

**Sister chromatids are often incompletely cohesed in meristematic and endopolyploid  
interphase nuclei of *Arabidopsis thaliana***

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## ABSTRACT

We analysed whether sister chromatids are continuously cohesed in meristematic and endopolyploid Arabidopsis interphase nuclei by studying sister chromatid alignment at various chromosomal positions.

FISH with individual BACs to flow-sorted 4C root and leaf nuclei frequently yielded more than two hybridization signals indicating incomplete or lacking sister chromatid alignment. Up to 100% of 8C, 16C and 32C nuclei showed no sister chromatid alignment at defined positions. Simultaneous FISH with BACs from different chromosomal positions revealed more frequent sister chromatid alignment in terminal than in mid arm positions. Centromeric positions were mainly aligned up to a ploidy level of 16C but became separated or dispersed in 32C nuclei. DNA hypomethylation (of the whole genome) and transcriptional activity (at *FWA* gene position) did not impair sister chromatid alignment. Only 6.1% of 4C leaf nuclei showed sister chromatid separation of entire chromosome 1 top arm territories. Homozygous transgenic tandem repeat (*lac* operator) arrays showing somatic homologous pairing more often than average euchromatic loci did not promote an increased frequency of sister chromatid alignment.

The high frequency of separated sister chromatid arm positions in  $\geq 4C$  nuclei suggests that sister chromatid cohesion is variable, dynamic and not obligatory along the entire chromosome arms in meristematic and differentiated Arabidopsis nuclei.

## INTRODUCTION

The co-linear alignment of sister chromatids is defined as “cohesion” (Maguire 1990; Miyazaki and Orr-Weaver 1994). It is widely assumed that sister chromatids are cohesed from replication in S phase until the onset of anaphase to ensure post-replicative recombination repair as well as correct segregation of eukaryotic nuclear genomes from cell to cell and from generation to generation. A ring-shaped complex of cohesin proteins apparently mediates cohesion of newly replicated sister chromatids until complete bi-polar orientation is achieved during metaphase (for recent reviews see Biggins and Murray 1999; Campbell and Cohen-Fix 2002; Haering and Nasmyth 2003; Hagstrom and Meyer 2003; Hirano 2000; Jessberger 2003; Koshland and Guacci 2000; Nasmyth, 2005; Nasmyth and Schleiffer 2004; Riedel et al. 2004; Uhlmann, 2003, 2004).

Distinct mechanisms to cohes/separate chromatids at specific chromosomal domains such as centromeres, telomeres and nucleolus organizing regions (NORs) (D'Amours et al. 2004; Dynek and Smith 2004; Pereira and Schiebel 2004; Rieder and Cole 1999; Sullivan et al. 2004; Tang et al. 2004; Warren et al. 2000; Watanabe 2005; Yalon et al. 2004) seem to be related to the different functions of these regions. Furthermore, cohesins are involved in chromosome condensation (together with condensins), regulation of gene expression, post-replicative recombination repair and meiotic recombination (Glynn et al. 2004; Hagstrom and Meyer 2003; Jessberger 2003; Lee and Orr-Weaver 2001; Lengronne et al. 2004; Morrison et al. 2003; Revenkova et al. 2004; van Heemst and Heyting 2000; Webber et al. 2004). For repair of double-strand breaks in G2 nuclei a *de novo* recruitment of cohesins to break positions seems to be required (Kim et al. 2002; Ünal et al. 2004; Ström et al. 2004). The knowledge on sister chromatid cohesion is mainly based on investigations in yeast, *Sordaria*, *Drosophila*, *Caenorhabditis*, *Xenopus*, chicken, mouse and human. Cohesin genes in plants acting during mitosis and meiosis were reported for *Arabidopsis* (Cai et al. 2003; Dong et al. 2001; Liu et al. 2002; Mercier et al. 2001; 2003, Lam et al. 2005).

Cohesins are not randomly distributed along chromosomes but rather located at specific loci. In yeast, these loci are represented mainly by intergenic A+T-rich regions and also by telomeric and centromeric regions. The average extension of cohesion sites is 0.8 – 1.0 kb (Blat and Kleckner 1999; Laloraya et al. 2000; Megee et al. 1999; Tanaka et al. 1999) separated by ~11 kb intervals. Activation of transcription mediates repositioning of cohesins (Glynn et al. 2004; Lengronne et al. 2004). Due to the close spacing of cohesion sites, FISH signals from sister chromatids can not be distinguished in yeast (Blat and Kleckner 1999; Guacci et al. 1994). However, in cultured human fibroblasts in G2, sister chromatids may occupy considerably distant positions when probed for distinct loci by FISH (Volpi et al. 2001). In *Drosophila*, in spite of somatic pairing of homologues, chromatin tagging with a recombinant GFP-Lac repressor protein revealed regular sister chromatid separation at the tagged locus during mid-G2 before first male meiosis (Vazquez et al. 2002). Applying the same chromatin tagging system to *Arabidopsis*, a higher frequency of homologous pairing was found in somatic 2C nuclei for the transgenic tandem repeats (lac operator arrays and also for HPT1 repeats) than for average euchromatin regions (Pecinka et al. 2005), but sister chromatid alignment in 4C nuclei was not investigated. The greater range of movement of GFP-tagged lac operator loci in endopolyploid than in 2C *Arabidopsis* nuclei (Kato and Lam 2003) could be considered as an indirect indication of incomplete sister chromatid alignment in endopolyploid nuclei.

During preliminary investigations we occasionally found three or four instead of one or two FISH signals for chromosome-specific ~100 kb segments in 4C nuclei of *A. thaliana* indicating that not only homologues but also sister chromatids may occupy separate positions within a nucleus. Therefore, we became motivated to study the degree of sister chromatid alignment (close spatial vicinity of identical segments) in *A. thaliana* nuclei of meristematic and differentiated tissues. For this purpose we used fluorescence in situ hybridization of DNA sequences from different positions along chromosomes. *A. thaliana* is an 'endopolyploid'

species, i.e., differentiated cells may undergo endoreplication cycles without nuclear division between replication phases. The 4C nuclei resulting from the first replication step could theoretically correspond to the mitotic G2 stage. Therefore, we tested meristematic and differentiated 4C nuclei as to differences in sister chromatid alignment and compared the results with those obtained for nuclei of higher endopolyploidy levels. The number of FISH signals was taken as a measure for sister chromatid alignment. An increase in number of FISH signals with the ploidy level indicates the absence of cohesion at the loci under study. Furthermore, we tested by FISH sister chromatid alignment at transgenic tandem repeat (*lac* operator) arrays which have a tendency for increased somatic pairing in Arabidopsis. Additionally, we looked for possible correlations of positional sister chromatid alignment with transcriptional activity and with the degree of overall DNA methylation.

## MATERIALS AND METHODS

**Plant material and preparation of nuclei:** Nuclei from young root tips or rosette leaves of *A. thaliana* accessions Columbia (Col) and Landsberg *erecta* (*Ler*), of the mutants *ddm1* in Col background (Vongs et al. 1993), and *fwa-1* in *Ler* background (Soppe et al. 2000) and of the transgenic line EL702C in Col background (Kato and Lam 2001) were isolated after formaldehyde fixation and flow-sorted on slides according to their ploidy level (2C to 8C) as described (Pecinka et al. 2004). Nuclei of 16C and 32C were isolated from stems.

