

# Organization and dynamics of plant interphase chromosomes

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**Eukaryotic chromosomes occupy distinct territories within interphase nuclei. The arrangement of chromosome territories (CTs) is important for replication, transcription, repair and recombination processes. Our knowledge about interphase chromatin arrangement is mainly based on results from *in situ* labeling approaches. The phylogenetic affiliation of a species, cell cycle, differentiation status and environmental factors are all likely to influence interphase nuclear architecture. In this review we survey current data about relative positioning of CTs, somatic pairing of homologs, and sister chromatid alignment in meristematic and differentiated tissues, using data derived mainly from *Arabidopsis thaliana*, wheat (*Triticum aestivum*) and their relatives. We discuss morphological constraints and epigenetic impacts on nuclear architecture, the evolutionary stability of CT arrangements, and alterations of nuclear architecture during transcription and repair.**

## Anaphase orientation may or may not be maintained during interphase

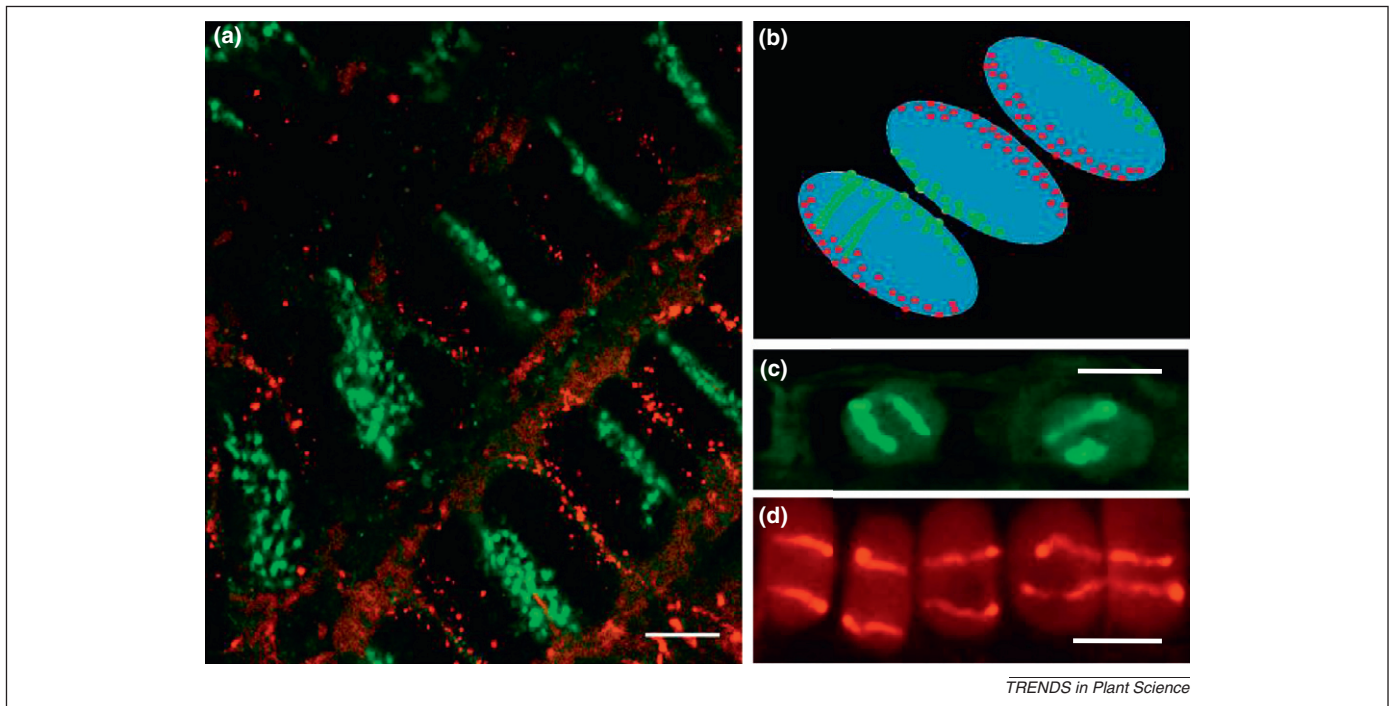
In 1885 C. Rabl [1] proposed that chromosomes are oriented in an arrangement reflecting anaphase, with centromeres and telomeres clustered at opposite nuclear poles, and T. Boveri [2] suggested that chromosomes occupied distinct chromosome territories (CT) during interphase. However, the introduction of FISH (fluorescence *in situ* hybridization) revolutionized our understanding of chromosomes, by enabling visualization for the first time of interphase chromosomes, rather than only mitotic chromosomes [3–5].

