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Alterations in DNA Replication and Histone Levels Promote Histone Gene Amplification in *Saccharomyces cerevisiae*

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ABSTRACT

Gene amplification, a process that increases the copy number of a gene or a genomic region to two or more, is utilized by many organisms in response to environmental stress or decreased levels of a gene product. Our previous studies in *Saccharomyces cerevisiae* identified the amplification of a histone H2A-H2B gene pair, *HTA2-HTB2*, in response to the deletion of the other H2A-H2B gene pair, *HTA1-HTB1*. This amplification arises from a recombination event between two flanking Ty1 elements to form a new, stable circular chromosome and occurs at a frequency higher than has been observed for other Ty1-Ty1 recombination events. To understand the regulation of this amplification event, we screened the *S. cerevisiae* nonessential deletion set for mutations that alter the amplification frequency. Among the deletions that increase *HTA2-HTB2* amplification frequency, we identified those that either decrease DNA replication fork progression (*rrm3*Δ, *dpb3*Δ, *dpb4*Δ, and *clb5*Δ) or that reduce histone H3-H4 levels (*hht2-hhf2*Δ). These two classes are related because reduced histone H3-H4 levels increase replication fork pauses, and impaired replication forks cause a reduction in histone levels. Consistent with our mutant screen, we found that the introduction of DNA replication stress by hydroxyurea induces the *HTA2-HTB2* amplification event. Taken together, our results suggest that either reduced histone levels or slowed replication forks stimulate the *HTA2-HTB2* amplification event, contributing to the restoration of normal chromatin structure.

CHANGES in gene copy number can cause significant phenotypic consequences. Gene amplification, a process that increases gene copy number to two or more, has been shown to occur as part of normal developmental processes, such as chorion gene amplification during oogenesis in *Drosophila melanogaster* (Spradling 1981; Orr *et al.* 2004; Claycomb *et al.* 2004; Tower 2004). In other cases, gene amplification has been shown to enhance growth under particular conditions; for example, amplification of a weakened *lac* operon in *Escherichia coli* allows improved growth on medium containing lactose as the carbon source (Hastings *et al.* 2004; Kugelberg *et al.* 2006). Recent studies have shown that changes in gene copy number are common in the human genome because copy number variants in humans occur genomewide, are polymorphic, and are sometimes associated with particular traits (McCarroll and Altshuler 2007). Moreover, gene amplification in humans is associated with some cancers (Stark and Wahl 1984; Hahn 1993; Graux *et al.* 2004; Vogt *et al.* 2004; Albertson 2006; Fridlyand *et al.* 2006).

One mechanism by which copy number changes occur is via recombination between repeated elements, such as transposable elements. In the yeast *Saccharomyces cerevisiae*, Ty retrotransposons have been shown to play a significant role in mediating genomic rearrangements, including gene amplifications (see Mieczkowski *et al.* 2006 for a review). These changes have been shown to occur under many different growth conditions, including selections for increased gene expression or drug resistance, after stress, or spontaneously (for examples, see Liebman *et al.* 1981; Brown *et al.* 1998; Rachidi *et al.* 1999; Hughes *et al.* 2000; Dunham *et al.* 2002; Koszul *et al.* 2004; Schacherer *et al.* 2004; Selmecki *et al.* 2006; Argueso *et al.* 2008). A recent study showed that Ty-mediated amplifications are a common occurrence when *S. cerevisiae* is grown under conditions limiting for glucose, phosphate, or sulfate (Gresham *et al.* 2008).

Several studies in *S. cerevisiae* have provided strong evidence that compromised DNA replication can increase the level of mitotic recombination and of Ty-mediated rearrangements. Cha and Kleckner (2002) showed that double-strand breaks in DNA occur at a greater frequency when replication is impaired by a *mec1-ts* mutation, thereby leading to increased mitotic recombination. Moreover, Vernon *et al.* (2008) demon-

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strated that in *mec1 tel1* strains, the breakpoints of interchromasomal genomic rearrangements are associated with Ty elements. In another study, reduced levels of the replicative DNA polymerase α were shown to slow DNA replication and to cause greatly elevated frequencies of rearrangements, with many occurring by recombination between Ty elements (Lemoine *et al.* 2005). Finally, in a recent study, segmental duplications, many Ty-mediated, were shown to occur in a DNA-replication-dependent fashion (Payen *et al.* 2008). *Schizosaccharomyces pombe* appears to be similar to *S. cerevisiae* because elevated levels of mitotic recombination were shown to occur when replication forks were blocked artificially (Lambert *et al.* 2005). Taken together, these studies suggest that impaired replication forks are prone to suffer double-strand breaks, which in turn promote mitotic recombination.

