Copyright © 2008 by the Genetics Society of America
DOI: 10.1534/genetics.108.088914

Histone H3 K56 Hyperacetylation Perturbs Replisomes and Causes DNA Damage

Ivana Celic,*,† Alain Verreault‡ and Jef D. Boeke*,†,1

**High Throughput Biology Center and †Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205 and ‡Institute for Research in Immunology and Cancer, Département de Pathologie et de Biologie Cellulaire, Université de Montreal, Montreal, Quebec H3C 3J7, Canada*

Manuscript received March 7, 2008
Accepted for publication May 7, 2008

Downloaded from https://academic.oup.com/genetics/article/179/4/1769/6064715 by guest on 23 April 2024

ABSTRACT

Deacetylation of histone H3 K56, regulated by the sirtuins Hst3p and Hst4p, is critical for maintenance of genomic stability. However, the physiological consequences of a lack of H3 K56 deacetylation are poorly understood. Here we show that cells lacking Hst3p and Hst4p, in which H3 K56 is constitutively hyperacetylated, exhibit hallmarks of spontaneous DNA damage, such as activation of the checkpoint kinase Rad53p and upregulation of DNA-damage inducible genes. Consistently, *hst3 hst4* cells display synthetic lethality interactions with mutations that cripple genes involved in DNA replication and DNA double-strand break (DSB) repair. In most cases, synthetic lethality depends upon hyperacetylation of H3 K56 because it can be suppressed by mutation of K56 to arginine, which mimics the nonacetylated state. We also show that *hst3 hst4* phenotypes can be suppressed by overexpression of the PCNA clamp loader large subunit, Rfc1p, and by inactivation of the alternative clamp loaders *CTF18*, *RAD24*, and *ELG1*. Loss of *CTF4*, encoding a replisome component involved in sister chromatid cohesion, also suppresses *hst3 hst4* phenotypes. Genetic analysis suggests that *CTF4* is a part of the K56 acetylation pathway that converges on and modulates replisome function. This pathway represents an important mechanism for maintenance of genomic stability and depends upon proper regulation of H3 K56 acetylation by Hst3p and Hst4p. Our data also suggest the existence of a precarious balance between Rfc1p and the other RFC complexes and that the nonreplicative forms of RFC are strongly deleterious to cells that have genomewide and constitutive H3 K56 hyperacetylation.

GENOMIC stability is maintained by a complex interplay of DNA replication, repair, and checkpoint signaling. These processes play central roles in the maintenance of genomic stability but their mode of action in the context of chromatin is poorly understood. Newly synthesized histones deposited during DNA replication are acetylated at their N termini and in the core region (Jackson *et al.* 1976; Sobel *et al.* 1995; Hyland *et al.* 2005; Masumoto *et al.* 2005; Ozdemir *et al.* 2005; Xu *et al.* 2005; Ye *et al.* 2005). Lysine K56 acetylation is present on newly synthesized histone H3 and implicated in the DNA damage response (Hyland *et al.* 2005; Masumoto *et al.* 2005). This modification accumulates during S phase and is then removed either prior to or during mitosis (Masumoto *et al.* 2005) in a process regulated by the redundant yeast sirtuins, Hst3p and Hst4p (Celic *et al.* 2006; Maas *et al.* 2006). In *hst3 hst4* mutants, K56 acetylation is observed in virtually 100% of histones throughout the cell cycle (Celic *et al.* 2006), although Sir2p does also contribute to deacetylation of H3 K56 in telomeric regions (Xu *et al.* 2007a).

Hst3p and Hst4p belong to a highly conserved family of NAD^+-dependent protein deacetylases, known as the Sir2 protein family or sirtuins (Brachmann *et al.* 1995; Imai *et al.* 2000; Landry *et al.* 2000; Smith *et al.* 2000). The importance of K56 deacetylation is evident from the high level of genomic instability observed in *hst3 hst4* cells. Cells lacking *HST3* and *HST4* show a plethora of chromatin-associated phenotypes (Brachmann *et al.* 1995) resulting from hyperacetylation of K56 in H3; mutation of K56 to arginine (K56R) suppresses nearly all these *hst3 hst4* phenotypes (Celic *et al.* 2006; Maas *et al.* 2006).

hst3 hst4 cells also accumulate spontaneous suppressors at a high rate (Brachmann *et al.* 1995) and the majority of these suppressors appear to adapt to the high level of K56 acetylation rather than preventing acetylation (Miller *et al.* 2006). We show here that *hst3 hst4* phenotypes are alleviated by overexpression of *RFC1*, encoding the large subunit of the clamp loader (Howell *et al.* 1994), supporting the notion that the inability to deacetylate K56 interferes with normal DNA replication. These phenotypes are also suppressed by

[1]*Corresponding author:* Johns Hopkins University School of Medicine, 339 Broadway Research Bldg., 733 N. Broadway, Baltimore, MD 21205. E-mail: jboeke@jhmi.edu

inactivation of alternative clamp-loading complexes and by deletion of *CTF4*. We propose that *CTF4*, together with *ASF1* (Celic *et al.* 2006; Recht *et al.* 2006), *RTT109* (Schneider *et al.* 2006; Driscoll *et al.* 2007; Han *et al.* 2007), and *HST3/HST4* define a K56 acetylation/deacetylation pathway important for the survival of replication-linked lesions induced by genotoxic agents or by collision of the replication fork with DNA-protein barriers that impinges upon clamp-loading complexes. We also show that cells lacking Hst3p and Hst4p activate a DNA damage checkpoint response due to the presence of chronic DNA damage. This is a direct consequence of K56 hyperacetylation. We show here that cells lacking Hst3p and Hst4p depend on a functional DNA damage checkpoint and a subset of repair factors for viability.

MATERIALS AND METHODS

Strains, growth conditions, and plasmids: All strains used in this work are described in Table 1. They were generated by standard methods and grown under standard conditions unless otherwise noted. The pCEN-*URA3-HST3* plasmid was previously described (Celic *et al.* 2006). YEP351/*RFC1* is YEP351 (Hill *et al.* 1986) carrying an insert corresponding to chromosome *XV* coordinates 748644–752328 (high-copy library isolate carrying the *RFC1* gene). Plasmid pJP16 is pCEN-LEU2-HHT2/HHF2. Derivative pCEN-LEU2-H3K56R was created by subcloning a *Sac*I/*Xho*I insert from pDM18K56R (Park *et al.* 2002) into pRS415 (Sikorski and Hieter 1989).

High-copy suppressor screen: The high-copy suppressor screen was done by transforming strain YCB828 (relevant genotype *hst1 hst2 hst3 hst4 sir2 leu2 ura3*) with a *Saccharomyes cerevisiae* genomic 2μ *LEU2* library and selecting transformants on synthetic complete (SC) −Ura −Leu. Transformants were subsequently replica-plated on SC −Leu plates to segregate the resident *CEN-URA3-HST3* vector and then replica-plated onto SC +5-FOA plates to select for the colonies that had lost the *CEN-URA3-HST3* vector, but were able to support growth due to the presence of the library vector. Leu^+, Ura^- colonies were additionally tested through a plasmid segregation test, colony PCR to eliminate high-copy plasmids containing *SIR2*, *HST3*, and *HST4*, and finally through a retransformation assay. With this procedure we screened ~20,000 Leu^+ Ura^+ colonies and isolated 82 5-FOA-resistant colonies. Twenty-five of these showed 5-FOA resistance dependent on the library plasmid. We obtained *HST3* seven times, *SIR2* six times, *RFC1* three times, *FKH1* two times, and *UBP10* two times as high-copy suppressors.

Cell synchronization and FACS analysis: Cells grown at 25° in YPD medium were arrested in G_1 using 0.3 μM α-factor for 3 hr. Cells were released into the cell cycle by washing with 3–4 culture volumes of YPD and resuspending in fresh YPD medium with 0.1 mg/ml pronase (Sigma). Aliquots were collected at the indicated time intervals. DNA content was determined by flow cytometry with propidium iodide (Haase and Lew 1997).

Immunofluorescence: Cells were processed as previously described (Pringle *et al.* 1991). Mouse anti-tubulin antibody (Sigma) was used at a 1:1000 dilution. Sheep anti-mouse secondary antibody (Amersham) was used at a 1:5000 dilution.

RNA isolation, Northern blot analysis, and microarray hybrdization: Total RNA was isolated using the hot-acid phenol method. Probes for Northern blot were prepared by a random priming method using the Prime-It II kit (Stratagene). Total RNA was separated on 1% agarose-formaldehyde gel and hybridized to the probe using Ultrahybe hybridization solution (Ambion) according to the manufacturer's instructions. Microarray hybridization and data analysis were performed at the Johns Hopkins Microarray Core Facility (http://www.microarray.jhmi.edu). The raw data are deposited at Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo); the accession number is pending. Whole-cell lysates for immunoblotting were prepared for SDS–PAGE using an alkaline method (Kushnirov 2000). For immunoblotting of Rad53p, lysates were prepared as described (Gardner *et al.* 2005). Depending on the experiment, lysates from 2.5×10^6 to 1×10^7 cells were resolved in SDS 4–20% (histones) or 8% (Rad53p) polyacrylamide gels and transferred to a PVDF membrane (Amersham). The blots were probed with rabbit polyclonal antibodies against the C terminus of histone H3 (Gunjan and Verreault 2003), K56-acetylated H3 (Masumoto *et al.* 2005), or Rad53p (Santa Cruz), followed by horseradish peroxidase (HRP)-conjugated antibody against rabbit IgGs (Amersham) and chemiluminescence detection (Amersham). As previously described (Masumoto *et al.* 2005), immunoblots to detect histone H3 K56 acetylation were performed with a 1000-fold molar excess of unacetylated peptide over the affinity-purified antibody to ensure that observed signals were specific for K56-acetylated histone H3.

RESULTS

Overexpression of Rfc1p, the large subunit of the DNA clamp loader, suppresses phenotypes of *hst3 hst4* cells: Considering that phenotypes of *hst3 hst4* cells arise from K56 hyperacetylation and that the majority of spontaneous suppressors appear to adapt to the high levels of K56 acetylation (K56Ac), we have performed a genetic screen to isolate *hst3 hst4* suppressors to uncover pathways that function aberrantly in the presence of K56 hyperacetylation, We conducted a high-copy screen for suppressors of the synthetic lethality of a *hst1 hst2 hst3 hst4 sir2* strain (Brachmann 1996) and isolated a plasmid that contained a full-length *RFC1* gene. Rfc1p is the large subunit of the "clamp loader," which loads the PCNA clamp onto DNA during replication (Howell *et al.* 1994). The only other full-length ORF within this clone was the dubious ORF *YOR218C*, overlapping the 3′ end of *RFC1*. We introduced a deletion in the *RFC1* ORF (from +230 to +1311) and showed that this clone lost the ability to suppress *hst1 hst2 hst3 hst4 sir2* synthetic lethality (data not shown), confirming that *RFC1* is indeed a high-copy suppressor of *hst1 hst2 hst3 hst4 sir2* synthetic lethality. We subsequently tested whether overexpression of *RFC1* suppresses the Ts phenotype of *hst3 hst4* mutant cells. *hst3 hst4* cells carrying the *HST3* gene on a *URA3*-marked plasmid do not grow at 37° on 5-FOA medium. This lack of growth was suppressed by a high-copy plasmid carrying *RFC1* (Figure 1A). In addition to the suppression of the Ts phenotype, overexpression of *RFC1* partially suppresses the sensitivity of *hst3 hst4* cells to several genotoxic agents. Overexpression of *RFC1* suppressed

Downloaded from https://academic.oup.com/genetics/article/179/4/1769/6064715 by guest on 23 April 2024