Because composition and organization of repetitive sequences within plant genomes differ from those of mammals and birds by a nearly homogenous distribution of all types of dispersed repeats along all types of chromosomes, chromosome painting (the use of labeled chromosome-specific DNA sequences to visualize specific chromosomes by FISH) in plants was impossible for a long time [6]. As an alternative approach [7,8], in interspecific plant hybrids carrying one or more pairs of ‘alien’ chromosomes from the pollinator species, chromosomes of either parent have been labeled by genomic *in situ* hybridization (GISH) with genomic DNA of the corresponding parental species, a technique first invented for mammals [9–11]. Whereas chromosome painting relies on exclusive hybridization of chromosome-specific sequences, GISH relies on hybridiza-

tion of species-specific dispersed repeats. Many wheat lines with additional barley (*Hordeum vulgare*) or rye (*Secale cereale*) chromosomes or substitution of the equivalent rye or barley chromosome for the native wheat chromosome are available. Three-dimensional GISH imaging of intact tissues of hexaploid wheat carrying various different substitutions has demonstrated that the chromosomes are in a regular Rabl configuration [12,13], confirmed by the localization of probes to the centromeres and telomeres at opposite nuclear poles (Figure 1). This arrangement is shared by barley and oats (*Avena sativa*) [14], and by the dicots *Pisum sativum* and *Vicia faba* [15], whereas other species, such as maize (*Zea mays*) and *Sorghum bicolor*, do not show the Rabl configuration [14]. *A. thaliana* (*Arabidopsis*) chromosomes do not exhibit the Rabl configuration, and in many cell types the centromeric heterochromatin is located at the nuclear periphery whereas the telomeres are located internally at the nucleolar periphery. Most somatic cells of rice (*Oryza sativa*) also lack a Rabl configuration [14], but premeiotic anther cells and xylem-vessel precursor cells in rice do show a Rabl configuration [16,17]. Whether the chromosomes adopt a Rabl configuration is therefore not a consequence of the genome organization, because rice chromosomes can either adopt it or not, depending on the cell type. Neither is the Rabl configuration a consequence of a large genome size, because some small genomes (*Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*) [18,19] show it, whereas the larger mammalian genomes do not (e.g. [20]).

The Rabl configuration might be regarded as the default interphase chromosome configuration, because it maintains anaphase chromosome orientation. Rather than ask why some species maintain this organization after cell division, we should probably ask why the other species or cell types lose it during interphase. In some karyotypes, the Rabl configuration might be disrupted by the presence of many acrocentric chromosomes, whose short-arm telomeres are close to the centromeres. *Arabidopsis* and wheat contain predominantly metacentric chromosomes, so the presence of Rabl organization in wheat and its absence in *Arabidopsis* cannot be explained by the effect of acrocentric chromosomes. The interphase CT organization must depend on: (i) the amount and genomic organization of the repetitive regions that form heterochromatin; (ii) the precise regions of the chromosomes that undergo decondensation on entry into interphase; (iii) the resulting freedom of movement of the chromosome segments and the characteristics of their diffusional motion; (iv) association with the

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**Figure 1.** Rabl chromosome organization in wheat root tissue. (a) Centromeres (green) and telomeres (red) are labeled by FISH and are located at opposite sides of the nuclei. (b) Diagrammatic interpretation of the organization in (a). (c) Introgressed pair of rye chromosomes in wheat labeled by GISH with total rye genomic probe confirms a Rabl organization of individual CTs. (d) Single rye arm translocation into wheat localized by GISH. Bars = 10  $\mu\text{m}$ .

nuclear envelope or with the nucleolus; (v) ectopic pairing between different regions of the genome; (vi) association with other sub-nuclear structures, subsequently operating on the chromosomes.