Meristematic nuclei were prepared from roots of 2 days old seedlings; ~0.5 cm long roots were incubated for 30 min in 100  $\mu$ M BrdUrd, 5  $\mu$ M Urd, 0.1  $\mu$ M FdUrd, fixed in 3:1 ethanol/acetic acid for at least 5 h and washed 3x20 min in 10 mM sodium citrate buffer (pH 4.8). After incubation for 35 min at 37° in 2% pectinase and 2% cellulase to soften the tissue, root tips were washed 3x20 min in 10 mM sodium citrate buffer (pH 4.8), transferred to 45 % acetic acid for 5-10 min and squashed in a drop of 45 % acetic acid. Coverslips were removed

after freezing on dry ice. Then slides were immediately dehydrated in a series of 70, 90 and 96% ethanol, air-dried and stored at 4°.

**Probe labelling and fluorescent in situ hybridisation:** BACs (bacterial artificial chromosomes) used for FISH were obtained from the Arabidopsis Biological Resource Center (Columbus, OH). The 178 bp *A. thaliana* centromere-specific sequence (pAL) was cloned by Martinez-Zapater et al (1986). DNA of individual clones was isolated as described by Birnboim and Doly (1979).

DNA was labeled by nick translation with digoxigenin-dUTP, biotin-dUTP or Cy3-dUTP according to Ward (2002). For painting of chromosome 1 top arm 15 pools of altogether 76 BACs (from T25K16 up to T9G5) were labeled as described (Pecinka et al. 2004). Post-hybridization washes and detection of FISH signals were performed according to Schubert et al. (2001). Biotin was detected by avidin conjugated with Texas Red (1:1000; 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. Nuclei and chromosomes were counterstained with DAPI (1  $\mu$ g/ml) in Vectashield (Vector Laboratories). BrdU incorporation was detected by rat-anti-BrdU (1:100; Abcam) and rabbit-anti-rat conjugated with Cy3 (1:100; Jackson Immunoresearch).

**Microscopic evaluation, image processing and statistics:** Analysis of fluorescence signals was performed with an epifluorescence microscope (Zeiss Axiophot) using a 100x/1.4 Zeiss plan apochromat objective and a Sony (DXC-950P) camera. Images were captured separately for each fluorochrome using the appropriate excitation and emission filters. The images were merged using Adobe Photoshop 6.0 (Adobe Systems) software. In 4C nuclei, the occurrence

of 3 or 4 FISH signals was considered to represent sister chromatid separation when their distance was larger than the signal diameter.

To distinguish in squashes from root tip meristems 4C nuclei without BrdU incorporation from 2C nuclei, areas of BrdU-labeled S phase nuclei were calculated on the basis of size measurements using a Digital Optical Microscope System (Schwertner GbR, Jena). Nuclei with an area larger than that of replicating, BrdU-labeled nuclei plus 95% confidence interval were regarded as 4C.

Fisher's exact test has been used to compare positional sister chromatid separation frequencies.

## RESULTS

**In differentiated 4C nuclei, sister chromatids are separated with variably high frequencies at various positions but rarely along entire chromosome arm territories:** To

analyse sister chromatid alignment in *A. thaliana* interphase nuclei, individual BACs (or BAC pairs with inserts of adjacent genomic sequences) from different positions along chromosomes 1 and 4 were hybridized to flow-sorted 4C nuclei. One FISH signal (pairing of both homologues) or two FISH signals per BAC (or BAC pair) were regarded as positional alignment at the corresponding region indicating that sister chromatids are cohesed. Three or four signals were considered to indicate sister chromatid separation (Figure 1). Positional sister chromatid separation occurred in 32.5% to 59.6% of nuclei (Figure 2).

Fig. 1,2

At three positions of chromosome 1 (BAC pairs T2P11/T7N9, F11P17/T1F9, T11I11/F3F9) the separation frequencies differed significantly ( $P < 0.001$ ) between leaf and root nuclei suggesting a tissue-specific degree of sister chromatid alignment. Even between individual mid arm positions a significant ( $P < 0.001$ ) variability of sister chromatid alignment may occur in 4C root nuclei (e.g. between BAC pairs T2P11/T7N9 and F11P17/T1F9).

The ratio of 4C root nuclei with 3 or 4 signals varied for chromosome 1 positions between 1.1:1 (T2P11/T7N9) and 2.7:1 (F6F3-F22L4). The 1.1:1 ratio is significantly different ( $P < 0.001$ ) from the random ratio of 2:1 (random separation involving one homologue should be twice as frequent as separation involving both homologues). The larger-than-expected proportion of nuclei with four signals might suggest a tendency for simultaneous separation of sister chromatids of both homologues at the respective homologous positions.

Using a FISH probe covering the top arm of chromosome 1, we analyzed the frequency of sister chromatid alignment for the entire arm. While sister chromatid separation at the segment corresponding to the BAC T2P11 within the top arm of chromosome 1 appeared in 38.1% of nuclei, completely separated sister chromatid arm territories were found only in 6.1% of 359 4C leaf nuclei (Figure 1B). In 4.7% of nuclei one and in 1.4% both homologous arms were separated.

**Neither DNA hypomethylation nor transcriptional activity impair positional sister chromatid alignment:** A comparison of sister chromatid separation frequencies between 4C nuclei of wild-type (accession Columbia) and of the hypomethylation mutant *ddm1* (Vongs et al. 1993) in Col background displayed no significant differences at the same chromosomal positions of chromosome 4. Hence, sister chromatid alignment is apparently not influenced by an overall DNA hypomethylation (Figure 2).

The flowering gene *FWA* residing in BAC M7J2 and mapped at the bottom arm of chromosome 4 is strongly methylated and not expressed in wild-type plants (accession *Ler*), but constitutively expressed and hypomethylated in leaf nuclei of the *fwa-1* mutant in *Ler* background (Soppe et al. 2000). FISH signals of M7J2 revealed separation of sister chromatids in 45.6% of 4C leaf nuclei (n=230) of wild-type *Ler* and in 33.9% of *fwa-1* nuclei (n=121), with a ratio of nuclei with 3 or 4 signals of 3.2:1 and 3.6:1, respectively. While the frequency of separation at the position of M7J2 in *Ler* nuclei is within the range observed for

various positions in Col, it is in *fwa-1* mutant nuclei even lower than found for wild-type *Ler* nuclei where the *FWA* gene is silent. These results indicate the lack of an activation-mediated reduction of sister chromatid alignment at the transcribed *FWA* locus.

**Nuclei with an endopolyploidy level above 4C showed up to 100% sister chromatid**

**separation at the tested positions:** In 8C leaf nuclei, sister chromatid separation varied between 74.4% and 99.1% along different positions of chromosome 1 (Figure 2). In 16C and 32C stem nuclei, BAC positions F2H10, T1F9 and T11I11 showed sister chromatid separation in nearly all tested nuclei. From 8C nuclei it became obvious that alignment is retained more often at very terminal (see values for the BAC pair F6F3/F22L4 located 104 kbp upstream the telomeric repeats, Figure 2) than at interstitial arm positions.

In some 8C, 16C and 32C nuclei, a number of separate FISH signals corresponding even to the maximum number of homologous chromatids was observed (Figure 3, B<sub>2</sub>, E).

Fig. 3

**Sister chromatid separation may vary along the same chromosome:** Simultaneous FISH with BACs from different positions along chromosome 1 has shown that sister chromatids in the same nucleus might be aligned at some and separated at other positions (Figure 3A). In 8C nuclei, the degree of sister chromatid alignment may vary also between mid arm positions (Figure 3E<sub>1</sub>). In highly endopolyploid nuclei (32C), sister chromatids were on average more frequently separated in mid arm and in distal than in pericentromeric positions (compare BACs F2H10, T1F9 and T11I11 in Figure 3, E<sub>2,3</sub>). Although it is difficult to count unambiguously individual signals in 16C and 32C nuclei, the high degree of separation becomes clearly evident from images such as shown in Figure 3D.

