**The distribution of α-kleisin during meiosis in the holocentromeric plant *Luzula* *elegans***

Wei Ma 1, Veit Schubert 1, Mihaela Maria Martis 2,6, Gerd Hause 3, Zhaojun Liu 1, Yi Shen 4, Udo Conrad 1, Wenqing Shi 4, Uwe Scholz 1, Stefan Taudien 5, Zhukuan Cheng 4, Andreas Houben 1,a

1 Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, 06466 Stadt Seeland, Germany

2 Institute of Bioinformatics and Systems Biology/Munich Information Center for Protein Sequences, Helmholtz Center Munich, German Research Center for Environmental Health, 85764 Neuherberg, Germany

3 Institute of Biology, Department of Genetics, Martin Luther University Halle-Wittenberg, 06120 Halle, Germany

4 State Key Laboratory of Plant Genomics, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, 100101 Beijing, China

5 Leibniz Institute on Aging - Fritz-Lipmann-Institut e.V. (FLI) Beutenbergstraße 11,

07745 Jena, Germany

6 Division of Cell Biology, Department of Clinical and Experimental Medicine, Bioinformatics Infrastructure for Life Sciences, Linköping University, 558185 Linköping, Sweden

a Corresponding author: houben@ipk-gatersleben.de (e-mail) / (0049) (0) 39482 5486 (Phone)

**Keywords**

Holocentric chromosome; α-kleisin; Synaptonemal complex; *Luzula elegans*

**Introduction**

Accurate chromosome transmission is required both for the proliferative cell divisions in mitosis and the two sequential divisions in meiosis to reduce the genome copy number from two in diploid germline cells to one in haploid gametes. During these cell divisions sister chromatids are held together at their arm and centromere regions by cohesin complexes. These complexes consist of different subunits: namely the SMC1 and SMC3 (Structural Maintenance of Chromosomes) proteins, the α-kleisin SCC1 (also named RAD21 or REC8, see also Table 1) and the SCC3 protein (Nasmyth 2011). The subunits have been extensively studied in yeast, animals and human, but also homologs in plants have been characterized (reviewed in Schubert 2009). Biochemical and structural studies demonstrated that SCC1 simultaneously binds to SMC1 and SMC3 to form a tripartite ring proposed to mediate sister chromatid cohesion by encircling sister chromatids (reviewed in Nasmyth and Haering 2005). In almost all eukaryotes SCC1 is present in the mitotic cohesin complex and replaced during meiosis by REC8 (Anderson et al. 2002; Cai et al. 2003; Golubovskaya et al. 2006; Pasierbek et al. 2001; Zhang et al. 2006). SCC1 is the main regulator of the cohesin complex at somatic anaphase.

During mitosis, most of the cohesins are degraded from the chromosome arms via phosphorylation of the SCC3 subunit by PLK1 before metaphase (Losada et al. 2002; Sumara et al. 2002). However, the centromeric cohesins are maintained until the anaphase onset when SCC1 is cleaved by the separase (Haering and Nasmyth 2003). In contrast to mitosis, during meiosis two rounds of chromosome segregation follow a single replication step to generate haploid gametes. Thus, sister chromatid cohesins must be released in two steps during meiosis in monocentric species. (i) loss of chromatid arm cohesion between both homologues to release chiasmata and to enable reductional segregation during meiosis I (Kudo et al. 2006; Kudo et al. 2009), (ii) the loss of sister centromere cohesion allows the sister chromatids to segregate during anaphase of meiosis II (Llano et al. 2008). However, the process of meiosis in organisms with holocentric chromosomes illustrates that our knowledge of meiotic chromosome arrangement and segregation based on observations of monocentric chromosomes may not apply to all organisms (Cabral et al. 2014; Heckmann et al. 2014a).

In principle there are two options to release cohesins during holocentric meiosis: (i) such as in the nematode *Caenorhabditis elegans*, at a cruciform bivalent with a short (mid-bivalent) and a long arm. Spindle fibers attach to a restricted terminal chromosome region during metaphase I allowing the degradation of cohesion at the mid-bivalent and retention at the long arms during anaphase I enabling homologue separation. During meiosis II, cohesion at the sister chromatid interface gets lost allowing sister chromatid separation (Albertson and Thomson 1993; Nabeshima et al. 2005; Kaitna et al. 2002); (ii) in other holocentric species such as the wood rush *Luzula elegans*, an inverted sequence of meiotic sister chromatid segregation occurs (Figure 3). In contrast to monopolar sister centromere orientation in monocentric species, the unfused holokinetic sister centromeres behave as two distinct functional units during meiosis I, resulting in sister chromatid separation. Homologous non-sister chromatids remain terminally linked after metaphase I until metaphase II. Then, they separate at anaphase II (Heckmann et al. 2014a). Therefore, it is expected that the degradation of cohesins during holocentric meiosis may deviate from that of monocentric species. However, the dynamics and function of cohesin during this process is not yet known.

Besides realising sister chromatid cohesion, cohesin complexes also participate in the assembly of the synaptonemal complex (SC) (Klein et al. 1999; Hartsuiker et al. 2001). The SC consists of a proteinaceous structure, the axial element (AE) mediating the association of each pair of homologous sister chromatids. After pairing, the axial elements become associated by transverse filaments to the central element (CE) to establish the tripartite SC. The SC provides the structural framework for synapsis, double strand-break repair and exchange between homologues (Henderson and Keeney 2005). However, unknown is whether the SC complex structure is conserved in holocentric species such as *L. elegans*.

In this work, we aimed to delve deeper into the organization of holocentric chromosomes. We found that α-kleisin at both mitotic and meiotic metaphase chromosomes colocalizes with the centromere and may contribute to the assembly of the centromere. This localization of α-kleisin and the formation of a tripartite SC structure indicate that the prophase I behaviour of *L. elegans* is similar as in monocentric species.

**Materials and Methods**

**Plant material and plant cultivation**

*Luzula elegans* Lowe (2n=6) (Vouchers at the Herbarium Gatersleben: GAT 7,852–7,856) plants were cultivated for 4 weeks under short-day conditions (8 h light/16 h dark, 20°C /18°C) and then vernalized (10 h light/14 h dark, 4°C) for at least 4 weeks. The plants were finally grown under the long-day conditions (16 h light, 22°C day/16°C night) and all experimental materials from different tissues were collected during this period.

