

Interphase chromosomes and the Rabl configuration: does genome size matter?

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Summary

It is now well established that the cereals share a common gene order or gene synteny. However, the cereal species encompass an enormous range of genome size, with wheat being one of the largest and rice one of the smallest. Here we describe the current state of knowledge of interphase chromosome structure within the cereal species. In wheat and its close relatives, the interphase chromosomes adopt a highly regular Rabl configuration, with the two chromosome arms lying next to each other and the centromeres and telomeres located at opposite poles of the nuclei. By contrast, the chromosomes in most rice nuclei clearly do not show a Rabl configuration. Surprisingly, the chromosomes in the endoreduplicated xylem vessel cells of rice do adopt a Rabl configuration. To explain this observation, we propose that endoreduplication may occur immediately after chromosome segregation in these cells, and that the new chromatin interactions, particularly at the centromeres, in the endoreduplicated chromosomes may stabilize the anaphase chromosome configuration.

Introduction

Wheat (*Triticum aestivum*) and rice (*Oryza sativa*) have been used as contrasting models in our laboratory to understand the three-dimensional (3D) interphase chromosome organization in plants. Hexaploid wheat (AABBDD) has a very large genome estimated at 17 000 Mbp (Heslop-Harrison, 1991) whereas the rice genome is about 430 Mbp (Arumuganathan & Earle, 1991). This huge difference in the amount of total DNA is partly due to the fact that wheat is a hexaploid species, but it is mainly due to the difference in the amount of the repetitive DNA sequences, which evolved and diverged during cereal speciation. This repetitive DNA does not show conservation between different cereal species, and is mainly seen in

the interphase nuclei as heterochromatin. Nevertheless, there is a high degree of conservation of the gene content of all the cereals, and a high degree of synteny in the gene order, so that a collinear map can be drawn that shows a correspondence of many genetic markers in the different cereal species (Gale & Devos, 1998). The significance of the wide variation in genome size is still an open question and it has been suggested that there is a correlation between the genome size and nuclear organization.

Genomic *in situ* hybridization (GISH) uses a total genomic probe to label introduced alien chromosomes or chromosome arms. It has been a useful approach to visualize interphase chromosomes in plants and thus to understand functional aspects of chromosome organization in interphase nuclei. For example, how are chromosomes arranged within the interphase nucleus, how are the chromosome arms arranged with respect to each other; and how do the chromosomes fit within the interphase nucleus? The observation of entire rye interphase chromosomes in the wheat genome showed that they appear as discrete and elongated domains spanning the width of the nucleus, usually parallel to one another with the two arms of each chromosome lying alongside each other and physically close together (Abranches *et al.*, 1998). More recently, in *Arabidopsis*, Fransch *et al.* (2002) have used a series of BAC (bacterial artificial chromosome) DNA probes covering a large fraction of chromosome 4, probes to 45S and 5S ribosomal DNA and antibodies to acetylated histones to show that the centromeric chromocentres, rich in repetitive DNA sequences, show heavy DNA methylation and low levels of histone H3 acetylation. They proposed a model in which gene-rich, transcriptionally active euchromatic loops with high levels of histone H3 acetylation emanate from the methylated chromocentres.

We have recently developed methods and *in situ* markers to analyse 3D chromosome organization in the model cereal species, rice. The chromosomes in most rice nuclei clearly do not show a Rabl configuration. Surprisingly, the chromosomes in the endoreduplicated xylem vessel cells of rice do adopt a Rabl configuration. To explain this observation, we propose that endoreduplication may occur immediately after chromosome

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segregation in these cells, and that the new chromatin interactions, particularly at the centromeres, in the endoreduplicated chromosomes may stabilize the anaphase chromosome configuration.

Results and discussion

In wheat, centromeres and telomeres are polarized in a classic Rabl configuration

In wheat, *in situ* hybridization with the centromeric sequence (CCS1) and the telomeric repeat (TTTAGGG) showed that the centromere and telomere sequences are clustered at opposite sides of the nuclear envelope, defining a clear nuclear polarization, a classic Rabl configuration, that is maintained through

several rounds of cell division (Abranches *et al.*, 1998) (Fig. 1). This organization is seen in all cell types of wheat that have been examined. It is also seen in many close relatives of wheat, both diploid and polyploid.

