Schizosaccharomyces pombe Histone Acetyltransferase Mst1 (KAT5) Is an Essential Protein Required for Damage Response and Chromosome Segregation

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ABSTRACT

Schizosaccharomyces pombe Mst1 is a member of the MYST family of histone acetyltransferases and is the likely ortholog of *Saccharomyces cerevisiae* Esa1 and human Tip60 (KAT5). We have isolated a temperature-sensitive allele of this essential gene. *mst1* cells show a pleiotropic phenotype at the restrictive temperature. They are sensitive to a variety of DNA-damaging agents and to the spindle poison thiabendazole. *mst1* has an increased frequency of Rad22 repair foci, suggesting endogenous damage. Two-hybrid results show that Mst1 interacts with a number of proteins involved in chromosome integrity and centromere function, including the methyltransferase Skb1, the recombination mediator Rad22 (Sc Rad52), the chromatin assembly factor Hip1 (Sc Hir1), and the Msc1 protein related to a family of histone demethylases. *mst1* mutant sensitivity to hydroxyurea suggests a defect in recovery following HU arrest. We conclude that Mst1 plays essential roles in maintenance of genome stability and recovery from DNA damage.

MANY events in DNA metabolism, including replication and repair, are modulated by the pattern of different histone modifications in the chromatin, leading to the speculation that a “histone code” provides epigenetic information that facilitates numerous chromatin functions (reviewed in JENUWEIN and ALLIS 2001; KOUZARIDES 2007). Histone acetylation is associated with diverse chromatin functions (reviewed in KOUZARIDES 2007; LEE and WORKMAN 2007). Histone acetylation changes association of DNA with the underlying nucleosomes (SHOGREN-KNAAK *et al.* 2006) and also creates specific binding sites for proteins involved in a variety of DNA transactions (reviewed in KOUZARIDES 2007; LEE and WORKMAN 2007). Histone acetyltransferase (HAT) enzymes can be separated into several sequence-specific subfamilies that differ in their substrates and in the composition of their associated complexes. The MYST family of HATs (reviewed by UTLEY and COTE 2003) has been extensively characterized in budding yeast, which has three members (reviewed in LAFON *et al.* 2007). We have reported two MYST family proteins in fission yeast (GÓMEZ *et al.* 2005): Mst2, the likely ortholog of *Saccharomyces cerevisiae* Sas2, is a nonessential gene required for normal telomere silencing (GÓMEZ *et al.* 2005). Mst1, the likely ortholog of *S. cerevisiae* Esa1, is an essential gene and is the subject of this report.

Work from several experimental systems shows that ScEsa1 histone acetyltransferase is a catalytic subunit of a large protein complex called NuA4 and a smaller subcomplex, Piccolo, that is part of NuA4 (ALLARD *et al.* 1999; BOUDREAULT *et al.* 2003; EBERHARTER *et al.* 2005; SELLECK *et al.* 2005). The NuA4 complex acetylates histone H4 and H2A (SMITH *et al.* 1998; ALLARD *et al.* 1999; GALARNEAU *et al.* 2000). It may also be associated with acetylation of the histone variant H2AZ (KEOGH *et al.* 2006; MILLAR *et al.* 2006). Esa1 family HATs contain a chromodomain, a motif associated with binding to methylated histones (reviewed in DE LA CRUZ *et al.* 2005). While this suggests that some aspect of Esa1 function is mediated by binding methylated histone(s), the target for such binding remains elusive. The mammalian homolog is Tip60 (reviewed in SQUATRITO *et al.* 2006). Under a new initiative to regularize nomenclature in the chromatin field, Mst1, Esa1, and Tip60 are defined as the KAT5 family of histone acetyltransferases (ALLIS *et al.* 2007).

ScEsa1 is an essential gene, and loss of function results in cell-cycle-specific arrest after replication but before mitosis (SMITH *et al.* 1998; CLARKE *et al.* 1999), although a recent report suggests that it may also be required for cell cycle entry (EARLY *et al.* 2004). The roles of ScEsa1 are diverse, and recent studies have also linked it to the spindle assembly checkpoint (LE MASSON *et al.* 2003) and the morphogenesis checkpoint (LE MASSON *et al.* 2003; RUAULT and PILLUS 2006). Genome-level analyses in budding yeast indicate that ScEsa1 activity is associated with active gene expression

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(GALARNEAU *et al.* 2000; VOGELAUER *et al.* 2002). Paradoxically, however, H4 acetylation is also associated with decreased gene expression (DECKERT and STRUHL 2001). Recent studies suggest that ScEsal may also repress activity of specific gene regions, particularly the heterochromatic domains of the telomeres and the rDNA (CLARKE *et al.* 2006).

Histone acetylation by the NuA4 complex has a role in DNA double-strand break (DSB) repair (reviewed in VAN ATTIKUM and GASSER 2005b; SQUATRITO *et al.* 2006). Increased histone acetylation near the damage site occurs (YU *et al.* 2005) and acetylation of H4 is linked to activation of homologous recombination and non-homologous end-joining (BIRD *et al.* 2002; TAMBURINI and TYLER 2005). ScEsal is recruited to DNA breaks (BIRD *et al.* 2002; TAMBURINI and TYLER 2005). One of the major responses to DNA double-strand breaks is phosphorylation of the histone variant H2AX by the damage checkpoint kinases ATM and ATR (reviewed in FOSTER and DOWNS 2005; MORRISON and SHEN 2005). H2AX phosphorylation and recruitment of NuA4 occur with similar timing (DOWNS *et al.* 2004). Interestingly, two chromatin-remodeling complexes, INO80 and SWR1, are also recruited to H2AX, at least in part through the common Arp4 subunit that they share with NuA4 (DOWNS *et al.* 2004; ROBERT *et al.* 2006). NuA4, INO80, and SWR1 are proposed to facilitate exchange of phospho-H2AX, ultimately downregulating the damage signal (MORRISON *et al.* 2004; TSUKUDA *et al.* 2005; VAN ATTIKUM and GASSER 2005b; PAPAMICHOS-CHRONAKIS *et al.* 2006). Recent studies have implicated the NuA4 complex in regulation and modification of an additional histone H2A variant called H2AZ (BABIARZ *et al.* 2006; KEOGH *et al.* 2006; MILLAR and GRUNSTEIN 2006). It has been suggested that the downregulation of the H2AX phosphorylation signal reflects exchange of phospho-H2AX with H2AZ (reviewed in VAN ATTIKUM and GASSER 2005a).

Tip60, the human ortholog of Mst1 and ScEsal, acetylates nonhistone proteins independently of the NuA4 complex with substrates including Notch (KIM *et al.* 2007), ATM (SUN *et al.* 2005; JIANG *et al.* 2006), p53 (SYKES *et al.* 2006; TANG *et al.* 2006), and DNA-PKcs (JIANG *et al.* 2006). Tip60-deficient cells exposed to ionizing radiation have defects in chromosome repair and reduced apoptosis (IKURA *et al.* 2000). This leads to the important conclusion that HATs are not limited to histones as substrates. These data suggest that KAT5 family HATs contribute to DNA damage response in two ways: first, by affecting DNA repair and recovery (*e.g.*, through histone modifications that recruit specific DNA damage response proteins) and, second, by affecting the checkpoint response (*e.g.*, through direct modification of p53 or ATM).

To gain further insight into the diverse function of the KAT5 protein family in DNA metabolism, we investigated the role of *Schizosaccharomyces pombe* Mst1 in the

tractable fission yeast system. In this report, we present initial genetic characterization of this gene. Mst1 is essential for viability, and mutants are sensitive to a variety of DNA-damaging agents, including hydroxyurea (HU), ultraviolet (UV) irradiation, methyl methanesulfonate (MMS), and bleomycin. Mutants are also sensitive to the spindle poison thiabendazole. Using a two-hybrid screen, we identify a number of potential interaction proteins involved in DNA dynamics, including chromatin proteins, transcription factors, and the recombination protein Rad22 (ScRad52). Analysis of the HU response suggests that Mst1 is required for normal recruitment of Rad22 in HU. Thus, Mst1 has multiple roles in maintenance of genome integrity in *S. pombe*.

MATERIALS AND METHODS

Strains, media, and manipulations: Strains used in this study are listed in Table 1. Strains were grown and maintained on yeast extract plus supplements (YES) or Edinburgh minimal media with appropriate supplements, using standard techniques (FORSBURG and RHIND 2006). Matings were performed on synthetic sporulation agar plates for 2–3 days at 25°. Temperature-sensitive (ts) cells were grown at 25° and non-ts cells at 32°. Transformations were carried out by electroporation. Double mutants were constructed by standard tetrad analysis or random spore analysis. G418 plates were YES supplemented with 100 µg/ml G418 (Sigma, St. Louis).

Plasmids and constructions: Disruption and tagging of *mst1+* is described in GÓMEZ *et al.* (2005). Site-directed mutants were constructed using the Quick-Change mutagenesis kit (Stratagene, La Jolla, CA).

Primers used were L-S/F (CATACAATTTTCTTATGAATC GACCAAGCGTGAGCACAAAC) and L-S/R GTTTGTGCTCA CGCTTGGTTCGATTCATAAGAAAATTGTATG and L-P/F GG TGTAGGAATATTTGTCTTCCGTCACACCTTTTCTGGATC AC and L-P/R GTGATCCAGAAAAAGTTTGGACGGAAGACAAATATTCCTACACC. Complementmentation and temperature sensitivity were assessed by transforming the expression plasmid into the heterozygous *mst1⁺/Δmst1::ura4⁺* diploid FY1754 and by isolating haploids that contain both *Δmst1::ura4⁺* and the plasmid markers. An integrant was constructed by moving the *Pnmt-mst1-L344S* cassette from plasmid pEBG99 into plasmid pJK148 to create plasmid pEBG98 and integrating this construct at the *leu1-32* locus in the diploid. Haploid temperature-sensitive segregants containing both *Δmst1::ura4⁺* and *leu1⁺::nmt-mst1-L344S* were isolated by random spore analysis.

Silencing was assessed by examining growth in the absence of uracil and low adenine in a strain with *ura4⁺* integrated into the centromere and *ade6⁺* integrated into the telomere of a minichromosome.

FACS: Samples were collected after 5 and 10 hr, ethanol fixed, and analyzed by flow cytometry as described previously (GÓMEZ and FORSBURG 2003).

