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RESEARCH ARTICLE

Interphase chromatin organisation in *Arabidopsis* nuclei: constraints versus randomness

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Abstract The spatial chromatin organisation and molecular interactions within and between chromatin domains and chromosome territories (CTs) are essential for fundamental processes such as replication, transcription and DNA repair via homologous recombination. To analyse the distribution and interaction of whole CTs, centromeres, (sub)telomeres and ~100-kb interstitial chromatin segments in endopolyploid nuclei, specific FISH probes from *Arabidopsis thaliana* were applied to 2–64C differentiated leaf nuclei. Whereas CTs occupy a distinct and defined volume of the nucleus and do not obviously intermingle with each other in 2–64C nuclei, ~100-kb sister chromatin segments within these CTs become more non-cohesive with increasing endopolyploidy. Centromeres, preferentially located at the nuclear periphery, may show ring- or half-moon like shapes in 2C and 4C nuclei. Sister centromeres tend to associate up to the 8C level. From 16C nuclei on, they become progressively separated. The higher the polyploidy level gets, the more separate chromatids are present. Due to sister chromatid separation in highly endopolyploid nuclei, the centromeric histone variant CENH3, the 180-bp centromeric repeats and pericentromeric heterochromatin form distinct subdomains at adjacent but not intermingling positions. The (sub)telomeres are frequently associated with each other and with the nucleolus and less often with centromeres. The extent of chromatid separation and of chromatin decondensation at subtelomeric chromatin segments varies between chromosome arms. A mainly random distribution and similar shapes of CTs even at higher ploidy levels indicate that in general no substantial CT reorganisation occurs during endopolyploidisation. Non-cohesive sister chromatid regions at chromosome arms and at the (peri) centromere are accompanied by a less dense chromatin conformation in highly endopolyploid nuclei. We discuss the possible function of this conformation in comparison to transcriptionally active regions at insect polytene chromosomes.

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Introduction

Interactions between chromatin segments in interphase nuclei are required for such basic biological processes as transcription, replication and DNA repair. Both transcription and replication are thought to proceed in distinct transcription and replication factories which require chromatin fibre movements. Depending on tissue and developmental stage, these processes may reorganise the 3D architecture of interphase nuclei (Chakalova et al. 2005; Chakalova and Fraser 2010; Ferrai et al. 2010; Misteli and Soutoglou 2009; Papantonis and Cook 2010). Additionally, the DNA quantity reflected by the ploidy level may also influence the interphase nuclei architecture. Specialised tissues in plants and animals may contain endopolyploid nuclei, however, even if endoploidisation was hypothesised to provide a mechanism for increasing cell size and gene transcription (Barow 2006; Sugimoto-Shirasu and Roberts 2003) the biological significance of endoreduplication is still under debate. Therefore, examination of interphase chromatin arrangement may contribute to better understand this phenomenon.

In all eukaryotes analysed so far by cytological methods, interphase chromosomes are arranged in distinct chromosome territories (CTs) (Cremer and Cremer 2010). Although mainly compactly organised, CTs may show fuzzy boarders and a certain degree of overlapping and/or intermingling with adjacent CTs (Branco and Pombo 2006) which may allow interchromosomal interactions.

Recent molecular studies based on three-dimensional genome-wide mapping of chromatin interactions (chromosome conformation capture, 3–5C) confirm the presence of CTs and interactions within and between them (Cope et al. 2010; Dekker et al. 2002; Lieberman-Aiden et al. 2009; Dostie et al. 2007; Zhao et al. 2006).

The fractal globule has been proposed as a model of chromatin architecture in interphase nuclei. It is the only statistical polymer model consistent with chromosome conformational capture data and observations obtained by fluorescence in situ hybridisation (FISH). It explains the formation of CTs and the occurrence of intra- and interchromosomal short- and long-range interactions (Mirny 2011).

Although chromosome organisation varies between different cell types (Parada et al. 2004), a radial arrangement of CTs with gene-dense chromosomes located more internally and gene-poor ones closer to the nuclear periphery was described for spherical nuclei (Boyle et al. 2001; Cremer et al. 2001; Croft et al. 1999). Interestingly, this organisation seems to be conserved in most vertebrates (Berchtold et al. 2011; Küpper et al. 2007; Koehler et al. 2009; Neusser et al. 2007). For mammals and *Arabidopsis thaliana*, the relative positioning of chromosomes was found to be partially transmitted through mitosis and maintained at least transiently in a mirror-symmetrical pattern in sister nuclei (Berr and Schubert 2007; Essers et al. 2005; Gerlich et al. 2003; Thomson et al. 2004; Walter et al. 2003).

Whether gene density reflects transcriptional activity and therefore influences CT positioning is still a matter of debate. Whereas several authors describe an internal positioning of actively transcribed genes (Kozubek et al. 2002; Lukasova et al. 2002; Scheuermann et al. 2004; Zink et al. 2004), the location of highly expressed genes was also found at the nuclear periphery (Brown et al. 2006; Küpper et al. 2007).

Several studies in mammals and plants indicate that transcriptional activation induces chromatin decondensation and out-looping of chromatin fibres from their CTs (Wegel and Shaw 2005; Wegel et al. 2005, 2009).

In addition to functional constraints, topological factors may influence chromosome configurations in interphase nuclei. In many eukaryotes as a relic of anaphase movement, centromeres cluster at one pole whereas telomeres localise at the opposite pole forming the so-called Rabl orientation (Rabl 1885). Rabl orientation as found in meristems and even in differentiated tissue of several Triticeae species (Dong and Jiang 1998; Schubert and Shaw 2011; Schubert et al. 2011) could mediate interaction of distinct homologous and heterologous chromatin regions.

Random but also preferential associations between homologues were reported in many eukaryotes. A close spatial association of homologues was found in somatic cells of *Drosophila* (Csink and Henikoff 1998; Fung et al. 1998; Hiraoka et al. 1993) possibly as a prerequisite for transvection (Coulthard et al. 2005; Duncan 2002). A non-random association was reported for a pair of barley substitution chromosomes in wheat tapetum and premeiotic nuclei (Aragon-Alcaide et al. 1997) and for homologues in specific differentiated human cell types (Chandley et al. 1996; Nagele et al. 1999). Furthermore, in murine hematopoietic cells, homologues also tend to associate (Rajapakse et al. 2009). Conversely, rye chromosome pairs added to hexaploid wheat are mostly not associated in root tip cells (Corredor et al. 2005), and homologous CTs in human cancer cells are clearly apart from each other (Heride et al. 2010).

In *Arabidopsis* species, CT arrangement and somatic homologous pairing in interphase nuclei occur mainly at random. Only the NOR-bearing CTs associate more often than random due to the formation of a joint nucleolus (Berr et al. 2006; Pecinka et al. 2004).

Berr and Schubert (2007) demonstrated a similar arrangement of whole *Arabidopsis* CTs in differentiated and meristematic cells, that it is not significantly influenced by nuclear shape, nucleolar volume and/or the level of endopolyploidy.

A random positional homologous pairing of single copy homologous sequences along euchromatic chromatin segments has been reported for somatic cells in both *A. thaliana* and *Arabidopsis lyrata* (Berr et al. 2006; Pecinka et al. 2004). Pericentromeric regions containing 5S rRNA genes are also randomly distributed in *A. thaliana* nuclei (Saez-Vasquez and Gadal 2010).

The collinear alignment of sister chromatids defined as cohesion (Maguire 1990; Miyazaki and Orr-Weaver 1994) is required for correct chromosome segregation during cell division as well as for DNA recombination repair and transcription (Onn et al. 2008; Uhlmann 2008). In yeast, the close distances between cohesion sites (~11 kb) along chromosomes (Glynn et al. 2004; Laloraya et al. 2000) do not allow to distinguish sister chromatids by FISH at the resolution of light microscopy (Guacci et al. 1994). In contrast, in human nuclei allelic loci of sister chromatids may occupy distant positions when probed by FISH (Selig et al. 1992; Volpi et al. 2001).

Similar observations were made in *Arabidopsis*. The high frequency (more than 30 %) of positional sister chromatid separation at ~100-kb mid-arm positions, the absence of preferential cohesion sites along a ~1.2-Mb euchromatic segment and the variable extension of cohesion or separation (<500 kb to 14.2 Mb) along sister chromatid arms,

suggest that sister chromatid cohesion in higher plants is highly dynamic and may therefore influence the interphase chromatin architecture (Berr et al. 2006; Schubert et al. 2006, 2007, 2008).

Based on chromosome conformation capture techniques, maps of spatial chromosome interactions in interphase nuclei have already been constructed for human and yeast. These allow to analyse dynamic and functional conformations of whole genomes (Duan et al. 2010; Lieberman-Aiden et al. 2009). For plants such tools are not yet available. Therefore, we used specific DNA sequences to label whole CTs and distinct eu- and heterochromatic segments along chromosomes by FISH. Homologous and/or heterologous associations, the extend of sister chromatid cohesion and chromatin condensation allow to trace the behaviour of distinct chromatin domains. We tested whether or not CTs and various chromatin domains behave similar or different in specific differentiated 2C and 4C nuclei versus highly endopolyploid (up to 64C) nuclei, to see whether endopolyploidy levels have an impact on chromatin organisation, similar or different from that of polyteny.

The biological significance of endopolyploidy is still under debate. The understanding of changes in chromatin organisation that may occur during endoreduplication can be helpful to clarify the phenomenon. For example, in *Drosophila* salvary gland cells endoreduplicated sister chromatids stay cohesive and form polytene chromosomes whose centromeres associate (Zhimulev et al. 2004). In contrast, we found that in *Arabidopsis* with rising endopolyploidisation sister chromatids become separated. Non-cohesive chromatids, possibly corresponding to puffs of polytene chromosomes, are probably more accessible, e.g. for the transcription machinery, than strictly cohered ones.