In rice, centromeres and telomeres are not polarized in diploid root cells

Analysis of rice centromeric sequences indicated that the centromere is a complex region with stretches of tandemly repeated sequences intermixed with middle repetitive elements (Dong & Jiang, 1998). At least seven centromeric repetitive DNA families have been described in the rice centromere, six middle repetitive sequences (50–300 copies) and one consisting of four copies of a tandemly arranged repeat with a

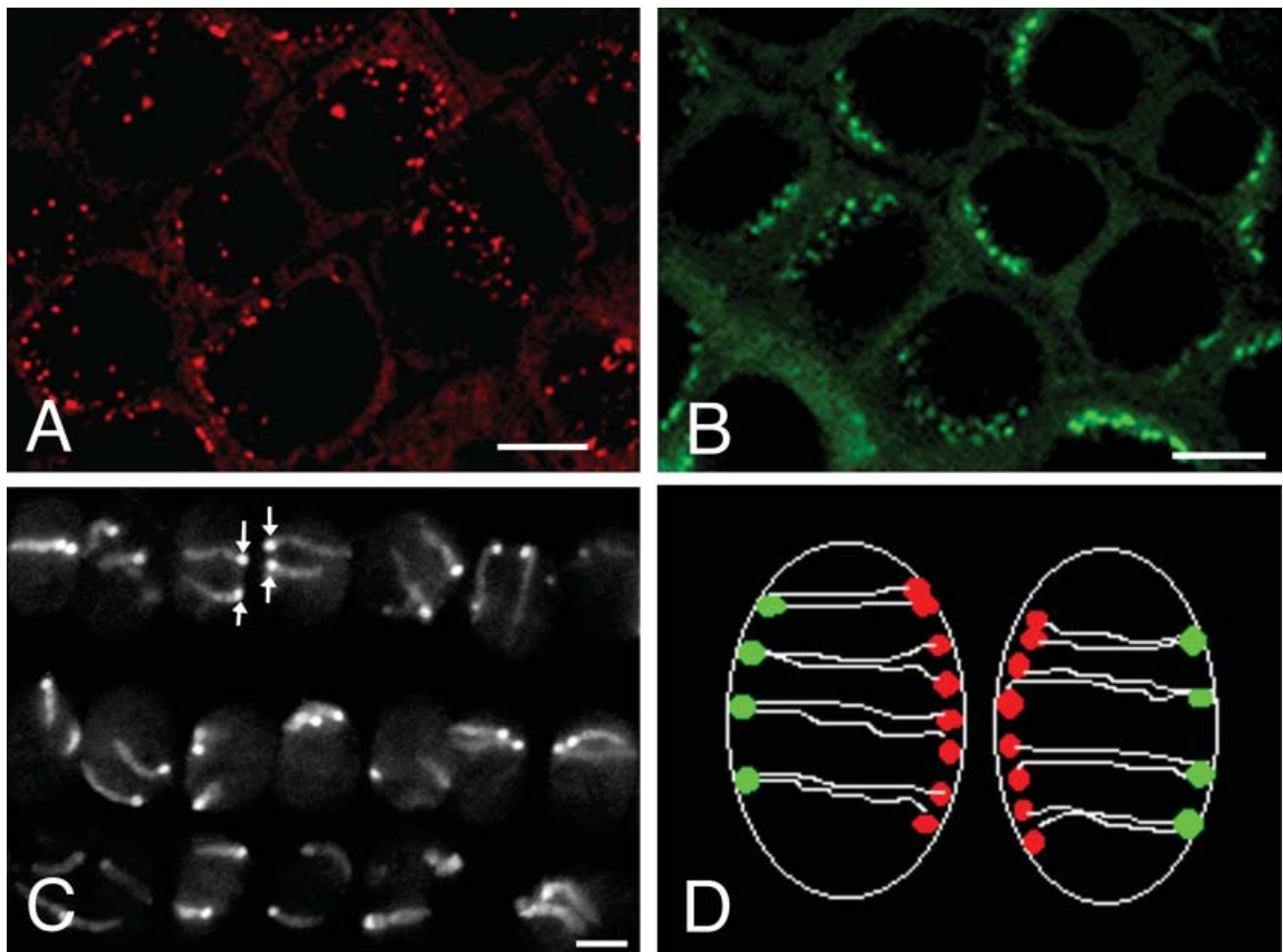


Fig. 1. The interphase nuclei of wheat root meristematic cells show a Rabl configuration. Individual confocal images are shown in A–C. Confocal image stacks were recorded with a section spacing of 1 μm . Telomeres (A, labelled in red) and centromeres (B, labelled in green) are localized in opposite sides of the interphase nucleus. (C) Genomic *in situ* hybridization in intact root tissue sections from a wheat/rye translocation line ($1A^L/1R^S$) was used to visualize the rye chromosome arms ($1R^S$) in which heterochromatic blocks at subtelomeric regions can be seen as