Immunoprecipitation: Samples were prepared as described in GÓMEZ *et al.* (2002). For co-immunoprecipitation with MCM proteins, we used anti-HA antibody 12CA5 (Abcam) and antiV5 (Invitrogen, San Diego). For histone acetylation assays, the dried beads were treated with 20 µl of a histone acetylation mix containing 5 µM C14-Acetyl CoA (ICN), 0.7 µg of core histones, 50 mM HEPES (pH 7.9), 10% glycerol, and 1 mM DTT. The reaction was incubated 1 hr 30 min at 30°. Beads

TABLE 1
Strains used in this study

Strain	Genotype	Source
11	<i>h⁻ ade6-M210</i>	Our stock
261	<i>h⁻ ura4-D18 leu1-32 ade6-M210 can1-1</i>	Our stock
865	<i>h⁻ Δ<i>cds1</i>::<i>ura4 ura4-D18 leu1-32</i></i>	T. Wang
1104	<i>h⁻ Δ<i>rad3</i>::<i>ura4⁺ ura4 leu1-32 ade6-M210</i> (?)</i>	Our stock
1107	<i>h⁻ Δ<i>rad3</i>::<i>ura4⁺ ura4-D18 leu1-32 ade6-M216</i></i>	Our stock
1256	<i>h⁻ Δ<i>mad2</i>::<i>ura4⁺ ade6M210 leu1-32 ura4D18</i></i>	S. Sazer
1552	<i>h⁻ Δ<i>bub1</i>::<i>ura4⁺ ade6M210 leu1-32 ura4D18</i></i>	K. Hardwick
1754	<i>h⁺/h⁻ Δ<i>mst1</i>::<i>ura4⁺/+ ura4-D18/ura4-D18 leu1-32/leu1-32 ade6-M216/ade6-M210</i></i>	GÓMEZ <i>et al.</i> (2005)
2180	<i>h⁺ cbh1::GFP-pBSIIS-ura4⁺-chh1C leu1-32 ura4-D18</i>	BAUM and CLARKE (2000)
2192	<i>h⁻ Δ<i>chk1</i>::<i>ura4⁺ ura4-D18 ade6-M216</i></i>	Our stock
2217	<i>h⁻ Δ<i>cbh1</i>::<i>LEU2 ura4-D18 ade6-M216 leu1-32</i></i>	Y. Murakami
2339	<i>h⁺ Δ<i>mst1</i>::<i>ura4⁺ leu1⁺ ::mst1-L344S ura4-D18 ade6-M216</i> (#7)</i>	This study
2396	<i>h⁻ Δ<i>mst1</i>::<i>ura4⁺ leu1⁺ ::mst1-L344S ura4-D18 ade6-M210</i></i>	This study
2449	<i>h⁺ Δ<i>mst1</i>::<i>kanMX6 leu1⁺ ::mst1-L344S⁺ ura4-D18 ade6-M210</i></i>	This study
2450	<i>h⁻ Δ<i>mst1</i>::<i>kanMX6 leu1⁺ ::mst1-L344S⁺ ura4-D18 ade6-M210</i></i>	This study
2495	<i>h⁺ Δ<i>taz1</i>::<i>ura4⁺ Δmst1::kanMX6 leu1⁺ ::mst1-L344Sura4-D18 leu1-32 ade6-M210</i></i>	This study
2498	<i>h⁻ Δ<i>rhp51</i>::<i>ura4⁺ Δmst1::kanMX6 leu1⁺ ::mst1-L344Sura4-D18 leu1-32 ade6-M210</i></i>	This study
2499	<i>h⁺ Δ<i>rad50</i>::<i>kanMX6 Δmst1::ura4⁺ leu1⁺ ::mst1-L344S⁺ ura4-D18 leu1-32 ade6-M21?</i></i>	This study
2501	<i>h⁺ Δ<i>rad32</i>::<i>ura4⁺ mst1::kanMX6 leu1⁺ ::mst1-L344S⁺ ura4-D18 leu1-32 ade6-M21?</i></i>	This study
2505	<i>h⁺ mst2::ura4⁺ mst1::kanMX6 leu1⁺ ::mst1-L344S⁺ ura4-D18 leu1-32 ade6-M210</i>	This study
2506	<i>h⁻ mst2::ura4⁺ mst1::kanMX6 leu1⁺ ::mst1-L344S⁺ ura4-D18 leu1-32 ade6-M210</i>	This study
2508	<i>h⁺ swi6::ura4⁺ mst1::kanMX6 leu1⁺ ::mst1-L344S⁺ ura4-D18 leu1-32 ade6-M210</i>	This study
2525	<i>h⁻ hsk1-1312 mst1::kanMX6 leu1⁺ ::mst1-L344S⁺ leu1-32 ade6-M21?</i>	This study
2535	<i>h⁺ Δ<i>mst1</i>::<i>kanMX6 leu1⁺ ::mst1-L344S⁺ ade6-M210 ura4-D18</i></i>	
2562	<i>h⁺ Δ<i>rad12</i>::<i>ura4⁺ Δmst1::kanMX6 leu1⁺ ::mst1-L344S⁺ ade6-M210 ura4-D18</i></i>	This study
2566	<i>h⁻ Δ<i>clr4</i>::<i>ura4⁺ Δmst1::kanMX6 leu1⁺ ::mst1-L344S⁺ ade6-M210 ura4-D18 (otrR(SphI)::ade6⁺) ?</i></i>	This study
2569	<i>h⁺ orp1-4 Δmst1::ura4⁺ leu1⁺ ::mst1-L344S⁺ ade6-M210 ura4-D18 (can1-1)?</i>	This study
2570	<i>h⁺ Δ<i>chr3</i>::<i>kanMX6 Δmst1::ura4⁺ leu1⁺ ::mst1-L344S⁺ ade6-M210 ura4-D18 (can1-1)?</i></i>	This study
2572	<i>h⁺ mcm7-98 Δmst1::ura4⁺ leu1⁺ ::mst1-L344S⁺ ade6-M216 ura4-D18 (can1-1)?</i>	This study
2574	<i>h⁺ cdc19-P1 Δmst1::ura4⁺ leu1⁺ ::mst1-L344S⁺ ade6-M210 ura4-D18</i>	This study
2576	<i>h⁺ pol1-1 Δmst1::ura4⁺ leu1⁺ ::mst1-L344S⁺ ade6-M210 ura4-D18</i>	This study
2578	<i>h⁺ cdc21-M68 Δmst1::ura4⁺ leu1⁺ ::mst1-L344S⁺ ade6-M216 ura4-D18 (can1-1)?</i>	This study
2601	<i>h⁺ Δ<i>skb1</i>::<i>ura4⁺ mst1::kanMX6 leu1⁺ ::mst1-L344S⁺ ura4-D18 ade6-M210</i></i>	This study
2535	<i>h⁺ Δ<i>mst1</i>::<i>kanMX6 leu1⁺ ::mst1-L344S⁺ ade6-M210 ura4-D18</i></i>	This study
3135	<i>h⁻ ade6-M210 leu1-32 ura4-D1 Δ<i>hip</i>::<i>ura4⁺</i></i>	S. Whitehall
3286	<i>h⁻ Δ<i>cds1</i>::<i>ura4⁺ rad22YFP::KanMX6 ura4-D18 leu1-32 ade6?</i></i>	This study
3369	<i>h⁺ Δ<i>mst1</i>::<i>ura4⁺ leu1⁺ ::mst1-L344S⁺ ura4-D18 ade6-M21? rad22-YFP:kanMX4</i></i>	This study
3436	<i>h⁻ rad22-YFP:kanMX4 ade6-M210 ura4-D18 leu1-32</i>	This study
3479	<i>h⁺ Δ<i>msc1</i>::<i>kanMX6 leu1-32</i></i>	N. Walworth
3480	<i>h⁺ msc1-HA-kanMX6 leu1-32</i>	N. Walworth
3553	<i>h? Δmst1::kanMX6 leu1⁺ ::mst1-L344S⁺ otr1L(dh/HindIII)::ura4⁺ leu1-32 ura4ΔS/E ade6ΔN/N [ch16 M23::LEU2⁺ tel::ade6⁺]</i>	This study
3563	<i>h? Δ<i>cbh1</i>::<i>LEU2 Δmst1::kanMX6 leu1⁺ ::mst1-L344S⁺ ade6-M21? ura4-D18</i></i>	This study
3562	<i>h? Δmst1::kanMX6 leu1⁺ ::mst1-L344S⁺ ade6-M210 ura4-D18 Δ<i>hip</i>::<i>ura4⁺</i></i>	This study

were spun down, and supernatant was recovered and boiled in SDS-Laemmli loading buffer before electrophoresis in 15% SDS-polyacrylamide gel electrophoresis (PAGE). The gel was fixed and treated with fluorography-enhancing solution (NEN Life Science) before drying and autoradiography.

For co-immunoprecipitation with Rad22-YFP or Cbh1-GFP (strains 3436, 3480), we prepared soluble protein lysates from fission yeast cells in B88 buffer as in MORENO *et al.* (1991). A total of 1.0 mg protein was used per immunoprecipitation. Lysates were precleared with sepharose A beads (Repligen) for 1 hr. One microliter of anti-GFP (Abcam 290) was added and left to rotate overnight at 4°. Proteins were analyzed by 8%

SDS-PAGE, followed by immunoblotting with anti-V5 (Invitrogen) at 1:2500 dilution and with anti-GFP (living colors, Clontech) at a 1:1000 dilution. Crosslinking immunoprecipitations were done as previously described (AUGER *et al.* 2008). Briefly, cells were crosslinked with 1% formaldehyde (Sigma) for 50 min on ice. Harvested cells were vortexed with glass beads in IP buffer (50 mM HEPES-KOH, pH 1.6, 500 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate). Extracts were treated with DNase I to reverse crosslink. A total of 1.0 mg protein was used per immunoprecipitation. Whole-cell extracts and immunoprecipitates were incubated with SDS sample buffer for 1 hr at 90° before Western blot analysis.

FIGURE 1.—Spore germination. (A) Wild-type diploid strain (*ura4⁺/ura4-D18*) and (B) heterozygous disruption mutant (*mst1⁺/Δmst1::ura4⁺*) were sporulated and processed for random spore analysis. Spores were inoculated into media lacking uracil and sampled for flow cytometry to monitor entry into S phase and the process of DNA replication. Cell morphology was visualized by staining for DNA with DAPI.

