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Ycs4 is Required for Efficient Double-Strand Break Formation and Homologous Recombination During Meiosis

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Condensin is not only responsible for chromosome condensation, but is also involved in double-strand break (DSB) processing in the cell cycle. During meiosis, the condensin complex serves as a component of the meiotic chromosome axis, and mediates both proper assembly of the synaptonemal complex and DSB repair, in order to ensure proper homologous chromosome segregation. Here, we used the budding yeast *Saccharomyces cerevisiae* to show that condensin participates in a variety of chromosome organization processes and exhibits crucial molecular functions that contribute to meiotic recombination during meiotic prophase I. We demonstrate that Ycs4 is required for efficient DSB formation and establishing homolog bias at the early stage of meiotic prophase I, which allows efficient formation of interhomolog recombination products. In the Ycs4 meiosis-specific allele (*ycs4S*), interhomolog products were formed at substantial levels, but with the same reduction in crossovers and non-crossovers. We further show that, in prophase chromosomal events, *ycs4S* relieved the defects in the progression of recombination interactions induced as a result of the absence of Rec8. These results suggest that condensin is a crucial coordinator of the recombination process and chromosome organization during meiosis.

Keywords: Ycs4, cohesin, *Saccharomyces cerevisiae*, cell cycle

Introduction

Condensins are evolutionarily conserved multisubunit protein complexes that are responsible for stable chromosome condensation and large-scale changes in chromosome structure. They associate with chromosomes to allow proper segregation during mitosis and meiosis [3, 36]. In budding yeast, the condensin complex consists of five subunits: Smc2, Smc4, Ycs4, Ycg1, and Brn1 [8, 23], and manifests as a rod-shaped structure in electron micrographs [1], where Brn1, Ycs4, and Ycg1 connect to form the stable condensin complex [11].

Recent studies show that condensin complexes participate in a variety of chromosome dynamics during meiosis [3]. Condensin subunits are expressed throughout the entire meiotic cell cycle, and are involved in chromosome processes that are essential for sporulation of budding yeast [36]. They are also required for the synaptonemal complex (SC), a protein complex that forms between homologous chromosomes during meiosis, and mediates double-strand break (DSB) repair *via* homologous recombination [36]. During meiosis in budding yeast, homologous recombination occurs *via* the generation of programmed DSB formation, predominantly mediated by meiosis-specific expression of the topoisomerase-like protein Spo11 [17, 18, 27, 28, 31] and mediators Rec114, Mer2, and Mei4 [16, 31]. After DSB formation, Exo1/Dna2 and the Mre11/Rad50/Xrs2 complex (MRX complex) resect single-stranded DNA (ssDNA) to expose the 3′ ssDNA tail, which is involved in the search for a homologous partner or sister template [6, 9]. Two distinct pathways repair meiotic DSBs: the crossover (CO) and non-crossover (NCO) pathways. The RecA homologs Rad51 and Dmc1 bind to the ssDNA tail, and assist in searching for and exchanging a partner template *via* formation of a nascent D-loop [7, 10, 13]. In the CO pathway, the first DSB end extends the strand with its partner template by polymerase, and forms single-end invasions (SEIs) and double-Holliday junctions (dHJs), where one SEI and the

second DSB are engaged [16, 30]. The resulting dHJs are resolved to crossover with the aid of the enzyme resolvase as well as Sgs1, Mus81-Mms4, Slx1-Slx4, Yen1, Mlh1, and Mlh3 proteins [26, 30, 32, 38, 39]. NCO pathways are considered to involve a synthesis-dependent strand-annealing mechanism, in which the nascent D-loop is extended by DNA synthesis and then dissociated from the homolog template before formation of dHJs [16, 25].

Another suggested meiotic function for condensin is that it supports the chromosome axial elements Red1 and Hop1, and repairs DSBs in Ycs4 meiotic null mutants even in the absence of Dmc1, suggesting that Rad51 and its accessory factors mediate DSB repair in the absence of Ycs4 and Dmc1 during meiosis [37]. Therefore, the functions of condensins may not be limited to chromosome assembly and segregation during the cell cycle. In the present study, we used a meiosis-specific allele of the Ycs4 subunit labeled with a 12-Myc tag at its C-terminus to assess the molecular role of condensins in the regulation of homologous recombination initiated by programmed DSBs during meiosis. Our data suggest that Ycs4 plays roles in meiotic DSB formation, the establishment of bias early in prophase I, and the progression of recombination interactions.

Materials and Methods

Strains

All strains of *Saccharomyces cerevisiae* used in our analyses are derivatives of SK1 [15, 20]. The *HIS4LEU2*/*his4XLEU2-URA3* locus has been previously described [15, 20]. The meiosis-specific allele *ycs4S* used contains a 12-Myc tag (*YCS4-12Myc*), which has also been previously described [21, 36].

