Hsk1-Dfp1/Him1, the Cdc7-Dbf4 Kinase in Schizosaccharomyces pombe, Associates with Swi1, a Component of the Replication Fork Protection Complex*§

Seiji Matsumoto1, Keiko Ogino1, Eishi Noguchi1*, Paul Russell¶, and Hisao Masai‡

From the 4Genome Dynamics Project, Tokyo Metropolitan Institute of Medical Science, Tokyo 113-8613, Japan, 8Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, Pennsylvania 19102, and the 6Departments of Molecular Biology and Cell Biology, The Scripps Research Institute, La Jolla, California 92037

The protein kinase Hsk1 is essential for DNA replication in Schizosaccharomyces pombe. It associates with Dfp1/Him1 to form an active complex equivalent to the Cdc7-Dbf4 protein kinase in S. cerevisiae. Hsk1 and Swi1 are subunits of the replication fork protection complex in S. pombe that is homologous to the Tof1-Csm3 complex in S. cerevisiae. The fork protection complex helps to preserve the integrity of stalled replication forks and is important for activation of the checkpoint protein kinase Cds1 in response to fork arrest. Here we describe physical and genetic interactions involving Swi1 and Hsk1-Dfp1/Him1. Dfp1/Him1 was identified in a yeast two-hybrid screen with Swi1. Hsk1 and Dfp1/Him1 both co-immunoprecipitate with Swi1. Swi1 is required for growth of a temperature-sensitive hsk1 (hsk1ts) mutant at its semi-permissive temperature. Hsk1ts cells accumulate Rad22 (Rad52 homolog) DNA repair foci at the permissive temperature, as previously observed in swi1 mutants, indicating that abnormal single-stranded DNA regions form near the replication fork in hsk1ts cells. hsk1ts cells were also unable to properly delay S-phase progression in the presence of a DNA alkylating agent and were partially defective in mating type switching. These data suggest that Hsk1-Dfp1/Him1 and Swi1-Swi3 complexes have interrelated roles in stabilization of arrested replication forks.

Schizosaccharomyces pombe Hsk1 kinase (the fission yeast CDC7 homologue) forms a complex with Dfp1/Him1 (the fission yeast DBF4 homologue) and regulates the initiation of DNA replication (1, 2). The expression of Dfp1/Him1 is cell cycle regulated, peaking at the G1/S transition when the protein kinase activity of Hsk1-Dfp1/Him1 is maximal and coinciding with the phosphorylation of its presumptive substrates, mini-chromosome maintenance (MCM)2 proteins (3, 4). Hsk1-Dfp1/Him1 has been implicated in DNA replication checkpoint signaling that occurs in response to replication fork arrest. Dfp1/Him1 was also genetically identified as a radiation-sensitive rad25 mutant. Mutations in the N-terminal conserved domain of Dfp1/Him1 render cells sensitive to a variety of genotoxic agents, including hydroxyurea (HU), a compound that stalls replication forks (5, 6). The C-terminal domain of Dfp1/Him1 (Dbf4-motif-c), which is required for full activation of Hsk1, is also conserved and proposed to play an important role in the response to DNA damage in S-phase (6). Both Hsk1 and Dfp1/Him1 are hyper-phosphorylated in response to HU treatment in a manner dependent on the checkpoint kinase Cds1 (4, 7, 8). This hyperphosphorylation is eliminated in a temperature-sensitive mutant, hsk1–89 (8). Furthermore, it was reported that cds1α partially suppresses the temperature-sensitive growth of hsk1–1312 cells, suggesting that Cds1 may negatively regulate Hsk1 (7). On the other hand, we previously reported that HU-mediated activation of Cds1 is substantially impaired in a hsk1–89 mutant grown at its permissive temperature (8).

