The double-bromodomain proteins Bdf1 and Bdf2 modulate chromatin structure to regulate S-phase stress response in *Schizosaccharomyces pombe*

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ABSTRACT

Bromodomain proteins bind acetylated histones to regulate transcription. Emerging evidence suggests that histone acetylation plays an important role in DNA replication and repair, although its precise mechanisms are not well understood. Here we report studies of two double bromodomain-containing proteins, Bdf1 and Bdf2, in fission yeast. Loss of Bdf1 or Bdf2 led to a reduction in the level of histone H4 acetylation. Both \textit{bdf1}\textDelta and \textit{bdf2}\textDelta cells showed sensitivity to DNA damaging agents including camptothecin that cause replication fork breakage. Consistently, Bdf1 and Bdf2 were important for recovery of broken replication forks and suppression of DNA damage. Surprisingly, deletion of \textit{bdf1} or \textit{bdf2} partially suppressed sensitivity of various checkpoint mutants including \textit{swi1}\textDelta, \textit{mrc1}\textDelta, \textit{cds1}\textDelta, \textit{crb2}\textDelta, \textit{chk1}\textDelta, and \textit{rad3}\textDelta, to hydroxyurea, a compound that stalls replication forks and activates the Cds1-dependent S-phase checkpoint. This suppression was not due to reactivation of Cds1. Instead, we found that \textit{bdf2} deletion alleviates DNA damage accumulation caused by defects in the DNA replication checkpoint. We also show that hydroxyurea sensitivity of \textit{mrc1}\textDelta and \textit{swi1}\textDelta was suppressed by mutations in histone H4 acetyltransferase subunits or histone H4. These results suggest that the double bromodomain-containing proteins modulate chromatin structure to coordinate DNA replication and S-phase stress response.
INTRODUCTION

Eukaryotic DNA is packaged within the nucleus through its association with histones, forming nucleosomes (KORNBERG 1974; KORNBERG and LORCH 1999; LUGER et al. 1997). Histones are subject to various post-translational modifications, including acetylation, methylation, phosphorylation and ubiquitination (KOUZARIDES 2007; Li et al. 2007), which affect chromatin structures to coordinate most DNA metabolism including transcription, DNA replication and repair programs (ANNUNZIATO 2005; GROTH et al. 2007; HENIKOFF et al. 2004). Such modifications create a “histone code” that is proposed to generate binding sites for recruitment of non-histone chromatin associated proteins (JENUWEIN and ALLIS 2001; STRAHL and ALLIS 2000; TURNER 2000). These proteins often contain protein recognition modules, such as the bromodomain and chromodomain, which bind acetylated lysines and methylated lysines, respectively, and control gene expression (LEE and WORKMAN 2007). However, how these proteins also affect DNA replication and repair programs is not well understood.

The bromodomain is an evolutionarily conserved motif found in various chromatin-associated proteins, and has been shown to bind acetyl-lysines in N-terminal tails of histone H3 and H4 in vitro (SANCHEZ and ZHOU 2009; ZENG and ZHOU 2002). Among other bromodomain containing proteins, Bdf1 in *Saccharomyces cerevisiae* contains two bromodomains and is localized throughout the genome at sites enriched with acetylated histones (DURANT and PUGH 2006; KURDISTANI et al. 2004). Bdf1 appears to be important during early steps of transcription because it recruits the TFIID general transcription factor to acetylated histones (HUISINGA and PUGH 2004; MATANGKASOMBUT et al. 2000). Further studies show that another double bromodomain containing protein, Bdf2, shares functions with Bdf1 and is localized on chromatin at distinct locations from Bdf1 (DURANT and PUGH 2007). However, the deletion of the bdf1 gene leads to accumulation of the Bdf2 protein in Bdf1-vacated sites, indicating that Bdf1 and Bdf2 have redundant functions (DURANT and PUGH 2007). Bdf1 has TFIID independent roles. For example, Bdf1 has been shown to be a component of the SWR1-C chromatin-remodeling complex, which is responsible to facilitate an exchange of histone H2A
with H2AZ, a histone H2A variant (KOBOR et al. 2004; KROGAN et al. 2003; MIZUGUCHI et al. 2004). Importantly, the recruitment of Bdf1 and H2AZ is regulated by acetylation of histone H4, which is controlled by the NuA4 histone acetyltransferase (BABIARZ et al. 2006; BONENFANT et al. 2006; BRUCE et al. 2005; KEOGH et al. 2006; KIM et al. 2009; KURDISTANI et al. 2004; MILLAR et al. 2006; RAISNER et al. 2005; REN and GOROVSKY 2001; ZHANG et al. 2005). In the current model, NuA4-dependent acetylation of H4 results in recruitment of Bdf1, which in turn recruits SWR1-C to facilitate deposition of H2AZ and subsequent acetylation of H2AZ (LU et al. 2009). H2AZ has also a role in preservation of genomic integrity (AHMED et al. 2007; KEOGH et al. 2006; KIM et al. 2009; KOBOR et al. 2004; KROGAN et al. 2004; MIZUGUCHI et al. 2004; RANGASAMY et al. 2004; ZHANG et al. 2004). In yeast, mutations in H2AZ or SWR1-C render cells sensitive to genotoxic agents, including hydroxyurea (HU) and methyl methanesulfonate (MMS) (KEOGH et al. 2006; KOBOR et al. 2004; KROGAN et al. 2004; MIZUGUCHI et al. 2004). It is also known that bdf1Δ cells are sensitive to MMS in budding yeast (CHANG et al. 2002; CHUA and ROEDER 1995), suggesting the importance of the double bromodomain proteins in DNA damage response. In budding yeast, NuA4 consists of a number of subunits including the MYST family protein Esa1 (the catalytic subunit) (DOYON and COTE 2004). Mutations in NuA4 subunits including Esa1, Vid21/Eaf1, Eaf2, Yaf9, Epl1, and Yng2 render cells sensitive to HU and MMS (AUGER et al. 2008; BIRD et al. 2002; CHOY and KRON 2002; GAME et al. 2005; GOMEZ et al. 2008; KOBOR et al. 2004; ZHANG et al. 2004). Thus, histone acetylation and H2AZ regulation play important roles in DNA damage response.

