveri T: Die Blastomerenkerne von *Ascaris megalocephala* und die Theorie der Chromosomenindividualität. *Arch Zellforsch* 3:181–268 (1909).
- Brown JW, Shaw PJ: The role of the plant nucleolus in pre-mRNA processing. *Curr Top Microbiol Immunol* 326:291–311 (2008).
- Buser C, Fleischer F, Mertens T, Michel D, Schmidt V, Walther P: Quantitative investigation of murine cytomegalovirus nucleocapsid interaction. *J Microsc* 228:78–87 (2007).
- Campbell BR, Song Y, Posch TE, Cullis CA, Town CD: Sequence and organization of 5S ribosomal RNA-encoding genes of *Arabidopsis thaliana*. *Gene* 112:225–228 (1992).
- Castel SE, Martienssen RA: RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. *Nat Rev Genet* 14:100–112 (2013).
- Ceccarelli M, Morosi L, Cionin PG: Chromocenter association in plant cell nuclei: determinants, functional significance, and evolutionary implications. *Genome* 41:96–103 (1998).
- Chen K, Gao C: TALENs: customizable molecular DNA scissors for genome engineering of plants. *J Genet Genomics* 40:271–279 (2013).
- Chen M: Phytochrome nuclear body: an emerging model to study interphase nuclear dynamics and signaling. *Curr Opin Plant Biol* 11:503–508 (2008).
- Chen M, Chory J: Phytochrome signaling mechanisms and the control of plant development. *Trends Cell Biol* 21:664–671 (2011).
- Cheutin T, Cavalli G: Progressive polycomb assembly on H3K27me3 compartments generates polycomb bodies with developmentally regulated motion. *PLoS Genet* 8:e1002465 (2012).
- Chubb JR, Liverpool TB: Bursts and pulses: insights from single cell studies into transcriptional mechanisms. *Curr Opin Genet Dev* 20: 478–484 (2010).
- Chupeau MC, Granier F, Pichon O, Renou JP, Gaudin V, Chupeau Y: Characterization of the early events leading to totipotency in an *Arabidopsis* protoplast liquid culture by temporal transcript profiling. *Plant Cell* 25:2444–2463 (2013).
- Chytilova E, Micas J, Galbraith DW: Green fluorescent protein targeted to the nucleus, a transgenic phenotype useful for studies in plant biology. *Ann Bot* 83:645–654 (1999).
- Ciska M, Masuda K, Moreno Diaz de la Espina S: Lamin-like analogues in plants: the characterization of NMCP1 in *Allium cepa*. *J Exp Bot* 64:1553–1564 (2013).
- Cisse II, Izeddin I, Causse SZ, Boudarene L, Senecal A, et al: Real-time dynamics of RNA polymerase II clustering in live human cells. *Science* 341:664–667 (2013).
- Cloix C, Tutois S, Yukawa Y, Mathieu O, Cuvillier C, et al: Analysis of the 5S RNA pool in *Arabidopsis thaliana*: RNAs are heterogeneous and only two of the genomic 5S loci produce mature 5S RNA. *Genome Res* 12: 132–144 (2002).
- Collier S, Pendle A, Boudonck K, van Rij T, Dolan L, Shaw P: A distant coilin homologue is required for the formation of Cajal bodies in *Arabidopsis*. *Mol Biol Cell* 17:2942–2951 (2006).
- Copenhaver GP, Pikaard CS: Two-dimensional RFLP analyses reveal megabase-sized clusters of rRNA gene variants in *Arabidopsis thaliana*, suggesting local spreading of variants as the mode for gene homogenization during concerted evolution. *Plant J* 9:273–282 (1996).
- Copenhaver GP, Nickel K, Kuromori T, Benito MI, Kaul S, et al: Genetic definition and sequence analysis of *Arabidopsis* centromeres. *Science* 286:2468–2474 (1999).
- Costa S, Shaw P: Chromatin organization and cell fate switch respond to positional information in *Arabidopsis*. *Nature* 439:493–496 (2006).

- Cowan CR, Carlton PM, Cande WZ: The polar arrangement of telomeres in interphase and meiosis. *Rabl organization and the bouquet*. *Plant Physiol* 125:532–538 (2001).
- Cremer T, Cremer M: Chromosome territories. *Cold Spring Harb Perspect Biol* 2:a003889 (2010).
- Davidson S, Macpherson N, Mitchell JA: Nuclear organization of RNA polymerase II transcription. *Biochem Cell Biol* 91:22–30 (2013).
- de Graaf CA, van Steensel B: Chromatin organization: form to function. *Curr Opin Genet Dev* 23:185–190 (2013).
- Dekker J, Marti-Renom MA, Mirny LA: Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data. *Nat Rev Genet* 14:390–403 (2013).
