LETTERS

nature cell biology

Fused sister kinetochores initiate the reductional division in meiosis I

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During meiosis I the genome is reduced to the haploid content by a coordinated reductional division event. Homologous chromosomes align, recombine and segregate while the sister chromatids co-orient and move to the same pole[1,2]. Several data suggest that sister kinetochores co-orient early in metaphase I and that sister chromatid cohesion (which requires Rec8 and Shugoshin) supports monopolar orientation. Nevertheless, it is unclear how the sister kinetochores function as single unit during this period. A gene (monopolin)[3] with a co-orienting role was identified in *Saccharomyces cerevisiae;* however, it does not have the same function in fission yeast[4] and no similar genes have been found in other species. Here we pursue this issue using knockdown mutants of the core kinetochore protein MIS12 (minichromosome instability 12). MIS12 binds to base of the NDC80 complex, which in turn binds directly to microtubules[5–7]. In maize plants with systemically reduced levels of MIS12, a visible MIS12–NDC80 bridge between sister kinetochores at meiosis I is broken. Kinetochores separate and orient randomly in metaphase I, causing chromosomes to stall in anaphase due to normal cohesion, marked by Shugoshin, between the chromatids. The data establish that sister kinetochores in meiosis I are fused by a shared microtubule-binding face and that this direct linkage is required for reductional division.

Meiosis I is built on the basic mechanics of mitosis with the fundamental modifications that homologous chromosomes recombine with each other and that sister chromatids remain together (Fig. 1a)[1]. A structure called the synaptonemal complex mediates both events by simultaneously linking homologues, promoting crossing over, and sealing sister chromatids along their axis. The combination of crossover points (chiasmata), cohesion between sister chromatids and the fusion of sister kinetochores forms bivalents that promote bipolar spindle interactions. The final step of coordinating sister chromatids gives meiosis its 'reductional' name; without this coordination, the pairing information from prophase is lost and chromosomes segregate randomly, as in mitosis.

Using an unknown mechanism, the bound sister kinetochores present a single binding face that interacts with one pole only. It is known that the cohesin subunit Rec8 is involved in this process, and in fission yeast *Schizosaccharomyces pombe*, Rec8 is regulated within the kinetochore by a protein called Moa1 (Monopolar attachment 1, ref. 8). Rec8 and Moa1 influence the behaviour of kinetochores in metaphase by binding chromatids at their base during prophase[9]. In the budding yeast *S. cerevisiae*, a protein with a direct role in coordinating sister kinetochore co-orientation, monopolin[3], functions in a Rec8-independent manner. In *S. pombe*, however, loss of monopolin has only a minor affect on meiosis I and a severe impact on the accuracy of mitotic segregation[4]. It has been proposed that monopolin organizes bundles of microtubules at the kinetochore interface[4]. Accordingly, in budding yeast (which has only one microtubule per kinetochore) monopolin is only required at meiosis but in more complex eukaryotes (with 4–11 microtubules per kinetochore) it functions to maintain kinetochore integrity in general. Monopolin homologues have not been found in species other than fungi. A conserved kinetochore 'sister clamp' has been postulated[4], but the protein and underlying molecular mechanism have remained elusive.

In all eukaryotes, microtubules attach to kinetochores by the four-protein NDC80 complex. NDC80 interacts directly with the MIS12 complex, which interacts indirectly with the DNA-binding proteins CENH3 (centromeric histone H3) and CENPC (centromere protein C)[7]. MIS12 is required to maintain the structural integrity of mitotic kinetochores[6] and as such is one candidate for the clamping function that mediates sister kinetochore co-orientation at meiosis I. We chose to study MIS12 in maize, which is known for its exceptional chromosome cytology. Like many other genes in maize, there are two ancient copies of *Mis12*: *Mis12-1* and *Mis12-2*. The two cDNAs are 89% identical, but *Mis12-1* produces a shorter protein product (223 amino acids) than *Mis12-2* (244 amino acids) due to an early stop codon. Quantitative reverse transcription-PCR (qRT-PCR) revealed that both genes are expressed, with *Mis12-2* being particularly abundant. MIS12 antisera were prepared to analyse the protein product in wild-type and mutant individuals. The antibodies recognized both MIS12-1 and MIS12-2 as purified proteins, but identified a single band with a relative molecular mass of ~26,000 (M_r ~26K) in protein extracts from maize shoots and roots (Fig. 2a).

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Received 23 March 2009; accepted 1 June 2009; published online 16 August 2009; DOI: 10.1038/ncb1923

