### Title

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ORIGINAL ARTICLE

# Out-of-position telomeres in meiotic leptotene appear responsible for chiasmate pairing in an inversion heterozygote in wheat (*Triticum aestivum* L.)

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## Abstract

Chromosome pairing in meiosis usually starts in the vicinity of the telomere attachment to the nuclear membrane and congregation of telomeres in the leptotene bouquet is believed responsible for bringing homologue pairs together. In a heterozygote for an inversion of a rye (*Secale cereale* L.) chromosome arm in wheat, a distal segment of the normal homologue is capable of chiasmate pairing with its counterpart in the inverted arm, located near the centromere. Using 3D imaging confocal microscopy, we observed that some telomeres failed to be incorporated into the bouquet and occupied various positions throughout the entire volume of the nucleus, including the centromere pole. Rye telomeres appeared ca. 21 times more likely to fail to be included in the telomere bouquet than wheat telomeres. The frequency of the out-of-bouquet rye telomere position in leptotene was virtually identical to the frequency of telomeres deviating from Rabl's orientation in the nuclei of somatic cells, and was similar to the frequency of synapsis of the normal and inverted chromosome arms, but lower than the MI pairing frequency of segments of these two arms normally positioned across the volume of the nucleus. Out-of-position placement of the rye telomeres may be responsible for reduced MI pairing of rye chromosomes in hybrids with wheat and their disproportionate contribution to aneuploidy, but appears responsible for initiating chiasmate pairing of distantly positioned segments of homology in an inversion heterozygote.

**Keywords** Telomere · Centromere · Leptotene bouquet · Pairing initiation · 3D FISH

## Introduction

Meiotic metaphase I pairing of homologues is critical for proper reduction of the chromosome number, hence efficient production of functional gametes. The process of chromosome pairing is logistically complex, as homologues have to find each other, verify their identity, and align properly to initiate the process of chiasma formation, regardless of the total number of chromosomes present in the nucleus. A failure of the metaphase I (MI) pairing results in random segregation of homologues, which in most cases produces gametes with abnormal chromosome numbers. Gametes which deviate from the standard haploid chromosome number are either non-functional or transmit such chromosome aberrations to progeny.

The complex process of homologue search is simplified by the polarity of the nucleus, the so-called Rabl's orientation (Fussel 1987) which in essence preserves the chromosome arrangement from the previous anaphase, with all centromeres congregating on one pole of the nucleus and telomeres on the other. This reduces a three-dimensional problem of homologue search and alignment to two dimensions. Moreover, in most species, in early meiotic prophase, telomeres sliding along the nuclear membrane cluster in a narrow space of the telomere pole forming the so-called leptotene (or telomere) bouquet (Dawe 1998; Harper et al. 2004). Telomere clustering and bouquet formation appear to occur only if telomeres are

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recruited to the nuclear envelope. At the onset of meiosis, chromosomes are linked to the nuclear membrane and through the nuclear envelope proteins (LINC, NEAP, and others) to the cytoskeleton (reviewed in Alleva and Smolikove 2017). Transformation studies clearly demonstrate that it is the telomeric repeat that is responsible for the migration of telomeres into the bouquet (Carlton and Cande 2002).

The leptotene bouquet is believed critical for homologue identification and offers sufficient proximity of homologues to initiate the process of synapsis (Bass et al. 2000; Bass 2003; Harper et al. 2004; Scherthan 2001, 2007). Misalignment of telomere regions of homologous pairs in the leptotene bouquet dramatically reduces the MI pairing frequency, or may prevent it completely (Moens et al. 1989). In hexaploid wheat, heterozygosity for a deficiency of ca. 34% of a chromosome arm (missing terminal 34% of the arm) reduced MI pairing from normal to 3%; heterozygosity for even longer segments prevented all MI pairing (Curtis et al. 1991). At the same time, homozygosity for deletions (missing proximal segments of chromosome arms) of similarly long segments had little effect on MI pairing. This effect was equally striking for arms of asymmetrical isochromosomes (Lukaszewski 1997a).

