Untimely expression of gametogenic genes in vegetative cells causes uniparental disomy

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Uniparental disomy (UPD), in which an individual contains a pair of homologous chromosomes originating from only one parent, is a frequent phenomenon that is linked to congenital disorders and various cancers. UPD is thought to result mostly from pre- or post-zygotic chromosome missegregation. However, the factors that drive UPD remain unknown. Here we use the fission yeast *Schizosaccharomyces pombe* as a model to investigate UPD, and show that defects in the RNA interference (RNAi) machinery or in the YTH domain-containing RNA elimination factor Mmi1 cause high levels of UPD in vegetative diploid cells. This phenomenon is not due to defects in heterochromatin assembly at centromeres. Notably, in cells lacking RNAi components or Mmi1, UPD is associated with the untimely expression of gametogenic genes. Deletion of the upregulated gene encoding the meiotic cohesin Rec8 or the cyclin Crs1 suppresses UPD in both RNAi and mmi1 mutants. Moreover, overexpression of Rec8 is sufficient to trigger UPD in wild-type cells. Rec8 expressed in vegetative cells localizes to chromosomal arms and to the centromere core, where it is required for localization of the cohesin subunit Psc3. The centromeric localization of Rec8 and Psc3 promotes UPD by uniquely affecting chromosome segregation, causing a reductional segregation of one homologue. Together, these findings establish the untimely vegetative expression of gametogenic genes as a causative factor of UPD, and provide a solid foundation for understanding this phenomenon, which is linked to diverse human diseases.

The genetic tractability and small karyotype of *S. pombe* provide an outstanding model for studying chromosome segregation. The three *S. pombe* chromosomes contain discrete blocks of facultative heterochromatin as well as constitutive heterochromatin domains such as at centromeres and telomeres. RNAi-dependent and -independent mechanisms control gene expression and direct heterochromatin assembly. Chromodomain proteins such as heterochromatin protein 1 (HP1) bound to methylated histone H3 lysine9 (H3K9me) recruit effectors involved in gene silencing and proper chromosome segregation. Indeed, cells lacking Clr4 (a homologue of mammalian SUV39H), HP1 or RNAi machinery show lagging chromosomes resulting from defects in heterochromatin-dependent cohesin localization at centromeres.

Notably, RNAi mutants also exhibit a distinct chromosome segregation defect. We found that RNAi mutant diploids consistently display asymmetric segregation of alleles, such that otherwise heterozygous diploids become homozygous for genetic markers mapping to one of the chromosomes, thus resulting in loss of heterozygosity (LOH). To investigate this phenotype further, we designed an assay to measure LOH quantitatively. Heterozygous diploids carrying markers distributed on different chromosomes were constructed by mating wild-type or mutant haploid strains (Fig. 1a, b). Diploids maintained by selecting for markers on one chromosome were scored for LOH of markers on another chromosome (see Methods). Using our assay, we observed higher levels of LOH for the pericentromeric *lys1* locus in diploids lacking RNAi components Dicer (dcr1), Argonaute (ago1) or the RNA-directed RNA polymerase (rdp1) as compared to wild type (Fig. 1c).

To confirm LOH further, we sporulated diploids and performed tetrad analysis and random spore analysis (Fig. 1d, bottom; Extended Data Fig. 1a, b). We also used live-cell imaging to monitor the *lys1* locus, which was tagged with lacO arrays labelled with green fluorescent protein (GFP)-tagged Lacl (Fig. 1d, top). Diploids lacking RNAi machinery frequently yielded asymmetric 4:0 or 0:4 segregation of genetic markers instead of the expected 2:2 segregation (Fig. 1d; Extended Data Fig. 1b), which is indicative of LOH occurring during the growth of diploid cells. Together, these results show a high incidence of LOH in RNAi mutant diploids.

We wondered whether the LOH phenotype resulted from UPD, in which an entire chromosome becomes homozygous, presumably owing to missegregation. To test this, we developed a quantitative assay based on visual scoring of colony colour and monitoring of genetic markers (Fig. 1e and Extended Data Fig. 1c). This assay revealed UPD events in *dcr1*Δ since markers on both arms of chromosome III (ChrIII) were homozygosed (Fig. 1e). Our quantitative analysis confirmed significantly higher UPD frequencies in *dcr1*Δ than in wild type (Fig. 1f). Moreover, we found that UPD can occur with either of the homologues, which we further confirmed by tetrad analysis (Extended Data Fig. 2a, b). The ability of diploids to undergo meiosis and sporulation was not crucial for UPD, because non-sporulating *dcr1*Δ diploids obtained by either mating or protoplast fusion displayed higher frequencies of UPD than their wild-type counterpart (Extended Data Fig. 2c, d). These analyses show that defects in RNAi result in high levels of UPD.