A tight connection has also been established between histone levels, impaired DNA replication, and genome stability. In *S. cerevisiae*, early studies established that histone levels are correlated with DNA synthesis (Hereford *et al.* 1981; Osley and Hereford 1982). Furthermore, altered histone levels have been shown to cause problems with chromatin structure, chromosome segregation, transcription, and other aspects of cell growth (Meeks-Wagner and Hartwell 1986; Norris and Osley 1987; Clark-Adams *et al.* 1988; Norris *et al.* 1988). More recent evidence suggests that reduced histone levels in *S. cerevisiae* cause problems with DNA replication because repression of histone H4 gene transcription results in the elevation of replication-stress-induced transcripts, thereby suggesting that H4 depletion causes an impairment of fork movement (Wyrick *et al.* 1999). Consistent with this finding, another study showed that decreased histone H4 levels increases stalled replication forks, thereby increasing the frequency of homologous recombination (Prado and Aguilera 2005). Conversely, treatment of yeast cells with agents that damage DNA or that stall or slow replication forks decreases histone mRNA levels in both yeast and mammalian cells (Lycan *et al.* 1987; Gasch *et al.* 2001; Su *et al.* 2004), likely reflecting the effects of these agents on progression through the cell cycle. Overall, these studies have highlighted the importance of controlling histone levels to maintain genome stability (Gunjan *et al.* 2005).

Our previous work showed that one mechanism by which *S. cerevisiae* maintains proper histone levels is by amplification of a histone H2A-H2B locus, *HTA2-HTB2* (Libuda and Winston 2006). This amplification was shown to occur in response to reduced H2A-H2B levels caused by deletion of the other H2A-H2B locus, *HTA1-HTB1*. Our work demonstrated that *HTA2-HTB2* amplification occurs by formation of a small circular chromosome, the result of mitotic recombination between two Ty1 elements that flank a 40-kb region of chromosome II that contains *HTA2-HTB2*, *CEN2*, and three origins of replication. Furthermore, the amplification occurs at a much higher frequency than other Ty-Ty recombination events. To understand circumstances in which *HTA2-HTB2* amplification might normally occur, we have performed a genetic screen to identify regulators of the *HTA2-HTB2* amplification event. Among the genes identified in our screen, we identified factors required for the normal progression of DNA replication forks. On the basis of this finding, we also show that treating cells with hydroxyurea (HU) stimulates *HTA2-HTB2* amplification. In addition, our mutant screen shows that alterations in histone stoichiometry stimulate the amplification event. Finally, our analysis has shown that a greatly reduced expression of the genes encoding histones H3 and H4 significantly increases the level of DNA replication-stress-induced transcripts. On the basis of these results, we propose a model in which environmental conditions that reduce histone levels, and hence impair replication fork movement, enhance the formation of the *HTA2-HTB2* amplification to aid in the recovery and restoration of normal chromatin structure.

MATERIALS AND METHODS

***S.cerevisiae* strains and media:** All *S. cerevisiae* strains used in this study (Table 1) are isogenic with a *GAL2*$^+$ derivative of S288C (Winston *et al.* 1995). Standard methods for mating, sporulation, transformation, and tetrad analysis were used, and all media were prepared as described previously (Rose *et al.* 1990). Strains were constructed by either crosses or transformations and were grown at 30°. Gene deletions made as part of this study removed the following sequences (coordinates given from 5′ of the ATG to 3′ of the termination codon): *rrm3*Δ, 12 bp 5′ to 152 bp 3′; *dpb3*Δ, 12 bp 5′ to 34 bp 3′; *dpb4*Δ, 102 bp 5′ to 1 bp 3′; *clb5*Δ, 305 bp 5′ to 418 bp 3′; *dun1*Δ, 40 bp 5′ to 40 bp 3′; *mec1*Δ, 1 bp to 6884 bp of the ORF; and *sml1*Δ, 39 bp 5′ to 71 bp 3′. Sequences of oligonucleotides used are in the supporting information, Table S1. The *(hht1-hhf1)*Δ mutation removes the *HHT1-HHF1* locus from 100 bp 3′ of the *HHT1* ORF to 250 bp 3′ of the *HHF1* ORF. The *(hht2-hhf2)*Δ mutation removes the *HHT2-HHF2* locus from 26 bp 3′ of the *HHT2* ORF to 18 bp 3′ of the *HHF2* ORF. The *coq1::HIS3* reporter was integrated by transformation with *Bsu*361-digested pDL1 (Libuda and Winston 2006). FY2784 was created by transforming FY1990 with pDM10, selection on 5-fluoroorotic acid (5-FOA) medium to lose pDM1, transformation with pMR102, and selection on 5-fluoroanthranilic acid media with 2% galactose to lose pDM10.