Recent studies have identified a group of proteins that are required for activation of replication checkpoint in fission yeast. Mrc1, a mediator of the replication checkpoint, is essential for Cds1 activation in a Rad3-dependent manner (9, 10). Swi1, a Tof1/Tim1-related protein, which is required for a programmed fork-pausing event necessary for mating type switching (11), is also known to be required for proficient activation of Cds1 (12). Interestingly, Swi1, Tof1 (the budding yeast homologue of Swi1), and budding yeast Mrc1 are localized at the replication fork and are thought to be components of the replication machinery (12–14). Moreover, Swi1, together with Swi3, is proposed to form a replication fork protection complex (FPC) that is required for stabilization of forks in a configuration that is recognized by replication checkpoint sensors (14). Here, we show that Swi1 genetically and physically interacts with Hsk1. Hsk1 appears to be required for proper arrest of the replication fork and its stabilization in conjunction with Swi1.

EXPERIMENTAL PROCEDURES

General Techniques—Methods for genetic and biochemical analyses of fission yeast have been described previously (15, 16). For immunoblotting, extracts from ~107 cells were made by the “boiling method” as described previously (3).

Co-immunoprecipitation—Cells expressing Swi1–3FLAG and/or Dfp1–13myc at their own genomic loci were grown to 1 × 107 cells/ml in 50 ml of yeast extract-supplemented (YES) medium. Cells were collected and washed in phosphate-buffered saline and resuspended in 2 ml of SP buffer (1.2 M sorbitol, 0.1 M KPO4 (pH 6.5), and 2 mM phenylmethylsulfonyl fluoride) containing 0.2% 2-mercaptoethanol. Spheroplasts were prepared by adding 0.5 ml of SP buffer containing 2 mg/ml zymolysate-100T, followed by incubation at 30 °C for 30 min. The spheroplasts were pelleted, washed twice with SP buffer, and resuspended in 1.4 ml of IP buffer (20 mM HEPES (pH 7.0), 50 mM potassium acetate, 5 mM magnesium acetate, 100 mM sorbitol, 0.1% Triton X-100, 2 mM dithiothreitol, 50 mM sodium orthovanadate, 50 mM β-glycerophosphate, and protease inhibitors (number 8215; Sigma) containing 2

* This work was supported by grants-in-aid from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (to H. M.), by National Institutes of Health Grant GM059447 (to P. R.), and by Drexel University start-up funds (to E. N.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. 1S and 2S.

‡ To whom correspondence should be addressed. Tel.: 81-3-5685-2264; Fax: 81-3-5685-2932; E-mail hmasai@rinshoken.or.jp.

§ This work was supported by grants-in-aid from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (to H. M.), by National Institutes of Health Grant GM059447 (to P. R.), and by Drexel University start-up funds (to E. N.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: MCM, mini-chromosome maintenance; HU, hydroxyurea; FPC, fork protection complex; YES, yeast extract-supplemented medium; YFP, yellow fluorescent protein.
Role of Hsk1 Kinase in Replication Fork Stabilization

RESULTS

Physical and Genetic Interactions Involving Swi1 and Hsk1-Dfp1/Him1—To better understand how the Swi1-Swi3 complex participates in replication fork stabilization, we used yeast two-hybrid screening to detect interactions of these proteins could be co-immunoprecitated, indicating that the complexes are unstable and/or transient

Because Swi1 interacts both physically and genetically with Hsk1 and Hsk1–89,chk1 double mutant displayed synthetic lethality at 37 °C (Fig. 3A). This synthetic growth defect would suggest that efficient growth of hsk1–89 requires Swi1 function. The kinase activity of Hsk1–89 protein is severely compromised but can be activated to some extent in the presence of Dfp1/Him1 subunit in vitro. In vivo, the temperature-sensitive growth of hsk1–89 can be partially suppressed by overexpression of Dfp1/Him1 protein (8).

Interestingly, the hsk1–89,swi1Δ double mutant was viable at 25 °C, but at 37 °C it showed a severe growth reduction compared with the single mutants (Fig. 2C). This synthetic growth defect would suggest that efficient growth of hsk1–89 mutant at 37 °C requires Swi1 function.