Since RNAi is required for centromeric heterochromatin assembly, we wondered whether UPD is caused by defects in heterochromatin-dependent loading of cohesin at pericentromeric regions. The loss of heterochromatin assembly factor Clr4 abolishes cohesin localization at centromeres (Extended Data Fig. 3a, b), but caused only a minor increase in LOH and UPD as compared to RNAi mutants (Fig. 1c, f). By contrast, *dcr1*Δ showed only a partial defect in cohesin localization at centromeres, but exhibited high levels of UPD (Fig. 1f and Extended Data Fig. 3a, b). Cells lacking Dcr1 showed no major changes in cohesin localization at other heterochromatic loci including telomeres (Extended Data Fig. 3c), and cohesin distribution on chromosomal arms was comparable between *dcr1*Δ and wild type (Extended Data Fig. 3d). These results indicate that additional factors other than defects in heterochromatin-dependent cohesin loading at centromeres must be responsible for the UPD in RNAi mutants.

Independently of heterochromatin assembly, RNAi silences meiotic genes during vegetative growth. The untimely expression of gametogenic genes, which has been found to occur in tumours in humans and to induce tumorigenesis in *Drosophila*, might be linked to aneuploidy observed in cancer cells. However, the link between chromosome segregation defects and misregulation of gametogenic genes, including meiotic genes, has not been directly tested.
Confirmation by live-cell imaging and tetrad analysis. Top, distribution of high frequency of UPD and chromosome segregation defects such as minichromosome loss and sensitivity to spindle-poison drug thiabendazole (TBZ) (Fig. 3a and Extended Data Fig. 4). Therefore, we used mmi1Δ to test specifically the role of candidate factors in UPD without the caveats associated with defects in heterochromatin assembly at centromeres. We assembled a group of 27 candidate factors consisting of gametogenic genes, most of which are required for proper meiotic chromosome segregation and are upregulated in both mmi1Δ and RNAi mutants (Supplementary Table 1). Deletions of candidate genes were combined with mmi1Δ to assess the effect on chromosome segregation. Among all of the tested candidate genes, only the deletion of rec8, which encodes meiotic cohesin15,16, or crs1, a meiosis-specific cyclin gene17, suppressed TBZ sensitivity and resulted in stable propagation of a monochromosome in mmi1Δ (Fig. 3b). Loss of either of these factors also suppressed UPD in mmi1Δ (Fig. 3c). Furthermore, rec8Δ, and crs1Δ albeit to a lesser extent, suppressed UPD in dcr1Δ (Fig. 3d). Together, these results suggest that Rec8 and Crs1 contribute to UPD in both mmi1Δ and RNAi mutants.

To establish whether upregulation of rec8 resulted in increased protein expression, we determined Rec8 protein levels in mmi1Δ and RNAi mutant cells. As expected, Rec8 was barely detectable in wild type (Fig. 3e, f). However, the loss of Mmi1 or RNAi factors (Ago1 or Dcr1) caused a major increase in Rec8 levels as compared to wild type (Fig. 3e, f). Therefore, derepression of rec8 in mmi1Δ and RNAi mutants correlates with the increased level of UPD that we observed.
We wondered whether Rec8 expression in vegetative cells promotes UPD by causing aberrant cohesin distribution. In vegetative cells, mitotic cohesin containing Rad21 and Psc3 is preferentially enriched across heterochromatin domains including at centromeres, and shows distinct peaks at specific sites on chromosomal arms.\(^{5,6,9,21}\) As cells enter meiosis, Rec8–Rec11 replaces Rad21–Psc3 on arms, while Rec8 partners with Psc3 at centromeres.\(^{22,23}\) Notably, we found that Rec8 and its interaction partner Rec11 were loaded onto chromosomal arms in \(\text{mmi}1\Delta\) cells, at sites normally occupied by mitotic cohesin (Extended Data Fig. 5), and their localization correlated with a proportional decrease in both Rad21 and Psc3 at these sites (Extended Data Figs. 6 and 7). As in meiotic cells, Rec11 was not detected at centromeres in \(\text{mmi}1\Delta\) cells (Extended Data Fig. 5b, e). By contrast, Rec8 and Psc3 showed abnormal localization at centromeres and were highly enriched, particularly at the central core (Fig. 4a–c; Extended Data Figs. 5c, 6d), which is the site of kinetochore assembly.\(^{22,23}\) This aberrant localization of Psc3 at the central core in vegetative cells requires Rec8, as Psc3 was not enriched at the central core in \(\text{mmi}1\Delta\) \(\text{rec8}\Delta\) cells (Fig. 4b, c). Taken together, our results reveal a composite pattern of cohesin distribution in \(\text{mmi}1\Delta\) that shares features of both mitotic and meiotic cohesin localization at centromeres and chromosomal arms.