Microscopy: Whole fixed cells were stained as described for DAPI and Calofluor (GÓMEZ and FORSBURG 2003). Cells were visualized with a Leica DMR microscope. Objectives used were Leica 100X/1.30, PLFL, NA = 1.30, and Leica 63X/1.32, PLApO, NA = 1.32. Images were captured with a Hamamatsu digital camera and Improvision Openlab software (Improvision, Lexington, MA). Images were assembled using Canvas (ACD/Deneba) and adjusted for contrast. Nuclear spreads were performed as in PANKRATZ and FORSBURG (2005) with the following modifications. Rad22YFP was detected using Abcam 290-50 rabbit anti-GFP, 1:2000 in 5% BSA (Sigma), and secondary donkey anti-rabbit cy3 1:500 DNA was stained with 1× Hoechst (Invitrogen). The objective used was Leica 63X/1.32, PLApO, NA = 1.32. Images were captured and assembled as above.

Damage assays: Cells were grown to $A_{595} = 1$, serially diluted fivefold, and spotted onto YES plates or YES plates containing 10 μ g/ml thiabendazole (TBZ), 5 mM HU, or 0.005, 0.007, or 0.01% MMS, and incubated for 3–5 days at 25°, 29°, or 32°, as indicated. For UV irradiation assay, 1000 cells were plated in duplicate onto YES and immediately treated with the indicated doses of UV irradiation using a Stratalinker (Stratagene). Control plates were left untreated. Plates were incubated at 32° for 2–3 days. Colonies were counted and the percentage survival was obtained relative to the number of colonies grown on the untreated control plates.

Two-hybrid strains: The *mst1⁺* open reading frame was isolated by PCR from plasmid pEBG72 with primers HAT-3 forward (AGATCTCGCCAAAACTTGCTCAATCTTCTTCC) and HAT-3 reverse (GGTACCCGACATAGACTGGCACATTA CATCC) and cloned into the two-hybrid vector pDBLeu cleaved with *Nco*I and *Nof*I to create plasmid pEBG91. Budding-yeast two-hybrid strain AH109 (Clontech) with *HIS3*, *ADE2*, and *lacZ* reporters downstream of heterologous *GAL4*-responsive promoter elements was transformed with an *S. pombe* Matchmaker cDNA library (Clontech) and screened for interactions as described in LEVERSON *et al.* (2002). Plasmids from positive clones were isolated and verified by retransformation, and the identity of the interacting open reading frame was determined by sequence.

RESULTS

***Δmst1* cells undergo disordered mitosis:** Previously, we identified two members of the MYST family of histone acetyltransferases in fission yeast. Our analysis indicated that Mst1 is the likely ortholog of *S. cerevisiae* Esa1, and like *ScEsa1*, is a chromatin-bound protein that is essential for viability (GÓMEZ *et al.* 2005). Data from

metazoans suggest that the Hbo1 histone acetyltransferase, which is also a MYST family protein, may function in DNA replication (IIZUKA and STILLMAN 1999; BURKE *et al.* 2001; IIZUKA *et al.* 2006). To investigate whether *S. pombe* Mst1 contributes to DNA replication, we examined the phenotype of cells containing the *Δmst1* allele. Mutant diploids heterozygous for *Δmst1::ura4⁺* and wild-type diploids heterozygous for *ura4⁺* were induced to sporulate. We inoculated purified spores from both diploids into medium lacking uracil to carry out bulk spore germination. Only spores containing the *ura4⁺* gene are able to germinate in these conditions, and their DNA content was determined by flow cytometry. Spores containing *Δmst1::ura4⁺* synthesized DNA to an approximately 2C DNA content, with timing similar to that of wild type (Figure 1). Half of each population contains nongerminating *ura4-D18* spores, which persist as a 1C population. Upon microscopic examination, we observed that many of the *Δmst1::ura4⁺* cells showed highly disordered mitosis, including a high fraction of *cut* phenotypes. This suggests defects in chromosome segregation, which might result from mitotic defects or from replication defects and checkpoint disruption.

Isolation and characterization of a temperature-sensitive allele: To better characterize Mst1, we constructed a temperature-sensitive allele based on temperature-sensitive alleles in *S. cerevisiae* *ESA1* (CLARKE *et al.* 1999). We tested two mutations: L271P (corresponding to L254P in *ScEsa1*) and L344S (corresponding to L267S in *ScEsa1*). Constructs containing these were placed in a *LEU2* plasmid under control of the *nmt1* promoter and transformed into the *Δmst1::ura4⁺* diploid. Following sporulation, we assessed recovery of spores at 25° containing both the *ura4⁺* marker (marking *Δmst1*) and the *LEU2* marker (marking the plasmid). Both mutants were able to complement the disruption at 25°. However, upon shift to 36°, only the *mst1-L344S* strain was temperature sensitive.

We attempted to replace the endogenous *mst1⁺* allele with the *L344S* allele, without success. We reasoned that, when integrated under the native promoter, a single

FIGURE 2.—Characterization of temperature-sensitive allele. (A–D) Comparison of growth rate and FACS for wild-type (FY11) and *mst1-L344S* cells (FY 2396) grown in the presence of thiamine at 25° or 36°. (Top) Colony formation on plates, where colony size is proportional to growth rate. (Bottom) FACS analysis following shift to 36°. (E) *mst1-L344S* cells at 25° (left) or 36° at 6 (middle) and 8 (right) hr after shift. Cells were stained with DAPI and Calcofluor. (F) Fraction of cells with indicated phenotypes during a temperature-shift experiment.

copy of *mst1-L344S* might be insufficient for viability. Therefore, we integrated *mst1-L344S* under control of the *nmt1*⁺ promoter into the *leu1-32* locus of the heterozygous diploid and sporulated to create the haploid strain $\Delta mst1::ura4^+ leu1^+::P_{nmt1}-mst1-L344S$. Upon shift to 36° and addition of thiamine to shut off the *nmt1* promoter, this strain was unable to grow. However, cells were able to grow in the presence of thiamine at 25° or 32°, indicating that the protein is functional at lower temperatures. There is sufficient background expression of the *nmt1*⁺ promoter even the presence of thiamine for stable proteins to be maintained (Figure 2, A–D). An equivalent strain with a *kanMX* marker at $\Delta mst1$ behaved similarly (data not shown).

We examined the phenotype of cells containing *mst1-L344S* by shifting asynchronous cells to the restrictive temperature. The increase of cell number was blocked within 2 hr. However, cell mass measured by A_{595} continued to increase for ~8 hr. DNA content, measured by flow cytometry, showed no change from the 2C peak characteristic of exponentially growing wild-type fission yeast, although later time points showed a flattening of the profile and an increase of cells with higher DNA content. This is common in heterogeneous, elongated cells and is typical of most *cdc* mutants.

We examined cells for nuclear and septum morphology by staining with DAPI and Calcofluor, respectively, and observing under fluorescence (Figure 2E, quanti-

fied in Figure 2F). Strikingly, the number of cells staining with Calcofluor significantly increased in the *mst1-L344S* strain at restrictive temperature, with nearly 50% of cells arrested with septa by 6 hr. Some of these were mononucleate, showing asymmetric nuclear segregation. Additionally, there was an increase in the overall number of binucleate cells relative to wild type. *S. pombe* has an unusual cell cycle in which cytokinesis is completed during the subsequent nuclear cell cycle. Thus, cells with a 2C DNA content by FACS may be mononucleate cells in G₂ or M phase or binucleated cells in which the nuclei are in G₁ or S phase and cytokinesis is not complete. Therefore, arrest as a binucleate septated cell may represent a defect entering DNA replication in the ensuing cell cycle. Alternatively, it could represent a defect in cytokinesis that delays entry into the next cell cycle.

This phenotype is different from the disordered mitosis seen in the $\Delta mst1$ mutant spores. Because germinating spores arise from stationary or G₁ cells, while exponentially growing *S. pombe* are predominantly G₂ with a 2C DNA content, we reasoned that the different phenotypes could represent the effect of inactivating Mst1 at different cell cycle stages. Alternatively, they could suggest that the *mst1-L344S* allele creates a hypomorph rather than a complete inactivation of the gene. We attempted to resolve whether the mutation affects S phase by synchronizing the *mst1-L344S* cells in G₁,

TABLE 2

Genetic interactions between *mst1-L344S* and other mutants

Mutation	Protein affected	Double-mutant phenotype
DNA replication		
<i>mcm2ts</i>	MCM helicase	Reduced growth temperature
<i>mcm4ts</i>	MCM helicase	Reduced growth temperature
<i>mcm7ts</i>	MCM helicase	Reduced growth temperature
<i>orp1ts</i>	ORC subunit	Reduced growth temperature
<i>pol1ts</i>	DNA polymerase α	Reduced growth temperature
<i>hsk1ts</i>	Cdc7 kinase	Reduced growth temperature
$\Delta rad2$	FEN endonuclease	No effect
DSB repair		
$\Delta rad32$	MRE11	No effect
$\Delta rad50$	RAD50	Reduced growth temperature
$\Delta rhp51$	RAD51	Reduced growth temperature
$\Delta rqh1$	SGS1/Blm helicase	No effect
$\Delta rti1$	Rad52 homolog	No effect
Centromere/heterochromatin		
$\Delta cbh1$	CENP-B homolog	No effect
$\Delta clr3$	Histone deacetylase	Reduced growth temperature
$\Delta clr4$	Histone methyltransferase	Reduced growth temperature
$\Delta hip1$	HIRA homolog	No effect
$\Delta msc1$	Putative demethylase	No effect
$\Delta mst2$	MYST acetyltransferase	No effect
$\Delta swi6$	HP1 homolog	Reduced growth temperature
Other		
$\Delta skb1$	Arginine methyltransferase	No effect
$\Delta taz1$	Telomere-binding protein	No effect

Double mutants were constructed and growth rate relative to the single mutants was assessed by streaking at different temperatures.

using nitrogen starvation (supplemental Figure 1). When released from nitrogen starvation into complete medium, *mst1-L344S* cells completed replication with similar timing to wild type, also suggesting that inactivation of *mst1* by the *mst1-L344S* allele or by the $\Delta mst1$ allele is not sufficient for cells to block DNA replication in the first cell cycle.