TABLE 1

Strain list

Strain	Genotype
YCB617	MAT**a** his3Δ200 leu2Δ::TRP1 lys2Δ202 trp1Δ63 ura3-52 (Brachmann *et al.* 1995)
YCB470	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::TRP1 (Brachmann *et al.* 1995)
YCB575	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst4Δ::TRP1 (Brachmann *et al.* 1995)
YCB828	MATα ura3-52 trp1Δ63 leu2Δ1 his3Δ200 lys2-801 ade2Δ::hisG sir2Δ2::TRP1 hst1Δ3::TRP1 hst2Δ2::TRP1 hst3Δ3::TRP1 hst4Δ1::TRP1 pCAR202 pCEN-URA3-HST3 (Brachmann 1996)
ICY48	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 bar1Δ::hygMX (Celic *et al.* 2006)
ICY49	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 bar1Δ::hygMX hst3Δ::HIS3 hst4Δ::TRP1
ICY188	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 pRS416 YEP351
ICY189	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 pRS416 YEP351/RFC1
ICY190	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 pCEN-URA3-HST3 YEP351
ICY191	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 pCEN-URA3-HST3 YEP351/RFC1 isolate 1
ICY192	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 pCEN-URA3-HST3 YEP351/RFC1 isolate 2
ICY230	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 dun1Δ::kanMX4 pCEN-URA3-HST3
ICY252	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::TRP1 hst4Δ::TRP1 pCEN-URA3-HST3
ICY342	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::TRP1 hst4Δ::TRP1 rad24Δ::kanMX4 pCEN-URA3-HST3
ICY351	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 sml1Δ::kanMX4 rad53Δ::hygMX pCEN-URA3-HST3
ICY356	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 pRS416 (Celic *et al.* 2006)
ICY356a	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 pRS415 pRS416
ICY410	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::TRP1 hst4Δ::TRP1 bub2Δ::kanMX4 pCEN-URA3-HST3
ICY430	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 sml1Δ::kanMX4 pCEN-URA3-HST3
ICY431	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 sml1Δ::kanMX4 mec1Δ::hygMX pCEN-URA3-HST3
ICY431a	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 sml1Δ::kanMX4 mec1Δ::hygMX pCEN-URA3-HST3 pRS415
ICY431b	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 sml1Δ::kanMX4 mec1Δ::hygMX pCEN-URA3-HST3 pJP16
ICY431c	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 sml1Δ::kanMX4 mec1Δ::hygMX pCEN-URA3-HST3 pCEN-LEU2-H3K56R
ICY449	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::TRP1 hst4Δ::TRP1 rad9Δ::kanMX4 pCEN-URA3-HST3
ICY610	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::kanMX4 pCEN-URA3-HST3 (Celic *et al.* 2006)
ICY674	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 pol2::pol2-11::TRP1 pCEN-URA3-HST3
ICY676	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 pol2::pol2-11::TRP1 hst3Δ::HIS3 pCEN-URA3-HST3
ICY680	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 pol2::pol2-11::TRP1 hst4Δ::kanMX4 pCEN-URA3-HST3
ICY682	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 pol2::pol2-11::TRP1 hst3Δ::HIS3 hst4Δ::kanMX4 pCEN-URA3-HST3
ICY682a	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::kanMX4 pol2::pol2-11::TRP1 pCEN-URA3-HST3 pRS415
ICY682b	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::kanMX4 pol2::pol2-11::TRP1 pCEN-URA3-HST3 pJP16
ICY682c	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::kanMX4 pol2::pol2-11::TRP1 pCEN-URA3-HST3 pCEN-LEU2-H3K56R
ICY703	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 pCEN-URA3-HST3 (Celic *et al.* 2006)
ICY703a	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 pCEN-URA3-HST3 pRS415
ICY703b	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 pCEN-URA3-HST3 pJP16
ICY703c	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 pCEN-URA3-HST3 pCEN-LEU2-H3K56R
ICY773	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::TRP1 hst4Δ::TRP1 rad9Δ::kanMX4 rad24Δ::hygMX pCEN-URA3-HST3 hst4Δ::natMX/HST4
ICY793	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 mad2Δ::natMX pCEN-URA3-HST3
ICY819	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 chk1Δ::kanMX4 pCEN-URA3-HST3
ICY975	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 rad52Δ::kanMX4 pCEN-URA3-HST3
ICY980	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 rad51Δ::kanMX4 pCEN-URA3-HST3
ICY986	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 rad54Δ::kanMX4 pCEN-URA3-HST3
ICY992	MAT**a** his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 rad55Δ::kanMX4 pCEN-URA3-HST3

(*continued*)

Downloaded from https://academic.oup.com/genetics/article/179/4/1769/6064715 by guest on 23 April 2024

TABLE 1

(Continued)

ICY995	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 rad57Δ::kanMX4 pCEN-URA3-HST3
ICY997	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 mre11Δ::kanMX4 pCEN-URA3-HST3
ICY1002	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 xrs2Δ::kanMX4 pCEN-URA3-HST3
ICY1008	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 rad50Δ::kanMX4 pCEN-URA3-HST3
ICY1013	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 srs2Δ::kanMX4 pCEN-URA3-HST3
ICY1036	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 slx4Δ::kanMX4 pCEN-URA3-HST3
ICY1216	MATa his3Δ200 leu2Δ::TRP1 lys2Δ202 trp1Δ63 ura3-52 asf1Δ::kanMX4 adh4::URA3-TEL (Celic et al. 2006)
ICY1488	MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 (Celic et al. 2006)
ICY1492	MATa ade2-101 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hht2-hhf2Δ::kanMX6 (Celic et al. 2006)
ICY1497	MATa ade2-101 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hht2-hhf2Δ::kanMX6 hht1::hht1-K56R::TRP1 (Celic et al. 2006)
ICY1501	MATa ade2-101 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hht2-hhf2Δ::kanMX6 hst3Δ::$his5^+$ hst4Δ::natMX (Celic et al. 2006)
ICY1506	MATa ade2-101 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hht2-hhf2Δ::kanMX6 hht1::hht1-K56R::TRP1 hst3Δ::$his5^+$ hst4Δ::natMX (Celic et al. 2006)
ICY1514	MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hst3Δ::$his5^+$ hst4Δ::natM4X (Celic et al. 2006)
ICY1518	MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hht1::hht1-K56R::TRP1 hst3Δ::$his5^+$ hst4Δ::natMX (Celic et al. 2006)
ICY1528	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 rad24Δ::kanMX4 pCEN-URA3-HST3
ICY1534	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 elg1Δ::kanMX4 pCEN-URA3-HST3
ICY1537	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 ctf18Δ::kanMX4 pCEN-URA3-HST3
ICY1544	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 mec3Δ::kanMX4 pCEN-URA3-HST3
ICY1550	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 rad17Δ::kanMX4 pCEN-URA3-HST3
ICY1556	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 ddc1Δ::kanMX4 pCEN-URA3-HST3
ICY1566a	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 rad52Δ::kanMX4 pCEN-URA3-HST3 pRS415
ICY1566b	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 rad52Δ::kanMX4 pCEN-URA3-HST3 pJP16
ICY1566c	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 rad52Δ::kanMX4 pCEN-URA3-HST3 pCEN-LEU2-H3K56R
ICY1568a	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 mre11Δ::kanMX4 pCEN-URA3-HST3 pRS415
ICY1568b	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 mre11Δ::kanMX4 pCEN-URA3-HST3 pJP16
ICY1568c	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 mre11Δ::kanMX4 pCEN-URA3-HST3 pCEN-LEU2-H3K56R
ICY1570a	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 xrs2Δ::kanMX4 pCEN-URA3-HST3 pRS415
ICY1570b	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 xrs2Δ::kanMX4 pCEN-URA3-HST3 pJP16
ICY1570c	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 xrs2Δ::kanMX4 pCEN-URA3-HST3 pCEN-LEU2-H3K56R
ICY1572a	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 rad50Δ::kanMX4 pCEN-URA3-HST3 pRS415
ICY1572b	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 rad50Δ::kanMX4 pCEN-URA3-HST3 pJP16
ICY1572c	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 rad50Δ::kanMX4 pCEN-URA3-HST3 pCEN-LEU2-H3K56R
ICY1574a	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 srs2Δ::kanMX4 pCEN-URA3-HST3 pRS415
ICY1574b	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 srs2Δ::kanMX4 pCEN-URA3-HST3 pJP16
ICY1574c	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 srs2Δ::kanMX4 pCEN-URA3-HST3 pCEN-LEU2-H3K56R
ICY1576a	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 slx4Δ::kanMX4 pCEN-URA3-HST3 pRS415
ICY1576b	MATa his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 slx4Δ::kanMX4 pCEN-URA3-HST3 pJP16

(*continued*)

Downloaded from https://academic.oup.com/genetics/article/179/4/1769/6064715 by guest on 23 April 2024

TABLE 1

(Continued)

ICY1576c	*MAT***a** *his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 slx4Δ::kanMX4* pCEN-URA3-HST3 pJP16 pCEN-LEU2-H3K56R
ICY1601	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ctf18Δ::hygMX*
ICY1605	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hht2-hhf2Δ::kanMX6 ctf18Δ::hygMX*
ICY1607	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hht2-hhf2Δ::kanMX6 hht1::hht1-K56R::TRP1 ctf18Δ::hygMX*
ICY1613	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hst3Δ::his5⁺ hst4Δ::natMX ctf18Δ::hygMX*
ICY1618	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad24Δ::hygMX*
ICY1646	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hst3Δ::his5⁺ hst4Δ::natMX rad24Δ::hygMX*
ICY1653	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 elg1Δ::hygMX*
ICY1664	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hst3Δ::his5⁺ hst4Δ::natMX elg1Δ::hygMX*
ICY1676	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ctf4Δ::hygMX*
ICY1684	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hst3Δ::his5⁺ hst4Δ::natMX ctf4Δ::hygMX*
ICY1688	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hht2-hhf2Δ::kanMX6 ctf4Δ::hygMX*
ICY1692	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hht2-hhf2Δ::kanMX6 hht1::hht1-K56R::TRP1 ctf4Δ::hygMX*
ICY1795	*MAT***a** *his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 dcc1Δ::kanMX4* pCEN-URA3-HST3
ICY1797	*MAT***a** *his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 ura3-52 hst3Δ::HIS3 hst4Δ::TRP1 ctf8Δ::kanMX4* pCEN-URA3-HST3

hydroxyurea (HU), methyl methane sulphonate (MMS), camptothecin (CPT), and ultraviolet radiation (UV) sensitivity in a dose-dependent manner in *hst3 hst4* cells (Figure 1B). We have previously shown that the Ts phenotype and sensitivity to genotoxic agents of *hst3 hst4* cells are suppressed by the K56R mutation. This suggests that overexpression of *RFC1* directly or indirectly counteracts K56 hyperacetylation in *hst3 hst4* cells.

Suppression of the *hst3 hst4* Ts phenotype by inactivation of alternative clamp loading complexes: Rfc1p, together with Rfc2-5p, forms a heteropentameric complex called RFC that loads the homotrimeric PCNA ring onto DNA during replication (Tsurimoto and Stillman 1990; Fien and Stillman 1992; Cullmann *et al.* 1995). In addition to RFC, there are three "alternative" RFC-like complexes that share the Rfc2-5p subunits, but differ in the nature of their large subunit: Rad24p–RFC, Elg1p–RFC and Ctf18p–RFC. The function of these complexes has been linked to the DNA damage response and for Ctf18p, to sister chromatid cohesion (Shimomura *et al.* 1998; Green *et al.* 2000; Mayer *et al.* 2001; Naiki *et al.* 2001; Ben-Aroya *et al.* 2003). The Rad24p–RFC complex loads a specialized heterotrimeric (non-PCNA) clamp encoded by *MEC3*, *RAD17*, and *DDC1*, referred to as the 9-1-1 complex (Kondo *et al.* 1999).