intensely labelled regions at one end of each arm, located at the nuclear periphery (arrows). (D) An interpretation of A, B and C showing chromosomes in the Rabl configuration and the centromeres and telomeres in opposite sides of the nucleus. Scale bars = 10 μm .

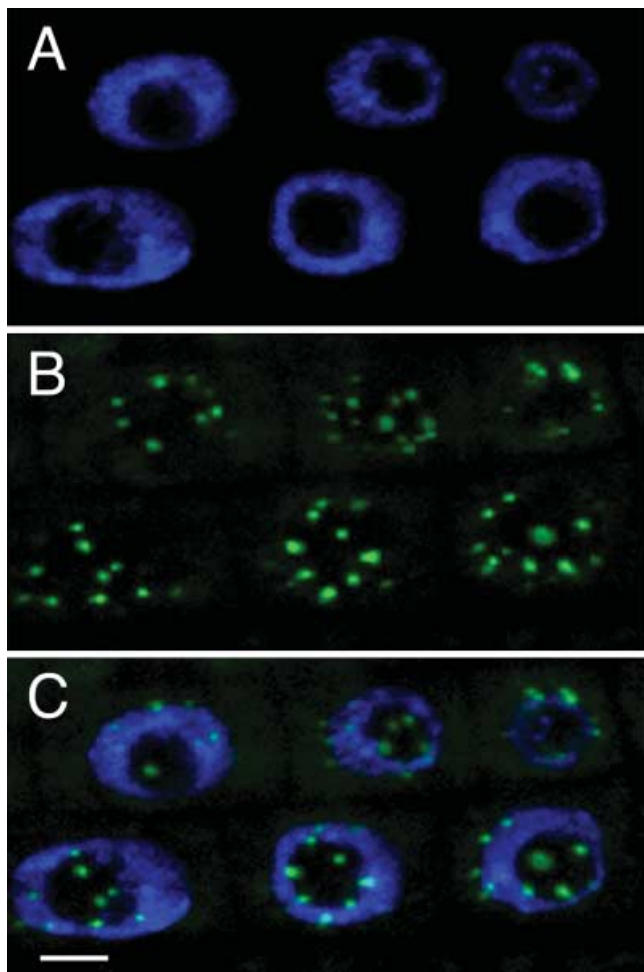


Fig. 2. Arrangement of centromeres in interphase nuclei of diploid cells of rice root sections. Individual confocal images are shown. (A) DAPI staining of DNA; (B) the centromeres (labelled in green) are dispersed around the periphery of the nucleus, encircling the nucleolus. (C) Merged image. Scale bar = 5 μm .

consensus sequence of 168 bp (RCS2). This sequence is unique to the centromeric regions on all rice chromosomes (Jiang *et al.*, 1996). We used probes to the telomeric and RCS2 centromeric regions for *in situ* hybridization experiments. Figure 2 gives an example of labelling of the rice centromeres, showing that these parts of the chromosomes are dispersed around the periphery of the nucleus, encircling the nucleolus. Thus, these nuclei clearly do not show a Rabl configuration.