#### Interphase CT positioning in *Arabidopsis* and relatives is random

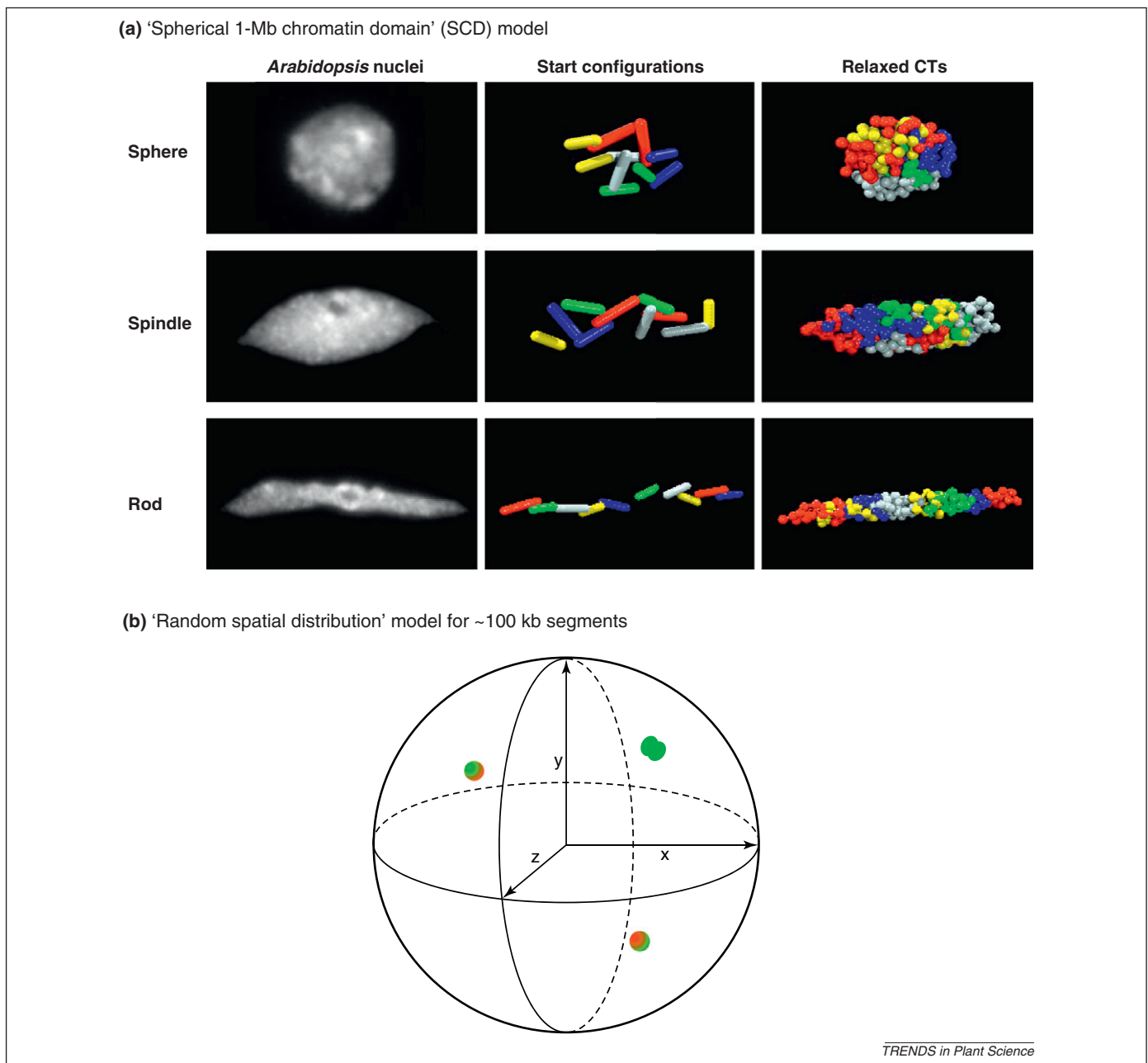
Chromosome paints have been developed in *Arabidopsis* and related species [21–24] by combining BAC clones individually selected for the absence of dispersed repeats. Segmental chromosome painting suggested that chromosome arms of *Arabidopsis* are organized as loops of different sizes emanating from pericentromeric heterochromatin within their CTs [25]. Simultaneous painting of the five *Arabidopsis* chromosomes in 4C leaf nuclei showed that the spatial association of homologous and heterologous CTs was not significantly different from random, according to a modified ‘spherical 1-Mb chromatin domain’ (SCD) model (Figure 2a) [22,26,27], nor was there any preferential radial arrangement of the chromosomes. In mammals [26–31] and birds [32] gene-dense and/or smaller chromosomes usually occupy a more central position than gene-poor and/or larger chromosomes. The lack of any preferential arrangement of *Arabidopsis* CTs could be due to the presence of only five chromosomes of similar size and gene density, with repeats mainly around the heterochromatic pericentromeric chromocenters that are located at the nuclear periphery [24,25].

Painting of each arm of individual chromosomes in a different color allowed tracing of the spatial association of individual chromosome arms. Independent of the nuclear shape, the observed association frequency for the homologous arm territories of the metacentric chromosomes 1, 3,

and 5 was random, whereas the values for the asymmetric, nucleolus-organising region (NOR)-bearing chromosomes were significantly higher [22]. The frequent association of the NOR-bearing chromosomes is explained by attachment of both homologs to a single nucleolus in more than 90% of nuclei. The CT arrangement was similar for meristematic root nuclei and for flow-sorted nuclei of differentiated leaf cells [23]. A less condensed chromatin structure and a lower density of chromosome 1 arm territories than in 2C leaf nuclei was found in 3C endosperm nuclei [33].

In another set of experiments the pairing of individual loci at ten positions along chromosomes 1, 3 and 5 was tested by localizing single BAC probes, each detecting approximately 100 kb. On average, only about 5% of 2C or 4C nuclei showed homologous chromosome pairing, and this corresponds to random pairing according to the ‘random spatial distribution’ model (Figure 2b), that predicts 6–8% pairing for random arrangements [22]. In addition to paired loci, the FISH foci from unpaired individual homologous loci were often found less than half the signal diameter apart from each other. Such closely neighboring loci can be brought together by Brownian motion to allow for homologous recombination and repair, similarly to paired loci [22]. Finally, individual segments of the chromosome can pair while the chromosome as a whole remains unpaired.