We examined the effect of *RAD24*, *ELG1*, and *CTF18* deletion on *hst3 hst4* cells and found that deletions of the genes encoding large subunits of alternative clamp loaders efficiently suppress the Ts phenotype of *hst3 hst4* cells (Figure 2A). In addition, deletion of *MEC3*, *RAD17*, and *DDC1*, encoding the subunits of the Rad24p-specific clamp and *CTF8* and *DCC1*, which encode additional subunits of the Ctf18p–RFC complex (Bermudez *et al.* 2003) also suppress the Ts phenotype of *hst3 hst4* cells (Figure 2A). We have observed suppression of the *hst3 hst4* Ts phenotype by *rad24*Δ even in a *rad9*Δ background (Figure 2B). Rad9p is an important mediator of DNA damage checkpoints (Aboussekhra *et al.* 1996; De La Torre-Ruiz *et al.* 1998) that functions in parallel with Rad24p. We have previously shown that deletion of *RAD9* increases UV sensitivity of *hst3 hst4* cells (Brachmann *et al.* 1995) and reduces the DNA damage checkpoint response in *hst3 hst4* cells (see Figure 6, A and B) indicating that spontaneous DNA damage in *hst3 hst4* cells (see below) is partially recognized by Rad9p-mediated DNA damage checkpoint. The suppression of the *hst3 hst4* Ts phenotype by *rad24*Δ even in a *rad9* background suggests that inactivation of the Rad24p-clamp loader eliminates a requirement for *RAD9*-mediated checkpoint function by reducing K56 hyperacetylation-induced spontaneous DNA damage that is recognized by the Rad9p-mediated DNA damage checkpoint.

Deletion of *CTF4* suppresses *hst3 hst4* phenotypes: The strongest suppression that we observed resulted from deletion of components of the Ctf18p–RFC clamp loader (Ctf18p, Ctf8p, and Dcc1p). The function of Ctf18p is related to that of Ctf4p, as both were genetically defined as chromosome transmission fidelity mutants (Spencer *et al.* 1990). Ctf4p is a replication fork-associated β-propeller protein (Jawad and Paoli 2002; Gambus *et al.* 2006; Lengronne *et al.* 2006) required for maintenance of genomic stability and sister chromatid cohesion (Kouprina *et al.* 1992; Miles and Formosa 1992; Hanna *et al.* 2001). This prompted us to test what effect *ctf4*Δ has in a *hst3 hst4* background. Indeed, *ctf4*Δ strongly suppressed the Ts and partially suppressed the HU sensitivity phenotype of *hst3 hst4*

Downloaded from https://academic.oup.com/genetics/article/179/4/1769/6064715 by guest on 23 April 2024

FIGURE 1.—Overexpression of *RFC1* suppresses the growth defect, Ts phenotype, and sensitivity to genotoxic agents of *hst3 hst4* cells. (A) Serial dilutions (1:5) of strains ICY188 (WT + YEP351), ICY189 (WT + YEP351/*RFC1*), ICY190 (*hst3 hst4* + YEP351), and ICY191 (*hst3 hst4* + YEP351/*RFC1*) were spotted on SC −Leu −Ura and SC −Leu +5-FOA and grown at the indicated temperatures for 2–3 days. (B) After shuffling out a *URA3*-marked plasmid on 5-FOA and an additional round of 5-FOA selection, the strains ICY188, ICY190, and ICY191 were spotted in serial dilutions (1:5) on SC −Leu and grown at 30° as indicated.

Downloaded from https://academic.oup.com/genetics/article/179/4/1769/6064715 by guest on 23 April 2024

cells (Figure 3A). The strong suppression of *hst3 hst4* phenotypes by *ctf4*Δ suggests that *CTF4* may function directly in the K56Ac pathway. If this hypothesis is correct, deletion of *CTF4* in K56R cells should not lead to any additional increase in sensitivity to DNA damaging agents (above the sensitivity observed with either single mutant) and we indeed observed very little increase in sensitivity in this double mutant (Figure 3B). The increase in sensitivity is at most fivefold, as determined by assaying phenotypes over a range of HU and MMS concentrations (data not shown). This result suggests that *CTF4* and K56 acetylation may have some interdependent function in the maintenance of genomic integrity.

In striking contrast to the *ctf4* deletion, a strong synergistic interaction was observed between the *ctf18* and *H3 K56R* mutations (Figure 3B). These results suggest that *CTF4* and K56 acetylation have a common function in the response to genotoxic agents, whereas *CTF18* clearly acts via a separate pathway.

Analysis of K56 acetylation in *hst3 hst4* suppressors: Our results demonstrate strong genetic links between DNA replication clamp loaders, and the cohesion protein *CTF4* on the one hand and the K56 acetylation/deacetylation pathway on the other. Overexpression of *RFC1* could suppress *hst3 hst4* phenotypes either by reducing K56 acetylation or by allowing *hst3 hst4* cells to adapt to the high level of K56 acetylation. To determine which mechanism is in play, we analyzed K56 acetylation levels in *hst3 hst4* cells carrying a high-copy *RFC1* plasmid by immunoblotting with a K56Ac-specific antibody (MASUMOTO *et al.* 2005). Histone H3 K56Ac levels were equally high in *hst3 hst4* cells carrying either a high-copy *RFC1* plasmid or an empty vector (Figure 4A). Therefore, Rfc1p overexpression suppresses the phenotypes of *hst3 hst4* mutant cells by allowing them to survive despite the persistence of K56 hyperacetylation. Next we examined the K56 hyperacetylation in *ctf4* and other suppressors (*ctf18*, *rad24*, and *elg1*) of *hst3 hst4* mutants and found that in all of these, K56 acetylation remained

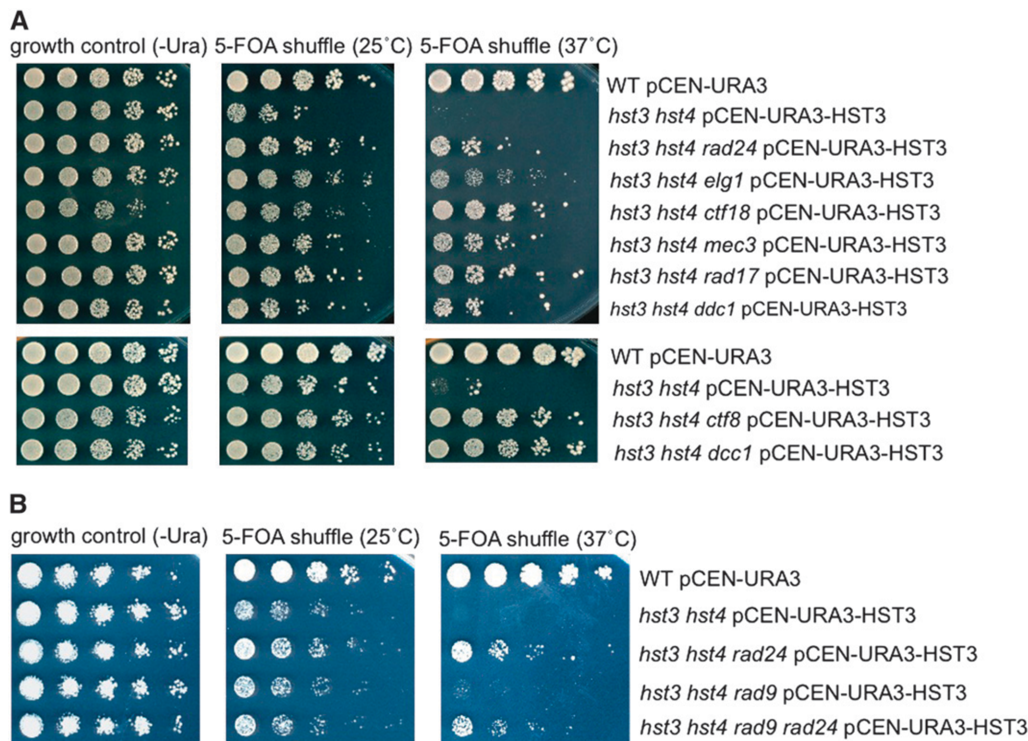


Figure 2.—Suppression of the *hst3 hst4* growth defect and Ts phenotype by inactivation of alternative RFC complexes. (A) Serial dilutions (1:5) of strains ICY356 (WT), ICY703 (*hst3 hst4*), ICY1528 (*hst3 hst4 rad24*), ICY1534 (*hst3 hst4 elg1*), ICY1537 (*hst3 hst4 ctf18*), ICY1544 (*hst3 hst4 mec3*), ICY1550 (*hst3 hst4 rad17*), ICY1556 (*hst3 hst4 ddc1*), ICY1797 (*hst3 hst4 ctf8*), ICY1795 (*hst3 hst4 dcc1*) were spotted on SC −Ura or SC +5-FOA and grown for 3 days at the indicated temperatures. (B) Serial dilutions (1:5) of strains ICY356 (WT), ICY252 (*hst3 hst4*), ICY342 (*hst3 hst4 rad24*), ICY449 (*hst3 hst4 rad9*), and ICY773 (*hst3 hst4 rad9 rad24*) were spotted on SC −Ura (25°), SC +5-FOA (25°), and SC +5-FOA (37°) and grown for 3 days at the indicated temperatures.

Downloaded from https://academic.oup.com/genetics/article/179/4/1769/6064715 by guest on 23 April 2024

as high as in *hst3 hst4* cells (Figure 4B). This suggests that *CTF4* functions downstream of K56 acetylation and that mutations in *CTF4* and *RFC1* paralogs suppress *hst3 hst4* phenotypes by allowing *hst3 hst4* cells to adapt to constitutive K56 hyperacetylation.

Spontaneous DNA damage checkpoint activation in *hst3 hst4* cells: Although *hst3 hst4* cells are sensitive to a wide spectrum of genotoxic agents that damage DNA during replication, this cannot be explained by a defect in the S-phase DNA damage checkpoint (Figure 5, A and B). Normal cells respond to DNA damage during S phase by slowing down DNA synthesis and spindle elongation, while cells defective in checkpoint functions progress through the cell cycle in the presence of damage with ultimately catastrophic consequences (Allen *et al.* 1994; Weinert *et al.* 1994; Navas *et al.* 1995; Paulovich and Hartwell 1995). We have analyzed DNA content in MMS-treated wild-type (WT) and *hst3 hst4* cells. Wild-type and mutant cells were synchronized in G_1 with α-factor, released into medium with or without 0.03% MMS, and DNA content was analyzed by fluorescence-activated cell sorting (FACS). *hst3 hst4* cells slow down DNA replication in response to MMS treatment at a rate comparable to the wild type (Figure 5A), suggesting that the MMS-induced checkpoint is functional in *hst3 hst4* cells. We next tested the effect of the replication inhibitor HU on cell-cycle progression and spindle elongation in *hst3 hst4* and wild-type cells (Figure 5, B and C). Both wild-type and mutant cells did not elongate their spindle when treated with HU (Figure 5C). To gain further insights into the consequences of K56 hyperacetylation, we compared the genomewide transcriptional profiles in wild-type and *hst3 hst4* cells. *HUG1* and *RNR3,* two genes that are highly induced by DNA damage (Elledge and Davis 1990; Basrai *et al.* 1999), were the most highly upregulated genes in *hst3 hst4* cells. Induction of *HUG1* and *RNR3* in *hst3 hst4* cells suggests the activation of a chronic DNA damage response in the absence of exogenous damage. We have confirmed the microarray results by RNA blot analysis. In addition to *hst3 hst4* cells showing strong upregulation of *RNR3* and *HUG1* (Figure 6A), we observed weaker induction of these genes in the *hst3* single mutant (but no signal in the *hst4* single mutant). This suggests that *hst3* cells experience a low level of spontaneous DNA damage and that the double mutant is more severely affected. These results help explain synthetic fitness interactions observed between *hst3* (but not *hst4*) mutants and several mutants affecting DNA metabolism (Tong *et al.* 2001; Suter *et al.* 2004; Pan *et al.* 2006) and suggest that *HST3* has the more dominant role in regulation of genomic stability. This is also consistent with our observation that K56 acetylation is elevated in *hst3* but not *hst4* single mutants and is maximally elevated, to ∼100%, in *hst3 hst4* double mutants (Celic *et al.* 2006). The checkpoint response to DNA damage or inhibition of DNA replication leads to Rad53p phosphorylation (Sanchez *et al.* 1996). In addition to upregulation of *HUG1* and *RNR3*, Rad53p is hyperphosphorylated in normally growing *hst3 hst4* cells (Figure 6B), further demonstrating activation of the checkpoint response in *hst3 hst4* cells, presumably due to a form of spontaneous DNA damage.