**The majority of sister centromeres appears to be aligned up to a DNA content of 16C:**

Most of the (tandem)repetitive sequences of *A. thaliana* occur around centromeres and at the

NORs on the short arm termini of chromosomes 2 and 4. Therefore, up to 14 strongly DAPI-stained chromocenters per nucleus should appear if sister chromatids are cohesed. However, because of frequent association of NORs with the corresponding pericentromeres (forming short-arm loops) and because of frequent association of homologous pericentromeric regions, nuclei from squashed organs revealed between 4 and 10 (most frequently 7 or 8)

Fig. 4

chromocenters (Fransz et al. 2002). To find out whether sister chromatid alignment at centromeres is retained after endopolyploidization, we investigated flow-sorted nuclei of 4C, 8C (root), 16C and 32C (stem) DNA content (Figure 4). After FISH with the tandem-repetitive centromere-specific 178 bp sequence (pAL) we found, compared with 2C nuclei, a shift towards more nuclei with up to 10 signals in 4C, 8C and 16C nuclei. This shift indicates a lower degree of interchromosomal centromere associations, but not necessarily separation of sister chromatids. Of the 16C nuclei 22.3% showed 11 or more centromeric signals, the same was true for 68.2% of 32C nuclei. Additionally, 18.3% of 32C nuclei yielded dispersed FISH signals with pAL (Figure 4C<sub>3</sub>). In 32C nuclei these findings were paralleled by an increased number and/or diffuse appearance of DAPI-intense chromocenters. Thus, separation of sister centromeres is not evident up to an 8C DNA content, it appears to a low extent in 16C and increases strongly in 32C nuclei.

#### **Sister chromatid alignment is not increased at tandem repetitive *lac* operator array**

**insertion sites:** In 2C leaf nuclei of homozygous transgenic plants with two insertion loci of *lac* operator (*lac* O) sequences on top arm of chromosome 3 (line EL702C, Kato and Lam 2001), a significant increase of somatic homologous pairing frequency of the transgene loci in comparison to average euchromatic regions was demonstrated (Pecinka et al. 2005). On the

Fig. 5

other hand, in *Drosophila* in which a regular development-specific somatic pairing occurs (Hiraoka et al. 1993), GFP-tagged *lac* O arrays allowed to trace separation of homologues and even of sister chromatids in male pre-meiotic mid-G2 nuclei (Vazquez et al. 2002). Therefore,

we have tested fixed 4C leaf nuclei of homozygous EL702C plants by FISH with *lac O* sequences and with BACs containing sequences that flank the *lac O* arrays as to the occurrence of sister chromatid separation at or adjacent to the transgene loci (Figure 5). The two *lac O* loci at the chromosome 3 top arm may pair in allelic as well as in ectopic manner. Therefore, signal configurations, which cannot clearly be identified as alignment or separation, may appear (Figure 5B<sub>6</sub>). Configurations unambiguously indicating sister chromatid separation (Figure 5B<sub>5</sub>) were identified at the *lac O*/MGL6 and at the *lac O*/F18C1 locus. Sister chromatid separation was observed in 40.9% of each of the two loci in 44 nuclei. In addition, 20.5% and 18.2% equivocal configurations, respectively, were found (Figure 5B<sub>6</sub>). Thus, sister chromatid separation at the *lac O* insertion sites occurred at about the same frequency as found for the different single copy euchromatic positions in wild-type plants and is not counteracted by the tendency for homologous pairing at the tandem repeat loci. Life observation of GFP spots was not done because of uncertainties regarding the appearance of individual spots and their tendency to fuse with each other (Pecinka et al. 2005).

**Positional sister chromatid separation is also evident in meristematic cells:** Because leaf and root nuclei with a DNA content of 4C or higher are typical for differentiated cells with no need for further mitotic division, sister chromatid cohesion might be dispensable in such nuclei. Therefore, we studied meristematic root tip nuclei after incorporation of bromodeoxyuridine. Separation of sister chromatids at a distal position of chromosome 1 top arm (F22L4) occurred in 30.9% of 55 replicating nuclei (Figure 6) and in 34.4% of 64 4C root tip nuclei and hence not significantly less often than in 4C nuclei of differentiated tissues.

Fig. 6

## DISCUSSION

Our results show that FISH is effective to prove positional sister chromatid separation as a common feature in S, G2 and endopolyploid nuclei of *A. thaliana*.

**Sister chromatid separation is frequent, position-specific and dynamic:** According to a model of Koshland and Guacci (2000) chromosome condensation towards nuclear division is mediated by 'coalescence of cohesion sites' along paired sister chromatids. Between the coalescent sites symmetric loops are formed. The size of the presumed loops depends on the distance of cohesion sites and determines the degree of compaction. If the degree of compaction increases with genome size (Vinogradov 2005), larger genomes might have fewer cohesion sites and larger loops between them, possibly yielding positional separation along sister chromatids with a higher probability than for instance in budding yeast (Koshland and Guacci 2000). However, during prophase sister chromatids are continuously aligned and the distance between FISH signals on sister chromatids becomes very small or not resolvable by light microscopy. Assuming that the number and distance of cohesion sites along chromosome arms is not increased from replication up to mitosis, the distance of FISH signals for identical positions on sister chromatids in interphase nuclei should not exceed that observed during the highest compaction at metaphase if coalescence of cohesion sites would be the main reason for mitotic chromosome condensation. However, frequently three or four FISH signals with distances of sometimes about half the nuclear diameter were observed in 4C nuclei. Recruitment of condensins towards mitosis might mediate an intense folding of sister chromatid loops between cohesion sites, thus leading to the appearance of closely aligned sister chromatids of pro- and metaphase chromosomes (Haering and Nasmyth 2003; Hagstrom and Meyer 2003, Hirano 2005). Metaphase chromosomes with chromatid deletions mediated by erroneous repair, can be observed after genotoxin exposure. Chromatin deleted from an interstitial position of one sister chromatid remains attached to the homologous region of its undamaged sister (see Fig. 7 and Schubert et al. 1994). This observation confirms a closer proximity of identical sister chromatids during pro- and metaphase than during preceding G2 when sister chromatids are frequently separated at various positions.

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The highest degree of alignment along interphase chromosome arms occurs around centromeres up to an endopolyploidy level of 16C. It remains an open question whether for correct segregation during nuclear divisions strict cohesion at centromeric regions is sufficient or whether a premitotic increase of homologous alignment along chromosome arms is required. Since even in late S and early G2 of meristematic cells positional sister chromatid separation is evident, sparse cohesion along arms (and a somewhat more dense cohesion at telomeres) might be sufficient until the onset of prophase when condensins enforce sister chromatid alignment. The observations from meristematic nuclei are in accordance with a tight connection of sister chromatid exchange (requiring a close vicinity of homologous sister regions for recombination) with DNA replication when sister chromatids are just emerging. In yeast, cohesion sites occupy the boundaries of transcriptionally silenced regions (Laloraya et al. 2000), are usually not transcribed (Tanaka et al. 1999) and shift away from transcribing regions (Glynn et al. 2004; Lengronne et al. 2004). In comparison to Arabidopsis wild-type nuclei, constitutive expression of the *FWA* gene in *fwa-1* mutant nuclei did not cause increased sister chromatid separation at this locus, indicating that there is no cohesion site in the vicinity to interfere with transcription at this locus. Further comparative studies with other gene regions in silent *versus* active state are needed for conclusions as to whether or not transcriptional activity has an impact on cohesion in Arabidopsis.