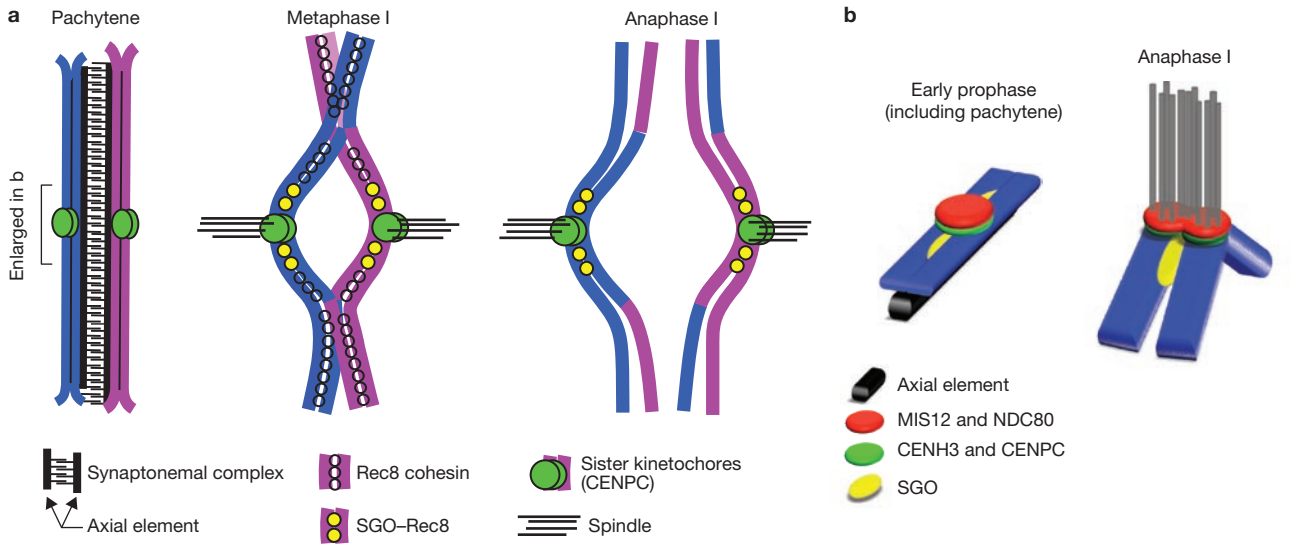

Figure 1 Meiosis I. (**a**) An overview of the reductional division event, showing the roles of kinetochores, Rec8 and Shugoshin (SGO). By the time chromosomes align at metaphase I, homologous chromosomes have already paired and recombined to form crossover points (chiasmata). Chiasmata are held in place by a cohesion complex, containing Rec8, between sister chromatids. Rec8 is destroyed at anaphase to release chiasmata. Shugoshin protects Rec8 in pericentromeric regions until meiosis II. Kinetochores are presumed to initiate segregation. (**b**) A three-dimensional perspective highlighting the role of the MIS12–NDC80 bridge. The left panel shows that meiotic kinetochores are formed in prophase I when a single axial element underlies the sister chromatids. The MIS12–NDC80 domain is shown in red and the centromeric regions (marked by CENH3 and CENPC) are shown in green. At this stage, Shugoshin is visible in pericentromeric regions (see Fig. 4a)[17]. Axial elements are removed in late prophase but the MIS12–NDC80 bridge, which binds directly to microtubules, remains intact during metaphase I and early anaphase I.

We interpret the single band to indicate that one of the two proteins, presumably MIS12-2, is abundant in these tissues.

Immunolocalization revealed that MIS12 is present at kinetochores during all stages of the cell cycle (Fig. 2b–e), as are the maize homologues of CENPC, CENH3 and NDC80 (refs 10–12). When kinetochores are under tension at metaphase, it is apparent that MIS12 localizes to an outer region (Fig. 2f) that contains maize NDC80 (Fig. 2d)[10]. We also found that both MIS12 and NDC80 show a specialized staining pattern at metaphase I: a continuous domain of staining that links the two sister kinetochores (Fig. 2f, g). In contrast, staining by anti-CENH3 and anti-CENPC clearly resolves the individual kinetochores[11,12]. Although MIS12 staining is weaker between sisters than it is over them, the bridge-like pattern is highly reproducible and qualitatively different from CENPC staining. The novel staining pattern of MIS12 and NDC80 will be referred to as the MIS12–NDC80 bridge.

To test MIS12 function, we used RNAi to knockdown *Mis12-1* and *Mis12-2* gene expression. The respective cDNAs were cloned into RNAi vectors, transformed into maize and the progeny scored over several generations (Supplementary Information, Fig. S1). Expression assays in *Mis12-1* RNAi lines revealed that mRNA levels were reduced by 40–80% relative to wild type, and that both the *Mis12-1* and *Mis12-2* genes were suppressed by the single RNAi construct (Supplementary Information, Fig. S2). We also measured the reduction of MIS12 protein directly on kinetochores, using CENPC as an internal control (MIS12/CENPC ratio). The data reveal that in both *Mis12-1* and *Mis12-2* RNAi lines, MIS12 abundance is reduced by 20–40% (Supplementary Information, Table S1). As both the *Mis12-1* and *Mis12-2* RNAi constructs systemically reduced total MIS12 levels, we interpret the experiments as replicate treatments, although they were carried out for different durations and at different times of year.

We observed no whole-plant (mitotic) phenotypes in primary transformants or first-generation (F_1) progeny, presumably because the MIS12 knockdown of ~25% was relatively mild. Nevertheless, we anticipated that larger segregating populations in different genetic backgrounds would reveal noticeable defects. Hundreds of F_2 progeny from *Mis12-1* lines were planted under field conditions, where sporadic and severe dwarfing features were observed (Supplementary Information, Fig. S3). The sporadic and severe nature of the phenotypes suggested that aneuploid progeny were produced by meiotic errors in the F_1 parents. We did not test this interpretation directly because the affected plants were sterile and aneuploidy is difficult to assay on mature plants. Instead, parents or direct relatives of the lines producing small plants were re-grown under greenhouse conditions and assayed at meiosis (Supplementary Information, Fig. S1).

Severe meiotic failures were observed in all of the families chosen for detailed study (Fig 3; Table 1). Although prophase and recombination (chiasmata) frequencies were normal in *Mis12* RNAi lines (Table 1), chromosome alignment and segregation at meiosis I was aberrant. Metaphase I chromosomes failed to align in the centre of the spindle and produced an uneven mass of chromosomes. The irregular metaphase was followed by incomplete anaphase I segregation (Fig. 3a, b) where chromosomes stalled and remained in the midzone as the new cell plate formed (Fig. 3c, d). In meiosis II, the isolated chromosomes formed independent nuclei that organized their own mini-spindles (Fig. 3e). Such severe abnormalities produced aberrantly shaped tetrads with small nuclei scattered in the cytoplasm of daughter cells (Fig. 3f–h). Approximately 2.17% of tetrad cells contained mini-nuclei or other abnormalities in mutant lines (s.d. ± 1.35%; n = 10,353 from 11 plants), whereas in wild-type segregants the mini-nuclei frequency was 0.06% (s.d. ± 0.03%; n = 2,479 from 3 plants). We also observed a

nearly identical suite of phenotypes in the independent *Mis12-2* RNAi experiment (Table 1). The combined data show that visible errors from metaphase I to the tetrad stage are nearly 30 times higher than those observed in wild-type cells. Most of these events, if not all, can be traced to defects in anaphase I disjunction, which produce lagging chromosomes that cause errors in meiosis II spindle morphogenesis and tetrad formation. The large difference between the observed error rate at metaphase and anaphase (20–40%) and the mini-nuclei frequency (2.17%) can be attributed to the fact that most errors are ultimately resolved (cytologically) by premature disjunction or random migration to one pole and that the mini-nuclei assay is very conservative. Only complete failures that leave a chromosome isolated from the spindle and at a wide distance from the forming telophase nuclei are observed as mini-nuclei.