Meiotic Time Course

A meiotic time course was performed as previously described [20]. Cells were plated on a YPG plate (1% yeast extract, 2% peptone, 3% glycerol, and 2% bacto-agar) and incubated for 1 day, separated by streaking, and incubated for another 2 days at 30°C. A single colony was inoculated in a glass test tube containing 2 ml of YPD liquid medium and incubated for 1 day at 30°C in a shaking incubator. To obtain cells at the G1 stage, the cells were transferred to SPS medium (1% potassium acetate, 1% peptone, 0.5% yeast extract, 0.17% yeast nitrogen base without amino acids, 0.5% ammonium sulfate, and 0.05 M potassium biphthalate, pH 5.5) at a 1:500 dilution and incubated for 18 h at 30°C. Cells were then washed with pre-warmed SPM medium (0.2% potassium acetate, 0.02% raffinose, and 2 drops/l antifoam (Sigma)), harvested at specific time points (0, 2.5, 3.5, 4, 5, 6, 7, 8, 10, and 24 h) and then cross-linked with psoralen (Sigma) under UV light at 365 nm (6 mW/Cm^2) for 15 min. To measure meiotic division, cells were fixed in a buffer containing 40% ethanol and 0.1 M sorbitol, and then stained with 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI).

Genomic DNA Preparation

Cell samples from the meiotic time course were treated with psoralen and UV light (365 nm) to crosslink DNA and stabilize joint molecules. Genomic DNA preparation from meiotic cultures was performed as previously described [13, 15, 20].

Physical Analysis and Southern Blotting

DNA physical assays were performed as previously described [15, 20]. For one-dimensional (1D) gel analysis, 2 μg of genomic DNA was digested by the *Xho*I restriction enzyme (Enzynomics) for 3 h at 37°C. DNA samples were then electrophoresed using a 0.6% Seakem LE agarose gel (Lonza) in 1× TBE buffer without ethidium bromide (EtBr) at 2 V/cm for 24 h. For CO/NCO gel analysis, genomic DNA was digested with *Xho*I and *Ngo*MIV restriction enzymes. For two-dimensional (2D) gel analysis, 2.5 μg of genomic DNA was digested with 80 units of *Xho*I. In the first gel electrophoresis, DNA samples were electrophoresed using a 0.4% gold agarose gel (Lonza) in 1× TBE buffer without EtBr at 1 V/cm for 21 h. The gel was then stained with 0.5 g/ml EtBr for 30 min, and then sliced from the top down to 1 kb, and placed in 2D gel trays. The second gel electrophoresis was performed using a 0.8% Seakem LE agarose gel (Lonza) with 0.5 g/ml EtBr in 1× TBE buffer. Electrophoresis was carried out under 6 V/cm for 6 h at 4°C. The 2D gel was washed in a 0.25 M HCl solution for 20 min, followed by a 0.4 M NaOH buffer for 30 min. Southern blotting was performed using a Zeta probe membrane and ^{32}P-dCTP-labeled radioactive nucleotides, which were reacted with a Random Primer Labeling Kit (Agilent Technologies). The radioactive signal was scanned using a Bio-Rad phosphorimager, and the signals were quantified using the Quantity One software (Bio-Rad).

Results and Discussion

Ycs4 Is Involved in the Formation of Double-Strand Breaks

Condensins are important for efficient homolog pairing and DSB repair during meiosis [36]. However, the role of condensins in the regulation of DSB formation is not well understood. To determine whether Ycs4 is involved in meiotic DSB formation, we performed 1D gel analysis. Genomic DNA was collected from the *rad50S* strain background at specific time points during meiosis. This background strain was used because DSBs accumulate owing to a block at the ssDNA resection step. We assayed DSBs in *HIS4LEU2*, *BUD23*, and *CYS3* hotspots by standard Southern hybridization analysis, using a suitable DNA fragment as the hybridization probe (Fig. 1A). In *rad50S* cells, DSBs accumulated to 24.53% for *HIS4LEU2*, 8.44%