**RNA extraction, RT-PCR and qRT-PCR**

Total RNA was extracted from leaves, stems and flower buds by the TRIzol method (Life Technologies). The RNA samples were treated with RNA-free DNase I (Ambion TURBO DNase; Invitrogen) before cDNA synthesis. The absence of genomic DNA was confirmed by PCR with *LeGAPDH*-specific primers G1F and G1R (Supplemental Table 1). All cDNAs (20 μl) were generated from 1 μg DNase I-treated RNA, using the Reverse Aid H Minus First Strand cDNA Synthesis Kit (Fermentas). cDNA used for 5’- and 3’- RACE PCR were synthesized from mRNA of flower buds according to the SMART RACE cDNA Amplification Kit (Clontech, Palo Alto, CA) manual protocol.

25 μl PCR reaction mixtures contained: 1 μl cDNA, 10 μM of each forward and reverse primers (Supplemental Table 1), 5 mM of each deoxynucleotide triphosphates, 2.5 μl 10xPCR reaction buffer and 1 unit of *Taq* polymerase (Qiagen). The cycling protocol was: 94°C for 3 min, 35 cycles at (94°C for 40s, 58°C for 40 s, 1 min/kb elongation at 72°C), 72°C final elongation for 10 min. 25 cycles PCR were run with *LeGAPDH*-specific primers (G1F and G1R, Supplemental Table 1) to quantify the abundance of transcripts. 5’- and 3’- RACE PCR were performed according to the SMART RACE cDNA Amplification Kit (Clontech, Palo Alto, CA) manual protocol.

qRT-PCR was performed using the SYBR Green Master (Applied Biosystems) on the 7900HT Fast Real-Time PCR System (Applied Biosystems). 10 μl of PCR mixture contained 0.2 μl of cDNA template, 5 μl of 2× Power SYBR Green PCR Master Mix (Applied Biosystems), and 0.33 mM of the forward and reverse primers (Supplemental Table 1) for each gene. The amplification conditions were one cycle at 95°C for 10 min, 40 cycles of two consecutive steps at 95°C for 15 s and at 60°C for 60s. *LeGAPDH*-specific primers G2F and G2R (Supplemental Table 1) were used as endogenous control.

**RNAseq and *de novo* assembly**

Total RNA was isolated from *L. elegans* pollen mother cells using the SpectrumTM plant total RNA kit (Sigma) according to manufacturer’s instruction followed by cDNA Illumina paired-end library preparation (Vertis Biotechnologie AG, Freising, Germany). The library was sequenced (1 lane, 2x 100 bp) on an Illumina HiSeq 2000, resulting in 81.2 million read pairs which were assembled. The pollen mother cell transcriptome of *L. elegans* can be used for BLAST search under: <http://webblast.ipk-gatersleben.de/luzula/>.

**Sequence analysis**

DNA fragments were sequenced by the service facility of the IPK (Gatersleben, Germany). Sequences were analyzed by Sequencher 5.2.4 (Gene Codes Corporation Inc), assembled using Seqman pro 12.0.0 (DNASTAR, Inc) and processed by EditSeq and MegAlign Lasergene 8 (DNASTAR, Inc). Reference IDs for the phylogenetic analysis of the α-kleisinsequences used in this study are available in Supplemental Table 2, CENH3 sequences used for comparison are described in (Marques et al. 2015). Phylogenetic trees were constructed by the software Geneious (version 7.0.6; <http://www.geneious.com>).

**Total protein extraction and Western blot analysis**

For isolation of total *L. elegans* proteins 200 mg of grinded flower buds were suspended in 250 μl extraction buffer (112 mM Na2CO3, 112 mM DTT, 4% SDS, 24% sucrose, 4 mM EDTA and 1 mg 3,3,5,5-tetrabromophenolsulfonephthalein) and kept at 65℃ for 20 minutes. After centrifugation at 14,000 rpm for 5 min at 4°C the supernatant contained the total soluble proteins.

The proteins were separated by 10% (wt/vol) polyacrylamide gels according to Schägger and Von Jagow (1987), then the gels were blotted on Immobilon PVDF membranes (Millipore). These membranes were incubated first with primary antibodies (1:1,000 rabbit anti-LeCENH3, 1:5,000 rabbit anti-histone H3 (Sino Biological Inc., 100005-MM01-50) and 1:5,000 mouse anti-α tubulin (clone DM 1A, Sigma) and then with the corresponding secondary antibodies [1:5,000 anti-rabbit IgG IRDye800CW (LI-COR, 925-32213) or 1:5,000 anti-mouse IgG IRDye 680RD (LI-COR, 926-32222)]. The immunoblots were imaged using a LI-COR Odyssey Imager. Histone H3 and α-tubulin signals were used as controls.

**Antibody production**

To generate antibodies against Leα-kleisin, a 1017-bp fragment of Leα-kleisin (primers R1F and R1R, Supplemental Table 1) was amplified from flower bud cDNA. The fragments were cloned into the vector pSC-A-amp/kan using the StrataClone PCR cloning kit (Stratagene), sequenced and then sub-cloned into the expression vector pET-23a-d(+) (Novagen). The resulting pET-23a-Leα-kleisin construct was transformed into *Escherichia coli* BL21 (DE3) and the expression of proteins was induced by 1 mM isopropylthio-beta-D-galactoside (IPTG). The Leα-kleisin recombinant proteins were purified under native condition on Ni-NTA agaroses (Qiagen), then confirmed by Western blot using mouse monoclonal anti-His-tag (1:1,000, Millipore, 05-949) and 1:5,000 anti-mouse IgG IRDye 680RD (LI-COR, 926-32222) antibodies. A polyclonal rabbit anti-Leα-kleisin antibody was produced by Pineda (Antikörper-Service, Berlin, Germany). The specificity of anti-Leα-kleisin antibody (1:1,000) was checked on a Western blot with recombinant proteins. The method for Western blot was discussed above.

For the generation of LeCENH3-specific antibodies an epitope corresponding to the N-terminal end of LeCENH3 (3-RTKHFSNRKSIPPKKQTPAK-23) was identified. Peptide synthesis, immunization of rabbits, and peptide affinity purification of antisera were performed by LifeTein LLC (South Plainfield, NJ, USA).