The reduction in MIS12 caused by *Mis12-1* and *Mis12-2* RNAi causes approximately 30% of the sister kinetochores to separate and align equationally at meiosis I (Figs 3; Table 1; Supplementary Information, Fig. S4). On equationally orientated chromosomes, the sister kinetochores are wholly separate and distinct, as measured by CENPC, MIS12 or NDC80 antisera, align with the spindle axis and organize distinct microtubule bundles (kinetochore fibres) that emanate to opposite poles (see Fig. 3a,-b for detail; Supplementary Information, Fig. S4). Although CENPC staining usually disjoins cleanly, MIS12 staining often seems to stretch and separate unevenly (Fig. 3a, kinetochores 2 and 4). When MIS12 separates largely towards one kinetochore, there is a corresponding increase in the size of the attached microtubule bundle (Supplementary Information, Fig. S4D). In rare cases, single kinetochores form two microtubule-binding faces that orient to opposite poles (merotelic attachment; Fig. 3c). The merotelic phenotype was common in severe *Mis12* knockdown mutant lines that affect human mitosis[6] but was unusual in our analysis. In a sample of 28 anaphase I cells from a *Mis12-1* RNAi line (XL373), sister separation followed by equational alignment was observed 145 times, but sister separation followed by merotelic alignment was observed only 13 times (~8% of the errors and < 3% overall). These data rule out merotelic alignment as a cause[13] of the *Mis12* RNAi equational disjunction phenotype. Although the MIS12–NDC80 bridge was particularly sensitive to reductions in MIS12 abundance, we presume that more severe *Mis12* knockdowns would have caused more frequent merotelic alignments and pronounced defects in mitosis. Such a phenotype is unlikely to have survived our transformation protocol, which requires full plant regeneration from cultured cells.

The onset of meiotic anaphase I is regulated by proteins that destroy the cohesion between sister chromatids (Fig. 1a). When the chromosomes have adopted metaphase positions that impart tension on the kinetochores, an anaphase-promoting complex causes the removal of Rec8, a meiosis specific cohesin component[1]. This breaks chiasma bonds so that chromosomes can pull away from each other. A specialized protein called Shugoshin ('guardian spirit'; SGO) protects Rec8 in the vicinity of centromeres so that sisters remain together during anaphase I (refs 14, 15). Our observations revealed that Shugoshin is localized to pericentromeric chromatin during prophase (Fig. 4a) and behind the kinetochores at early prometaphase I (Fig. 4b, c). When *Mis12* RNAi weakens the MIS12–NDC80 bridge and kinetochores separate, Shugoshin lies in the regions between the sister chromatids (Fig. 4d), presumably restraining the chromosomes and causing the abundance of lagging chromosomes observed in anaphase I. As expected, Shugoshin staining intensity did not differ between *Mis12* mutant and wild-type cells (Supplementary Information, Table S1). Taken together, the data indicate that Shugoshin is not sufficient for kinetochore co-orientation, confirming the results of Shugoshin knockout mutations in maize and other species[14–18].