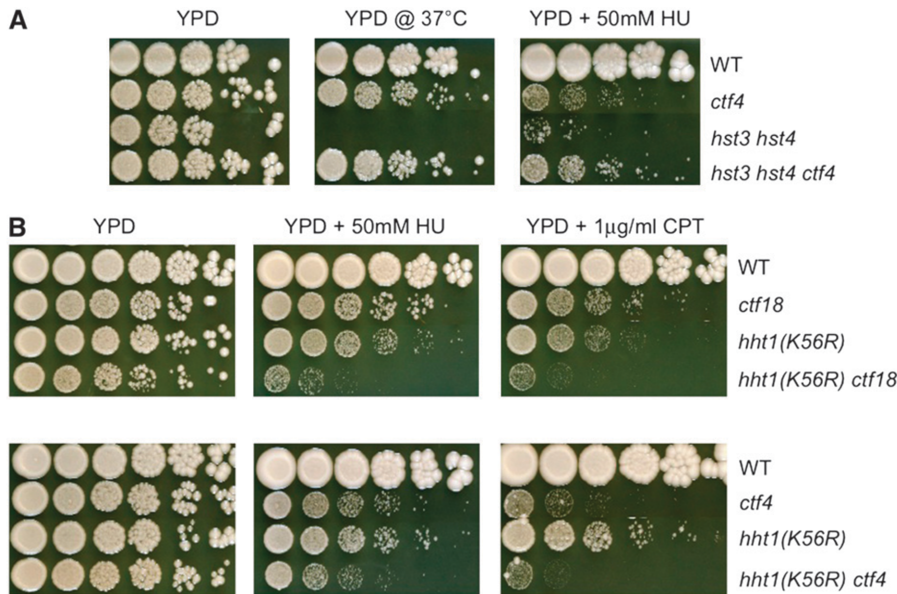


Figure 3.—Genetic interaction with *ctf4* and *ctf18*. (A) Suppression of the *hst3 hst4* Ts phenotype and HU sensitivity by deletion of *CTF4*. Serial dilutions (1:5) of strains ICY1488 (WT), ICY1676 (*ctf4*), ICY1514 (*hst3 hst4*), and ICY1684 (*hst3 hst4 ctf4*) were spotted on YPD and YPD + 100 mm HU and grown for 3 days at 25° and 37° (YPD) and 5 days at 25° (YPD + HU). (B) Analysis of genetic interaction between *ctf4*, *ctf18*, and *hht1*(*K56R*). Serial dilutions (1:5) of strains ICY1492 (*hht2-hhf2*), ICY1688 (*hht2-hhf2 ctf4*), ICY1605 (*hht2-hhf2 ctf18*), ICY1497 [*hht2-hhf2 hht1*(*K56R*)], ICY1692 [*hht2-hhf2 hht1*(*K56R*) *ctf4*], and ICY1607 [*hht2-hhf2 hht1*(*K56R*) *ctf18*] were spotted on YPD, YPD + 50 mm HU, and YPD + 1 µg/ml CPT and grown for 3 days (YPD) and 5 days (YPD + HU; YPD + CPT) at 25°.

RAD9 and *RAD24* control two separate pathways required for induction of *RNR3* and *HUG1* and for Rad53p phosphorylation in response to DNA damage (Aboussekhra *et al.* 1996; De La Torre-Ruiz *et al.* 1998). We deleted *RAD9* and *RAD24* in *hst3 hst4* cells and examined the levels of *RNR3* and *HUG1* mRNA and of Rad53p phosphorylation. Deletion of either *RAD9* or *RAD24* in *hst3 hst4* cells resulted in reduction of *RNR3* and *HUG1* mRNA (Figure 6A) and Rad53p phosphorylation (Figure 6B) with *RAD9* having the greater effect on *HUG1* mRNA level and Rad53p phosphorylation than *RAD24*. Deletion of both genes showed a further reduction of, but did not completely abolish *RNR3* expression, suggesting that there may be an additional pathway(s) required for residual upregulation of *RNR3*. Importantly, the lack of *RNR3*/*HUG1* RNAs in *hst3 hst4 rad9 rad24* mutant cells does not imply that deletion of *RAD9* and *RAD24* necessarily suppressed spontaneous DNA damage in *hst3 hst4* mutant cells. These effects are likely due to the overlapping roles of Rad9p and Rad24p in activating Rad53p, which, in turn, is necessary for DNA damage-induced expression of *RNR3* and *HUG1*. Induction of the latter genes is not observed in a histone H3 K56R mutant according to an expression study done on this mutant (Xu *et al.* 2005), suggesting the damage response of nonacetylatable chromatin is distinct from that of hyperacetylated chromatin.

K56 hyperacetylation is responsible for most if not all phenotypes observed in *hst3 hst4* mutants (Celic *et al.* 2006). We next examined whether the presence of a single copy of a histone H3 gene with a K56R mutation would reduce Rad53p phosphorylation in *hst3 hst4* mutants. Indeed, *hst3 hst4 hht1K56R* cells show reduced Rad53p phosphorylation, comparable to that of wild-type cells (Figure 6C). Even in *hst3 hst4 hht2-hhf2 hht1K56R* cells, in which the only source of histone H3 is H3K56R, the mutation reduced Rad53p phosphorylation, although not to the level of wild type, but only to the same level observed in a *hht2-hhf2 hht1K56R* strain, which itself shows a mildly elevated level of Rad53p phosphorylation; it is much less dramatic than in *hst3 hst4* cells.

The *hst3 hst4* mutant depends on the Mec1p-mediated checkpoint for viability: In addition to recognition of DNA damage by the Rad9p- or Rad24p-dependent checkpoint, there is an additional checkpoint response that recognizes stalled replication forks (Navas *et al.* 1995). We investigated whether this checkpoint is activated in *hst3 hst4* cells. To eliminate this DNA replication fork integrity checkpoint, we introduced the *pol2-11* allele in *hst3 hst4* mutant cells. This allele generates a truncated version of the DNA polymerase ε-catalytic subunit, which participates in leading strand replication (Pursell *et al.* 2007). The Pol2-11 protein is functional with respect to replication function at the permissive temperature, but allegedly loses a replication checkpoint function (Navas *et al.* 1995). We generated this mutation in *hst3 hst4* mutant cells "covered" by a *URA3*-marked plasmid containing a wild-type copy of *HST3*. *hst3 hst4* cells can readily lose the *HST3* plasmid at the permissive temperature, because *HST3* and *HST4* are nonessential. If there is a synthetic lethality interaction between *hst3 hst4* and the third gene, triple-mutant cells cannot grow on 5-FOA medium because they cannot lose the *HST3* plasmid. After introducing the *pol2-11* allele, the *hst3 hst4* mutant cells became unable to segregate the *HST3* plasmid (Figure 7A) at the permissive temperature, indicating that the triple-mutant combination is lethal and that the *hst3 hst4* mutant potentially depends on a functional replication fork integrity checkpoint for viability. Although able to

Downloaded from https://academic.oup.com/genetics/article/179/4/1769/6064715 by guest on 23 April 2024

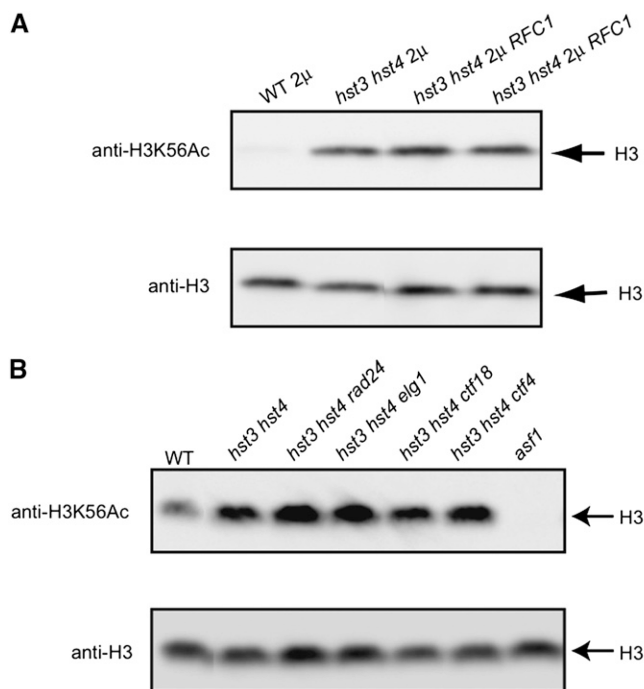
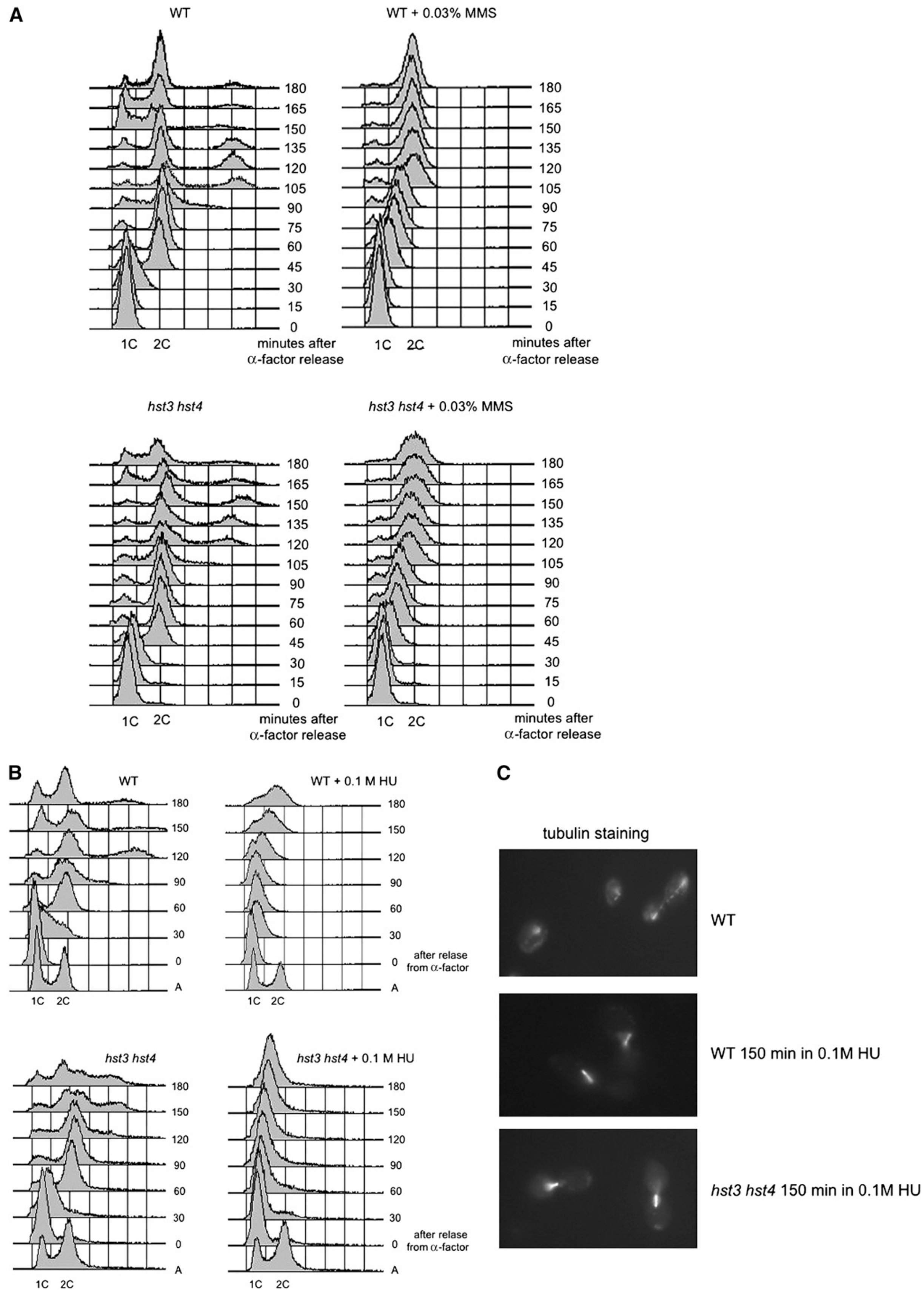