**Indirect immunostaining and light microscopy**

Indirect immunostaining of *L. elegans* was performed as described by Heckmann et al. (2014a), of *Hordeum vulgare* and *Vicia faba* as described by Schubert et al. (1993). The following primary antibodies were used: rabbit anti-Leα-kleisin (1:100), mouse anti-OsSgo1 (1:200) (Wang et al. 2011), guinea pig anti-ZmZYP1 (1:100) (Golubovskaya et al. 2011), rabbit anti-grass CENH3 (1:300) (Sanei et al. 2011) and rabbit anti-LeCENH3 (1:100). Texas red-conjugated anti-rabbit antibodies (1:400) (Molecular Probes), fluorescein isothiocyanate-conjugated anti-mouse antibodies (1:300) (Molecular Probes) and Alexa 488 conjugated anti-guinea pig (1:300) (Dianova) antibodies were used as secondary antibodies. Anti-LeCENH3 and anti-grass CENH3 antibodies were directly labelled by the Fluorescein Labeling Kit-NH2 (Dojindo, LK01-10).

Images were collected in gray scale using an Olympus BX61 microscope (Olympus; <http://www.olympus.com>) and an ORCA-ER CCD camera (Hamamatsu; <http://www.hamamatsu.com>), then pseudocoloured and merged with Adobe Photoshop CS5 (Adobe). To achieve a lateral optical resolution of ∼120 nm (super-resolution, obtained with a 488 nm laser), we applied structured illumination microscopy (SIM) using a 63x/1.4 Oil Plan-Apochromat objective of an Elyra PS.1 microscope system and the software ZEN (Carl Zeiss GmbH). Images were captured separately for each fluorochrome using the 561 nm, 488 nm and 405 nm laser lines for excitation and appropriate emission filters (Weisshart et al. 2016).

**Electron microscopy**

For transmission electron microscopy cut-opened anthers undergoing prophase I were fixed for 4 h in 3% glutaraldehyde (Sigma, Taufkirchen, Germany) in 0.1 M sodium cacodylate buffer pH 7.2 (SCB), washed, postfixed for 1 hour with 1% osmiumtetroxide (Carl Roth, Karlsruhe, Germany) in SCB, dehydrated in a graded series of ethanol and embedded in epoxy resin according to Spurr (1969). Ultrathin sections (70 nm) were transferred to formvar coated grids and poststained with uranyl acetate and lead citrate. Subsequently the grids were observed with an EM 900 (Carl Zeiss Microscopy, Oberkochen, Germany) transmission electron microscope (acceleration voltage 80 kV). Electron micrographs were taken with a slow scan camera (Variospeed SSCCD camera SM-1k-120, TRS, Moorenweis, Germany) using the iTEM software from Olympus SIS (Münster, Germany).

**Accession numbers**

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers PRJEB12761, *LeCENH3* gDNA (KT932953), *LeCENH3.1* mRNA (KT932953), *LeCENH3.2* mRNA (KT932954) *Leα-kleisin-1* mRNA (KT932948), *Leα-kleisin-2* mRNA (KT932949), *Leα-kleisin-3* mRNA (KT932950) and *Leα-kleisin-4* mRNA (KT932951).

**Results**

**Identification of the centromere-specific histone H3 variant CENH3 in *L. elegans***

First a *L. elegans*-specific centromere antibody recognizing the centromere-specific histone H3 (CENH3) was established. Both CENH3 variants of *Luzula nivea* (GenBank BAE026 and ADM18965) (Nagaki et al. 2005; Moraes et al. 2011) were used as query to identify the corresponding gene in the established RNAseq database of *L. elegans* pollen mother cells (<http://webblast.ipk-gatersleben.de/luzula/>). To determine the start and end of the *LeCENH3* transcript, 3’-RACE and 5’-RACE experiments were performed based on a 55 amino acids fragment showing high similarity to the C-terminal part of *L. nivea* CENH3 (Supplemental Figures 1A, 1B). Cloning of the 5’-RACE products revealed two gene splicing variants (called *LeCENH3.1* and *LeCENH3.2*). *LeCENH3.2* differs from *LeCENH3.1* by having 21 bp- and 3 bp-long deletions near the 5’-terminal part (Supplemental Figure 1A). The full sequences of *LeCENH3.1* and *LeCENH3.2* were confirmed after PCR and RT-PCR using the primer pair C4F/C4R (Supplemental Figure 1A). Phylogenetic analysis grouped both LeCENH3 variants together with CENH3s of other *Juncaceae* species in a sister branch of monocots (Supplemental Figure 1C).

Both *LeCENH3* splicing variants show a higher expression in flower buds than in stems and leaves as revealed by quantitative RT-PCR using the primer combinations (C2F/C2R and C3F/C2R). *LeCENH3.1* exhibited a higher expression than *LeCENH3.2* in flower buds and stems. In leaves the activity of both was almost identical (Figure 1A). But a diverging expression was found in anthers by sequencing the cloned 5’-RACE products. 74% and only 26% of the products (n=38) originated from *LeCENH3.1* and *LeCENH3.2*, respectively.

Next, a rabbit anti-LeCENH3 antibody was raised against a synthetic peptide containing the N-terminal 20 amino acid residues of both CENH3s (Supplemental Figure 1B). To determine the antibody specificity, a Western blot assay was performed using the affinity purified antibodies as probe on total protein extracts from flower buds of *L. elegans*. The major band identified fitted to the expected size of 20 kD (Supplemental Figure 1D).

**Identification of the *L. elegans* α-kleisins**

To identify the α-kleisin subunits of *L. elegans* cohesin we searched by BLASTP in the *L. elegans* RNAseq database using the Rad21/Rec8-like sequences of rice (Zhang et al. 2004) as query, and identified *in silico* four α-kleisin-like genes. The phylogenetic analysis of the different mono- and eudicot Rad21/Rec8 proteins indicated that each of the four *L. elegans* α-kleisin-like proteins were categorized into different subfamilies (Supplemental Figure 2A), namely Leα-kleisin-1, Leα-kleisin-2, Leα-kleisin-3 and Leα-kleisin-4. The alignment of these four incomplete proteins revealed an overall similarity of only 8.3% to 36.8% (Supplemental Table 3). However, the conserved N-terminal regions showed a higher similarity with 25.8% to 46.8% (Supplemental Figure 2B).