We then asked whether Rec8 is expressed in wild-type diploid cells. We used a strain in which \(\text{rec8}\Delta\) expression is driven by the constitutive \(\text{adh}1\) promoter, leading to increased \(\text{rec8}\Delta\) levels (Fig. 4e). Unlike \(\text{crs}1\Delta\) overexpression that arrests the cell cycle,\(^{20}\) cells overexpressing \(\text{rec8}\Delta\) (\(\text{rec8}\Delta-\text{OE}\)) are viable, as previously observed,\(^{23}\) but show TBZ sensitivity (Extended Data Fig. 8a). We observed a high level of \(\text{rec8}\Delta-\text{OE}\) cells (Fig. 4f and Extended Data Fig. 8b), indicating that expression of Rec8 alone is sufficient to trigger UPD. Indeed, the rate of UPD was approximately 25-fold higher in \(\text{rec8}\Delta-\text{OE}\) cells than in the wild-type diploids, indicating that expression of Rec8 alone is sufficient to trigger UPD. Indeed, the rate of UPD was approximately 25-fold higher in \(\text{rec8}\Delta-\text{OE}\) cells than in the wild-type diploids, indicating that expression of Rec8 alone is sufficient to trigger UPD.
centromere central core (Fig. 4h). Thus, Rec8 overexpression in vegetative cells causes altered cohesin distribution, similar to the pattern observed in mmi1Δ.

Since cohesin at the central core during meiosis is critical for cohesion and mono-orientation\(^2\), we wondered whether aberrant centromeric localization of Rec8–Psc3 in vegetative cells could promote stochastic ‘pseudomeiosis’ events (for example, reductional segregation of sister chromatids). To test this, we examined segregation events using two assays. In one assay, we visually monitored diploids in which centromere 2 (cen2) of both homologues are marked: one with lacO–GFP and the other with tetO–Tomato. Additionally, we investigated UPD of both homologues of ChrIII, which each carried a distinct ade6 allele. One homologue was marked with ade6-210 (red) and the other with ade6-216 (pink), allowing segregation to be determined by scoring colony colour. Coordinated UPD of both homologues would generate half-red and half-pink diploid colonies (meiosis I-like segregation), whereas half-white and half-red or pink colonies would be expected for a diploid undergoing UPD of only one homologue. Notably, we found a high frequency of reductional segregation in rec8-OE, involving only one of the homologues per event in both assays (Fig. 4i, Extended Data Fig. 10a, b and Supplementary Video 1). Consistent with this result, homologue pairing and chiasmata, which are expected to be crucial for reductional segregation of both homologues, seemed dispensable for UPD. Combining a deletion of the gene encoding Rec12\(^{apo1}\), which abrogates double-strand break formation and recombination, with either dcr1Δ, mmi1Δ or rec8-OE did not suppress UPD (Extended Data Fig. 10c).

We also investigated the potential contribution of other factors associated with meiotic chromosome segregation in UPD. Shugosin 1 (Sgo1), which prevents the cleavage of centromeric Rec8 during meiosis\(^1\), was not required for UPD in rec8-OE. Although coexpression of Sgo1 and Rec8 was previously shown to induce chromosome missegregation\(^2\), we observed similar UPD frequencies in both rec8-OE and rec8-OE sgo1Δ (Extended Data Fig. 10d). Furthermore, we observed no effect on UPD in rec8-OE upon the loss of Moa1 (Extended Data Fig. 10d), which is required for mono-orientation of kinetochores during meiosis I (ref. 28). By contrast, we observed increased UPD in rec8-OE cells lacking Sgo2 (Extended Data Fig. 10d), which recruits the chromosome passenger complex to correct erroneous microtubule–kinetochore attachments\(^2\), indicating that missegregation events contribute to UPD. Taken together, these results strongly suggest that UPD originates from stochastic, abnormal reductional events that occur during mitosis rather than from programmed meiosis I segregations.