Although the karyotype of *Arabidopsis lyrata* differs from that of *A. thaliana* by a 50% larger genome, a higher chromosome number ( $2n = 16$ ), and a more pronounced size difference between the chromosomes, both showed a random positional pairing frequency except for NOR-bearing chromosomes that were more frequently associated than expected at random [24]. Therefore, this nuclear architecture might also be conserved in other related Brassicaceae



**Figure 2.** Models to simulate interphase arrangement of chromosome territories and of specific chromosome segments. (a) Simulation of random chromosome territory (CT) arrangement in spherical, spindle-shaped, and rod-shaped 2C *Arabidopsis* nuclei of about  $30 \mu\text{m}^3$ . The 'spherical 1-Mb chromatin domain' model considers each CT to be a chain of ~1-Mb chromatin domains of ~500-nm diameter. The model assumes, as a starting configuration, compressed cylinders corresponding to mitotic chromosomes. These cylinders are allowed to relax until a thermodynamic equilibrium is reached after ~200 000 Monte Carlo cycles, that is the nucleus is filled uniformly. CTs are considered associated if their boundaries are less than 500 nm apart (courtesy of Ales Pecinka, Gregor Kreth and Armin Meister). (b) The 'random spatial distribution' model simulates homologous pairing of ~100-kb chromosome-arm segments (corresponding to an average BAC insert) as spheres with randomly determined coordinates within a virtual nucleus of average dimension. The frequency of overlapping of homologous (green) spheres indicates random positional (single-point) pairing (courtesy of Armin Meister, Marco Klatté, Ales Pecinka and Veit Schubert).

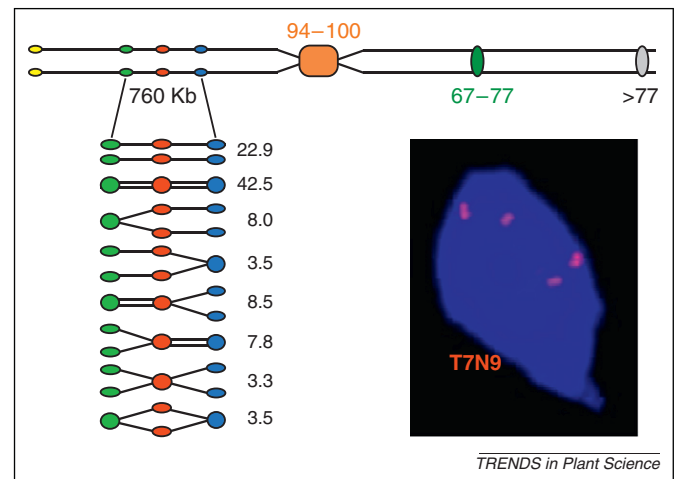
species. The preservation of CT arrangement during the cell cycle and in differentiated cells has been studied in nuclei of root-tip meristems and of guard cells of *Arabidopsis* [23]. The mirror symmetry of homologous CTs found immediately after mitosis decays with time, and is no longer obvious between adjacent but non-sister nuclei or between sister nuclei of fully differentiated stomatal guard cells. The maintenance of mirror symmetry between sister nuclei, for a brief period after mitosis, has been reported in mammalian cells [32,34–37]. The results suggest that phylogenetic affiliation is more important for the mode

of interphase CT arrangement in plants than genome size, sequence organization and chromosome number. Furthermore, a mirror image CT arrangement between daughter nuclei of cycling plant and animal cells up to the next prometaphase [38] seems to be the result of the symmetric movement of anaphase chromosome sets.

**Alignment of sister chromatids is variable and dynamic**  
Collinear alignment of sister chromatids, defined as 'cohesion' [39,40], has been assumed to be established during replication and to last until chromatid separation at ana-

phase [41–43]. Close sister chromatid alignment is important for post-replication repair of double-strand breaks (DSBs) through homologous recombination with the undamaged sister chromatid as a template in S and G2 phase, and, together with the spindle checkpoint control, for the correct segregation of the sister chromatids to daughter nuclei. In yeast, cohesin binding sites of 0.8–1 kb are separated by ~11 kb on average and are enriched about threefold around centromeres [44–47]. Owing to the high density of cohesin sites, FISH signals cannot distinguish sister chromatids in yeast 4C nuclei [48]. By contrast, in human fibroblast G2 nuclei, FISH signals on sister chromatids can be distinguished [49]. Clear separation of sister chromatid loci has also been observed in 4C interphase nuclei of meristematic and differentiated cells of mono- and dicot plants, preferentially in mid-arm positions [50,51]. In endopolyploid 8C *Arabidopsis* nuclei, all homologous positions can display sister separation. At chromosome termini, sister separation is less frequent, and at centromeric positions only endopolyploid nuclei greater than 16C frequently show separated FISH signals [50]. Although sister chromatid alignment apparently does not depend on the DNA sequence, high-copy-number arrays (centromeric repeats or rDNA) are aligned at up to 100% of homologous positions [24,50,51]. Sister chromatid alignment is independent of the CpG methylation level, because separation of sister chromatids in the hypomethylation mutant *ddm1* [52] was no more frequent than in the wild-type background [50]. An *Arabidopsis* mutant of the p150 subunit of the chromatin assembly factor CAF1 (*fas1-4*) showed up to a 100-fold increase of (intrachromosomal) somatic homologous recombination, but did not display a significantly altered sister chromatid cohesion or somatic pairing compared to wild-type *Arabidopsis* nuclei [53]. Possibly, the more relaxed chromatin structure in *fas1-4* nuclei enabled preferentially intrachromosomal homologous recombination *in cis*.