***mst1-L344S* genetically interacts with replication, recombination, and heterochromatin mutants:** We examined the phenotypes of double mutants, which contained *mst1-L344S* as well as mutations in genes required for DNA replication, double-strand break repair, and heterochromatin assembly (Table 2, Figure 3). We tested the double-mutant strains for their tem-

perature sensitivity. We defined a genetic interaction as occurring when a double-mutant strain was unable to grow at temperatures where both parents were growth proficient (“reduced growth temperature”). There were modest genetic interactions with many replication initiation mutants. Additionally, we observed that the maximum growth temperature was reduced when *mst1* was combined with mutations $\Delta rhp51$ (ScRad51), $\Delta rad50$, and $\Delta rad22$, which affect homologous DNA recombination. The $\Delta rad22$ *mst1-L344S* strain grew extremely slowly even at 25°; however, because of the high frequency of spontaneous suppressors in $\Delta rad22$ backgrounds (OSMAN *et al.* 2005), we cannot be confident that this strain is suppressor free.

Because *mst1-L344S* cells show defects in chromosome segregation and are sensitive to thiabendazole (see Figure 5), we examined interactions with genes required for centromere heterochromatin assembly (Table 2, centromere). We observed that maximum-growth temperature was strikingly lower than that of the parents when *mst1-L344S* was combined with $\Delta swi6$, $\Delta clr3$, and $\Delta clr4$, all of which define genes required to establish and maintain centromeric heterochromatin (reviewed in EKWALL 2007). However, we observed no defects in centromere or telomere silencing in *mst1-L344S* cells (data not shown). This suggests that the heterochromatin itself is intact. We examined additional chromatin-related proteins. No effect is observed when *mst1-L344S* is combined with mutation of histone acetyltransferase $\Delta mst2$ or the telomere-binding protein $\Delta taz1$.

Identification of potential Mst1 interaction partners by two-hybrid screening: SpMst1 interacts with the actin-related protein SpAlp5/ScAtp4, a known subunit of the NuA4 complex (MINODA *et al.* 2005). Because evidence suggests that Tip60 may function independently of the NuA4 complex (see Introduction), we sought to identify additional proteins that interact with Mst1, using a two-hybrid screen. We isolated a number of interacting gene products, summarized in Table 3.

We isolated Rad22, the *S. pombe* ortholog of homologous DNA recombination protein Rad52. Rad22 promotes the strand-invasion step of homologous recombination. Fission yeast has an additional Rad22 family member, called Rti1. We constructed plasmids to test whether Mst1 interacted in the two-hybrid system with Rti1 or with Rhp51. Although Rhp51 and Rad22 themselves interact in two-hybrid screens (*e.g.*, CATLETT and FORSBURG 2003), we observed no evidence for interaction between Mst1 and Rhp51 or between Mst1 and Rti1, suggesting that this interaction is specific (Figure 4A). Immunoprecipitation of Rad22YFP using a cross-reacting anti-GFP antibody precipitates Mst1-V5 (Figure 4B); however, immunoprecipitation of Mst1-V5 does not precipitate Rad22-YFP (data not shown).

We isolated three chromatin-related proteins as potential Mst1 partners. The first, Hip1, is a putative histone chaperone most closely related to ScHir1 and human

FIGURE 3.—Genetic interactions. Double mutants were constructed and serial dilutions were performed at different temperatures to characterize any synthetic interactions. Equivalent numbers of exponentially growing cells were diluted fivefold and incubated at the indicated temperatures.

HIRA (BLACKWELL *et al.* 2004). The second, Cbh1, is one of the three CenpB homologs in fission yeast associated with centromere assembly (BAUM and CLARKE 2000; NAKAGAWA *et al.* 2002). We tested all three CenpB homologs for interaction with Mst1. Only Cbh1 showed a specific interaction (Figure 4). We observed no co-immunoprecipitation between Cbh1 and Mst1 from soluble or crosslinked lysates (data not shown). The third gene that we isolated was Msc1. Msc1 was identified as a high-copy suppressor of the checkpoint mutation $\Delta chk1$; it has E3 ligase activity and homology to a family of demethylases, and mutation disrupts histone acetylation (AHMED *et al.* 2004; DUL and WALWORTH 2007). We constructed double mutants containing *mst1-L344S* with $\Delta hip1$, $\Delta cbh1$, or $\Delta msc1$ and tested growth at different temperatures. There was no difference in the growth characteristics of the double mutants compared to *mst1-L344S* cells alone. We were unable to detect Msc1 in soluble extracts and our crosslinking protocol was unsuccessful on this protein (data not shown).

The most frequently isolated two-hybrid clone was *skb1*⁺, which encodes an arginine methyltransferase, an ortholog of *S. cerevisiae* Hsl7 (FUJITA *et al.* 1999; SHULEWITZ *et al.* 1999; LEE *et al.* 2000). We observed no obvious genetic interactions between $\Delta skb1$ and *mst1-L344S*, in contrast to

budding yeast where *hsl7* and *esal* mutants have been shown to have synthetic interactions (RUAULT and PILLUS 2006). Two putative transcription factors were also isolated. The *res2/pct1*⁺ gene has been studied extensively and it is required for G₁/S-specific transcription (TANAKA *et al.* 1992; ZHU *et al.* 1994). *taf111*⁺ (also known as *taf1*⁺) is a putative TFIID subunit required for induction of genes during nitrogen starvation and sexual development (UENO *et al.* 2001). The remaining two-hybrid isolates included *bud6*⁺, which is also associated with actin function (GLYNN *et al.* 2001), and a *SEC18* homolog likely involved in vesicular transport (SPAC1834.11c). The only previously described fission yeast Mst1 interactor, Alp5 (MINODA *et al.* 2005), was not isolated in our screen, nor were any other subunits of the NuA4 complex.

We observed very little Mst1 (or Mst2) protein in the soluble fraction (supplemental Figure 2a). This may explain why our attempts to confirm the two-hybrid interactions using co-immunoprecipitation in fission yeast were for the most part unsuccessful. Upon overproduction of Mst1, it is possible to observe some interactions with other proteins; for example, we found that Mcm2 can interact with Mst1 or Mst2 but only when both partners are overproduced (supplemental Figure 2b). However, we observed no interactions with endogenous levels of MCM proteins (data not shown), even

TABLE 3
Genes isolated by two-hybrid screening

β -Galactosidase	Gene	Function
+	<i>bud6/fat1⁺</i>	Actin-interacting protein (ScBUD6/AIP3 homolog) (GLYNN <i>et al.</i> 2001)
+	<i>cbh1⁺</i>	One of three fission yeast CENP-B proteins. Required for heterochromatin function. No <i>S. cerevisiae</i> homolog (BAUM and CLARKE 2000; NAKAGAWA <i>et al.</i> 2002).
+	<i>hip1⁺</i>	HIRA histone chaperone (ScHIR1 homolog) (BLACKWELL <i>et al.</i> 2004)
++	<i>msc1⁺</i>	High-copy suppressor of Δ <i>chk1</i> E3 ubiquitin ligase and putative demethylase (AHMED <i>et al.</i> 2004; DUL and WALWORTH 2007). No <i>S. cerevisiae</i> homolog.
++	<i>rad22⁺</i>	Homologous recombination protein (ScRAD52 homolog) (OSTERMANN <i>et al.</i> 1993)
+	<i>res2/pct1⁺</i>	Transcription factor; forms a complex with Cdc10 (ScMBP1 homolog) (MIYAMOTO <i>et al.</i> 1994).
+	<i>sec18⁺</i>	Putative vesicle membrane fusion protein; uncharacterized ScSec18 homolog. ORF: SPAC1834.11c.
++	<i>skb1⁺</i>	Arginine methyltransferase (ScHSL7 homolog) (GILBRETH <i>et al.</i> 1996)
++	<i>taf111⁺</i>	Transcription factor; putative TFIID subunit required for sexual development SPAC2G11.14 (UENO <i>et al.</i> 2001).

β -Galactosidase is a qualitative measure of the degree of activation in the two-hybrid system.

though MCMs themselves can be coprecipitated under those conditions (*e.g.*, SHERMAN *et al.* 1998; GÓMEZ *et al.* 2002). We completed our initial characterization of Mst1 protein by showing that acetylation of the four core histones can be observed in an IP–acetylation assay (supplemental Figure 3).

***mst1-L344S* cells are damage sensitive:** Data suggest that the NuA4 complex in other species is recruited to double-strand breaks through association with phosphorylated histone H2AX (DOWNS *et al.* 2004; ROBERT *et al.* 2006). Our data showing an interaction both genetically and physically with Rad22 suggested that Mst1 may contribute to repair of DSBs by influencing homologous recombination. Rad22 has also been implicated in recovery from HU (MEISTER *et al.* 2005; BAILIS *et al.* 2008). Therefore, we examined *mst1-L344S* mutants for evidence of a role in the damage response.

We first examined growth of *mst1-L344S* in response to chronic exposure to low levels of DNA-damaging agents (Figure 5A). We examined MMS (alkylating agent delays replication), HU (inhibits ribonucleotide reductase and causes fork stalling), and camptothecin (CPT; blocks topoisomerase and causes DSBs). *mst1-L344S* cells were sensitive to HU and MMS, but not to CPT at 29°. *mst1-L344S* was also sensitive to UV irradiation (200 J/m²), but not to γ -irradiation (400 Gy) at 25° (not shown). We observed that sensitivity to these agents increased with temperature (data not shown). The *mst1-L344S* mutant was extremely sensitive to bleomycin at 32° (radio-mimetic; Figure 5B). These results are consistent with known roles for Mst1 family acetyltransferases in some forms of DSB repair. We also observed substantial sensitivity to thiabendazole, a microtubule-destabilizing drug (Figure 5C). Mutations affecting

centromere or kinetochore function are often TBZ sensitive; this may relate to the synthetic interactions that we observed with the heterochromatin mutants (Table 2) and to the physical interactions with Cbh1 and Msc1, which affect centromere function (Table 3).