There is a Rabl configuration in rice xylem vessel cells

Endoreduplication occurs in certain cell types, and consists of replication of the genome without mitosis, resulting in an increase in ploidy. We have shown previously that endoreduplication occurs in the files of developing xylem vessel cells in wheat roots (Martinez-Perez *et al.*, 2001). In rice, too, the xylem vessel cell nuclei are seen to undergo endoredu-

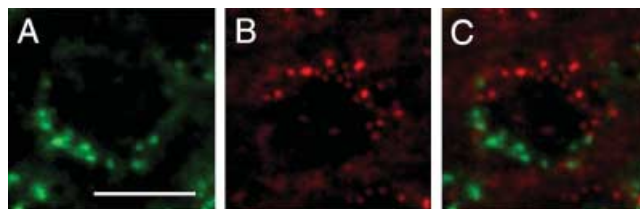


Fig. 3. Xylem vessel precursor cell in rice root tissue. The centromeres are labelled in green, the telomeres in red. In the endoreduplicated xylem vessel nuclei, the chromosomes adopt a clear Rabl configuration. Scale bar = 5 μm . (Figure courtesy of Pilar Prieto.)

plication, and are clearly recognizable by their increased size. Figure 3 shows centromeres and telomeres in a rice xylem vessel cell. It is clear that the chromosomes have adopted a new arrangement, which must be a Rabl configuration, because centromeres and telomeres are seen on opposite sides of the nuclear periphery.

There is no pairing of homologous chromosome regions in diploid root cells of rice, but centromeres pair in the xylem cells

As a first step to making a chromosome paint for rice, we have used *in situ* hybridization with probes made from selected BACs. Based on the physical map for rice, the predicted size of chromosome 1 of rice is about 44 Mb (Chen *et al.*, 2002). We selected a set of 35 rice BAC clones covering a large fraction of chromosome 1 and performed *in situ* hybridization on intact rice root vibratome sections. We initially tested individually the 35 selected rice BAC clones to determine whether the presence of repetitive sequences in the BAC clones would generate high levels of non-specific hybridization. We identified two discrete hybridization signals in 14 of the 35 rice BAC clones tested, showing specific hybridization to unique chromosomal locations (Fig. 4). The remaining clones either showed labelling of several sites or widespread non-specific labelling, which we presume was due to the repetitive sequences in the BACs.

In situ hybridization with these BAC clones was used as a tool to investigate the occurrence of associations between homologous chromosomes. In all cases in the diploid root nuclei, we observed two well-separated foci showing that homologous pairing was not occurring (Fig. 4, Table 1). Very often, each of the two foci was resolvable into two closely adjacent spots, corresponding to the two chromatids in G2 nuclei (Fig. 4B). We obtained the same results with probes either to the centromeric or telomeric regions or to regions in the middle of the chromosome arms. However, when we examined the xylem vessel cells, we found that the two signals corresponding to BACs close to the telomeres or the centromeres were significantly closer together than in the diploid root cells (decreasing from a mean distance apart of about 4 μm to about 2 μm – P. Prieto *et al.*, unpubl. data). This suggests that there is a tendency for homologues to associate in the endoreduplicated