- Delest A, Sexton T, Cavalli G: Polycomb: a paradigm for genome organization from one to three dimensions. *Curr Opin Cell Biol* 24:405–414 (2012).
- Delgado M, Frescatada M, Ribeiro-Varandas E, Viegas W, Jones RN: Dynamics of functional heterochromatic domains in the plant interphase nucleus. *Cytogenet Genome Res* 129:64–71 (2010).
- de Nooijer S, Wellink J, Mulder B, Bisseling T: Non-specific interactions are sufficient to explain the position of heterochromatic chromocenters and nucleoli in interphase nuclei. *Nucleic Acids Res* 37:3558–3568 (2009).
- de Wit E, de Laat W: A decade of 3C technologies: insights into nuclear organization. *Genes Dev* 26:11–24 (2012).
- Diggle PJ: *Statistical Analysis of Spatial Point Patterns*, ed 2 (Arnold Publication, London 2003).
- Dion V, Gasser SM: Chromatin movement in the maintenance of genome stability. *Cell* 152:1355–1364 (2013).
- Dittmer TA, Stacey NJ, Sugimoto-Shirasu K, Richards EJ: LITTLE NUCLEI genes affecting nuclear morphology in *Arabidopsis thaliana*. *Plant Cell* 19:2793–2803 (2007).
- Dong F, Jiang J: Non-Rabl patterns of centromere and telomere distribution in the interphase nuclei of plant cells. *Chromosome Res* 6:551–558 (1998).
- Douet J, Blanchard B, Cuvillier C, Tourmente S: Interplay of RNA Pol IV and ROS1 during post-embryonic 5S rDNA chromatin remodeling. *Plant Cell Physiol* 49:1783–1791 (2008).
- Douet J, Tutois S, Tourmente S: A Pol V-mediated silencing, independent of RNA-directed DNA methylation, applies to 5S rDNA. *PLoS Genet* 5:e1000690 (2009).
- Dundr M: Nuclear bodies: multifunctional companions of the genome. *Curr Opin Cell Biol* 24:415–422 (2012).
- Eils R, Dietzel S, Bertin E, Schrock E, Speicher MR, et al: Three-dimensional reconstruction of painted human interphase chromosomes: active and inactive X chromosome territories have similar volumes but differ in shape and surface structure. *J Cell Biol* 135:1427–1440 (1996).
- Fang Y, Spector DL: Centromere positioning and dynamics in living *Arabidopsis* plants. *Mol Biol Cell* 16:5710–5718 (2005).
- Fang Y, Spector DL: Identification of nuclear dicing bodies containing proteins for microRNA biogenesis in living *Arabidopsis* plants. *Curr Biol* 17:818–823 (2007).
- Feng CM, Qiu Y, Van Buskirk EK, Yang EJ, Chen M: Light-regulated gene repositioning in *Arabidopsis*. *Nat Commun* 5:3027 (2014).
- Floutsakou I, Agrawal S, Nguyen TT, Seoighe C, Ganley AR, McStay B: The shared genomic architecture of human nucleolar organizer regions. *Genome Res* 23:2003–2012 (2013).
- Fong KW, Li Y, Wang W, Ma W, Li K, et al: Whole-genome screening identifies proteins localized to distinct nuclear bodies. *J Cell Biol* 203:149–164 (2013).
- Franklin AE, Cande WZ: Nuclear organization and chromosome segregation. *Plant Cell* 11:523–534 (1999).
- Franz P, Armstrong S, Alonso-Blanco C, Fischer TC, Torres-Ruiz RA, Jones G: Cytogenetics for the model system *Arabidopsis thaliana*. *Plant J* 13:867–876 (1998).
- Franz P, Armstrong S, de Jong JH, Parnell LD, van Druenen C, et al: Integrated cytogenetic map of chromosome arm 4S of *A. thaliana*: structural organization of heterochromatic knob and centromere region. *Cell* 100:367–376 (2000).
- Franz P, De Jong JH, Lysak M, Castiglione MR, Schubert I: Interphase chromosomes in *Arabidopsis* are organized as well defined chromocenters from which euchromatin loops emanate. *Proc Natl Acad Sci USA* 99:14584–14589 (2002).
- Franz P, Soppe W, Schubert I: Heterochromatin in interphase nuclei of *Arabidopsis thaliana*. *Chromosome Res* 11:227–240 (2003).
- Fussner E, Ching RW, Bazett-Jones DP: Living without 30 nm chromatin fibers. *Trends Biochem Sci* 36:1–6 (2011).
- Gaj T, Gersbach CA, Barbas CF 3rd: ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol* 31:397–405 (2013).
- Gaspin C, Rami JF, Lescure B: Distribution of short interstitial telomere motifs in two plant genomes: putative origin and function. *BMC Plant Biol* 10:283 (2010).