During routine screening of some cytogenetic stocks of wheat, rye chromosome 1R was identified with an inversion of almost an entire long arm. Observations of meiotic behavior of this chromosome offered several interesting insights into pairing initiation and chiasma formation and distribution in wheat. These observations demonstrated that only distal ca. half of the arm was capable of crossing over (Lukaszewski 2008) and that a gradient of increasing crossover frequency toward the telomere persisted after the arm was inverted (Valenzuela et al. 2012), suggesting segment-specific crossover frequencies independent of the segment's location on the telomere–centromere axis. Successful chiasmate pairing of normal and inverted arms suggests that a mechanism of homologue search and pairing/synapsis initiation different from the leptotene bouquet must also exist. In an inversion heterozygote, the crossover-capable segment of the inverted arm, now in direct proximity to the centromere, formed chiasmata with its counterpart in the normal arm, in the vicinity of the telomere (Fig. 1) with consistent frequency. This implies either that the centromere regions of chromosomes can occasionally migrate to the telomere pole of the nucleus, or that telomeres may do so into the centromere pole. In studies of the role of the centromeres in pairing initiation in wheat (Corredor et al. 2007) and in maize (Bass et al. 2000; Carlton and Cande 2002), no observations of such centromere migration have been reported.

Since the first 3D fluorescence in situ hybridization (3D FISH) experiments were performed on tissues of *Arabidopsis thaliana* (Bauwens and Van Oostveldt 1996), rapid and efficient development of confocal microscopy has made it possible to capture high-quality images of separate optical sections and to study the 3D structure of a fragile interphase nucleus (Brakenhoff et al. 1985; Eckardt 2008). The 3D FISH technology has become an excellent tool to understand the spatial and time-based control of chromosomes in biologically important stages of meiosis. 3D telomere FISH was applied to establish the timing of the telomere bouquet formation during meiotic prophase I in maize, and in polyploid species (Bass et al. 1997, 2000; Moore 2002; Schubert et al. 2007). This study was undertaken to identify the mechanism behind occasional pairing of segments of inverted and normal chromosome arms, located on the opposite poles of the nucleus, using 3D microscopy.

## Materials and methods

### Plant material

The leptotene part of this study was conducted on plants of hexaploid wheat (*Triticum aestivum* L.) cv. Pavon 76 heterozygous for chromosome 1R of rye with inverted long arm ($1R_{inv}$) and telocentric long arm of rye chromosome 1R (1RL, normal orientation of the arm) (Fig. 2), as described by Lukaszewski (2008). $1R_{inv}$ was identified during a study involving wheat-rye translocation 1RS.1BL (Lukaszewski 1997b); it was designated $1R_{inv}$ and it is present in substitution for wheat chromosome 1A. Plants with disomic substitution $1R_{inv}(1A)$ were crossed to a ditelosomic substitution line 1RL(1A). Confirmed heterozygotes for chromosomes 1RL and $1R_{inv}$ were grown in the greenhouse of the University of California, Riverside, or in the greenhouse at the Centre for Agricultural Research, Hungarian Academy of Sciences, Agricultural Institute, Martonvásár, Hungary. As a control, telomere positioning in the nuclei of somatic cells was studied in a line of wheat homozygous for a wheat-rye translocation 1AS.1RL. Except for the inversion in $1R_{inv}$, chromosome arm 1RL in all three stocks is identical.

### Three-dimensional fluorescence in situ hybridization

Meiotic disposition of rye telomeres was studied using the 3D FISH (whole-mount ISH) protocol according to Bauwens and Van Oostveldt (1996) with minor modifications making it suitable for *Triticeae.* At meiosis, one of the three anthers in each tested flower was stained in 1% acetocarmine and checked under a microscope. If the early prophase (leptotene) stages of meiosis were present, the remaining two anthers of the flower were fixed in a 3:1 mixture of glacial absolute ethanol and acetic acid and stored at 4 °C overnight. From the following morning, anthers were passaged through 3 days of mild chemical treatments as listed in the original protocol (Bauwens and Van Oostveldt 1996). These treatments preserved the 3D structure of nuclei while making the