We also show that, in addition to a random CT arrangement, similar preferential homologous and heterologous associations and degrees of chromatin condensation may occur at identical chromatin domains in differentiated leaf nuclei of different endopolyploidy levels. In this respect, no obvious chromatin rearrangements occur during endopolyploidisation. The findings suggest that structural similarities are essential to maintain similar functions within a tissue of varying endopolyploidy.

Materials and methods

Preparation of nuclei, probe labelling, immunostaining and FISH

A. thaliana (accession Columbia) and *A. lyrata* plants were grown under short-day conditions (8-h light/16-h dark) at 21 °C.

Differentiated endopolyploid nuclei which no longer perform mitosis were isolated and flow-sorted from rosette leaves after formaldehyde fixation using a FACS Aria (BD Biosciences) according to their ploidy level as described by Pecinka et al. (2004).

The *A. thaliana* BACs were obtained from the Arabidopsis Biological Resource Center (Columbus, OH, USA).

The 45S rDNA-specific probe was prepared from the *A. thaliana* BAC clone T15P10 bearing the 45S rDNA genes.

The 180-bp centromeric repeat probe (pAL) (Martinez-Zapater et al. 1986) was generated by PCR as previously described (Kawabe and Nasuda 2005). The telomere-specific probe was generated by PCR in the absence of template DNA using primers $(TAAACCC)_7$ and $(GGGTTTA)_7$ (Ijdo et al. 1991). Probes specific for the three centromeric repeat families (pAa, pAge1 and pAge2) of *A. lyrata* were prepared from PCR products (Berr et al. 2006).

For painting of the chromosome 1 top arm (CT1top) and the chromosome 3 bottom arm (CT3bottom) (Fig. 3a) 17 pools of in total 87 BACs (from T25K16 to F12K21) and 12 pools of in total 46 BACs (from T5C2 to F16M2), respectively, were labelled with biotin-dUTP and digoxigenin-dUTP as described (Pecinka et al. 2004).

BAC DNA from positions along chromosomes 1, 3 and 5 (Fig. 1) was labelled by nick translation with digoxigenin-dUTP, biotin-dUTP, or Cy3-dUTP according to Ward (2002). Biotin was detected by avidin conjugated with Texas Red (1:1,000; Vector Laboratories), goat-anti-avidin conjugated with biotin (1:200; Vector Laboratories) and again with avidin conjugated with Texas Red; digoxigenin by mouse-anti-digoxigenin (1:250; Roche) and goat-anti-mouse conjugated with Alexa-488 (1:200; Molecular Probes). Cy3 was observed directly.

FISH was performed according to Schubert et al. (2001). Nuclei and chromosomes were counterstained with DAPI (1 μg/ml) in Vectashield (Vector Laboratories).

For colocalisation of CENH3 immunosignals with centromeric FISH signals, immunostaining and FISH were performed subsequently. After immunostaining, nuclei were fixed in 4 % paraformaldehyde/3.6 % sucrose. Immunostaining of nuclei was performed as described (Jasencakova et al. 2000). CENH3 was detected with rabbit polyclonal antisera against *A. thaliana* CENH3 (1:500) (Talbert et al. 2002) and goat anti-rabbit rhodamine (1:100; Jackson Immuno Research Laboratories).

Microscopic evaluation, image processing and statistics

Analysis of FISH signals was performed with an epifluorescence microscope (Zeiss Axiophot) using a 100×/1.45 Zeiss α plan-fluar objective and a 3-chip Sony (DXC-950P) colour camera. Images were captured separately for each fluorochrome using appropriate excitation and emission filters. Images were merged using Adobe Photoshop 6.0 software (Adobe Systems, San Jose, USA).

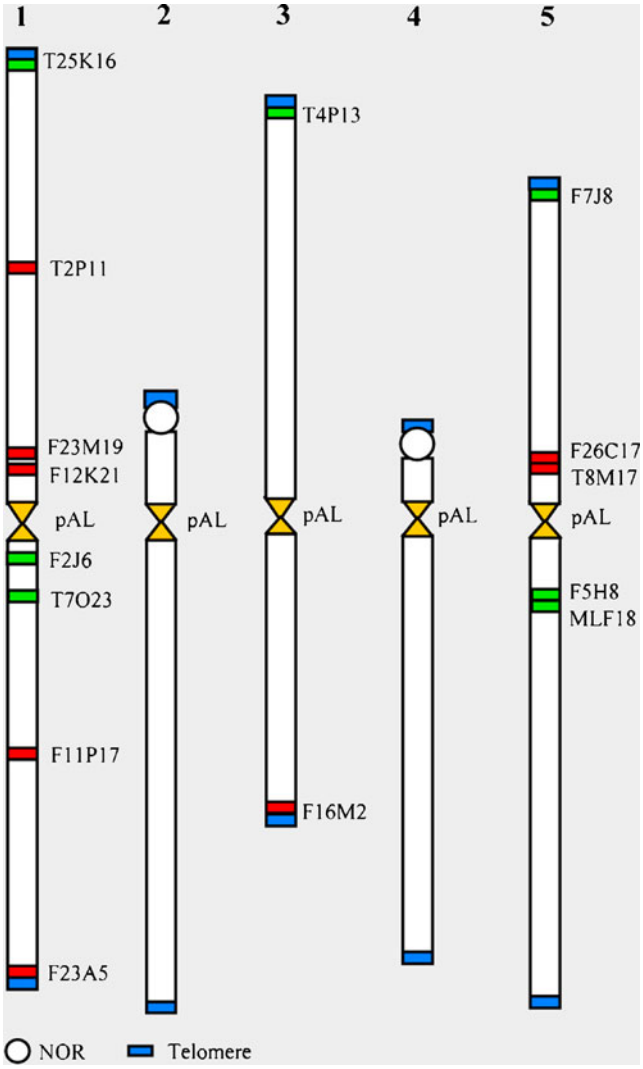


Fig. 1 Scheme of *A. thaliana* chromosomes indicating the chromosomal localisation of centromeric (*pAL*), subtelomeric and interstitial BAC probes used to label *A. thaliana* interphase nuclei by FISH

The DOM Laycheck software (Confovis, Jena) was used to measure 2C interphase nuclei (x=6.7, y=3.0 and z=2.4 μm) and the corresponding BAC (diameter, 0.2 μm) and CT (x=2.4, y=1.4 and z=1.7 μm) dimensions. On this basis, the "random spatial distribution" (RSD) model (Schubert et al. 2007) was modified to simulate round-shaped homologous and heterologous chromosome segments (corresponding to BAC and CT FISH signal areas) with coordinates determined randomly in a virtual interphase nucleus. The frequency of attachment and overlapping of two BAC areas, taken as homologous or heterologous association, is considered to be random (Fig. 2). The differences between simulated values and those obtained experimentally from the FISH experiments were compared by the two-sided Fisher exact test.

The cohesion frequency of sister CTs was calculated per homologue. One FISH signal cluster and overlapping signals per homologue were regarded as cohesion, two signal clusters as separated. The cohesion of ~100-kb BAC segments was evaluated as described (Schubert et al. 2008).

CTs covering more than 50 % of the nucleus area were regarded as completely dispersed (Fig. 3b, c).

The association frequencies of chromosome termini with centromeres and NORs, respectively, were compared with expected values using the Chi² test.

Calculation of telomere associations with centromeres and NORs

The theoretically expected relation of telomere association with centromeres and NORs was estimated in that way that we consider only those telomeres which are associated with at least one NOR or one centromere (9.9 % in *A. thaliana* and 9.8 % in *A. lyrata*, respectively, are without any association).

In *A. thaliana* 2C nuclei 4 (on top arms of chromosomes 2 and 4) of the 20 telomeres (=20 %) are physically close to a NOR region. The remaining 16 telomeres (80 %) should be randomly associated to 14 chromatin segments totally, namely to 4 NORs (represented by a single nucleolus) and to 10 centromeres (4+10=14) according to their frequencies (4 of 14 of telomeres should be associated with NORs and 10 of 14 of them with centromeres). This results in 20 % (a priori localised to nucleolus)+4/14×80 %=42.9 % telomere–nucleolus association. The telomere–centromere association amounts to 10/14×80 %=57.1 %.

For *A. lyrata* (32 telomeres, 10 of them close to the nucleolus and 16 centromeres) the analogous calculation results in an expected association frequency of 57.7 % for telomere–nucleolus association and of 42.3 % for telomere–centromere association.

Results

CTs are randomly arranged in highly endopolyploid nuclei

To analyse the organisation of CTs in 2C to 64C differentiated nuclei, we investigated by FISH the distribution of labelled BAC contigs for CT1top and CT3bottom in different colours (Fig. 3). The frequency of homologous association of ~43–60 % in 2C to 8C nuclei was similar for both CTs to that observed by Pecinka et al. (2004). We obtained now similar values for 16–64C nuclei (Table 1). Only in 64C nuclei the homologous association of CT1top was decreased to 36.7 %. This suggests a random

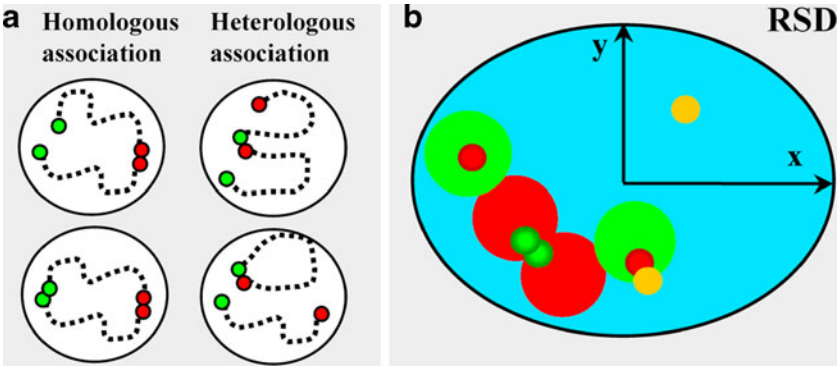


Fig. 2 Homologous and heterologous association configurations and its computer simulation in the modified "random spatial distribution" (*RSD*) model. **a** Scheme showing the main four configurations of homologous and heterologous associations in 2C interphase nuclei. **b** The RSD model simulates random homologous and heterologous association of ~100-kb chromosome arm segments via two small spheres in the same colour corresponding to two homologous loci with coordinates determined randomly in a virtual interphase nucleus (*blue ellipsoid*) of average dimensions. It is assumed that the ~100-kb segments can occupy any position within its chromosome arm territory (*large red* and *green sphere*). Segments of other arms (*yellow*) can occupy any other position within the nucleus. Under these limitations the coordinates of the small spheres are calculated as random values. The frequency of overlapping of two small spheres of the same colour (*green spheres*) and of different colours (*small red* and *yellow spheres*) are considered as random single-point homologous and heterologous association, respectively. The *small red* and *green spheres* belong to two different arms (*large red* and *green spheres* illustrating CTs) of the same chromosome. Therefore, these small spheres are linked and pair more often with each other than with the *yellow spheres*

homologous association of CTs also in highly endopolyploid nuclei.