In living G2 spermatocytes of *Drosophila* GFP-tagging revealed positional sister chromatid separation at *lacO* loci (Vazquez et al. 2002). Similarly, sister chromatid separation at transgenic tandem repeat loci of 4C nuclei of Arabidopsis appears with a 'normal' frequency, albeit these loci are more often homologously paired than average euchromatic regions (Pecinka et al. 2005). While somatic pairing frequency of *lacO* repeat arrays decreases with decreasing methylation at CpG sites (Watanabe et al. in press), overall sister chromatid alignment is not significantly less frequent in the background of the DNA hypomethylation mutant *ddm1* than in 4C wild-type nuclei.

Reproducibly different sister chromatid alignment frequencies along chromosome arms suggest a non-uniform distribution of cohesion sites that, at least in part, may differ between tissues indicating a developmentally regulated dynamics of sister chromatid cohesion (Figures 2 and 8).

Fig. 8

**Separation of identical chromatids increases with the endopolyploidy level:** Contrary to the situation observed in 4C nuclei, positional sister chromatid separation outside the pericentromeres may concern up to 100% of >4C nuclei. This observation contradicts the results of Esch et al. (2003). These authors reported a very high association frequency of homologous positions in endopolyploid *A. thaliana* nuclei resulting in only one GFP spot per nucleus after lac operator/GFP-lac repressor chromatin tagging. However, transgenic repeat loci have a strong tendency for homologous pairing which is not typical for average euchromatic regions (Pecinka et al. 2004; 2005). Sister centromeres tend to separate from the 16C level on in stem nuclei. Individual ~100 kb regions are of a spread appearance within highly endopolyploid nuclei (Figure 3B<sub>2</sub>, D<sub>2-3</sub>). In general, the degree of positional alignment decreases with increasing ploidy level (as to be expected when chromatids do not form polytene chromosomes) and is lower at interstitial than at centromeric or terminal positions. Non-dividing endopolyploid nuclei are less endangered by unrepaired double-strand breaks than dividing cells, therefore, nuclei may possibly possess or acquire less cohesion sites with increasing endopolyploidy level. Whether the lower degree of sister chromatid alignment correlates with a higher level of transcription in endopolyploid nuclei requires further investigation. However, at least our data obtained for the FWA gene are not in favor of a transcription-mediated reduction of sister chromatid alignment.

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## LITERATURE CITED

- BIGGINS, S., AND A. W. MURRAY, 1999 Sister chromatid cohesion in mitosis. *Curr. Opin. Genet. Dev.* **9**: 230-236.
- BIRNBOIM, H. C., AND J. DOLY, 1979 A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* **7**: 1513-1523.
- BLAT, Y., AND N. KLECKNER, 1999 Cohesin bind to preferential sites along yeast chromosome III, with differential regulation along arms versus the centric region. *Cell* **98**: 249-259.
- CAI, X., F. G. DONG, R. E. EDELMANN, AND C. A. MAKAROFF, 2003 The *Arabidopsis* SYN1 cohesin protein is required for sister chromatid arm cohesion and homologous chromosome pairing. *J. Cell Sci.* **116**: 2999-3007.
- CAMPBELL, J. L., AND O. COHEN-FIX, 2002 Chromosome cohesion: ring around the sisters? *Trends Biochem. Sci.* **27**: 492-495.
- D'AMOURS, D., F. STEGMEIER, AND A. AMON, 2004 Cdc14 and condensin control the dissolution of cohesin-independent chromosome linkages at repeated DNA. *Cell* **117**: 455-469.
- DONG, F., X. CAI, AND C. A. MAKAROFF, 2001 Cloning and characterization of two *Arabidopsis* genes that belong to the RAD21/REC8 family of chromosome cohesin proteins. *Gene* **271**: 99-108.
- DYNEK, J. N., AND S. SMITH, 2004 Resolution of sister telomere association is required for

- progression through mitosis. *Science* **304**: 97-100.
- ESCH, J. J., M. CHEN, M. SANDERS, M. HILLESTAD, S. NDKIUM *et al.*, 2003 A contradictory GLABRA3 allele helps define gene interactions controlling trichome development in *Arabidopsis*. *Development* **130**: 5885-5894.
- FRANSZ, P., J. H. DE JONG, M. LYSAK, M. RUFFINI CASTIGLIONE, AND I. SCHUBERT, 2002 Interphase chromosomes in *Arabidopsis* are organized as well defined chromocenters from which euchromatin loops emanate. *Proc. Natl. Acad. Sci. USA* **99**: 14584-14589.
- GLYNN, E. F., P. C. MEGEE, H. G. YU, C. MISTROT, E. UNAL *et al.*, 2004 Genome-wide mapping of the cohesin complex in the yeast *Saccharomyces cerevisiae*. *PLoS. Biol.* **2**: 1325-1339.
- GUACCI, V., E. HOGAN, AND D. KOSHLAND, 1994 Chromosome condensation and sister chromatid pairing in budding yeast. *J. Cell Biol.* **125**: 517-530.
- HAERING, C. H., AND K. NASMYTH, 2003 Building and breaking bridges between sister chromatides. *BioEssays* **25**: 1178-1191.
- HAGSTROM, K. A., AND B. J. MEYER, 2003 Condensin and cohesin: more than chromosome compactor and glue. *Nature Rev. Genet.* **4**: 520-534.
- HIRANO, T., 2000 Chromosome cohesion, condensation, and separation. *Annu. Rev. Biochem.* **69**: 115-144.
- HIRANO, T., 2005 Condensins: organizing and segregating the genome. *Curr. Biol.* **15**: R265-R275.
- HIRAOKA, Y., A. F. DERNBURG, S. J. PARMELEE, M. C. RYKOWSKI, D. A. AGARD, AND J. W.

- SEDAT, 1993 The onset of homologous chromosome pairing during *Drosophila melanogaster* embryogenesis. *J. Cell Biol.* **120**: 591-600.
- JESSBERGER, R., 2003 SMC proteins at the crossroads of diverse chromosomal processes. *IUBMB Life* **55**: 643-652.
- KATO, N., AND E. LAM, 2001 Detection of chromosome tagged with green fluorescent protein in live *Arabidopsis thaliana* plants. *Genome Biol.* **2**: research0045.
- KATO, N., AND E. LAM, 2003 Chromatin of endoreduplicated pavement cells has greater range of movement than that of diploid guard cells in *Arabidopsis thaliana*." *J. Cell Sci.* **116**: 2195-2201
- KIM, J.-S., T. B. KRASIEVA, V. LAMORTE, A. M. R. TAYLOR, AND K. YOKOMORI, 2002 Specific recruitment of human cohesion to laser-induced DNA damage. *J. Biol. Chem.* **277**: 45149-45153.
- KOSHLAND, D. E., AND V. GUACCI, 2000 Sister chromatid cohesion: the beginning of a long and beautiful relationship. *Curr. Opin. Cell Biol.* **12**: 297-301.
- LALORAYA, S., V. GUACCI, AND D. KOSHLAND, 2000 Chromosomal addresses of the cohesin component Mcd1p. *J. Cell Biol.* **151**: 1047-1056.
- LAM, W. S., X. YANG, AND C. A. MAKAROFF, 2005 Characterization of *Arabidopsis thaliana* SMC1 and SMC3: evidence that AtSMC3 may function beyond chromosome cohesion. *J. Cell Sci.* **118**: 3037-3048.
- LEE, J. Y., AND T. L. ORR-WEAVER, 2001 The molecular basis of sister-chromatid cohesion. *Annu. Rev. Cell Dev. Biol.* **17**: 753-777.