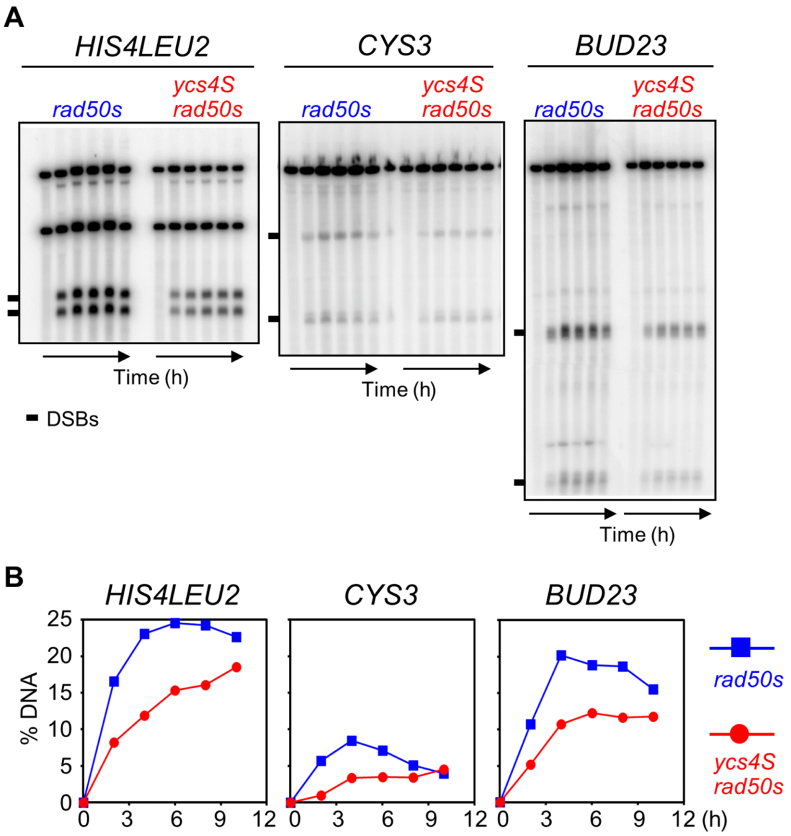

Fig. 1. Analysis of meiotic DSB formation in *rad50S* and *ycs4S rad50S*.
(**A**) 1D gel analysis of the *HIS4LEU2*, *CYS3*, and *BUD23* loci. DNA from *rad50S* strains was isolated at 0, 2.5, 4, 6, 8, and 10 h after initiation of meiosis and analyzed using the Southern hybridization technique. (**B**) Quantitative analysis of DSB formation.

for *CYS3*, and 18.81% for *BUD23* (Fig. 1B). At the well-characterized *HIS4LEU2* hotspot, *rad50S* DSB levels were reduced to 18.5% in *ycs4S*, a meiosis-specific allele (Fig. 1B). Comparable DSB formation defects were also observed at the *CYS3* (4.51%) and *BUD23* (12.23%) sites (Fig. 1B). In all strains, no joint molecules (JMs) were observed at time points ranging from 0 to 10 h; however, with this time frame, both meiotic divisions were completed in wild-type cells (Fig. 1A). Therefore, we conclude that Ycs4, a condensin subunit, is required for efficient DSB formation during meiosis.

Recombination Phenotypes in *ycs4S*, a Meiosis-Specific Allele

Recombination assay system. The *HIS4LEU2* locus at chromosome 3 has been used extensively for the physical analysis of homologous recombination in diploid mitotic or meiotic cell cycles [5, 13, 14, 15, 20, 33, 34]. For genetic studies, recombination frequency can be measured by assessing the link between the *his4X* allele and the *URA3* gene [15]. Here, meiotic recombination was monitored over time in sporulation medium undergoing synchronous meiosis at 30°C. Cell samples from the time course were

Table 1. Yeast strains used in this study.

Strain[a]	Genotype
KKY276	*MATa/MATα HIS4::LEU2-(BamHI)/his4x::LEU2-(NgoMIV)--URA3*
KKY1288	*MATa/MATα HIS4::LEU2-(BamHI)/his4x::LEU2-(NgoMIV)--URA3, ycs4-12myc/ycs4-12myc*
KKY1080	*MATa/MATα HIS4::LEU2-(BamHI)/his4x::LEU2-(NgoMIV)--URA3, rec8Δ::KanMX4/rec8Δ::KanMX4*
KKY1425	*MATa/MATα HIS4::LEU2-(BamHI)/his4x::LEU2-(NgoMIV)--URA3, ycs4-12myc/ycs4-12myc, rec8Δ::KanMX4/rec8Δ::KanMX4*

[a]All strains are isogenic derivatives of the SK1 background.