Requirement of Swi1 for Survival of hsk1–89 Is Independent of Chk1—Because Swi1 interacts both physically and genetically with Hsk1 and both Swi1 and Hsk1 are required for proficient activation of the Cds1 kinase in response to replication fork arrest (8, 12), we examined genetic interactions of hsk1–89 with checkpoint mutations. The cds1Δ mutation very weakly suppressed the growth defect of hsk1–89 at 30 °C, a finding consistent with studies of the hsk1–1312 allele (7), and cds1Δ,hsk1–89 cells grew as efficiently as hsk1–89 cells at 37 °C (Fig. 3B). Interestingly, the hsk1–89,chk1Δ double mutant displayed synthetic lethality at 37 °C (Fig. 3A). This growth defect was more severe than that of hsk1–89,swi1Δ cells (Fig. 3B). A similar genetic interaction was reported for chk1Δ and hsk1–1312 mutations (7). It is likely that

hsk1–89 is partially compromised at 37 °C (Fig. 2A). Indeed, we have previously reported that hsk1–89 cells have an abnormal nuclear morphology at 37 °C. The kinase activity of Hsk1–89 protein is severely compromised but can be activated to some extent in the presence of Dfp1/Him1 subunit in vitro. In vivo, the temperature-sensitive growth of hsk1–89 can be partially suppressed by overexpression of Dfp1/Him1 protein (8).
Role of Hsk1 Kinase in Replication Fork Stabilization

hsk1–89 cells at 37 °C sustain spontaneous DNA damage that must be repaired, thereby invoking a requirement for the Chk1-dependent DNA damage checkpoint. Indeed, we have detected an increased amount of the hyperphosphorylated, activated form of Chk1 in hsk1–89 cells at 37 °C.3

The similar genetic interactions involving swi1Δ or chklΔ with hsk1–89 led us to compare the morphology of hsk1–89,swi1Δ and hsk1–89,chklΔ cells shifted to 37 °C for 6 h. As expected, hsk1–89,chklΔ cells appeared to be short in their cell length and frequently showed the cut phenotype (Fig. 4A). On the other hand, hsk1–89,swi1Δ cells were much longer and rarely showed the cut phenotype. In hsk1–89,chklΔ cells, cells with reduced DNA content (<1C genome content) gradually accumulated after the temperature shift to 37 °C (Fig. 4B). However, hsk1–89,swi1Δ cells did not show an accumulation of cells with less than 1C DNA content, but they did display an increase of 1C cells, indicative of a defect in DNA replication initiation (Fig. 2D). These data suggest that Swi1 is required for the growth of hsk1–89 cells at 37 °C in a manner independent of Chk1. Indeed, Chk1 activation is still observed in a hsk1–89,swi1Δ double mutant (data not shown), indicating that Chk1 activation does not require Swi1. We attempted to isolate a hsk1–89,swi1Δ,chk1Δ triple mutant to examine the effect of chklΔ on the phenotype of hsk1–89,swi1Δ at 37 °C and found that the hsk1–89,swi1Δ,chk1Δ triple mutant is lethal. This result further supports the idea that Swi1 and Chk1 are independently required for the growth of hsk1–89 mutant at 37 °C.

Formation of Rad22 DNA Repair Foci in hsk1–89 Cells—The synthetic growth defect and frequent appearance of the cut phenotype in hsk1–89,chk1Δ cells indicated that hsk1–89 cells experience spontaneous DNA damage that triggers a Chk1-dependent mitotic checkpoint, as previously demonstrated in swi1Δ cells (12). We investigated this possibility by analyzing the localization of Rad22-yellow fluorescent protein (YFP) fusion protein (17). Fission yeast Rad22 (also known as Rad22A) is a homologue of budding yeast Rad52 and forms nuclear foci at double-strand breaks and some other sites that have exposed single-stranded DNA segments (18, 19). A large increase in spontaneous Rad22-YFP foci was reported in swi1Δ cells (12). As shown in Fig. 5, the numbers of the cells carrying spontaneous Rad22-YFP nuclear foci was obviously increased in hsk1–89 cells relative to wild-type. The effect was similar to that observed in swi1Δ cells, indicating that hsk1–89 cells experience spontaneous DNA damage or other abnormal DNA structures that are bound by Rad22. It is possible that replication forks are not

3 S. Matsumoto and H. Masai, unpublished data.

FIGURE 2. Swi1 genetically interacts with Hsk1. A, wild-type (YM71) and hsk1–89 (KO147) cells were streaked on YES agar plates and incubated at 25, 30, and 37 °C for 5 days. B, hsk1–89 cells grown at 25 °C in YES were shifted to 30 °C. At the indicated time, cells were counted by Coulter counter, plated at 25 °C, and viability was calculated. C, genetic interaction between hsk1–89 and swi1Δ. 5-fold serial dilutions of exponentially growing cultures of the indicated genotypes were plated on YES agar and incubated at 25, 30, and 37 °C for 4 days. D, cells of the indicated genotypes were grown at 25 °C and shifted to 37 °C. Samples were taken at the indicated times after the shift up and were analyzed by fluorescence-activated cell sorter.