We chose Leα-kleisin-1for further analysis,because this protein possibly represents an ortholog of the *Arabidopsis thaliana* α-kleisin SYN4 required for cohesion along chromosome arms and at centromeres (Schubert et al. 2009). In order to determine the transcription dynamics of *Leα-kleisin-1*, cDNA derived from stems, leaves and flower buds were used to perform quantitative reverse transcription PCR (qRT-PCR, primers R2F/R2R) (Supplemental Figure 2C). As shown in Figure 1B, the highest level of expression was found in flower buds. This agrees to data obtained for Rad21-1 of rice (Zhang et al. 2004).

To test the chromosomal distribution of Leα-kleisin-1, rabbit polyclonal antibodies were raised against a partial recombinant Leα-kleisin-1 protein. We cannot exclude that these antibodies recognize also other members of the α-kleisin family since the N-terminal part is conserved. Therefore, we named the antibodies ‘anti-Leα-kleisin’. The molecular weight of the recombinant protein used for antibody production was ~55 kDa (Supplemental Figure 2D) although the expected size is 38 kDa. Such a difference was also observed for antibodies established against α-kleisin orthologs of mouse (Lee and Hirano 2011), *C. elegans* (Birkenbihl and Subramani 1995) and budding yeast (Michaelis et al. 1997), likely due to the high polarity of the proteins. Nevertheless, the cross-reaction of anti-Leα-kleisin with antigens producedby *E. coli* confirmed its specificity (Supplemental Figure 2E).

**Prophase I is conventional in *L. elegans***

Anti-Leα-kleisin staining was performed on pollen mother cell chromosomes to decipher the distribution of α-kleisin in prophase I. Leα-kleisin signals lined up into continuous structures during leptotene/zygotene (Figure 2A, 2B). Double immunostaining with ZmZYP1 and Leα-kleisin antibodies showed that Leα-kleisin mainly localized in the ZmZYP1 positive regions during zygotene/pachytene (Figure 2C). In addition, it was found that Leα-kleisin colocalizes to LeCENH3 during this stage (Supplemental Figure 3). Thus, the distribution of meiotic cohesin during prophase I seems to be as similar as reported for monocentric species (Qiao et al. 2011).

To decipher whether a tripartite structure of the synaptonemal complex can be observed in *L. elegans* we examined pachytene cells by transmission electron microscopy. We identified a 111.6 ± 10,6 nm (n=20) wide synaptonemal complex comprising a dense central region traversed by thin filaments (Figure 2D). Surrounded by chromatin, the synaptonemal complex lies “zipper-like” along the central axis of the bivalent (Figure 2D). These findings indicate that the synaptonemal complex structure of holocentric species is similar to those of monocentrics.

**Leα-kleisin colocalizes with the centromeres of condensed chromosomes**

In *L. elegans* sister chromatid cohesion becomes already resolved during metaphase I (Heckmann et al. 2014a). However, the dynamics and function of cohesin during meiosis is not yet known. Therefore, we investigated the distribution of Leα-kleisin by immunostaining, and found it present only in the centromere regions of metaphase I and II chromosomes (Figure 3B, 3C). Super-resolution microscopy of metaphase I and II chromosomes labelled with anti-Leα-kleisin and anti-LeCENH3 revealed a close proximity of both proteins. In addition, antibodies against rice shugoshin-specific (OsSGO1) were used as markers for cohesion. OsSGO1 stabilizes the synaptonemal complex and protects centromeric cohesion during the meiosis of rice (Wang et al., 2011). However, in *L. elegans* we found that SGO1 was exclusively located in the holocentromeres of metaphase II chromosomes (Supplemental Figure 4).

Leα-kleisin was also located at the holocentromeres of somatic *L. elegans* metaphase chromosomes, but not in regions where sister chromatids attach (Figure 4A). In monocentric metaphase chromosomes of *H. vulgare* (Figure 4B), Leα-kleisin signals appeared not only at the CENH3-positive regions, but also in between the sister chromatids. For *V. faba* (Supplemental Figure 5), two separate Leα-kleisin signals were only present in the primary constrictions.

In summary, the results suggest that the α-kleisins of *L. elegans* may not only realize sister chromatid cohesion, instead they colocalize with the position of the centromere. Additional experiments are required to prove the involvement of α-kleisins in the assembly of the centromeres in this species.

**Discussion**

**The CENH3 of *L. elegans***

Whereas in the closely related species *L. nivea* two *CENH3* isoforms are present (Nagaki et al. 2005; Moraes et al. 2011), only one was found in *L. elegans*. But interestingly, two different *LeCENH3* splicing variants with a tissue-specific expression pattern are evident. Similarly, two pearl millet (Ishii et al. 2015) and human CENH3 (also called CENP-A) (Gerhard et al. 2004) splicing variants were proven. However, no different functions of these variants have been determined until now. By immunostaining we confirm previous findings (Heckmann et al. 2011; Heckmann et al. 2014a) that somatic *L. elegans* chromosomes contain a CENH3-positive longitudinal centromere along each sister chromatid and that holocentricity is maintained, and no fusion of sister centromeres occurs throughout meiosis.

**α-kleisins colocalize with the centromere**

In *L. elegans* four α-kleisins (Leα-kleisin-1-4) were identified. Based on our phylogenetic analysis they correspond to those of other plants like *A. thaliana* (AtSYN1-4) and *O. sativa* (OsRad21-1-4) as follows: Leα-kleisin-4/AtSYN1/OsRad21-4, Leα-kleisin-2/AtSYN2/OsRad21-2, Leα-kleisin-3/AtSYN3/OsRad21-3 and Leα-kleisin-1/AtSYN4/ OsRad21-1 (da Costa-Nunes et al. 2006; Dong et al. 2001; Zhang et al. 2004; Zhang et al. 2006; Tao et al. 2007; Gong et al. 2011), in which the Leα-kleisin-4/AtSYN1/OsRad21-4 α-kleisins act during meiosis.