- LENGRONNE, A., Y. KATOU, S. MORI, S. YOKOBAYASHI, G. P. KELLY *et al.*, 2004 Cohesin relocation from sites of chromosomal loading to places of convergent transcription. *Nature* **430**: 573-578.
- LIU, C. M., J. MCELVER, I. TZAFRIR, R. JOOSEN, P. WITTICH *et al.*, 2002 Condensin and cohesin knockouts in *Arabidopsis* exhibit a titan seed phenotype. *Plant J.* **29**: 405-415.
- MAGUIRE, M. P., 1990 Sister chromatid cohesiveness: vital function, obscure mechanism. *Biochem. Cell Biol.* **68**: 1231-1242.
- MEGEE, P. C., C. MISTROT, V. GUACCI, AND D. KOSHLAND, 1999 The centromeric sister chromatid cohesion site directs Mcd1p binding to adjacent sequences. *Mol. Cell* **4**: 445-450.
- MERCIER, R., S. J. ARMSTRONG, C. HORLOW, N. P. JACKSON, C. A. MAKAROFF *et al.*, 2003 The meiotic protein SWI1 is required for axial element formation and recombination initiation in *Arabidopsis*. *Development* **130**: 3309-3318.
- MERCIER, R., D. VEZON, E. BULLIER, J. C. MOTAMAYOR, A. SELIER *et al.*, 2001 SWITCH1 (SWI1): a novel protein required for the establishment of sister chromatid cohesion and for bivalent formation at meiosis. *Genes Dev.* **15**: 1859-1871.
- MIYAZAKI, W. Y., AND T. L. ORR-WEAVER, 1994 Sister-chromatid cohesion in mitosis and meiosis. *Annu. Rev. Genet.* **28**: 167-187.
- MORRISON, C., P. VAGNARELLI, E. SONODA, S. TAKEDA, AND W. C. EARNSHAW, 2003 Sister chromatid cohesion and genome stability in vertebrate cells. *Biochem. Soc. Trans.* **31**: 263-265.
- NASMYTH, K., 2005 How might cohesin hold sister chromatids together? *Philos. Trans. R.*

- Soc. Lond. B Biol. Sci. **360**: 483-496.
- NASMYTH, K., AND A. SCHLEIFFER, 2004 From a single double helix to paired double helices and back. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **359**: 99-108.
- PECINKA, A., N. KATO, A. MEISTER, A. V. PROBST, I. SCHUBERT, AND E. LAM, 2005 Tandem repetitive transgenes and fluorescent chromatin tags alter the local interphase chromosome arrangement in *Arabidopsis thaliana*. *J. Cell. Sci.* **118**: 3751-3758.
- PECINKA, A., V. SCHUBERT, A. MEISTER, G. KRETH, M. KLATTE *et al.*, 2004 Chromosome territory arrangement and homologous pairing in nuclei of *Arabidopsis thaliana* are predominantly random except for NOR-bearing chromosomes. *Chromosoma* **113**: 258-269.
- PEREIRA, G., AND E. SCHIEBEL, 2004 Cdc14 phosphatase resolves the rDNA segregation delay. *Nature Cell Biol.* **6**: 473-475.
- REVENKOVA, E., M. EIJEPE, C. HEYTING, C. A. HODGES, P. A. HUNT *et al.*, 2004 Cohesion SMC1 $\beta$  is required for meiotic chromosome dynamics, sister chromatid cohesion and DNA recombination. *Nature Cell Biol.* **6**: 555-562.
- RIEDEL, C. G., J. GREGAN, S. GRUBER, AND K. NASMYTH, 2004 Is chromatin remodeling required to build sister-chromatid cohesion? *Trends Biochem. Sci.* **29**: 389-392.
- RIEDER, C. L., AND R. COLE, 1999 Chromatid cohesion during mitosis: lessons from meiosis. *J. Cell Sci.* **112**: 2607-2613.
- SCHUBERT, I., P. F. FRANSZ, J. FUCHS, AND J. H. DE JONG, 2001 Chromosome painting in plants. *Methods Cell Sci.* **23**: 57-69.

- SCHUBERT, I., R. RIEGER, J. FUCHS, AND U. PICH, 1994 Sequence organization and the mechanism of interstitial deletion clustering in a plant genome (*Vicia faba*). *Mutat. Res.* **325**: 1-5.
- SOPPE, W. J. J., S. E. JACOBSEN, C. ALONSO-BLANCO, J. P. JACKSON, T. KAKUTANI *et al.*, 2000 The late flowering phenotype of *fwa* mutants is caused by gain-of-function epigenetic alleles of a homeodomain gene. *Mol. Cell* **6**: 791-802.
- STRÖM, L., H. B. LINDROOS, K. SHIRAHIGE, AND C. SJÖGREN, 2004 Postreplicative recruitment of cohesin to double-strand breaks is required for DNA repair. *Mol. Cell* **16**: 1003-1015.
- SULLIVAN, M., T. HIGUCHI, V. L. KATIS, AND F. UHLMANN, 2004 Cdc14 phosphatase induces rDNA condensation and resolves cohesin-independent cohesion during budding yeast anaphase. *Cell* **117**: 471-482.
- TANAKA, T., M. P. COSMA, K. WIRTH, AND K. NASMYTH, 1999 Identification of cohesion association sites at centromeres and along chromosome arms. *Cell* **98**: 847-858.
- TANG, Z. Y., Y. X. SUN, S. E. HARLEY, H. ZOU, AND H. T. YU, 2004 Human Bub1 protects centromeric sister-chromatid cohesion through Shugoshin during mitosis. *Proc. Natl. Acad. Sci. USA* **101**: 18012-18017.
- UHLMANN, F., 2003 Chromosome cohesion and separation: from men and molecules. *Curr. Biol.* **13**: R104-R114.
- UHLMANN, F., 2004 The mechanism of sister chromatid cohesion. *Exp. Cell Res.* **296**: 80-85.
- ÜNAL, E., A. ARBEL-EDEN, U. SATTLER, R. SHROFF, M. LICHTEN *et al.*, 2004 DNA damage

- response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. *Mol. Cell* **16**: 991-1002.
- VAN HEEMST, D., AND C. HEYTING, 2000 Sister chromatid cohesion and recombination in meiosis. *Chromosoma* **109**: 10-26.
- VAZQUEZ, J., A. S. BELMONT, AND J. W. SEDAT, 2002 The dynamics of homologous chromosome pairing during male *Drosophila* meiosis. *Curr. Biol.* **12**: 1473-1483.
- VINOGRADOV, A. E., 2005 Genome size and chromatin condensation in vertebrates. *Chromosoma* **113**: 362-369.
- VOLPI, E. V., D. SHEER, AND F. UHLMANN, 2001 Cohesion, but not too close. *Curr. Biol.* **11**: R378.
- VONGS, A., T. KAKUTANI, R. A. MARTIENSSSEN, AND E. J. RICHARDS, 1993 *Arabidopsis thaliana* DNA methylation mutants. *Science* **260**: 1926-1928.
- WARD, P. B., 2002 FISH probes and labelling techniques, pp. 5-28 in *FISH: a practical approach. (Practical approach series; 260)*, edited by B. BEATTY, S. MAI AND J. SQUIRE. Oxford Univ. Press, Oxford.
- WARREN, W. D., S. STEFFENSEN, E. LIN, P. COELHO, M.-L. LOUPART *et al.*, 2000 The *Drosophila* RAD21 cohesin persists at the centromere region in mitosis. *Curr. Biol.* **10**: 1463-1466.
- WATANABE, K., A. PECINKA, A. MEISTER, N. KATO, I. SCHUBERT, AND E. LAM, 2005 DNA hypomethylation impairs homologous pairing of transgenic tandem repeat arrays in somatic nuclei of *Arabidopsis thaliana*. *Plant J.*, in press.

