J. Microbiol. Biotechnol. (2017), 27(2), 405–411
https://doi.org/10.4014/jmb.1610.10074

jmb

Hed1 Promotes Meiotic Crossover Formation in *Saccharomyces cerevisiae*

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Received: October 28, 2016
Revised: November 30, 2016
Accepted: December 2, 2016

First published online
December 14, 2016

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pISSN 1017-7825, eISSN 1738-8872

Homologous recombination occurs between homologous chromosomes and is significantly involved in programmed double-strand break (DSB) repair. Activation of two recombinases, Rad51 and Dmc1, is essential for an interhomolog bias during meiosis. Rad51 participates in both mitotic and meiotic recombination, and its strand exchange activity is regulated by an inhibitory factor during meiosis. Thus, activities of Rad51 and Dmc1 are coordinated to promote homolog bias. It has been reported that Hed1, a meiosis-specific protein in budding yeast, regulates Rad51-dependent recombination activity. Here, we investigated the role of Hed1 in meiotic recombination by ectopic expression of the protein after pre-meiotic replication in *Saccharomyces cerevisiae*. DNA physical analysis revealed that the overexpression of Hed1 delays the DSB-to-joint molecule (JM) transition and promotes interhomolog JM formation. The study indicates a possible role of Hed1 in controlling the strand exchange activity of Rad51 and, eventually, meiotic crossover formation.

Keywords: Hed1, meiosis, recombination, *Saccharomyces cerevisiae*

During meiosis, homologous recombination leads to proper chromosome segregation and generates genetic diversity in most of the eukaryotic cells [1–3]. Meiotic recombination is initiated by programmed DNA double-strand break (DSB) formation by the topoisomerase-like protein, Spo11, along with its accessory proteins [4–7]. Mre11, Rad50, and Xrs2 (known as MRX; Nbs1 instead of Xrs2 in mammalian cells) form a heterotrimeric complex having 3′ to 5′ exonuclease activity and endonuclease activity, which was characterized from the Mre11 subunit [8, 9]. Sae2, along with the MRX complex, is involved in DNA resection processing, which activates Mre11 endonuclease as well as Dna2 and Exo1, which have 5′ to 3′ exonuclease activity and create long-range resections [10]. The exposed 3′ ssDNA tails are coated with replication protein A (RPA), which binds to the ssDNA prior to binding of Rad51. RPA-coated ssDNAs are displaced by Rad51, Rad52, and Rad55/57 in budding yeast [11–14]. In eukaryotes, two highly conserved recombinases, Rad51 and Dmc1, orthologs to RecA in bacteria, bind to the ssDNA in an ATP-dependent manner, forming a helical nucleofilament [15]. These recombinases catalyze homology search and DNA strand exchange between homologous chromosomes by repairing the DSBs [16, 17]. Rad51 mediates the identification of homologous sequences, followed by the exchange of strands in sister chromatids during mitosis [18]. In meiosis, however, Rad51-mediated strand exchange is stimulated by Dmc1 or other mediator proteins [19–22]. Dmc1, a meiosis-specific recombinase, forms nucleoprotein filaments with the ssDNA and promotes interhomolog recombination [23]. These active filaments perform DNA strand exchange in meiotic prophase I, and Rad51 plays as an accessory factor [19, 24, 25].

Hed1 is specifically expressed during the meiotic cell cycle [26, 27]. Although deletion of the *HED1* gene does not affect the progression of sporulation, spore viability is slightly decreased compared with the wild type (WT) [27]. It has been reported in a yeast two-hybrid assay that Hed1 physically interacts with Rad51 [27]. The Hed1 foci are almost completely abolished in the absence of Rad51 [27]. In budding yeast, a *dmc1Δ hed1Δ* mutant has been demonstrated to have significantly improved spore viability [27]. However, the *rad51Δ dmc1Δ hed1Δ* mutant exhibits defects in spore viability and sporulation [27]. Overproduction

of Rad51 increased the spore viability in *dmc1Δ* and *dmc1Δ hed1Δ* cells. Thus, Hed1 has a negative effect on Rad51 activity in meiotic recombination [27].