Thirteen ~100-kb fragments of a 1.2-Mb region of chromosome 1 showed individual sister chromatid alignment for more than two-thirds of homologs (Figure 3) [54]. These values do not differ significantly from each other indicating the absence of ‘hot spots’ or ‘cold spots’ of alignment within this euchromatic region of the *Arabidopsis* genome. The entire 1.2-Mb region displayed alignment in 69% of homologs; partial alignment or separation within this region occurred rarely [54]. The sister chromatids of the entire top arm of chromosome 1 (~14.2 Mb) were found to be separated in 3.8% of *Arabidopsis* 4C leaf nuclei [50]. Clearly, *Arabidopsis* sister chromatids can be separated or aligned within a range from <100 kb up to several Mb [54]. The average distances between aligned sites on plant chromosomes are much larger than the ~11-kb distances between cohesin binding sites in yeast. Random pairing of homologs and association of sister chromatid alignment of 60–70% along chromosome arm positions are apparently enough to ensure efficient homologous recombination and repair during interphase of cycling cells. Consistent sister centromere alignment alone seems to be sufficient for correct sister chromatid segregation. Chromosome condensation toward nuclear divisions brings separated sister chromatid regions into close proximity. Insertion mutations in all



**Figure 3.** Schematic of an *Arabidopsis* chromosome indicating the average frequency of sister-chromatid alignment per homolog (in %) at centromeric, mid-arm, and terminal positions. For a 760-kb region of chromosome 1, the frequencies per homolog (%) of the different alignment configurations at its central position and at the flanking positions are given below [49]. The image on the right shows a 4C leaf nucleus displaying separation of sister chromatids for both homologs of chromosome 1 at the position of a BAC (T7N9) insert (courtesy of Veit Schubert).

known cohesin genes of *Arabidopsis* impair sister chromatid alignment along chromosome arms and in some cases even at centromeres [55]. Some of these mutants show many ‘spontaneous’ anaphase bridges representing either asymmetric chromatid rearrangements or unresolved DNA concatenation.

The average sister chromatid alignment frequency (but not the pairing of homologs) increases significantly immediately after X-irradiation and returns to the basal level after 1 h of recovery, when most of the DSBs are repaired. This phenomenon is significantly less pronounced in mutants of genes encoding components of S phase-established cohesin or of the SMC5/6 complex, which is known to be recruited to DSBs [56]. Depletion of the MCM helicase-binding replication factor ETG1 of *Arabidopsis* also causes reduced sister chromatid cohesion, a phenotype which is more severe in combination with mutants of the cohesin SYN4 or the replication factor CTF18 [57]. These observations indicate movement of homologous sister loci toward each other to favor homologous recombination after DNA breakage.

### Transgenic tandem repeat arrays can alter interphase chromatin arrangement

Whereas FISH techniques are useful to study chromosome and chromatin arrangement in fixed nuclei, fluorescent protein tags [58–61] are often used to analyze individual chromatin sites and their motility *in vivo*. The most frequently used system uses binding of green fluorescent protein (GFP)-linked bacterial Lac-repressor protein (GFP-lacI) to transgenic *lacO* arrays, for review [62,63]. In a transgenic *Arabidopsis* line harboring two *lacO* arrays of >10 kb on chromosome 3 ~4.2 Mb apart from each other, the chromatin regions carrying tandem repetitive *lacO* sequences paired more frequently and were more often associated with heterochromatic chromocenters than the respective regions of wild-type nuclei, whether or not the fluorescent protein tag was expressed [64]. Similar results