Our results uncover a mechanism contributing to chromosome segregation defects in RNAi mutants, and establish an unambiguous connection between the untimely expression of gametogenic genes and UPD. Specifically, we find that meiotic cohesin Rec8 promotes UPD in mmi1Δ and RNAi mutants. Additional factors such as Crs1 also affect UPD. However, its mode of action is probably distinct, because Crs1 is not required for UPD caused by Rec8 expression (Extended Data Fig. 10d). In vegetative cells expressing Rec8, the aberrant localization of meiotic cohesin at the centromere core may promote mono-orientation of sister kinetochores, or alternatively, Rec8–Psc3 could be inefficiently cleaved, thus triggering a stochastic reductional segregation of an individual homologue. Regardless of the exact mechanism, our findings have important implications for understanding UPD in humans, in which constitutive and acquired UPD have been linked to various cancers\(^2\). The expression of germline genes, including some with roles in meiosis, is associated with cancer in somatic cells\(^2\), and we propose that aberrant activation of the meiotic program might induce UPD and cause aneuploidy in these cells. Finally, our results may advance the application of UPD as a ‘chromosome therapy’ tool to correct chromosomal aberrations\(^8\), and lay the groundwork for further detailed study of this important phenomenon.

### Online Content

Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions H.D.F. and S.I.S.G. conceived the project and designed experiments. H.D.F. performed live cell imaging, UPD and other genetic assays, V.R.C. and H.D.F. conducted ChIP–chip and ChIP–qPCR, T.S. performed western blots, G.T. conducted bioinformatics analyses, M.Z. and V.C. performed RNA-seq, and V.B., J.D., H.D.F., T.S. and T.M. constructed strains. All authors contributed to data interpretation. H.D.F. and S.I.S.G. wrote the manuscript with input from all authors.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.I.S.G. (grewals@mail.nih.gov).

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METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized and investigators were not blinded to allocation during experiments and outcome assessment.

Yeast strains and methods. Standard procedures were used for fixation yeast cell culture and genetic manipulations. S. pombe strains used in this study are listed in Supplementary Table 2. Strains bearing Rec8-GFP or Psc3-GFP were gifts from Y. Watanabe. Deletion strains for the genes summarized in Supplementary Table 1 were obtained from the Bioneer haploid deletion library version 4.0. The following arrays, gene fusions and mutant alleles were previously described: lacO at pericentromeric regions lys1 and cen2-D107 and Lac−GFP at his7 loci32, tetO at cen2 and TetR-Tomato adjacent to zfl1 (Z locus)33, Pad1−rec8−3HA and Pad1−rec8−GFP35, mei4ΔNC−NAT4, and rec12Δ (ref. 33). Deletion of mni1 was performed in cells carrying a previously described truncated non-functional mei4 (mei4Δ−NAT) allele to alleviate growth defects caused by derepression of mei4 in mitotic cells42.

Construction of the HA–Rec8 strain. Haemaggulutinin (HA) tagging at the N terminus of rec8 was performed by the pop-in/pop-out approach34. In brief, the rec8 fragment (−496 to 509) was cloned into the EcoRI site of pST650, pBluescript SKI (−) containing the ura4 marker gene at its Nael site35, and then the 3′ HA tag was introduced by a PCR-based method. mni1Δ cells were transformed with the resultant plasmid, pST650-3′-HA–Rec8, and transformants were selected on minimal media lacking uracil. After confirmation of the proper integration of the plasmid to the rec8 locus, the transformants were subjected to western blot analysis to test the 3′ HA–Rec8 expression. Positive clones that expressed 3′ HA–Rec8 were grown in rich media and then plated on counter-selective medium containing 5-FOA to select for cells in which the ura4−containing plasmid had popped out, and clones retaining the 3′ HA tag sequence were isolated. One of the 3′ HA–Rec8 mni1Δ strains was stained with a wild-type strain, and 3′-HA–Rec8 strains with or without mni1 were obtained. These strains were further tested by western blotting to confirm 3′ HA–Rec8 expression.