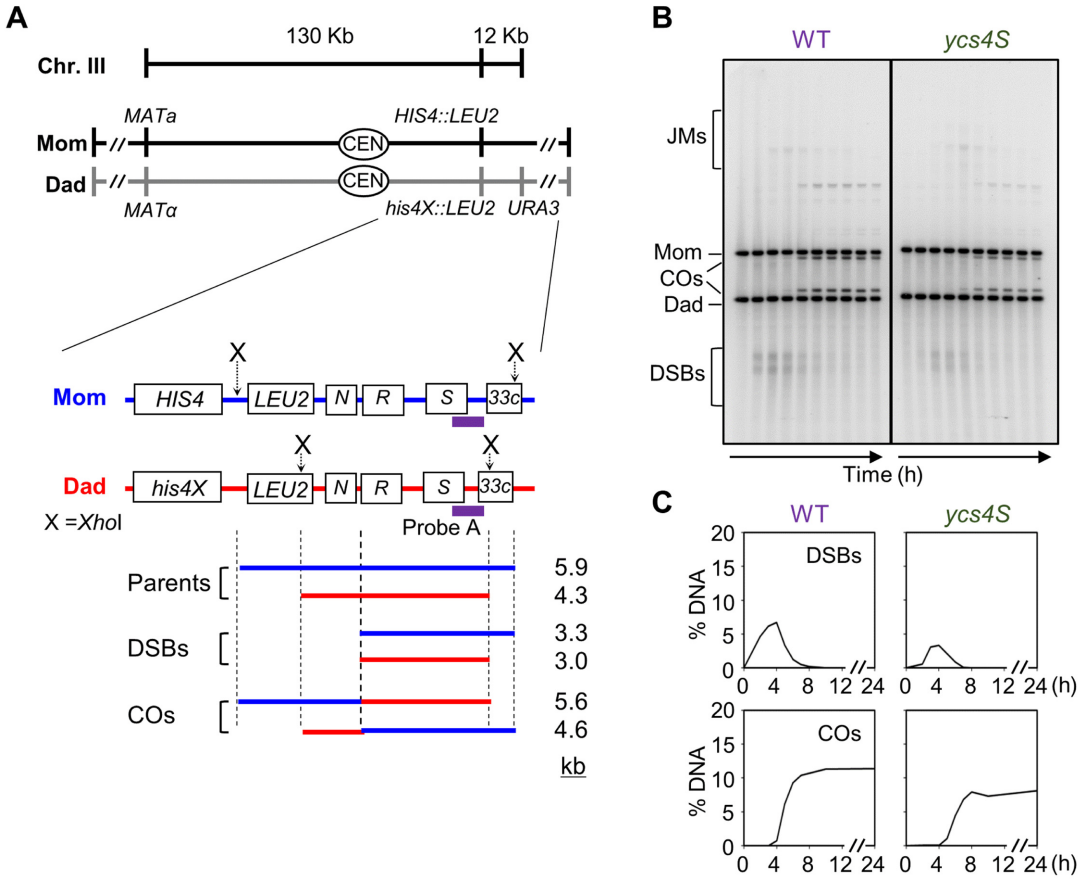

Fig. 2. Physical assay system and 1D gel analysis in WT and *ycs4S*.
(**A**) Map of the *HIS4LEU2* hotspot on chromosome 3 showing diagnostic restriction site polymorphisms and the position of probe A in *Saccharomyces cerevisiae*. (**B**) Image of 1D Southern blot analysis showing parental chromosomes, COs, and DSBs in WT and *ysc4S*. (**C**) Quantitative analysis of DSB and CO formation.

immediately treated with psoralen, and then exposed to 365 nm UV light to induce interstrand crosslinks, thus stabilizing branched JM structures, which are recombination intermediates [14, 15, 34]. Total genomic DNA was then extracted and digested with *Xho*I enzyme, and recombination species were quantitatively detected *via* Southern hybridization using a probe specific for both parent chromosomes (Figs. 2A and 2B). The kinetics of DSBs and recombination products were evaluated by 1D gel analysis [13, 15, 20, 34]. The recombination intermediates, SEIs and dHJs, were clearly resolved, as determined by native-native 2D gel electrophoresis, where JMs were separated first according to molecular weight and then according to secondary structure (Figs. 3A and 3B). JMs move slowly on agarose gels compared with linear DNA in the second-dimensional gel electrophoresis. Increasing the voltage and concentration of agarose in the presence of EtBr enhances this anomalous migration. SEIs and dHJs were quantified by Southern hybridization analysis (Figs. 3A and 3B).

Formation of DSBs and COs. Meiotic DSB formation is initiated by the Spo11 protein. This involves concomitant release of the Spo11-5′ termini of broken DNA strands by endonucleolytic cleavage facilitated by the MRX complex and Sae2. DNA 5′-to-3′ resection, mediated by the Exo1 exonuclease, exposes the 3′ single-stranded tail [19, 39]. Rad51 might be expected to be expressed and accumulated during meiosis. Thus, we analyzed the abundance of Rad51 in the meiotic cell cycle. Interestingly, *ycs4S* cells had slightly reduced Rad51 expression (Fig. S1). In wild-type (WT) meiosis, DSBs designated to become COs are first converted to SEIs, then to dHJs, and finally to COs [15, 16, 35]. Since condensins are involved in chromosome condensation and DSB formation, we examined whether condensins are required for the processing of DSBs into COs. WT and *ycs4S* cultures were synchronized to induce meiosis, and physical analysis at *HIS4LEU2* was performed