were obtained for a large transgenic multicopy hygromycin phosphotransferase locus [65], suggesting that such ectopic pairing can be induced by many repeated transgene arrays. The transgenic *lacO* repeats were strongly methylated; such methylation is correlated with frequent homologous pairing of the transgenic loci but not with their association with heterochromatin [66]. In contrast to these observations, which were made on fixed leaf nuclei, *in vivo* fluorescent chromatin tagging using similar T-DNA constructs (*lacO* or *tet*-operator, *tetO*, sequences) did not show more frequent pairing of the transgene-bearing chromatin regions in *Arabidopsis* root nuclei [67]. To interpret these contradictory results, several transgenic lines harboring one or more large (>9.3 kb) or small (~2 kb) *lacO* or *tetO* (4.8 kb) repeat arrays were used in FISH experiments to analyze homologous positional pairing of the insert sites and their association frequency with heterochromatin in flow-sorted 2C leaf and root nuclei [68] (Gabriele Jovtchev, Branmira E. Borisova-Todorova, Markus Kuhlmann, Koichi Watanabe, Ingo Schubert, Michael F. Mette, unpublished data). This study showed: (i) The frequency of somatic homologous pairing (allelic as well as ectopic) is higher at insertion sites of large (>9.3 kb) repeat arrays, if these are strongly CpG-methylated and more than one homozygous array per genome is present. (ii) Depletion of the heterochromatin-specific mark H3K9me2 or of non-CpG methylation has no obvious impact on the pairing behavior of the large repeat arrays. (iii) The frequency of a repeat array's association with chromocenters depends on insert size (i.e. size and number of repeat units per locus), but not on their methylation status. Possibly, somatic association between chromosomes is in general favored by large repeat arrays. (iv) One or more low-copy inserts (independent of their transcriptional activity) do not significantly alter interphase chromatin arrangement, even if strongly methylated. Therefore, low-copy arrays seem to be more suitable for chromatin tagging than multi-copy arrays.

### Genes become reorganized on transcriptional activation

In a given cell type thousands of genes are expressed, but labeling of the nascent transcripts by immunofluorescence detection of *in vivo* incorporated BrUTP reveals only a few hundred rather bright foci in the nucleoplasm of animals and plants (e.g. [13,69–73]). One possible explanation is that transcription takes place at specific 'transcription factories' with several genes being recruited to each of these sites [70,74]. Although thousands of genes and many other sequences are transcribed in any given cell type, the number of genes transcribed by a given nucleus at a given moment is much less certain. It is likely that genes are activated stochastically and those transcribed at low levels are switched on in some and switched off in other cells.

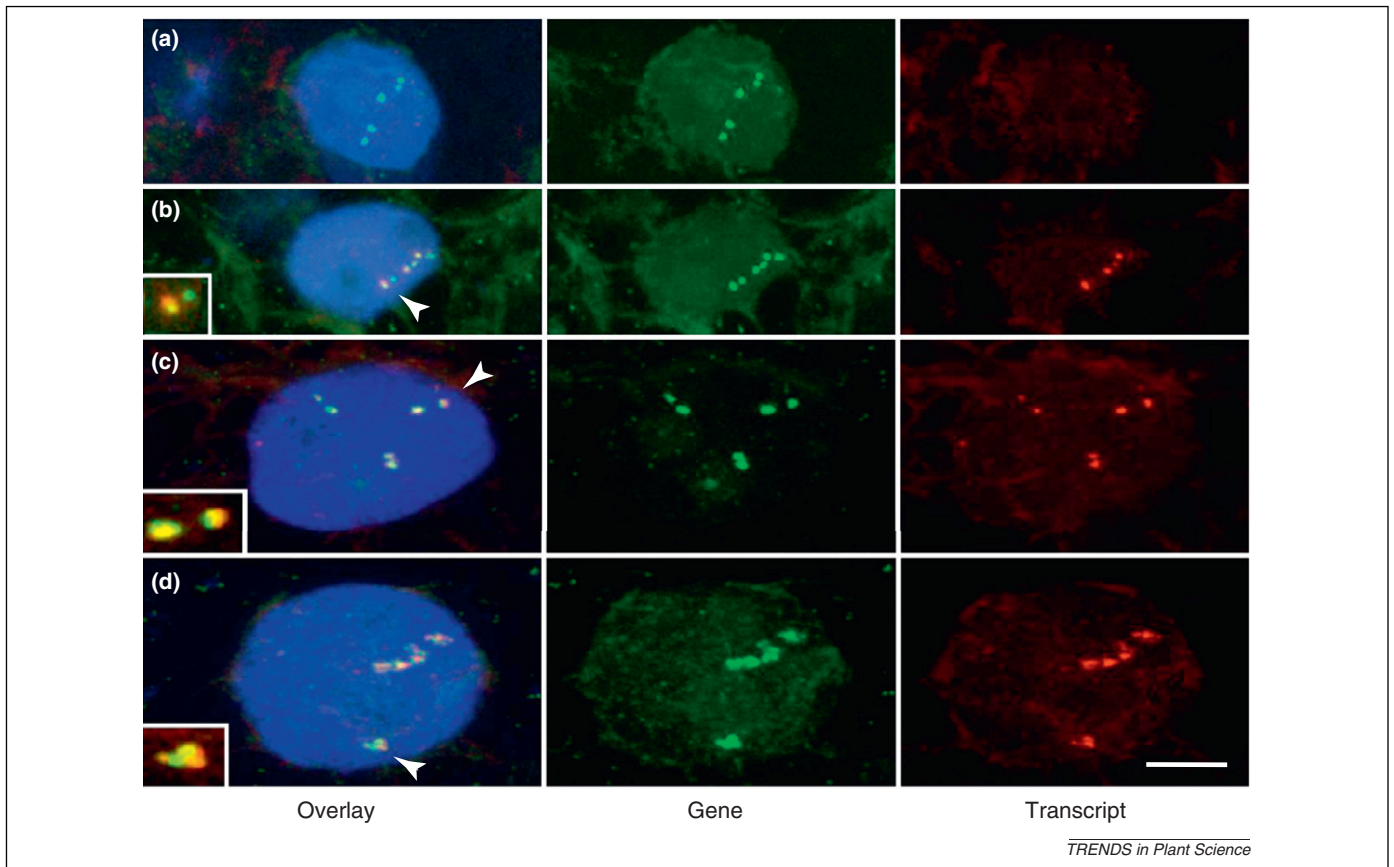
Transcription sites, whether organized as factories or not, have been proposed to be located at the periphery of CTs and the transcripts to be transported through an interchromosome space [75–77]. For example, euchromatin and gene regions have been shown to be located at the nuclear periphery in rod cell nuclei from mammalian species adapted to nocturnal lifestyle [78]. In wheat, interphase chromosomes have a very distinctive organization,