Sister CTs, although sometimes separated are seldom dispersed and intermingled among each other in nuclei up to 64C

In *A. thaliana* 4C nuclei, a ~1.2-Mb chromatin segment at an interstitial position of chromosome 1 bottom arm is often (42.5 %) completely cohered. A complete sister chromatid separation of CT1top (14.2 Mb) may occur at ~4 % of homologues (Schubert et al. 2006, 2008). Here, we show that with increasing ploidy level whole CT1top and CT3bottom chromatids tend to be separated, but this separation does not exceed 15.5 % of homologues as found in 64C nuclei for CT1top (Table 1; Fig. 3c). Concurrent FISH of CT1top and the mid-arm segment T2P11 revealed that ~100-kb chromatin segments within the compact CTs are often separated in endopolyploid nuclei (Fig. 3d).

The low frequency (2.4–14.4 %) of dispersed CTs (covering >50 % of the nuclear area) in 2C to 16C nuclei even decreased to 1.0–2.3 % in 32C and 64C nuclei (Table 1; Fig. 3b, c). Interestingly, double FISH with CT1top and CT3bottom showed that this dispersion did not occur simultaneously for both CTs in all nuclei. The frequency of nuclei with only one of the homologous CTs dispersed ranged from 12.5 to 57.1 %.

In contrast to (peri)centromeres (see below), most chromosomes form one compact CT. Interstitial identical segments within these CTs are mainly separated at high endopolyploidy levels.

Centromeric and CENH3 associated repeats become separated in nuclei >16C and form distinct subdomains at adjacent positions but do not intermingle

The close cohesion of sister centromeres is essential for their bipolar orientation and subsequent segregation to opposite poles during nuclear division. The centromeric histone variant CENH3 is required to initiate the formation of kinetochores. To investigate centromere cohesion in differentiated leaf nuclei of different ploidy levels, we combined immunostaining and FISH to analyse the spatial distribution of CENH3 and of chromatin comprising 180-bp centromeric repeats (pAL).

The evaluation of 3D image stacks showed that the centromeric sequences are localised close to the nuclear periphery. In 2C, 4C and 8C nuclei, sister centromeres are often associated (Schubert et al. 2006) and in 2C and 4C nuclei the centromeres appear mainly as a compact FISH signal, suggesting tightly arranged chromatin. In some nuclei, more decondensed centromeres appear as ring- or half-moon-like structures (Fig. 4a). In 8C nuclei, centromeres start to split and are frequently separated in 32C and 64C nuclei (Figs. 3b and 4b).

Centromeric signal numbers higher than 32 and 64 in 32C and 64C nuclei, respectively (Fig. 3b), indicate increased sister centromere separation at higher ploidy levels. This conclusion is supported by the observation that also DAPI stained chromocenters appeared to be disintegrated.

When nuclei were hybridised with the pAL probe after immunostaining with antibodies against the centromeric histone variant CENH3, most nuclei showed co-localisation

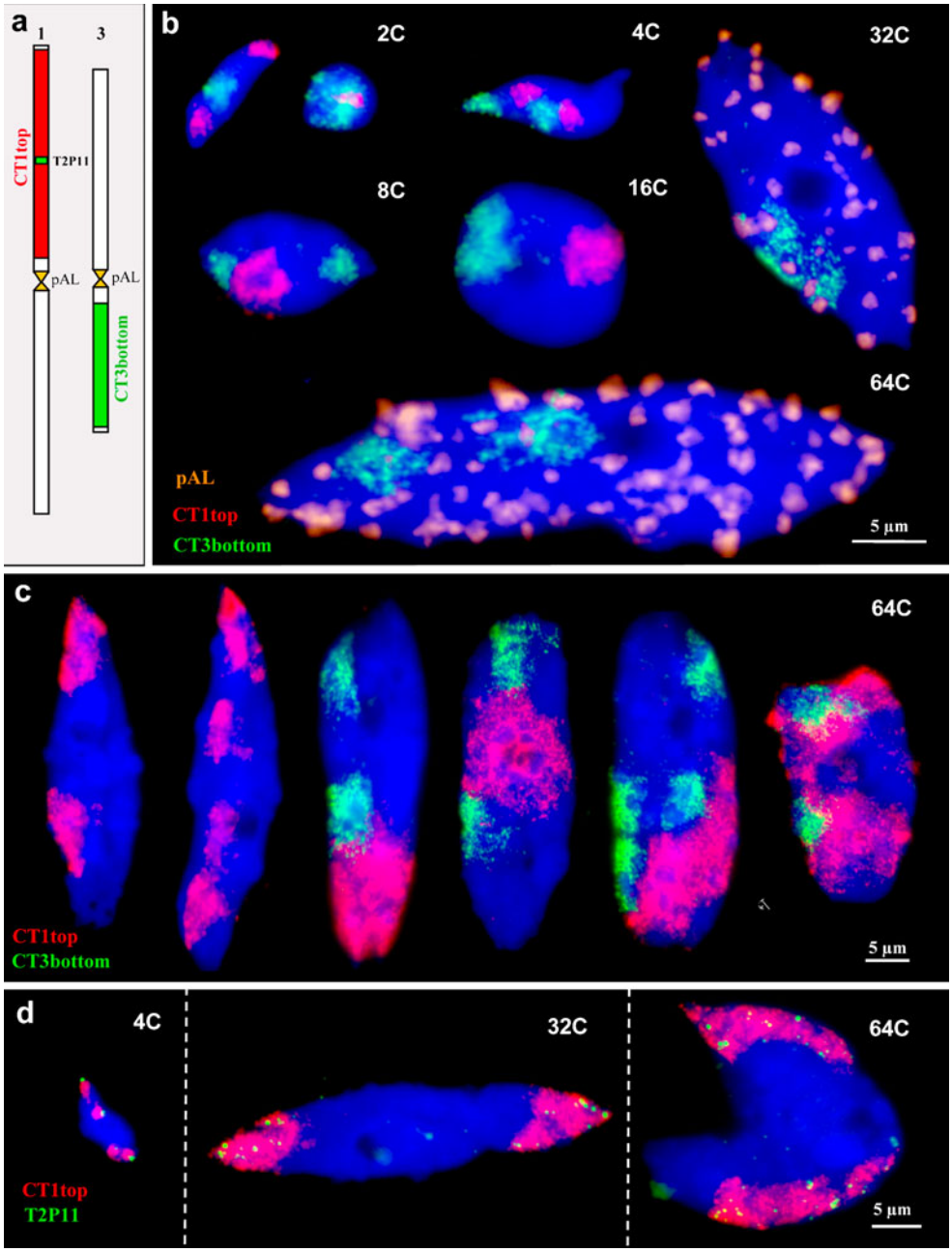


Fig. 3 Chromosome territory (*CT*), ~100-kb interstitial chromatin segment and centromeric pAL DNA sequence arrangement in differentiated *A. thaliana 2–64C* interphase nuclei. **a** Scheme of differential labelling of *A. thaliana* chromosome regions. **b** Arrangement of CT1top and CT3bottom in *2–64C* nuclei. *2C* nuclei, both compact CT3bottom arms associate whereas the compact CT1top arms are separated (*left*). The right nucleus shows dispersed CT3bottom arms but the CT1top arms are compact and associated. *4C* nucleus, both arms are compact and separated. *8C* nucleus, association of compact CT1top arms and separation of compact CT3bottom arms. *16C* nucleus, both CT1top and CT3bottom arms are compact and associated. *32C* and *64C* nuclei, whereas the centromeric pAL sequences stay cohesive in 4C nuclei (see Fig. 4a) signal numbers >32 and >64 indicate sister centromere separation. In both nuclei, the CT3bottom arms appear compact. **c** CT arrangements in *64C* nuclei. *From left to right*: two separated compact CT1top arms; four separated compact CT1top arms; two separated compact CT3bottom arms in combination with two compact associated CT1top arms; two separated compact CT3bottom arms in combination with two partially dispersed associated CT1top arms; four separated compact CT3bottom arms in combination with two partially dispersed associated CT1top arms; two separated compact CT3bottom arms in combination with completely dispersed CT1top arms. **d** Compact arrangement of CT1top arms in combination with positionally separated ~100-kb mid-arm chromatin segments in *4C*, *32C* and *64C* nuclei. Both homologous CT1top arms are separated in the *4C* nucleus but cohesive in the *32C* and *64C* nuclei, respectively

of CENH3 signals with FISH signals for pAL repeats. In nuclei, with a ploidy level higher than 4C (Fig. 4b) some 180-bp centromeric repeats were obviously not connected to CENH3 and vice versa. Limited association of CENH3 with

Table 1 Homologous association, sister chromatid separation and degree of chromatin dispersion of CTs1top and CTs3bottom in 2–64C differentiated leaf nuclei

Ploidy	No. of nuclei	Homologous association per nucleus (%)	Separation per homologue (%)	CTs dispersed[a] (%)
CT1top				
2C	686	60.1	0	5.4 (40.0)
4C	1840	60.5	1.0	2.4 (12.5)
8C	203	47.8	2.5	10.8 (31.8)
16C	463	49.0	8.9	6.9 (34.6)
32C	611	48.1	5.2	1.0
64C	360	36.7	15.5	1.1
CT3bottom				
2C	146	49.3	0	14.4 (57.1)
4C	159	43.4	3.3	10.7 (17.6)
8C	203	43.8	6.4	12.3 (40.0)
16C	254	43.3	5.5	11.4 (41.4)
32C	214	49.3	3.5	1.4
64C	171	46.2	8.2	2.3

[a]In parentheses, per cent of nuclei with dispersed CTs where only one of both CTs appeared dispersed. For CT1top, the calculation based on nuclei numbers denoted for CT3bottom

some 180-bp repeat forming knobs along extended chromatin fibres has also been proven in *A. thaliana* cell cultures (Shibata and Murata 2004).