In response to DNA damage or stalled replication forks, eukaryotic cells activate the cell cycle checkpoint to allow time for DNA repair. Central to this system are protein kinases such as human ATM (Ataxia telangiectasia mutated) and ATR (Ataxia telangiectasia and Rad3 related), fission yeast Rad3, and budding yeast Mec1 (ABRAHAM 2001; BODDY and RUSSELL 2001; NYBERG et al. 2002; OSBORN et al. 2002; ROUSE and JACKSON 2002). These protein kinases are required for all known DNA structure checkpoint pathways and for activation of downstream effector kinases by phosphorylation. In fission yeast, Rad3 activates downstream
kinases Cds1 and Chk1 to promote phosphorylation of Cdc25, thereby inhibiting the Cdc2 (Cdk1) kinase to arrest the cell cycle and to facilitate DNA repair and recombination pathways (BODDY and RUSSELL 2001; CARR 2002; NYBERG et al. 2002). Full activation of Cds1 requires additional factors to be recruited to stalled replication forks. These factors include Mrc1 and Swi1, both of which are known to be integral components of the replisome complex (MATSUMOTO et al. 2005; NOGUCHI et al. 2003; NOGUCHI et al. 2004; TANAKA and RUSSELL 2001). Consistently, fission yeast cells deleted of mrc1 or swi1 display strong sensitivity to HU, a ribonucleotide reductase inhibitor, which leads to arrest of replisome progression (NOGUCHI et al. 2003; TANAKA and RUSSELL 2001). In contrast, when cells experience DNA damage outside of S-phase, a damage checkpoint mediator Crb2 is recruited to the site of DNA damage to promote the Chk1-dependent DNA damage checkpoint (DU 2003; NYBERG et al. 2002; O’CONNELL et al. 2000).

It is widely understood that multiple factors are involved in the regulation of checkpoint activities and DNA damage response during DNA replication. In this report, we identified two double-bromodomain factors, Bdf1 and Bdf2, as fine tuners of S-phase checkpoints in fission yeast. We show that Bdf1 and Bdf2 are involved in efficient acetylation of histone H4, tolerance due to DNA damaging agents, and recovery of collapsed DNA replication forks. Our results suggest that Bdf1 and Bdf2 modulate chromatin structures to regulate S-phase stress response. Although previous studies have reported the role of Bdf1 and Bdf2 in transcriptional regulation in budding yeast, how these proteins function in response to replication stress is unknown. Emerging evidence suggests that chromatin modifying enzymes and chromatin remodeling factors are also involved in DNA replication and repair programs. Thus, our results contribute to the understanding of how cells coordinate chromatin structures with DNA replication stress responses.

MATERIALS AND METHODS
General Techniques

The methods used for genetic and biochemical analyses of fission yeast have been described previously (ALFA 1993; MORENO et al. 1991). PCR amplification of DNA was done using EXtaq DNA polymerase (TaKaRa, Ohtsu, Japan). Accurate PCR reactions were confirmed by DNA sequencing analyses. Oligonucleotide primers used in this study are listed in Supplementary Table S1. Western blotting, Cds1 kinase assay, drug sensitivity assays, and pulsed-filed gel electrophoresis (PFGE) were performed as described in our earlier studies (ANSBACH et al. 2008; NOGUCHI et al. 2009). Detection of Rad22-YFP and 2YFP-Crb2 foci has been described previously (ANSBACH et al. 2008; DU 2003; NOGUCHI et al. 2009). For Western blotting, FLAG fusion proteins were probed with the anti-FLAG M2 monoclonal antibody (Sigma-Aldrich, St. Louis, MO). TAT-1 (WOODS et al. 1989) was used to detect tubulin. Microscopic analyses of yellow fluorescent proteins (YFP) were performed using Olympus PROVIS AX70 microscope equipped with a Retiga EXi camera (QImaging, Surrey, BC, Canada). Images were acquired with Ivision software (BioVision Technologies, Exton, PA).

Detection of histone H4 acetylation

Crude histones were prepared as described previously (PIDOUX et al. 2004). Five µg of histones were separated on 5% SDS-polyacrylamide gels and electroblotted onto polyvinylidene difluoride membranes. The blots were blocked with 3% BSA in Tris-buffered saline supplemented with 0.1% Tween 20 and incubated with rabbit antibodies against histone H4 acetylated at position K5, K8, K12 and K16 (06-866, Milipore, Billerica, MA). The anti-histone H4 antibody (ab10158, Abcam, Cambridge, MA) was used to detect total levels of histone H4. Quantification of histone H4 acetylation was performed using EZ Quant-Gel 2.1 software from (EZQuant LTD, Tel-Aviv, Israel), normalizing the H4 acetylation level to the total histone H4 amount in each strain. Relative acetylation of histone H4 was calculated by setting wild-type level to 1.
**Plasmids and site-directed mutagenesis**

Genomic DNA was isolated from *S. pombe* cells containing the *mst1-5FLAG:hphMX6* gene. The 2.7 kb genomic fragment including the *mst1* 3'-terminal coding region (615 bp) fused to 5FLAG, *hphMX6* and the *mst1* 3'-untranslated region (239 bp) was amplified by PCR from the genomic DNA preparation, and subsequently cloned into the XbaI/EcoRV site of pBluescript II TKS (+), to generate pTKS-mst1CT-5FLAG-hphMX6. The *mst1-L344S* mutation was introduced by Kunkel site-directed mutagenesis (KUNKEL 1985) in pTKS-mst1CT-5FLAG-hphMX6. The 2.7 kb XbaI-EcoRV *mst1CT-L344S-5FLAG-hphMX6* fragment from the mutagenized plasmid was used to generate the *mst1-L344S* strain as described below. The 3.1 kb Genomic DNA fragment containing the *hht2* (histone H3.2) and *hhf2* (histone H4.2) genes were amplified form genomic DNA isolated from wild-type *S. pombe* cells, cloned into the KpnI/SacI site of pBluescript II SK (+), to generate pBluescript II SK (+)-hht2-hhf2. The 1.1kb *natMX6* fragment was amplified from pCR2.1-nat (SATO et al. 2005) and inserted into the Ndel site of pBluescript II SK (+)-hht2-hhf2, to generate pBluescript II SK (+)-hht2-hhf2-natMX6. The *hhf2-KR* (K5/8/12/16R) mutation was introduced by Kunkel site-directed mutagenesis (KUNKEL 1985) in pBluescript II SK (+)-hht2-hhf2-natMX6. The 4.2 kb EcoRI-SacI *hht2-hhf2-KR-natMX6* fragment from the mutagenized plasmid was used to generate the *hhf2-KR* strain as described below.