- Gaudin V, Libault M, Pouteau S, Juul T, Zhao G, et al: Mutations in LIKE HETEROCHROMATIN PROTEIN 1 affect flowering time and plant architecture in *Arabidopsis*. *Development* 128:4847–4858 (2001).
- Geyer PK, Vitalini MW, Wallrath LL: Nuclear organization: taking a position on gene expression. *Curr Opin Cell Biol* 23:354–359 (2011).
- Ghaffari R, Cannon EK, Kanizay LB, Lawrence CJ, Dawe RK: Maize chromosomal knobs are located in gene-dense areas and suppress local recombination. *Chromosoma* 122:67–75 (2013).
- Graumann K, Runions J, Evans DE: Nuclear envelope proteins and their role in nuclear positioning and replication. *Biochem Soc Trans* 38:741–746 (2010).
- Grimaud C, Bantignies F, Pal-Bhadra M, Ghana P, Bhadra U, Cavalli G: RNAi components are required for nuclear clustering of Polycomb group response elements. *Cell* 124:957–971 (2006).
- Guelen L, Pagie L, Brasset E, Meuleman W, Faza MB, et al: Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. *Nature* 453:948–951 (2008).
- Hall AE, Kettler GC, Preuss D: Dynamic evolution at pericentromeres. *Genome Res* 16:355–364 (2006).
- Heitz E: Das Heterochromatin der Moose. *Jahrb Wiss Bot* 69:762–819 (1928).
- Heslop-Harrison JS, Schwarzacher T: Organization of the plant genome in chromosomes. *Plant J* 66:18–33 (2011).
- Heslop-Harrison JS, Murata M, Ogura Y, Schwarzacher T, Motoyoshi F: Polymorphisms and genomic organization of repetitive DNA from centromeric regions of *Arabidopsis* chromosomes. *Plant Cell* 11:31–42 (1999).
- Heslop-Harrison JS, Brandes A, Schwarzacher T: Tandemly repeated DNA sequences and centromeric chromosomal regions of *Arabidopsis* species. *Chromosome Res* 11:241–253 (2003).
- Hovel I, Louwers M, Stam M: 3C technologies in plants. *Methods* 58:204–211 (2012).
- Isono K, Endo TA, Ku M, Yamada D, Suzuki R, et al: SAM domain polymerization links subnuclear clustering of PRC1 to gene silencing. *Dev Cell* 26:565–577 (2013).
- Jovtchev G, Watanabe K, Pecinka A, Rosin FM, Mette MF, et al: Size and number of tandem repeat arrays can determine somatic homologous pairing of transgene loci mediated by epigenetic modifications in *Arabidopsis thaliana* nuclei. *Chromosoma* 117:267–276 (2008).
- Kato N, Lam E: Detection of chromosomes tagged with green fluorescent protein in live *Arabidopsis thaliana* plants. *Genome Biol* 2:RESEARCH0045 (2001).
- Kato N, Lam E: Chromatin of endoreduplicated pavement cells has greater range of movement than that of diploid guard cells in *Arabidopsis thaliana*. *J Cell Sci* 116:2195–2201 (2003).
- Koornneef M, Franz P, de Jong H: Cytogenetic tools for *Arabidopsis thaliana*. *Chromosome Res* 11:183–194 (2003).
- Kreth G, Finsterle J, von Hase J, Cremer M, Cremer C: Radial arrangement of chromosome territories in human cell nuclei: a computer model approach based on gene density indicates a probabilistic global positioning code. *Biophys J* 86:2803–2812 (2004).
- Lamb JC, Yu W, Han F, Birchler JA: Plant chromosomes from end to end: telomeres, heterochromatin and centromeres. *Curr Opin Plant Biol* 10:116–122 (2007).

- Lermontova I, Schubert V, Fuchs J, Klatte S, Macas J, Schubert I: Loading of *Arabidopsis* centromeric histone CENH3 occurs mainly during G2 and requires the presence of the histone fold domain. *Plant Cell* 18:2443–2451 (2006).
- Lermontova I, Rutten T, Schubert I: Deposition, turnover, and release of CENH3 at *Arabidopsis* centromeres. *Chromosoma* 120:633–640 (2011).
- Lermontova I, Kuhlmann M, Friedel S, Rutten T, Heckmann S, et al: *Arabidopsis* kinetochore null2 is an upstream component for centromeric histone H3 variant cenH3 deposition at centromeres. *Plant Cell* 25:3389–3404 (2013).
- Libault M, Tessadori F, Germann S, Snijder B, Franz F, Gaudin V: The *Arabidopsis* LHP1 protein is a component of euchromatin. *Plant* 222:910–925 (2005).
- Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, et al: Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* 326:289–293 (2009).
- Lindhout BI, Franz P, Tessadori F, Meckel T, Hooykaas PJ, van der Zaal BJ: Live cell imaging of repetitive DNA sequences via GFP-tagged polydactyl zinc finger proteins. *Nucleic Acids Res* 35:e107 (2007).
