

## Roles of Budding Yeast Hrr25 in Recombination and Sporulation

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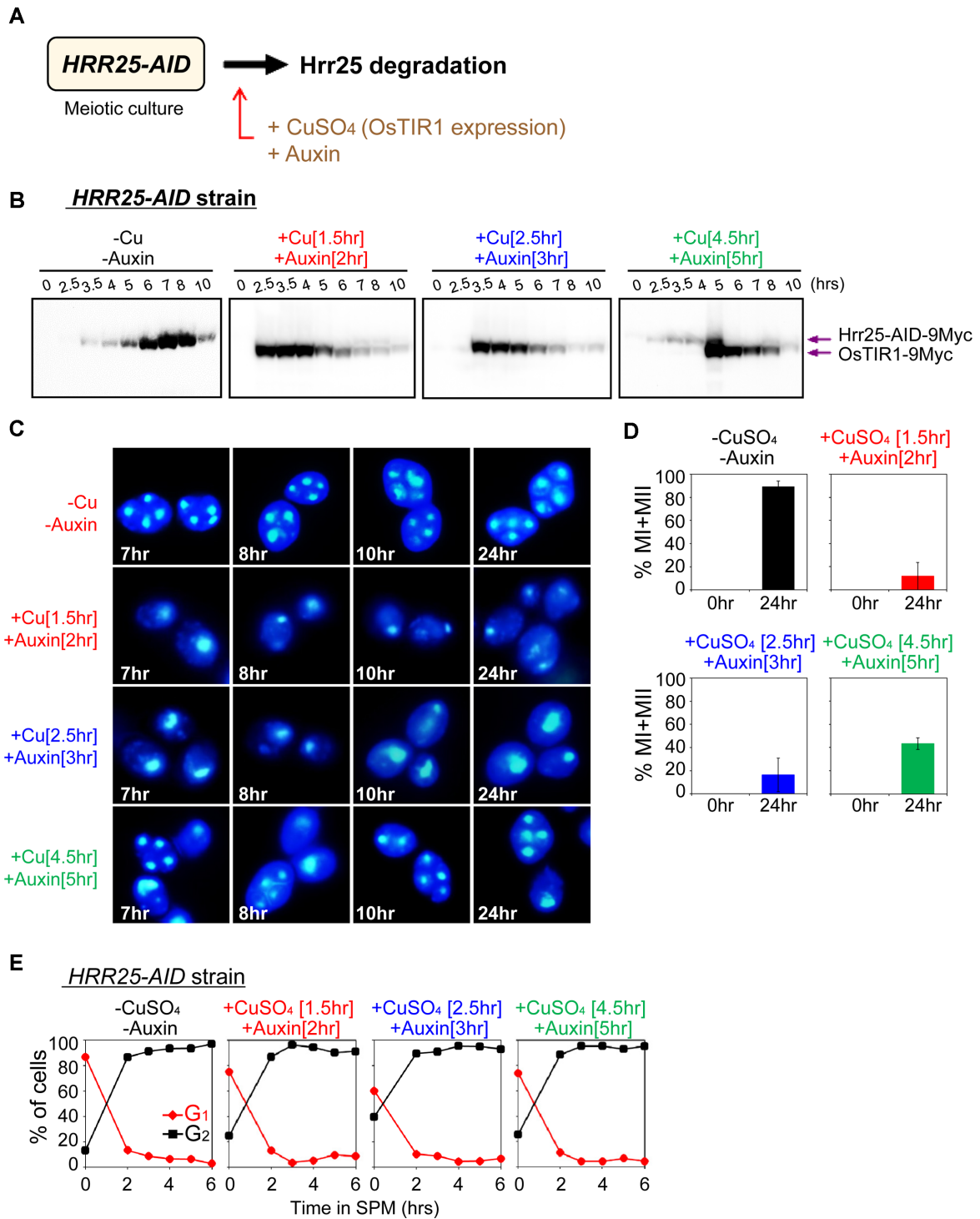
Hrr25, a casein kinase 1  $\delta/\epsilon$  homolog in budding yeast, is essential to set up mono-orientation of sister kinetochores during meiosis. Hrr25 kinase activity coordinates sister chromatid cohesion via cohesin phosphorylation. Here, we investigated the prophase role of Hrr25 using the auxin-inducible degron system and by ectopic expression of Hrr25 during yeast meiosis. Hrr25 mediates nuclear division in meiosis I but does not affect DNA replication. We also found that initiation of meiotic double-strand breaks as well as joint molecule formation were normal in *HRR25*-deficient cells. Thus, Hrr25 is essential for termination of meiotic division but not homologous recombination.