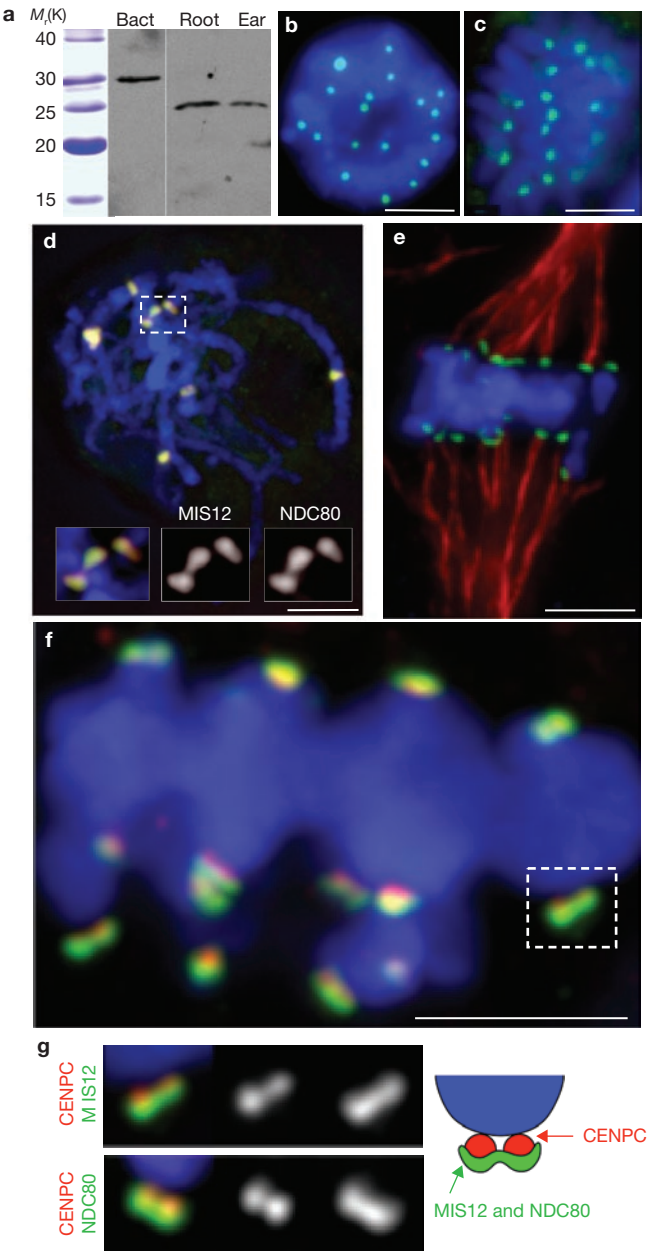

Figure 2 Wild-type MIS12 staining. (**a**) Protein analysis of MIS12-2 antibodies. The bacterially expressed protein (bact) is larger because of a His tag. In maize roots and young ears, the antibodies recognize a band of relative molecular mass (M_r) 25–27K. (**b**, **c**) Staining for MIS12 (green) and chromosomes (blue) of a cell in mitotic interphase (**b**) and a cell in mitotic prophase (**c**). (**d**) MIS12 (red) and NDC80 (green) at meiotic prophase (pachytene). The cell is a full projection with insets showing the staining for each protein. MIS12 and NDC80 co-localize well, and are shown in yellow in the main image. (**e**) Cell in meiotic metaphase (spindle in red and MIS12 in green). (**f**) A prometaphase I cell double stained for MIS12 (green) and CENPC (red). The boxed pair of kinetochores is also shown in **g**. (**g**) The MIS12–NDC80 bridge. The upper panel shows CENPC (red) and MIS12 (green) from the cell in **f**. The lower panel shows a pair of sister kinetochores from a different prometaphase cell stained for CENPC (red) and NDC80 (green). Scale bars, 5 μm.