According to Nagaki et al. (2003), ~15 % of the 180-bp repeats are connected to CENH3 nucleosomes. Transposable elements, such as *Athila, Tat, Tim, Copia*, additionally accumulated at the centromeric regions (Arabidopsis Genome Initiative 2000) are associated to CENH3 with a frequency of up to 4.5 %. CENH3 nucleosomes not colocalising with pAL may associate with these transposable elements.

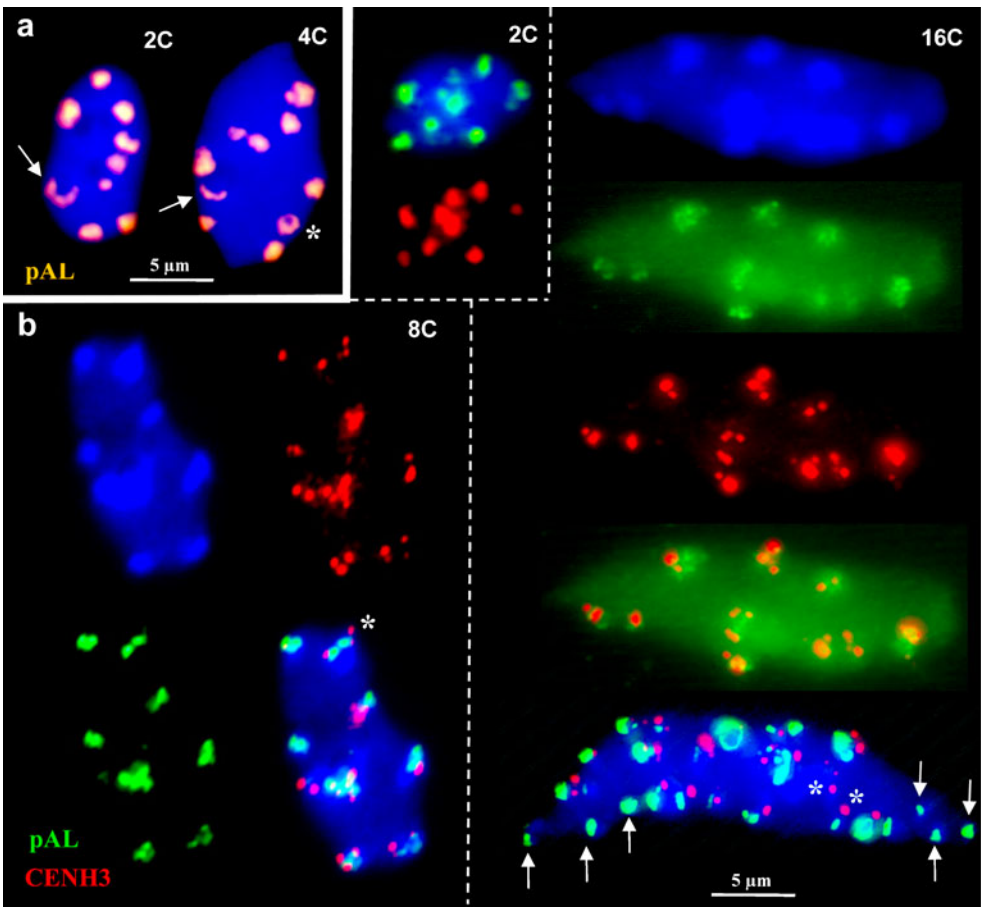


Fig. 4 Arrangement of (peri) centromeric regions in *A. thaliana 2–16C* interphase nuclei. **a** Nine (two of them associated) and ten centromeric FISH signals (*pAL*) in *2C* and *4C* nuclei, respectively. The mainly compact round shape centromeric signals are similar in *2C* and in *4C* nuclei. Some less compact centromeres are *ring-* (*asterisk*) or *half-moon shaped* (*arrows*). **b** Due to chromatin decondensation and sister chromatid separation in *8C* and *16C* nuclei, the centromeric histone variant CENH3 and the centromeric DNA signals form mostly contiguous subdomains within or adjacent to pericentromeric heterochromatin (bright DAPI signals). Some CENH3 (*asterisks*) and pAL (*arrows*) signals do no longer attach or overlap each other

Several centromere organisation models suggest the coalescence of CENH3-containing nucleosomes to form subdomains at the inner kinetochores of both sister chromatids (Verdaasdonk and Bloom 2011). These subdomains seem to be also maintained in differentiated nuclei even at higher ploidy levels where the amount of CENH3 is not proportionally increasing with the amount of 180-bp repeats (Lermontova et al. 2006, 2007).

Similar as in *A. thaliana*, CENH3 forms subdomains and colocalises closely with the centromeric repeat Bilby in 2C and 4C differentiated nuclei of diploid rye (Schubert and Houben, unpublished).

Telomeres tend to associate but sister telomeres may be separated

The NORs on *A. thaliana* chromosomes 2 and 4 (Fig. 1) form a joint nucleolus in most of the nuclei (>90 %) surrounded by the majority of telomeres (Berr et al. 2006). To precisely define the spatial positioning of telomeres, telomeric DNA probes were localised by FISH on isolated leaf nuclei of *A. thaliana* ($2n=10$) and *A. lyrata* ($2n=16$) (Figs. 5 and 6).

On average, 11.4 (4 to 14) and 15.7 (8 to 25) telomeric FISH signals were observed in *A. thaliana* and *A. lyrata* 2C interphase nuclei, respectively. In both species, telomere signal numbers varied between individual interphase nuclei, with an average number lower than the expected maximum number per species, implying that telomere association occurs (Figs. 5a and 6a). In *A. thaliana*, 4C nuclei showed more FISH signals than 2C nuclei (Fig. 5a) suggesting that sister telomeres can separate.

Telomeres associate more often to NORs than to centromeres

In nuclei of *Arabidopsis* species, we frequently observed telomeric FISH signals associated with the nucleolus (Fig. 5c). To analyse the spatial distribution of telomeres within interphase nuclei, telomeric DNA probes, 45S rDNA and centromeric probes were hybridised simultaneously to 2C interphase nuclei. In *A. thaliana* 62.9 % of telomeric FISH signals were found to be associated with 45SrDNA and 37.1 % with centromeres, whereas in *A. lyrata* 73.5 % of telomeric signals were associated with nucleoli and 26.5 % with centromeres (Figs. 5b and 6b). In *A. thaliana*, two of the five chromosomes (AT2 and AT4) bear NORs (Fransz et al. 1998) while in *A. lyrata*, five of the eight chromosomes (AL1, AL3, AL4, AL5 and AL7) contain NORs (Berr et al. 2006). Thus, considering 2C nuclei, four out of 20 in *A. thaliana* and ten out of 32 in *A. lyrata* are physically close to 45S rDNA repeats resulting in the observed preferential association of telomeric FISH signals with NORs, but the actual percentage of telomere-NOR associations is much higher in both species.