Plasmids: Plasmids were prepared from *E. coli* strains DH5α or MH1 according to standard procedures. The plasmids B155 (Libuda and Winston 2006), pSAB6 (Hirschhorn *et al.* 1995), pDL1 (Libuda and Winston 2006), pDL2 (Libuda and Winston 2006), pMR102 (Mann and Grunstein 1992), pHB59 (Hirschhorn *et al.* 1992), pCC64 (Clark-Adams *et al.* 1988), and pCC65 (Clark-Adams *et al.* 1988) have been described previously. pDM1 was constructed by amplifying the *HHT2-HHF2* locus from plasmid pCC65 (Clark-Adams *et al.* 1988) and ligating it into pRS416 (Brachmann *et al.* 1998) after digestion with *Hin*dIII and *Bam*HI. pDM10 was constructed by amplifying the *HHT1-HHF1* locus from plasmid pCC64 (Clark-Adams *et al.* 1988) and ligating it into pRS414 (Brachmann *et al.* 1998) after digestion with *Hin*dIII and *Bam*HI.

TABLE 1

***S. cerevisiae* strains used in these studies**

Strain	Genotype
FY4	*MAT***a**
FY2509	*MAT***a**/*MAT*α *(hta1-htb1)*Δ*::LEU2/HTA1-HTB1 his3*Δ*200/his3*Δ*200 ura3*Δ*0/ura3*Δ*0 leu2*Δ*0/leu2*Δ*0 lys2-128*δ*/lys2-128*δ
FY2510	*MAT***a** *(hta1-htb1)*Δ*::LEU2 his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2512	*MAT*α *(hta1-htb1)*Δ*::LEU2 his3*Δ*200 ura3-52 leu2*Δ*1 lys2-128*δ *trp1*Δ*63* <pSAB6>
FY2749	*MAT***a**/*MAT*α *(hta1-htb1)*Δ*::LEU2/HTA1-HTB1 dpb3*Δ*::HIS3/dpb3*Δ*::kanMX his3*Δ*200/his3*Δ*200 leu2*Δ*0/leu2*Δ*0 ura3*Δ*0/ura3*Δ*0 lys2-128*δ*/lys2-128*δ
FY2750	*MAT*α *(hta1-htb1)*Δ*::LEU2 dpb3*Δ*::kanMX his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2751	*MAT*α *dpb3*Δ*::HIS3 his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2752	*MAT***a**/*MAT*α *(hta1-htb1)*Δ*::LEU2/HTA1-HTB1 dpb4*Δ*::HIS3/dpb4*Δ*::kanMX his3*Δ*200/his3*Δ*200 leu2*Δ*0/leu2*Δ*0 ura3*Δ*0/ura3d0 lys2-128*δ*/lys2-128*δ
FY2753	*MAT***a** *dpb4*Δ*::kanMX his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2754	*MAT***a** *(hta1-htb1)*Δ*::LEU2 dpb4*Δ*::HIS3 his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2755	*MAT*α *dpb4*Δ*::kanMX his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2756	*MAT***a**/*MAT*α *(hta1-htb1)*Δ*::LEU2/HTA1-HTB1 rrm3*Δ*::HIS3/rrm3*Δ*::kanMX his3*Δ*200/his3*Δ*200 leu2*Δ*0/leu2*Δ*0 ura3*Δ*0/ura3*Δ*0 lys2-128*δ*/lys2-128*δ
FY2757	*MAT***a** *rrm3*Δ*::HIS3 his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2758	*MAT*α *(hta1-htb1)*Δ*::LEU2 rrm3*Δ*::kanMX his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2759	*MAT*α *rrm3*Δ*::HIS3 his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2760	*MAT***a**/*MAT*α *(hta1-htb1)*Δ*::LEU2/ HTA1-HTB1 clb5*Δ*::HIS3/CLB5 his3*Δ*200/his3*Δ*200 leu2*Δ*0/leu2*Δ*0 ura3*Δ*0/ura3*Δ*0 lys2-128*δ*/lys2-128*δ
FY2761	*MAT***a** *clb5*Δ*::HIS3 his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2762	*MAT***a** *clb5*Δ*::HIS3 (hta1-htb1)*Δ*::LEU2 his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2763	*MAT***a** *coq1::HIS3* FB118*::YDRWTy1-5 his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-218*δ *trp1*Δ*63*
FY2764	*MAT***a** *coq1::HIS3* FB118*::YPLWTy1-1 his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-218*δ *trp1*Δ*63*
FY2765	*MAT*α *coq1::HIS3* FB118*::YDRWTy1-5 his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-218*δ *trp1*Δ*63*
FY2766	*MAT*α *coq1::HIS3* FB118*::YPLWTy1-1 his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-218*δ *trp1*Δ*63*