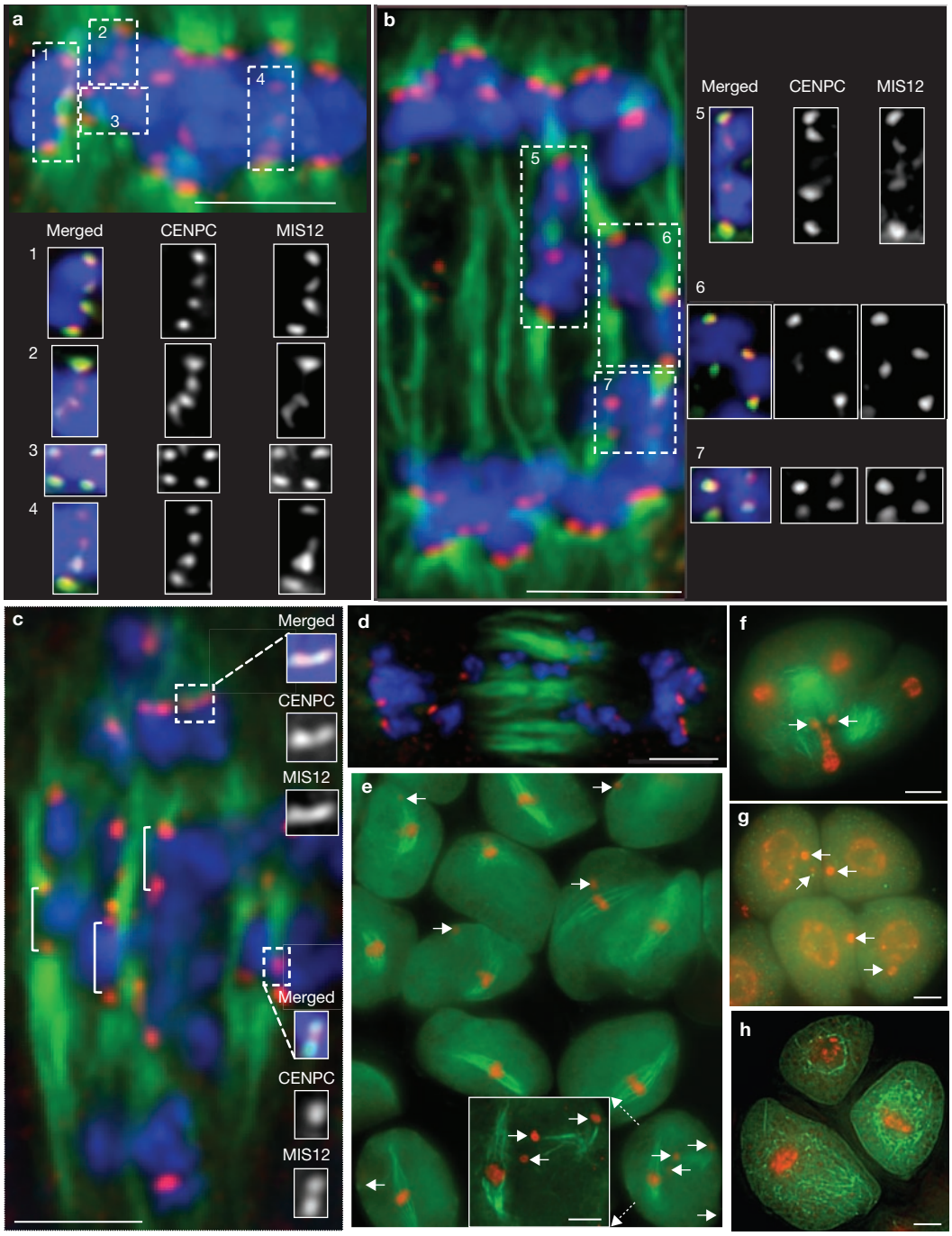

Figure 3 Meiotic defects in *Mis12* RNAi cell lines. All images are optical sections from 3D data sets. (**a**) Metaphase I cell showing that sister kinetochores prematurely separate and bi-orient following *Mis12* RNAi. This cell was stained for both CENPC and MIS12, but only CENPC is shown on the main image. At least eight sets of bi-oriented sister kinetochores are indicated with numbered boxes. Each numbered box is shown below the main image with CENPC in red and MIS12 in green. Single-channel images showing CENPC and MIS12 alone are shown the right of each inset. (**b**) Anaphase I cell showing chromosomes lagging in the spindle midzone. On the main image only CENPC is shown. Three pairs of homologous chromosomes showing premature kinetochore separation and equational alignment are shown. As in **a**, the affected chromosomes are indicated with numbered boxes and are reproduced on the right to reveal CENPC and MIS12 staining. (**c**) A different anaphase I cell showing a particularly severe phenotype (CENPC, red). Sister kinetochores are separated and aligned equationally on nearly all chromosomes (three in the focal plane are indicated with brackets). In addition, three single kinetochores are aligned merotelically (one is highlighted, lower right). An example of normal co-orientation with a remaining MIS12 bridge is highlighted, upper right. MIS12 is shown in green in merged insets. (**d**) Late telophase cell showing lagging chromosomes trapped in the emerging cell plate (MIS12, red). (**e**) A field of metaphase II cells illustrating how lagging chromosomes from meiosis I affect meiosis II spindle morphology (nuclei, red). The inset is an enlarged view of multiple mini-nuclei and mini-spindles. (**f**–**h**) Tetrad defects caused by multiple spindles and lagging chromosomes. Mini-nuclei are indicated with arrows. DNA is shown in red and tubulin in green. Scale bars, 5 μm.

Table 1 Quantification of meiotic errors in *Mis12* RNAi mutant plants

	Diakinesis	Prometaphase/metaphase I	Anaphase I	Metaphase II	Anaphase II
		***Mis12-1* RNAi**			
WT	0/110 (0)	0/125 (0)	1/142 (0.7%)	2/181 (1.1%)	150/0 (0)
XL48	0/107 (0)	68/216 (32%)	108/139 (78%)	72/193 (37%)*	12/38 (32%)*
XL370	0/102 (0)	60/146 (42%)	49/121 (41%)	70/184 (38%)	41/114 (36%)
XL373	0/115 (0)	41/137 (30%)	38/103 (37%)	64/150 (43%)	44/121 (36%)
		***Mis12-2* RNAi**			
WT	0/145 (0)	0/114 (0)	0/121 (0)	1/110 (0.9%)	0/123 (0)
XL346	0/118 (0)	25/98 (26%)	43/126 (34%)	73/221 (33%)	35/121 (29%)
XL361	0/120 (0)	26/106 (25%)	24/101 (24%)	31/110 (28%)	31/118 (26%)

Data are represented as number of cells with errors per number of cells counted. The errors counted were: diakinesis, unpaired chromosomes; prometaphase/metaphase I, sister kinetochore separation; anaphase I, lagging chromosomes; metaphase II, multiple nuclei and anaphase II, spindle abnormalities. Seventeen wild-type (WT) plants were scored to confirm that meiotic errors were limited to transgene-containing lines; two wild-type plants are featured to illustrate the natural error rate. Twenty-eight mutant plants were observed and all showed meiotic defects. The five scored in the table were among those with severe phenotypes. Pedigree information for the lines used (XL numbers) is shown in Supplementary Information, Fig. S1. *Data from sibling line XL43.

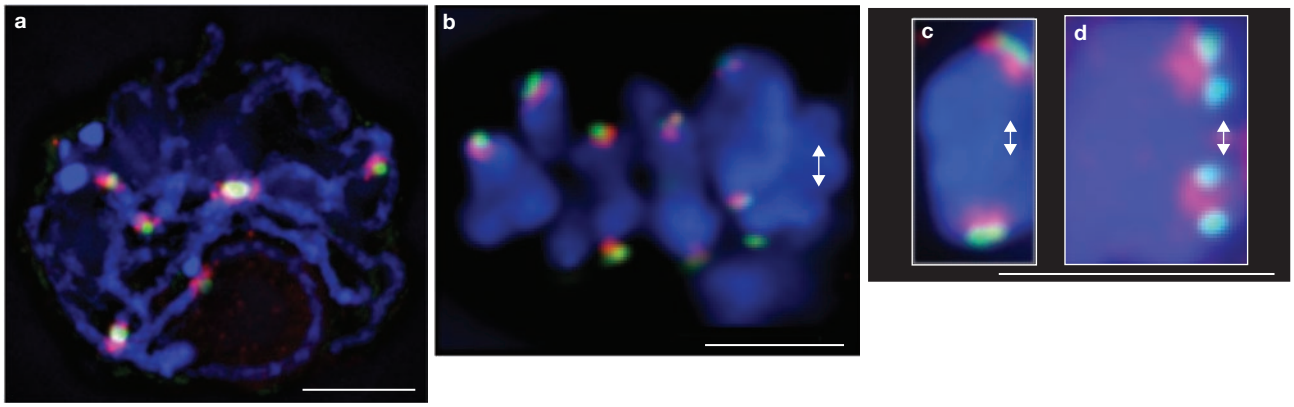

Figure 4 Shugoshin (SGO) stains pericentromeric regions and remains in place when sister kinetochores separate in *Mis12* RNAi mutants. (**a**) Meiotic prophase (pachytene) cell showing CENPC (green) and maize SGO (red). This image is a full projection showing all the chromosomes in the cell. (**b**) Optical section of a prometaphase I cell showing SGO staining trailing CENPC (green). (**c**) A close-up of one set of homologous chromosomes oriented properly. (**d**) A metaphase I chromosome from a *Mis12* RNAi line showing both sets of sisters separated and aligned with the spindle axis. SGO remains between the separated sister kinetochores. Arrows show spindle axis. Scale bars, 5 μm.

On the basis of these data we propose a model for the mechanism of sister kinetochore co-orientation and reductional chromosome segregation in meiosis I (Fig. 1b). Axial elements of the synaptonemal complex hold sister chromatids together in early prophase I (refs 19–21), and provide the axes for fused kinetochore assembly at the centromere core[9,22]. By the time the spindles have begun to form, the unified kinetochore has become the leading structure and initiates reductional segregation by a single microtubule-binding face. Axial elements must be either disassembled[23] or otherwise subordinate to kinetochore function at metaphase and anaphase I, as *Mis12* RNAi alone can cause premature sister separation. Nevertheless, sister kinetochores may naturally separate as anaphase progresses[21,24], and under these conditions we presume that pericentromeric Shugoshin becomes the primary means of holding the sister chromatids together. Therefore, we propose that the MIS12–NDC80 bridge and Shugoshin cooperate to cause reductional segregation. This model accommodates the fact that loss of Rec8 causes the full disjunction of sister chromatids[1,8,9,25], as Rec8 is required to organize the axial elements[25–27] that support kinetochore formation and is the binding substrate of Shugoshin[14,15]. We emphasize the nature and timing of kinetochore formation, and do not mean to imply that MIS12 is sufficient for sister kinetochore co-orientation (although MIS12 is a particularly important structural protein)[6]. It is possible that knockdown of NDC80 or other critical proteins in the central domain[7] may have similar meiotic phenotypes.