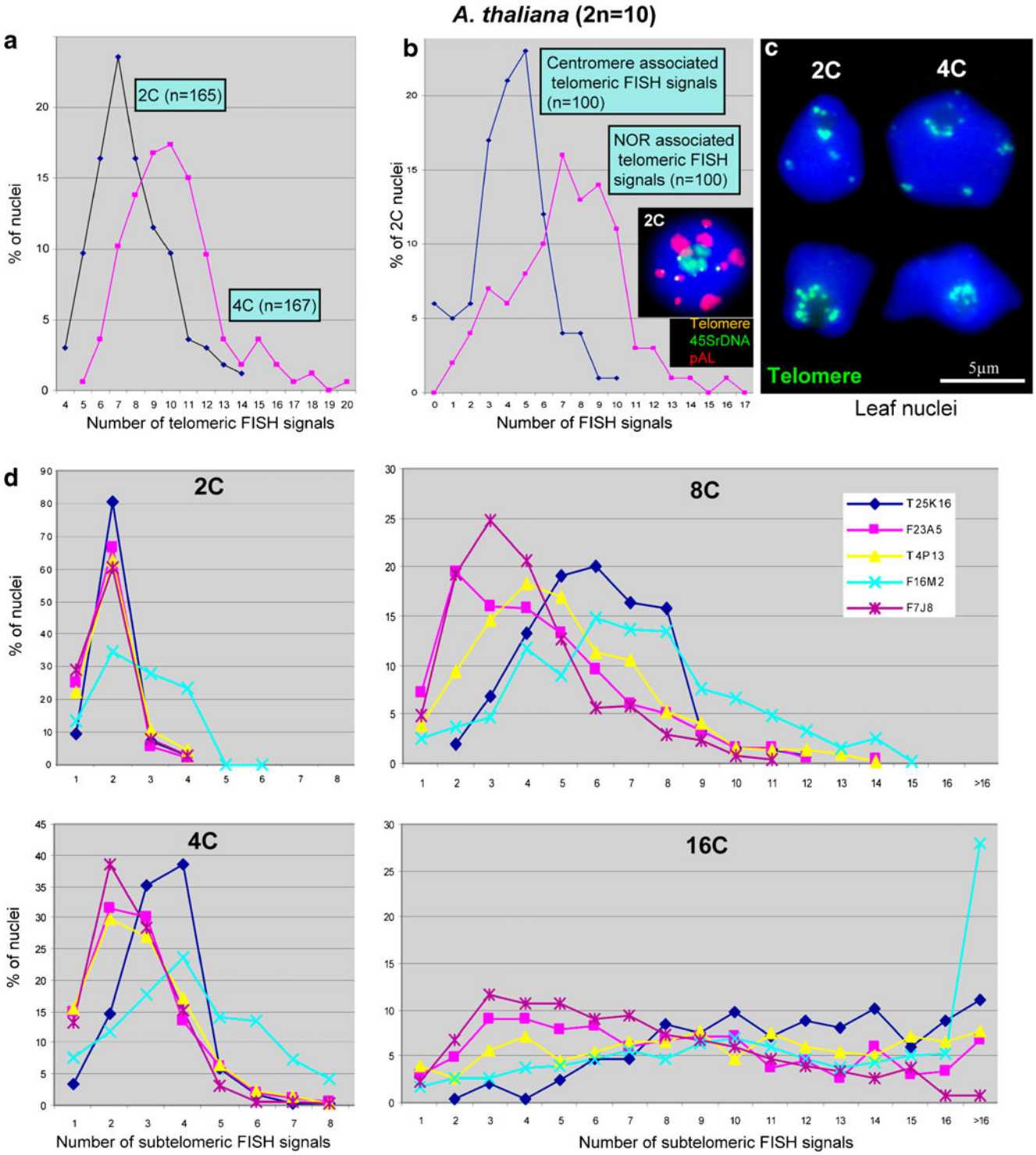
Fig. 5 Arrangement of (sub)telomeric regions in *A. thaliana* interphase nuclei. **a** Number of telomeric FISH signals in *2C* and *4C* interphase nuclei. **b** Number of centromere and NOR associated telomeric FISH signals in *2C* nuclei. *Insert* shows as an example nucleus with telomeric signals associated to 45SrDNA (*NOR*) and pAL (*centromeric*) signals. **c** Partial (*top*) and complete (*bottom*) association of telomeric signals with the single nucleolus in *2C* and *4C* nuclei. **d** FISH signal frequencies of subtelomeric chromatin fragments labelled by BACs T25K16, F23A5, T4P13, F16M2 and F7J8 in *2–16C* nuclei. The higher than expected FISH signal numbers for F16M2, and to a lower extent for T25K16, indicate subtelomeric chromatin decondensation. **e** Arrangement of homologous and heterologous subtelomeric segments in *2–16C* nuclei. *2C* nuclei, homologous and heterologous association (see Fig. 2a) at subtelomeric segments (*left*) labelled by BACs F23A5, F16M2 and T7J8. The right nucleus shows homologous association but heterologous separation. The segment labelled by BAC F16M2 shows more than two signals and is therefore decondensed. Heterologous association of two (*left*), twice two (*middle*) and of all (*right*) subtelomeric loci labelled with BACs T4P13 and F16M2. *4C* nuclei, cohesion and homologous association of subtelomeric segments of both chromosome 3 arms with that of CT5top (*left*) and three nuclei showing subtelomeric segment configurations of both chromosome 1 arms. *8C* nucleus, arrangement of subtelomeric segments from both chromosome 1 arm ends, with the complete cohesion and homologous association for F23A5 (*bottom arm*) and complete separation for T25K16 (*top arm*). *16C* nucleus, similar configuration as in the *8C* nucleus but partial separation at F23A5

In *A. thaliana*, the observed associations of telomeres to the nucleolus amount to 63.3 % (719 of 1,137; expected, 42.9 %) and to centromeres 36.8 % (418 of 1,137; expected, 57.1 %). In *A. lyrata*, we found 73.5 % (1,133 of 1,541; expected, 57.7 %) telomere–nucleolus associations and 26.5 % (408 of 1,541; expected, 42.3 %) telomere–centromere associations.

The statistical comparison of the expected (for calculations see "Materials and methods") with the observed frequencies of telomere associations with centromeres and NORs by the Chi² test results in highly significant ($P<0.001$) differences, indicating a preferential association of telomeres to the nucleolus. The observation that ~10 % of telomeric signals were associated neither to NORs nor to centromeres in *A. thaliana* and *A. lyrata* suggest that these associations are not compulsory.

Both preferential and random chromatin associations appear at subtelomeres and at pericentromeres

To test the frequencies of homologous and heterologous associations between subtelomeric chromatin segments in *A. thaliana*, we performed simultaneous FISH with differently labelled probes hybridising closely to the top and bottom arm ends of chromosomes 1, 3 and 5 (Table 2; Figs. 1, 2a and 5e). A preferential (sub)telomere-specific homologous and/or heterologous association could indicate a specific arrangement of distinct chromatin domains potentially linked with their gene expression status.



Compared with the RSD model simulations assuming exemplarily a random distribution of six spheres for three arbitrarily selected subterminal regions in a virtual interphase nucleus (Fig. 2b), a significant increase of homologous association at all subtelomeric segments analysed in 2C nuclei was proven (Fig. 5e). Only the subtelomeric association at top arm of chromosome 1 (BAC T25K16) was relatively low with 9.5 %.

Also, heterologous interchromosomal associations were found to be significantly increased at most tested subtelomere combinations. Only the combination of BACs T25K16-F7J8 associated more seldom than expected at random.

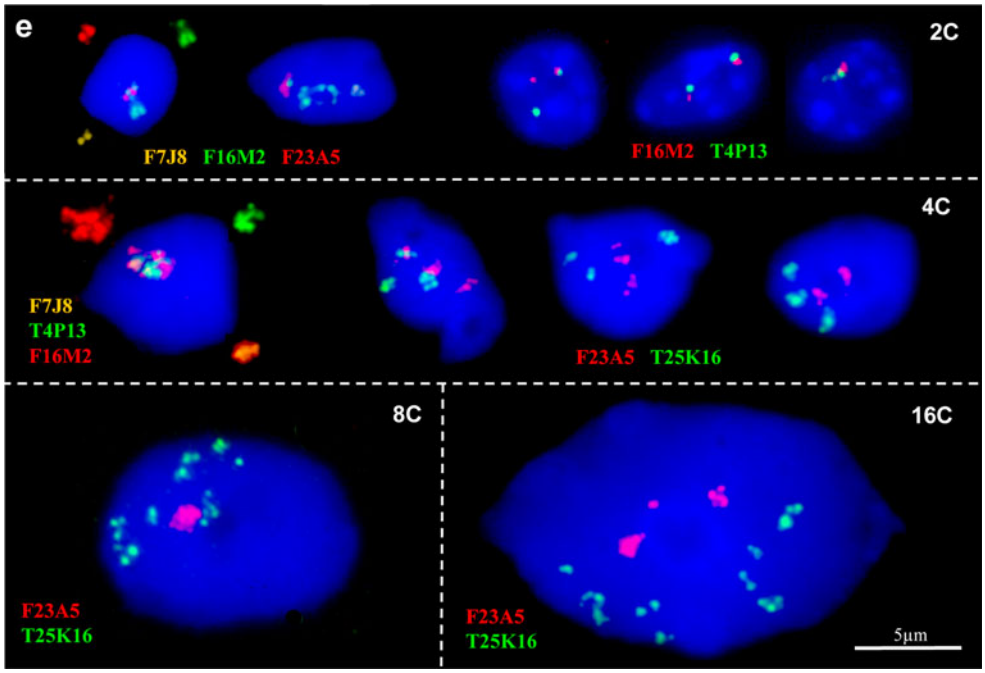


Fig. 5 (continued)

A highly significant increase of intrachromosomal subtelomere association was found for chromosome 3. In contrast, for chromosome 1 the intrachromosomal subtelomeric association was less frequent than random.

Chromatin segments located at opposite arms of chromosome 1 close to the pericentromere associated in 2C nuclei more often than expected at random, while this was not true for chromosome 5. The tendency toward lower or higher homologous and heterologous association frequencies was similar in endopolyploid nuclei of 4–16C.

In summary, we conclude that in contrast to other interstitial euchromatic segments of *A. thaliana* and *A. lyrata* (Berr et al. 2006; Pecinka et al. 2004) preferential homologous and heterologous associations may occur around subtelomeres and at pericentromeres which might indicate joint gene activity patterns in these regions within nuclei of different endopolyploidy level.