**S. pombe strains**

The *S. pombe* strains used in this study were constructed using standard techniques (ALFA 1993), and their genotypes are listed in Supplementary Table S2. *bdf1Δ* (*bdf1::kanMX6*) was generated by a two-step PCR method (KRAWCHUK and WAHLS 1999) using primers P824, P825, P828, and P829 to replace the *bdf1* open reading frame with the *kanMX6* gene. *bdf2Δ* (*bdf2::hphMX6*) was generated by a two-step PCR method (KRAWCHUK and WAHLS 1999) using primers P831, P832, P835, and P836 to replace the *bdf2* open reading frame with the *hphMX6* gene. *bdf1Δ* (*bdf1::hphMX6*) and *bdf2Δ* (*bdf2::kanMX6*) were generated by a one-step marker switch method (SATO et al. 2005) using the *bdf1::kanMX6* and *bdf2::hphMX6* strains, respectively. A two-step
PCR method (Krawchuk and Wahls 1999; Noguchi et al. 2008) was used to construct a 5xFLAG tag at the C terminus of bdf1, bdf2, and mst1, generating bdf1-5FLAG (bdf1-5FLAG:hpMX6), bdf2-5FLAG (bdf2-5FLAG:hpMX6), and mst1-5FLAG (mst1-5FLAG:hpMX6), respectively. Primers used to construct these 5xFLAG tagged strains are P826, P827, P828, P829, P833, P834, P835, P836, P959, P960, 961 and P962.

To generate the mst1-L344S mutant (mst1-L344S-5FLAG:hpMX6), the wild-type mst1+ gene was replaced with mst1CT-L344S-5FLAG-hpMX6 at the mst1 locus by a standard transformation method. hht2/hhf2Δ (hht2/hhf2::kanMX6) was generated by a two-step PCR method (Krawchuk and Wahls 1999) using primers hhf2-T1, hhf123-B4, hhf123-T5, and hhf2-B6 to replace the hht2 and hhf2 open reading frames with the kanMX6 gene. To generate the hhf2-KR mutant (hhf2-KR-natMX6) and hhf2-natMX6 strain, the hht2/hhf2::kanMX6 gene was replaced with hht2-hhf2-KR-natMX6 and hht2-hhf2-natMX6 at the hht2/hhf2 locus, respectively.

nmt81-vid21 (nmt81-3FLAG-vid21:kanMX6) was generated by a two-step PCR method (Krawchuk and Wahls 1999; Noguchi et al. 2008) using primers P933, P934, P935, and P936 to construct a 3xFLAG tag at the N-terminus of vid21 and to replace the vid21 promoter with the nmt81 promoter. To visualize Rad22-YFP in various mutants, pJK210-Rad22YFP-CT (Rapp et al. 2010) was integrated at the rad22 locus of the mutant strains.

Mutations and epitope-tagged genes have previously been described for swi1Δ (swi1::kanMX6) (Noguchi et al. 2003), cds1Δ(cds1::kanMX6), chk1Δ(chk1::kanMX6), rad3Δ (rad3::kanMX4) (Ansbach et al. 2008), crb2Δ(crb2::ura4+) (Du 2003), mrc1Δ(mrc1::kanMX6) (Tanaka and Russell 2001), cdc25-22 (Fantes 1979), and 2YFP-crb2 (2xYFP-crb2-leu1+) (Du 2003).

RESULTS

Inactivation of Bdf1 or Bdf2 suppresses hydroxyurea sensitivity of various checkpoint mutants
We have previously shown that Swi1 is involved in efficient activation of the Cds1 checkpoint kinase, which is an essential component of the S-phase checkpoint (NOGUCHI et al. 2003). Consistently, swi1Δ cells were reported to show sensitivity to HU (NOGUCHI et al. 2003), a compound that inhibits ribonucleotide reductase, thereby arresting replication fork progression and activating the Cds1-dependent checkpoint (BODDY et al. 1998; LINDSAY et al. 1998). To identify additional pathways involved in the regulation of the S-phase stress response, we performed genetic crosses between a swi1Δ strain and various strains lacking proteins implicated in checkpoint response, DNA replication, DNA repair, and/or chromatin regulation.

Among many interesting genetic interactions with swi1Δ, we found that deletion of SPAC631.02 (referred to as bdf2; see below) partially rescued HU sensitivity of swi1Δ cells (Figure 1A). S. pombe SPAC631.02 encodes a 769 amino-acid protein that contains two bromodomains (the double-bromodomain) and is closely related to S. cerevisiae Bdf1 and Bdf2, which also possess the double-bromodomain and are known to be involved in gene regulation (DURANT and PUGH 2007; MATANGKASOMBUT and BURATOWSKI 2003). S. pombe also has another double-bromodomain containing protein, which is encoded by the SPCC1450.02 gene. This gene is annotated to bdf1+ at GeneDB (Sanger Institute), and its 578 amino-acid gene product, Bdf1, has been indentified as a component of the SWR1-C complex in S. pombe (SHEVCHENKO et al. 2008). Therefore, we referred to SPAC631.02 as S. pombe Bdf2. Intriguingly, bdf2 deletion also suppressed HU sensitivity of various cell cycle checkpoint mutants including mrc1Δ, crb2Δ (Figure 1B), chk1Δ, cds1Δ, rad3Δ (Figure 1C), and cds1Δ chk1Δ (Figure 1D), suggesting that Bdf2 play an important role in DNA replication stress responses.

EMBOSS Pairwise Alignment analysis (European Bioinformatics Institute) revealed that S. pombe Bdf1 (Bdf1sp) had 44.9% similarity to S. cerevisiae Bdf1 (Bdf1sc) and 43.6% similarity to S. cerevisiae Bdf2 (Bdf2sc), while S. pombe Bdf2 (Bdf2sp) had 37.7% similarity to Bdf1sc and 34.4% similarity to Bdf2sc (Figure S1). In addition, S. pombe Bdf1 and Bdf2 were similar each other (48.3% similarity), as is the case for the S. cerevisiae homologues (48.2%
similarity between Bdf1sc and Bdf2sc). Furthermore, these four proteins were closely related to human Brd2 that also has the double-bromodomain (Figure S1). These results suggest that these double-bromodomain factors have diverged from a single ancestor protein during evolution and that these proteins have redundant functions.

*bdf1Δ* and *bdf2Δ* cells were viable and showed no significant growth defect in YES medium at normal growth temperatures (Figure 1A), although they show significant sensitivity to some S-phase stressing agents as described later. However, none of *bdf1Δ bdf2Δ* double mutant strains grew after tetrad analyses (Figure S2). Therefore, as in the case of *S. cerevisiae* results (Matangkasombut et al. 2000), our data established that *bdf1Δ* is synthetically lethal with *bdf2Δ* in fission yeast, suggesting that Bdf1 and Bdf2 have redundant cellular functions.

To determine whether *bdf1* deletion also suppresses HU sensitivity of checkpoint mutants, we also examined genetic interaction between *bdf1Δ* and checkpoint mutations in HU sensitivity assays. Although the level of suppression was weaker, *bdf1Δ* cells reproducibly suppressed HU sensitivity of *swi1Δ* (Figure 1A), *mrc1Δ*, and *crb2Δ* (Figure S3A), and *chk1Δ* (Figure S3B) cells. Thus we concluded that Bdf1 and Bdf2 have a negative role in the cellular survival of HU when cell cycle checkpoints are compromised.