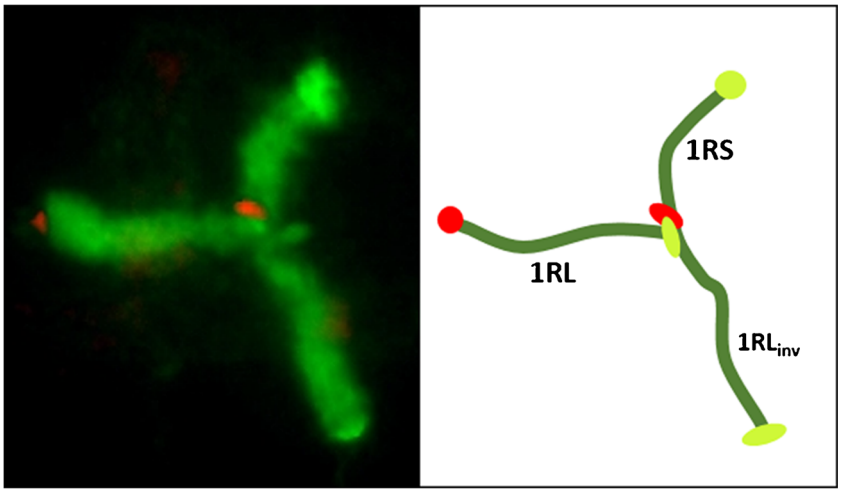



**Fig. 1** An early MI bivalent of telocentric 1RL and 1R$_{inv}$. Centromeres are labeled red; telomeres are lighter green. On the right, schematic interpretation of the configuration: terminal region of 1RL is paired with the centromeric region of 1R$_{inv}$. Telomeres are represented by light green ovals, centromeres by red dots. Modified from Lukaszewski (2008)

cell wall permeable to DNA probes. After final fixation in PBT/1% formaldehyde solution, in situ hybridization was performed on intact anthers. The final volume of 500 μl of the hybridization solution contained 50% formamide, 10% 20× SSC, 2 ng/μl each of the labeled probes used in any given experiment, and distilled water. After pre-incubation at room temperature for 2 h, target and probe DNA were denatured at 90 °C for 4 min and placed on ice for 3 min. Incubation was carried out for 60 h at 37 °C. After post-hybridization washes, digoxigenin and biotin were detected with anti-digoxigenin-Rhodamine Fab fragments (Roche) and Alexa Fluor-488 Streptavidin (Invitrogen) and incubated at 15 °C overnight in the dark. For post-detection washes, 4× SSC containing 0.05% (*v*/*v*) Tween 20 was used for 4 × 15 min at room temperature. Single anthers were placed on a microscope slide and squashed gently to release intact meiocytes retaining their 3D structure, and immediately frozen in liquid nitrogen.

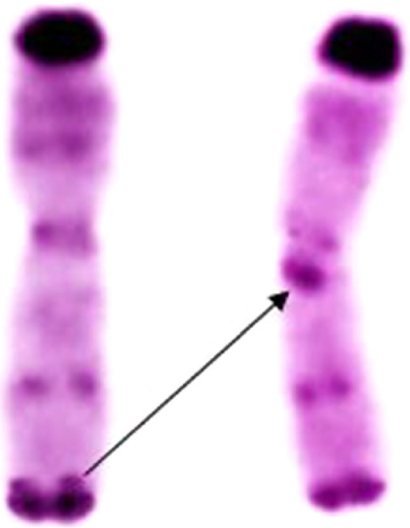


**Fig. 2** Chromosomes 1R and 1R$_{inv}$ after C-banding. Normal chromosome 1R on the left; chromosome 1R$_{inv}$ on the right; arrow points to the extent of the inversion

Counterstaining the chromatin of the nuclei was performed with 0.2 μg/ml DAPI (4′-6-diamino-2-phenylindole) in the VECTASHIELD antifade mounting medium (Vector Laboratories, Burlingame, USA).

Probe labeling was carried out according to Linc et al. (2012). The HT100.3 telomere repeat (TTTAGGG)n sequences were originally isolated and amplified from *A. thaliana* L. (Juchimiuk-Kwasniewska et al. 2011). The centromere repeat probe pAet6-J9 from *Aegilops squarrosa* labels all centromeres in wheat (Zhang et al. 2004). Rye centromere-specific probe pAWRC1 of Francki (2001) was used to visualize rye centromeres and total rye genomic DNA was used to visualize rye chromatin. In the present experiments, repetitive DNA sequences were amplified and labeled by PCR either with biotin-11-dUTP (Roche Applied Science, USA) or with biotin-14-dATP (Invitrogen); rye genomic DNA was labeled with digoxigenin-11-dUTP (Roche Applied Science, USA) by nick translation using standard kits and following manufacturer-recommended protocols. Confocal imaging was carried out with a Leica TCS SP8 confocal laser scanning microscope (Leica, Germany) capturing DAPI, Rhodamine, and FITC signals. Images were processed with the Imaris 3/4D image visualization and analysis software, using a series of 40–60 optical sections of a confocal image stack (0.4 μm each). Images presented here were manipulated to enhance contrast.