Chromatin segments close to centromeres, telomeres and at mid-arm positions show a different extent of sister chromatid cohesion and chromatin condensation

Analysing the degree of condensation and sister chromatid cohesion at distinct chromatin segments could provide a hint as to the presence of transcriptionally active chromatin. If BACs of ~100-kb yield only one FISH signal per chromatid, the maximum signal number per nucleus should correspond to the ploidy level. However, depending on the chromatin segments analysed, also higher signal numbers appeared indicating chromatin decondensation. This has been found

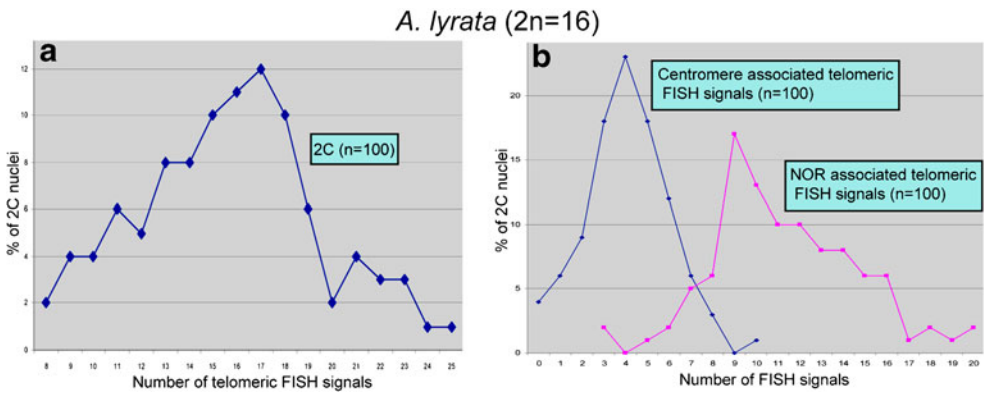


Fig. 6 Telomere arrangement in *A. lyrata* interphase nuclei. **a** Number of telomeric FISH signals and **b** their association with centromeric and NOR signals in *2C* nuclei

Table 2 Homologous/heterologous association and sister chromatid separation at different BAC positions along chromosomes 1 (grey), 3 (green) and 5 (yellow) in 2–16C differentiated leaf nuclei (*P<0.05; **P<0.01;***P<0.001; number of investigated nuclei/loci in parentheses)

BAC	Chromosome arm	Position	Size (bp)	Chromatin elongation (%)[a]				Homologous association per nucleus (%)[b]				Positional separation per homologue (%)[c]			Heterologous association per locus (%)[d]				
				2C	4C	8C	16C	2C	4C	8C	16C	4C	8C	16C	with	2C	4C	8C	16C
T25K16	1top	adjacent to telomer	86436	10.0 (401)	8.0 (513)	5.8 (430)	6.1 (297)	9.5** (401)	3.5 (513)	0 (430)	0.3 (297)	64.2*** (1026)	94.8 (860)	98.7 (594)	F23A5	9.2*** (402)	8.6 (626)	8.9 (550)	12.3 (252)
															T4P13	9.2 (326)	7.7 (350)	9.2 (292)	6.9 (394)
															F16M2	15.1 (126)	16.7 (150)	17.4 (138)	11.9 (252)
T2P11	1top	mid-arm	85372	19.0 (100)	1.6 (255)	23.0 (100)	20.0 (70)	9.0 (100)	3.5 (255)	1.0 (100)	0 (70)	51.0 (510)	98.5 (200)	100 (140)	F11P17	14.5* (200)	10.8 (510)	18.5 (200)	21.4 (140)
F23M19	1top	close to centromer	88401	1.0 (100)	3.0 (202)	3.0 (263)	0.9 (112)	6.0 (100)	1.5 (202)	1.0 (263)	0 (112)	62.9*** (404)	95.4 (526)	99.1 (224)	T7O23	53.0*** (100)	18.3 (404)	24.7 (526)	22.4 (224)
F12K21	1top	close to centromer	117585	78.1 (73)	49.0 (100)	45.0 (100)	17.8 (45)	4.1 (73)	1.0 (100)	1.0 (100)	0 (45)	81.0*** (200)	96.0 (200)	100 (95)	F2J6	28.8*** (146)	42.5 (200)	36.5 (200)	32.2 (90)
F2J6	1bottom	close to centromer	108061	63.0 (73)	51.0 (100)	38.0 (100)	15.6 (45)	4.1 (73)	0 (100)	0 (100)	0 (45)	82.0*** (200)	95.0 (200)	100 (95)					
T7O23	1bottom	close to centromer	72832	6.0 (100)	2.0 (202)	1.5 (263)	0.9 (112)	3.0 (100)	2.0 (202)	0 (263)	0 (112)	60.9** (404)	87.3 (526)	98.7 (224)					
F11P17	1bottom	mid-arm	103338	17.0 (100)	1.6 (255)	24.0 (100)	35.7 (70)	9.0 (100)	3.5 (255)	0 (100)	0 (70)	51.8 (510)	96.0 (200)	100 (140)					
F23A5	1bottom	adjacent to telomer	109694	7.8 (638)	10.1 (496)	7.6 (446)	6.8 (264)	25.4*** (638)	14.9 (496)	7.2 (446)	3.0 (264)	38.6*** (992)	65.4 (892)	87.5 (528)	T4P13	18.0*** (200)	24.5 (200)	30.5 (200)	41.2 (148)
															F7J8	19.0*** (200)	24.7 (166)	31.9 (144)	38.3 (128)
T4P13	3top	adjacent to telomer	93735	15.5 (376)	10.4 (395)	9.6 (436)	7.7 (336)	22.3*** (376)	15.4 (395)	3.9 (436)	3.9 (336)	41.1*** (790)	79.4 (872)	90.6 (672)	F16M2	38.5*** (278)	42.4 (340)	44.4 (500)	49.8 (434)
F16M2	3bottom	close to telomer	75106	51.7 (366)	39.0 (428)	26.7 (404)	28.0 (300)	13.7** *(366)	7.7 (428)	2.5 (404)	1.7 (300)	71.5*** (856)	91.5 (808)	94.3 (600)	F7J8	22.7*** (352)	31.2 (356)	32.2 (506)	36.1 (310)
															F23A5	16.5** (200)	21.1 (166)	24.3 (144)	35.2 (128)
F7J8	5top	adjacent to telomer	118507	10.4 (376)	3.4 (507)	4.8 (558)	0.7 (300)	29.0*** (376)	13.2 (507)	4.8 (558)	2.3 (300)	33.9*** (1014)	63.4 (1116)	85.2 (600)	T4P13	27.2*** (552)	31.2 (590)	35.9 (716)	45.6 (472)
															T25K16	2.0*** (200)	4.0 (200)	6.5 (154)	3.5 (142)
F26C17	5top	close to centromer	53537	4.0 (124)	3.5 (142)	10.8 (158)	5.7 (35)	13.7*** (124)	4.2 (142)	1.3 (158)	0 (35)	44.0 (284)	90.8 (316)	100 (70)	MLF18	8.5*** (248)	10.9 (284)	11.1 (316)	11.4 (70)
T8M17	5top	close to centromer	114695	7.0 (71)	3.2 (93)	8.0 (100)	13.0 (100)	8.5 (71)	3.2 (93)	1.0 (100)	0 (100)	39.2** (186)	89.0 (200)	100 (200)	F5H8	12.7 (142)	6.5 (186)	9.5 (200)	16.0 (200)
F5H8	5bottom	close to centromer	80098	25.4 (71)	12.9 (93)	20.0 (100)	11.0 (100)	4.2 (71)	3.2 (93)	1.0 (100)	0 (100)	50.0 (186)	92.5 (200)	100 (200)					
MLF18	5bottom	close to centromer	84842	4.8 (124)	4.2 (142)	8.9 (158)	14.3 (35)	4.8 (124)	4.3 (142)	0.6 (158)	0 (35)	42.3* (284)	96.2 (316)	100 (70)					
RSD								5.8 (10^5)								9.9/17.2 (10^5)			

[a] Per cent of nuclei showing more than the expected maximum signal number

[b] Compare 2C values with the simulated random values according to the RSD model

[c] Separation means that at least three FISH signals were present per nucleus. Compare 4C values with the value of BAC T2P11

[d] Compare 2C values with the simulated random values according to the RSD model for loci at different arms of the same chromosome (17.2 %) and for loci located at different chromosomes (9.9 %; red)

especially adjacent to the pericentromeric heterochromatin of chromosome 1 (labelled by BACs F12K21 and F2J6) and at the subtelomere of the bottom arm of chromosome 3 (labelled by BAC F16M2) in nuclei of endopolyploidy levels from 2–16C (Table 2; Fig. 5d, e). Other segments although of similar size did not show this phenomenon.

Sister chromatids are often not cohesive at mid-arm positions in higher plants (Berr et al. 2006; Schubert et al. 2006, 2007). Here, we show that also sister termini can be separated in differentiated 4C leaf nuclei. To test whether the frequency of sister chromatid separation varies between different subtelomeres and between pericentromeric chromatin segments, we calculated the separation frequencies in 4–16C nuclei. In comparison to a mid-arm segment of chromosome 1, labelled by BAC T2P11, significantly increased (BACs T25K16 and F16M2) and decreased (BACs F23A5 and T4P13) separation at subtelomeres was observed in 4C nuclei. Significantly increased separation was also evident for chromatin segments close to the pericentromeric heterochromatin of chromosome 1 but not of chromosome 5 (Table 2).

Up to 100 % sister chromatid separation was observed in 8C and 16C nuclei (Table 2; Fig. 5e). Thus, depending on the chromosomal position, the degree of chromatin condensation and sister chromatid cohesion can vary possibly in correlation with gene activity.

Discussion

There is increasing evidence of functional and topological constraints restricting a random spatial arrangement of chromatin in interphase nuclei (Lanctot et al. 2007; Misteli 2007). Chromosome size, gene density and expression during different developmental stages are factors which may constrain the random positioning of chromatin within interphase nuclei.

A network of co-regulated gene expression causing chromatin interactions during differentiation seems to result in the self-organisation of cell lineage-specific chromatin topologies. Self-organisation and fractal globule formation are promising models to explain the spatial distribution of chromatin segments and its dynamics in interphase nuclei (McNally and Mazza 2010; Misteli 2007, 2009; Rajapakse et al. 2009).

In addition to *A. thaliana*, a suitable model organism to analyse higher order chromatin organisation (Saez-Vasquez and Gadal 2010), we analysed comparatively the closely related species *A. lyrata*.

We show that in higher plants, in addition to random arrangement, preferential and dynamic chromatin association may occur within nuclei of different ploidy levels. Our data are mainly based on a defined developmental stage and tissue (nuclei isolated from rosette leaves) of *Arabidopsis*. To clarify whether preferential associations are connected to gene expression and whether they differ between various tissues and developmental stages, further investigations are required.