The observation that MIS12 has an important role in connecting sister chromatids at meiosis I helps to resolve the long-standing question of whether kinetochores have active or passive roles in reductional segregation[1,20,21,28]. Our results broadly support a previous proposal that sister centromeres do not replicate in meiosis I (ref. 28). Although centromeres do replicate, the functional kinetochore domains do not. Similarly, our data support previous interpretations from yeast, where different proteins are involved (the monopolin complex) but a similar mechanism operates[4]. Like MIS12 and NDC80 in maize, monopolin organizes bundles of microtubules to ensure the co-orientation of sister kinetochores. The implications also extend to medicine and the underpinnings of aneuploid diseases. In human females, most errors in meiosis occur during ovulation as chromosomes align and segregate at meiosis I (ref. 28). Our data show that quantitative reductions of a key kinetochore structural protein lead to premature separation of sister chromatids, a main cause of human aneuploidy[29]. Age-dependant loss of kinetochore proteins may provide a mechanical basis for many of these meiotic errors. □

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturecellbiology/.

Accession codes. GenBank: *Mis12-1,* FJ971487; *Mis12-2,* FJ971488.

Note: Supplementary Information is available on the Nature Cell Biology website.

ACKNOWLEDGEMENTS

We thank X. Zhang for her support and help with RT-PCR and image analysis, H. Tang for help with statistics, R. Wang for providing Shugoshin antibodies, A. Luce for technical support and C. Topp and L. Kanizay for helpful comments. This study was supported by a grant from the National Science Foundation to R.K.D. (0421671).

AUTHOR CONTRIBUTIONS

X.L. performed experimental work and data analysis. R.K.D. focused on planning and interpretation.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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METHODS

Identification of maize *Mis12* genes and comparison of their mRNA abundance. A presumed *Mis12* homologue from *Glycine max* (sp43a06.y1; ref. 5) was used as a query to search maize sequence databases. Another research group followed the same reasoning and identified *Arabidopsis* MIS12 (ref. 30). Primers homologous to two maize sequences (MAGI4_132977 and MAGI4_143787; AC155386.2) were used to identify full-length *Mis12* cDNAs from inbred B73 ear tissue. To compare relative expression levels, specific primers were used in a quantitative reverse transcription-PCR (qRT-PCR) assay with Ubiquitin as an internal control. Primers used were: *Mis12-1*, 5′-GAAGAGTCGGAAGAAGAAGCGGGCG-3′ (forward) and 5′-TAATCTCAGTCCTTCTC TGATTTGCA-3′ (reverse); *Mis12-2*, 5′-GCCCCCCCACAAATCCACAATCCAA-3′ (forward) and 5′-ATTTTCTGCCGCAATGCCGGTATTG-3′ (reverse); maize Ubiquitin-1 *(Ubi1)*, 5′-TAAAGACCCTGACTGGAAAA (forward) and 5′-ACGACCCATGACTTACTGAC-3′ (reverse).

Protein analysis. The complete *Mis12-2* coding sequence was cloned into a pET-28a expression vector (Novagen) and expressed in bacteria. The His-tagged MIS12-2 protein was purified using Ni-NTA agarose. Anti-MIS12 antibodies were prepared in rabbit and affinity-purified by Strategic Biosolutions. The Mis12-2 antiserum was used for all images except Fig. 2d. To achieve the double staining shown in this figure, a MIS12-1 antiserum (which recognizes both MIS12 proteins; data not shown), made in the same manner but prepared in rat, was used as the MIS12 label. For protein blotting, nuclear protein from fresh root tips (~3 mm in length) and young ears (~7 cm in length) was extracted and blotted as described previously[31].

Indirect immunostaining of male meiotic cells. Male meiocytes were prepared[31] from *Mis12* transgenic lines and wild-type siblings. Cells were incubated with rat anti-MIS12-1 (1:100), rabbit anti-MIS12-2 (1:100), rabbit anti-NDC80 (1:50; ref. 10), chicken anti-CENPC (1:100; ref. 11) rabbit anti-Shugoshin (1:50; ref. 17) or mouse anti-tubulin (1:500).

Image analysis. Data were collected and analysed using a Zeiss Axioimager and Slidebook software (Intelligent Imaging Innovations). For quantification of MIS12 and Shugoshin signal intensity, wild-type and mutant cells were spotted to the same slide to reduce experimental variation. The total signal intensity from all kinetochores in a cell was subtracted from the background signal intensity.

Transgenic plant production and propagation. *Mis12-1* cDNA was cloned into pMCG7942 (ref. 32) such that the maize Ubiquitin-1 promoter drove expression over two inverted copies of the same sequence. The *Mis12-1* RNAi construct was transformed into a hybrid line HiII by biolistic bombardment at Iowa State University. The *Mis12-2* RNAi construct was prepared in a similar vector (pMCG1005) and transformed into HiII by *Agrobacterium*-mediated transformation. Nineteen *Mis12-1* RNAi lines and 9 *Mis12-2* RNAi lines were crossed and studied. *Mis12-1* RNAi lines were screened at the UGA Plant Sciences farm (summer, 2007), other plants were grown in greenhouses.