In the present study, DNA physical analysis was carried out to understand how Hed1 regulates the meiotic recombination in the budding yeast *Saccharomyces cerevisiae*. We show that although Hed1 does not affect sporulation, it delays nuclear division. In recombination analysis, we show that Hed1 is not essential for the formation of DSB. Noticeably, Hed1 was also found to regulate proper homolog bias.

In the budding yeast, the endogenous Hed1 protein is expressed during meiosis, and it regulates meiotic recombination by down-regulation of the activity of Rad51 in the absence of Dmc1 [27]. Although *hed1Δ* does not affect sporulation, spore viability was slightly reduced compared with WT in the SK1 strain background [27]. To observe the meiotic prophase roles of Hed1, stain *pCUP1-HED1* was constructed using a PCR-based method, followed by generation of diploid cells [28]. The endogenous Hed1 promoter was replaced with the *CUP*1 promoter, enabling the expression of the protein with an N-terminal 3-hemagglutinin (HA)-tag in the presence of copper [29, 30]. For synchronization of pre-meiotic culture at the G1 phase, the cells were grown in supplemented pre-sporulation (SPS) medium, and were then transferred to sporulation medium (SPM) to initiate meiosis. The expression of Hed1 protein was induced by the addition of 50 μM $CuSO_4$ to *pCUP1-HED1* culture after 2.5 h of SPM culture (Fig. 1A).

To analyze whether Hed1 normally progresses meiotic division, the nuclei were stained with DAPI for observation under the fluorescence microscope (Figs. 1B and 1C). The *pCUP1-HED1* cells were delayed about 1 h compared with WT cells. However, addition of copper did not affect the meiotic nuclear division in the *pCUP1-HED1* strains. Nuclear divisions were initiated after 5 h of SPM culture, and the strains produced normal four nuclei as shown in WT (Figs. 1B and 1C). Thus, these results imply that chromosome segregation was normal in the presence or absence of Hed1.

As shown in Fig. 1B, suppression of sporulation was not observed in *pCUP1-HED1* cells, regardless of the presence of copper. A homozygous *pCUP1-HED1* diploid strain was constructed, and genetic analysis was carried out using the *HIS4LEU2* physical assay system [19, 29–37]. 1D gel