WATANABE, Y., 2005 Sister chromatid cohesion along arms and at centromeres. *Trends Genet.* **21**: 405-412.

WEBBER, H. A., L. HOWARD, AND S. E. BICKEL, 2004 The cohesion protein ORD is required for homologue bias during meiotic recombination. *J. Cell Biol.* **164**: 819-829.

YALON, M., S. GAL, Y. SEGEV, S. SELIG, AND K. L. SKORECKI, 2004 Sister chromatid separation at human telomeric regions. *J. Cell Sci.* **117**: 1961-1970.

**FIGURE 1.** -Sister chromatid alignment/separation of homologous ~100 kb segments and of entire of chromosome arms in 4C nuclei. **A** FISH with different BAC pairs. **A<sub>1</sub>** sister chromatid alignment at non-paired homologous position, **A<sub>2</sub>** sister chromatid alignment at paired homologous positions, **A<sub>3</sub>** sister chromatid separation at one of the homologous positions, and **A<sub>4</sub>** positional sister chromatid separation at both homologues. **B<sub>1</sub>** Scheme of chromosome 1 with the top arm labeled by a contig of BACs (red) and a 85.3 kbp sequence cloned in BAC T2P11 (green), **B<sub>2</sub>** separation of the sister chromatid arm territories of one homologue (arrows), **B<sub>3</sub>** positional sister chromatid separation (T2P11 green) within both homologous chromosome arm territories. Nuclei were counterstained with DAPI. Bars = 3  $\mu$ m

**FIGURE 2.** Positional separation frequencies in % (number of investigated nuclei in parenthesis) analysed in accession 'Columbia' and in the DNA hypomethylation mutant *ddm1* in Col background after FISH with differentially labelled BACs from chromosomes 1 and 4. \*F2H10 was tested only in 16C and 32C nuclei. \*\*M7J2 was tested in 4C leaf nuclei of accession *Ler* (and of mutant *fwa-1* in *Ler* background, for mutant data see text). In 8C leaf nuclei, BACs were not hybridized as adjacent pairs but in various combinations. Only 4C leaf nuclei of the *ddm1* mutant were studied.

**FIGURE 3.** Positional sister chromatid alignment/separation as identified after FISH with BACs from different positions along chromosome 1. **A** Alignment versus separation at BAC insert positions (T2P11 and T1F9) of both homologues in a 4C and **B<sub>1-3</sub>** in a 8C leaf nucleus; note eight signal groups (encircled) in **B<sub>2</sub>**. **C<sub>1-4</sub>** Alignment/separation at a distal (T11I11), an intermediate (T1F9) and a proximal (F2H10) position of chromosome 1 bottom arm in a 16C and **D<sub>1-4</sub>** in a 32C nucleus. **E** Proportion of 8C, 16C and 32C nuclei with varying numbers of FISH signals. **E<sub>1</sub>** Two BACs from the middle of the top and the bottom-arm of chromosome 1

(Fig. 2) showing a different degree of sister chromatid alignment in 8C nuclei. **E<sub>2,3</sub>** The centromere-adjacent position F2H10 is more often aligned (i.e. showing less signals) than the other two positions in 16C (**E<sub>2</sub>**) and in 32C (**E<sub>3</sub>**) nuclei. Bars = 5  $\mu$ m

**FIGURE 4.** Centromeric/pericentromeric sister chromatid alignment.

**A** Scheme of an *A. thaliana* chromosome with the 178 bp centromere-specific sequence (pAL) surrounded by pericentromeric heterochromatin that together form DAPI-intense chromocenters. **B** Alignment of centromeric regions in a 4C and **C<sub>1</sub>** in a 32C nucleus, **C<sub>2</sub>** partial separation/dispersion in a 32C and **C<sub>3</sub>** extended centromere dispersion in a 32C nucleus as visualised by DAPI-staining (top), FISH with pAL (middle) and after merging (bottom). Chromocenters not co-localizing with pAL signals represent NOR-specific heterochromatin of chromosomes 2 and 4. **D** Number of centromeric FISH signals in nuclei of different ploidy level. From 4C to 8C, nuclei display mainly alignment of centromeres ( $\leq 10$  pAL signals). In contrast, some 16C and the majority (86.5%) of 32C nuclei show clear separation, i.e.  $>10$  centromeric signals, or signal dispersion. Bar = 5  $\mu$ m

**FIGURE 5.** Positional sister chromatid alignment and/or separation at the *lacO* insertion sites in 4C leaf nuclei of *A. thaliana* line EL702C. **A** Scheme of chromosome 3 of line EL702C showing the *lacO* insertion sites (green) and the flanking regions MGL6 (red) and F18C1 (blue). **B** Selected images of nuclei after FISH with *lacO*, MGL6 and F18C1 and schematic interpretations below. **B<sub>1</sub>** Separation of homologous chromosomes but alignment of sister chromatids of both homologues. **B<sub>2</sub>** Allelic pairing and sister chromatid alignment of both homologues. **B<sub>3</sub>** Ectopic pairing within one homologue and sister chromatid alignment concerning both homologues. **B<sub>4</sub>** Allelic and ectopic pairing and sister chromatid alignment of both homologues. **B<sub>5</sub>** Ectopic pairing and sister chromatid separation at one insertion site on both homologues (unambiguous as to separation). **B<sub>6</sub>** Either allelic and ectopic pairing

between both loci of both homologues and sister chromatid separation at the distal locus of one homologue or allelic pairing between the proximal loci of both homologues, additional ectopic pairing involving one homologue and sister chromatid alignment of both homologues (ambiguous situation as to sister chromatid separation, see text). Bars = 3  $\mu\text{m}$

**FIGURE 6.** Positional separation of sister chromatids of one homologue at position F22L4 (green) of chromosome 1 in a replicating meristematic root nucleus after BrdU pulse labelling (red). Bar = 5  $\mu\text{m}$ .

**FIGURE 7.** Presumed cohesion (red) along an undamaged metaphase chromosome of the field bean (left) and along a homologue with a circular interstitial chromatid deletion of (darkly stained) heterochromatic material from the left chromatid (right) imaged after Giemsa banding. The ring-shaped deleted chromatin remained attached to the homologous region of the undamaged sister chromatid (modified after Schubert et al. 1994). Bar = 1  $\mu\text{m}$

**FIGURE 8.** Various possibilities of sister chromatid alignment/separation at different positions along chromosomes of an 8C nucleus.

Figure 1.

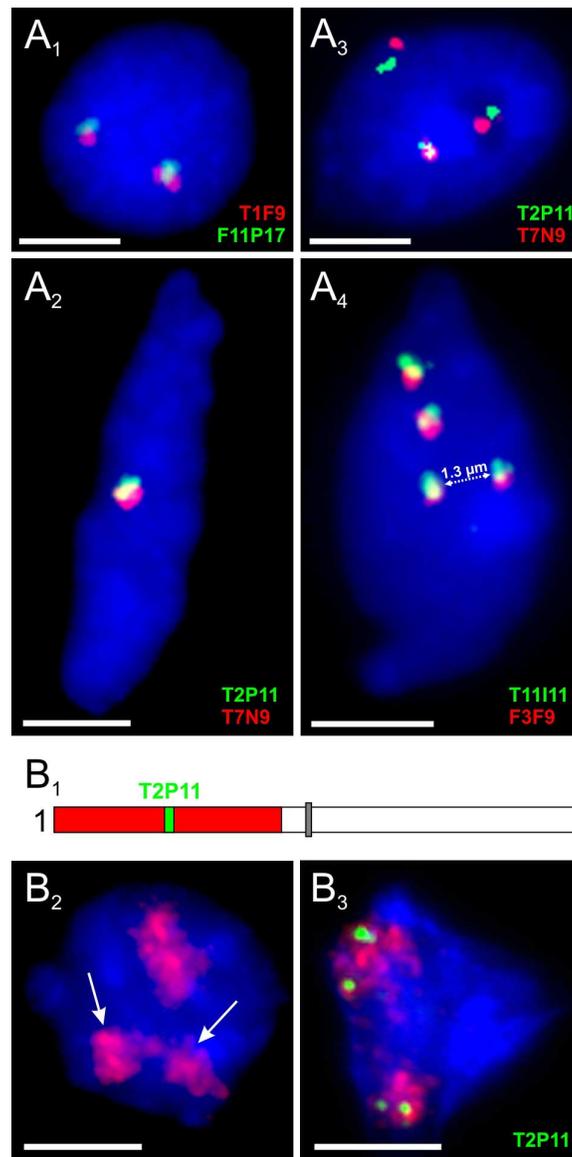


Figure 2.