*bdf2Δ* suppresses HU sensitivity of checkpoint mutants in a manner independent of the replication checkpoint kinase Cds1

HU activates Cds1 in order for the cell to stabilize replication forks (Boddy et al. 1998; Lindsay et al. 1998; Noguchi et al. 2003). It has been shown that *mrc1Δ* cells are defective for Cds1 activity in response to HU (Tanaka and Russell 2001). Therefore, it was possible that *bdf2* deletion might cause elevation of Cds1 activity in *mrc1Δ* cells, leading to the suppression of HU sensitivity. However, *bdf2* deletion also suppressed HU sensitivity of *cds1Δ* cell (Figure 1C), excluding this possibility. Indeed, the defect of HU-dependent Cds1 activation in *mrc1Δ* cells was not recovered in *mrc1Δ bdf1Δ* or *mrc1Δ bdf2Δ* cells (Figure 2). In addition, *bdf1Δ* and *bdf2Δ* cells had Cds1 activity similar to wild-type cells (Figure 2). Thus, *bdf1Δ*- or *bdf2Δ*-dependent
suppression of HU sensitivity of checkpoint mutants is not due to re-activation of Cds1.

**Role of Bdf1 and Bdf2 in S-phase DNA damage tolerance**

To further understand the role of Bdf1 and Bdf2 in the S-phase stress response, we examined sensitivity of \( bdf1\Delta \) and \( bdf2\Delta \) to various genotoxic agents other than HU, including ultraviolet light (UV), methyl methanesulfonate (MMS), camptothecin (CPT), and bleomycin (Bleo) (Figure 3A). UV causes the formation of a variety of DNA lesions and replication fork blockage, while MMS generates alkylation damage on template DNA and stalls replication forks. CPT specifically induces replication fork breakage, leading to double-stranded breaks (DSBs) during S-phase, and the radiomimetic drug, bleomycin also causes DSBs. \( bdf1\Delta \) cells were slightly sensitive to MMS and significantly sensitive to CPT, while there is no apparent growth defect in response to UV or Bleo (Figure 3A). In contrast, \( bdf2\Delta \) cells had sensitivity to UV, CPT and Bleo, while they were not sensitive to MMS (Figure 3A). In addition, \( bdf1\Delta \) cells were sensitive to 10 mM HU, whereas \( bdf2\Delta \) cells grew at the wild-type level in the presence of HU (Figure 1C, Figure S3B). These results suggest that the two double-bromodomain proteins are involved in damage tolerance due to genotoxic agents that induce DNA damage. Our data also suggest that cells utilize Bdf1 or Bdf2 for damage tolerance depending on the type of DNA damage.

Next, we performed epistasis analysis between \( bdf1\Delta \) and checkpoint kinase mutations including \( rad3\Delta \), \( chk1\Delta \), and \( cds1\Delta \) in survival assays to various genotoxic agents (Figure 3B). As shown in Figure 3B, \( bdf1\Delta \ rad3\Delta \) cells were significantly more sensitive to UV, MMS, CPT and Bleo than either single mutant. Rad3 is the master kinase of DNA maintenance checkpoints and known to regulate the downstream Cds1 and Chk1 kinases (BODDY and RUSSELL 2001; CARR 2002; NYBERG et al. 2002), suggesting that \( bdf1\Delta \) cells accumulate DNA damage that must be repaired through checkpoint pathways. Interestingly, deletion of \( bdf1 \) significantly suppressed UV sensitivity of \( chk1\Delta \), and to a lesser extent of \( cds1\Delta \) cells (Figure 3B). In addition, \( bdf1\Delta \) weakly suppressed HU sensitivity of \( chk1\Delta \) cells as described above (Figure S3B). UV and HU are known to cause arrest of replication forks, which in turn activate the S-phase
checkpoint that is dependent primarily on Cds1 and partly on Chk1. Therefore, our findings suggest that Bdf1 might modulate chromatin structures to negatively affect stabilization of stalled replication forks generated by UV or HU. In contrast, \textit{bdf1Δ chk1Δ} and \textit{bdf1Δ cds1Δ} cells were more sensitive to MMS, CPT and Bleo when compared to corresponding single mutants (Figure 3B). There was also synergistic genetic interaction between \textit{bdf1Δ} and \textit{mrc1Δ} in CPT sensitivity assay (Figure S3C). MMS, CPT and Bleo can cause DSBs, suggesting that Bdf1 might be important for efficient repair of DSBs when checkpoints are abrogated.

Next, we performed epistasis analysis between \textit{bdf2Δ} and checkpoint mutations (Figure 3C). There is no significant genetic interaction between \textit{bdf2Δ} and \textit{rad3Δ} in UV and MMS. However, \textit{bdf2Δ rad3Δ} cells were more sensitive to CPT and Bleo than either single mutant. There was also synergistic genetic interaction between \textit{bdf2Δ} and \textit{chk1Δ} in sensitivity to UV and CPT, and \textit{bdf2Δ cds1Δ} cells were more sensitive to UV, MMS, CPT and Bleo. In addition, \textit{bdf2Δ mrc1Δ} cells were much more sensitive to CPT than either single mutant (Figure S3D). These results suggest the importance of Bdf2 in S-phase DNA damage responses. However, as described above, \textit{bdf2Δ} significantly suppressed HU sensitivity of \textit{swi1Δ}, \textit{mrc1Δ}, \textit{crb2Δ}, \textit{cds1Δ}, \textit{chk1Δ} and \textit{rad3Δ} cells (Figure 1), suggesting a negative role for Bdf2 in stabilization of replication forks. Taken together, our results indicate that Bdf1 and Bdf2 play a significant role in S-phase stress and DNA damage responses.

**Bdf1 and Bdf2 are involved in recovery of replication after fork breakage**

CPT sensitivity of \textit{bdf1Δ} and \textit{bdf2Δ} cells suggests the roles of the double-bromodomain proteins in recovery of broken replication forks during DNA replication. Therefore, we analyzed chromosomal DNA isolated from \textit{S. pombe} mutant cells and investigated the effect of \textit{bdf1} and \textit{bdf2} deletion on replication fork recovery after fork breakage induced by CPT (Figure 4A). Chromosome samples were prepared before and at 3 h after CPT treatment, and at different time points during recovery after the removal of CPT. These chromosomes were then analyzed by pulsed-field gel electrophoresis (PFGE), which allows only a fully replicated chromosomes to
appear in the gel. Intact chromosomes from exponentially growing cells (Log) in wild-type, \textit{bdf1}Δ and \textit{bdf2}Δ strains migrated into the gel. CPT treatment causes replication fork breakage, leading to the reduction in the amount of intact chromosomes that migrated into the gel in all strains. When cells were returned into fresh medium without CPT, intact chromosomes from wild-type cells re-appeared in the gel at 1.5 h after CPT removal due to the completion of DNA replication. However, far less chromosomes from both \textit{bdf1}Δ and \textit{bdf2}Δ cells migrated into the gel at 1.5 h and 3 h during recovery (Figure 4A). In contrast, we did not observe significant defects in recovery of HU-treated chromosomes (Figure 4B). Thus our data is consistent with the notion that Bdf1 and Bdf2 are required for the recovery of DNA replication after fork breakage. We have noticed that \textit{bdf1}Δ cells used in this experiment harbor a longer chromosome III that contains ribosomal DNA repeats in \textit{S. pombe}. Although it is not the focus of this study, this phenotype is probably due to an increased mitotic recombination rate at the rDNA repeats, which may be caused by genomic instability in \textit{bdf1}Δ cells.