- Liu Q, Shi L, Fang Y: Dicing bodies. *Plant Physiol* 158:61–66 (2012).
- Lopez E, Pradillo M, Oliver C, Romero C, Cunado N, Santos JL: Looking for natural variation in chiasma frequency in *Arabidopsis thaliana*. *J Exp Bot* 63:887–894 (2012).
- Luger K, Dechassa ML, Tremethick DJ: New insights into nucleosome and chromatin structure: an ordered state or a disordered affair? *Nat Rev Mol Cell Biol* 13:436–447 (2012).
- Luo S, Preuss D: Strand-biased DNA methylation associated with centromeric regions in *Arabidopsis*. *Proc Natl Acad Sci USA* 100:11133–11138 (2003).
- Lysák M: Comparative cytogenetics of wild crucifers, in Group TF (ed): *Biology and Breeding of Crucifers*, pp 177–205 (CRC Press, Neuvenden 2009).
- Lysak MA, Fransz PF, Ali HB, Schubert I: Chromosome painting in *Arabidopsis thaliana*. *Plant J* 28:689–697 (2001).
- Lysak MA, Pecinka A, Schubert I: Recent progress in chromosome painting of *Arabidopsis* and related species. *Chromosome Res* 11:195–204 (2003).
- Ma J, Wing RA, Bennetzen JL, Jackson SA: Plant centromere organization: a dynamic structure with conserved functions. *Trends Genet* 23:134–139 (2007).
- Machyna M, Heyn P, Neugebauer KM: Cajal bodies: where form meets function. *Wiley Interdisc Rev RNA* 4:17–34 (2013).
- Mansfield SG, Briarty LG: Cotyledon cell development in *Arabidopsis thaliana* during reserve deposition. *Can J Botany* 70:151–164 (1992).
- Marshall WF, Straight A, Marko JF, Swedlow J, Dernburg A, et al: Interphase chromosomes undergo constrained diffusional motion in living cells. *Curr Biol* 7:930–939 (1997).
- Matera AG, Izaguirre-Sierra M, Praveen K, Rajendra TK: Nuclear bodies: random aggregates of sticky proteins or crucibles of macromolecular assembly? *Dev Cell* 17:639–647 (2009).
- Mathieu O, Jasencakova Z, Vaillant I, Gendrel AV, Colot V, et al: Changes in 5S rDNA chromatin organization and transcription during heterochromatin establishment in *Arabidopsis*. *Plant Cell* 15:2929–2939 (2003).
- Matzke AJ, van der Winden J, Matzke M: Tetra-cycline operator/repressor system to visualize fluorescence-tagged T-DNAs in interphase nuclei of *Arabidopsis*. *Plant Mol Biol Rep* 21:9–19 (2003).
- Matzke AJ, Huettel B, van der Winden J, Matzke M: Use of two-color fluorescence-tagged transgenes to study interphase chromosomes in living plants. *Plant Physiol* 139:1586–1596 (2005).
- McClintock B: The relationship of a particular chromosomal element to the development of the nucleoli in *Zea mays*. *Zeit Zellforsch Mikrosk Anat* 21:294–328 (1934).
- McManus KJ, Stephens DA, Adams NM, Islam SA, Freemont PS, Hendzel MJ: The transcriptional regulator CBP has defined spatial associations within interphase nuclei. *PLoS Comput Biol* 2:e139 (2006).
- Mine-Hattab J, Rothstein R: Increased chromosome mobility facilitates homology search during recombination. *Nat Cell Biol* 14:510–517 (2012).
- Mine-Hattab J, Rothstein R: DNA in motion during double-strand break repair. *Trends Cell Biol* 23:529–536 (2013).
- Mirny LA: The fractal globule as a model of chromatin architecture in the cell. *Chromosome Res* 19:37–51 (2011).
- Mittelsten Scheid O, Probst AV, Afsar K, Paszkowski J: Two regulatory levels of transcriptional gene silencing in *Arabidopsis*. *Proc Natl Acad Sci USA* 99:13659–13662 (2002).
- Miyinari Y, Ziegler-Birling C, Torres-Padilla ME: Live visualization of chromatin dynamics with fluorescent TALEs. *Nat Struct Mol Biol* 20:1321–1324 (2013).
- Morimoto M, Boerkoel CF: The role of nuclear bodies in gene expression and disease. *Biology* 2:976–1033 (2013).
- Murata M, Heslop-Harrison JS, Motoyoshi F: Physical mapping of the 5S ribosomal RNA genes in *Arabidopsis thaliana* by multi-color fluorescence in situ hybridization with cosmid clones. *Plant J* 12:31–37 (1997).
- Nemeth A, Langst G: Genome organization in and around the nucleolus. *Trends Genet* 27:149–156 (2011).