Figure 4.—Analysis of K56 acetylation. (A) Total protein extracts were prepared from strains ICY188 (WT + YEP351), ICY190 (*hst3 hst4* + YEP351), ICY191 (*hst3 hst4* + YEP351/*RFC1*), and ICY192 (*hst3 hst4* + YEP351/*RFC1*) after two rounds of 5-FOA selection to lose the *URA3* (WT) and *URA3-HST3* (*hst3 hst4* strains) plasmids, and the acetylation of histone H3 K56 was analyzed by immunoblotting with a K56Ac-specific antibody. The membrane was stripped and reprobed with an antibody specific for the C terminus of H3. (B) Total protein extracts from strains ICY1488 (WT), ICY1514 (*hst3 hst4*), ICY1646 (*hst3 hst4 rad24*), ICY1664 (*hst3 hst4 elg1*), ICY1613 (*hst3 hst4 ctf18*), ICY1684 (*hst3 hst4 ctf4*), and ICY1216 (*asf1*) were separated by SDS–PAGE and immunoblotted with a K56Ac specific antibody. The membrane was stripped and reprobed with an antibody specific for the C terminus of H3.

replicate at the permissive temperature, *pol2-11* cells are likely to be somewhat deficient in DNA replication, on the basis of their FACS profile (Navas *et al.* 1995). Thus, the lethality of *hst3 hst4 pol2-11* cells could result from sensitivity of *hst3 hst4* cells to subtle perturbations in leading strand synthesis. Indeed, we have also observed lethality between *hst3 hst4* and epitope-tagged alleles of otherwise wild-type replication proteins (Table 2). This indicates that *hst3 hst4* cells are extremely sensitive to subtle perturbations in DNA replication that are well tolerated by wild-type cells or even the *hst3* or *hst4* single mutants. Lethality of *hst3 hst4 pol2-11* cells was confirmed by generating a triply heterozygote diploid strain and performing tetrad analysis. In addition to the lethality of the triple mutant, we observe a synthetic growth defect between *hst3* and *pol2-11*, but not between *hst4* and *pol2-11* (Figure 7A). This is further evidence that Hst3p plays the more prominent role in deacetylation of H3 K56. Mec1p is a central transducer of DNA damage signals, whether originating from breaks in DNA or stalled replication forks (Weinert *et al.* 1994). Mec1p activates Rad53p in response to DNA damage or replication blocks (Sanchez *et al.* 1996). This leads to the activation of the protein kinase Dun1p and transcriptional induction of numerous DNA repair genes (Zhou and Elledge 1993; Allen *et al.* 1994; Gasch *et al.* 2001). In a parallel pathway, Mec1p activates Chk1p, which leads to stabilization of the anaphase inhibitor Pds1p and arrest of the cell cycle at the metaphase–anaphase transition (Cohen-Fix and Koshland 1997; Gardner *et al.* 1999; Sanchez *et al.* 1999). Deletion of *MEC1* in *hst3 hst4 sml1* cells resulted in synthetic lethality (Figure 7B); this genetic interaction was confirmed by tetrad analyses. Surprisingly, deletion of *RAD53* in *hst3 hst4* cells did not result in lethality (Figure 7B), even though Rad53p is the direct target of Mec1p in both the DNA damage and DNA replication checkpoints (Sanchez *et al.* 1996; Sun *et al.* 1996). We also tested the effect of *dun1Δ* in *hst3 hst4* cells and here results were mixed. We observed synthetic lethality in one strain background (the "FY" strains directly derived from S288C), but not in a related strain background (the "YPH" background derived from S288C by backcrossing into a different strain background (Kumar *et al.* 2003). The basis for these differences is unknown. In contrast to *pol2-11*, we did not observe synthetic fitness defects between *hst3* and *mec1 sml1* or *hst3* and *dun1* (in the FY background). Deletion of *CHK1*, which mediates the DNA damage response in parallel to *RAD53*, had no detectable effect on fitness of *hst3 hst4* cells (Table 2). Similarly, elimination of the spindle or mitotic exit checkpoints by deletion of *MAD2* and *BUB2* (Gardner and Burke 2000) had no significant impact on *hst3 hst4* cells (Table 2). The data presented suggest that although multiple pathways sensing DNA damage are activated in the absence of *HST3* and *HST4*, the most important pathway required for survival of *hst3 hst4* mutant cells is the DNA damage checkpoint mediated through *MEC1*.

***hst3 hst4* cells require a subset of DNA repair proteins for viability:** The presence of spontaneous DNA damage in *hst3 hst4* cells prompted us to examine genetic interaction between *hst3 hst4* and various repair proteins. If *hst3 hst4* cells require particular DNA repair pathways, one would expect to see genetic fitness or lethality interactions between *hst3 hst4* mutations and those in the relevant DNA repair pathway. We have deleted several DNA repair proteins in *hst3 hst4* strains. As described above, triple-mutant cells were grown on 5-FOA medium, allowing *HST3* plasmid-free cells to grow. We observed synthetic lethality interactions between *hst3 hst4* and *rad52*. Interestingly, *hst3 hst4* cells do not require several other genes in the *RAD52* epistasis group for viability, including *RAD51*, *RAD54*, *RAD55*, and *RAD57* (Figure 8; Table 2).

Downloaded from https://academic.oup.com/genetics/article/179/4/1769/6064715 by guest on 23 April 2024



Downloaded from https://academic.oup.com/genetics/article/179/4/1769/6064715 by guest on 23 April 2024

FIGURE 5.—Like wild-type cells, *hst3 hst4* cells slow down DNA replication and spindle elongation when exposed to MMS and HU, respectively. (A) ICY48 (*bar1*) and ICY49 (*bar1 hst3 hst4*) cells were arrested with α-factor and released into medium with and without MMS. Aliquots of the cells were taken at indicated time points and analyzed by FACS. (B–C) ICY48 (*bar1*) and ICY49 (*bar1 hst3 hst4*) cells were arrested with α-factor and released into medium with and without 100 mM HU. Aliquots of the cells were taken at indicated time points and analyzed by FACS (B) and immunofluorescence for tubulin staining (C).

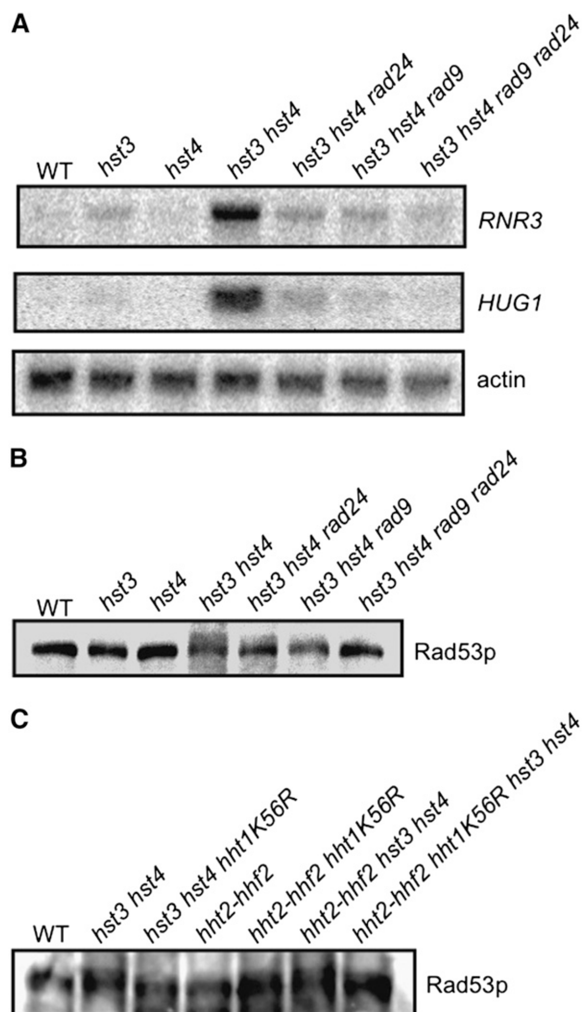


Figure 6.—Induction of *RNR3* and *HUG1* and hyperphosphorylation of Rad53p in *hst3 hst4* cells. (A) Total RNA was isolated from strains YCB617 (WT), YCB470 (*hst3*), YCB575 (*hst4*), ICY252 (*hst3 hst4*), ICY342 (*hst3 hst4 rad24*), ICY449 (*hst3 hst4 rad9*), and ICY773 (*hst3 hst4 rad9 rad24*) and hybridized to *RNR3*, *HUG1*, and actin-specific probes. Prior to the experiment, strains ICY252, 342, 449, and 773 were grown on 5-FOA to shuffle out a *URA3* plasmid carrying the *HST3* gene. (B) Rad53p is hyperphosphorylated in a Rad24p- and Rad9p-dependent manner in *hst3 hst4* cells, but not in the single mutants. Total protein extracts from the strains YCB617 (WT), YCB470 (*hst3*), YCB575 (*hst4*), ICY252 (*hst3 hst4*), ICY342 (*hst3 hst4 rad24*), ICY449 (*hst3 hst4 rad9*), and ICY773 (*hst3 hst4 rad9 rad24*) were separated by SDS–PAGE and immunoblotted with an antibody specific for Rad53p. Prior to the experiment, strains ICY252, 342, 449, and 773 were grown on 5-FOA to shuffle out a *URA3* plasmid carrying the *HST3* gene. (C) H3 K56R mutation reduces Rad53p phosphorylation in *hst3 hst4* strains. Total protein extracts were prepared from strains ICY1488 (WT), ICY1514 (*hst3 hst4*), ICY1518 (*hst3 hst4 hht1K56R*), ICY1492 (*hht2-hhf2*), ICY1497 (*hht2-hhf2 hht1K56R*), ICY1501 (*hht2-hhf2 hst3 hst4*), and ICY1506 (*hht2-hhf2 hht1K56R hst3 hst4*) and analyzed for Rad53p phosphorylation using a Rad53p-specific antibody.

hst3 hst4 cells require the MRX complex for viability (Figure 8; Table 2) and show synthetic lethality with mutations affecting all three members of this complex (*xrs2*, *rad50*, and *mre11*). Additionally, we observed synthetic lethality interactions with *slx4* and *srs2*. These results demonstrate that *hst3 hst4* require functional DNA repair for viability, consistent with the histone H2A S128 hyperphosphorylation observed in these cells (Celic *et al.* 2006), which suggests the presence of elevated levels of DNA double-strand breaks (DSBs). The lethality observed with a specific subset of repair genes suggests that *hst3 hst4* cells are particularly susceptible to the absence of a specific repair pathway and hints at the existence of specific type(s) of DNA lesions caused by K56 hyperacetylation. Except for *hst3 hst4 srs2*, the triple-mutant lethalities that we observed can all be partially suppressed by a K56R mutation (Figure 8; Table 2).