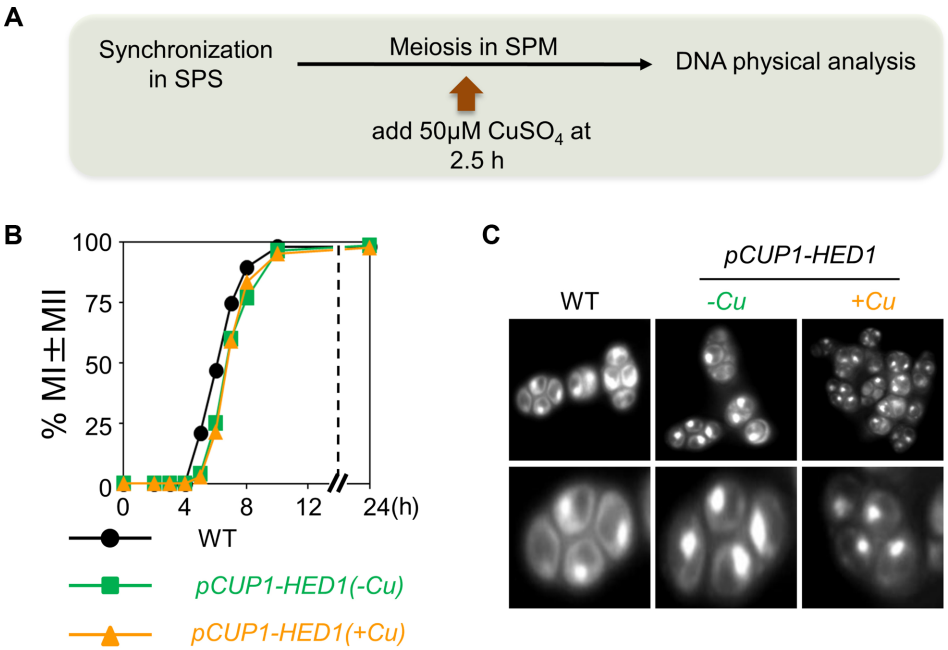

Fig. 1. Schematic representation of the experimental design and depiction of nuclear division in the *pCUP1-HED1* strains.
(**A**) Diagrammatic representation of the experimental outline. A single colony was inoculated in 2 ml of YPD liquid medium. For arresting the cells at G1 phase, the cells were cultured in supplemented pre-sporulation (SPS) medium. Meiosis was initiated in the sporulation medium (SPM). For overexpression of Hed1 protein, the cultures were induced with 50 μM $CuSO_4$ at 2.5 h. This was followed by the DNA physical analysis of mutants. (**B**) The samples were stained with DAPI at every time point, and the number of DAPI foci and meiosis I ± meiosis II (MI ± MII) was counted. Hed1 expression was delayed in the copper-induced and uninduced *pCUP1-HED1* cells by 1 h as compared with the WT. WT: Black; *pCUP1-HED1(-Cu)*: Green; *pCUP1-HED1(+Cu)*: Orange. (**C**) Fluorescence microscopy image of 24 h sample stained with DAPI.

electrophoresis was applied to analyze the formation of DSBs and crossovers (COs) between parental chromosomes at the *HIS4LEU2* hotspot on chromosome III (Fig. 2A). To stabilize the recombination intermediates, DNA samples were cross-linked using Psoralen on UV light, and total genomic DNA samples were purified by phenol-guanidine extraction. DNA samples from each time course were digested with *Xho*I, and the resulting fragments were separated by agarose gel (0.6%) electrophoresis, followed by Southern blotting experiment using Probe A (Fig. 2A). "Mom" allele and "Dad" allele chromosomes were detected at 5.9 and 4.3 kb, respectively, at the *HIS4LEU2* locus (Fig. 2A). COs were detected at 5.6 and 4.6 kb. DSB signals from Mom and Dad were observed at 3.3 and 3 kb by 1D gel analysis (Figs. 2A and 2B). In *pCUP1-HED1* cells, when $CuSO_4$ was absent, DSBs appeared at 2.5 h after the induction of meiosis and they were gradually repaired to form recombinants from 4 h (Figs. 2B and 2C). COs appeared at 5 h and were present until the termination of meiosis (Figs. 2B and 2C). When $CuSO_4$ was present in meiotic cultures of *pCUP1-HED1* cells, early DSB formation was observed. However, DSBs gradually progressed in comparison with the cells grown in the absence of $CuSO_4$. The expression of Hed1 during meiosis led to approximately 14% increase in the CO formation (Fig. 2C).