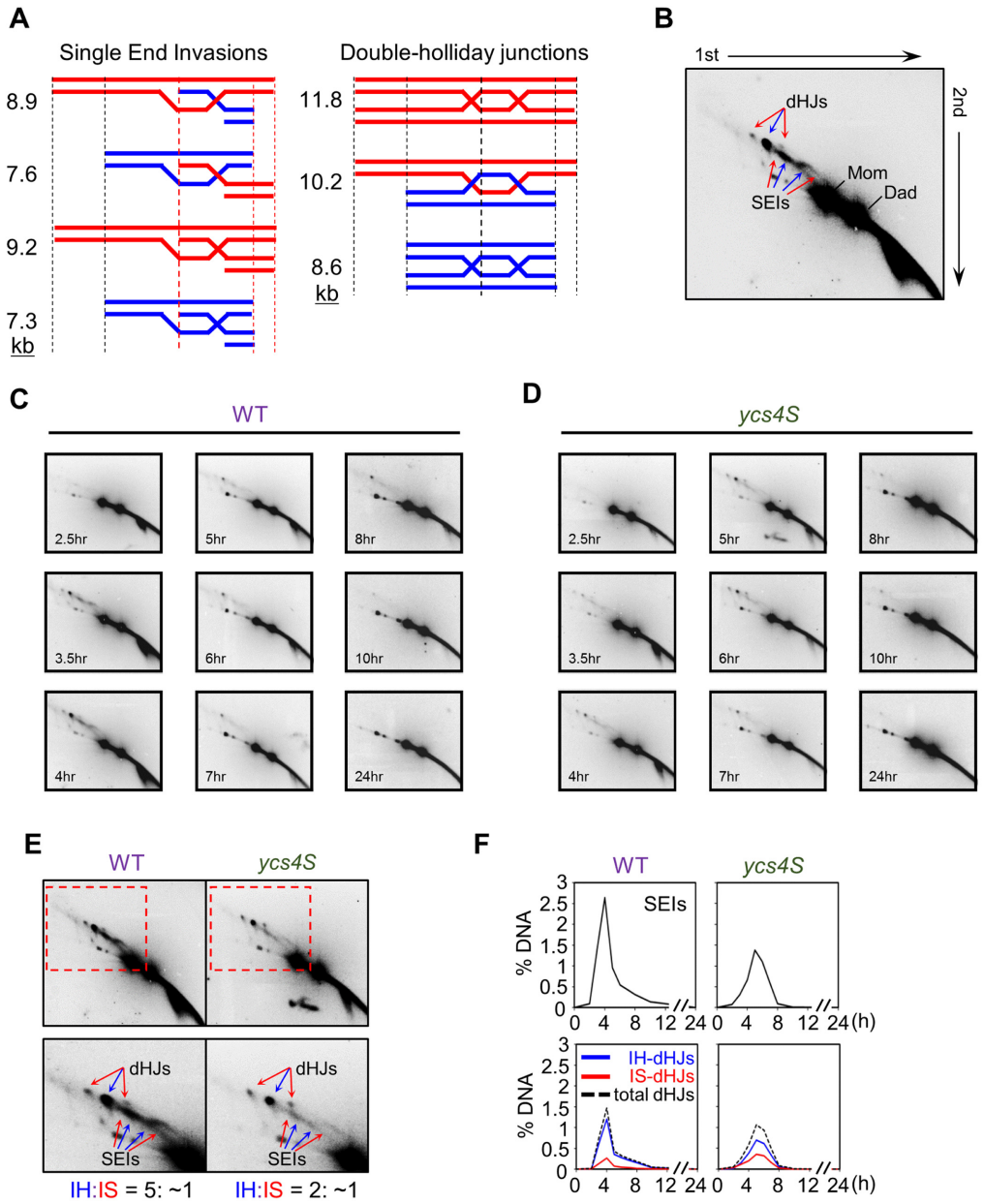

Fig. 3. Structures of JMs and 2D gel analysis in WT and *ycs4S*.

(**A**) Major JM species detected by 2D gel analysis [15, 20]. (**B**) Image of Southern 2D gel analysis showing JM and parental species detailed in (**A**). (**C** and **D**) 2D gel analysis of JM in WT and *ycs4S*. (**E**) Representative 2D gel images showing magnification of the JM regions. (**F**) Quantitative analysis of JM formation.

to observe DSBs and COs in the *HIS4LEU2* hotspot (Fig. 2C). In WT cells, DSBs first appeared 2.5 h after meiosis induction in SPM culture and reached peak levels by 4 h. CO levels plateaued at 12% of chromosomes after 8 h (Fig. 2C). In *ycs4S* cells, DSB formation was delayed by approximately 1 h, and the levels were reduced approximately 2-fold compared with that in WT (Fig. 2B). These data are consistent with the published effects of *ycs4S* mutation on DSB processing at other time intervals [36]. The formation of CO products in *ycs4S* cells was delayed by approximately 1.5 h relative to WT cells (Figs. 2B and 2C). In addition, *ycs4S* cells exhibited a 70% reduction in COs at the *HIS4LEU2*

hotspot (Figs. 2B and 2C). Zip1 is a meiosis-specific protein required for the synaptonemal complex (SC) and CO-designated recombination [16]. In WT cells, Zip1 was assembled in pachytene chromosomes, whereas *ycs4S* cells exhibited significantly low levels of Zip1 assembly and high levels of aggregated Zip1 protein polycomplexes [36]. These results indicate that Ycs4 is required for the formation of a stable SC, and that defects in SC formation in *ycs4S* may result in inefficient homolog pairing and proper homologous recombination during meiosis.