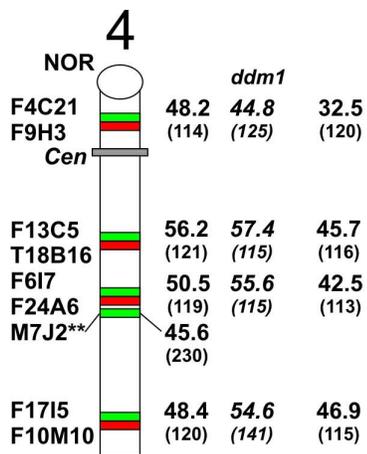
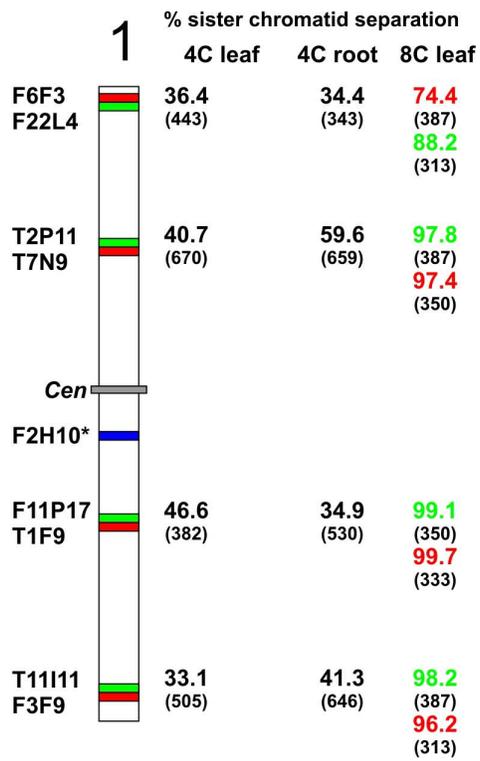


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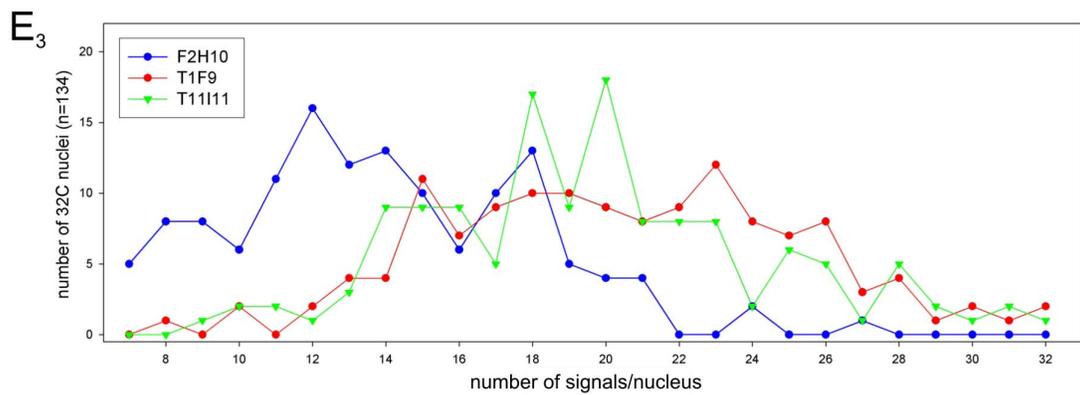
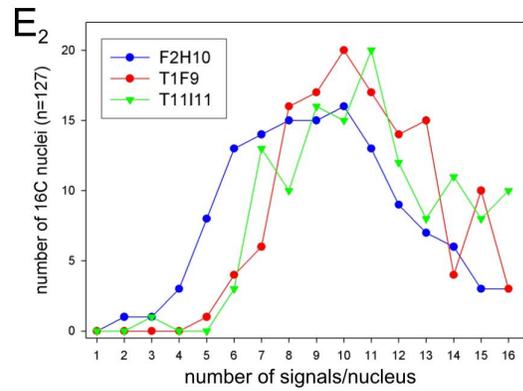
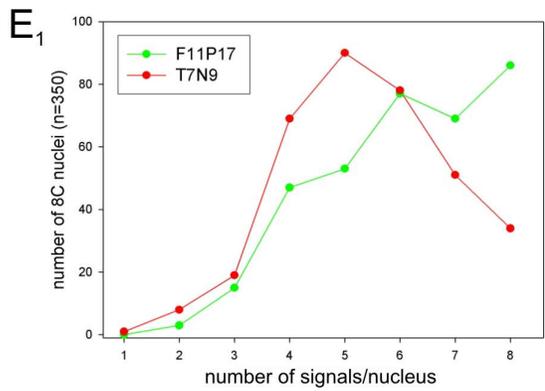
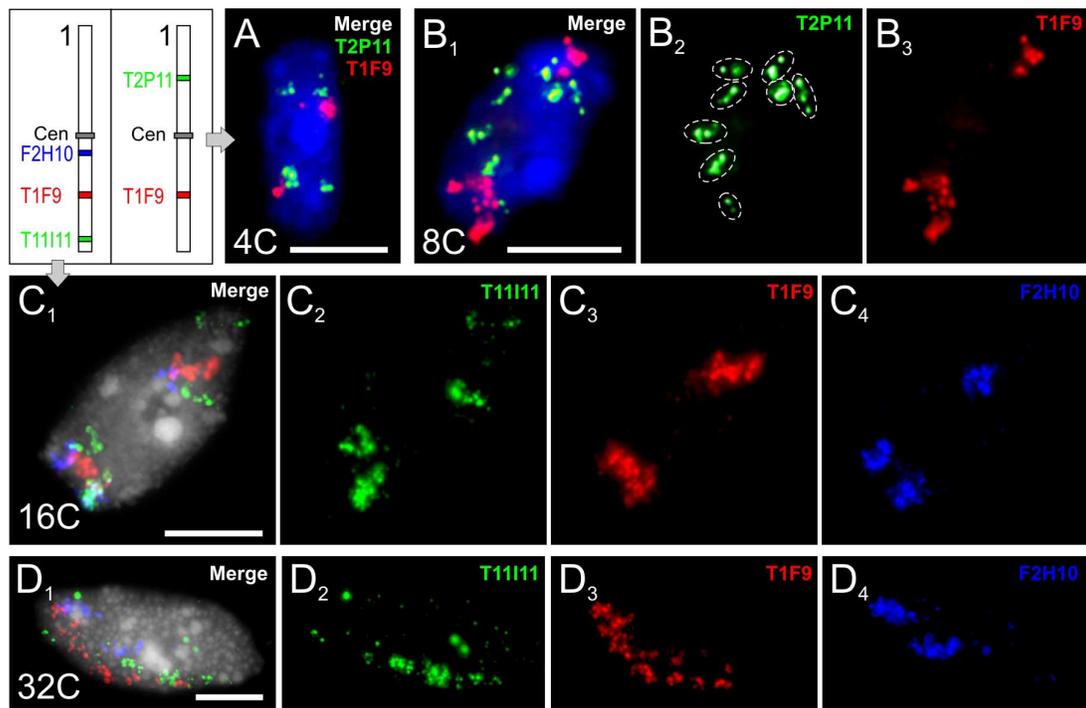


Figure 4.