Mst1 is required for recovery from HU arrest: HU causes nucleotide depletion, which results in replication fork stalling early in S phase. Defects in HU response may reflect a failure to arrest the cell cycle during the acute treatment or an ability to restore replication and recover after HU is removed (reviewed in BRANZEI and FOIANI 2007). As shown in Figure 6, A, B, and D, *mst1-L344S* mutants arrest with a 1C DNA content and modestly elongated morphology, as do wild-type and Δ *cds1* cells, indicating that checkpoint-mediated cell cycle arrest is intact. As seen in the time course in Figure 6, A and B, wild-type cells complete S phase within 30 min of removing HU, while Δ *cds1* mutants are severely delayed, consistent with their recovery defect. Wild-type cells also proceed to division and enter the next cell cycle, as seen in the increase of 4C population (reflecting binucleate cells that complete S phase before completing the previous cytokinesis). This population is not seen in the Δ *cds1* mutants, which are defective for recovery and show a broad, heterogeneous peak of DNA content at the later time points. The *mst1* cells resemble wild type, suggesting that the mutants maintain and restart the replication fork normally. This suggests that the sensitivity that we observed in *mst1* cells in HU reflects defects in a later stage of recovery. Similar results have been seen for Δ *rad22* and Δ *rhp51* (MEISTER *et al.* 2005; BAILIS *et al.* 2008). Interestingly, although *mst1-L344S* cells were sensitive to HU during chronic treatment (Figure 5),

FIGURE 4.—Two-hybrid interactions. (A) A prey construct containing Mst1 was tested for interaction with full-length Rad22, Rti1, Rhp51, Cbh1, Cbh2, and Abp1 (see MATERIALS AND METHODS). *S. cerevisiae* cells containing the indicated plasmids were tested for growth on SD media lacking histidine and adenine (right), compared to control media containing both supplements. Growth on the selective media indicates a successful two-hybrid interaction. (B) Physical interaction between Mst1-V5 and Rad22-GFP. One milligram of protein was immunoprecipitated using 1 μ l of anti-GFP antibody. Input lanes: 50 μ g of total protein extract. GFP was detected by ECL+ incubation for 30 min. After 30 min, V5 blots were exposed to film after ECL+ (Pierce) incubation for 30 sec.

viability was relatively unaffected by an acute treatment in the HU block-and-release protocol (Figure 6C). This suggests that prolonged exposure to HU leads to an accumulation of growth defects, whereas transient exposure for a single generation causes relatively little problem.

One measure of DNA damage is provided by recruitment of recombination proteins to repair centers (CASPARI *et al.* 2002; DU *et al.* 2003; MEISTER *et al.* 2005). During HU treatment, Rad22 focus formation can distinguish two functions. In wild-type cells, Rad22 is recruited in a burst during the recovery phase after cells are released from HU. In contrast, in checkpoint mutants that fail to respond properly to HU, collapsed replication forks cause DNA breaks and Rad22 is recruited during HU treatment (CASPARI *et al.* 2002; DU *et al.* 2003; MEISTER *et al.* 2005; BAILIS *et al.* 2008). To determine whether reduced Mst1 function causes DNA damage, we examined Rad22YFP focus formation in wild-type and *mst1-L344S* cells at 32° (Figure 6, E and F). Due to background fluorescence observed in *mst1-L344S* mutant cells, we had to detect Rad22YFP using indirect immunofluorescence on nuclear spreads. We observed a modest increase in Rad22 focus formation in *mst1-L344S* cells without HU treatment, relative to wild-type cells (compare 0-hr time point of *mst1* and wild type in Figure 6F). This suggests that the cells suffer some amount of endogenous damage. During acute HU treatment, Δ *cds1* cells show a dramatic increase in Rad22YFP focus formation, consistent with replication fork collapse and double-strand breaks (MEISTER *et al.* 2005; BAILIS *et al.* 2008). There is no change in the Rad22YFP foci in *mst1-L344S*, again consistent with the model that replication forks are intact.

DISCUSSION

The MYST family of histone acetyltransferases is associated with diverse functions (reviewed in UTLEY and COTE 2003). Previously, we showed that *S. pombe mst1*⁺ is an essential gene that encodes a protein constitutively bound to the chromatin (GÓMEZ *et al.* 2005). In this report, we have characterized the phenotypes associated with deletion of *mst1*⁺ and a temperature-sensitive allele.

S. pombe Mst1 is the ortholog of *S. cerevisiae* Esa1 and human Tip60, the catalytic subunit of the NuA4 family of histone acetyltransferases, which is now referred to as

FIGURE 5.—Damage sensitivity of *mst1-L344S*. (A) *mst1* sensitivity relative to DNA-damaging agents. Exponentially growing cells were diluted fivefold on YES plates with the indicated drugs and grown 4 days at 29°. (B) *mst1* is sensitive to bleomycin. Exponentially growing cells were diluted fivefold on YES plates and grown 3 days at 32°. (C) *mst1* is sensitive to the spindle poison TBZ. Exponentially growing cells were diluted fivefold on YES plates and grown 3 days at 32°.

FIGURE 6.—*mst1-L344S* has HU recovery defect. (A and B) Time course of DNA content following release from HU at 32° (A) and 36° (B) for cells in YES. Strains: wild type (FY11), *mst1-L344S* (FY2396), $\Delta cds1$ (FY865). (C) Viability of cells following treatment with 12 mM HU at 32°. Circles, wild type; triangles, $\Delta cds1$; squares, *mst1*. Open symbols, no HU; solid symbols, 12 mM HU. (D) Morphology of cells in C released before (–HU), during (4 hr HU), and after release (2.5 hr rel). Cells were fixed in ethanol and stained with DAPI. Bar, 10 μ m. (E) Rad22YFP foci in wild-type, $\Delta cds1$, or *mst1* cells after 2 hr in HU. Rad22YFP was detected by immunofluorescence with anti-GFP antibody, which recognizes the YFP variant. (F) Fraction of cells with Rad22YFP foci at 0 or 2 hr of incubation in HU. –, negative control with no Rad22YFP. Strains: wild type (FY261), wild type with Rad22YFP (FY3436), *mst1* Rad22YFP (FY3369), $\Delta cds1$ Rad22YFP (FY3286).

the KAT5 family (reviewed in SQUATRITO *et al.* 2006; ALLIS *et al.* 2007). In addition to its presumed effects in transcriptional regulation (SMITH *et al.* 1998; ALLARD *et al.* 1999; CLARKE *et al.* 1999, 2006; DURANT and PUGH 2006), this family is implicated in checkpoint control by

direct acetylation of ATM and p53 (SUN *et al.* 2005; JIANG *et al.* 2006; SYKES *et al.* 2006; TANG *et al.* 2006), as well as in repair by direct recruitment to phosphorylated histone H2AX at double-strand breaks (BIRD *et al.* 2002; DOWNS *et al.* 2004; TAMBURINI and TYLER 2005;

TABLE 4
Summary of processes associated with *mst1*⁺ function

Associated genes	Function	<i>mst1ts</i> genetic interactions	Mst1 physical interactions
Process: DNA double-strand break repair			
Phenotypes: <i>mst1ts</i> mutant sensitivity to DNA-damaging agents MMS, bleomycin, and CPT			
<i>rhp51</i> ⁺	Homologous recombination	Yes	None (Y2H)
<i>rad22</i> ⁺	Homologous recombination	Yes	Yes (Y2H, IP)
Process: replication fork stability/recovery			
Evidence: <i>mst1ts</i> mutant is sensitive to chronic HU exposure. Increased Rad22 foci even under permissive conditions			
<i>mcm2</i> ⁺ , <i>mcm4</i> ⁺ , <i>mcm7</i> ⁺	MCM helicase, required for replication fork assembly and elongation, and recovery from HU	Yes (all)	Mcm2 (coIP)
<i>hsk1</i> ⁺	MCM kinase, required for replication initiation and HU recovery	Yes	ND
<i>pol1</i> ⁺	DNA polymerase α	Yes	ND
Process: centromere function			
Evidence: $\Delta mst1$, <i>mst1ts</i> mutants have chromosome segregation defects. <i>mst1ts</i> mutant is TBZ sensitive. No silencing defect suggests that the heterochromatin is intact in <i>mst1ts</i> .			
<i>clr3</i> ⁺	Pericentromere heterochromatin assembly, silencing, centromere cohesion, assembly of Cnp1 histone in central core	Yes (all)	ND
<i>clr4</i> ⁺			
<i>swi6</i> ⁺			
<i>cbh1</i> ⁺	Pericentromere heterochromatin assembly, assembly of Cnp1 histone in central core	None	Yes (Y2H)
<i>msc1</i> ⁺	Assembly of Cnp1 histone in central core, kinetochore assembly	None	Yes (Y2H)
<i>hir1</i> ⁺	Pericentromere heterochromatin silencing, kinetochore assembly	None	Yes (Y2H)

IP, co-immunoprecipitation. Y2H, yeast two-hybrid interaction. ND, not done.

ROBERT *et al.* 2006). Data also suggest that this family of HAT proteins acetylates histone H2AZ (BABIARZ *et al.* 2006; KEOGH *et al.* 2006; MILLAR *et al.* 2006); this histone variant is paradoxically associated with both regions of active gene transcription, as well as with centromeres and silent heterochromatin (reviewed in GUILLEMETTE and GAUDREAU 2006).

We characterized two mutations affecting *mst1*⁺ function. As we reported previously, the $\Delta mst1$ mutation causes lethality (GÓMEZ *et al.* 2005). In this report, we show that spores bearing the disruption are able to germinate and complete DNA replication. However, they show a highly disordered mitosis. We also constructed the temperature-sensitive mutant *mst1-L344S*, which contains the same lesion as a temperature-sensitive budding-yeast mutant. At the restrictive temperature, this strain is also proficient for replication, whether it is shifted asynchronously (Figure 2), released from nitrogen starvation in G₁ (supplemental Figure 1), or released from hydroxyurea in S phase (Figure 6). We observed pleiotropic phenotypes, including disordered mitosis, irregular segregation, and a high fraction of cell cycle arrest with septa. Cells arrest within one to two cell cycles with this mixed phenotype.

Our genetic analysis and subsequent two-hybrid screen suggest that Mst1 impacts three pathways (Table 4): damage response, which is consistent with the physiological analysis of the *mst1-L344S* mutant and

data from other systems; centromere function; and control of mitotic entry.