FY2767	*MAT***a**/*MAT*α *(hta1-htb1)*Δ*::LEU2/HTA1-HTB1 mec1*Δ*::HIS3/mec1*Δ*::HIS3 sml1*Δ*::kanMX/sml1*Δ*::kanMX his3*Δ*200/his3*Δ*200 ura3*Δ*0/ura3*Δ*0 leu2*Δ*0/leu2*Δ*0 lys2-128*δ*/lys2-128*δ
FY2768	*MAT***a**/*MAT*α *(hta1-htb1)*Δ*::LEU2/HTA1-HTB1 dun1*Δ*::kanMX/dun1*Δ*::kanMX his3*Δ*200/his3*Δ*200 ura3*Δ*0/ura3*Δ*0 leu2*Δ*0/leu2*Δ*0 lys2-128*δ*/lys2-128*δ
FY2770	*MAT***a**/*MAT*α *(hta1-htb1)*Δ*::LEU2/HTA1-HTB1 sml1*Δ*::kanMX/sml1*Δ*::kanMX his3*Δ*200/his3*Δ*200 ura3*Δ*0/ura3*Δ*0 leu2*Δ*0/leu2*Δ*0 lys2-128*δ*/lys2-128*δ
FY2771	*MAT***a** *mec1*Δ*::HIS3 sml1*Δ*::kanMX his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2772	*MAT***a** *mec1*Δ*::HIS3 sml1*Δ*::kanMX (hta1-htb1)*Δ*::LEU2 his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2774	*MAT***a** *sml1*Δ*::kanMX his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2775	*MAT***a** *sml1*Δ*::kanMX (hta1-htb1)*Δ*::LEU2 his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2776	*MAT***a** *dun1*Δ*::kanMX his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2777	*MAT*α *dun1*Δ*::kanMX (hta1-htb1)*Δ*::LEU2 his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2778	*MAT***a**/*MAT*α *his3*Δ*200/his3*Δ*200 leu2*Δ*0/leu2*Δ*0 ura3*Δ*0/ura3*Δ*0 lys2-128*δ*/lys2-128*δ *(hta1-htb1)*Δ*::LEU2/HTA1-HTB1 (hhf1-hht1)*Δ*::G418/(hhf1-hht1)*Δ*::G418*
FY2779	*MAT***a**/*MAT*α *his3*Δ*200/his3*Δ*200 leu2*Δ*0/leu2*Δ*0 ura3*Δ*0/ura3*Δ*0 lys2-128*δ*/lys2-128*δ *(hta1-htb1)*Δ*::LEU2/HTA1-HTB1 (hhf2-hht2)*Δ*::G418/(hhf2-hht2)*Δ*::G418*
FY2780	*MAT*α *(hhf1-hht1)*Δ*::kanMX his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2781	*MAT*α *(hhf1-hht1)*Δ*::kanMX (hta1-htb1)*Δ*::LEU2 his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2782	*MAT***a** *(hhf2-hht2)*Δ*::kanMX (hta1-htb1)*Δ*::LEU2 his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2783	*MAT*α (*hhf2-hht2)*Δ*::kanMX his3*Δ*200 ura3*Δ*0 leu2*Δ*0 lys2-128*δ
FY2784	*MAT*α *his3*Δ*200 leu2*Δ*1 lys2-128*δ *ura3-52 trp1*Δ*63 (hht1-hhf1)*Δ*::LEU2 (hht2-hhf2)*Δ*::kanMX4* <pMR102>
FY2789	*MAT*α *(hta1*−*htb1)*Δ*::LEU2 leu2*Δ*0 lys2*−*128*δ *ura3*Δ*0 can1*Δ*::STE2pr*−*HIS3 his3*Δ*200* <pSAB6>

A screen to identify mutations that increase or decrease the frequency of *HTA2-HTB2* amplification: To identify mutations that alter the frequency of *HTA2-HTB2* amplification, we relied on the observation that amplification of *HTA2-HTB2* suppresses the inviability caused by *(hta1-htb1)*Δ (Libuda and Winston 2006). First, we crossed the *S. cerevisiae* nonessential gene deletion set (Giaever *et al.* 2002) by strain FY2789, which contains both *(hta1-htb1)*Δ and plasmid pSAB6 (with *HTA1-HTB1*). After selecting for diploids, we replica plated strains to 5-FOA to lose pSAB6 and then sporulated the strains as

described previously (Giaever *et al.* 2002). To assess the level of viable progeny when *(hta1-htb1)*Δ was combined with each deletion, progeny were replica plated to select for haploids (Can^R), the *(hta1-htb1)*Δ mutation (Leu^+), and the deletion set mutation ($G418^R$). From this primary screen, a large number of gene deletions were found to either increase or decrease the level of *(hta1-htb1)*Δ papillae relative to wild type. These candidates were then retested by repeating the screen for the subset of deletion set strains identified in the primary screen.