DISCUSSION

The yeast sirtuins Hst3p and Hst4p are important for maintaining genomic stability and recent findings demonstrate that their role in regulating genomic stability is directly linked to regulation of histone H3 K56 deacetylation (Brachmann *et al.* 1995; Celic *et al.* 2006; Maas *et al.* 2006). Newly synthesized histone H3 molecules are acetylated at K56 and incorporated into DNA during S phase (Masumoto *et al.* 2005). K56Ac histone H3 incorporated into chromatin is then deacetylated in an Hst3p/Hst4p-dependent manner. Failure to deacetylate K56 has detrimental consequences for yeast cells and the resulting K56 hyperacetylation leads to accumulation of spontaneous damage and genomic instability. To gain insight into the consequences of K56 hyperacetylation, we performed a high-copy suppressor screen and isolated *RFC1*, the large subunit of the clamp loader that loads PCNA onto DNA during replication. Analysis of the K56 acetylation level indicated that, rather than causing a decrease in K56Ac, overexpression of *RFC1* allowed cells to adapt to elevated levels of K56 acetylation. Similar results were observed upon deletion of *CTF18*, *ELG1*, and *RAD24*, which encode large subunits of alternative clamp loaders. Since yeast clamp loaders share four small subunits, Rfc2–5p, our suppression data suggest that persistent K56 acetylation negatively affects Rfc1p–RFC function. Suppression observed by deletion of alternative clamp loader large subunits would increase a pool of available small subunits and tip the equilibrium between different clamp loaders toward the formation of Rfc1p–RFC. Although deletion of *CTF18*, *ELG1*, and *RAD24* suppressed the Ts phenotype of *hst3 hst4* cells, we did not observe suppression of sensitivity to genotoxic agents in these deletion mutants, rather increased sensitivity of *hst3 hst4* cells to genotoxic agents was observed (data not shown). We imagine that the increased availability of the small RFC subunits upon deletion of *CTF18*, *ELG1*, and *RAD24* is sufficient to suppress the growth defect generated by K56 hyperacetylation.

Downloaded from https://academic.oup.com/genetics/article/179/4/1769/6064715 by guest on 23 April 2024

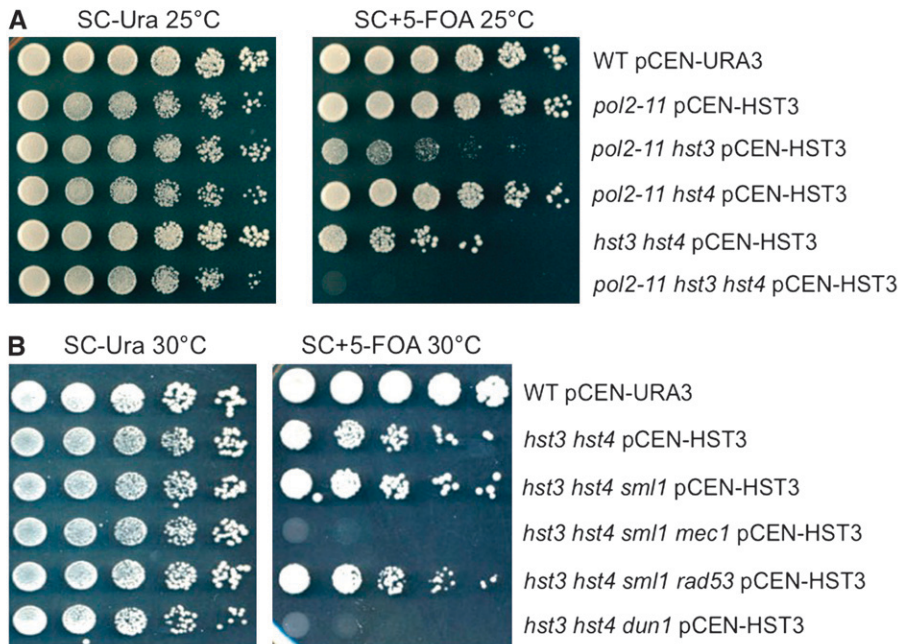


Figure 7.—*hst3 hst4* cells require a Mec1p-dependent function for viability. (A) Synthetic lethality between *hst3 hst4* and *pol2-11*. Serial dilutions (1:5) of strains ICY356 (WT), ICY674 (*pol2-11*), ICY676 (*pol2-11 hst3*), ICY680 (*pol2-11 hst4*), ICY610 (*hst3 hst4*), and ICY682 (*pol2-11 hst3 hst4*) were spotted on SC −Ura and SC +FOA and grown for 3–4 days at 25°. (B) Synthetic lethality between *hst3 hst4* and *mec1* and *dun1*. Serial dilutions (1:5) of strains ICY356 (WT), ICY703 (*hst3 hst4*), ICY430 (*hst3 hst4 sml1*), ICY431 (*hst3 hst4 sml1 mec1*), ICY351 (*hst3 hst4 sml1 rad53*), and ICY230 (*hst3 hst4 dun1*) were spotted on SC −Ura and SC +FOA and grown for 3–4 days at 30°.

The growth defect and the Ts phenotype of *hst3 hst4* cells are caused, at least in part, by spontaneous DNA damage. On the other hand, treatment with genotoxic agents may create distinct DNA lesions that qualitatively differ from the consequences of K56 hyperacetylation. Under those conditions, the contribution of alternative clamp loaders to DNA repair and checkpoint signaling may be more important for the survival of *hst3 hst4* cells than their ability to antagonize the Rfc1p–RFC. We have also found that deletion of *CTF4* strongly suppresses the Ts phenotype of *hst3 hst4* cells. In contrast to all of the other "knockout mutation" suppressors (*ctf18*, *elg1*, and *rad24*), only *ctf4* suppressed sensitivity to HU, indicating a closer link between the response to K56 *hyperacetylation* and Ctf4p. For normal cellular growth, *CTF4* was genetically defined as part of the K56 *acetylation* pathway (Collins *et al.* 2007), together with *RTT109*, *ASF1*, *RTT101*, *MMS1*, and *MMS22*. Our genetic analysis reinforces this notion and suggests that, for cellular resistance to genotoxic agents, K56 acetylation and *CTF4* function together in a pathway that is parallel to and distinct from the *CTF18* pathway. These pathways converge on the replication fork and promote molecular events that are necessary to rescue replication forks damaged by genotoxic agents. Ctf18p may not function strictly in parallel to Ctf4p, but may be partially controlled by Ctf4p as recruitment of Ctf18p to replication forks partially depends on Ctf4p (Lengronne *et al.* 2006). Ctf4p, a large β-propeller protein with many potential binding sites, could accommodate multiple functions. Considering that K56 acetylation levels are unchanged in *hst3 hst4 ctf4* cells relative to *hst3 hst4* cells, we believe *CTF4* actually functions downstream of K56 acetylation, similarly to *RTT101*, *MMS1*, and *MMS22*, deletion of which does not affect K56Ac levels in *hst3 hst4* cells (Collins *et al.* 2007). Ctf4p is a part of a large replisome progression complex (RPC) (Gambus *et al.* 2006) that includes the GINS complex (Kanemaki *et al.*

TABLE 2

Synthetic lethal analysis with *hst3 hst4*

Mutant	*hst3*[a]	*hst4*[a]	*hst3 hst4*[a]	K56R suppression[b]
rad52	+	+	SL	+
rad50	+	+	SL	+
pol2-11	+	+	SL	+
mec1[c]	+	+	SL	+
mre11	+	+	SL	+
xrs2	+	+	SL	+
srs2	+	+	SL	—
slx4	+	+	SL	+
CDC45-Myc$_{13}$[d]	+	+	SL	NT
POL30-HA$_3$[d]	+	+	SL	NT
rad53[c]	+	+	+	NA
chk1	+	+	+	NA
mad2	+	+	+	NA
bub2	+	+	+	NA
rad51	+	+	+	NA
rad54	+	+	+	NA
rad55	+	+	+	NA
rad57	+	+	+	NA

[a] +, growth of double or triple mutant; SL, triple mutant is synthetic lethal by plasmid shuffle assay.
[b] +, addition of a K56R allele suppresses *hst3 hst4* synthetic lethality; NT, not tested; NA, not applicable.
[c] Performed in *sml1* background.
[d] Wild-type alleles with epitope tags that fully complement deletion mutations in these essential genes.

Downloaded from https://academic.oup.com/genetics/article/179/4/1769/6064715 by guest on 23 April 2024

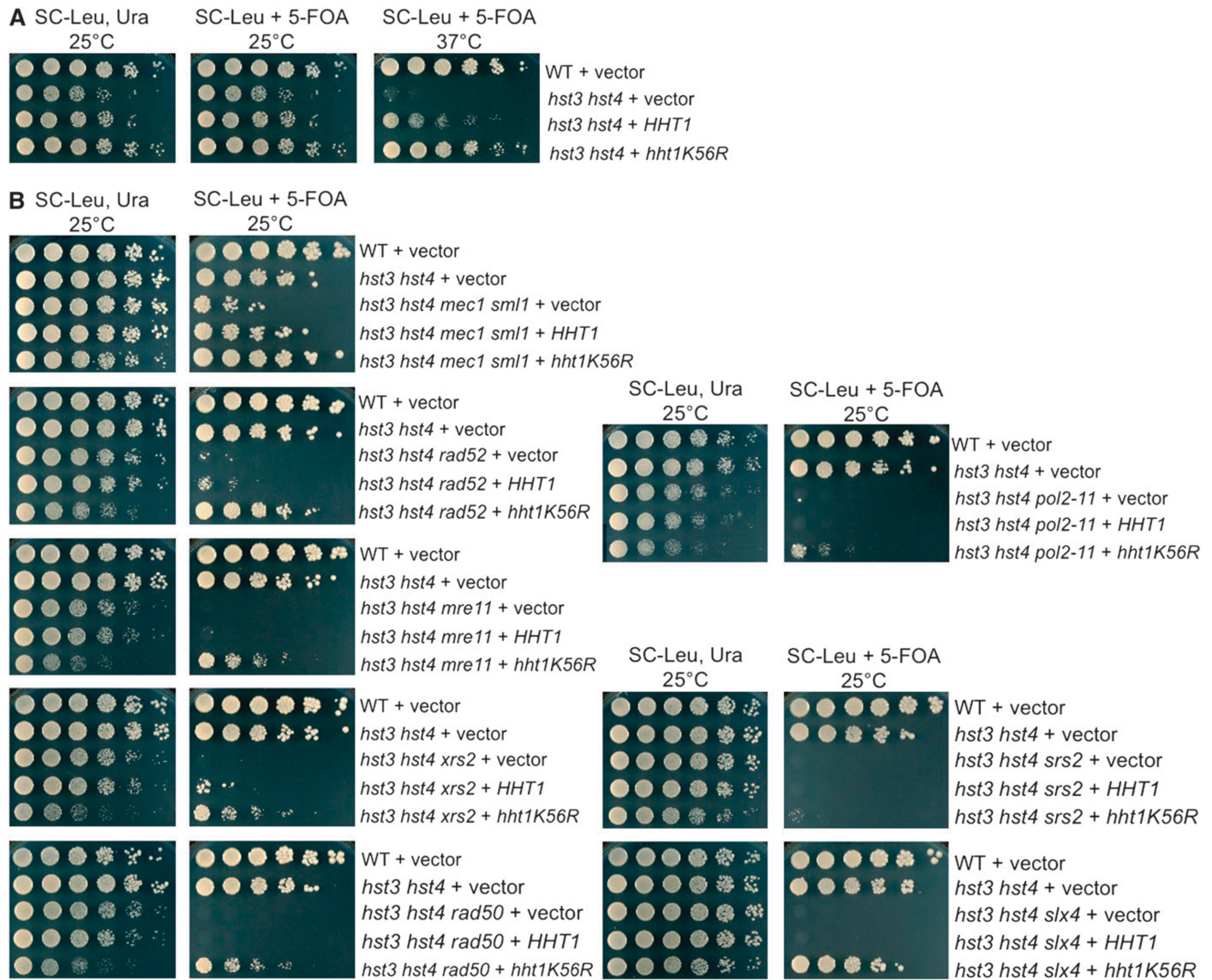


FIGURE 8.—Synthetic lethality analysis with *hst3 hst4* and suppression with H3 K56R. (A) Serial dilutions (1:5) of strains ICY356a (WT + vector), ICY703a (*hst3 hst4* + vector), ICY703b (*hst3 hst4* + *HHT1*), and ICY703c (*hst3 hst4* + *hht1K56R*) were spotted on SC −Leu −Ura and SC −Leu +5-FOA and grown at the indicated temperatures for 2 and 3 days, respectively. (B) Serial dilutions (1:5) of strains ICY356a (WT + vector), ICY703a (*hst3 hst4* + vector), ICY431a, -b, and -c, ICY1566a, -b, and -c, ICY1568a, -b, and -c, ICY1570a, -b, and -c, ICY1572a, -b, and -c, ICY682a, -b, and -c, ICY1574a, -b, and -c, and ICY1576a, -b, and -c were spotted on SC −Leu −Ura and SC −Leu +5-FOA and grown at the indicated temperatures for 2 and 3 days, respectively.