As a control, behavior of telomeres of rye chromosome arm 1RL in the nuclei of somatic tissues was done in isolated nuclei of root tips. Nuclei isolation and 3D FISH were done according to Howe et al. (2014) and Phillips et al. (2010) with minor modifications. Root tips from young seedlings were collected and fixed in 2% (*v*/*v*) formaldehyde in the meiocyte buffer A for 20 min at 5 °C. After fixation, root tips were washed and meristem tissue was cut and transferred into a 5-ml sample tube containing 400 μl of buffer A, and homogenized. The homogenate was filtered through 20-μm nylon mesh into a 5-ml polystyrene tube. The nuclear suspension

was stained with 2 μg/ml DAPI (4′,6-diamidino-2-phenylindole) and nuclei in $G_1$ were sorted using a FACSAria II SORP flow cytometer (BD Biosciences, San Jose, CA, USA). Total genomic DNA of rye was labeled with TRITC using a Nick translation kit (Roche Applied Science, Penzberg, Germany) according to manufacturer's instructions, and applied as a probe. For centromere, an oligonucleotide probe based on the sequence of clone pHind258 (Ito et al. 2004) was used. Total genomic DNA of wheat was sheared to 200–500-bp fragments by boiling and used as blocking DNA. Total genomic DNA of rye labeled the rye chromosome arms light red, and their telomeres dark red, so independent labeling of telomeric repeats was not required. FISH mixture included probes, blocking DNA, 30% formamide, and 0.1× saline sodium citrate (SSC). The nuclei were counterstained with 1.5 μg/ml DAPI in VECTASHIELD antifade mounting medium (Vector Laboratories, Burlingame, USA). Probed nuclei were optically sectioned using an inverted microscope Observer Z1 (ZEISS, Germany) with a spinning disk system coupled with the high-resolution Evolve 512 EM CCD camera and ZEN Blue 2012 software. For each nucleus, 80–120 optical sections in 200-nm steps were collected and merged into a 3D model. Subsequent analyses were performed using the Imaris 9.0.2 software (Bitplane, Zurich, Switzerland).

## Results and discussion

Tight clustering of telomeres at one pole of the nuclei was taken as indicative of the leptotene stage (Fig. 3). In all 183 pollen mother cells (PMCs) scored at this stage (Table 1), telomeres and centromeres always occupied opposite poles of the nucleus, but the congregation of the centromeres was not particularly tight, in some PMCs spreading over up to one third of the nuclear volume. As in previous studies (Harper et al. 2004), clustering of telomeres was much tighter than that of the centromeres. In 69.9% of the PMCs, all telomeres were included in the bouquet; in the remaining 30.1% of the PMCs, one and occasionally more, telomeres were outside the bouquet, occupying different positions in the volume of the nucleus (Fig. 3). Of those 30.1% of PMCs, one telomere was clearly present in the centromere pole of the nucleus in 38 cases (20.8% of all nuclei scored). The remaining 9.3% were nuclei with a single telomere, or in several instances two, positioned somewhere between the two poles of the nucleus: the centromere pole and the pole occupied by the cluster of telomeres (Fig. 4). Among the telomeres located between the centromere and the telomere poles, two were clearly located in the interior of the nuclear volume with no apparent contact with the nuclear membrane.

Repeated attempts were made to identify the genomic origin of the telomeres located away from the bouquet itself. This was done with a low concentration of the total genomic rye DNA probe visualized with Alexa-488. In most cases, labeling was either too strong and obscured other signals, or too weak to trace the chromosome. Successful balance was achieved in only 13 nuclei in which an away-from-bouquet telomere was present. Seven of those were rye telomeres (Fig. 4), suggesting that about one half of the out-of-position telomeres belonged to the two rye chromosomes. Given that analyzed plants had 42 chromosomes each, 40 chromosomes of wheat with 80 telomeres and two rye chromosomes with four telomeres, it appears that rye telomeres were ca. 21 times more likely to be out of position (away from the bouquet) than the telomere of an average wheat chromosome. If the sample of leptotene nuclei with out-of-position rye telomeres (7 out of 13) is representative of the entire sample of such nuclei (30.1% of 183 nuclei scored), the probability of a single rye telomere being out of position was 4.2%.