Minichromosome maintenance assays. Minichromosomes Ch16 and pNBg were previously described36,37. In brief, the large linear Ch16 minichromosome (530kb) contains the entire centromere 3 and an ade6-M216 allele that complements the ade6-M210 allele in the host strain to yield an ade6− phenotype. By contrast, the small circular pNBg minichromosome (27 kb) plasmid carries ura4− and the opal suppressor mRNA sup3-5 selection systems. Cells without ura4− cannot grow on plates that lack uracil (−Ura), whereas sup3-5 suppresses a premature stop in the chromosomal ade6-704 mutation, allowing growth on −Ade plates. For the experiments shown in Fig. 3, cells containing Ch16 or pNBg were grown on Pombe minimal glutamate (PMG) – Ade, or PMG – Ura media, respectively, and subsequently plated on YE low adenine medium. Cells containing minichromosomes generate white colonies, whereas minichromosome loss results in red or colored colonies. Screening for genes (Supplementary Table 1) that suppress minichromosome loss in mni1Δ cells was performed as follows: a mat1M-smt0 mep1 mni1 mep1Δ strain containing pNBg was crossed to the h′ strain containing the gene deletion of interest. Random spores were germinated in minimal medium (PMG – Ura – Ade) selective for minichromosome retention. Then, replica plating onto appropriate media was performed to assess the potential enhanced minichromosome stability in double mutants, as denoted by white/sectored colonies instead of red colonies that are observed in the mep1 mni1Δ control.

LOH and UPD assays. LOH and UPD assays consist of several steps that are performed at 33°C unless otherwise stated. The total duration for each assay was approximately 3 weeks.

The growth media used for each assay is as follows. LOH assay: ‘diploidizing’, AA − Ade + NAT; ‘amplifying’, AA − Ade + NAT; ‘testing’, AA − Ade. UPD assay A: ‘diploidizing’, AA − Ade + NAT; ‘amplifying’, AA − Ade + NAT; ‘testing’, PMG – Lys + NAT + low Ade. UPD assay B: ‘diploidizing’, yeast extract plus adenine (YEZA) + NAT + Hyg; ‘amplifying’, AA − Ade; ‘testing’, YE + NAT + Hyg; UPD assay C: ‘diploidizing’, AA − Ade; ‘amplifying’, PMG – His − Ura – Ade; ‘testing’, PMG – His − Ura – Ade. Live-cell imaging of azygotic asci (Fig. 1d) and nonsporulating diploids (Fig. 4i and Supplementary Video 1) was performed on a DeltaVision Elite microscope. For microarray, untreated ChIP–chip analyses using a custom 4 × 4K oligonucleotide array (Agilent).

Western blotting. The sample preparation for western blotting was performed using an alkaline protein extraction method41. Anti-GFP (7.1 and 13.1, Roche), anti-HA (12CA5, Roche), anti-HA (16B12, BioLegend), anti-GFP (ab290, Abcam) antibodies were used for immunoprecipitation. Each experiment used two or more biological replicates per genotype of interest. Oligonucleotides used for ChIP–qPCR at central core (ccl3), act1 and fbp1 were previously described40, whereas the following oligonucleotides were used for arm locations Chr1 1.92 Mb (5′-ACACATGAGAACTGTGGC-3′ and 5′-AGGAACAGAGAGTACAAGGC-3′), Chr1 3.50 Mb (5′-CGCAT TATGTCTTTTAATCCC-3′ and 5′-GAATCAAGAATATCGTCGTG-3′) and Chr3 0.36 Mb (5′-TAGCTTCGGAGGAGTACAC-3′ and 5′-TTCTGG TACGCGCACCTCTC-3′). DNA isolated from immunoprecipitated chromatin or from whole-cell extracts was labelled with Cy5/Cy3 for microarray-based ChIP–chip analyses using a custom 4 × 4K oligonucleotide array (Agilent).

RNA-seq library construction and analyses. RNA-seq library preparation, sequencing, and data analysis were performed as previously described42. In brief, the MasterPure Yeast RNA Purification Kit (Epicentre) was used to purify RNA for the construction of the RNA-Seq library from exponentially growing cells. rRNA was removed using the Ribosomal RNA Removal Magnetic Kit (Epicentre) before library construction using the ScriptSeq v2 RNA-Seq Library Preparation Kit (Epicentre). Libraries were analysed using an Agilent 2100 BioAnalyzer and sequenced on the Illumina MiSeq platform. TopHat was used to align sequenced reads to the referenced S. pombe (ASM2394v2) genome. Cufflinks was used to assemble mapped reads into the final transcriptome and to calculate fragments per kilobase of transcript per Million (FPKM) mapped reads. Genes were classified as either meiotic or non-miotic. The area proportional Euler Venn diagram for meiotic gene overlap was constructed using EulerAPE.