- Nemeth A, Conesa A, Santoyo-Lopez J, Medina I, Montaner D, et al: Initial genomics of the human nucleolus. *PLoS Genet* 6:e1000889 (2010).
- Ng CK, Kinoshita T, Pandey S, Shimazaki K, Assmann SM: Abscisic acid induces rapid sub-nuclear reorganization in guard cells. *Plant Physiol* 134:1327–1331 (2004).
- Noordmans HJ, van der Kraan K, van Driel R, Smeulders AW: Randomness of spatial distributions of two proteins in the cell nucleus involved in mRNA synthesis and their relationships. *Cytometry* 33:297–309 (1998).
- Ogrocka A, Polanska P, Majerova E, Janeba Z, Fajkus J, Fojtova M: Compromised telomere maintenance in hypomethylated *Arabidopsis thaliana* plants. *Nucleic Acids Res* 42:2919–2931 (2013).
- Onodera Y, Haag JR, Ream T, Nunes PC, Pontes O, Pikaard CS: Plant nuclear RNA polymerase IV mediates siRNA and DNA methylation-dependent heterochromatin formation. *Cell* 120:613–622 (2005).
- Otsu N: A threshold selection method from gray-level histograms. *IEEE Transactions on Systems, Man, and Cybernetics* 9:62–66 (1979).
- Parada L, Misteli T: Chromosome positioning in the interphase nucleus. *Trends Cell Biol* 12:425–432 (2002).
- Pavet V, Quintero C, Cecchini NM, Rosa AL, Alvarez ME: *Arabidopsis* displays centromeric DNA hypomethylation and cytological alterations of heterochromatin upon attack by *Pseudomonas syringae*. *Mol Plant Microbe Interact* 19:577–587 (2006).
- Pecinka A, Schubert V, Meister A, Kretsch G, Klatte M, et al: Chromosome territory arrangement and homologous pairing in nuclei of *Arabidopsis thaliana* are predominantly random except for NOR-bearing chromosomes. *Chromosoma* 113:258–269 (2004).
- Pecinka A, Kato N, Meister A, Probst AV, Schubert I, Lam E: Tandem repetitive transgenes and fluorescent chromatin tags alter local interphase chromosome arrangement in *Arabidopsis thaliana*. *J Cell Sci* 118:3751–3758 (2005).
- Pecinka A, Dinh HQ, Baubec T, Rosa M, Lettner N, Scheid OM: Epigenetic regulation of repetitive elements is attenuated by prolonged heat stress in *Arabidopsis*. *Plant Cell* 22:1–12 (2010).
- Pendle AF, Clark GP, Boon R, Lewandowska D, Lam YW, et al: Proteomic analysis of the *Arabidopsis* nucleolus suggests novel nucleolar functions. *Mol Biol Cell* 16:260–269 (2005).
- Pickersgill H, Kalverda B, de Wit E, Talhout W, Fornerod M, van Steensel B: Characterization of the *Drosophila melanogaster* genome at the nuclear lamina. *Nat Genet* 38:1005–1014 (2006).
- Pirrotta V, Li HB: A view of nuclear Polycomb bodies. *Current Opin Gen Dev* 22:101–109 (2012).
- Platani M, Goldberg I, Swedlow JR, Lamond AI: In vivo analysis of Cajal body movement, separation, and joining in live human cells. *J Cell Biol* 151:1561–1574 (2000).

- Poggio L, Gonzalez G, Confalonieri V, Comas C, Naranjo CA: The genome organization and diversification of maize and its allied species revisited: evidences from classical and FISH-GISH cytogenetic analysis. *Cytogenet Genome Res* 109:259–267 (2005).
- Pontes O, Pikaard CS: siRNA and miRNA processing: new functions for Cajal bodies. *Curr Opin Genet Dev* 18:197–203 (2008).
- Prieto P, Santos AP, Moore G, Shaw P: Chromosomes associate premeiotically and in xylem vessel cells via their telomeres and centromeres in diploid rice (*Oryza sativa*). *Chromosoma* 112:300–307 (2004).
- Probst AV, Fransz PF, Paszkowski J, Mittelsten Scheid O: Two means of transcriptional reactivation within heterochromatin. *Plant J* 33:743–749 (2003).
- Probst AV, Fagard M, Proux F, Mourrain P, Boutet S, et al: *Arabidopsis* histone deacetylase HDA6 is required for maintenance of transcriptional gene silencing and determines nuclear organization of rDNA repeats. *Plant Cell* 16:1021–1034 (2004).
- Puchta H, Fauser F: Synthetic nucleases for genome engineering in plants: prospects for a bright future. *Plant J* [Epub ahead of print] (2013).
- Rabl C: Über Zellteilung. *Morphol Jahrbuch* 10:214–330 (1885).