Leptotene behavior of telomeres of normal rye chromosome arms in wheat has been studied in considerable detail (for review, see Naranjo 2018) and so it was assumed that controls made of normal 1RL arms would not be particularly informative in this study, especially given high regularity of their MI pairing (Lukaszewski 2008). Instead, behavior of such arms in somatic cells (root meristems) was analyzed. Labeling was with total genomic DNA and only positions of rye chromosome arms with their telomeres were determined. In a majority of the 106 nuclei scored, rye telomeres were clearly located at one pole of the nuclei, opposite from the centromere pole. However, in nine nuclei, a rye telomere was out of normal position, and located either between telomere pole and centromere pole or at the centromere pole itself (Fig. 5). Given that these plants had two rye telomeres each, the probability of out-of-position placement of any individual rye telomere was 4.25%. Among those nine out-of-position telomeres, one was clearly located in the interior of the nuclear volume. The remaining eight were at the nuclear membrane, either in the centromeric pole (two cases, see Fig. 5) or somewhere between the two poles of the nucleus (six cases). These observations on nuclei of somatic cells indicate that the failure of rye telomeres to assume standard positions in a nucleus is not limited to PMCs but may be systemic in nature. However, apart from similar frequencies, there are no indications at this point of a direct connection between the failure to assume the Rabl's orientation in somatic tissue and failure to incorporate into the bouquet configuration in the leptotene. Still, a point must be made that standard (non-inverted) arms 1RL, identical to those observed here, in the same configuration (complete 1R + telocentric 1RL) and in the same background, paired with 92.6% frequency (Lukaszewski 2008, Table 1), which is quite similar to the 91.5% frequency of somatic nuclei with rye telomeres in a standard Rabl's orientation. Interestingly, the average frequency of the out-of-position location of rye telomeres in the native environment of somatic nuclei of diploid rye is 0.20% (Kolackova and Kopecky,