FIGURE 3. Genetic interactions between hsk1–89 and checkpoint mutations. A, hsk1–89 and chk1Δ show synthetic lethality at 37 °C. Cells were streaked on YES agar plates and were incubated at 25, 30, and 37 °C for 5 days. B, 5-fold serial dilutions of exponentially growing cultures of the indicated genotypes were plated on YES agar plates and were incubated at 25, 30, and 37 °C for 4 days. hsk1–89,chk1Δ showed more severe growth defect than hsk1–89,swi1Δ, and cds1Δ partially suppressed the growth defect of hsk1–89 at 30 °C.
stabilized in hsk1–89 cells, generating single-stranded DNA segments due to abortive fork unwinding, as was reported for the tof1 mutant in budding yeast (13). At 25 and 30 °C, the efficiency of Rad22 foci formation was not greatly increased in a swi1/hsk1–89 double mutant, although some additive effects of swi1 and hsk1–89 mutations on Rad22 foci formation were observed at 37 °C (Fig. 5B). These results indicate that Swi1 and Hsk1 proteins largely work in the same pathway for suppression of spontaneous DNA damages during cell proliferation.

Synergistic Interaction between hsk1–89 and swi1 in Survival of Genotoxic Agents—The genetic interactions involving Swi1 and Hsk1 were investigated in more detail by analyzing the response of mutant strains to HU, a compound that causes replication fork arrest by deple- tion of deoxyribonucleotides. As shown in Fig. 6A, the hsk1–89 mutant was only weakly HU sensitive at its permissive temperature of 25 °C. The swi1 mutant was more sensitive to HU than the hsk1–89 mutant and was less sensitive to HU than cds1Δ. Again, there was a synergistic interaction between hsk1–89 and swi1 in HU sensitivity. The hsk1–89/swi1Δ mutant was more sensitive to HU than either single mutant (Fig. 6A), suggesting that Hsk1 and Swi1 may act in distinct pathways of HU resistance. A synergistic interaction was also observed between hsk1–89 and swi1Δ in sensitivity to the alkylating agent methyl methanesulfonate (MMS). Both hsk1–89 and swi1Δ cells were sensitive to MMS, but the hsk1–89/swi1Δ double mutant was more sensitive to MMS than either single mutant or the chk1Δ mutant (Fig. 6B). These data suggest that Hsk1 and Swi1 act in distinct pathways in MMS resistance even though these proteins can associate in a complex.
Role of Hsk1 Kinase in Replication Fork Stabilization

FIGURE 7. Ectopic expression of Swi1 is deleterious for hsk1–89 mutant cells. Wild-type (hsk1+), or hsk1–89 cells harboring either pREP1, PE132 (expressing Swi1 on pREP1, indicated as Swi1), or pREP41-hsk1/3 (expressing Hsk1 on pREP41, indicated as Hsk1) were streaked on Edinburgh minimal medium agar with (upper) or without (lower) thiamine. Addition of thiamine represses the expression from the nmt1 promoter present on pREP1 or pREP41 (25).

Together, our finding suggests that Swi1 has both Hsk1-dependent and -independent functions in the survival of MMS treatment.