with interphase CTs arranged as parallel cylinders spanning the nucleus. The transcription sites would therefore be expected to be arranged to reflect this configuration; transcription sites were observed throughout the CTs, however [13]. The gradient of gene concentration along the wheat chromosomes with more genes in distal regions (closer to the telomeres) than in proximal parts (nearer the centromeres) should result in a gradient of transcription site concentration, but a uniform density of transcription sites throughout the nucleoplasm was observed in wheat and in *Zingieria kochii* [13,79]. Similar results showing both active and silent genes distributed throughout the CTs have been also found in animal cells [80]. Thus, transcription sites are organized on different principles than is chromosomal location within the nucleus and genes can move some distance within the nucleus to their transcription sites. There is currently a debate whether the interchromatin compartment is just intermingling chromatin fibers [81], or whether it is a defined, functional domain linking the nuclear interior with the nuclear pores and is where transcription, splicing, repair and replication complexes are located [82,83].

Examination of transgenic wheat lines for the location of the transgenes within the nucleus [84] revealed that many of these lines contained multiple transgenes that were inserted at different locations within single chromosomes, in some cases on opposite chromosome arms. FISH showed that these copies were brought together during interphase and were often seen as a single labeled focus. One possible explanation would be that the different copies are recruited to a common transcription factory. The Rab1 configuration of the chromosomes would explain how sites on opposite chromosome arms could be close together in interphase. Another explanation could be ectopic pairing of the identical multiple copies of the integrated DNA, as has been observed with *lacO* and *tetO* integrations (see above).

All of these transgenes were transcriptionally silenced to some extent [85]. The silencing could be reduced by treatment with azacytidine, that reduces DNA methylation, or with trichostatin A, that inhibits histone deacetylases and thus increases levels of histone acetylation associated with transcriptional activation. Both these treatments caused the transgene copies to move apart, so that they could be seen individually by *in situ* labeling. This result argues against recruitment to a common transcription factory as the cause of gene colocalization and suggests the transgene association is correlated with epigenetic modifications and transcriptional silencing, although a direct causal relationship is hard to prove. GISH labeling of CTs after the two drug treatments also revealed some marked differences [85]. The Rab1 configuration was maintained, but the chromosome arms lost their smooth outline and appeared as smaller blocks separated by gaps, consistent with a chromosome organization containing large stretches of heterochromatin interspersed with gene-rich islands that can become decondensed [86].

Other transgenic wheat lines carried multiple tandem insertions of the high-molecular-weight glutenin gene and its flanking genomic DNA [87]. This gene is exclusively expressed in the endosperm from 8 days after anthesis until the end of grain filling. The flanking genomic



**Figure 4.** Activation of transcription of glutenin genes in (triploid) wheat endosperm. This line carries about 20 glutenin transgenes in two tandem arrays. (a) Before transcription the genes are seen as two sets of condensed sites (green), without associated transcript (red). (b) As transcription begins, transcript RNA (red) is seen, but no significant decondensation of the gene arrays is visible, and not all loci become activated to the same extent. (c,d) As transcription progresses, the gene arrays become decondensed, but transcript RNA is still not equally associated with all gene foci. Bar = 10  $\mu\text{m}$ .

sequences should ensure that the temporal and spatial regulation of expression of the transgene copies were the same as those of the endogene. In root tissue or at earlier stages in endosperm development, the  $\sim 20$  transgene copies in tandem were condensed into one or two large foci. They began to decondense as transcription began, as judged by double labeling for the genes and transcripts (Figure 4). The first transcripts at the gene sites were visible before any significant decondensation. When the locus decondensed more gene foci appeared. In some nuclei the foci extended in linear patterns, showing that segments of  $\sim 200$  kb can extend almost across the nucleus, probably outside their CTs. Many but not all foci were associated with transcripts, suggesting a stochastic induction of transcription.