In all the experiments, the kinetics of DSB repair was

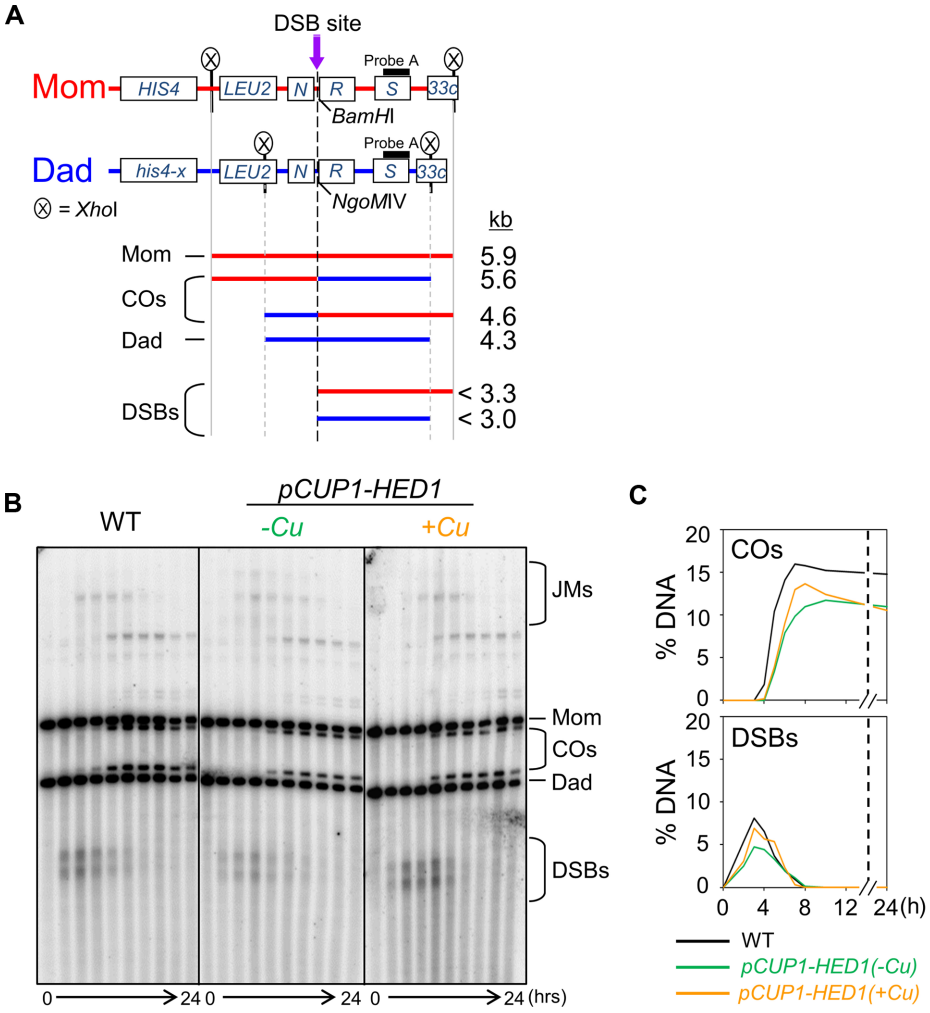

Fig. 2. DNA analysis of WT and *pCUP1-HED1* strains by 1D gel electrophoresis.
(**A**) *HIS4LEU2* hot spot is located on chromosome III. The construct had two different structures that contain sites for DSBs, a selection marker, the enzyme sites (*BamH*I, *NgoM*IV), probe A, and *Xho*I sites in *Saccharomyces cerevisiae*. Digestion with *Xho*I resulted in distinct fragments. Mom: mom species; Dad: dad species; COs: crossover species; DSBs: double-strand breaks. (**B**) 1D gel analysis showing all the WT and *pCUP1-HED1* strains grown in the presence and absence of $CuSO_4$. The samples were collected at different time points (0, 2.5, 3.5, 4, 5, 6, 7, 8, 10, and 24 h). (**C**) Quantification of the COs and DSBs at each time point.