Even at permissive temperatures, *mst1-L344S* cells are sensitive to a variety of agents that disrupt the cell cycle, including bleomycin and MMS, which cause DNA damage and double-strand breaks; hydroxyurea, which causes replication fork arrest; and thiabendazole, which causes spindle depolarization. The damage sensitivity was expected because data from numerous sources link members of this family to the double-strand break response. The recruitment of KAT5 orthologs to double-strand breaks is thought to modulate the break response and perhaps downregulate the signal associated with H2AX phosphorylation by recruiting the remodelers INO80 and SWR1 and the alternative histone H2AZ (MORRISON *et al.* 2004; TSUKUDA *et al.* 2005; VAN ATTIKUM and GASSER 2005b; PAPAMICHOS-CHRONAKIS *et al.* 2006).

When *mst1-L344S* is combined with the mutations $\Delta rhp51$ (Rad51), $\Delta rad50$, or $\Delta rad22$, cells have a severe synthetic growth phenotype in which they grow more poorly than either parent. This is particularly apparent in $\Delta rad22$ *mst1-L344S*; however, because $\Delta rad22$ alone is prone to accumulate suppressors in the *fbh1*⁺ gene (OSMAN *et al.* 2005), we cannot be sure that our double mutant is not also carrying a suppressor. It is possible that $\Delta rad22$ *mst1-L344S* will be synthetically lethal but we have not been able to test this convincingly. These

genetic data suggest that *mst1-L344S* requires a functional recombination pathway for viability. To assess whether *mst1-L344S* causes damage itself, we examined focus formation of Rad22-YFP in *mst1-L344S* cells. There is an increase in foci in *mst1* compared to wild type at 32°, even in the absence of any damaging treatment (Figure 6F). This suggests that attenuation of Mst1 function results in some form of damage that requires Rad22 for repair, but also suggests that Rad22-YFP can be recruited to sites of damage in the absence of Mst1.

We observed a physical association between Mst1 and Rad22 in two-hybrid analysis and in co-immunoprecipitation. This interaction is specific to Rad22, as neither the Rad22 homolog Rtl1 nor the Rad51 homolog Rhp51 associate with Mst1 in two-hybrid tests (Figure 4). This could reflect a role in recruitment. Another possibility is that Mst1 acetylates Rad22 itself, as Tip60 does to ATM or p53, to modulate its behavior, and we will investigate this in the future.

Unexpectedly, *mst1-L344S* cells are also sensitive to hydroxyurea, which causes replication fork arrest. The mutant cells arrest properly with a 1C content without Rad22-YFP foci and appear to complete S phase following release, indicating that the checkpoint functions and the replication fork are intact; instead, they have modestly reduced viability reminiscent of $\Delta rhp51$ or $\Delta rad22$ mutants (MEISTER *et al.* 2005; BAILIS *et al.* 2008). We observe that HU sensitivity of *mst1-L344S* to HU differs if cells are exposed to low levels over a prolonged time, which causes cell death (chronic exposure, shown in Figure 5), or whether they are exposed to higher levels over a single generation (acute exposure, shown in Figure 6). Similar results have been observed for the fork protection mutant $\Delta swi1$ (ScTof1), which survives acute exposure but dies during chronic exposure (SOMMARIVA *et al.* 2005). It is also possible that this HU sensitivity is related to the interaction between *mst1* and mutations in recombination genes; however, Rad22-YFP recruitment appears largely normal in *mst1* cells treated with HU.

A second broad functional area defined by our two-hybrid and genetic interactions is centromere assembly. Fission yeast centromeres are large and complex elements, with a central core surrounded by flanking repeats that form peri-centromeric heterochromatin (reviewed in EKWALL 2007; GREWAL and JIA 2007). Mutations disrupting the heterochromatin, the central core, or the kinetochore typically show sensitivity to spindle poisons such as TBZ, and a high fraction of chromosome loss and disordered mitoses, as we observed (Figures 1, 2, and 5). Our genetic analysis suggests that mutations that disrupt normal pericentromeric heterochromatin function such as $\Delta clr3$ (histone deacetylase), $\Delta clr4$ (methyltransferase), or $\Delta swi6$ (heterochromatin protein 1) reduce the growth temperature of *mst1-L344S* quite substantially. The simplest interpretation of this epistasis experiment is that *mst1*⁺ affects a separate pathway from Clr4 and Swi6 in cen-

tromere function, leading to a synthetic effect in the double mutant. We see no loss of silencing at centromeres or telomeres in a *mst1* mutant, which suggests that Swi6-formed heterochromatin is largely intact, and some other aspect of centromere assembly and function is disrupted. A good candidate is the central core. Three of the proteins that we identified in the two-hybrid tests are linked to central core and kinetochore function:

1. We isolated Msc1, which was first identified as a high-copy suppressor of a strain lacking the checkpoint kinase Chk1 (AHMED *et al.* 2004). A recent study shows that overexpression of Msc1 rescues mutations of *cnp1* (the specialized H3-Cen/CenP of the centromere central core) in addition to $\Delta chk1$ and that this suppression requires the H2AZ histone variant encoded by *pht1*⁺ (AHMED *et al.* 2007). H2AZ is linked to heterochromatin assembly and centromere function (reviewed in GUILLEMETTE and GAUDREAU 2006) and H2AZ is a known substrate of KAT5 family HATs (BABIARZ *et al.* 2006; KEOGH *et al.* 2006; MILLAR *et al.* 2006; AUGER *et al.* 2008).
2. We isolated Cbh1, which is one of the three CenpB homologs in fission yeast (BAUM and CLARKE 2000; NAKAGAWA *et al.* 2002). The CenpB homologs are proposed to affect centromere structure (NAKAGAWA *et al.* 2002) and, in metazoans, CenpB is involved in recruitment of H3-Cen/CenP (reviewed in MEL-LONE and ALLSHIRE 2003).
3. We isolated Hip1, the HIRA/Hir1 homolog. HIR proteins are histone chaperones required for assembly of chromatin outside of S phase (reviewed in GUNJAN *et al.* 2005). In fission yeast, deletion of Hip1 causes chromosome loss, TBZ sensitivity, and disruption of centromere silencing (BLACKWELL *et al.* 2004).

HIR proteins in *S. cerevisiae* are implicated in normal kinetochore function (SHARP *et al.* 2002). In contrast to the heterochromatin mutants, we see no synthetic growth phenotypes when *mst1* is combined with mutants $\Delta cbh1$, $\Delta msc1$, or $\Delta hip1$. This would be consistent with *mst1*⁺ acting in a pathway with these genes. Together with the segregation defects of *mst1-L344S*, these two hybrid interactions suggest that Mst1 may affect the centromere central core. We are examining mitotic phenotypes of *mst1* in more detail to determine how this may occur and the role of H2AZ in this process.

There is indirect evidence that Mst1 may affect mitotic entry. We isolated the Skb1 methyltransferase in our two-hybrid screen. Budding yeast Hsl7, the ortholog of Skb1, was identified because mutations of *hsl7* are synthetically lethal with histone tail mutants (MA *et al.* 1996); genetic interactions between *ScΔhsl7* and *ScEsa1* suggest that histone tail modifications are important for normal mitosis when Swe1 kinase is activated (RUAULT and PILLUS 2006). While we observed no genetic interaction between *SpΔskb1* and our

temperature-sensitive allele of *mst1*, the physical interaction that we observed, together with the genetic data from budding yeast, suggests that there is a role for histone tail modification in the proper regulation of mitotic entry in both species. Another link to CDK activation is provided by our isolation of Hip1 as a putative interactor. The Δ *hip1* mutation is synthetically lethality with *cdc25-22*, which is a temperature-sensitive mutation in the mitotic inducer Cdc25 that opposes Wee1 (ScSwe1) (BLACKWELL *et al.* 2004; reviewed in KARLSSON-ROSENTHAL and MILLAR 2006).

We did not isolate any of the NuA4 complex proteins in our two-hybrid screen, although the previously characterized SpAlp5/ScArp4 is known to interact with Mst1 by co-immunoprecipitation (MINODA *et al.* 2005). This is not surprising as the two-hybrid screen identifies binary associations, and many of the interactions in the large NuA4 complex are likely to be indirect (AUGER *et al.* 2008). We speculate that the structure of the two-hybrid bait construct, in which the amino-terminus of Mst1 is fused to the DNA-binding domain of Gal4, may interfere with the assembly of NuA4 subunits with Mst1. Conversely, we did not observe co-immunoprecipitation of epitope-tagged Mst1 with most of the two-hybrid clones. This could reflect chromatin association of the proteins involved, temporally or spatially limiting association, or could indicate that the C-terminal epitope tag on Mst1 is not available when the protein is in a complex with other factors. A more detailed structure-function analysis and localization studies are in progress to resolve these observations.

The interactions that we observed, in addition to the phenotypes associated with the *mst1* mutants, suggest that Mst1 affects a variety of cell functions. Importantly, the recent discovery that Tip60 can acetylate nonhistone proteins in the absence of the NuA4 complex (*e.g.*, SUN *et al.* 2005; SYKES *et al.* 2006; TANG *et al.* 2006; KIM *et al.* 2007) indicates that the identification of Mst1 as a histone acetyltransferase may be too limiting a definition. We speculate that the pleiotropic effects of *mst1* mutants indicate a variety of functions that may be directly modulated by Mst1 catalytic activity, not necessarily linked to histone acetylation. Our study suggests that Mst1 may have functions in replication fork stability and in centromere assembly, and experiments are underway to determine whether these effects are mediated by histone modifications or by other aspects of Mst1 function.