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nature cell biology

DOI: 10.1038/ncb1923

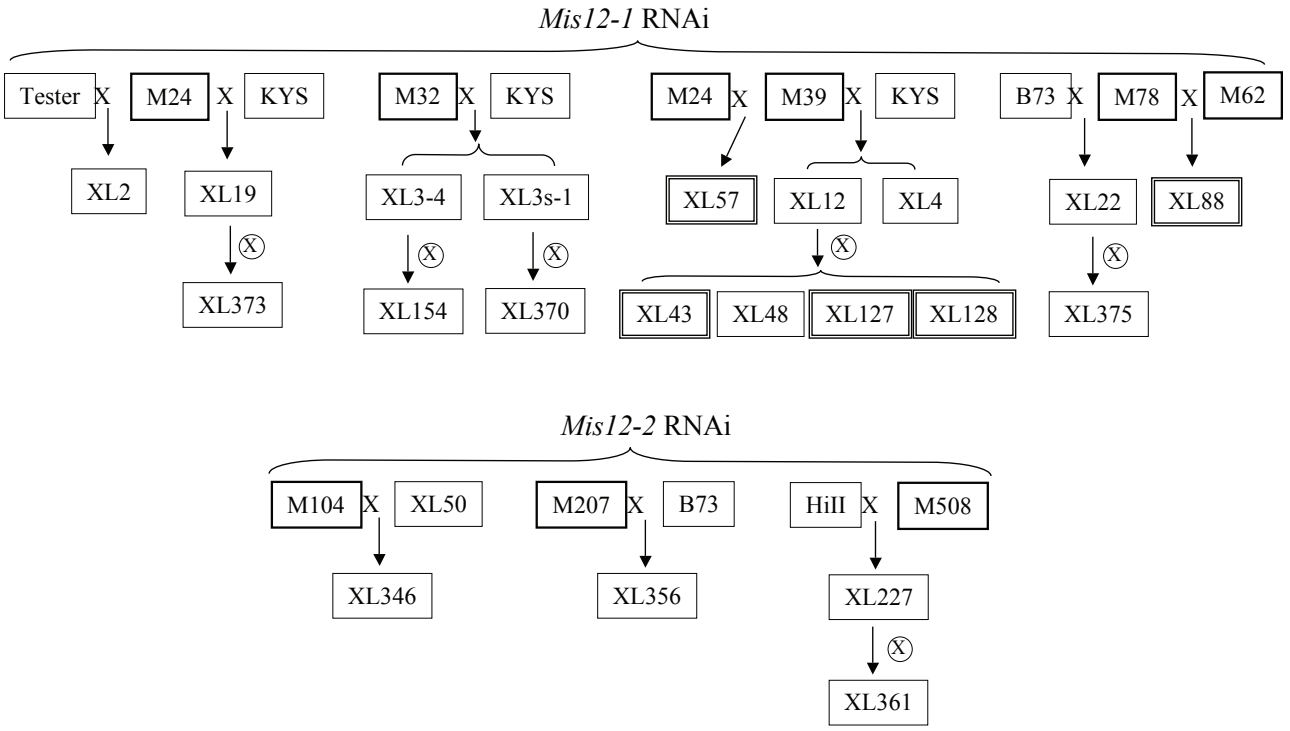

Figure S1 Pedigree of the *Mis12* RNAi lines used in this study. Families beginning with 'M' and outlined in bold are primary transformants. Transformants were first crossed to inbred lines (KYS or B73) or hybrid (HiII or lab tester), then self crossed for further analysis. Families marked by double outline segregated sporadic dwarf plants.

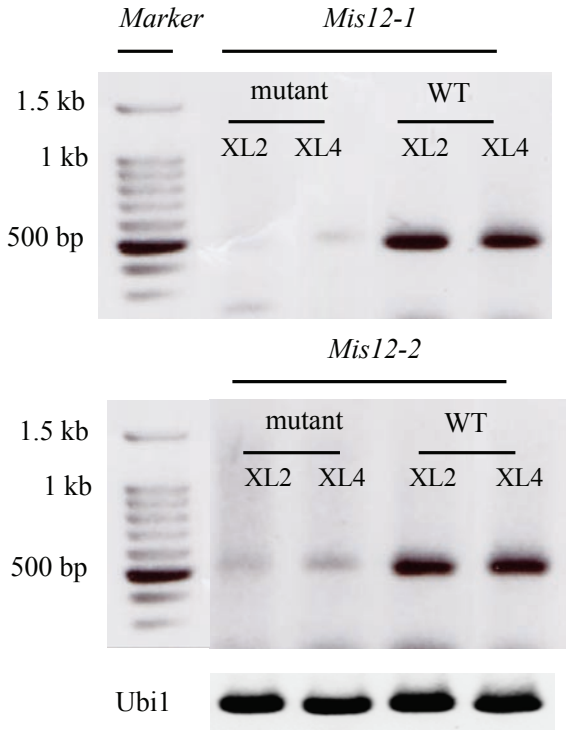

Figure S2 *Mis12-1* RNAi reduces the accumulation of both *Mis12-1* and *Mis12-2* mRNA. The gels show the results of a quantitative RTPCR experiment using primers specific to the individual genes. mRNA reduction varied from 40-80% in different experiments. The origin of the RNAi lines used (XL numbers) can be found in Suppl. Figure 1.