Overexpression of Swi1 Impedes the Growth of hsk1–89 Cells—Because the growth of the hsk1–89 mutant at 37 °C is highly dependent on the function of Swi1, we examined whether overexpression of Swi1 can suppress the growth defect of hsk1–89 at the non-permissive temperature of 30 °C. The swi1+ gene was placed under the control of the thiamine-repressible nmt1 promoter. As shown in Fig. 7, overexpression of Swi1 could not suppress the growth defect of the hsk1–89 mutant at 30 °C. On the contrary, at 37 °C, the hsk1–89 mutant bearing an nmt1-swi1+ plasmid could not form colonies when Swi1 was overexpressed in medium that lacked thiamine. Even in the presence of thiamine, which permits leaky expression of Swi1 from the nmt1 promoter, we observed very poor growth in the hsk1–89 background mutant at 37 °C. This acute sensitivity of hsk1–89 cells to even moderate overexpression of Swi1 is interesting in view of the genetic and physical interactions involving Swi1, Hsk1-Dbf1/Him1, and MCM (23). The overexpressed Swi1 protein may interfere with the MCM helicase activity, presumably located at the replication fork for continuous unwinding of the duplex DNA. The increased sensitivity of hsk1–89 to overexpression of Swi1 may reflect the attenuated MCM helicase in the mutant cells, because Hsk1 kinase is likely to play a crucial role in activating the MCM helicase at the replication fork (1).

MMS-induced Slowing of S-phase Requires Hsk1—These results were consistent with the suggestion that Hsk1 is required for the proper arrest of replication forks, an event that also requires the Swi1-Swi3 complex. We examined this idea by measuring DNA replication in hsk1–89 cells exposed to the DNA-damaging agent MMS. Because hsk1–89 cells are not acutely sensitive to HU at 25 °C, we synchronized both wild-type and hsk1–89 cells in early S by HU and then released them into the cell cycle in medium without HU. We added 0.015% MMS or mock treated and used flow cytometry to monitor S-phase progression. In the absence of MMS, wild-type cells completed S-phase within 5 h after release (Fig. 8). In the presence of MMS, the rate of DNA replication was significantly slowed in wild-type, and most cells were still in S-phase at 2 h after release and S-phase was not completed even at 3 h. In hsk1–89 cells, S-phase progression was completed within 2 h after release in the absence of MMS. In the presence of MMS, the delay was very subtle; S-phase was nearly completed in 2 h and was completed by 3 h (Fig. 8). Thus, these results indicate that fork progression in the presence of DNA damage is accelerated in hsk1–89, suggesting that Hsk1 is required for the stable arrest of DNA replication forks and intra-S-phase checkpoint.

It was previously reported that Swi1 is required for slowing of S-phase in response to DNA damages (21), and we confirmed this result by analyzing S-phase progression in release from HU arrest in the presence of MMS (supplemental Fig. S1). We then analyzed S-phase progression in hsk1–89,swi1Δ double mutant in the presence and absence of MMS. The rate of S-phase progression was significantly reduced in hsk1–89,swi1Δ cells even in the absence of MMS (requiring 4 h for completion). In the presence of MMS, unexpectedly, S-phase was slowed down in the double mutant (supplemental Fig. S1), requiring 5 h for completion. It is possible that hsk1–89,swi1Δ cells suffer excessive DNA damage in MMS, resulting in widespread physical impediment of replication forks.

Mating Type Switching Is Partially Impaired in hsk1–89 Cells—Swi1-Swi3 complex is required for the programmed fork-pausing events that lead to mating type switching in fission yeast (11). To further explore the relationship between Swi1 and Hsk1, we attempted to determine whether mating type switching requires Hsk1. Switching-proficient strains mate and form spores that can be stained with iodine vapors. Heterothallic h+ strains do not mate and remain unstained. Switching-defective swi1Δ cells switch mating type at a very low frequency and produced dark lines or patches where rare events of mating-switch had occurred. In contrast, the wild-type and hsk1–89 colonies produced uniformly intense iodine staining at 25 °C, suggesting that the hsk1–89 mutant is switching proficient at its permissive temperature of 25 °C (Fig. 9A). At a semi-permissive temperature of 28.5 °C, colonies of h80 hsk1–89 were still stained fairly dark by iodine, but dark lines or patches...
Role of Hsk1 Kinase in Replication Fork Stabilization

point kinase Cds1 in response to fork arrest, suggesting that Swi1-mediated fork stabilization is required for Cds1 activation. We previously reported that Cds1 activation in response to fork arrest is significantly impaired in hsk1 Δ cells (8), leading us to speculate that Swi1 and Hsk1 may be involved in a common pathway leading to activation of Cds1 kinase after fork arrest.