To further evaluate the roles of Bdf1 and Bdf2 in DNA damage response during S-phase, we examined the cellular amounts of Bdf1 and Bdf2 during cell cycle progression (Figure 4C). A \textit{cdc25-22} temperature sensitive strain was engineered to express Bdf1-5FLAG or Bdf2-5FLAG from its own promoter. These strains showed no significant changes in DNA damaging sensitivity (data not shown), indicating that Bdf1-5FLAG and Bdf2-5FLAG are functional. The \textit{cdc25-22} allele was used to synchronize cells at the G2-M boundary at the restrictive temperature, and cells were released into the cell cycle at the permissive temperature. Although the level of Bdf1 stayed similar during the cell cycle, we observed a significant oscillation in the cellular amounts of Bdf2 (Figure 4C). After the cells were released into the cell cycle, Bdf2 levels quickly dropped down at 30 min. However, the Bdf2 level rose as the septation index increased (60 to 90 min), and dropped down again (150 to 180 min). The second increase in the Bdf2 amount also coincided with the second increase of the septation index (Figure 4C). In \textit{S. pombe}, septation emergence coincides with the onset of S-phase. Therefore, our results suggest that Bdf2 levels increase during S- and G2-phases and decrease in M- and G1-phases, which is
consistent with the notion that Bdf2 is involved in the S-phase DNA damage response.

**Bdf1 and Bdf2 are required to prevent DNA damage**

Next, we monitored the formation of Rad22-YFP DNA repair foci in the absence of genotoxic agents (Figure 5A). Rad22 is a homolog of budding yeast Rad52 and has been shown to bind ssDNA at the site of DNA damage (LISBY et al. 2003; LISBY et al. 2001). Depletion of Swi1 or Swi3 was shown to be associated with replication fork abnormalities, resulting in the strong accumulation of spontaneous Rad22-YFP foci during unperturbed S-phase (NOGUCHI et al. 2003; NOGUCHI et al. 2004). We observed significantly elevated levels of Rad22-YFP foci formation in bdf1Δ and bdf2Δ mutants (Figure 5A). In addition, we have also observed a significant increase in the number of 2YFP-Crb2 foci when bdf1 or bdf2 was deleted (Figure 5B). Crb2 is also known to be recruited to sites of DNA damage (DU 2003). Therefore, our data suggest that bdf1Δ and bdf2Δ cells experience DNA damage even in the absence of genotoxic agents. In addition, the results suggest that damaged DNA in bdf1Δ and bdf2Δ cells were still able to recruit DNA damage response proteins.

**bdf2 deletion alleviates DNA damage accumulation in mrc1Δ cells.**

Aforementioned results suggest that Bdf2 plays a positive role during DNA damage response. However, we also found that Bdf2 has a toxic effect when checkpoint mutants were treated with HU, a compound that stalls replication forks (Figure 1). Therefore, it is likely that Bdf2 may function as a fine tuner during replication stress responses. Interestingly, accumulation of DNA damage in mrc1Δ cells was significantly alleviated by bdf2 deletion (Figure 5C). As shown in Figure 5C, mrc1Δ cells displayed a dramatic accumulation of Rad22-YFP foci as expected from the role of Mrc1 in replication fork stabilization. However, mrc1Δ bdf2Δ cells contained much less Rad22-YFP foci compared to mrc1Δ cells. It should also be noted that the number of nuclei with multiple Rad22-YFP foci was greatly reduced in bdf2Δ mrc1Δ cells when compared to mrc1Δ cells (Figure 5C, 2 or more foci). Since Mrc1 is known to be involved in fork stabilization
our results suggest that Bdf2 has a toxic effect when cells contain unstable replication forks. To further test this possibility, we investigated chromosomal DNA isolated from mrc1Δ, mrc1Δ bdf1Δ and mrc1Δ bdf2Δ cells (Figure 5D). These cells were treated with HU for 3 h and released into fresh medium. As expected, mrc1Δ cells showed accumulation of fragmented DNA and were unable to resume DNA replication after fork arrest provoked by HU. However, we observed a great reduction in the level of fragmented chromosomes in mrc1Δ bdf2Δ cells and a mild reduction in mrc1Δ bdf1Δ cells (Figure 5D). Consistently, bdf2 deletion significantly suppressed HU sensitivity of various replication checkpoint mutants including mrc1Δ (Figure 1B). Taken together, our data are consistent with the notion that Bdf2 plays a negative role when cells contain stalled replication forks, while Bdf2 contributes to the recovery of DNA damage during normal S-phase.

**Reduction in the level histone acetylation is involved in the suppression of HU sensitivity of mrc1Δ and swi1Δ cells**

Our findings suggest that the double-bromodomain proteins function as a fine tuner of S-phase stress response. The bromodomain is thought to have affinity with acetylated histones (SANCHEZ and ZHOU 2009; ZENG and ZHOU 2002). Indeed, in *S. cerevisiae*, Bdf1 binds acetylated histone H3 and H4 to stabilize them in vitro (MATANGKASOMBUT and BURATOWSKI 2003; PAMBLANCO *et al.* 2001). Therefore, we examined the levels of acetylated histone H4 in bdf1Δ and bdf2Δ cells in *S. pombe*. As shown in Figure 6A, the level of acetylated histone H4 was greatly reduced in bdf2Δ and moderately reduced in bdf1Δ.