- Rajapakse I, Groudine M: On emerging nuclear order. *J Cell Biol* 192:711–721 (2011).
- Ravi M, Chan SW: Haploid plants produced by centromere-mediated genome elimination. *Nature* 464:615–618 (2010).
- Ravi M, Shibata F, Ramahi JS, Nagaki K, Chen C, et al: Meiosis-specific loading of the centromere-specific histone CENH3 in *Arabidopsis thaliana*. *PLoS Genet* 7:e1002121 (2011).
- Reddy AS, Day IS, Gohring J, Barta A: Localization and dynamics of nuclear speckles in plants. *Plant Physiol* 158:67–77 (2012).
- Regad F, Lebas M, Lescure B: Interstitial telomeric repeats within the *Arabidopsis thaliana* genome. *J Mol Biol* 239:163–169 (1994).
- Roberts NY, Osman K, Armstrong SJ: Telomere distribution and dynamics in somatic and meiotic nuclei of *Arabidopsis thaliana*. *Cytogenet Genome Res* 124:193–201 (2009).
- Robinett CC, Straight A, Li G, Willhelm C, Sudlow G, et al: In vivo localization of DNA sequences and visualization of large-scale chromatin organization using lac operator/repressor recognition. *J Cell Biol* 135:1685–1700 (1996).
- Ronneberger O, Baddeley D, Scheipl F, Verveer PJ, Burkhardt H, et al: Spatial quantitative analysis of fluorescently labeled nuclear structures: problems, methods, pitfalls. *Chromosome Res* 16:523–562 (2008).
- Rosa S, De Lucia F, Mylne JS, Zhu D, Ohmido N, et al: Physical clustering of *FLC* alleles during Polycomb-mediated epigenetic silencing in vernalization. *Genes Dev* 27:1845–1850 (2013).
- Rosin FM, Watanabe N, Cacas JL, Kato N, Arroyo JM, et al: Genome-wide transposon tagging reveals location-dependent effects on transcription and chromatin organization in *Arabidopsis*. *Plant J* 55:514–525 (2008).
- Russell RA, Adams NM, Stephens DA, Batty E, Jensen K, Freemont PS: Segmentation of fluorescence microscopy images for quantitative analysis of cell nuclear architecture. *Biophys J* 96:3379–3389 (2009).
- Sakamoto Y, Takagi S: LITTLE NUCLEI 1 and 4 regulate nuclear morphology in *Arabidopsis thaliana*. *Plant Cell Physiol* 54:622–633 (2013).
- Sanchez-Moran E, Armstrong SJ, Santos JL, Franklin FC, Jones GH: Variation in chiasma frequency among eight accessions of *Arabidopsis thaliana*. *Genetics* 162:1415–1422 (2002).
- Santos AP, Shaw P: Interphase chromosomes and the Rabl configuration: does genome size matter? *J Microsc* 214:201–206 (2004).
- Sato H, Shibata F, Murata M: Characterization of a MIS12 homologue in *Arabidopsis thaliana*. *Chromosome Res* 13:827–834 (2005).
- Schonrock N, Exner V, Probst A, Grussem W, Hennig L: Functional genomic analysis of *CAF-1* mutants in *Arabidopsis thaliana*. *J Biol Chem* 281:9560–9568 (2006).
- Schubert I, Shaw P: Organization and dynamics of plant interphase chromosomes. *Trends Plant Sci* 16:273–281 (2011).
- Schubert V, Klatte M, Pecinka A, Meister A, Jasencakova Z, Schubert I: Sister chromatids are often incompletely aligned in meristematic and endopolyploid interphase nuclei of *Arabidopsis thaliana*. *Genetics* 172:467–475 (2006).
- Schubert V, Kim YM, Berr A, Fuchs J, Meister A, et al: Random homologous pairing and incomplete sister chromatid alignment are common in angiosperm interphase nuclei. *Mol Genet Genomics* 278:167–176 (2007).
- Schubert V, Weissleder A, Ali H, Fuchs J, Lermontova I, et al: Cohesin gene defects may impair sister chromatid alignment and genome stability in *Arabidopsis thaliana*. *Chromosoma* 118:591–605 (2009).
- Schubert V, Meister A, Tsujimoto H, Endo TR, Houben A: Similar rye A and B chromosome organization in meristematic and differentiated interphase nuclei. *Chromosome Res* 19:645–655 (2011).
- Schubert V, Berr A, Meister A: Interphase chromatin organisation in *Arabidopsis* nuclei: constraints versus randomness. *Chromosome Res* 121:369–387 (2012).
- Schubert V, Lermontova I, Schubert I: The *Arabidopsis* CAP-D proteins are required for correct chromatin organisation, growth and fertility. *Chromosoma* 122:517–533 (2013).
- Schuettengruber B, Cavalli G: Recruitment of polycomb group complexes and their role in the dynamic regulation of cell fate choice. *Development* 136:3531–3542 (2009).
- Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS: In situ localization of parental genomes in a wide hybrid. *Ann Bot* 64:315–324 (1989).
- Shaw PJ, Brown JW: Plant nuclear bodies. *Curr Opin Plant Biol* 7:614–620 (2004).
- She W, Grimanelli D, Rutowicz K, Whitehead MW, Puzio M, et al: Chromatin reprogramming during the somatic-to-reproductive cell fate transition in plants. *Development* 140:4008–4019 (2013).
- Shiels C, Adams NM, Islam SA, Stephens DA, Freemont PS: Quantitative analysis of cell nucleus organisation. *PLoS Comput Biol* 3:e138 (2007).
- Shu H, Wildhaber T, Siretskiy A, Grussem W, Hennig L: Distinct modes of DNA accessibility in plant chromatin. *Nat Commun* 3:1281 (2012).
- Simon JA, Kingston RE: Occupying chromatin: polycomb mechanisms for getting to genomic targets, stopping transcriptional traffic, and staying put. *Mol Cell* 49:808–824 (2013).
- Smigova J, Juda P, Cmarko D, Raska I: Fine structure of the ‘PcG body’ in human U-2 OS cells established by correlative light-electron microscopy. *Nucleus* 2:219–228 (2011).
- Soppe WJ, Jasencakova Z, Houben A, Kakutani T, Meister A, et al: DNA methylation controls histone H3 lysine 9 methylation and heterochromatin assembly in *Arabidopsis*. *EMBO J* 21:6549–6559 (2002).
- Spector DL, Lamond AI: Nuclear speckles. *Cold Spring Harb Perspect Biol* 3pii: a000646 (2011).
- Stack SM, Clark CR: Chromosome polarization and nuclear rotation in *Allium cepa* roots. *Cytologia* (Tokyo) 39:553–560 (1974).
- Strayer C, Oyama T, Schultz TF, Raman R, Somers DE, et al: Cloning of the *Arabidopsis* clock gene *TOC1*, an autoregulatory response regulator homolog. *Science* 289:768–771 (2000).
- Stroud H, Otero S, Desvoves B, Ramirez-Parra E, Jacobsen SE, Gutierrez C: Genome-wide analysis of histone H3.1 and H3.3 variants in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* 109:5370–5375 (2012).
- Tachiwana H, Kurumizaka H: Structure of the CENP-A nucleosome and its implications for centromeric chromatin architecture. *Genes Genet Syst* 86:357–364 (2011).
- Tachiwana H, Kagawa W, Shiga T, Osakabe A, Miya Y, et al: Crystal structure of the human centromeric nucleosome containing CENP-A. *Nature* 476:232–235 (2011).
- Taddei A, Gasser SM: Structure and function in the budding yeast nucleus. *Genetics* 192:107–129 (2012).
- Talbert PB, Masuelli R, Tyagi AP, Comai L, Henikoff S: Centromeric localization and adaptive evolution of an *Arabidopsis* histone H3 variant. *Plant Cell* 14:1053–1066 (2002).
- Tao LZ, Cheung AY, Nibau C, Wu HM: RAC GTPases in tobacco and *Arabidopsis* mediate auxin-induced formation of proteolytically active nuclear protein bodies that contain AUX/IAA proteins. *Plant Cell* 17:2369–2383 (2005).

- Tessadori F, Chupeau MC, Chupeau Y, Knip M, Germann S, et al: Large-scale dissociation and sequential reassembly of pericentric heterochromatin in dedifferentiated *Arabidopsis* cells. *J Cell Sci* 120:1200–1208 (2007a).
- Tessadori F, Schulkes RK, van Driel R, Franz P: Light-regulated large-scale reorganization of chromatin during the floral transition in *Arabidopsis*. *Plant J* 50:848–857 (2007b).
- Tessadori F, van Zanten M, Pavlova P, Clifton R, Pontvianne F, et al: Phytochrome B and histone deacetylase 6 control light-induced chromatin compaction in *Arabidopsis thaliana*. *PLoS Genet* 5:e1000638 (2009).
- Tiang CL, He Y, Pawlowski WP: Chromosome organization and dynamics during interphase, mitosis, and meiosis in plants. *Plant Physiol* 158:26–34 (2012).
- Towbin BD, Gonzalez-Sandoval A, Gasser SM: Mechanisms of heterochromatin subnuclear localization. *Trends Biochem Sci* 38:356–363 (2013).
- Turck F, Roudier F, Farrona S, Martin-Magniette ML, Guillaume E, et al: *Arabidopsis* TFL2/LHP1 specifically associates with genes marked by trimethylation of histone H3 lysine 27. *PLoS Genet* 3:e86 (2007).