Data availability statement. The microarray and sequencing data that support the findings of this study are available at the NCBI Gene Expression Omnibus (GEO) repository under the accession number GSE77050. Uncropped blots and unprocessed data from LOH and UPD assays are provided as source date files. All other data are available from the corresponding author upon reasonable request.

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Extended Data Figure 1 | Additional validation of LOH and schematic of UPD assays. a, Sporulation efficiency of the resulting diploid colonies from LOH assays. Resulting diploid colonies from starting heterozygous diploids were scored for sporulation by iodine staining (iodine stains the starch-like compound produced by sporulating cells). Note that for each dcr1Δ diploid, the fraction of iodine positive colonies is similar in both the resulting LOH and heterozygous diploid populations, indicating that meiosis efficiency is independent of LOH (resulting heterozygous diploids n > 100; resulting LOH diploids n ≥ 7). b, Resulting dcr1Δ heterozygous diploids (AB) or progeny homozygosed for ChrI (AA and BB) were obtained from LOH assays and subjected to random spore analysis. Six diploids from each class were sporulated and plated onto rich medium for germination. Subsequently, colonies were genotyped by replica plating. Note that markers on ChrI were homozygosed in LOH diploids whereas markers on ChrIII show normal segregation. Over 80 haploid colonies were counted per diploid. c, Haploid parental S. pombe chromosomes showing relevant markers for UPD assays. Starting heterozygous diploids obtained by mating or fusing parental strains A and B were used to quantify UPD frequency. Diploids were maintained by selecting for heterozygosis of ChrI (assays A and B) or ChrII (assay C), whereas ChrIII was used as a reporter for UPD. The chromosome containing no relevant markers for the respective assay is shaded. UPD frequencies were calculated using the indicated formula and were adjusted using the validation coefficient (that is, the fraction of candidates exhibiting the expected homozygous markers on both arms of ChrIII; see Supplementary Table 3). Marker denotes various genetic markers at ura4 and mni1 loci for assays A-B and C, respectively. Detailed information is provided in the Methods.
Extended Data Figure 2 | Characterization and additional validation of UPD in dcr1Δ mutants. a, Left, distribution of resulting UPD diploids homozygosed for ChrIII (AA and BB) per starting heterozygous sporulating diploid. Right, validation coefficients per single starting diploid used for adjustment of UPD frequencies. More than 12 UPD candidates from each starting diploid were validated. b, Tetrad dissection analyses of dcr1Δ diploids (AA and BB) homozygosed for ChrIII, obtained from UPD assays. A schematic of the S. pombe chromosomes in the haploid parental strains A and B, indicating the location of the relevant markers, is depicted at the top. Note the asymmetric (4:0 or 0:4) segregation at ChrIII, in contrast to the (2:2) segregation at ChrI and ChrII. The yellow arrowhead denotes dead cells carried over from the master plate during replica plating. c, d, Quantification of UPD in the indicated mat2-102/mat1M-smt0 (c) and h−/h− (d) nonsporulating diploids obtained by mating (c) and protoplast fusion (d). Note that clr4Δ caused only a modest increase in UPD compared to RNAi mutant dcr1Δ as observed in Fig. 1c, f. These results are explained by the fact that RNAi mutants show more penetrance than clr4Δ in meiotic gene misregulation. Each filled red circle represents the UPD frequency of an independent starting heterozygous diploid. More than 100 colonies were scored for each diploid. Filled bars and error bars are mean and s.d. **P < 0.01; ****P < 0.0001 (Mann–Whitney U test).
Pericentromeric cohesin is reduced but not abolished in the
*dcr1*Δ mutant. a, Distribution of the Psc3 subunit of cohesin as determined by ChIP–chip. Psc3–GFP localization along ChrI is shown for the indicated strains. Note that Psc3 localization in *clr4*Δ was specifically affected at heterochromatic regions (grey shaded), but not at chromosome arm regions. On the other hand, Psc3 centromeric localization was reduced but not abolished in *dcr1*Δ. b, c, Psc3–GFP localization to heterochromatin coated centromere 2 (*cen2*; b) and to telomere 1 left (*tel1L*; c). d, Psc3–GFP localization to euchromatic chromosome arm regions. Enrichments along a 200-kb region of the right arm of ChrII are shown. Green bars represent open reading frames according to the 2007 *S. pombe* genome assembly. The fold enrichment of Psc3–GFP (*y* axis) is plotted at the indicated chromosome positions (*x* axis).
Extended Data Figure 4 | Centromeric heterochromatin is maintained in mmi1Δ. a, Tenfold serial dilutions of each strain were plated on YEA rich media containing the indicated concentrations of the spindle poison TBZ, and were grown at 33 °C. b, H3K9me2 enrichments in the indicated strains were determined by ChIP–chip analysis. The fold enrichment of H3K9me2 (y axis) is plotted at the indicated chromosome position (shown at top). H3K9me2 distribution at the mat locus is shown in addition to cen1L. c, Tenfold serial dilutions of strains containing a ura4Δ insertion at the outer repeats of centromere 1 were plated on the indicated PMG minimal media and grown at 33 °C. Note that mmi1Δ is lethal but can be rescued by loss of function of Mei4, a meiotic transcription factor. mei4 mmi1Δ is compared to appropriate mei4 and wild-type controls.
Extended Data Figure 5 | Rec8 is enriched at centromeres and colocalizes with Rec11 on chromosome arms in mmi1Δ.