Formation of single-end invasions and double-Holliday junctions. The formation of JMs has been examined in derivatives of the marked tester strain heterozygous for *HIS4LEU2/his4XLEU2-URA3* [13, 15, 20, 24, 29, 34]. A detailed kinetic analysis of meiotic DNA events was performed by quantifying the levels of various DNA species over time. JMs, SEIs, and dHJs formed at the *HIS4LEU2* locus were monitored by 2D gel electrophoresis, which distinguishes branched structures in the second dimension (Fig. 3) [13, 15, 20, 29, 34]. Four types of JMs can be distinguished using this approach: interhomolog-SEIs (IH-SEIs) and interhomolog-dHJs (IH-dHJs) between homologous chromosomes, as well as intersister-SEIs (IS-SEIs) and intersister-dHJs (IS-dHJs) between sister chromatids [15, 20]. The majority of dHJs eventually matured into COs, and in WT cells, both SEIs and dHJs occurred simultaneously at 2.5 h and peaked at 4 h. A >5-fold increase in the formation of IH-dHJs was observed compared with IS-dHJs, and the ratio of IH:IS-dHJ was about 5:~1. Unexpectedly, *ycs4S* cells demonstrated slightly higher than normal levels of IS-dHJ (the ratio of IH:IS-dHJ was 2:~1), and disappearance of JMs was delayed 1 h compared with WT cells. This reduced ratio of IH:IS dHJ could be due to establishment of, or maintenance defects in, homolog bias [20]. Furthermore, total dHJ accumulation levels were reduced 2-fold compared with WT cells (Figs. 3C−3F). These results indicate that the greatly reduced DSB levels observed in *ycs4S* cells impact JM formation. Recombination analysis in *ycs4S* demonstrated that progression is slightly delayed, since DSBs, SEIs, and dHJs occurred at lower than normal levels at later time points, indicating delayed/defective turnover. Moreover, considering that both IH-COs and IH-NCOs were reduced in *ycs4S* cells (Figs. 2 and 5C), we also conclude that Ycs4 promotes an early step in meiotic recombination; that is, the establishment of homolog bias.

ycs4S Relieves Progression Recombination Defects in the Absence of Meiotic Cohesin

Both cohesion and condensation regulators (cohesin and condensin complexes, respectively) have diverse roles in chromosome dynamics, and are crucial for separating identical copies of chromosomes into daughter cells during mitotic and meiotic cell division [12, 22, 37]. Previous studies have shown that condensins are associated with components of the axial elements Hop1 and Red1 [36]. Condensins are also needed for cohesin removal prior to anaphase I in yeast [37]. To assess whether condensins and cohesin function coordinately or independently to promote homologous recombination, we analyzed recombination phenotypes for *ycs4S* and *rec8Δ* single mutants, along with the double-mutant *ycs4S rec8Δ* (Fig. 4). DSBs, JMs, and COs were analyzed in parallel cultures of *rec8Δ* and *ycs4S rec8Δ* cells (Fig. 4). In *rec8Δ* cells, DSBs occurred at 2.5 h and peaked at 5 h after transfer to sporulation medium; however, steady-state levels of DSBs were observed until 10 h. In *ycs4S rec8Δ* cells, most DSBs were repaired within 8 h, and their disappearance was concurrent with the formation of SEIs and dHJs. In *rec8Δ* cells, COs were achieved in approximately 7% of chromosomes at 24 h. The timing of COs in *ycs4S rec8Δ* cells was earlier than in *rec8Δ* cells. Similarly, *ycs4S rec8Δ* cells exhibited fast turnover of COs compared with *rec8Δ* cells.

To detect recombination intermediates in *rec8Δ* and *ycs4Srec8Δ* cells, genomic DNA from each strain was extracted at different time points and analyzed by native-native 2D gel electrophoresis. The absence of Rec8 leads to defects in maintenance of homolog bias at the SEI-to-dHJ transition during meiotic recombination [13, 20]. The ratio of IH:IS dHJs was 1:1 in the *rec8Δ* strain, compared with 5:1 in the wild-type strain. This 1:1 dHJ ratio in *rec8Δ* could be due to a defective Rec8-mediated second-DSB end complex (or programmed quiescence complex), mediated by the Rec8-antagonizing factors Red1/Mek1 [13, 20]. In *rec8Δ* cells, the maximum levels of SEI and dHJ were 5-fold higher than in *ycs4S rec8Δ*, and JMs were detectable until 24 h. However, in *ycs4S rec8Δ* cells, SEI and dHJ signals rapidly disappeared, indicating that the lifespan of recombination intermediates might be shorter in *ycs4S rec8Δ* than in *rec8Δ* cells. In contrast, CO levels were slightly reduced to 5% in *ycs4S rec8Δ* (7% in *rec8Δ*). Therefore, we conclude that *ycs4S* prevented the delay of CO formation in *rec8Δ* cells. The absence of Rec8 delays the formation of COs, which appears to result primarily from delayed DSB-through-CO transition. The extent of this delay, as demonstrated by the accumulation of SEIs/dHJs, was greater than that observed in other mutants defective at this step. A steady-state level of some DSBs was observed in *rec8Δ* cultures, indicating controlled organization of the chromosome domain to