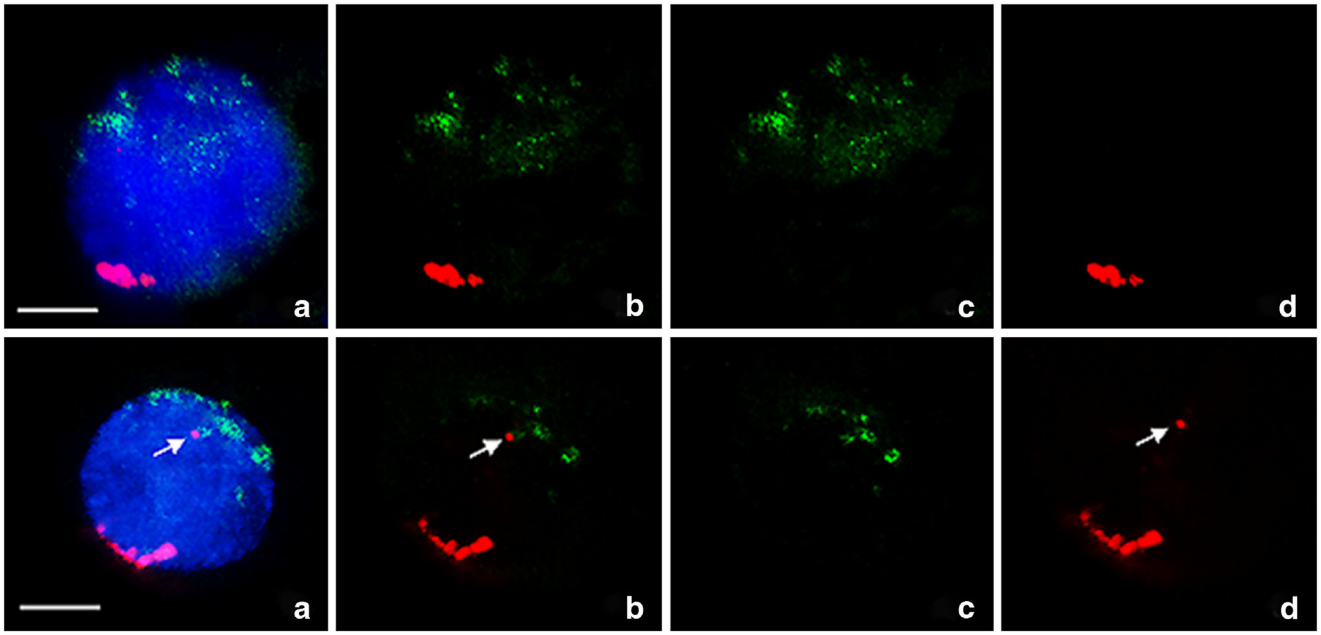



**Fig. 3** 3D FISH images of nuclei at meiotic prophase leptotene. The top row represents a typical leptotene bouquet; all telomeres (red) are clustered on one side of the nucleus; centromeres (green) occupy the other side of the nucleus. Bottom row, one telomere signal is out of the bouquet formation (arrowed). Telomere signals (red) are visualized with digoxigenin-Rhodamine Fab fragments and centromere signals (green) with Alexa Fluor-488 Streptavidin. Meiotic cells are counterstained with DAPI (4′-6-diamino-2-phenylindole). **a** DAPI, telomere, centromere. **b** Telomere, centromere. **c** Centromere. **d** Telomere. Bars represent 5 μm

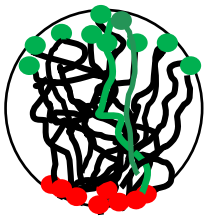
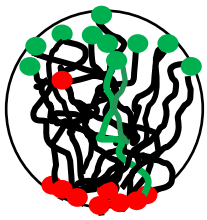
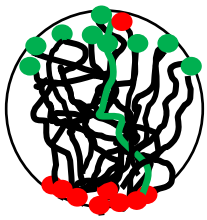
unpublished data) which is very similar to the out-of-position location of wheat telomeres in this study, and to the average MI pairing failure of rye chromosomes in population rye.

All observations in this study were made on fixed material so it cannot be ascertained if the observed telomere positions are static, or represent movement frozen in space at the moment of fixation, even if the latter appears more plausible. Chromosome movements in meiotic prophase are well documented, and formation of the bouquet is only a part of such movements (for review, see Alleva and Smolikove 2017). The meiocyte data presented here include only the nuclei with tight grouping of telomeres, believed to be the bouquet stage, and this may not represent the entire range of telomere movement/positions. The movement of telomere sequences during meiotic prophase in maize, humans, and mice is consistent with a model in which the telomeres attach to the nuclear envelope (NE) randomly during leptotene and then move around the inner surface of the NE until they approach each other (Bass et al. 1997; Scherthan et al. 1996).

MI chromosome arm pairing frequencies were scored previously in plants with the same chromosomes as those analyzed in this study (Lukaszewski 2008). On average, 96.4% of wheat chromosome arms were paired and no wheat univalents were observed. Rye chromosome arm pairing was considerably lower, with 9.2% pairing frequency for the long arm. All cases of the long arm pairing were in the configuration of the telomeric region of the non-inverted arm paired with the centromere region of the inverted arm (see Fig. 1). Interestingly, in pachytene, the two arms were synapsed in 15.1% of cases, indicating that in this configuration even correctly synapsed chromosome arms may not form a chiasma. In inversion heterozygotes, crossovers in the inverted region lead to bridge + fragment configurations in meiotic anaphases. Valenzuela et al. (2012) used this feature to show that the pattern of crossing over in the inverted arm was also inverted. In normal 1RL, the highest density of crossovers (per unit of length) occurs in the euchromatic segment between the terminal and subterminal C-bands (Lukaszewski 1992). In the inverted arm, the highest crossover density was in the same segment, but now immediately adjacent to the centromere (Valenzuela et al. 2012).

The frequency of chiasmate MI pairing of these two chromosomes ($1R_{inv}$ + 1RL) (Lukaszewski 2008) is higher than the frequency of the out-of-bouquet position of the telomeres observed here. This difference may be due to such a simple effect as observations made in different growing seasons and places, or may be an artifact of gauging the proper stage of meiosis. Leptotene, apart from the bouquet formation, lacks clearly identifiable boundaries and it is possible that this study, by selecting meiocytes with a very tight bouquet formation, focused only on a fraction of all meiocytes with active telomere movement. Search for homologues may in fact last much longer, and stretch into the zygotene. Rapid chromosome movements in zygotene, but not in leptotene, were observed in maize (Sheehan and Pawlowski 2009). These movements may reflect ongoing adjustment of homologue positions during progression of synapsis, or repeated attempts at homologue recognition after the bouquet formation has failed.