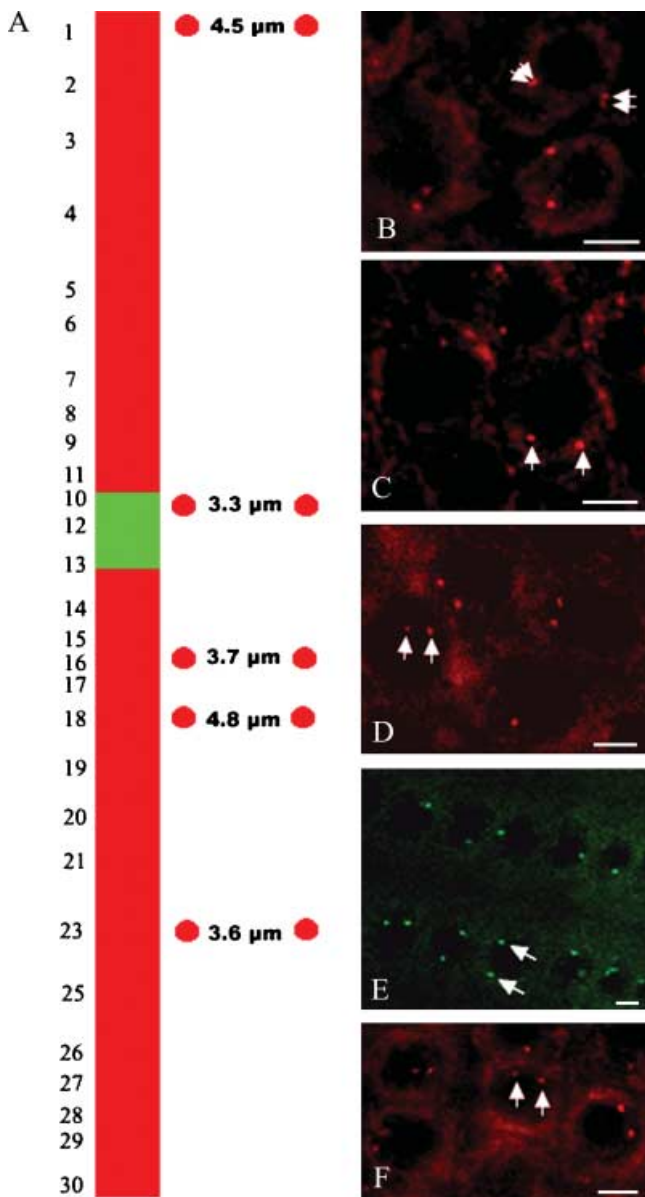


Fig. 4. Fluorescence *in situ* hybridization with bacterial artificial chromosomes (BACs) clones, which identify specific chromosome regions in interphase nuclei of diploid cells of rice root sections. (A) Physical map of chromosome 1 (~44 Mb, total size); the numbers represent the contigs that cover the chromosome, and the centromere region is indicated in green (adapted from Chen *et al.*, 2002; www.genome.clemson.edu/projects/rice/fpc/integration). (B–F) Individual confocal sections labelled with the following BAC probes: (B) a0002C21 – contig 1; (C) a0091E23 – contig 10; (D) a0040G11 – contig 16; (E) a0085L12 – contig 18; (F) a0019J20 – contig 23. Two discrete hybridization signals are indicated by arrows for each BAC. Two closely adjacent dots for each chromatid in a G2 phase cell can also be discriminated, as indicated by double arrows in B. The average of the linear distance between two hybridization signals (μm) for each BAC is indicated between the red circles.

xylem cells. This was confirmed by counting the number of centromeres seen per nucleus. This reduced to half the number seen in the diploid root cells – i.e. the haploid number – in the xylem vessel cells (P. Prieto *et al.*, unpubl. data). As yet we do not know the extent of the endoreduplication in the xylem vessel cells, neither do we know whether the Rabl configuration is adopted immediately on going from 2C to 4C, or requires a higher ploidy level.

What is the biological significance of the Rabl configuration?

The contrast between the rice and wheat chromosome architecture is striking. The further change in chromosome conformation in the endoreduplicated xylem vessel nuclei in rice is even more intriguing. It immediately raises the question of what causes the presence or absence of the Rabl conformation.

Dong & Jiang (1998) surveyed a variety of plant species using chromosome squashes, and also reported that rice did not adopt a Rabl configuration, in common with sorghum and maize. The Rabl configuration was observed in barley and oats (Dong & Jiang, 1998). It is also seen in *Drosophila melanogaster* (Hochstrasser *et al.*, 1986; Marshall *et al.*, 1996; Marshall & Sedat, 1999), in *Schizosaccharomyces pombe* (Funabiki *et al.*, 1993) and *Sacharomyces cerevisiae* (Gilson *et al.*, 1993; Palladino *et al.*, 1993; Jin *et al.*, 1998). It has been suggested that the difference between species that show a Rabl configuration and those that do not lies in the relative genome sizes and chromosome lengths (Dong & Jiang, 1998). However, there are clearly other factors, because *S. pombe* and *S. cerevisiae* have very small genomes and small chromosomes, and yet show the Rabl configuration, whereas other species such as mouse (Billia & De Boni, 1991) and human (Manuelidis, 1984, 1985; Manuelidis & Borden, 1988; Ferguson & Ward, 1992; Vourc'h *et al.*, 1993; Croft *et al.*, 1999; Bridger *et al.*, 2000) have large genomes and long chromosomes, but do not show the Rabl configuration.

The Rabl configuration may be regarded as the default chromosome configuration, as it is the direct consequence of the anaphase configuration of the chromosomes. We should probably ask why not all species and cell types adopt this chromosome configuration, rather than trying to explain why some do. The simplest hypothesis then might be that in all nuclei the chromosomes enter interphase in a conformation resembling the Rabl configuration, but then decondense into their interphase conformations. In some species the usual interphase configurations resemble the anaphase chromosome configuration, and thus a Rabl configuration is produced in the nucleus as a whole. This is clearly the case for wheat, rye, barley and closely related cereals, as well as for *Drosophila*. In other cases, the change into interphase chromosome structure changes the conformation of each chromosome so that it is no longer in the 'hairpin' anaphase conformation. This is clearly the case with human chromosomes. Thus, we predict this will be the case with rice chromosomes, although to date there are no data on the detailed interphase chromosome territorial organization

Table 1. Distance between two BAC signals (μm) in root diploid cells. The standard deviation (SD) is given in parentheses.