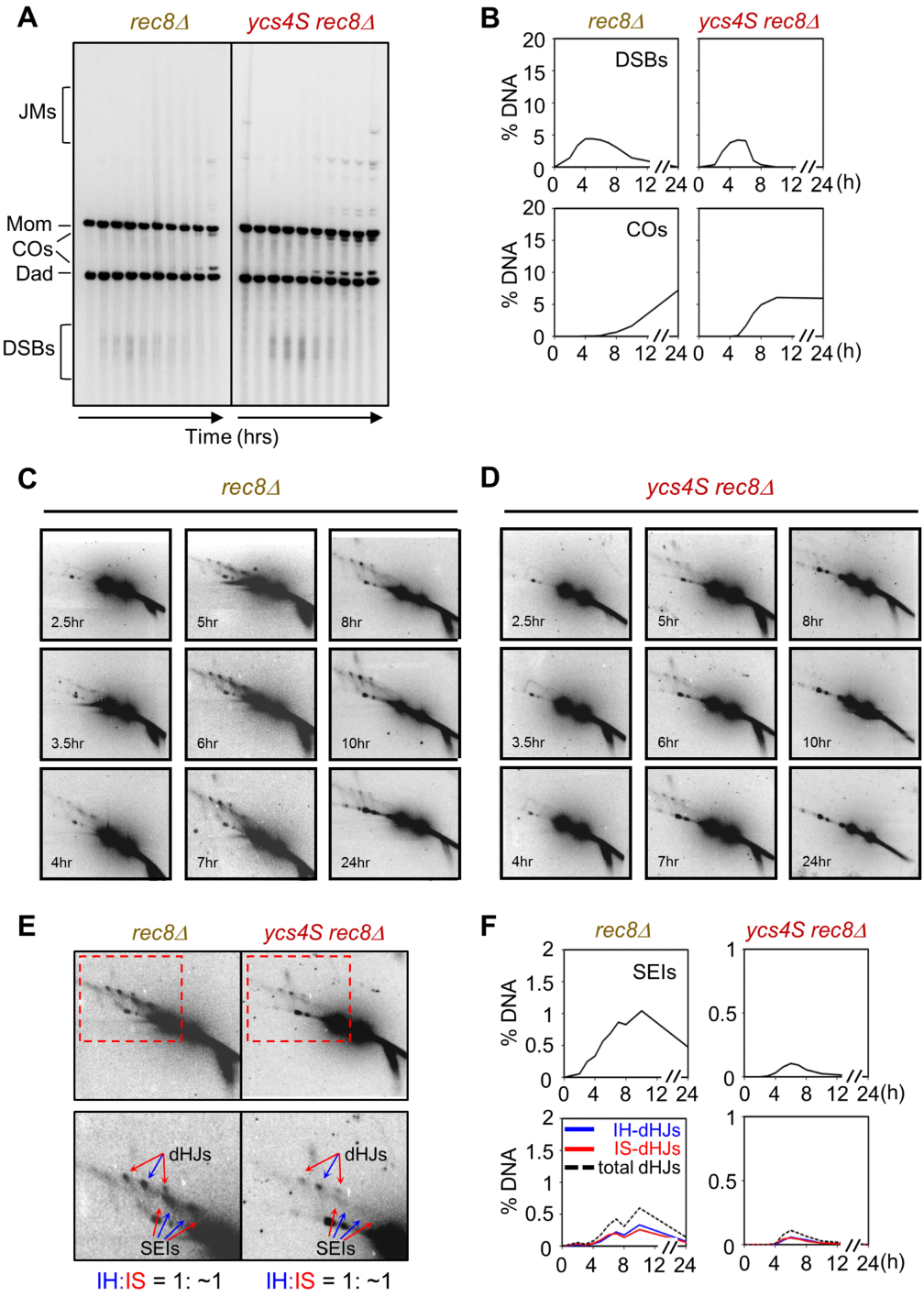

Fig. 4. Physical analysis of recombination in *rec8Δ* and *ycs4S rec8Δ*.
(**A**) Image of Southern 1D gel analysis showing DSBs, COs, and parental species of *rec8Δ* and *ycs4S rec8Δ*. (**B**) Quantitative analysis of DSB and CO formation. (**C** and **D**) 2D gel analysis of JM formation. (**E**) Representative 2D gel images showing magnification of the SEI and dHJ regions. (**F**) Quantitative analysis of JM formation.

reflect either a delayed post-DSB stage or a delay in strand invasion of the "second end" of a DSB to form mature SEIs (Figs. 4A–4D) [20]. In *rec8Δ* and *ycs4S rec8Δ* mutants, the ratio of IH:IS dHJs was 1:1 (2:1 in *ycs4S* and 1:1 in *rec8Δ*).