observed at the *HIS4LEU2* hotspot that is flanked by *Xho*I restriction enzyme sites on chromosome III [19, 29–37]. Both DSBs and COs bands can be detected by Southern blot analysis in 1D gel. Psoralen-UV crosslinks DNA and stabilizes the recombination intermediates, single-end invasions (SEIs) and double Holliday junctions (dHJs), also known as joint molecules (JMs) [19, 29–37]. Genomic DNA was digested with *Xho*I and the DNA fragments were analyzed by 0.8% agarose gel electrophoresis, followed by Southern blotting using Probe A (Figs. 3A and 3B). The structure of JMs was further analyzed by 2D gel electrophoresis, wherein the fragments were separated by different mobility based on their shape and molecular weight (Fig. 3B) [19, 29–37]. Mom-Mom, Mom-Dad, and Dad-Dad dHJs were detected at 11.8, 10.2, and 8.6 kb, respectively, at the *HIS4LEU2* hot spot, and SEI signals were detected at 9.2, 8.9, 7.6, and 7.3 kb by 2D gel analysis (Fig. 3B). To determine whether Hed1 affects the progression of homologous recombination after DSBs formation, Hed1 expression was induced with $CuSO_4$ and formation of JMs was observed by 2D gel electrophoresis (Figs. 3B and 3C). 2D gel electrophoresis enables clear visualization of JMs [19, 29–37]. Consistent with previous studies, a strong homolog bias was observed in WT cells (Fig. 3C) [19, 37].

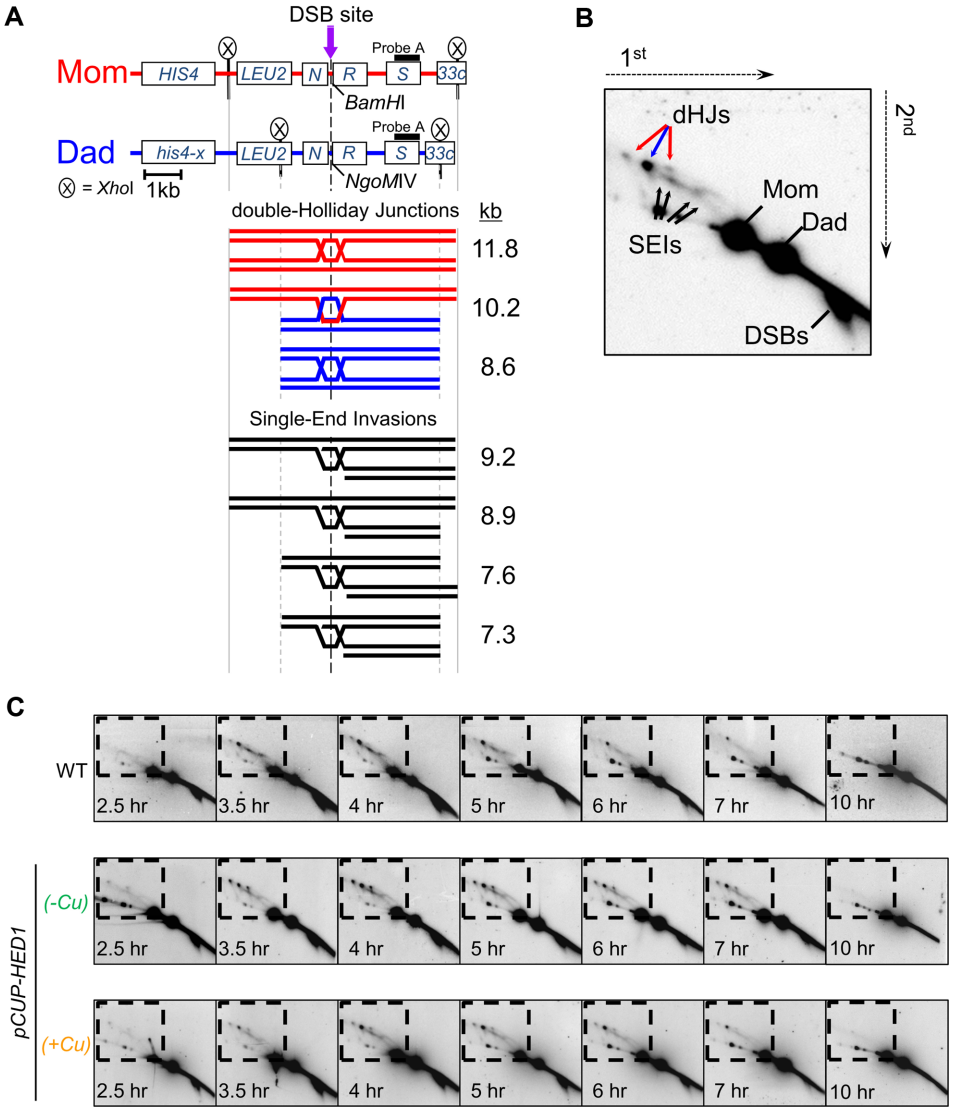

Fig. 3. 2D gel analysis of the WT and *pCUP1-HED1* strains.