In the holocentric nematode *C. elegans*, also four different α-kleisin proteins (COH-1, COH-2, COH-3, and the meiotic REC-8 α-kleisin) were identified (Mito et al. 2003). In contrast, yeast contains only two α-kleisins, the mitotic SCC1 and the meiosis-specific variant REC8 (Lee and Orr-Weaver 2001). In mammals, three α-kleisins, RAD21, REC8 and RAD21L were reported (Ishiguro et al. 2011; Nasmyth 2011). In *A. thaliana*, it was proven that the four α-kleisin proteins have different functions (reviewed in (Schubert 2009). SYN1 mediates cohesion during meiosis (Bhatt et al. 1999; Cai et al. 2003) and in differentiated interphase nuclei (Schubert et al., 2009). SYN2 and SYN3, mainly expressed in meristematic tissues, seem to be mitotic α-kleisins (Dong et al. 2001). SYN3 is enriched in the nucleolus, therefore, its additional involvement in controlling rDNA structure and transcription and its involvement in rRNA processing has been suggested (Jiang et al. 2007). SYN3 and SYN4 also support sister chromatid cohesion in differentiated interphase nuclei (Schubert et al., 2009). In agreement with the findings in vertebrates (Waizenegger et al. 2000) here we show that α-kleisins may mediate sister chromatid cohesion during mitosis in monocentric species as *H. vulgare,* since we observed that α-kleisin remained between the sister centromeres during metaphase. Previous studies (Suzuki et al. 2013) did not prove plant cohesins at somatic metaphase chromosomes, which may be caused by an insufficient sensitivity of the antibodies used, the image acquisition applied, or by the preparation methods employed.

We found that α-kleisin is present along each metaphase sister centromere in *L. elegans*. This is in agreement with the distribution of RAD21L in mice, where two separate signals appear at the primary constrictions during metaphase II (Herran et al. 2011). Therefore, we support the assumption of Herran et al. (2011) that the enrichment of α-kleisin at centromeres may contribute to the assembly of the inner centromere and that it may play role in promoting the bi-orientation of kinetochores (Sakuno et al. 2009).

In *L. elegans* between metaphase I and II the chromosomal termini of the homologous non-sister chromatids are connected to each other by chromatin threads.This allows to proceed an inverted sequence of meiotic sister chromatid segregation, and it was assumed that cohesins are involved in this end-to-end association (Heckmann et al. 2014b). However, here we show that α-kleisin-containing cohesin complexes obviously are not involved in maintaining these connections.

**The meiotic prophase I is conventional in *L. elegans***

Here we report that Leα-kleisins localize exclusively from leptotene to pachytene along the axial and lateral elements of the synaptonemal complex. This is consistent with the finding that REC8 and HIM3, components of the chromosomes axes are required for meiotic synapsis in holocentric nematodes during leptotene, zygotene and pachytene (Zetka et al. 1999). This suggests that REC8 is a component of axial/lateral elements (Pasierbek et al. 2001). In plants, a specific and intermittent localization of SMC3 in the axial/lateral elements has been observed in tomato by electron microscopy in microsporocytes during zygotene, similar to that observed by light microscopy after the immunolabeling of SMC1, SMC3, SCC3 and REC8, although not all subunits presented the same pattern of accumulation and appearance during prophase I (Qiao et al. 2011). Also, a correlation between the progression of axial or lateral element formation and synapsis, and the localization of several cohesin subunits was observed in many different monocentric species (Calvente and Barbero 2012). Although till now, no functional analysis regarding the participation of cohesin during synaptonemal complex formation and synapsis is available in *L. elegans*, the sequential α-kleisin loading indicates a role in the correct progression of synapsis.

We report here that the synaptonemal complex of *L. elegans* is similar in structure and function as in other species (Goldstein 1987; Sym et al. 1993; Page and Hawley 2003). The measurement of the width of the central region of the synaptonemal complex is a ~111 nm in *L. elegans*. This is consistent with the data reported for other plants (Westergaard and von Wettstein, 1972) and of *C. elegans* (Smolikov et al. 2008). Because the Leα-kleisins show a similar dynamic pattern during prophase I as monocentric species (Table 1), we conclude that their function during the synaptonemal complex formation is also conserved in holocentrics.

**Acknowledgements**

We are grateful to all members of the Chromosome Structure & Function laboratory (IPK Gatersleben), Ingo Schubert (IPK) and Eva Tomaštíková (Centre of the Region Haná for Biotechnological and Agricultural, Research, Institute of Experimental Botany, Olomouc, Czech Republic) for fruitful discussions; to Karla Meier, Katrin Kumke, Oda Weiß, Isolde Tillack and Gresch Ulrike (IPK) for excellent technical assistance; to Anne Fiebig for sequence submission and to Karin Lipfert (IPK) for help with artwork. This work was supported by the China CSC scholarship, the Deutsche Forschungsgemeinschaft (SPP 1384, HO 1779/17-1) and the IPK Gatersleben.

**References**

Albertson DG, Thomson JN (1993) Segregation of holocentric chromosomes at meiosis in the nematode, *Caenorhabditis elegans*. Chromosome Research 1: 15-26.

Anderson DE, Losada A, Erickson HP et al (2002) Condensin and cohesin display different arm conformations with characteristic hinge angles. The Journal of Cell Biology 156: 419-424.

Bhatt AM, Lister C, Page T et al (1999) The DIF1 gene of *Arabidopsis* is required for meiotic chromosome segregation and belongs to the REC8/RAD21 cohesin gene family. The Plant Journal 19: 463-472.

Birkenbihl RP, Subramani S (1995) The rad21 gene product of *Schizosaccharomyces pombe* is a nuclear, cell cycle-regulated phosphoprotein. Journal of Biological Chemistry 270: 7703-7711.

Cai X, Dong F, Edelmann RE, Makaroff CA (2003) The *Arabidopsis* SYN1 cohesin protein is required for sister chromatid arm cohesion and homologous chromosome pairing. Journal of Cell Science 116: 2999-3007.

Cabral G, Marques A, Schubert V et al (2014) Chiasmatic and achiasmatic inverted meiosis of plants with holocentric chromosomes. Nature communications 5: 5070.

Calvente A, Barbero JL (2012) Cohesins and Cohesin-Regulators in Meiosis. INTECH Open Access Publisher.

Calvente A, Viera A, Parra MT, et al (2013) Dynamics of cohesin subunits in grasshopper meiotic divisions. Chromosoma 122: 77-91.

da Costa-Nunes JA, Bhatt AM, O'Shea S et al (2006) Characterization of the three *Arabidopsis thaliana* RAD21 cohesins reveals differential responses to ionizing radiation. Journal of Experimental Botany 57: 971-983.

de Carvalho CE, Zaaijer S, Smolikov S, et al (2008) LAB-1 antagonizes the Aurora B kinase in *C. elegans*. Genes & development 22: 2869-2885.