**Table 1** Frequencies of the telomere–centromere positions in the leptotene stage of meiosis in inversion heterozygotes $1R_{inv}$ + 1RL in wheat. Telomeres represented by red dots, centromeres by green dots, rye chromosome arms by green lines

| Telomere-centromere position | Number of PMCs (Σ183) | Proportions (%) of various configurations |
|---|---|---|
|  | 128 | 69.9 |
|  | 17 | 9.3 |
|  | 38 | 20.8 |

The observations made here can also be explained from a different perspective. Among 183 leptotene meiocytes examined, 16.2% (7/13 of 30.1%) had at least one rye telomere located away from the bouquet. This produces a figure surprisingly close to the frequency of synapsis of the normal and inverted arms (15.1%, Lukaszewski 2008). Rye telomeres not included in the bouquet may, therefore, represent a pairing potential with the segment located in the centromere pole of the nucleus, here captured either at the start of the migration to the other pole, or at the end of it. While this study offers more questions than answers, it does suggest that positioning of telomeres away from the bouquet, or a failure to migrate into the bouquet, may be responsible for recognition of homologous segments and the establishment of chiasmate MI pairing even across the diameter of the nucleus.

The attachment of telomeres to the nuclear envelope and migration along that membrane during formation of the telomere bouquet appears to be the standard operating procedure in the initiation of synapsis and, eventually, chiasmate pairing in most species studied. The movement of chromosomes into the bouquet is driven by the meiotic cytoskeleton (both microtubules and actin) via the machinery of nuclear envelope proteins called LINC (linker of nucleoskeleton and cytoskeleton). Two main classes of LINC were identified in plants: SUN (inner membrane proteins) and KASH (outer membrane proteins). SUN proteins interact with other proteins, such as NEAPs involved in the attachment of the chromosome ends to the cytoskeleton (Graumann et al. 2010; Pawar et al. 2016). Double mutants for SUN1 and SUN2 display severe meiotic defects including a delay in the progression of meiosis, absence of full synapsis, and reduction in chiasma frequency (Varas et al. 2015). Similarly, telomere clustering was reduced in the mutants for KASH domain protein Ksm1 (Shimanuki et al. 1997). The most drastic effect of depleting LINC complex in meiosis was observed in mice, where Sun1 knockout leads to the pachytene arrest and massive apoptosis (Ding et al. 2007). Defective SUN proteins in the *dy* (desynaptic) mutant in maize produce a striking phenotype where a large

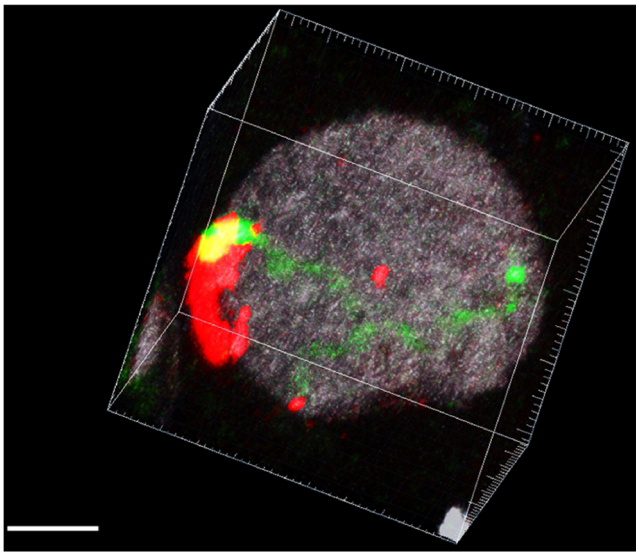


**Fig. 4** 3D reconstruction of a PMC nucleus in leptotene. One wheat telomere and one rye telomere signals are located outside the leptotene bouquet. Rye genomic DNA (green) is visualized with Alexa Fluor-488 Streptavidin and telomeres (red) are detected by digoxigenin-Rhodamine Fab fragments. Bar represents 5 μm

proportion of telomeres in leptotene are scattered throughout the volume of the nucleus, with such consequences as low MI pairing, high proportion of univalents, laggards in anaphase, and reduced fertility (Murphy and Bass 2012). This and Naranjo's (2018) observations on rye chromosomes in wheat link telomere behavior in early meiosis and the MI