(**A**) Structure of single-end invasion (SEI) and double Holliday junction (dHJ). (**B**) Representative image of native/native 2D gels in the WT strain. The image shows three spots for dHJs and two arcs for SEIs, which appear on the DNA. Blue line: IH-dHJs; Red line: IS-dHJs; Black line: SEIs. (**C**) 2D gel images of WT and *pCUP1-HED1* strains in the presence and absence of copper.

Interestingly, higher levels of IS-dHJs were observed in the *pCUP1-HED1* strain after 2.5 h than in WT. After addition of $CuSO_4$, the level of IS-dHJs was decreased in *pCUP1-HED1* cultures since 3.5 h. Subsequently, the formation of IH-dHJs was initiated in *pCUP1-HED1* in the presence or absence of $CuSO_4$. Thereafter, a gradual reduction in JMs occurred, followed by a complete disappearance at 10 h in WT and *pCUP1-HED1* cells.

Our previous study suggested that Hed1 might affect homolog bias (Fig. 3C) [19]. This aspect was investigated in detail using 2D gel electrophoresis (Fig. 4). The presence of JMs (IH-SEIs and IH-dHJs) was observed in the WT cells (Fig. 4A). All JMs were also present in *pCUP1-HED1* cells, grown in the absence of $CuSO_4$. However, the levels of IH-dHJs were reduced and those of IS-dHJs were increased ~ 4-fold as compared with WT (Fig. 4B). However, IS-dHJs were strongly reduced after the induction of Hed1 at 2.5 h. This result suggests that Hed1 could be involved in the regulation of homolog bias. The appearance of JMs was delayed by ~1 h in the presence of $CuSO_4$ as compared with the untreated cells (Fig. 4B). A previous study has reported that the IH-dHJ:IS-dHJ ratio in the WT and *hed1Δ* mutant cells were 5:1 and 2:1, respectively [19]. In the *pCUP1-HED1* cells grown in the absence of $CuSO_4$ (*pCUP1-HED1* (-Cu)), the level of IS-dHJs was about 0.67%. Moreover, the level of IH-dHJs was reduced to ~0.9%. The ratio of IH:IS dHJs in these cells decreased from 5:1 to 1.1:1 (Fig. 4C). In *pCUP1-HED1* (+Cu) cells, the maximum level of IS-dHJs was reduced to 50% compared with those of *pCUP1-HED1* (-Cu) cells. Furthermore, the ratio of IH:IS dHJs was 3.5:1 (Fig. 4C). The pick of dHJs was delayed 1 h, and the SEI lifespan was delayed in *pCUP1-HED1* (+Cu) (Figs. 4A and 4B). These observations suggest that appropriate expression of Hed1 protein might be required to regulate