Interaction of Hsk1-Dfp1/Him1 and Swi1 May Facilitate Stabilization of Arrested Replication Forks—We have discovered that Swi1 interacts with Dfp1/Him1 and Hsk1, suggesting a possibility that Hsk1-Dfp1/Him1 may facilitate stabilization of arrested replication forks in conjunction with Swi1-Swi3 FPC. This possibility was supported by the following findings. 1) Rad22-YFP foci, which are constitutively observed in swi1 Δ cells, accumulated in hsk1 Δ cells even at its permissive temperature (Fig. 5), suggesting the accumulation of single-stranded DNA regions. This may be caused by destabilization of spontaneously arrested replication forks, as is the case for swi1 Δ and tof1 Δ (12, 13). 2) Progression of DNA replication forks is not properly slowed down by alkylating DNA damages in hsk1 Δ cells (Fig. 8), consistent with the notion that Hsk1 functions for the temporal arrest of moving replication forks. 3) Mating type switching is partially impaired in hsk1 Δ cells (Fig. 9). While we were preparing this report, Sommariva et al. (21) reported that Hsk1 and the Swi1-Swi3 complex function in the same pathway in checkpoint responses to alkylating reagents. Our finding of physical and genetic interactions between swi1 Δ and hsk1 Δ extends their observations and strongly indicates that there is functional interplay between Hsk1 and the Swi1-Swi3 fork protection complex for responses to arrested replication forks. However, unlike their studies, we have found that mutations in the swi1 and hsk1 genes display additive or synergistic interactions in MMS and HU survival assays (Fig. 6). The reason(s) for these discrepancies are unknown. They might be attributable to the properties of different hsk1 alleles or experimental protocols, but our data clearly indicate that Hsk1-Dfp1/Him1 and Swi1-Swi3 complexes have both interdependent and independent roles in the response to replication fork arrest.

Swi1-Hsk1 Interaction May Play Roles Both in Initiation and in Responses to Replication Fork Block—Swi1 is critically important for the survival of hsk1 Δ cells at their semi-permissive temperature of 37 °C (Fig. 2C). Although Swi1 is required for proficient activation of Cds1 kinase (12), Cds1 function is not required for the viability of hsk1 Δ cells at 37 °C (Fig. 3). On the contrary, cds1 Δ partially suppresses the growth defect of hsk1 Δ cells at 30 °C (Fig. 3), as was reported previously for hsk1Δ1312 (7). Although Cds1 activation is significantly impaired in hsk1 Δ (8), Chk1 is constitutively activated in hsk1 Δ cells (data not shown), presumably functioning for repression of M-phase progression. In fact, hsk1 Δ, chk1 Δ cells incubated at 37 °C for 6 h were short, and many cells exhibited a cut phenotype (Fig. 4A). The growth defect of hsk1 Δ, chk1 Δ cells was more severe than that of hsk1 Δ, swi1 Δ cells (Fig. 3). Snith et al. (7) previously reported the synthetic lethality of chk1 Δ with hsk1 Δ. They also reported that Chk1 is constitutively activated in hsk1Δ1312 cells. On the other hand, we observed that hsk1Δ1-89,swi1 Δ cells were elongated with very few cut cells at 37 °C (Fig. 4A) and showed accumulation of cells with unrepli-
dated DNA (Fig. 2D). Thus, the requirement for Swi1 in hsk1 Δ cells is not dependent on Chk1, but Swi1 may assist Hsk1 for S-phase functions. Swi1 might activate Hsk1 by recruiting Dfp1/Him1 for initiation of DNA replication or facilitate the phosphorylation of its critical target. Because we did not detect a decrease of Hsk1 kinase activity in the extract from swi1 Δ cells (data not shown), it is more likely that Swi1 stimulates Hsk1-mediated phosphorylation of its target proteins, such as the subunits of the MCM complex. Indeed, a defect in entry into