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LITERATURE CITED

- AHMED, S., C. PALERMO, S. WAN and N. C. WALWORTH, 2004 A novel protein with similarities to Rb binding protein 2 compensates for loss of Chk1 function and affects histone modification in fission yeast. *Mol. Cell. Biol.* **24**: 3660–3669.
- AHMED, S., B. E. DUL, X. QIU and N. C. WALWORTH, 2007 Msc1 acts through histone H2A.Z to promote chromosome stability in *Schizosaccharomyces pombe*. *Genetics* **177**: 1487–1497.
- ALLARD, S., R. T. UTLEY, J. SAVARD, A. CLARKE, P. GRANT *et al.*, 1999 NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related co-factor Tralp. *EMBO J.* **18**: 5108–5119.
- ALLIS, C. D., S. L. BERGER, J. COTE, S. DENT, T. JENUWIEN *et al.*, 2007 New nomenclature for chromatin modifying enzymes. *Cell* **131**: 633–636.
- AUGER, A., L. GALARNEAU, M. ALTAFF, A. NOURANI, Y. DOYON *et al.*, 2008 Eaf1 is the platform for NuA4 molecular assembly that evolutionarily links chromatin acetylation to ATP-dependent exchange of histone H2A variants. *Mol. Cell. Biol.* **28**: 2257–2270.
- BABIARZ, J. E., J. E. HALLEY and J. RINE, 2006 Telomeric heterochromatin boundaries require NuA4-dependent acetylation of histone variant H2A.Z in *Saccharomyces cerevisiae*. *Genes Dev.* **20**: 700–710.
- BAILIS, J. M., D. D. LUCHE, T. HUNTER and S. L. FORSBURG, 2008 MCM proteins interact with checkpoint and recombination proteins to promote S phase genome stability. *Mol. Cell. Biol.* **28**: 1724–1738.
- BAUM, M., and L. CLARKE, 2000 Fission yeast homologs of human CENP-B have redundant functions affecting cell growth and chromosome segregation. *Mol. Cell. Biol.* **20**: 2852–2864.
- BIRD, A. W., D. Y. YU, M. G. PRAY-GRANT, Q. QIU, K. E. HARMON *et al.*, 2002 Acetylation of histone H4 by Esa1 is required for DNA double-strand break repair. *Nature* **419**: 411–415.
- BLACKWELL, C., K. A. MARTIN, A. GREENALL, A. PIDOUX, R. C. ALLSHIRE *et al.*, 2004 The *Schizosaccharomyces pombe* HIRA-like protein Hip1 is required for the periodic expression of histone genes and contributes to the function of complex centromeres. *Mol. Cell. Biol.* **24**: 4309–4320.
- BOUDREAULT, A. A., D. CRONIER, W. SELLECK, N. LACOSTE, R. T. UTLEY *et al.*, 2003 Yeast enhancer of polycomb defines global Esa1-dependent acetylation of chromatin. *Genes Dev.* **17**: 1415–1428.
- BRANZEI, D., and M. FOIANI, 2007 Interplay of replication checkpoints and repair proteins at stalled replication forks. *DNA Rep.* **6**: 994–1003.
- BURKE, T. W., J. COOKS-GOWEN, M. ASANO and J. R. NEVINS, 2001 Replication factors MCM2 and ORC1 interact with the histone acetyltransferase HBO1. *J. Biol. Chem.* **276**: 15397–15408.
- CASPARI, T., J. M. MURRAY and A. M. CARR, 2002 Cdc2-cyclin B kinase activity links Crb2 and Rqh1-topoisomerase III. *Genes Dev.* **16**: 1195–1208.
- CATLETT, M. G., and S. L. FORSBURG, 2003 *Schizosaccharomyces pombe* Rdh54 (TID1) acts with Rhp54 (RAD54) to repair meiotic double-strand breaks. *Mol. Biol. Cell* **14**: 4707–4720.
- CLARKE, A. S., J. E. LOWELL, S. J. JACOBSON and L. PILLUS, 1999 Esa1p is an essential histone acetyltransferase required for cell cycle progression. *Mol. Cell. Biol.* **19**: 2515–2526.
- CLARKE, A. S., E. SAMAL and L. PILLUS, 2006 Distinct roles for the essential MYST family HAT Esa1p in transcriptional silencing. *Mol. Biol. Cell* **17**: 1744–1757.
- DECKERT, J., and K. STRUHL, 2001 Histone acetylation at promoters is differentially affected by specific activators and repressors. *Mol. Cell. Biol.* **21**: 2726–2735.
- DE LA CRUZ, X., S. LOIS, S. SANCHEZ-MOLINA and M. A. MARTINEZ-BALBAS, 2005 Do protein motifs read the histone code? *Bio-Essays* **27**: 164–175.

- DOWNES, J. A., S. ALLARD, O. JOBIN-ROBITAILLE, A. JAVAHERI, A. AUGER *et al.*, 2004 Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. *Mol. Cell* **16**: 979–990.
- DU, L. L., T. M. NAKAMURA, B. A. MOSER and P. RUSSELL, 2003 Retention but not recruitment of Crb2 at double-strand breaks requires Rad1 and Rad3 complexes. *Mol. Cell. Biol.* **23**: 6150–6158.
- DUL, B. E., and N. C. WALWORTH, 2007 The plant homeodomain fingers of fission yeast Msc1 exhibit E3 ubiquitin ligase activity. *J. Biol. Chem.* **282**: 18397–18406.
- DURANT, M., and B. F. PUGH, 2006 Genome-wide relationships between TAF1 and histone acetyltransferases in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **26**: 2791–2802.
- EARLY, A., L. S. DRURY and J. F. DIFFLEY, 2004 Mechanisms involved in regulating DNA replication origins during the cell cycle and in response to DNA damage. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **359**: 31–38.
- EBERHARTER, A., R. FERREIRA and P. BECKER, 2005 Dynamic chromatin: concerted nucleosome remodelling and acetylation. *Biol. Chem.* **386**: 745–751.
- EKWALL, K., 2007 Epigenetic control of centromere behavior. *Annu. Rev. Genet.* **41**: 63–81.
- FORSBURG, S. L., and N. RHIND, 2006 Basic methods for fission yeast. *Yeast* **23**: 173–183.
- FOSTER, E. R., and J. A. DOWNES, 2005 Histone H2A phosphorylation in DNA double-strand break repair. *FEBS J.* **272**: 3231–3240.
- FUJITA, A., A. TONOUCHI, T. HIROKO, F. INOSE, T. NAGASHIMA *et al.*, 1999 Hsl7p, a negative regulator of Ste20p protein kinase in the *Saccharomyces cerevisiae* filamentous growth-signaling pathway. *Proc. Natl. Acad. Sci. USA* **96**: 8522–8527.
- GALARNEAU, L., A. NOURANI, A. A. BOUDREAU, Y. ZHANG, L. HELIOT *et al.*, 2000 Multiple links between the NuA4 histone acetyltransferase complex and epigenetic control of transcription. *Mol. Cell* **5**: 927–937.
- GILBRETH, M., P. YANG, D. WANG, J. FROST, A. POLVERINO *et al.*, 1996 The highly conserved skb1 gene encodes a protein that interacts with Shk1, a fission yeast Ste20/PAK homolog. *Proc. Natl. Acad. Sci. USA* **93**: 13802–13807.
- GLYNN, J. M., R. J. LUSTIG, A. BERLIN and F. CHANG, 2001 Role of bud6p and tealp in the interaction between actin and microtubules for the establishment of cell polarity in fission yeast. *Curr. Biol.* **11**: 836–845.
- GÓMEZ, E. G., and S. L. FORSBURG, 2003 Analysis of *Schizosaccharomyces pombe* cell cycle, pp. 93–111 in *Cell Cycle Checkpoint Protocols*, edited by H. G. LIEBERMAN. Humana Press, Tatawa, NJ.
- GÓMEZ, E. G., M. G. CATLETT and S. L. FORSBURG, 2002 Different phenotypes *in vivo* are associated with ATPase motif mutations in *Schizosaccharomyces pombe* minichromosome maintenance proteins. *Genetics* **160**: 1305–1318.
- GÓMEZ, E. B., J. ESPINOSA and S. L. FORSBURG, 2005 *S. pombe* mst2+ encodes a MYST-family histone acetyltransferase required for telomere silencing. *Mol. Cell. Biol.* **25**: 8887–8903.
- GREWAL, S. I., and S. JIA, 2007 Heterochromatin revisited. *Nat. Rev. Genet.* **8**: 35–46.
- GUILLETTE, B., and L. GAUDREAU, 2006 Reuniting the contrasting functions of H2A.Z. *Biochem. Cell Biol.* **84**: 528–535.
- GUNJAN, A., J. PAIK and A. VERREAU, 2005 Regulation of histone synthesis and nucleosome assembly. *Biochimie* **87**: 625–635.
- IIZUKA, M., and B. STILLMAN, 1999 Histone acetyltransferase HBO1 interacts with the ORC1 subunit of the human initiator protein. *J. Biol. Chem.* **274**: 23027–23034.
- IIZUKA, M., T. MATSUI, H. TAKISAWA and M. M. SMITH, 2006 Regulation of replication licensing by acetyltransferase Hbo1. *Mol. Cell. Biol.* **26**: 1098–1108.
- IKURA, T., V. V. OGRYZKO, M. GRIGORIEV, R. GROISMAN, J. WANG *et al.*, 2000 Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis. *Cell* **102**: 463–473.
- JENUWEIN, T., and C. D. ALLIS, 2001 Translating the histone code. *Science* **293**: 1074–1080.
- JIANG, X., Y. SUN, S. CHEN, K. ROY and B. D. PRICE, 2006 The FATC domains of PIKK proteins are functionally equivalent and participate in the Tip60-dependent activation of DNA-PKcs and ATM. *J. Biol. Chem.* **281**: 15741–15746.
- KARLSSON-ROSENTHAL, C., and J. B. MILLAR, 2006 Cdc25: mechanisms of checkpoint inhibition and recovery. *Trends Cell Biol.* **16**: 285–292.
- KEOGH, M. C., T. A. MENNELLA, C. SAWA, S. BERTHELET, N. J. KROGAN *et al.*, 2006 The *Saccharomyces cerevisiae* histone H2A variant Htz1 is acetylated by NuA4. *Genes Dev.* **20**: 660–665.
- KIM, M. Y., E. J. ANN, J. Y. KIM, J. S. MO, J. H. PARK *et al.*, 2007 Tip60 histone acetyltransferase acts as a negative regulator of Notch1 signaling by means of acetylation. *Mol. Cell. Biol.* **27**: 6506–6519.
- KOUZARIDES, T., 2007 Chromatin modifications and their function. *Cell* **128**: 693–705.
- LAFON, A., C. S. CHANG, E. M. SCOTT, S. J. JACOBSON and L. PILLUS, 2007 MYST opportunities for growth control: yeast genes illuminate human cancer gene functions. *Oncogene* **26**: 5373–5384.
- LEE, J. H., J. R. COOK, B. P. POLLACK, T. G. KINZY, D. NORRIS *et al.*, 2000 Hsl7p, the yeast homologue of human JBP1, is a protein methyltransferase. *Biochem. Biophys. Res. Commun.* **274**: 105–111.
- LEE, K. K., and J. L. WORKMAN, 2007 Histone acetyltransferase complexes: one size doesn't fit all. *Nat. Rev. Mol. Cell Biol.* **8**: 284–295.
- LE MASSON, I., D. Y. YU, K. JENSEN, A. CHEVALIER, R. COURBEYRETTE *et al.*, 2003 Yaf9, a novel NuA4 histone acetyltransferase subunit, is required for the cellular response to spindle stress in yeast. *Mol. Cell. Biol.* **23**: 6086–6102.
- LEVERSON, J. D., H. K. HUANG, S. L. FORSBURG and T. HUNTER, 2002 The *Schizosaccharomyces pombe* aurora-related kinase Ark1 interacts with the inner centromere protein Pic1 and mediates chromosome segregation and cytokinesis. *Mol. Biol. Cell* **13**: 1132–1143.
- MA, X. J., Q. LU and M. GRUNSTEIN, 1996 A search for proteins that interact genetically with histone H3 and H4 amino termini uncovers novel regulators of the Swe1 kinase in *Saccharomyces cerevisiae*. *Genes Dev.* **10**: 1327–1340.
- MEISTER, P., A. TADDEI, L. VERNIS, M. POIDEVIN, S. M. GASSER *et al.*, 2005 Temporal separation of replication and recombination requires the intra-S checkpoint. *J. Cell Biol.* **168**: 537–544.
- MELLONE, B. G., and R. C. ALLSHIRE, 2003 Stretching it: putting the CEN(P-A) in centromere. *Curr. Opin. Genet. Dev.* **13**: 191–198.
- MILLAR, C. B., and M. GRUNSTEIN, 2006 Genome-wide patterns of histone modifications in yeast. *Nat. Rev. Mol. Cell Biol.* **7**: 657–666.
- MILLAR, C. B., F. XU, K. ZHANG and M. GRUNSTEIN, 2006 Acetylation of H2AZ Lys 14 is associated with genome-wide gene activity in yeast. *Genes Dev.* **20**: 711–722.
- MINODA, A., S. SAITOH, K. TAKAHASHI and T. TODA, 2005 BAF53/Arp4 homolog Alp5 in fission yeast is required for histone H4 acetylation, kinetochore-spindle attachment, and gene silencing at centromere. *Mol. Biol. Cell* **16**: 316–327.
- MIYAMOTO, M., K. TANAKA and H. OKAYAMA, 1994 res2+, a new member of the *cdc10*⁺/SWI4 family, controls the 'start' of mitotic and meiotic cycles in fission yeast. *EMBO J.* **13**: 1873–1880.
- MORENO, S., A. KLAR and P. NURSE, 1991 Molecular genetic analysis of the fission yeast *Schizosaccharomyces pombe*. *Methods Enzymol.* **194**: 795–823.
- MORRISON, A. J., and X. SHEN, 2005 DNA repair in the context of chromatin. *Cell Cycle* **4**: 568–571.
- MORRISON, A. J., J. HIGHLAND, N. J. KROGAN, A. ARBEL-EDEN, J. F. GREENBLATT *et al.*, 2004 INO80 and gamma-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair. *Cell* **119**: 767–775.
- NAKAGAWA, H., J. K. LEE, J. HURWITZ, R. C. ALLSHIRE, J. NAKAYAMA *et al.*, 2002 Fission yeast CENP-B homologs nucleate centromeric heterochromatin by promoting heterochromatin-specific histone tail modifications. *Genes Dev.* **16**: 1766–1778.
- OSMAN, F., J. DIXON, A. R. BARR and M. C. WHITBY, 2005 The F-Box DNA helicase Fbh1 prevents Rhp51-dependent recombination without mediator proteins. *Mol. Cell. Biol.* **25**: 8084–8096.
- OSTERMANN, K., A. LORENTZ and H. SCHMIDT, 1993 The fission yeast rad22 gene, having a function in mating-type switching and repair of DNA damages, encodes a protein homolog to rad52 of *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **21**: 5940–5944.
- PANKRATZ, D. G., and S. L. FORSBURG, 2005 Meiotic S-phase damage activates recombination without checkpoint arrest. *Mol. Biol. Cell* **16**: 1651–1660.
- PAPAMICHOS-CHRONAKIS, M., J. E. KREBS and C. L. PETERSON, 2006 Interplay between Ino80 and Swr1 chromatin remodeling enzymes regulates cell cycle checkpoint adaptation in response to DNA damage. *Genes Dev.* **20**: 2437–2449.