2003; KUBOTA *et al.* 2003; TAKAYAMA *et al.* 2003), Mcm2-7p helicase, Cdc45p, Tof1p–Csm3p complex, the histone chaperone FACT, Mcm10p, and Top1p. As part of the RPC that moves with replication forks, Ctf4p is ideally positioned to modulate replication fork integrity with the help of K56 acetylation. For instance, the loss of histone–DNA interactions mediated by H3 K56 acetylation (MASUMOTO *et al.* 2005; DRISCOLL *et al.* 2007) may facilitate the action of Ctf4p at damaged replication forks. Alternatively, Ctf4p itself or an associated protein may contain a "reading head" that directly binds to K56-acetylated nucleosomes at damaged replication forks. Rtt101p is a yeast cullin implicated in promoting replication through MMS-alkylated DNA and natural pause sites. It has been proposed (COLLINS *et al.* 2007) that Rtt101p functions in the same pathway as K56 acetylation by targeting a protein whose degradation is important to allow replisome progression through genomic regions that are inherently difficult to replicate. Our suppression analysis suggests that Rfc1p function is limiting in *hst3 hst4* cells that have constitutive K56 acetylation throughout the genome. However, Rfc1p levels were not significantly affected in *hst3 hst4* cells (data not shown). The K56 acetylation pathway may regulate, either directly or indirectly, Rfc1p complex formation rather than the actual protein level or the activity of the Rfc1p–RFC complex. An obvious consequence of negative regulation of Rfc1p–RFC by K56

Downloaded from https://academic.oup.com/genetics/article/179/4/1769/6064715 by guest on 23 April 2024

hyperacetylation in *hst3 hst4* mutants would be reduced loading efficiency of PCNA at replication forks and this may lead to defects in DNA replication and spontaneous DNA damage. However, we did not find evidence (by ChIP using anti-PCNA) that loading of bulk PCNA onto DNA was affected in *hst3 hst4* cells. This may reflect the fact that Ctf18p–RFC also uses PCNA as a clamp. The nature of the clamp is unknown for Elg1p–RFC, but could be PCNA. Although overall PCNA loading appears unaltered in *hst3 hst4* cells, either PCNA-associated proteins or posttranslational modifications of PCNA (Naryzhny and Lee 2004) may actually differ in the mutant cells. We hypothesize that the K56 acetylation pathway, together with Ctf4p assists Rfc1p–RFC in rescuing stalled or collapsed DNA replication forks resulting from lesions or protein barriers tightly bound to DNA. In wild-type cells, it is not known yet whether deacetylation of K56 happens immediately after fork passage or genomewide in G_2. Since *hst3 hst4* cells are viable, but extremely sick, the negative effect of K56 hyperacetylation on Rfc1p–RFC cannot be absolute; Rfc1p–RFC function is either modestly reduced overall or significantly reduced but only in specific genomic regions.

Another interpretation of these interactions is that even in wild-type cells, there is ongoing competition and a precarious balance between Rfc1p and the other RFC complexes. This functional antagonism between the different RFC complexes may interfere with smooth progression of replication forks. This is not a major problem for wild-type cells, but because *hst3 hst4* mutant cells are acutely sensitive to subtle perturbations in DNA replication, the competition between the different RFC complexes is a serious threat to *hst3 hst4* cells.

In any case, our data argue that *hst3 hst4* cells replicate the genome under suboptimal conditions. This is consistent with the presence of spontaneous DNA damage in *hst3 hst4* cells and their synthetic lethality observed specifically with mutations in genes implicated in DNA replication and repair. The lethality of *hst3 hst4* cells occurs even with very subtle perturbations of DNA replication. For instance, a tagged but otherwise wild-type *CDC45* allele that has no detectable phenotype in a wild-type cell is lethal in combination with *hst3 hst4*. In addition to synthetic lethality observed with DNA replication and repair genes, we have observed synthetic lethality between *hst3 hst4* and some components of the DNA replication checkpoint. Interestingly, deletion of *MEC1* results in synthetic lethality with *hst3 hst4*. Ironically, Hst3p is subjected to Mec1p-dependent degradation when cells are exposed to DNA damage (Thaminy *et al.* 2007). Thus it appears that Mec1p has multiple roles in the K56 acetylation/deacetylation cycle.

Interestingly, a recent report suggested that *hst3 hst4* cells have a defect in sister chromatid cohesion (Thaminy *et al.* 2007). Conceivably, this defect could explain both the Ts phenotype and the genotoxic agent sensitivity of *hst3 hst4* cells, since cohesion facilitates DNA double-strand break repair (Strom *et al.* 2004). However, the *hst3 hst4* genetic interactions reported here are not fully consistent with this model. Ctf4p has been clearly implicated in sister chromatid cohesion (Hanna *et al.* 2001). Thus, the loss of Ctf4p would be expected to exacerbate the cohesion defect of *hst3 hst4* cells but, contrary to this expectation, we find that *CTF4* deletion rescues their Ts phenotype. Moreover, *CTF4*, *CSM3*, and *TOF1* belong to the same epistasis group for sister chromatid cohesion (Xu *et al.* 2007b). However, while deletion of *CTF4* suppresses *hst3 hst4* phenotypes, deletion of other RPC subunits, like *TOF1* and *CSM3*, actually results in synthetic lethality with *hst3 hst4* (data not shown; Thaminy *et al.* 2007). Hopefully, a detailed molecular analysis of replisome architecture in cells lacking K56Ac or in *hst3 hst4* cells that have constitutive K56 acetylation will reveal the detailed mechanism by which the cycle of K56 acetylation/deacetyation regulates genomic stability and whether or not this cycle is important uniformly throughout the genome.

The authors thank Steve Elledge for the *pol2-11* construct, Boeke lab members for discussions, and Emerita Caputo for technical support. Research in A.V.'s laboratory is funded by Fonds de al Recherche en Santé Québec and the Canadian Institutes of Health Research (R0014340). This work was supported in part by a Roadmap grant from the National Institutes of Health (RR020839) to J.B.

LITERATURE CITED

Aboussekhra, A., J. E. Vialard, D. E. Morrison, M. A. de la Torre-Ruiz, L. Cernakova *et al.*, 1996 A novel role for the budding yeast RAD9 checkpoint gene in DNA damage-dependent transcription. EMBO J. **15:** 3912–3922.

Allen, J. B., Z. Zhou, W. Siede, E. C. Friedberg and S. J. Elledge, 1994 The SAD1/RAD53 protein kinase controls multiple checkpoints and DNA damage-induced transcription in yeast. Genes Dev. **8:** 2401–2415.

Basrai, M. A., V. E. Velculescu, K. W. Kinzler and P. Hieter, 1999 NORF5/HUG1 is a component of the MEC1-mediated checkpoint response to DNA damage and replication arrest in Saccharomyces cerevisiae. Mol. Cell. Biol. **19:** 7041–7049.

Ben-Aroya, S., A. Koren, B. Liefshitz, R. Steinlauf and M. Kupiec, 2003 ELG1, a yeast gene required for genome stability, forms a complex related to replication factor C. Proc. Natl. Acad. Sci. USA **100:** 9906–9911.

Bermudez, V. P., Y. Maniwa, I. Tappin, K. Ozato, K. Yokomori *et al.*, 2003 The alternative Ctf18-Dcc1-Ctf8-replication factor C complex required for sister chromatid cohesion loads proliferating cell nuclear antigen onto DNA. Proc. Natl. Acad. Sci. USA **100:** 10237–10242.

Brachmann, C. B., 1996 The *SIR2/HST* gene family. Ph.D. Thesis. Johns Hopkins University, Baltimore.

Brachmann, C. B., J. M. Sherman, S. E. Devine, E. E. Cameron, L. Pillus *et al.*, 1995 The SIR2 gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression, and chromosome stability. Genes Dev. **9:** 2888–2902.

Celic, I., H. Masumoto, W. P. Griffith, P. Meluh, R. J. Cotter *et al.*, 2006 The sirtuins hst3 and Hst4p preserve genome integrity by controlling histone h3 lysine 56 deacetylation. Curr. Biol. **16:** 1280–1289.

Cohen-Fix, O., and D. Koshland, 1997 The anaphase inhibitor of Saccharomyces cerevisiae Pds1p is a target of the DNA damage checkpoint pathway. Proc. Natl. Acad. Sci. USA **94:** 14361–14366.

Collins, S. R., K. M. Miller, N. L. Maas, A. Roguev, J. Fillingham *et al.*, 2007 Functional dissection of protein complexes involved

Downloaded from https://academic.oup.com/genetics/article/179/4/1769/6064715 by guest on 23 April 2024