**Keywords:** Hrr25, meiosis, homologous recombination

During meiosis, homologous chromosomes are physically connected via chiasmata to allow combining of genetic information. Homologous chromosomes are segregated to opposite spindle poles during meiosis I, and following degradation of the cohesin complex at the centromere, sister chromatids are segregated during meiosis II [1–3]. After pre-meiotic replication, the cohesin complex holds the sister chromatids to prevent their premature segregation [1, 2]. Chromosome segregation is triggered by separase-mediated cohesin cleavage at the metaphase to anaphase stages [4]. The separase Esp1 is activated by degradation of the securin Pds1, which is inactivated via ubiquitination by the anaphase-promoting complex [5]. The cohesin complex is cleaved by separase during meiosis I, but the complex at centromeres is protected from separase, which recognizes the meiosis-specific  $\alpha$ -kleisin subunit Rec8 that is dephosphorylated by Sgo1 and protein phosphatase 2A during anaphase I [6]. At the onset of chromosome segregation during mitosis and meiosis, microtubules originating from opposite spindle poles attach to the kinetochore on the sister chromatids [7], a process that depends on sister chromatid cohesion, which stabilizes kinetochore microtubules on the monopolin complex; this regulates chromosome-microtubule attachment via crosslinked kinetochore components [8].

Meiotic recombination is initiated by programmed double-strand breaks (DSBs) formed by the topoisomerase-like protein Spo11 [9]. DSBs are processed by the Exo1, Dna2, and Mre11/Rad50/Xrs2 complex to expose 3' single-stranded DNA. The 3' ssDNA overhangs interact with two RecA homolog proteins, Rad51 and Dmc1, that mediate homologous recombination [10, 11]. These proteins generate nucleofilaments and then invade the homologous template (a nascent D-loop) [11, 12], generating single-end invasion (SEI) that engages in inter-homolog (IH) crossovers (COs); the remaining species are processed as IH non-crossovers via a separate pathway. SEIs and double-Holliday junctions (dHJs) occur in the synaptonemal complex, a highly conserved structure formed by a connection between maternal and paternal chromosomes during meiotic prophase I [13].

Casein kinase 1  $\delta/3$  (Hrr25 in budding yeast [14]) is a subunit of the monopolin complex that ensures segregation of sister kinetochores to the same pole during meiosis [5, 15]. Hrr25 mediates chromosome segregation by monopolar attachment to sister kinetochores during meiosis I and is localized at the mitotic spindle in a cell cycle-dependent manner [15, 16]. Moreover, Hrr25 coordinates exit from meiosis II by activating a pathway that induces inactivation of M phase promoting kinase [17]. Although Hrr25 has been implicated in meiotic prophase I, it is not well