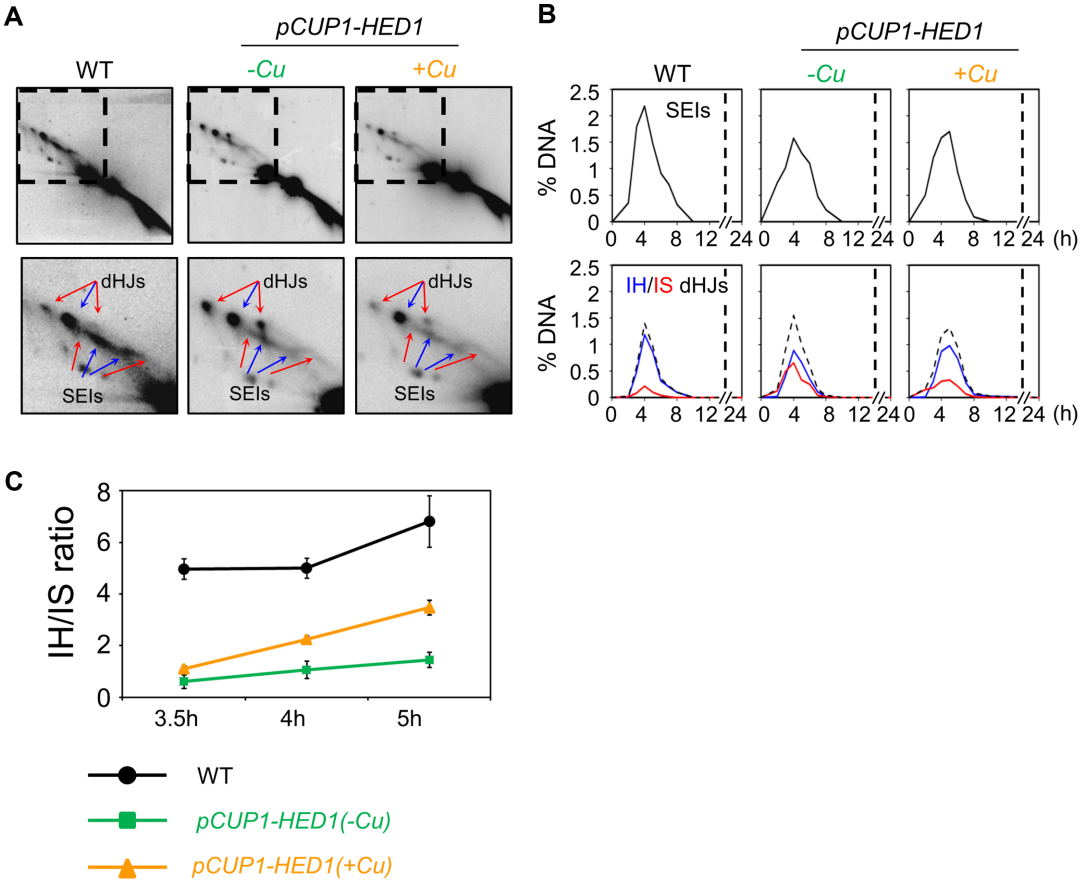

Fig. 4. Regulation of joint molecules (JM) formation by Hed1 in *pCUP1-HED1* strains.
(**A**) Representative image of 2D gel at different time points in WT and *pCUP1-HED1* cells grown with and without $CuSO_4$. The images are the same as Fig. 3C. (**B**) Quantification of the JMs (single-end invasions (SEIs) and double Holliday junctions (dHJs)). Blue line: IH-dHJs; Red line: IS-dHJs; Black dotted line: total dHJs; Black line: SEIs. (**C**) Ratios of IH/IS-dHJs in WT and *pCUP1-HED1* strains grown with and without $CuSO_4$ at 3.5, 4, and 5 h (n = 3). Black line: WT; Green line: (-)$CuSO_4$, Orange line: (+)$CuSO_4$.

the lifespan of SEIs and dHJs.

Meiotic recombination is initiated by generation of a programmed DSB during prophase I. Subsequently, the DSBs mediate strand search and exchange to form nascent D-loop and JMs (SEIs and dHJs), followed by onset of recombination. The course of recombination involves the formation of D-loop and JMs (SEIs/dHJs). In this study, we demonstrated that the presence of Hed1 regulates the level of interhomolog CO formation. The expression of Hed1 promotes proper formation of IH-JMs. Thus, Hed1 could act as a negative regulator for the strand exchange activity mediated by Rad51 during meiosis.

Acknowledgments

This work was supported by the Chung-Ang University Graduate Research Scholarship in 2015 and by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIP) (No. 2014R1A2A1A11051584).

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