The defect in proper histone H4 acetylation in bdf2Δ cells promoted us to test whether reduction of H4 acetylation could also suppress HU sensitivity of checkpoint mutant cells. For this purpose, we generated *S. pombe* mutants defective for the NuA4 acetyltransferase, which is known to acetylate chromatin-bound histone H4. It has been shown that *S. pombe* contains homologs of *S. cerevisiae* NuA4 subunits including Mst1 (the catalytic subunit) and Vid21 (a
regulatory subunit) (GOMEZ et al. 2008; SHEVCHENKO et al. 2008). To downregulate Mst1 activity, we constructed a temperature sensitive mutant of mst1 by introducing the genomic L344S mutation (mst1-L344S). In addition, we replaced the vid21 promoter with the thiamine-repressible nmt81 promoter to control the level of Vid21 expression (nmt81-vid21) (Figure S4). Mst1 was inhibited by shifting the culture to a restrictive temperature (35˚C), while Vid21 was downregulated by the addition of thiamine (B1) to the growth medium. As expected, we observed a strong reduction in levels of histone H4 acetylation in mst1-L344S cells at 35˚C and in nmt81-vid21 cells in the presence of an excess amount of thiamine (B1) (Figure 6A). Similar but a weaker reduction in H4 acetylation was also observed in mst1-L344S cells at a permissive temperature (25˚C) and in nmt81-vid21 cells in normal condition (Figure 6A). Importantly, mst1-L344S mutations significantly suppressed HU sensitivity of mrc1Δ (Figure 6B) and swi1Δ cells (Figure 6C). This experiment was done at 25˚C since mst1-L344S cells show a growth defect at a restrictive temperature (GOMEZ et al. 2008). However, as described above, H4 acetylation is significantly reduced in mst1-L344S cells at 25˚C (Figure 6A), suggesting that histone H4 acetylation has negative impact on replication fork stabilization in the absence of Mrc1 or Swi1. Similarly, nmt81-vid21 also suppressed HU sensitivity of mrc1Δ (Figure 6B) and swi1Δ cells (Figure 6C), strengthening the idea that histone H4 regulation plays an important role in replication fork stabilization.

To further test this possibility, we introduced genomic mutations in hhf2, one of three histone H4 genes in S. pombe. Since NuA4 acetylates lysine residues at positions 5, 8, 12 and 16 within the N-terminal domain of nucleosomal histone H4 (ALLARD et al. 1999; CLARKE et al. 1999; REID et al. 2000; SMITH et al. 1998), we have changed these four residues to arginines, which cannot be acetylated (hhf2-KR). Intriguingly, hhf2-KR mutations significantly suppressed HU sensitivity of mrc1Δ (Figure 6B, lower panels). Interestingly, swi1Δ hhf2-KR double mutants displayed a slow growth phenotype in the absence of HU probably due to increased genomic instability. However, swi1Δ hhf2-KR cells were significantly less sensitive to a high does of HU (5 mM) than swi1Δ cells (Figure 6C, bottom panels). Thus, we concluded that histone H4
acetylation has negative role in replication fork stabilization in the absence of Mrc1 or Swi1. In addition, our data are consistent with the notion that histone H4 acetylation is an important player in the regulation of S-phase stress responses.

**DISCUSSION**

In this report, we have described genetic interactions between double bromodomain factors and checkpoint genes. Interestingly, \textit{bdf2} or \textit{bdf1} deletion partially suppressed HU sensitivity of various checkpoint mutants. These results suggest that the activities of the double bromodomain proteins are toxic when cells experience problems in stabilizing stalled replication forks. Our investigation suggests that \textit{bdf2} deletion confers survival advantages on checkpoint mutants in the presence of HU. These advantages include alleviation of DNA damage in response to HU. Considering that \textit{bdf2} deletion results in reduction of H4 acetylation, and that H4 acetylation defects also suppress the HU sensitivity of \textit{mrc1}\textsuperscript{\Delta} and \textit{swi1}\textsuperscript{\Delta} cells, our data suggest that double bromodomain factors modulate chromatin structures to affect a variety of transactions that take place on chromatin.

*Role of double-bromodomain factors in coupling DNA replication and transcription*

In budding yeast, the replication checkpoint is reported to repress transcription of tRNA genes to prevent fork collapse at these sites (Nguyen et al. 2010). The study also showed that \textit{mrc1}\textsuperscript{\Delta} cells have increased level of tRNA transcription (Nguyen et al. 2010). Therefore, it is possible that accumulation of Rad22-YFP in \textit{mrc1}\textsuperscript{\Delta} cell is in part due to DNA damage at tRNA loci scattered throughout the genome. Interestingly, aside from its role in recruiting TFIID, Bdf1 is known to have a TFIID-independent role in euchromatin maintenance and antisilencing of genes located at heterochromatin-euchromatin boundaries (Ladurner et al. 2003). It has also been reported that tRNA genes contribute to the boundary functions (Donze and Kamakaka 2001). Therefore, it is possible that Bdf1 and/or Bdf2 are also involved in the elevated tRNA transcription in \textit{mrc1}\textsuperscript{\Delta} cells. Such a transcriptional upregulation would be toxic because the replication fork can collide
with the large transcription complex, leading to fork collapse in mrc1Δ cells. Consistently, we observed a significant reduction of DNA damage accumulation in bdf2Δ mrc1Δ cells when compared to mrc1Δ cells (Figure 5C and 5D). Another interesting mechanism at tRNA loci may involve Swi1, a component of the replication fork protection complex (Noguchi et al. 2004). Rozenzhak et al. reported an increase in ATR-dependent histone H2A phosphorylation at tRNA loci in fission yeast swi1Δ cells (Rozenzhak et al. 2010). This result is consistent with the notion that there is increased recombination activity possibly due to collisions between the replisome and transcription machinery at tRNA loci (Pryce et al. 2009). Therefore, it is possible that Mrc1 and Swi1 prevent chromosome breakage at tRNA loci by controlling tRNA transcription and smooth passage of replication forks, respectively (Figure 7).

It is also important to note that bdf2Δ cells have more DNA damage than wild-type cells (Figure 5A and 5B). Therefore, Bdf2 can function both as a damage inducer and preventer depending on the cellular situation. In wild-type cells, Bdf2 may be required for DNA repair and/or for preventing DNA damage. Indeed, bdf2Δ cells displayed significant defects in recovery of replication fork breakage induced by CPT treatment (Figure 4A). It is possible that Bdf2 modulates chromatin structures to facilitate DNA repair processes (Figure 7). In contrast, Bdf2-dependent transcriptional activation would be a significant impediment for normal replication fork progression especially when transcription levels are elevated in mutants defective for the replication checkpoint (Figure 7). Thus, it is highly possible that double-bromodomain factors are involved in proper coordination of DNA replication with transcription programs.