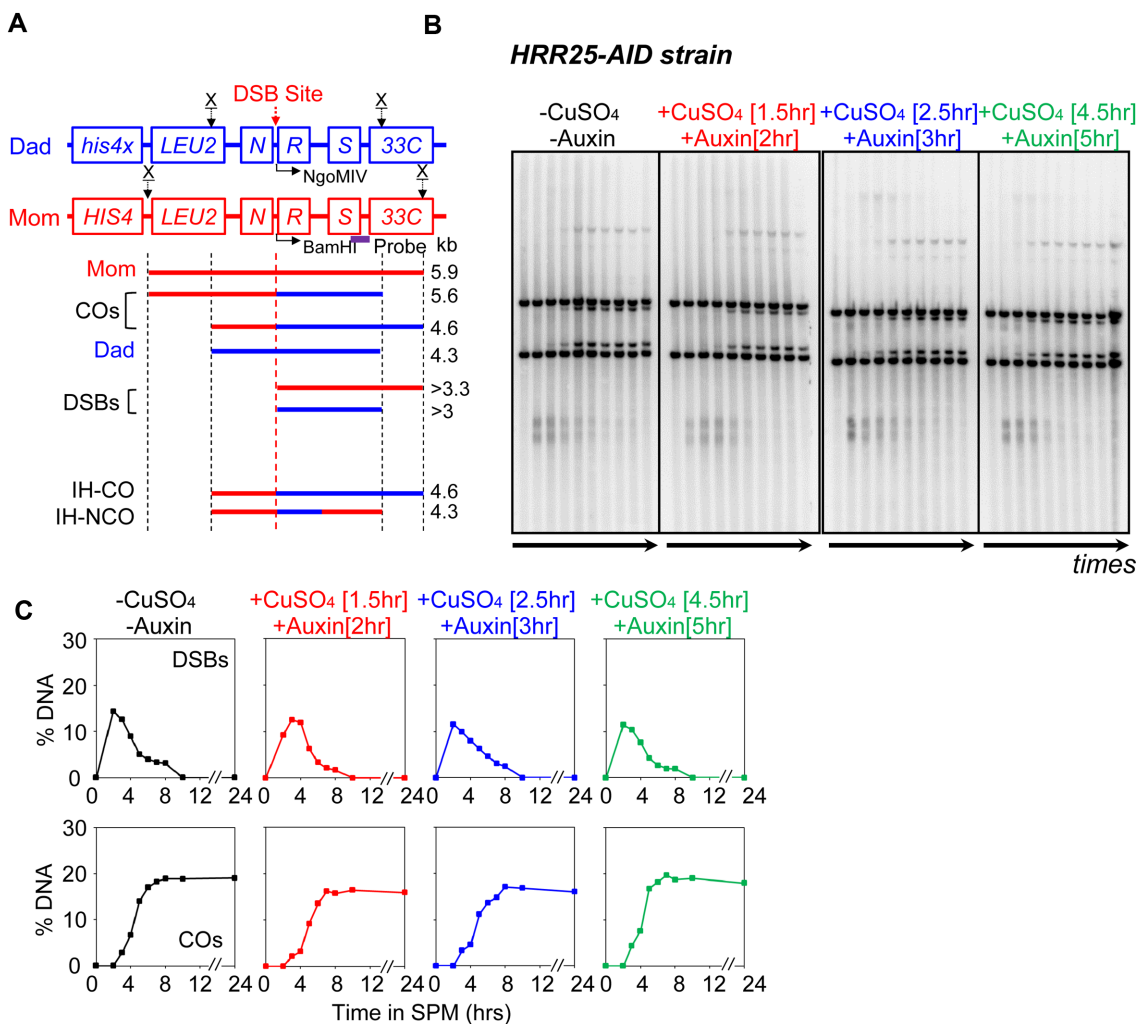


**Fig. 1.** Schematic overview of the *HRR25-AID* system and effects of Hrr25 expression. (A) Schematic overview of the *HRR25-AID* system during meiosis. (B) Expression of Hrr25-AID-9Myc and OsTIR1-9Myc proteins at indicated time points, as determined by western blotting. Copper ( $t = 1.5, 2.5,$  and  $4.5$  h) and auxin ( $t = 2, 3,$  and  $5$  h) were added. Protein positions are indicated on the right side. (C) Representative images of DAPI staining in the absence or presence of Hrr25. (D) Number of DAPI-positive foci during meiosis I and II (MI+MII) under indicated conditions. (E) Cell cycle analysis of Hrr25-expressing cells. Red and black squares indicate G<sub>1</sub> and G<sub>2</sub> phases, respectively. Wild type (black): copper added at  $t = 1.5$  h, auxin added at  $t = 2$  h; (red): copper added at  $t = 2.5$  h, auxin added at  $t = 3$  h; (blue): copper added at  $t = 4.5$  h, auxin added at  $t = 5$  h (green).

understood in meiotic recombination. In the present study, we applied the auxin-inducible degron (AID) system to conditionally deplete Hrr25 in the presence of auxin and OsTIR1 [18, 19]. We also ectopically expressed Hrr25 by replacing the native Hrr25 promoter with the *CUP1* promoter, which is strongly activated by addition of  $\text{CuSO}_4$  [20].