a–c, Distribution of the Rec8 and Rec11 subunits of cohesin as determined by ChIP–chip. HA–Rec8 and Rec11–GFP localization along the *S. pombe* genome (a, b) and centromere 2 (cen2) (c) is shown for the indicated strains. Note that Rec8 localization is highly enriched at centromeres (grey shaded) and colocalizes with Rec11 at chromosomal arms in mmi1Δ. An enhanced view of the left arm of ChrI (indicated by the dotted lines) is shown. d, Increased localization of Rec8 and Rec11 to chromosomal arms correlates with known mitotic cohesin peaks. Rec8, Rec11 and Psc3 enrichment along a 200-kb region of the left arm of ChrI is shown. ChIP–chip analysis of Psc3 (brown), Rec8 (green) and Rec11 (grey) was performed in wild-type or mmi1Δ strains as indicated. The fold enrichment of Psc3–GFP, HA–Rec8 and Rec11–GFP (y axis) is plotted at the indicated chromosome position (x axis). Note that the regions displaying higher enrichment of Rec8 (shaded grey) are correspondingly enriched for Rec11 and Psc3. e, Rec11 enrichment at the indicated chromosomal arm locations and centromere central core (cc1/3) were determined by ChIP–qPCR. The mmi1Δ strain used in this study carries a truncated non-functional allele of mei4.
Chromosomal localization of Psc3 and Rad21 in mmi1Δ. a, b, Distribution of Rad21 and the Psc3 subunit of cohesin as determined by ChIP–chip. Rad21–GFP (a) or Psc3–GFP (b) localization along the S. pombe genome is shown for the indicated strains. Enrichments at mae1 and mae2, marked by asterisks, reflect cross-hybridization of these loci to subtelomeric sequences. c, d, Rad21 (c) or Psc3 (d) localization to cen2. The fold enrichment of Rad21–GFP or Psc3–GFP (y axis) is plotted at the indicated chromosome position (x axis). Note the abnormally high enrichment of Psc3 but not Rad21 at the central core in mmi1Δ (blue arrow). The mmi1Δ strain used in this study carries a truncated non-functional allele of mei4.
Extended Data Figure 7 | Increased localization of Rec8 and Rec11 to chromosomal arms results in decreased Psc3 and Rad21. a, Distribution of Rec8 (green), Rec11 (grey), Psc3 (brown) and Rad21 (blue) subunits of cohesin as determined by ChIP–chip in mmi1Δ and wild-type strains. Enrichments along two 200-kb regions of ChrI (left arm) and ChrII (right arm) are shown. The fold enrichment of the indicated proteins in mmi1Δ, calculated by subtraction of wild-type, is plotted (y axis) at the indicated chromosome positions (x axis). Note that the regions showing high Rec8 enrichment in mmi1Δ (grey shaded) are also enriched for Rec11, but are depleted of Psc3 and Rad21. b, Boxplots showing ChIP enrichments of the indicated proteins at 133 Rec8-enriched chromosomal arm locations in wild-type and mmi1Δ strains. **P < 0.01; ****P < 0.0001 (one-way ANOVA plus Bonferroni post-tests). The mmi1Δ strain used in this study carries a truncated non-functional allele of mei4.
Extended Data Figure 8 | Additional characterization of rec8-OE diploids from UPD assays. a, Serial dilution growth assay of the indicated strains. Tenfold serial dilutions were spotted. Cells were grown for 3 days at 32 °C in YEA rich medium with or without TBZ (15 μg ml⁻¹). Note that like mei4 mmi1Δ, rec8-OE exhibits TBZ sensitivity. b, Distribution of resulting UPD diploids (AA and BB) and validation coefficients used for adjustment of UPD frequencies in rec8-OE diploids. At least 12 UPD candidates per starting diploid were validated. c, Tetrad dissection analyses were performed with rec8-OE diploids obtained from UPD assays that were heterozygous (AB) or homozygosed for ChrIII (AA and BB). A schematic of the S. pombe chromosomes in haploid parental strains A and B, indicating the location of the relevant markers, is depicted at the top. Note the asymmetric (4:0 or 0:4) segregations observed for ChrIII, in contrast to the normal (2:2) segregations observed for ChrI and ChrII. d, Left, quantification of asci based on the number of viable spores. Right, spore viability quantified by tetrad dissection analysis of rec8-OE diploids shown in c. In each set, data correspond to a total of ≥59 ascis from ≥7 independent diploids. NS, P > 0.1 (multiple t-tests).
Extended Data Figure 9 | See next page for caption.
Extended Data Figure 9 | Overexpression of Rec8 (rec8-OE) in wild-type cells phenocopies mmi1Δ.