CTs and centromeric chromatin mostly maintain a compact structure even in highly endopolyploid nuclei

Fritsch and Langowski (2011) suggest that the viscoelasticity of chromatin during decondensation in interphase is changed by chromatin cross-linking and loop formation in such a way that chromatin can rapidly decondense and then consolidate to prevent its homogeneous distribution. Here, we showed that in most of the *A. thaliana* 2–64C nuclei the CTs maintain their distinct compact structure. Also the 180-bp centromeric and CENH3 containing chromatin segments appear as distinct adjacent sub-domains even in nuclei >16C where sister chromatids close to the centromeres start to separate. In 2C and 4C nuclei, the 180-bp centromeric repeats can form characteristic ring- or half-moon-like structures. The occurrence of CENH3 subdomains suggests that centromeric repeats form loops or solenoids with CENH3 nucleosomes always in the opposite orientation than H3-containing nucleosomes. Shibata and Murata (2004) found that on extended chromatin fibres CENH3 nucleosomes are formed only at some of the 180-bp repeats.

Experimental stretching of human and *Drosophila* centromeres revealed that the arrays of CENH3 nucleosomes which coalesce in nuclei are interrupted by blocks of H3-containing nucleosomes (Blower et al. 2002). This coalescence is the basic assumption of several centromere organisation models: the loop model, the solenoid model and the sinusoidal patch model (Santaguida and Musacchio 2009; Verdaasdonk and Bloom 2011). CENH3 nucleosome coalescence seems to be present also in differentiated endopolyploid *A. thaliana* nuclei where no further CENH3 loading occurs (Lermontova et al. 2006). However, it cannot yet been decided which of the models (if any) is true for higher plants.

The mainly random CT arrangement in *Arabidopsis* interphase nuclei is modified by structural and functional constraints

By computer simulation de Nooijer et al. (2009) showed that non-specific chromatin interactions in *A. thaliana* interphase nuclei are sufficient to explain the position of nucleoli and of chromocenters and that chromatin fibre looping might be responsible for CT formation. On the other hand, Andrey et al. (2010) conclude that conserved constraints influence the distribution of centromeres and chromocenters in nuclei of differentiated cells because they found that in distantly related species with different genome size and chromosome number such as *A. thaliana* (1C=125 Mb, n=5) and rabbit (1C=2770 Mb, n=22) a more regular distribution than expected at random was evident.

Previously we showed that CTs are mainly randomly distributed within interphase nuclei of two *Arabidopsis* species, independent of chromosome size and nuclear shape and that the formation of a single nucleolus may induce preferential CT association of NOR-bearing chromosomes (Berr et al. 2006; Pecinka et al. 2004). Homologous transgenic tandem repetitive sequences pair more often with each other and associate with chromocenters in *A. thaliana* nuclei than flanking euchromatin (Jovtchev et al. 2008, 2011; Pecinka et al. 2005). Similarly, sister chromatid cohesion at endogenous centromeric repetitive sequences is increased compared with euchromatic sequences in *Arabidopsis*. Also, more distantly related plant species (*Brachycome*, rye and maize) show a high frequency of cohesion at repetitive chromatin segments (Schubert et al. 2006, 2007).

Now, we demonstrate that in addition to a random CT organisation and random chromatin associations also preferential homologous and heterologous associations between euchromatic segments close to centromeric heterochromatin and at the (sub)telomeres may occur in *A. thaliana*.

Fang and Spector (2005) showed a cell type dependent distribution of *A. thaliana* centromeres in endoreduplicated nuclei with predominant clustering in root epidermal cells and dispersion in leaf epidermal cells. These authors found no precise transmission of centromere positions from the

mother cell to daughter cells during mitosis, but Berr and Schubert (2007) showed transient mirror-image symmetry between meristematic daughter nuclei.

In contrast to polyploid wheat (Maestra et al. 2002; Martinez-Perez et al. 1999, 2000), in diploid rice a homologous association of centromeres and telomeres was found in root xylem and undifferentiated anther cells (Prieto et al. 2004).

Apparently, in plant species with relatively small chromosomes, different patterns of telomere distribution in interphase nuclei may occur (Fransz and De Jong 2011): in tomato at the edge of heterochromatin close to the centromere (Fransz 2004); in rice around the nuclear periphery (Prieto et al. 2004); and in budding yeast a Rabl-like conformation is present (Bystricky et al. 2005; Jin et al. 2000; Saez-Vasquez and Gadal 2010). In yeast, it was also shown that telomeres form clusters (Gotta et al. 1996) but it is not known whether these clusters include preferential association of homologous termini (as we found in *A. thaliana)* or not. Some telomere clustering has also been described in human cells with a higher frequency in differentiated than in cycling cells (Nagele et al. 2001).

Chromosome arm territories, similar as centromeric chromatin (see above) seem to be organised in a loop-like manner (Mateos-Langerak et al. 2009; Munkel et al. 1999). In maize, a loop of ~100 kb has been proven (Louwers et al. 2009) and in *A. thaliana* the size of loops emanating from heterochromatic chromocenters may vary between 100 kb and 2 Mb (Fransz et al. 2002; Fransz and De Jong 2011).

Here we show that not all chromatin fibres form loops that return to the chromocenters because in *A. thaliana* and *A. lyrata* only ~37 % and 26 % of telomeres, respectively, are located close to centromeres. The remaining telomeres mainly surround the nucleolus. Armstrong et al. (2001) suggest that nucleolus-associated telomere clustering is a prerequisite to establish synapsis during meiosis in *A. thaliana*. Whether the (sub)telomere associations we observed in somatic leaf nuclei are connected to transcription remains an open question.

To find out whether a transcribed gene loops out from its CT and/or influences the degree of sister chromatid cohesion, we tested a chromatin fragment of ~80 kb bearing the flowering gene FWA in nuclei of *A. thaliana* by FISH. Mutants where the gene is constitutively expressed in leaf tissue (Soppe et al. 2002) did not display significantly increased out-looping or decreased cohesion of the FWA region in 4C nuclei (Pecinka et al. 2004; Schubert et al. 2006) as expected if expression would be correlated with a higher degree of chromatin decondensation and an interaction with other chromatin segments. However, the frequencies of out-looping and cohesion may be influenced by the different expression levels of the other 13 genes located at the tested fragment. Here, we show that chromatin relaxation, potentially increasing interactions between gene loci, do not occur synchronously for different CTs within the same nucleus (Fig. 3b, c).

Along chromosomes different degrees of chromatin condensation may occur. We found frequent decondensation of a chromatin segment at the subtelomere of bottom arm of *A. thaliana* chromosome 3. Similarly, a single chromosome of *Aegilops markgrafii* (Greuter) Hammer contains such a stretched domain visible at an interstitial position during meiosis (Schubert 2011). In both cases it is not yet clear whether chromatin relaxation is related to transcriptional activity. In human nuclei nucleolus-associated chromatin alters its spatial distribution upon transcriptional changes (Nemeth et al. 2010). Constrained Brownian motion of chromatin could be responsible for short range chromatin movements (Chakalova and Fraser 2010). Therefore, most interactions are limited to genes on the same chromosome arm (Tolhuis et al. 2011) and occur at random. Nevertheless, long range chromatin interactions seem occasionally to play a role for regulation of gene expression (Schoenfelder et al. 2010).

Sister chromatid cohesion in *A. thaliana* is increased when induced double strand breaks have to be repaired (Watanabe et al. 2009). However, repair processes are not necessarily responsible for the observed variation of cohesion at subtelomeres and close to the centromeric heterochromatin. Alternatively, increased site-specific cohesion frequencies could be connected to a tissue and development-specific gene expression in transcription factories comprising the corresponding regions (Eskiw et al. 2011).

During differentiation heterochromatin becomes more condensed in mammals (Meshorer and Misteli 2006). In contrast, rye chromosomes showing Rabl orientation and a condensed string-like structure in meristematic nuclei become more relaxed in differentiated 2C and 4C nuclei (Schubert et al. 2011). *Arabidopsis* heterochromatin decondenses when differentiated mesophyll cells are transformed into protoplasts (Tessadori et al. 2007a). Stronger condensation of heterochromatin occurs a few days after germination (Mathieu et al. 2003; van Zanten et al. 2011) and during the floral transition (Tessadori et al. 2007b). Biotic and abiotic stress factors such as bacterial infection (Pavet et al. 2006), reduced light (Tessadori et al. 2009; van Zanten et al. 2010) and high temperature (Pecinka et al. 2010) may also induce chromatin condensation.

Regarding these observations it is important to analyse features of interphase chromatin architecture under standardised and reproducible conditions. In summary, we conclude that in nuclei of higher plants the mainly random chromatin arrangement is plastic during ontogenesis.

Endopolyploidy is accompanied by non-cohesive chromatid arrangement

Endopolyploidy occurring in plants and in animals results from amplification of sister chromatids without preceding nuclear division. It has been suggested that endopolyploidy

is important to provide high DNA amounts for increased transcriptional activities in specialised cells and to compensate the lack of DNA in species with small genomes (Galitski et al. 1999; Kondorosi and Kondorosi 2004; Nagl 1976). An increased gene copy number could be helpful to protect the genome against environmental stress, e.g. the exposure with ultraviolet B light (Hase et al. 2006; Radziejwoski et al. 2011).

There are findings against the hypothesis that endoreduplication is involved in the regulation of transcription by increasing the availability of DNA templates for gene expression. Leiva-Neto et al. (2004) found that a lower degree of endoreduplication in maize endosperm did not influence the starch and protein contents and also not the corresponding transcript levels. Similarly, gene over-expression in tomato fruits could not be attributed to the degree of endoreduplication (Chevalier et al. 2011).

The widespread occurrence of endopolyploidy in seed plants and the positive correlation between DNA content and cell volume of endopolyploid cells suggest that endopolyploidy may accelerate plant growth and environmental adaption by larger cell volumes (Barow 2006; Bourdon et al. 2010; Galbraith et al. 1991; Jovtchev et al. 2006; Melaragno et al. 1993). However, recent studies in tomato revealed that cell size and fruit size can be uncoupled from the level of endopolyploidy (Chevalier et al. 2011; Nafati et al. 2011).