DISCUSSION

Both Hsk1 and FPC Are Required for Cds1 Activation after Fork Arrest—Fission yeast Hsk1-Dfp1/Him1 kinase plays an essential role in initiation and progression of S-phase (7, 8). In fact, it has been shown that budding yeast Cdc7 kinase is required throughout S-phase for activation of each replication origin (20). Recent studies of Swi1 in fission yeast and Tof1 in budding yeast have shown that they are components of the replisome and are needed to stabilize stalled forks caused by inadequate supplies of deoxyribonucleotide triphosphates (dNTPs) (12, 13). Swi1 forms a replication FPC with a 181-amino acid protein, Swi3, and Swi1-Swi3 FPC plays a crucial role in stabilization of arrested forks (14). Swi1 is required for proficient activation of the replication check-

were observed, albeit at a frequency lower than in swi1 Δ cells (Fig. 9B), suggesting that mating type switching is partially abrogated in hsk1 Δ cells. This result suggests that Hsk1 is involved in the programmed fork-pausing event necessary for mating type switching, further supporting the notion that Hsk1 is required for the stable arrest of DNA replication forks.

FIGURE 9. Mating type switching is partially abrogated in hsk1 Δ cells. A heterothallic strain, h+/h wild-type (YM71), and three homothallic strains, h+/h wild-type (Y933), h+/h Δ (Y400), and h+/h Δ (N278), were streaked on Edinburgh minimal medium agar with supplements, incubated at 25 °C (A) or 28.5 °C (B) for 7 days, and then stained by iodine vapor to detect spores. A, colonies of h+/h Δ were stained uniformly dark by iodine just like h+/h wild-type colonies, indicating that h+/h Δ cells are proficient in mating type switching at 25 °C. As previously reported (12), swi1 Δ mutant colonies showed a mottled phenotype, indicating a defect in mating type switching. B, although colonies of h+/h Δ were stained dark by iodine at a semi-permissive temperature of 28.5 °C, dark lines or patches were observed, albeit at a frequency lower than that in swi1 Δ colonies, indicating that mating type switching is partially abrogated in hsk1 Δ cells.

were observed, albeit at a frequency lower than in swi1 Δ cells (Fig. 9B), suggesting that mating type switching is partially abrogated in hsk1 Δ cells. This result suggests that Hsk1 is involved in the programmed fork-pausing event necessary for mating type switching, further supporting the notion that Hsk1 is required for the stable arrest of DNA replication forks.

DISCUSSION

Both Hsk1 and FPC Are Required for Cds1 Activation after Fork Arrest—Fission yeast Hsk1-Dfp1/Him1 kinase plays an essential role in initiation and progression of S-phase (7, 8). In fact, it has been shown that budding yeast Cdc7 kinase is required throughout S-phase for activation of each replication origin (20). Recent studies of Swi1 in fission yeast and Tof1 in budding yeast have shown that they are components of the replisome and are needed to stabilize stalled forks caused by inadequate supplies of deoxyribonucleotide triphosphates (dNTPs) (12, 13). Swi1 forms a replication FPC with a 181-amino acid protein, Swi3, and Swi1-Swi3 FPC plays a crucial role in stabilization of arrested forks (14). Swi1 is required for proficient activation of the replication check-
S-phase was reported in swi\textsubscript{1}/\textit{H9004} and swi\textsubscript{3}/\textit{H9004} cells released from a \textit{cdc10}-mediated G1 arrest (21). Interaction of MCM with FPC or with Mrc1 was reported (23), and we also observed coimmunoprecipitation of MCM subunits with Swi1 and Hsk1 (Fig. 1C and data not shown). Thus, Hsk1-Dfp1/Him1 may not only facilitate initiation of DNA replication through interaction with Swi1 but may also help maintain the integrity of the replication forks during the course of DNA replication through physical interactions with the replication fork machinery and with the FPC. These interactions may facilitate fork stabilization upon encounter of a fork block by inhibiting abortive unwinding of DNA. Although we did not detect apparent mobility shift of Swi1 protein on SDS-PAGE before or after HU treatment (supplemental Fig. S2), further works will be needed to determine whether Swi1 or other FPC components are targets of Hsk1 in a process that stabilizes replication forks.