a, Examples of UPD in rec8-OE strains. Schematic of fission yeast chromosomes of haploid parental strains A and B, indicating the locations of the relevant markers, is depicted at the top. Haploid parental and the indicated diploid strains are shown. Note that the genetic markers on both arms of ChrIII were homozygosed in AA and BB diploids, which indicates UPD.

b, Quantification of UPD in the indicated mat2-102/mat1M-smt0 non-sporulating diploid strains as depicted in Extended Data Fig. 1c (assay A). The data for wild-type were replotted from Extended Data Fig. 2c. Each filled red circle represents the UPD frequency of an independent starting heterozygous diploid. Over 100 colonies were scored for each diploid. Filled bars and error bars are mean and s.d. ****P < 0.0001 (Mann–Whitney U test).

c, d, Distribution of the Rec8 subunit of cohesin along the S. pombe genome (c) as determined by HA–Rec8 ChIP–chip. Note that Rec8 is highly enriched at centromeres (shaded grey) and at chromosome arms in rec8-OE. An enhanced view of the left arm of ChrI (indicated by the dotted lines) is also shown (d). e, Rec8 localization to centromere 1 (cen1) is shown for the indicated strains. The fold enrichment of HA–Rec8 is plotted (y axis) at the indicated chromosome positions (x axis). Green bars represent open reading frames according to the 2007 S. pombe genome assembly.
Extended Data Figure 10 | UPD is likely to be caused by a reductional event affecting one homologue, whereas the other segregates equational. a, Schematic of fission yeast chromosomes of haploid parental strains A and B, indicating the locations of relevant markers (top). Quantification of rec8-OE half-sectored resulting diploids from UPD assays shown in Fig. 4f, g. b, Random spore analysis of half-sectored resulting diploids. Eight half-sectored diploids, white/red (1–4) and white/pink (5–8), were sporulated. Note that the colonies formed by spores from the white sectors show normal distribution of markers, spores from red or pink sectors show normal segregation of ChrI-based markers but homozygosis of ChrIII-based markers. Over 50 haploid colonies were counted per sectored diploid. c, d, Quantification of UPD in the indicated diploid strains as depicted in Extended Data Fig. 1c. Note that, as observed in mmi1Δ (Fig. 4d), rec11Δ does not suppress UPD in rec8-OE confirming that the Rec8 along with interaction partner Psc3 at centromeres is the main driver of UPD. The data for wild type, dcr1Δ, mei4 mmi1Δ and rec8-OE were replotted from Figs 1f, 2e and Extended Data Figs 2c  and 9b. Each filled red circle represents the UPD frequency of an independent starting heterozygous diploid. Over 100 colonies were scored for each diploid. Filled bars and error bars are mean and s.d. ***P < 0.0001 (Mann–Whitney U test).