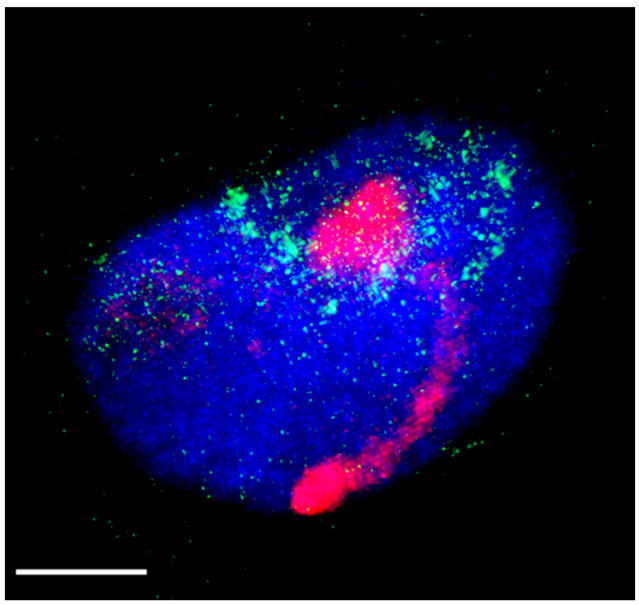


**Fig. 5** 3D reconstruction of a somatic nucleus of a root-tip cell in wheat-rye disomic translocation 1AS.1RL; rye genomic DNA was labeled with TRITC (red) and centromeres were visualized using oligonucleotide probe based on the sequence of clone pHind258 labeled with FITC (green). One of rye chromosome arms is in a typical Rabl's orientation; the second rye arm is folded in the centromere pole of the nucleus. Bar represents 5 μm

chromosome pairing success. Unfortunately, those studies were limited to PMCs. In this study, the frequencies of out-of-position telomeres in PMCs and in nuclei of somatic cells were practically identical suggesting that there may a systemic failure of some rye telomeres to properly position themselves in the nucleus. This effect may carry from somatic tissue into meiotic leptotene, leading to pairing failure. In this sense, making use of somatic nuclei as controls was quite informative.

A majority of out-of-position rye telomeres observed here appeared to be in direct contact with the nuclear membrane but whether they were attached to it or not is an open question. Many different proteins are involved in the attachment of chromosomes to the cytoskeleton among yeasts, plants, and animals. However, their common feature is that they all interact with telomeric repeats (Alleva and Smolikove 2017). Whether the telomeres play a specific active role in this process is unclear (Tiang et al. 2012). The fact that some rye telomeres in wheat often fail to enter the leptotene bouquet is already known and the frequencies of such failure appear related to chromosome structure (Naranjo et al. 2010; Naranjo 2014). For normal chromosome arms, such failure to enter the bouquet stage leads to pairing failure at MI. This may be responsible for reduced MI pairing frequency of rye chromosomes in wheat, for their disproportionate contribution to aneuploidy (Oleszczuk et al. 2011), and at times, elimination of the entire rye genome from wheat-rye amphiploids (Tsunewaki 1964). On the other hand, in an inversion heterozygote as the one studied here, out-of-position rye telomeres (and consequent aberrant positioning of the entire chromosome arm) appear to lead to contacts and chiasmate pairing of homologous segments which, by Rabl's orientation, should be positioned across the volume of the nucleus. In this sense, MI pairing of such distantly positioned segments of homology may not be a consequence of an active search for homology over large distances but a chance event brought about by aberrant positioning of some chromosome arms.

The resolution of the techniques used here offers no insight into the question of cause and effect. It is still unclear whether the absence of chiasmate pairing is a consequence of a failure of a telomere to migrate into the bouquet stage, or if the failure of the early stages of pairing initiation leads to migration of unassociated telomeres out of the bouquet and throughout the volume of the nucleus. What the study does establish is that aberrant positioning of telomeres (and, consequently, chromosome arms) may lead to contacts, probably random, between crossover-capable regions of homologues across the entire diameter of the nucleus. No evidence was observed here, in a reasonably sized sample of nuclei, that centromeres are capable of similar aberrant placement. Observations made here also imply that incorrect positioning of chromosomes may be systemic in nature and only carry from somatic tissue to meiocytes.

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**Authors' contribution** AJL and GL designed experiments; AJL, EG, and GL performed meiotic experiments; KP, OS, and DK performed mitotic experiments; AJL and GL analyzed data; AJL, GL, and DK wrote the manuscript. All authors read and approved the manuscript.

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## Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

**Conflict of interest** The authors declare that they have no conflict of interest.

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