Role of histone H4 acetylation in replication stress response

It has been reported that NuA4 acetylates histone H4, which in turn recruits double-bromodomain factors to chromatin (Babiarz et al. 2006; Bonenfant et al. 2006; Bruce et al. 2005; Keogh et al. 2006; Kim et al. 2009; Kurdistani et al. 2004; Millar et al. 2006; Raisner et al. 2005; Ren and Gorovsky 2001; Zhang et al. 2005). Interestingly, we observed
significant suppression of HU sensitivity of mrc1Δ and swi1Δ cells by mutations in mst1, vid21 or hhf2 (Figure 6). Mst1 and Vid21 are components of NuA4, and hhf2 encodes histone H4 (Doyon and Cote 2004; Matsumoto and Yanagida 1985). It should be noted that the bromodomain is known to interact with acetylated histones (Sanchez and Zhou 2009; Zeng and Zhou 2002). Therefore, it is possible that the bromodomain factors are partially dissociated from chromatin in NuA4 or H4 mutants, causing reduction of transcription and DNA damage at highly transcribed genome loci in the absence of the replication checkpoint. Interestingly, we observed a significant reduction in histone H4 acetylation in the absence of Bdf1 or Bdf2 (Figure 6A). Considering that the association of bromodomains and acetylated histones stabilizes the acetylation state and promotes chromatin-related activities (Fukuda et al. 2006), it is possible that bromodomain factors facilitate further acetylation of histones for transcriptional regulation. Indeed, in budding yeast, Bdf1 binds hypoacetylated forms of histone H4 at a low level (Matangkasombut and Buratowski 2003; Pamblanco et al. 2001). Therefore, the initial low amount of bromodomains might promote recruitment and/or stabilization of acetyltransferases, which results in hyperacetylation of histones and further recruitment of Bdf1/Bdf2 (Figure 7).

Currently, it remains unclear whether the loss of the bromodomain factors or loss of H4 acetylation primarily regulates S-phase stress response. However, our present results show a strong phenotypic correlation between bdf1Δ/bdf2Δ and histone H4 acetylation mutants in response to replication stress, suggesting that regulation of histone H4 acetylation plays an important role in coupling replication and transcription processes (Figure 7). Further investigation will clarify the mechanism of the histone acetylation-dependent replication stress response.

**Role of double-bromodomain factors in DNA damage response**

bdf1Δ and bdf2Δ cells displayed DNA damage accumulation, suggesting the role of the double-bromodomain factors in DNA damage response. Importantly, we found that Bdf2 is required for proper recovery of fork breakage after CPT treatment (Figure 4A). We also found
that Bdf2 is upregulated during S-phase (Figure 4C). These results suggest the role of Bdf2 in DNA repair processes during S-phase. In budding yeast, Bdf1 is a component of the SWR1-C chromatin-remodeling complex, which is required to deposit H2AZ onto chromatin (KOBOR et al. 2004; KROGAN et al. 2003; MIZUGUCHI et al. 2004). It has also been reported that Bdf2 contributes to H2AZ deposition (RAISNER et al. 2005; ZHANG et al. 2005). Considering that Bdf1 and Bdf2 have redundant functions and that H2AZ plays an important role in DSB repair (DURANT and PUGH 2007; KEOGH et al. 2006; KOBOR et al. 2004; KROGAN et al. 2004; MIZUGUCHI et al. 2004), bdf2Δ cells may have defects in H2AZ deposition in fission yeast, leading to defects in replication fork recovery. While H2AZ is required for DNA repair, H2AZ sites might be susceptible to the collision of forks. Since H2AZ is promoter-specific and H2AZ-dependent transcription activation accompanies a large chromatin conformational change and recruitment of the large transcription machinery (RAISNER et al. 2005; ZHANG et al. 2005), it is straightforward to suggest that H2AZ sites create a serious problem during DNA replication. Therefore, in the future, it would be interesting to investigate how cells coordinate replication fork progression at the H2AZ site to prevent genetic instability during replication.

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FIGURE LEGENDS

Figure 1. HU sensitivity of checkpoint mutants is partially suppressed by deletion of bdf1 or bdf2. Five-fold serial dilutions of cells of the indicated genotypes were incubated on YES agar medium supplemented with the indicated amounts of HU for 3 to 5 days at 25 or 32°C. Strain numbers and simplified genotypes are shown. Representative images of repeat experiments are shown.

Figure 2. Effects of bdf1 and bdf2 deletion on Cds1 kinase activity. Cells of the indicated genotypes were incubated in YES medium supplemented with 12 mM HU for 0 h (black), 2 h (red) and 4 h (blue) at 30°C. Cell extracts were prepared and protein concentrations equalized prior to immunoprecipitation of Cds1. Kinase activity of immunoprecipitated Cds1 was measured using myelin basic protein (MBP) as a substrate. The radiolabeled MBP was detected after gel electrophoresis (top panel). Then the MBP bands were excised form the gel, and the radioactivity levels (counts per minute, CPM) of MBP were determined in a liquid scintillation counter (bottom panel). Representative results of repeat experiments are shown.

Figure 3. Epistasis analyses between double-bromodomain and checkpoint genes. Five-fold serial dilutions of cells of the indicated genotypes were incubated on YES agar medium supplemented with the indicated amounts of HU, CPT, MMS, or Bleo, for 3 to 5 days at 25 or 32°C. For UV sensitivity assay, five-fold serial dilutions of cells were plated on YES agar medium and exposed to the indicated doses of UV. Strain numbers and simplified genotypes are shown. Representative images of repeat experiments are shown.

Figure 4. Effects of bdf1 and bdf2 deletions on the recovery of broken replication forks. (A, B) Chromosome samples from cells of the indicated genotypes were examined by PFGE. Cells were grown until mid-log phase (Log) and then incubated in the presence of 30 mM CPT (A) or 20 mM HU (B) for 3 h at 30°C. Cells were then washed and returned into fresh medium.
Chromosomal DNA samples were prepared at the indicated times. Representative results from repeat experiments are shown. (C) Cell cycle analysis of the Bdf1-5FLAG and Bdf2-5FLAG proteins. cdc25-22 cells were synchronized at the G2/M boundary by incubation at 36˚C for 3 h and then released into fresh YES medium. Whole cell extracts were prepared at the indicated time. An increase in the septation index indicates the onset of S phase. Western blotting of tubulin was also performed as a loading control. Representative results from repeat experiments are shown.

**Figure 5.** Effects of bdf1 and bdf2 deletions on DNA damage accumulation. (A, B, C). Cells of the indicated mutants were engineered to express Rad22-YFP and grown in YES medium at 25˚C until mid-log phase. The percentages of nuclei with one focus (x), 2 or more foci (y), and at least one focus (x + y) are shown. At least 200 cells were counted for each strain. Error bars correspond to standard deviations obtained from at least three independent experiments. This analysis shows that a significant increase in Rad22-YFP accumulation was observed in bdf1Δ, bdf2Δ and mrc1Δ cells. However, the increase in focus formation in mrc1Δ was alleviated by bdf2 deletion. (D) PFGE analysis of chromosome samples prepared from cells of the indicated genotypes. Cells were grown in the presence of 20 mM HU for 3 h and returned into fresh medium as described in Figure 4B. Representative results from repeat experiments are shown. mrc1Δ bdf2Δ cells have much less fragmented chromosomes than mrc1Δ cells.