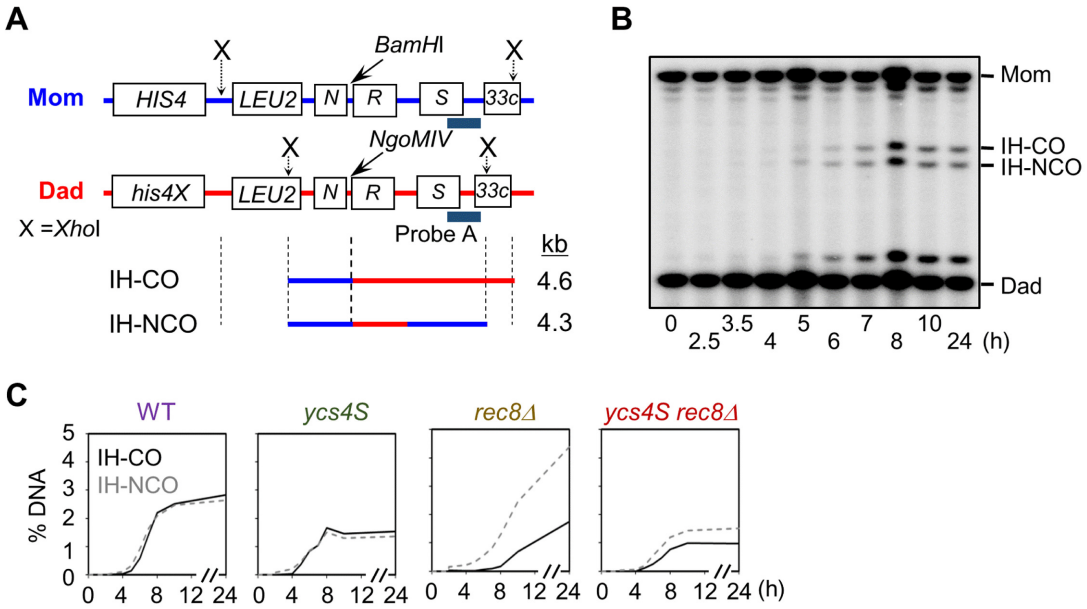

Fig. 5. Analysis of IH-COs and IH-NCOs.
(**A**) The IH-CO/IH-NCO tester construct in the *HIS4LEU2* hotspot. (**B**) Southern blot analysis after restriction digestion with *Xho*I and *Ngo*MIV detects IH-CO (4.6 kb) and IH-NCO (4.3 kb), respectively. (**C**) Quantification of IH-CO and IH-NCO formation in WT, *ycs4S*, *rec8Δ*, and *ycs4S rec8Δ*.

Therefore, in the absence of Rec8, Ycs4 is no longer relevant for homolog-versus-sister discrimination. Taken together, we conclude that the *ycs4S rec8Δ* double mutant results in a defect that is approximately the sum of that observed in the two single mutants (Fig. 4).

CO and NCO Levels Are Reduced in *ycs4S*

In WT meiosis, most DSBs interact with a homolog partner chromatid, with tightly controlled interactions that progress into IH-CO or IH-NCO types [2]. At the *HIS4LEU2* locus, total DSBs are quantitatively determined by the amount of IH recombination products, leaving only a few available for intersister CO or NCO. In *ycs4S* and *ycs4S rec8Δ* strains, both COs and NCOs were reduced; however, NCO levels were slightly higher than CO levels in *ycs4S rec8Δ* cells. The *ycs4S rec8Δ* mutant exhibited a *rec8Δ* phenotype with respect to the ratio of IH:IS dHJ. *ycs4S rec8Δ* cells exhibited reduced levels of both IH-COs and IH-NCOs, whereas *rec8Δ* exhibited high levels of IH-NCOs and reduced levels of IH-COs (Fig. 5C). Thus, at the early stage of prophase I (establishment of bias), IH-SEIs are slightly defective, whereas remaining SEIs progress efficiently to dHJs but undergo maintenance bias defect in the absence of Rec8 (Fig. 4) [20]. Taken together, our results suggest that condensins are specifically required for the establishment of homolog bias during meiotic recombination, and that this role is independent of the Rec8 cohesin.

The results presented in this study show that Ycs4 is involved in the regulation of DSB formation and promotes establishment of bias in meiotic recombination. When prophase chromosomal events are adversely impacted by the absence of Rec8, *ycs4S* leads to the progression of recombination interactions *via* Red1/Hop1 checkpoint-related pathways. These results provide new insight into recombination regulation along chromosome condensation, as well as confirm previous indications that Ycs4 plays important roles in axis formation and SC assembly. In that context, the results also provide an understanding of the control of recombinatorial progression and its relationship to chromosome organization. Finally, these findings provide further information concerning how condensins exert their effects under aberrant meiotic recombination conditions.

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