Here, we show that in endopolyploid *A. thaliana* nuclei the CTs formed by euchromatic chromosome arms maintain mostly the structure they have in 2C nuclei. This can be explained by the fractal globule model of chromatin. Only in 1–2 % of 32C and 64C nuclei we found dispersed CTs as assumed by the equilibrium globule model that describes the mixing of chromatin fibres (Lieberman-Aiden et al. 2009; Mirny 2011). Also, the occurrence of centromeric and pericentromeric sequences as small subdomains support the idea that chromatin is folded in fractal globules.

Applying ~100-kb BAC probes for FISH, Bourdon et al. (2011) describe an almost complete separation of sister chromatids in endopolyploid pericarp nuclei of up to 128C of tomato fruits. Concordantly, we demonstrate a high degree of positional sister chromatid separation in endopolyploid nuclei along chromosome arms of *A. thaliana*. Thus, a higher endopolyploidy in higher plants seems to be accompanied by non-cohesive chromatids acquiring a less condensed chromatin conformation which potentially makes DNA more accessible for the transcription machinery (Lieberman-Aiden et al. 2009). Whether a decondensed chromatin conformation is indeed important for a higher accessibility of genes to the transcription machinery is however still an open question. At least Kato and Lam (2003) found that endoreduplicated pavement cells display a greater range of chromatin movement than diploid guard cells in *A. thaliana*. Such an increased mobility could be important to bring genes together for co-expression in transcription factories. The extension of sister chromatid separation to (peri)centromeric regions is possibly tolerable in highly endopolyploid nuclei due to their mitotic inactivity.

In *Drosophila*, a Rabl orientation was found in endoreduplicated polytene nuclei (Hochstrasser et al. 1986) but not in other cells (Csink and Henikoff 1998). However, we show dispersed distribution of centromeric sequences in endopolyploid nuclei, indicating that endoreduplicated nuclei do not acquire Rabl configuration in *A. thaliana*. This is plausible because during endopolyploidisation no anaphases that mediate Rabl orientation occur.

We found a random association of homologous *A. thaliana* CTs in highly endopolyploid nuclei (16–64C) similar as described for 2C nuclei (Pecinka et al. 2004). Also tendencies of lower or higher frequencies of homologous or heterologous associations, sister chromatid cohesion and chromatin condensation at specific chromosome regions were similar in nuclei of different ploidy level, possibly because we analysed nuclei of the same tissue and developmental stage. Thus, large-scale chromatin rearrangements do apparently not occur during the first endopolyploidisation steps (before centromere dispersion starts).

In summary, the significant feature of endopolyploidy seems to be decreased chromatid cohesion, inducing a decondensed chromatin conformation, rather than a preferential arrangement of CTs. This extended conformation along entire *Arabidopsis* chromosomes could be a functional counterpart correlated to the regionally decondensed chromatid fibres in "puffs" and "Balbiani rings" of polytene chromosomes in dipterans. "Puffs" and "Balbiani rings" are the morphological manifestation of gene activity related to a specific state of differentiation (reviewed in Zhimulev et al. 2004).

Whether the separated sister chromatids of highly endoreduplicated nuclei are also organised in loops emanating from the chromocenters as suggested for nuclei of low endopolyploidy (Fransz et al. 2002; Fransz and De Jong 2011) remains to be investigated. Another interesting question is whether intrachromosomal interactions within large chromatin loops cause smaller loops therein.

Chromatin arrangement in differentiated *Arabidopsis* interphase nuclei- the model

Here, we achieved results concerning the behaviour (homologous and heterologous associations, sister chromatid cohesion) of pericentromeric and (sub)telomeric chromatin segments. We also tested the distribution and the degree of CT condensation at different endopolyploidy levels. Based on these results and previously obtained data, we propose models of interphase chromatin arrangement in differentiated *Arabidopsis* leaf nuclei of lower and higher endopolyploidy level (Fig. 7). We distinguish a varying chromatin

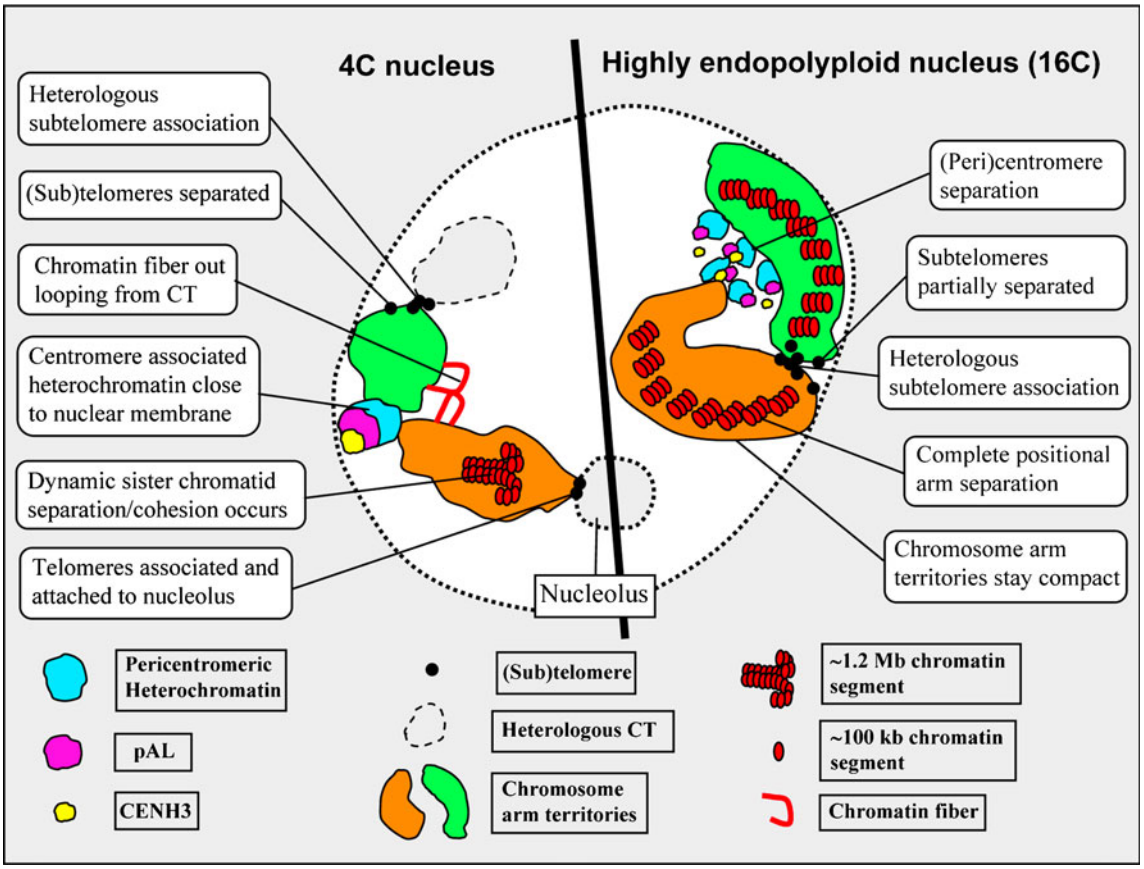


Fig. 7 Scheme of chromatin organisation in differentiated *Arabidopsis* cell nuclei. Compared with *2–8C* nuclei (exemplified for *4C*), a more relaxed chromatin structure is present at higher ploidy (*>16C*) without the disintegration of chromosome arm territories

organisation at heterochromatic (peri)centromeres, euchromatic chromosome arms and (sub)telomeres.

(Peri)centromeres

Centromeres located at the nuclear periphery may associate. Centromeric repeats, CENH3 associated repeats and pericentromeric heterochromatin form distinct co-localising subdomains. Sister centromeres separate increasingly from 16C to 64C nuclei, as their return into the mitotic cycle becomes more and more unlikely.

Euchromatic chromosome arm regions

In interphase nuclei, euchromatin seems to be organised as fractal globules forming loops of ~100 kb to 2 Mb. Euchromatic chromosome arm regions form mainly distinct CTs which do not obviously intermingle in endopolyploid nuclei. Sister chromatid cohesion/separation is variable along interphase chromosomes. Separation can reach several Mb so that whole euchromatic arm CTs may be detached. The minimum extension of cohesive sites or distances between them may fall below 500 kb in 4C nuclei. Independent of the endopolyploidy level, the chromosome arm CTs are mainly arranged randomly. Only NOR-bearing chromosomes are more often associated around a joint nucleolus. Complete dispersion of euchromatic chromosome arms appears at a low frequency and may occur not simultaneously at different CTs. Chromatin fibre out-looping from arm CTs is rare. In nuclei >8C, ~100-kb euchromatic sister chromatin segments within arm CTs are mostly not cohesive, reminiscent of transcriptionally active "puffs" along polytene chromosomes.

(Sub)telomeres

Although *Arabidopsis* telomeres frequently cluster (more often at NORs than at centromeres), sister telomeres can rarely be separated. Preferential as well as random association may occur between homologous and heterologous chromosome termini. The extent of positional sister chromatid separation and of chromatin condensation at subtelomeric chromatin segments varies between different chromosome arms. The frequency of homologous or heterologous association, of sister chromatid cohesion and of chromatin condensation at distinct subtelomeric segments is independent of the

ploidy level. A high degree of cohesion of telomeres and of centromeres, at least in nuclei of a lower endopolyploidy level, might serve as start points for SMC5/6 complex-mediated sister chromatid cohesion when needed for recombination repair of DNA damage (Watanabe et al. 2009). A general clustering and homologous association of telomeres might reflect the potential readiness for chromosome pairing when required, e.g. for meiosis.

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