Hrr25 has an important role in many cellular processes, such as cell cycle control and spindle formation during meiosis [6, 8, 15–17, 21]. To investigate the function of Hrr25 in meiosis using the AID system, the gene was C-terminally tagged with AID-9myc by PCR [22]; the *pCUP1*-OsTIR1 strain was generated by two-step PCR. OsTIR1

expression was induced in the presence of  $\text{CuSO}_4$ . After activation of the degron system by auxin, the target protein Hrr25 was rapidly degraded (Fig. 1A). Cells were synchronized at the  $G_1$  phase in pre-meiotic culture medium, and then transferred to sporulation medium to induce meiosis.  $\text{CuSO}_4$  (50  $\mu\text{M}$  at  $t = 1.5, 2.5,$  and  $4.5$  h) and auxin (3 mM at  $t = 2, 3,$  and  $5$  h) were added to the cultures, and cells were harvested at different time points (0, 2.5, 3.5, 4, 5, 6, 7, 8, and 10 h) (Figs. 1B and 1C). Hrr25-AID-9myc and OsTIR1 expression was confirmed by immunoblotting using an anti-Myc antibody. Hrr25 was expressed at an early time of prophase I; however, it was immediately degraded upon OsTIR1 expression and in the presence of



**Fig. 2.** Hrr25 does not affect double-strand break (DSB) and crossover (CO) formation.

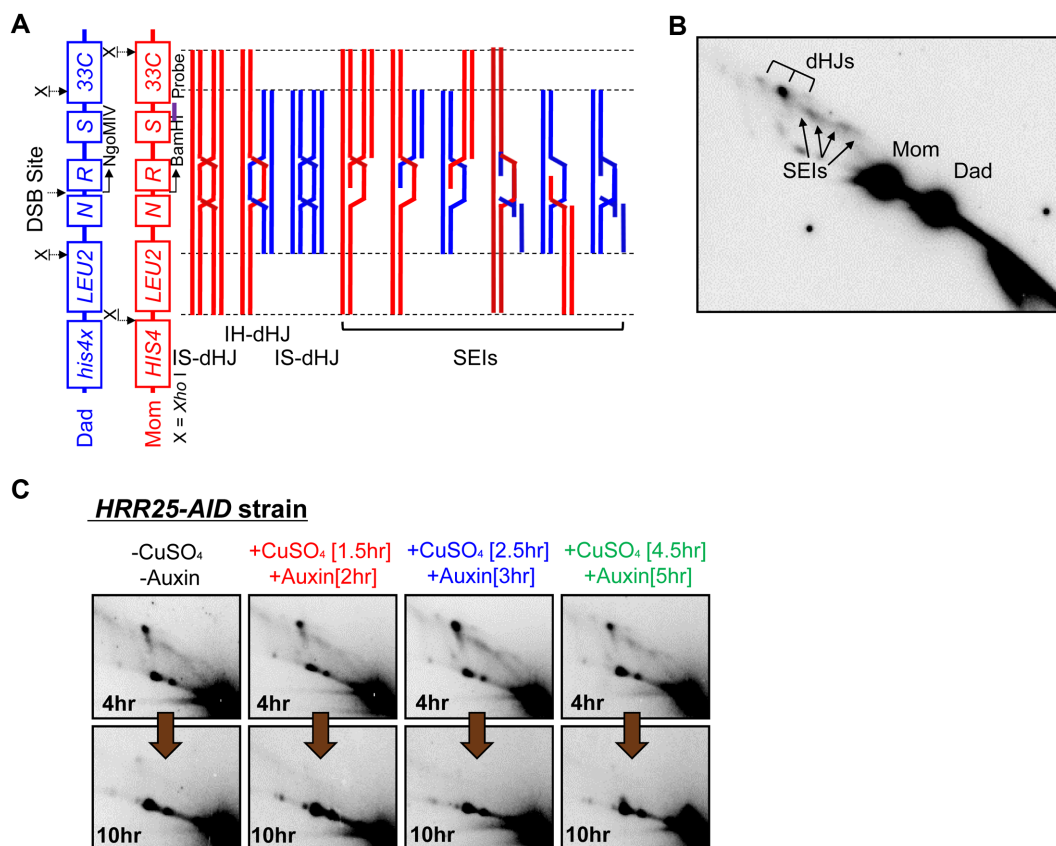
(A) Physical map of the *HIS4LEU2* locus on chromosome III. DSBs occur at the site indicated by a dotted arrow during meiosis. Maternal and paternal chromosomes are distinguishable by *Xho*I restriction site polymorphism (indicated by an X). Probe A was used to detect recombinants by Southern blot analysis. Maternal and paternal signals were detected at 5.9 and 4.6 kb, respectively; DSB signals were detected at 3 and 3.3 kb, respectively; and CO signals were detected at 4.6 and 5.6 kb, respectively. (B) One-dimensional gel analysis with or without addition of  $\text{CuSO}_4$  and auxin ( $t = 2, 3,$  and  $5$  h) in the *HRR25-AID* strain. (C) Quantitative analysis of the DSBs and COs shown in panel (B).