BAC	Average of the linear distance between two BAC signals (μm)	No. of cells analysed	BAC position in the chromosome contig no. (based on Chen <i>et al.</i> , 2002)
a0002C21	4.5 (\pm 2.1)	42	1
a0091E23	3.3 (\pm 1.7)	78	10
a0040G11	3.7 (\pm 1.5)	84	16
a0085L12	4.8 (\pm 1.5)	55	18
a0019J20	3.6 (\pm 1.3)	77	23

in rice. Whether the interphase conformation, or set of conformations, resembles the anaphase conformation probably depends on the distribution of heterochromatin, and its behaviour on entry into interphase. Thus in wheat, the chromosomes predominantly consist of heterochromatin, and the heterochromatin is distributed throughout the chromosomes. Probably only relatively minor changes affecting specific chromosome regions occur on entry into interphase. In *Arabidopsis*, by contrast, most of the heterochromatin is around the centromeres, and the remaining euchromatic parts of the chromosomes probably adopt a markedly different conformation on entry into interphase.

How then can we explain the change to a Rab1 configuration that we observe in the endoreduplicated rice xylem nuclei? Following the previous argument, we might suggest that in these nuclei, the final mitotic cell division is followed by a further round of replication, before the chromosomes have altered into their interphase configuration. This may have the effect of 'locking' some aspects of the chromosome configuration into the anaphase, Rab1 configuration by making new sets of interactions. There is good evidence that after many endoreduplication events, the replicated chromosomes remain together to a greater or lesser extent. This is seen most spectacularly in insect polytene nuclei, but is also clearly the case in wheat xylem vessels (Martinez-Perez *et al.*, 2001). In both these cases of polytene nuclei, the homologues also associate. In the case of wheat, the association seems to initiate at the centromeres, and is affected by the Ph1 locus (Martinez-Perez *et al.*, 2001). In *Drosophila*, the centromeres of all the chromosomes cluster into a single massive chromocentre. Thus, one of the most important factors in 'locking' the Rab1 configuration may be centromere interactions. This is logical; the major conformational change needed to change from the hairpin anaphase configuration to a more extended conformation is a hinging around the centromere. Thus, the conformation of the centromere present at anaphase may be stabilized by interaction between the endoreduplicated centromeres. A physical analogy is the way in which a woodworker constructs curved shapes by gluing layers of thin wood veneers together while they are held in a curved conformation; the glue between the veneers locks the curved conformation.

In addition to this, there may be other factors that stabilize the Rab1 configuration, such as specific interactions between

centromeres, telomeres and the nuclear envelope, for which there is evidence in plant nuclei. Furthermore, centromere clustering can take place independently of mitosis (Jin *et al.*, 2000; Goto *et al.*, 2001). In *S. cerevisiae*, centromere clustering was seen independently of anaphase chromosome polarization after mitosis was experimentally disrupted by the microtubule poison nocodazole. Following this treatment, centromere clustering was reconstituted in the absence of anaphase (Jin *et al.*, 2000).

Centromeres are clearly chromosomal sites that are capable of making strong associations with each other; the sister chromatids are strongly linked to each other at the centromeres prior to mitotic segregation, and such interaction sites must depend on an accessible centromere conformation as well as the presence of the necessary proteins. This may provide the beginnings of an explanation of the link between endoreduplication and centromere association. According to our hypothesis, endoreduplication can fix the anaphase chromosome and centromere configuration. This might also result in the maintenance of a suitable 'interacting' centromere conformation (together with the necessary proteins). In fact, it may increase the available interaction sites by the fact of endoreduplication. In this hypothesis, then, the more endoreduplication that occurs, the more likely it is that centromeres will interact with other centromeres. In the extreme case, this may cause the fusion of all the centromeres into a chromocentre, as in *Drosophila* salivary gland nuclei.

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