- ROBERT, F., S. HARDY, Z. NAGY, C. BALDEYRON, R. MURR *et al.*, 2006 The transcriptional histone acetyltransferase cofactor TRRAP associates with the MRN repair complex and plays a role in DNA double-strand break repair. *Mol. Cell. Biol.* **26**: 402–412.
- RUALT, M., and L. PILLUS, 2006 Chromatin-modifying enzymes are essential when the *Saccharomyces cerevisiae* morphogenesis checkpoint is constitutively activated. *Genetics* **174**: 1135–1149.
- SELLECK, W., I. FORTIN, D. SERMWITTAYAWONG, J. COTE and S. TAN, 2005 The *Saccharomyces cerevisiae* Piccolo NuA4 histone acetyltransferase complex requires the Enhancer of Polycomb A domain and chromodomain to acetylate nucleosomes. *Mol. Cell. Biol.* **25**: 5535–5542.
- SHARP, J. A., A. A. FRANCO, M. A. OSLEY and P. D. KAUFMAN, 2002 Chromatin assembly factor I and Hir proteins contribute to building functional kinetochores in *S. cerevisiae*. *Genes Dev.* **16**: 85–100.
- SHERMAN, D. A., S. G. PASION and S. L. FORSBURG, 1998 Multiple domains of fission yeast Cdc19p (MCM2) are required for its association with the core MCM complex. *Mol. Biol. Cell* **9**: 1833–1845.
- SHOGREN-KNAK, M., H. ISHII, J. M. SUN, M. J. PAZIN, J. R. DAVIE *et al.*, 2006 Histone H4–K16 acetylation controls chromatin structure and protein interactions. *Science* **311**: 844–847.
- SHULEWITZ, M. J., C. J. INOUE and J. THORNER, 1999 Hsl7 localizes to a septin ring and serves as an adapter in a regulatory pathway that relieves tyrosine phosphorylation of Cdc28 protein kinase in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **19**: 7123–7137.
- SMITH, E. R., A. EISEN, W. GU, M. SATTAR, A. PANNUTI *et al.*, 1998 ESA1 is a histone acetyltransferase that is essential for growth in yeast. *Proc. Natl. Acad. Sci. USA* **95**: 3561–3565.
- SOMMARIVA, E., T. K. PELLNY, N. KARAHAN, S. KUMAR, J. A. HUBERMAN *et al.*, 2005 *Schizosaccharomyces pombe* Swi1, Swi3, and Hsk1 are components of a novel S-phase response pathway to alkylation damage. *Mol. Cell. Biol.* **25**: 2770–2784.
- SQUATRITO, M., C. GORRINI and B. AMATI, 2006 Tip60 in DNA damage response and growth control: many tricks in one HAT. *Trends Cell Biol.* **16**: 433–442.
- SUN, Y., X. JIANG, S. CHEN, N. FERNANDES and B. D. PRICE, 2005 A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. *Proc. Natl. Acad. Sci. USA* **102**: 13182–13187.
- SYKES, S. M., H. S. MELLERT, M. A. HOLBERT, K. LI, R. MARMORSTEIN *et al.*, 2006 Acetylation of the p53 DNA-binding domain regulates apoptosis induction. *Mol. Cell* **24**: 841–851.
- TAMBURINI, B. A., and J. K. TYLER, 2005 Localized histone acetylation and deacetylation triggered by the homologous recombination pathway of double-strand DNA repair. *Mol. Cell. Biol.* **25**: 4903–4913.
- TANAKA, K., K. OKAZAKI, N. OKAZAKI, T. UEDA, A. SUGIYAMA *et al.*, 1992 A new cdc gene required for S phase entry of *Schizosaccharomyces pombe* encodes a protein similar to the *cdc10⁺* and *SWI4* gene products. *EMBO J.* **11**: 4923–4932.
- TANG, Y., J. LUO, W. ZHANG and W. GU, 2006 Tip60-dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis. *Mol. Cell* **24**: 827–839.
- TSUKUDA, T., A. B. FLEMING, J. A. NICKOLOFF and M. A. OSLEY, 2005 Chromatin remodelling at a DNA double-strand break site in *Saccharomyces cerevisiae*. *Nature* **438**: 379–383.
- UENO, M., R. KUROKAWA, H. RENAULD, K. WATANABE, T. USHIMARU *et al.*, 2001 *Schizosaccharomyces pombe* taf1+ is required for nitrogen starvation-induced sexual development and for entering the dormant GO state. *Curr. Genet.* **38**: 307–313.
- UTLEY, R. T., and J. COTE, 2003 The MYST family of histone acetyltransferases. *Curr. Top. Microbiol. Immunol.* **274**: 203–236.
- VAN ATTIKUM, H., and S. M. GASSER, 2005a ATP-dependent chromatin remodeling and DNA double-strand break repair. *Cell Cycle* **4**: 1011–1014.
- VAN ATTIKUM, H., and S. M. GASSER, 2005b The histone code at DNA breaks: A guide to repair? *Nat. Rev. Mol. Cell Biol.* **6**: 757–765.
- VOGELAUER, M., L. RUBBI, I. LUCAS, B. J. BREWER and M. GRUNSTEIN, 2002 Histone acetylation regulates the time of replication origin firing. *Mol. Cell* **10**: 1223–1233.
- YU, Y., Y. TENG, H. LIU, S. H. REED and R. WATERS, 2005 UV irradiation stimulates histone acetylation and chromatin remodeling at a repressed yeast locus. *Proc. Natl. Acad. Sci. USA* **102**: 8650–8655.
- ZHU, Y., T. TAKEDA, K. NASMYTH and N. JONES, 1994 *pct1⁺*, which encodes a new DNA-binding partner of p85^{cdc10}, is required for meiosis in the fission yeast *Schizosaccharomyces pombe*. *Genes Dev.* **8**: 885–898.

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