Dong F, Cai X, Makaroff C (2001) Cloning and characterization of two *Arabidopsis* genes that belong to the RAD21/REC8 family of chromosome cohesin proteins. Gene 271: 99-108.

Eijpe M, Offenberg H, Jessberger R, et al (2003) Meiotic cohesin REC8 marks the axial elements of rat synaptonemal complexes before cohesins SMC1β and SMC3. The Journal of Cell Biology 160: 657-670.

Garcia-Cruz R, Brieno MA, Roig I, et al (2010) Dynamics of cohesin proteins REC8, STAG3, SMC1β and SMC3 are consistent with a role in sister chromatid cohesion during meiosis in human oocytes. Human Reproduction: deq180.

Gerhard DS, Wagner L, Feingold EA, et al (2004) The status, quality, and expansion of the NIH full-length cDNA project. Genome Research 14: 2121-2127.

Goldstein P (1987) Multiple synaptonemal complexes (polycomplexes): origin, structure and function. Cell Biology International Reports 11: 759-796.

Golubovskaya IN, Hamant O, Timofejeva L et al (2006) Alleles of afd1 dissect REC8 functions during meiotic prophase I. Journal of Cell Science 119: 3306-3315.

Golubovskaya IN, Wang CJ, Timofejeva L, Cande WZ (2011) Maize meiotic mutants with improper or non-homologous synapsis due to problems in pairing or synaptonemal complex formation. Journal of Experimental Botany 62: 1533-1544.

Gong C, Li T, Li Q, Yan L, Wang T (2011) Rice OsRAD21-2 is expressed in actively dividing tissues and its ectopic expression in yeast results in aberrant cell division and growth. J Integr Plant Biol 53: 14-24.

Haering CH, Nasmyth K (2003) Building and breaking bridges between sister chromatids. BioEssays 25: 1178-1191.

Hartsuiker E, Vaessen E, Carr A, Kohli J (2001) Fission yeast Rad50 stimulates sister chromatid recombination and links cohesion with repair. The EMBO Journal 20: 6660-6671.

Heckmann S, Schroeder-Reiter E, Kumke K, Ma L, Nagaki K, Murata M, Wanner G, Houben A (2011) Holocentric chromosomes of *Luzula elegans* are characterized by a longitudinal centromere groove, chromosome bending, and a terminal nucleolus organizer region. Cytogenet Genome Res 134: 220-228.

Heckmann S, Jankowska M, Schubert V, Kumke K, Ma W, Houben A (2014a) Alternative meiotic chromatid segregation in the holocentric plant *Luzula elegans*. Nature Communications 5: 4979.

Heckmann S, Schubert V, Houben A (2014b) Holocentric plant meiosis: first sisters, then homologues. Cell Cycle 13: 3623-3624.

Henderson KA, Keeney S (2005) Synaptonemal complex formation: where does it start? BioEssays 27: 995-998.

Herran Y, Gutierrez-Caballero C, Sanchez-Martin M et al (2011) The cohesin subunit RAD21L functions in meiotic synapsis and exhibits sexual dimorphism in fertility. The EMBO Journal 30: 3091-3105.

Ishiguro K, Kim J, Fujiyama‐Nakamura S, Kato S, Watanabe Y (2011) A new meiosis‐specific cohesin complex implicated in the cohesin code for homologous pairing. EMBO Reports 12: 267-275.

Ishii T, Sunamura N, Matsumoto A, et al (2015) Preferential recruitment of the maternal centromere-specific histone H3 (CENH3) in oat (*Avena sativa* L.)× pearl millet (*Pennisetum glaucum* L.) hybrid embryos. Chromosome Research 23: 709-718.

Jiang L, Xia M, Strittmatter LI, Makaroff CA (2007) The *Arabidopsis* cohesin protein SYN3 localizes to the nucleolus and is essential for gametogenesis. The Plant Journal 50: 1020-1034.

Kaitna S, Pasierbek P, Jantsch M, Loidl J, Glotzer M (2002) The aurora B kinase AIR-2 regulates kinetochores during mitosis and is required for separation of homologous chromosomes during meiosis. Current Biology 12: 798-812.

Klein F, Mahr P, Galova M, Buonomo SB, Michaelis C, Nairz K, Nasmyth K (1999) A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell 98: 91-103.

Kudo NR, Wassmann K, Anger M et al (2006) Resolution of chiasmata in oocytes requires separase-mediated proteolysis. Cell 126: 135-146.

Kudo NR, Anger M, Peters AH et al (2009) Role of cleavage by separase of the Rec8 kleisin subunit of cohesin during mammalian meiosis I. Journal of Cell Science 122: 2686-2698.

Lee J, Hirano T (2011) RAD21L, a novel cohesin subunit implicated in linking homologous chromosomes in mammalian meiosis. The Journal of Cell Biology 192: 263-276.

Lee JY, Orr-Weaver TL (2001) The molecular basis of sister-chromatid cohesion. Annual Review of Cell and Developmental Biology 17: 753-777.

Lee J, Iwai T, Yokota T, et al (2003) Temporally and spatially selective loss of Rec8 protein from meiotic chromosomes during mammalian meiosis. Journal of Cell Science 116: 2781-2790.

Llano E, Gómez R, Gutiérrez-Caballero C et al (2008) Shugoshin-2 is essential for the completion of meiosis but not for mitotic cell division in mice. Genes & Development 22: 2400-2413.

Losada A, Hirano M, Hirano T (2002) Cohesin release is required for sister chromatid resolution, but not for condensin-mediated compaction, at the onset of mitosis. Genes & Development 16: 3004-3016.

Marques A, Ribeiro T, Neumann P et al (2015) Holocentromeres in *Rhynchospora* are associated with genome-wide centromere-specific repeat arrays interspersed among euchromatin. Proceedings of the National Academy of Sciences 112: 13633-13638.

Michaelis C, Ciosk R, Nasmyth K (1997) Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell 91: 35-45.

Mito Y, Sugimoto A, Yamamoto M (2003) Distinct developmental function of two *Caenorhabditis elegans* homologs of the cohesin subunit Scc1/Rad21. Molecular Biology of the Cell 14: 2399-2409.