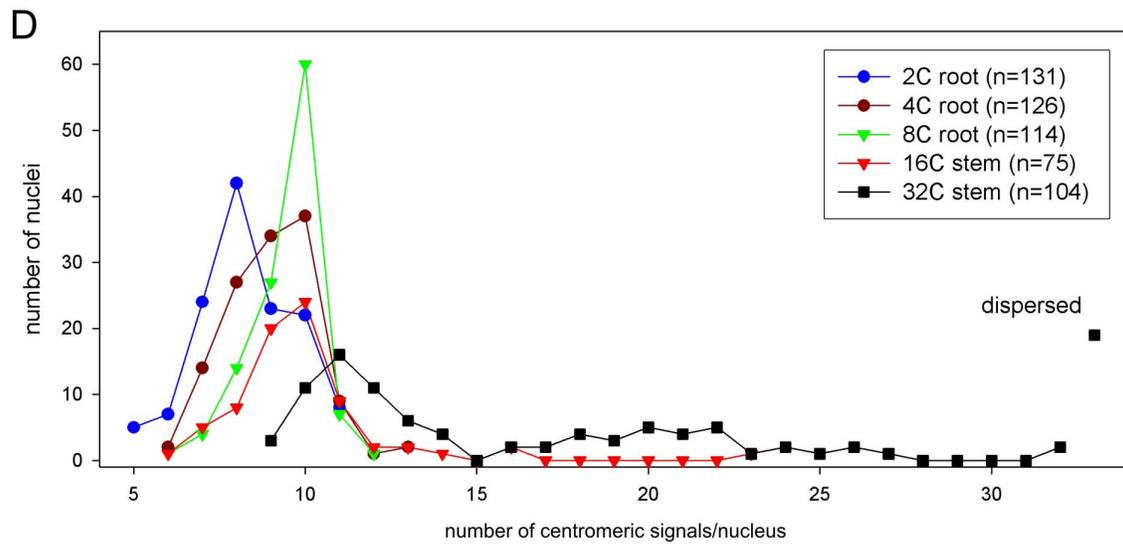
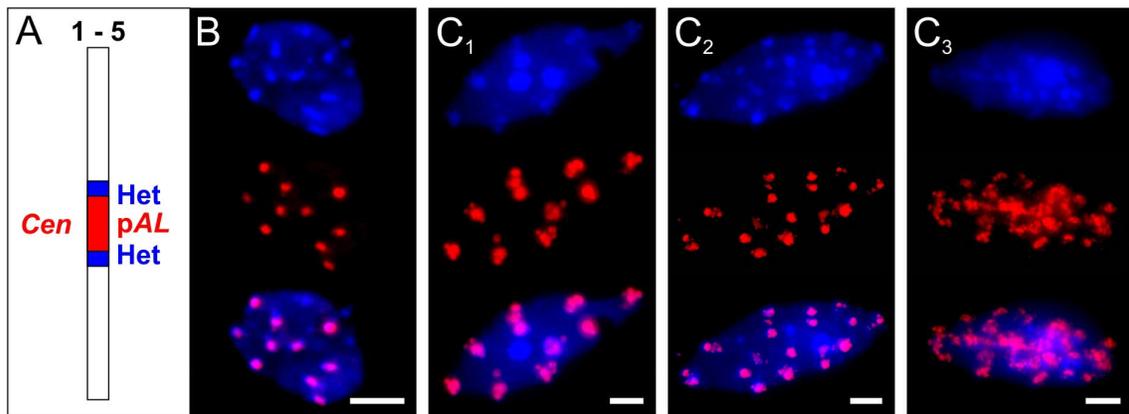


Figure 5.

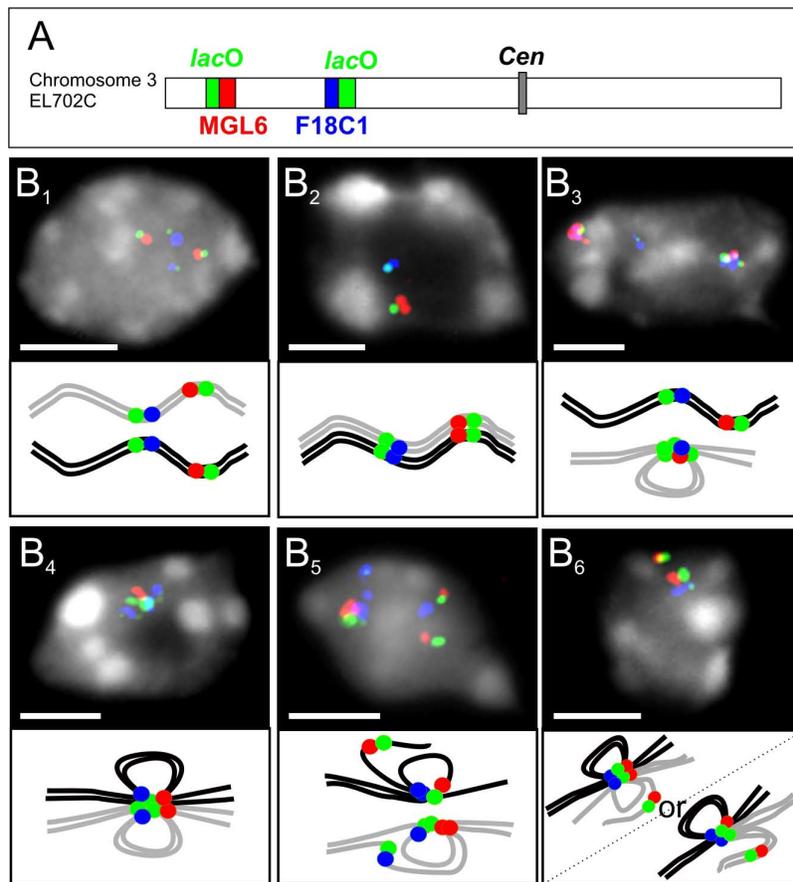


Figure 6.

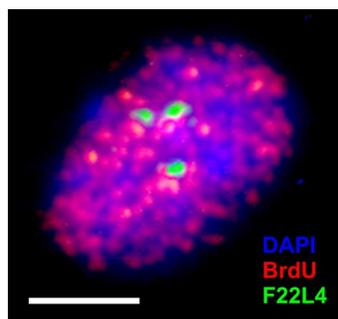


Figure 7.

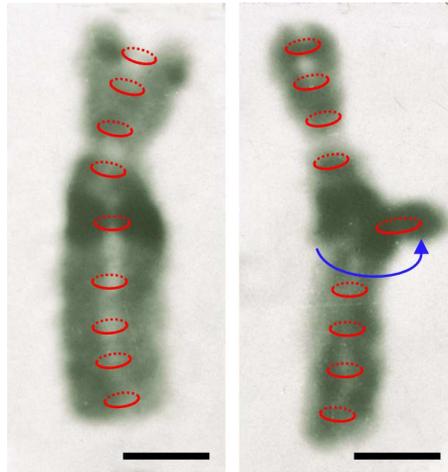


Figure 8.