REFERENCES
1. Masai, H., and Arai, K. (2002) \textit{J. Cell. Physiol.} \textbf{190}, 287–296
2. Sclafani, R. A. (2000) \textit{J. Cell Sci.} \textbf{113}, 2111–2117
3. Takeda, T., Ogino, K., Matsui, E., Chu, M. K., Kumagai, H., Miyake, T., Arai, K., and Masai, H. (1999) \textit{Mol. Cell. Biol.} \textbf{19}, 5535–5547
4. Brown, G. W., and Kelly, T. J. (1999) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{96}, 8443–8448
5. Ogino, K., Takeda, T., Matsui, E., Iiyama, H., Taniyama, C., Arai, K., and Masai, H. (2001) \textit{J. Biol. Chem.} \textbf{276}, 31376–31387
6. Fung, A. D., Ou, I., Bueler, S., and Brown, G. W. (2002) \textit{Mol. Cell. Biol.} \textbf{22}, 4477–4490
7. Snait, H. A., Brown, G. W., and Fordburg, S. L. (2000) \textit{Mol. Cell. Biol.} \textbf{20}, 7922–7932
8. Takeda, T., Ogino, K., Tatebayashi, K., Ikeda, H., Arai, K., and Masai, H. (2001) \textit{Mol. Biol. Cell} \textbf{12}, 1257–1274
9. Alcasabas, A. A., Osborn, A. J., Bachant, J., Hu, F., Werler, P. J., Bousset, K., Furuya, K., Diffley, J. F., Carr, A. M., and Elledge, S. J. (2001) \textit{Nat. Cell Biol.} \textbf{3}, 958–965
10. Tanaka, K., and Russell, P. (2001) \textit{Nat. Cell Biol.} \textbf{3}, 966–972
11. Dalgaard, J. Z., and Klar, A. J. (2000) \textit{Cell} \textbf{102}, 745–751
12. Ogino, E., Noguchi, C., Du, L.-L., and Russell, P. (2003) \textit{Mol. Cell. Biol.} \textbf{23}, 7861–7874
13. Katou, Y., Kanoh, Y., Bando, M., Noguchi, H., Tanaka, H., Ashikari, T., Sugimoto, K., and Shirahige, K. (2003) \textit{Nature} \textbf{424}, 1078–1083
14. Noguchi, E., Noguchi, C., McDonald, W. H., Yates, J. R., III, and Russell, P. (2004) \textit{Mol. Cell. Biol.} \textbf{24}, 8342–8355
15. Alfa, C., Fantes, P., Hyams, J., McLeod, M., and Warbrick, E. (1993) \textit{Experiments with Fission Yeast}, Cold Spring Harbor Press, Cold Spring Harbor, NY
16. Moreno, S., Klar, A., and Nurse, P. (1991) \textit{Methods Enzymol.} \textbf{194}, 795–823
17. Du, L.-L., Nakamura, T., Moser, B. A., and Russell, P. (2003) \textit{Mol. Cell. Biol.} \textbf{23}, 6150–6158
18. Ostermann, K., Lorentz, A., and Schmidt, H. (1993) \textit{Nucleic Acids Res.} \textbf{21}, 5940–5944
19. Mortensen, U. H., Bendixen, C., Sunjevaric, I., and Rothstein, R. (1996) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{93}, 10729–10734
20. Bousset, K., and Diffley, J. F. (1998) \textit{Genes Dev.} \textbf{15}, 480–490
21. Sommariva, E., Pelley, T. K., Karahan, N., Kumar, S., Huberman, J. A., and Dalgaard, J. Z. (2005) \textit{Mol. Cell. Biol.} \textbf{25}, 2770–2784
22. Masai, H., Miyake, T., and Arai, K. (1995) \textit{EMBO J.} \textbf{14}, 3094–3104
23. Nedelcheva, M. N., Roguev, A., Dolapchiev, L. B., Shevchenko, A., Taskov, H. B., Shevchenko, A., Stewart, A. F., and Stoynov, S. S. (2005) \textit{J. Mol. Biol.} \textbf{347}, 509–521
24. Grewal, S. I., and Klar, A. J. (1997) \textit{Genetics} \textbf{146}, 1221–1238
25. Maundrell, K. (1990) \textit{J. Biol. Chem.} \textbf{265}, 10857–10864