Moraes IC, Lermontova I, Schubert I (2011) Recognition of *A. thaliana* centromeres by heterologous CENH3 requires high similarity to the endogenous protein. Plant Molecular Biology 75: 253-261.

Nabeshima K, Villeneuve AM, Colaiácovo MP (2005) Crossing over is coupled to late meiotic prophase bivalent differentiation through asymmetric disassembly of the SC. The Journal of Cell Biology 168: 683-689.

Nagaki K, Kashihara K, Murata M (2005) Visualization of diffuse centromeres with centromere-specific histone H3 in the holocentric plant *Luzula nivea*. Plant Cell 17: 1886-1893.

Nasmyth K (2011) Cohesin: a catenase with separate entry and exit gates? Nature Cell Biology 13: 1170-1177.

Nasmyth K, Haering CH (2005) The structure and function of SMC and kleisin complexes. Annual Review of Biochemistry 74: 595-648.

Page SL, Hawley RS (2003) Chromosome choreography: the meiotic ballet. Science 301: 785-789.

Pasierbek P, Jantsch M, Melcher M, Schleiffer A, Schweizer D, Loidl J (2001) A *Caenorhabditis elegans* cohesion protein with functions in meiotic chromosome pairing and disjunction. Genes & Development 15: 1349-1360.

Qiao H, Lohmiller LD, Anderson LK (2011) Cohesin proteins load sequentially during prophase I in tomato primary microsporocytes. Chromosome Research 19: 193-207.

Sakuno T, Tada K, Watanabe Y (2009) Kinetochore geometry defined by cohesion within the centromere. Nature 458: 852-858.

Sanei M, Pickering R, Kumke K, Nasuda S, Houben A (2011) Loss of centromeric histone H3 (CENH3) from centromeres precedes uniparental chromosome elimination in interspecific barley hybrids. Proceedings of the National Academy of Sciences 108: E498-E505.

Schägger H, Von Jagow G (1987) Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Analytical Biochemistry 166: 368-379.

Schubert I, Dolezel J, Houben A, Scherthan H, Wanner G (1993) Refined examination of plant metaphase chromosome structure at different levels made feasible by new isolation methods. Chromosoma 102: 96-101.

Schubert V (2009) SMC proteins and their multiple functions in higher plants. Cytogenet Genome Res 124: 202-214.

Schubert V, Weissleder A, Ali H, Fuchs J, Lermontova I, Meister A, Schubert I (2009) Cohesin gene defects may impair sister chromatid alignment and genome stability in *Arabidopsis thaliana*. Chromosoma 118: 591-605.

Shao T, Tang D, Wang K, et al (2011) OsREC8 is essential for chromatid cohesion and metaphase I monopolar orientation in rice meiosis. Plant Physiology 156: 1386-1396.

Smolikov S, Schild-Prufert K, Colaiácovo MP (2008) CRA-1 uncovers a double-strand break-dependent pathway promoting the assembly of central region proteins on chromosome axes during *C. elegans* meiosis. PLoS Genet 4: e1000088.

Spurr AR (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. Journal of Ultrastructure Research 26: 31-43.

Sumara I, Vorlaufer E, Stukenberg PT et al (2002) The dissociation of cohesin from chromosomes in prophase is regulated by Polo-like kinase. Molecular Cell 9: 515-525.

Suzuki G, Nishiuchi C, Tsuru A, Kako E, Li J, Yamamoto M, Mukai Y (2013) Cellular localization of mitotic RAD21 with repetitive amino acid motifs in *Allium cepa*. Gene 514: 75-81.

Sym M, Engebrecht J, Roeder GS (1993) ZIP1 is a synaptonemal complex protein required for meiotic chromosome synapsis. Cell 72: 365-378.

Tao J, Zhang L, Chong K, Wang T (2007) OsRAD21-3, an orthologue of yeast RAD21, is required for pollen development in *Oryza sativa*. The Plant Journal 51: 919-930.

Valdeolmillos AM, Viera A, Page J, et al (2007) Sequential loading of cohesin subunits during the first meiotic prophase of grasshoppers. PLoS Genet 3: e28.

Wang M, Tang D, Wang K, Shen Y, Qin B, Miao C, Li M, Cheng Z (2011) OsSGO1 maintains synaptonemal complex stabilization in addition to protecting centromeric cohesion during rice meiosis. The Plant Journal 67: 583-594.

Waizenegger IC, Hauf S, Meinke A, et al (2000) Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. Cell 103: 399-410.

Weisshart, K., Fuchs, J. and Schubert, V. (2016) Structured illumination microscopy (SIM) and photoactivated localization microscopy (PALM) to analyze the abundance and distribution of RNA polymerase II molecules on flow-sorted *Arabidopsis* nuclei. Bio-protocol 6: e1725.

Westergaard M, D von Wettstein (1972) The synaptonemal complex. Annual Review of Genetics 60: 533-554.

Xu H, Beasley M, Verschoor S, et al (2004) A new role for the mitotic RAD21/SCC1 cohesin in meiotic chromosome cohesion and segregation in the mouse. EMBO Reports 5: 378-384.

Zetka MC, Kawasaki I, Strome S, Müller F (1999) Synapsis and chiasma formation in *Caenorhabditis elegans* require HIM-3, a meiotic chromosome core component that functions in chromosome segregation. Genes & Development 13: 2258-2270.

Zhang LR, Tao JY, Wang T (2004) Molecular characterization of OsRAD21-1, a rice homologue of yeast RAD21 essential for mitotic chromosome cohesion. Journal of Experimental Botany 55: 1149-1152.

Zhang L, Tao J, Wang S, Chong K, Wang T (2006) The rice OsRad21-4, an orthologue of yeast Rec8 protein, is required for efficient meiosis. Plant Molecular Biology 60: 533-554.

**Figure legends**

**Figure 1 Quantitative analysis of *LeCENH3* and *Leα*-kleisin-1 transcripts in different tissues.**

(A) The relative transcription level of *LeCENH3.1* and *LeCENH3.2* was measured by qRT-PCR. (B) The total transcription level of *Leα-kleisin-1* in leaves, stems and flower buds was measured by qRT-PCR. The number of biological replicates is indicated above the standard deviation bars.