In oats, two adjacent genes, both involved in the biosynthesis of the secondary metabolite avenacin, moved apart on activation from  $\sim 0.45$  to  $\sim 0.90$   $\mu\text{m}$  [88]. Labeling each half of one of the genes, *Sad1*, in different colors showed that the gene itself decondensed significantly, with FISH signals for the two ends moving apart from  $\sim 0.25$  to  $\sim 0.35$   $\mu\text{m}$  on transcriptional activation.

Painting of a CT in *Arabidopsis*, simultaneously with FISH labeling of a single BAC from the same region in a different color, showed signals for the BAC clearly outside the corresponding CT in 12.8% of nuclei [22]. To determine whether location of a single BAC outside its arm territory is related to the transcriptional activity of genes in the

BAC, a BAC from the bottom arm of chromosome 4 harboring the flowering gene *fwa* was hybridized to leaf nuclei of wild type and *fwa* mutant plants. In wild-type leaf nuclei, the gene is strongly methylated and not expressed, whereas it is hypomethylated and constitutively expressed in *fwa* mutant leaves [89]. For 2C nuclei, the BAC signals were found outside the arm territory in 4.2% of mutant and in 3.8% wild-type nuclei. In the case of 4C nuclei, the same BAC displayed out-looping in 10.7% of mutant and in 6.5% of wild-type nuclei. Thus out-looping was more frequent in FWA-expressing 4C nuclei; however, this difference was not significant at the  $P = 0.001$  level [22]. Of the other 21 genes of the *fwa*-containing BAC, seven are strongly transcribed in leaf cells; three of them within 20 kb upstream or downstream of *fwa* (<http://www.weigelworld.org>). A density of one active gene per 10 kb might be just at the limit for observation of a regular extension of a region out of its CT (e.g. [30]). Furthermore, a folded surface of the CT might permit a peripheral position of extended chromatin without the appearance of an obvious out-looping. The sister-chromatid alignment frequency at the position of the *fwa* gene-containing BAC was not significantly higher in 4C mutant (*fwa*) than in wild-type nuclei [50]. At present it is not clear for *Arabidopsis* whether out-looping of transcribed regions from CTs occurs, and whether active and silent sites are distributed throughout CTs as suggested for mammals and wheat [13,80].

Another *Arabidopsis* BAC containing the *GL2* locus, encoding a transcription factor which determines root hair formation, was hybridized to root epidermal cells [90]. In the non-root-hair-forming cells expressing *GL2*, clear FISH signals were seen, whereas in the hair-forming cells not expressing *GL2*, labeling intensity was much lower. This result shows that the transcriptionally inactive locus is less accessible to the probe due to a more compact chromatin structure. The differential labeling pattern and root-hair fate determination were disrupted in the *fas2* mutant, which is defective in CAF1, confirming the involvement of chromatin organization in the regulation of epidermal cell fate.

## Conclusions

In plant species with large genomes, the interphase CTs often adopt a Rabl configuration, whereas in *Arabidopsis* and other species they do not. There is no evidence for a preferred positioning of interphase CTs relative to each other, except for NOR-bearing chromosomes, which associate with the nucleoli. Within the physical constraints (e.g. nuclear shape or size and number of nucleoli), the arrangement of chromosome territories is mostly random. Somatic homologous pairing is the exception rather than the rule in plants.

The inconsistent sister-chromatid alignment in *Arabidopsis* clearly differs from the situation observed in yeast. A critical level of tandem repetitive sequences, epigenetic modifications, and DNA damage could alter random local chromatin arrangement. DSB ends move toward homologous sister regions. In wheat, transcriptional activation is linked with induced chromatin mobility and decondensation, whereas ectopic association of multiple transgenes seems to be linked with transcriptional silencing. Whether transcription-mediated chromatin mobility occurs in *Arabidopsis* is still unclear. The mechanisms that promote sister-chromatid alignment after DNA breakage or those that cause tandem repetitive domains to pair with each other and to associate with heterochromatin are poorly understood.

*In vivo* fluorescent labeling using systems such as GFP-LacO, FRAP (fluorescence recovery after photoactivation) and photoactivatable or photoswitchable fluorescent markers are increasing our understanding of chromatin dynamics. New approaches such as chromatin conformation capture are enabling us to study chromatin conformation at the DNA sequence level (for review see [91]). These methods allow an examination of which DNA sequences are located close to each other in 3D in the nucleus and will certainly soon be applied to plant genomes, extending our understanding of chromatin conformation. Finally, at least in large genome plant species, such as oat, changes in the length of a single gene on transcription are within conventional optical resolution limits. We can expect much progress from the application of recent optical 'super-resolution' techniques that enable dynamic molecular level imaging.

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