**Figure 6.** Mutations affecting H4 acetylation partially suppress HU sensitivity of swi1Δ cells. (A) Crude histone preparations from the indicated strains grown at the indicated temperature were examined by Western blotting using the anti-H4 and anti-acetyl (K5/8/12/16) histone H4 antibodies. Mutations in NuA4 (mst1, vid21) and bromodomain factors results in reduced levels of histone H4 acetylation. Vid21 was downregulated by adding 10 µg/ml of thiamin (+B1). Quantification of H4 acetylation was performed using EZ quant software, normalizing the H4 acetylation level to the total H4 amount in each strain. Relative acetylation of H4 was shown by
setting wild-type level to 1. Representative results of repeat experiments were shown. B1, thiamin. (B, C) Five-fold serial dilutions of cells of the indicated genotypes were incubated on YES agar medium supplemented with the indicated amounts of HU for 3 to 5 days at 25 or 32˚C. To deplete Vid21, cells were incubated in the presence of 10 μg/ml thiamine (+B1; lower panels in B and middle panels in C). Strain numbers and simplified genotypes are shown. Representative images of repeat experiments are shown.

**Figure 7.** Models for the roles of Bdf1 and Bdf2 in the coordination of DNA replication and transcription. Bdf1 and Bdf2 may upregulate the level of histone H4 acetylation to control transcriptional activities, which leads to collisions between the replication and transcription machinery. Mrc1 may negatively regulate transcription to avoid the collisions, while Swi1 may coordinate replication fork progression to prevent fork collapse at highly transcribed genomic regions. It is also possible that Bdf1 and Bdf2 directly regulate DNA repair processes through their roles in chromatin organization. See the text for details.
Figure 1

A
Y0001: wild-type
Y2239: bdf1Δ
Y2306: bdf2Δ
Y0211: swi1Δ
Y2463: bdf1Δ swi1Δ
Y2464: bdf2Δ swi1Δ

B
Y0001: wild-type
Y2306: bdf2Δ
Y0150: crb2Δ
Y0403: mrc1Δ
Y3260: bdf2Δ crb2Δ
Y3261: bdf2Δ crb2Δ
Y3265: bdf2Δ mrc1Δ
Y3266: bdf2Δ mrc1Δ

C
Y0001: wild-type
Y2306: bdf2Δ
Y1550: chk1Δ
Y0428: cdc1Δ
Y1567: rad3Δ
Y3493: bdf2Δ chk1Δ
Y2485: bdf2Δ cdc1Δ
Y2489: bdf2Δ rad3Δ

D
Y0001: wild-type
Y0266: chk1Δ cdc1Δ
Y1568: rad3Δ
Y3208: bdf1Δ chk1Δ cdc1Δ
Y2487: bdf1Δ rad3Δ
Y3219: bdf2Δ chk1Δ cdc1Δ
Y3220: bdf2Δ chk1Δ cdc1Δ
Y2489: bdf2Δ rad3Δ
Figure 3

A

Y0001: wild-type
Y2239: bdf1Δ
Y2306: bdf2Δ
Y0211: swt1Δ

Y0001: wild-type
Y2239: bdf1Δ
Y2306: bdf2Δ
Y0211: swt1Δ

Y0001: wild-type
Y2239: bdf1Δ
Y1551: chk1Δ
Y1565: cds1Δ
Y1567: rad3Δ
Y2479: bdf1Δ chk1Δ
Y2483: bdf1Δ cds1Δ
Y2487: bdf1Δ rad3Δ

Y0001: wild-type
Y2239: bdf1Δ
Y1551: chk1Δ
Y1565: cds1Δ
Y1567: rad3Δ
Y2479: bdf1Δ chk1Δ
Y2483: bdf1Δ cds1Δ
Y2487: bdf1Δ rad3Δ

1 μM CPT 2 μM CPT 5 μM CPT 10 μM CPT

0.00063% MMS 0.00125% MMS 0.00250% MMS 0.00100% MMS

0.5 μg/ml Bleo

B

Y0001: wild-type
Y2239: bdf1Δ
Y1551: chk1Δ
Y1565: cds1Δ
Y1567: rad3Δ
Y2479: bdf1Δ chk1Δ
Y2483: bdf1Δ cds1Δ
Y2487: bdf1Δ rad3Δ

Y0001: wild-type
Y2239: bdf1Δ
Y1551: chk1Δ
Y1565: cds1Δ
Y1567: rad3Δ
Y2479: bdf1Δ chk1Δ
Y2483: bdf1Δ cds1Δ
Y2487: bdf1Δ rad3Δ

0.00063% MMS 0.00125% MMS 0.00250% MMS 0.00100% MMS

0.5 μg/ml Bleo

C

Y0001: WT
Y2306: bdf2Δ
Y1550: chk1Δ
Y0428: cds1Δ
Y1567: rad3Δ
Y3493: bdf2Δ chk1Δ
Y2485: bdf2Δ cds1Δ
Y2489: bdf2Δ rad3Δ

Y0001: WT
Y2306: bdf2Δ
Y1550: chk1Δ
Y0428: cds1Δ
Y1567: rad3Δ
Y3493: bdf2Δ chk1Δ
Y2485: bdf2Δ cds1Δ
Y2489: bdf2Δ rad3Δ

0.00063% MMS 0.00125% MMS 0.00250% MMS 0.00100% MMS

0.5 μg/ml Bleo
**Figure 6**

A

<table>
<thead>
<tr>
<th></th>
<th>grown at 30°C</th>
<th>25°C</th>
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<td>Acetylated H4</td>
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<td>Relative Acetylation of H4</td>
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<td>1.0</td>
<td>1.0</td>
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B

Y0001: wild-type  
Y0403: mrc1Δ  
Y278: mst1-L344S  
Y3891: mst1-L344S mrc1Δ  
Y3892: mst1-L344S mrc1Δ

C

Y0001: wild-type  
Y0241: swi1Δ  
Y278: mst1-L344S  
Y2918: swi1Δ mst1-L344S  
Y2919: swi1Δ mst1-L344S

Y0001: wild-type  
Y225: mnt81-vid21  
Y272: hhh2-KR  
Y0256: swi1Δ  
Y2641: swi1Δ mnt81-vid21  
Y2642: swi1Δ mnt81-vid21

Y0001: wild-type  
Y225: mnt81-vid21  
Y272: hhh2-KR  
Y3480: swi1Δ hhh2-KR  
Y3481: swi1Δ hhh2-KR
Figure 7

NuA4

Bdf1/Bdf2

Histone H4 acetylation

chromatin structures

DNA repair

Mrc1

Swi1

transcription

collision between replication and transcription

??