**Figure 2 Prophase I is conventional in the holocentric species *L. elegans.***

(A-C) The distribution of Leα-kleisin from leptotene to late pachytene. (C) Colocalization of Leα-kleisin and ZmZYP1 at pachytene. (D) Electron micrographs of two *L. elegans* synaptonemal complexes (left), with the scheme of a synapsed homologous chromosome pair (right) in which the central element (CE), and putative transverse filaments (TF) indicated.

**Figure 3 Distribution of Leα-kleisin and LeCENH3 at meiotic metaphase I and II chromosomes of *L. elegans*.**

(A) Schematic model of meiosis in the holocentric species *L. elegans.* The U-shaped bivalents are aligned at metaphase I and the sister chromatids separate already during anaphase I. Homologous non-sister chromatids are connected at their termini until metaphase II. Then, they separate at anaphase II.(B) The colocalization of Leα-kleisin and LeCENH3 at metaphase I centromeres was identified by SIM after immunostaining (top). The middle panel show a region of interest (rectangle) further magnified. The quantification of centromeric fluorescence intensities of Leα-kleisin and anti-LeCENH3 from line scans of a single optical section is indicated (below). (C) The colocalization of Leα-kleisin and LeCENH3 at the centromeres of a single metaphase II daughter cell.

**Figure 4 Distribution of Leα-kleisin and CENH3 at mitotic metaphase chromosomes of *L. elegans* (A) and *H. vulgare* (B).**

(A) The holocentric species *L. elegans* shows clearly acolocalization of Leα-kleisin and LeCENH3 after immunostaining with specific antibodies. The below panels show regions of interest (rectangle) further magnified after applying SIM. (B) A monocentric *H. vulgare* chromosome acquired by SIM shows the Leα-kleisin in between of both sister centromeres, which are marked by two distinct CENH3 signals.

The schemata on the left side compare the centromere arrangement and localization of Leα-kleisin (red) and CENH3 (green) in *L. elegans* (A) and *H. vulgare* (B) chromosomes.

**Table 1**

**Temporal appearance of meiotic α-kleisin subunits during the meiosis in different species.**

**Supplemental Data**

**Supplemental Figure 1 CENH3 of *L. elegans***

(A) Gene structure model of *LeCENH3*, the positions of start/stop codons, and the *in silico* identified sequence. The obtained 5’ and 3’ RACE sequences and primer sites are indicated. (B) Alignment of CENH3 sequences from two *Luzula* species. The conserved domains of CENH3 are indicated by rectangle frames. (C) Phylogenetic analysis of CENH3 proteins from different species. (D) Western blot analysis using anti-LeCENH3, anti-histone H3 and anti-α-tubulin (as control) antibodies. The triangle indicates the band observed corresponding to the LeCENH3 protein. The total protein was extracted from *L. elegans* flower buds.

**Supplemental Figure 2 The α-kleisins of *L. elegans***

(A) Phylogenetic analysis of α-kleisin-like proteins from different plant species. Reference IDs for the phylogenetic analysis of the α-kleisinsequences used in this study are available in Supplemental Table 2. (B) Protein structure model of four *Luzula* α-kleisin-like proteins based on *in silico* identification. The similarity of N-terminal conserved regions (red) among each other is indicated. (C) Gene structure model of Leα-kleisin-1 transcripts. Positions of start/stop codon and primer sites are indicated. (D) The purified recombinant Leα-kleisinprotein was analyzed by comassie staining (blue gel on left) and Western blotting (black picture on right) with Anti-6X His tag antibodies. The major band observed corresponds to the Leα-kleisin protein (triangle). (E) The purified recombinant Leα-kleisinprotein was analyzed by Leα-kleisinrecombinant antibody. The major band observed corresponds to the Leα-kleisin protein (triangle).

**Supplemental Figure 3 The distribution of Leα-kleisin (red) and LeCENH3 (green)** **at pachytene of *L. elegans* wasidentified by SIM.**

The panels below show the regions of interest (rectangle) further magnified.

**Supplemental Figure 4 The distribution of anti-OsSGO1 (red) along metaphase II chromosomes of *L. elegans.***

**Supplemental Figure 5 The centromere localization of Leα-kleisin (red) in somatic metaphase chromosomes of *V. faba* was identified by SIM.**

**Supplemental Table1 List of primer sequences for PCR, RT-PCR and FISH**

**Supplemental Table2 List of sequence identifiers and description of α-kleisin sequences used for phylogenetic tree construction.**

**Supplemental Table3 The similarity of different Leα-kleisin protein sequences.**

**Table 1. Temporal appearance of meiotic α-kleisin subunits during the meiosis in different species.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Meiotic α-kleisin** | **Presence during meiosis** | **Reference** |
| **Common name** | **Scientific name** |
| Wood rush | *Luzula elegans* Lowe | Leα-kleisin | Leptotene to anaphase II | This study |
| [Thale cress](http://www.minnesotawildflowers.info/flower/mouse-ear-cress) | *Arabidopsis thaliana* (L.) Heynh. | SYN1 | Leptotene to metaphase I | Cai et al. 2003 |
| Tomato | *Solanum lycopersicum* (L.)H. Karst | REC8 | Leptotene to diplotene | Qiao et al. 2011 |
| Rice | *Oryza sativa* L. | REC8 | Leptotene to diplotene | Shao et al. 2011 |
| Nematode | *Caenorhabditis elegans* ([Maupas](https://en.wikipedia.org/wiki/%C3%89mile_Maupas%22%20%5Co%20%22%C3%89mile%20Maupas), 1900) | REC8 | Leptotene to the onset of anaphase I | de Carvalho et al. 2008 |
| Grasshopper | *Eyprepocnemis plorans* (Charpentier, 1825) | REC8 | Zygotene to metaphase I | Valdeolmillos et al. 2007; Calvente et al. 2013) |
| Mouse | *Mus musculus* L. | RAD21 | Leptotene to the end of anaphase II | Xu et al. 2004 |
| REC8 | Leptotene to metaphase II | Lee et al. 2003 |
| RAD21L | Leptotene to the end of pachytene | Lee and Hirano 2011 |
| Rat | *Rattus norvegicus* (Berkenhout, 1769) | REC8 | Leptotene to anaphase II | Eijpe et al. 2003 |
| Human | [*Homo sapiens*](https://en.wikipedia.org/wiki/Homo_sapiens)L. | RAD21L | Leptotene to anaphase II | Herrán et al. 2011 |
| REC8 | Leptotene to metaphase II | Garcia-Cruz et al. 2010 |