auxin (Fig. 1B). A previous study showed that the monopolar complex composed of Csm1, Hrr25, Lrs4, and Mam1 is required for segregation of homologous centromeres to opposite poles of a dividing cell during meiosis I [8]. In *hrr25* mutant cells, nuclear division is defective. [15]. Moreover, we observed that cell division was induced when  $\text{CuSO}_4$  and auxin were added at late but not early time points (Figs. 1C and 1D), indicating that Hrr25 regulates exit from the M phase of meiosis I.

We investigated cell division and DNA replication in the *HRR25-AID* strain by fluorescence-activated cell sorting [23]. There was no difference between *HRR25-AID* strains treated with or without  $\text{CuSO}_4$  and auxin, demonstrating that Hrr25 does not affect pre-meiotic replication (Fig. 1E). We also counted the number of 4',6-diamidino-2-phenylindole (DAPI)-positive foci to analyze nuclear divisions, meiosis I and II. Consistent with the above findings, the cell division rate reached to 50% when *HRR25-AID* cells were induced at a late time point with  $\text{CuSO}_4$  and auxin, whereas cells did not divide upon early activation of the degron system.

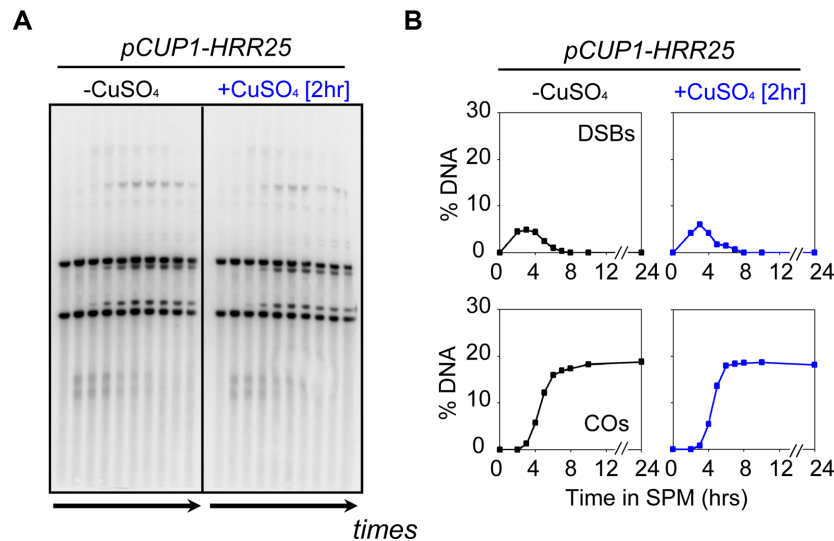
These results suggest that cell division is inhibited while DNA replication is unaffected on Hrr25 degradation at an early time point.