in yeast chromosome biology using a genetic interaction map. Nature **446:** 806–810.
Cullmann, G., K. Fien, R. Kobayashi and B. Stillman, 1995 Characterization of the five replication factor C genes of Saccharomyces cerevisiae. Mol. Cell. Biol. **15:** 4661–4671.
de la Torre-Ruiz, M. A., C. M. Green and N. F. Lowndes, 1998 RAD9 and RAD24 define two additive, interacting branches of the DNA damage checkpoint pathway in budding yeast normally required for Rad53 modification and activation. EMBO J. **17:** 2687–2698.
Driscoll, R., A. Hudson and S. P. Jackson, 2007 Yeast Rtt109 promotes genome stability by acetylating histone H3 on lysine 56. Science **315:** 649–652.
Elledge, S. J., and R. W. Davis, 1990 Two genes differentially regulated in the cell cycle and by DNA-damaging agents encode alternative regulatory subunits of ribonucleotide reductase. Genes Dev. **4:** 740–751.
Fien, K., and B. Stillman, 1992 Identification of replication factor C from Saccharomyces cerevisiae: a component of the leading-strand DNA replication complex. Mol. Cell. Biol. **12:** 155–163.
Gambus, A., R. C. Jones, A. Sanchez-Diaz, M. Kanemaki, F. van Deursen *et al.*, 2006 GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks. Nat. Cell Biol. **8:** 358–366.
Gardner, R. D., and D. J. Burke, 2000 The spindle checkpoint: two transitions, two pathways. Trends Cell Biol. **10:** 154–158.
Gardner, R., C. W. Putnam and T. Weinert, 1999 RAD53, DUN1 and PDS1 define two parallel G2/M checkpoint pathways in budding yeast. EMBO J. **18:** 3173–3185.
Gardner, R. G., Z. W. Nelson and D. E. Gottschling, 2005 Ubp10/Dot4p regulates the persistence of ubiquitinated histone H2B: distinct roles in telomeric silencing and general chromatin. Mol. Cell. Biol. **25:** 6123–6139.
Gasch, A. P., M. Huang, S. Metzner, D. Botstein, S. J. Elledge *et al.*, 2001 Genomic expression responses to DNA-damaging agents and the regulatory role of the yeast ATR homolog Mec1p. Mol. Biol. Cell **12:** 2987–3003.
Green, C. M., H. Erdjument-Bromage, P. Tempst and N. F. Lowndes, 2000 A novel Rad24 checkpoint protein complex closely related to replication factor C. Curr. Biol. **10:** 39–42.
Gunjan, A., and A. Verreault, 2003 A Rad53 kinase-dependent surveillance mechanism that regulates histone protein levels in S. cerevisiae. Cell **115:** 537–549.
Haase, S. B., and D. J. Lew, 1997 Flow cytometric analysis of DNA content in budding yeast. Methods Enzymol. **283:** 322–332.
Han, J., H. Zhou, B. Horazdovsky, K. Zhang, R. M. Xu *et al.*, 2007 Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication. Science **315:** 653–655.
Hanna, J. S., E. S. Kroll, V. Lundblad and F. A. Spencer, 2001 Saccharomyces cerevisiae CTF18 and CTF4 are required for sister chromatid cohesion. Mol. Cell. Biol. **21:** 3144–3158.
Hill, J. E., A. M. Myers, T. J. Koerner and A. Tzagoloff, 1986 Yeast/E. coli shuttle vectors with multiple unique restriction sites. Yeast **2:** 163–167.
Howell, E. A., M. A. McAlear, D. Rose and C. Holm, 1994 CDC44: a putative nucleotide-binding protein required for cell cycle progression that has homology to subunits of replication factor C. Mol. Cell. Biol. **14:** 255–267.
Hyland, E. M., M. S. Cosgrove, H. Molina, D. Wang, A. Pandey *et al.*, 2005 Insights into the role of histone H3 and histone H4 core modifiable residues in Saccharomyces cerevisiae. Mol. Cell. Biol. **25:** 10060–10070.
Imai, S., C. M. Armstrong, M. Kaeberlein and L. Guarente, 2000 Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature **403:** 795–800.
Jackson, V., A. Shires, N. Tanphaichitr and R. Chalkley, 1976 Modifications to histones immediately after synthesis. J. Mol. Biol. **104:** 471–483.
Jawad, Z., and M. Paoli, 2002 Novel sequences propel familiar folds. Structure **10:** 447–454.
Kanemaki, M., A. Sanchez-Diaz, A. Gambus and K. Labib, 2003 Functional proteomic identification of DNA replication proteins by induced proteolysis in vivo. Nature **423:** 720–724.
Kondo, T., K. Matsumoto and K. Sugimoto, 1999 Role of a complex containing Rad17, Mec3, and Ddc1 in the yeast DNA damage checkpoint pathway. Mol. Cell. Biol. **19:** 1136–1143.
Kouprina, N., E. Kroll, V. Bannikov, V. Bliskovsky, R. Gizatullin *et al.*, 1992 CTF4 (CHL15) mutants exhibit defective DNA metabolism in the yeast Saccharomyces cerevisiae. Mol. Cell. Biol. **12:** 5736–5747.
Kubota, Y., Y. Takase, Y. Komori, Y. Hashimoto, T. Arata *et al.*, 2003 A novel ring-like complex of Xenopus proteins essential for the initiation of DNA replication. Genes Dev. **17:** 1141–1152.
Kumar, C., R. Sharma and A. K. Bachhawat, 2003 Investigations into the polymorphisms at the ECM38 locus of two widely used Saccharomyces cerevisiae S288C strains, YPH499 and BY4742. Yeast **20:** 857–863.
Kushnirov, V. V., 2000 Rapid and reliable protein extraction from yeast. Yeast **16:** 857–860.
Landry, J., A. Sutton, S. T. Tafrov, R. C. Heller, J. Stebbins *et al.*, 2000 The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. Proc. Natl. Acad. Sci. USA **97:** 5807–5811.
Lengronne, A., J. McIntyre, Y. Katou, Y. Kanoh, K. P. Hopfner *et al.*, 2006 Establishment of sister chromatid cohesion at the S. cerevisiae replication fork. Mol. Cell **23:** 787–799.
Maas, N. L., K. M. Miller, L. G. DeFazio and D. P. Toczyski, 2006 Cell cycle and checkpoint regulation of histone H3 K56 acetylation by Hst3 and Hst4. Mol. Cell **23:** 109–119.
Masumoto, H., D. Hawke, R. Kobayashi and A. Verreault, 2005 A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response. Nature **436:** 294–298.
Mayer, M. L., S. P. Gygi, R. Aebersold and P. Hieter, 2001 Identification of RFC(Ctf18p, Ctf8p, Dcc1p): an alternative RFC complex required for sister chromatid cohesion in S. cerevisiae. Mol. Cell **7:** 959–970.
Miles, J., and T. Formosa, 1992 Evidence that POB1, a Saccharomyces cerevisiae protein that binds to DNA polymerase alpha, acts in DNA metabolism in vivo. Mol. Cell. Biol. **12:** 5724–5735.
Miller, K. M., N. L. Maas and D. P. Toczyski, 2006 Taking it off: regulation of H3 K56 acetylation by Hst3 and Hst4. Cell Cycle **5:** 2561–2565.
Naiki, T., T. Kondo, D. Nakada, K. Matsumoto and K. Sugimoto, 2001 Chl12 (Ctf18) forms a novel replication factor C-related complex and functions redundantly with Rad24 in the DNA replication checkpoint pathway. Mol. Cell. Biol. **21:** 5838–5845.
Naryzhny, S. N., and H. Lee, 2004 The post-translational modifications of proliferating cell nuclear antigen: acetylation, not phosphorylation, plays an important role in the regulation of its function. J. Biol. Chem. **279:** 20194–20199.
Navas, T. A., Z. Zhou and S. J. Elledge, 1995 DNA polymerase epsilon links the DNA replication machinery to the S phase checkpoint. Cell **80:** 29–39.
Ozdemir, A., S. Spicuglia, E. Lasonder, M. Vermeulen, C. Campsteijn *et al.*, 2005 Characterization of lysine 56 of histone H3 as an acetylation site in Saccharomyces cerevisiae. J. Biol. Chem. **280:** 25949–25952.
Pan, X., P. Ye, D. S. Yuan, X. Wang, J. S. Bader *et al.*, 2006 A DNA integrity network in the yeast Saccharomyces cerevisiae. Cell **124:** 1069–1081.
Park, J. H., M. S. Cosgrove, E. Youngman, C. Wolberger and J. D. Boeke, 2002 A core nucleosome surface crucial for transcriptional silencing. Nat. Genet. **32:** 273–279.
Paulovich, A. G., and L. H. Hartwell, 1995 A checkpoint regulates the rate of progression through S phase in S. cerevisiae in response to DNA damage. Cell **82:** 841–847.
Pringle, J. R., A. E. Adams, D. G. Drubin and B. K. Haarer, 1991 Immunofluorescence methods for yeast. Methods Enzymol. **194:** 565–602.
Pursell, Z. F., I. Isoz, E. B. Lundstrom, E. Johansson and T. A. Kunkel, 2007 Yeast DNA polymerase epsilon participates in leading-strand DNA replication. Science **317:** 127–130.
Recht, J., T. Tsubota, J. C. Tanny, R. L. Diaz, J. M. Berger *et al.*, 2006 Histone chaperone Asf1 is required for histone H3 lysine 56 acetylation, a modification associated with S phase in mitosis and meiosis. Proc. Natl. Acad. Sci. USA **103:** 6988–6993.
Sanchez, Y., B. A. Desany, W. J. Jones, Q. Liu, B. Wang *et al.*, 1996 Regulation of RAD53 by the ATM-like kinases MEC1

Downloaded from https://academic.oup.com/genetics/article/179/4/1769/6064715 by guest on 23 April 2024

and TEL1 in yeast cell cycle checkpoint pathways. Science **271:** 357–360.

Sanchez, Y., J. Bachant, H. Wang, F. Hu, D. Liu *et al.*, 1999 Control of the DNA damage checkpoint by chk1 and rad53 protein kinases through distinct mechanisms. Science **286:** 1166–1171.

Schneider, J., P. Bajwa, F. C. Johnson, S. R. Bhaumik and A. Shilatifard, 2006 Rtt109 is required for proper H3K56 acetylation: a chromatin mark associated with the elongating RNA polymerase II. J. Biol. Chem. **281:** 37270–37274.

Shimomura, T., S. Ando, K. Matsumoto and K. Sugimoto, 1998 Functional and physical interaction between Rad24 and Rfc5 in the yeast checkpoint pathways. Mol. Cell. Biol. **18:** 5485–5491.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae.* Genetics **122:** 19–27.

Smith, J. S., C. B. Brachmann, I. Celic, M. A. Kenna, S. Muhammad *et al.*, 2000 A phylogenetically conserved NAD+-dependent protein deacetylase activity in the Sir2 protein family. Proc. Natl. Acad. Sci. USA **97:** 6658–6663.

Sobel, R. E., R. G. Cook, C. A. Perry, A. T. Annunziato and C. D. Allis, 1995 Conservation of deposition-related acetylation sites in newly synthesized histones H3 and H4. Proc. Natl. Acad. Sci. USA **92:** 1237–1241.

Spencer, F., S. L. Gerring, C. Connelly and P. Hieter, 1990 Mitotic chromosome transmission fidelity mutants in *Saccharomyces cerevisiae.* Genetics **124:** 237–249.

Strom, L., H. B. Lindroos, K. Shirahige and C. Sjogren, 2004 Postreplicative recruitment of cohesin to double-strand breaks is required for DNA repair. Mol. Cell **16:** 1003–1015.

Sun, Z., D. S. Fay, F. Marini, M. Foiani and D. F. Stern, 1996 Spk1/Rad53 is regulated by Mec1-dependent protein phosphorylation in DNA replication and damage checkpoint pathways. Genes Dev. **10:** 395–406.

Suter, B., A. Tong, M. Chang, L. Yu, G. W. Brown *et al.*, 2004 The origin recognition complex links replication, sister chromatid cohesion and transcriptional silencing in *Saccharomyces cerevisiae.* Genetics **167:** 579–591.

Takayama, Y., Y. Kamimura, M. Okawa, S. Muramatsu, A. Sugino *et al.*, 2003 GINS, a novel multiprotein complex required for chromosomal DNA replication in budding yeast. Genes Dev. **17:** 1153–1165.

Thaminy, S., B. Newcomb, J. Kim, T. Gatbonton, E. Foss *et al.*, 2007 HST3 is regulated by MEC1-dependent proteolysis and controls the S phase checkpoint and sister chromatid cohesion by deacetylating histone H3 at lysine 56. J. Biol. Chem. **282:** 37805–37814.

Tong, A. H., M. Evangelista, A. B. Parsons, H. Xu, G. D. Bader *et al.*, 2001 Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science **294:** 2364–2368.

Tsurimoto, T., and B. Stillman, 1990 Functions of replication factor C and proliferating-cell nuclear antigen: functional similarity of DNA polymerase accessory proteins from human cells and bacteriophage T4. Proc. Natl. Acad. Sci. USA **87:** 1023–1027.

Weinert, T. A., G. L. Kiser and L. H. Hartwell, 1994 Mitotic checkpoint genes in budding yeast and the dependence of mitosis on DNA replication and repair. Genes Dev. **8:** 652–665.

Xu, F., K. Zhang and M. Grunstein, 2005 Acetylation in histone H3 globular domain regulates gene expression in yeast. Cell **121:** 375–385.

Xu, F., Q. Zhang, K. Zhang, W. Xie and M. Grunstein, 2007a Sir2 deacetylates histone H3 lysine 56 to regulate telomeric heterochromatin structure in yeast. Mol. Cell **27:** 890–900.

Xu, H., C. Boone and G. W. Brown, 2007b Genetic dissection of parallel sister-chromatid cohesion pathways. Genetics **176:** 1417–1429.

Ye, J., X. Ai, E. E. Eugeni, L. Zhang, L. R. Carpenter *et al.*, 2005 Histone H4 lysine 91 acetylation a core domain modification associated with chromatin assembly. Mol. Cell **18:** 123–130.

Zhou, Z., and S. J. Elledge, 1993 DUN1 encodes a protein kinase that controls the DNA damage response in yeast. Cell **75:** 1119–1127.

Communicating editor: M. Hampsey

Downloaded from https://academic.oup.com/genetics/article/179/4/1769/6064715 by guest on 23 April 2024