- Uchida W, Matsunaga S, Sugiyama R, Kawano S: Interstitial telomere-like repeats in the *Arabidopsis thaliana* genome. *Genes Genet Syst* 77:63–67 (2002).
- Vaillant I, Tutois S, Jasencakova Z, Douet J, Schubert I, Tourmente S: Hypomethylation and hypermethylation of the tandem repetitive 5S rRNA genes in *Arabidopsis*. *Plant J* 54:299–309 (2008).
- Van Buskirk EK, Decker PV, Chen M: Photobodies in light signaling. *Plant Physiol* 158:52–60 (2012).
- van Driel R, Franz P: Nuclear architecture and genome functioning in plants and animals: what can we learn from both? *Exp Cell Res* 296:86–90 (2004).
- van Koningsbruggen S, Gierlinski M, Schofield P, Martin D, Barton GJ, et al: High-resolution whole-genome sequencing reveals that specific chromatin domains from most human chromosomes associate with nucleoli. *Mol Biol Cell* 21:3735–3748 (2010).
- van Zanten M, Tessadori F, Bossen L, Peeters AJ, Franz P: Large-scale chromatin de-compaction induced by low light is not accompanied by nucleosomal displacement. *Plant Signal Behav* 5:1677–1678 (2010).
- van Zanten M, Koini MA, Geyer R, Liu Y, Brambilla V, et al: Seed maturation in *Arabidopsis thaliana* is characterized by nuclear size reduction and increased chromatin condensation. *Proc Natl Acad Sci USA* 108:20219–20224 (2011).
- van Zanten M, Tessadori F, Peeters AJ, Franz P: Shedding light on large-scale chromatin reorganization in *Arabidopsis thaliana*. *Mol Plant* 5:583–590 (2012a).
- van Zanten M, Carles A, Li Y, Soppe WJ: Control and consequences of chromatin compaction during seed maturation in *Arabidopsis thaliana*. *Plant Signal Behav* 7:338–341 (2012b).
- Vaquero-Sedas MI, Vega-Palas MA: Differential association of *Arabidopsis* telomeres and centromeres with histone H3 variants. *Sci Rep* 3:1202 (2013).
- Vaquero-Sedas MI, Luo C, Vega-Palas MA: Analysis of the epigenetic status of telomeres by using ChIP-seq data. *Nucleic Acids Res* 40:e163 (2012).
- Vazquez J, Belmont AS, Sedat JW: Multiple regimes of constrained chromosome motion are regulated in the interphase *Drosophila* nucleus. *Curr Biol* 11:1227–1239 (2001).
- Verdel A, Vavasseur A, Le Gorrec M, Touat-Todeschini L: Common themes in siRNA-mediated epigenetic silencing pathways. *Int J Dev Biol* 53:245–257 (2009).
- Vrbsky J, Akimcheva S, Watson JM, Turner TL, Daxinger L, et al: siRNA-mediated methylation of *Arabidopsis* telomeres. *PLoS Genet* 6:e1000986 (2010).
- Watson JM, Riha K: Comparative biology of telomeres: where plants stand. *FEBS Lett* 584:3752–3759 (2010).
- Weiss H, Maluszynska J: Molecular cytogenetic analysis of polyploidization in the anther tapetum of diploid and autotetraploid *Arabidopsis thaliana* plants. *Ann Bot* 87:729–735 (2001).
- Weston DJ, Adams NM, Russell RA, Stephens DA, Freemont PS: Analysis of spatial point patterns in nuclear biology. *PLoS ONE* 7:e36841 (2012).
- Xu X, Xu S, Jin L, Song E: Characteristic analysis of Otsu threshold and its applications. *Pattern Recogn Lett* 32:956–961 (2011).
- Xue JH, Zhang YJ: Ridler and Calvard's, Kittler and Illingworth's and Otsu's methods for image thresholding. *Pattern Recogn Lett* 33:793–797 (2012).
- Zellinger B, Riha K: Composition of plant telomeres. *Biochimica Biophysica Acta* 1769:399–409 (2007).
- Zemach A, Kim MY, Hsieh PH, Coleman-Derr D, Eshed-Williams L, et al: The *Arabidopsis* nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. *Cell* 153:193–205 (2013).
- Zhang H, Zhu JK: RNA-directed DNA methylation. *Curr Opin Plant Biol* 14:142–147 (2011).
- Zhang W, Lee HR, Koo DH, Jiang J: Epigenetic modification of centromeric chromatin: hypomethylation of DNA sequences in the CENH3-associated chromatin in *Arabidopsis thaliana* and maize. *Plant Cell* 20:25–34 (2008).
- Zhang X, Germann S, Blus BJ, Khorasanizadeh S, Gaudin V, Jacobsen SE: The *Arabidopsis* LHP1 protein colocalizes with histone H3 Lys27 trimethylation. *Nat Struct Mol Biol* 14:869–871 (2007).