To investigate whether Hrr25 regulates DSB and CO formation during meiotic recombination, we performed one-dimensional (1D) gel Southern blot analysis at the *HIS4LEU2* locus. Cells were synchronized at the  $G_1$  phase for initiation of meiosis. To stabilize the secondary structure of joint molecules (JMs), cell samples were treated with Psoralen and exposed to ultraviolet radiation. Genomic DNA was extracted and DNA samples were digested with XhoI [12, 13, 20, 24–27]. DNA fragments were separated by 0.6% agarose gel electrophoresis and DNA species were detected by Southern blotting (Fig. 2A). In *HRR25-AID* cells without  $\text{CuSO}_4$  and auxin, DSBs reached a peak at 2.5 h before gradually declining. Meanwhile, COs began to appear at 3.5 h and reached a peak at 8 h, remaining at the same level until 24 h (Figs. 2A and 2B). To deplete Hrr25 proteins,  $\text{CuSO}_4$  and auxin were added at 2, 3, and 5 h after induction of meiosis. DSBs appeared at 2.5 h and reached a



**Fig. 3.** Physical analysis of recombination in *HRR25-AID* cells.

(A) Physical map of the *HIS4LEU2* hot spot. SEIs, single-end invasions; dHJ, double-Holliday junction; DSB, double-strand break. (B) Representative image of 2D gel analysis. (C) Image of 2D gel analysis in the absence or presence of Hrr25 by adding  $\text{CuSO}_4$  and auxin.



**Fig. 4.** Analysis of double-strand breaks (DSBs) and crossovers (COs) in *pCUP1-HRR25* cells.

(A) One-dimensional gel analysis in the *pCUP1-HRR25* strain. To induce Hrr25 expression, 30  $\mu$ M  $\text{CuSO}_4$  was added at  $t = 2$  h after initiation of meiosis. (B) Quantification of DSBs and COs in the presence or absence of  $\text{CuSO}_4$ .

maximum level (~15%), which was similar to the non-degraded Hrr25 phenotype. COs were detected at 4 h after initiation of meiosis and persisted until its completion in Hrr25-degraded mutants (Fig. 2C). These results indicate that Hrr25 does not affect DSB formation/turnover and CO formation, and consequently the progression of meiotic recombination.

In budding yeast, Cdc5 is required for chromosome segregation by cleavage of the cohesin complex from the chromosome arms and for CO-fate JM resolution [20, 28–32]. To investigate whether the meiotic division defect is caused by the failure of JM resolution in Hrr25-deficient cells, JMs were distinguished by native–native 2D gel analysis. Maternal and paternal dHJs and SEIs at the *HIS4LEU2* locus of chromosome III were distinguishable based on their molecular weight and shape [24, 33, 34]. We found that meiotic division was inhibited in Hrr25-deficient cells in the presence of  $\text{CuSO}_4$  and auxin (Fig. 1C). When *HRR25-AID* cells were treated with  $\text{CuSO}_4$  and auxin ( $t = 2, 3,$  and  $5$  h), JMs were normally formed at 4 h and were completely resolved at 10 h in all the conditions. (Figs. 3A and 3B). The similarity of these phenotypes suggests that Hrr25 mediates meiotic division and that the defects in meiosis are unrelated to JM formation/resolution during homologous recombination (Fig. 3C).

Hrr25 is required for meiotic division; to identify whether Hrr25 overexpression affects meiotic recombination and cell division, we ectopically expressed Hrr25 from the *CUP1* promoter by adding  $\text{CuSO}_4$  and performed 1D gel

analysis (Fig. 4A). In *pCUP1-HRR25* ( $-\text{CuSO}_4$ ) cells, DSBs were initiated at 2.5 h and reached a maximum at 3.5 h (Fig. 4B). COs were detected at 4 h and reached a peak at 10 h, remaining at the same level up to 24 h (Fig. 4B). DSB turnover and CO formation in *pCUP1-HRR25* ( $+\text{CuSO}_4, t = 2$  h) cells showed similar patterns of phenotypes. Thus, ectopic expression of Hrr25 does not affect these processes in meiotic recombination; moreover, although Hrr25 is required for nuclear division in meiosis I, it cannot independently modulate JM progression and recombination progression.

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