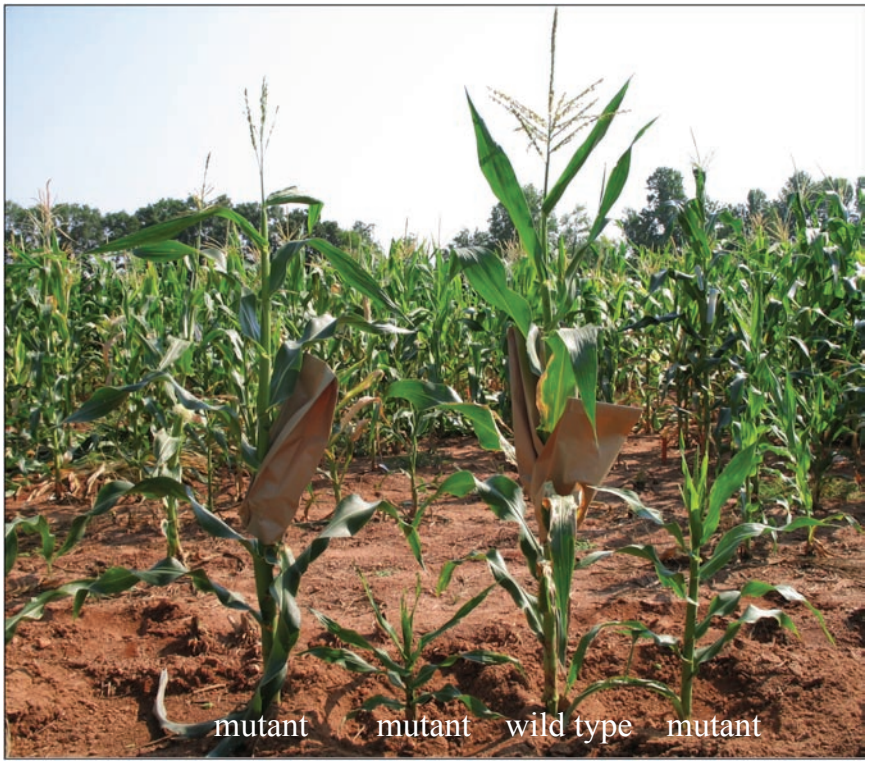

Figure S3 Dwarf plants co-segregate with *Mis12* RNAi in F2 progeny. Four plants from a family (XL88) segregating mutant and wild type plants are featured in the forefront. The shortest mutant plant is one ninth as tall as the wild type plant. However, many other mutant plants in the same family appeared normal. Brown bags are used to protect crossed ears.

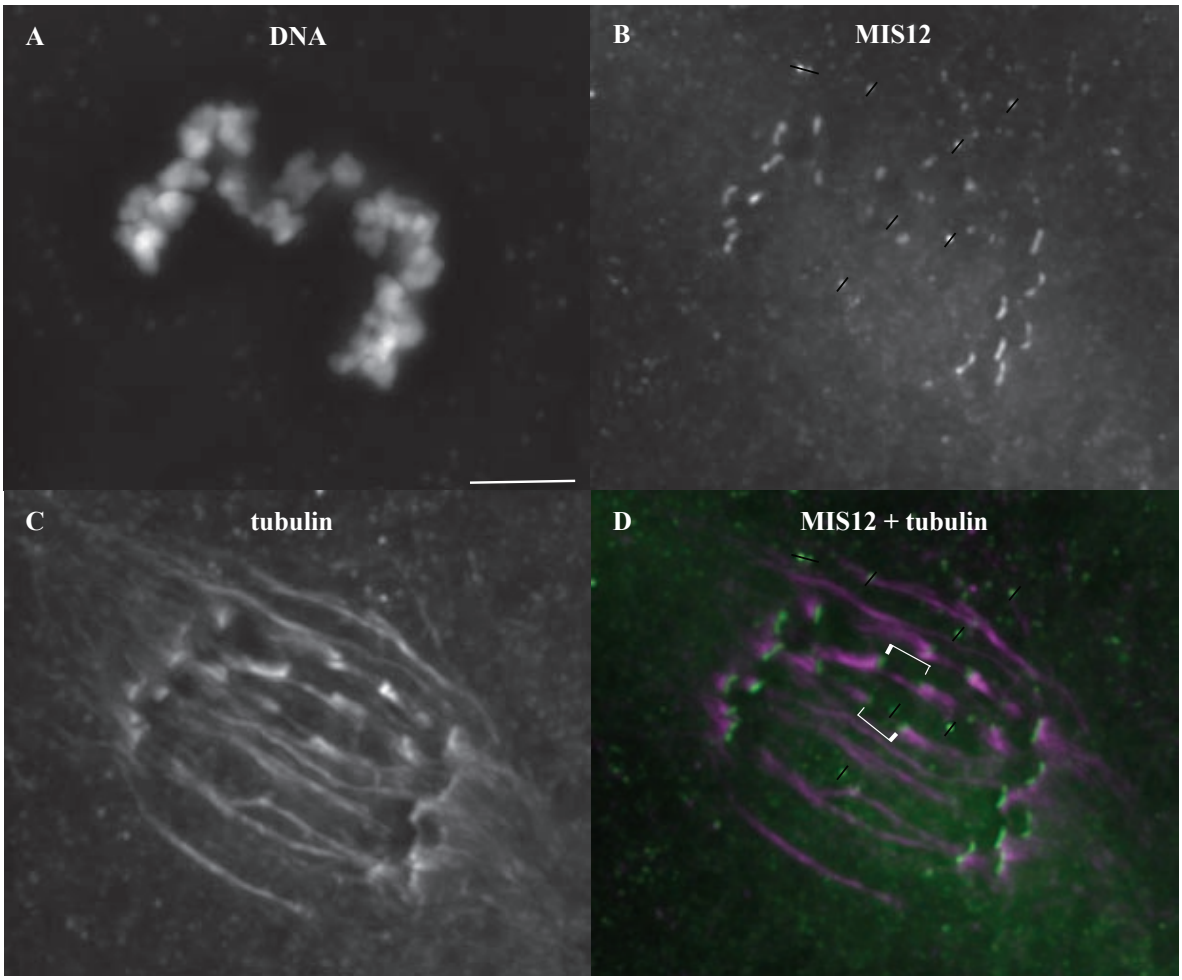

Figure S4 Full images of the cell shown in Figure 3B. Here, all optical sections from the cell are projected (added) to produce a single image. DNA (A), MIS12 (B) and tubulin (C) are shown individually, as well as double stain for MIS12 and spindle (D). Seven background spots in the MIS12 channel are struck with diagonal black lines for clarity. Background spots are not associated with either chromatin or spindle staining (similar spots are observed in wild type cells with this antibody). In D, two chromosomes with separated and equationally aligned sister kinetochores are indicated with white brackets. In these two cases, MIS12 separated unevenly such that staining is much brighter on one of the sister kinetochores. The size of the microtubule bundle corresponds closely to the size of the MIS12 domain. Scale bar=5 μm