Hydroxyurea and methyl methanesulfonate treatments: HU (Sigma-Aldrich) was dissolved in water and filter sterilized before adding to liquid YPD for a final concentration of 50, 100, or 200 mm. Methyl methanesulfonate (MMS) (Sigma-Aldrich) was dissolved in water and filter sterilized before adding to liquid YPD for a final concentration of 0.02%. Each solution was made fresh for each experiment. For the assays measuring Ty1-Ty1 recombination and *HTA2-HTB2* amplification frequencies, cells were initially grown to 1×10^8 cells/ml at 30° in SC-his to maintain the *coq1::HIS3* reporter. YPD containing the different concentrations of HU or MMS were inoculated with the SC-his cultures to a final concentration of 1×10^6 cells/ml. Following growth for 24 hr at 30°, cells were plated as described above. For Northern analysis of cells treated with HU, cells were grown in YPD at 30° to 1–2 $\times 10^7$ cells/ml. Then, HU was added for a final concentration of 200 mm. Total RNA was prepared at the time points shown in Figure 3 using methods described previously (Swanson *et al.* 1991).

Assays for measuring Ty1-Ty1 recombination and *HTA2-HTB2* amplification formation: The recombination frequency between two Ty1 elements was measured using Ty1-*URA3*-Ty1 recombination reporters at *YDRWTy1*-5 on chromosome IV and *YPLWTy1-1* on chromosome XVI (Libuda and Winston 2006). *HTA2-HTB2* amplification was measured using the *coq1::HIS3* construct (Libuda and Winston 2006), which is based on a method adapted from Chan and Botstein (1993). To measure Ty-Ty recombination and *HTA2-HTB2* amplification, strains containing both *coq1::HIS3* and one of the Ty1-Ty1 recombination reporters (FY2763, FY2764, FY2765, and FY2766) were grown to 1×10^8 cells/ml at 30° in SC-his medium to maintain the *coq1::HIS3* reporter. Cells were then shifted to either YPD or YPD containing either HU or MMS as described below. Following growth for 24 hr at 30°, cells were plated on (1) YPD to measure the number of total cells in the culture; (2) 5-FOA plates to measure the number of cells that had undergone Ty1-Ty1 recombination at the Ty1-*URA3*-Ty1 reporter; (3) YPglycerol to measure the number of cells that had undergone a recombination at the *coq1::HIS3* reporter to restore the *COQ1* gene; and (4) SC-his plates with glycerol as the carbon source (SC-his + glycerol) to measure the number of cells that either contained the *HTA2-HTB2* amplification or were disomic for chromosome II. These plating assays were done for the following number of independent cultures for each treatment: no treatment, 40 cultures; 50 mm HU, 9 cultures; 100 mm HU, 12 cultures; 200 mm HU, 22 cultures; and 0.02% MMS, 9 cultures. Contour-clamped homogeneous electric field (CHEF) gel Southern analysis was performed for His^+ Gly^+ colonies to determine the frequency of *HTA2-HTB2* amplification among His^+ Gly^+ colonies for each treatment. The number of His^+ Gly^+ isolates with the *HTA2-HTB2* amplification over the total number tested was as follows: (1) no treatment, 28/61; (2) 50 mm HU, 10/12; (3) 100 mm HU, 8/10; (4) 200 mm HU, 33/40; and (5) 0.02% MMS, 23/30. Ty1-Ty1 recombination and *HTA2-HTB2* amplification formation frequencies were calculated as described previously (Libuda and Winston 2006) but with the above frequencies for the *HTA2-HTB2* amplification among His^+ Gly^+ colonies factored in. The average frequency (amplification events/total cells) for each treatment was as follows: (1) no treatment—4.2×10^{-5}; (2) 50 mm HU—9.0×10^{-5}; (3) 100 mm HU—3.4×10^{-4}; (4) 200 mm HU—7.6×10^{-4}; and (5) 0.02% MMS—1.4×10^{-4}. The fold changes represent the average increase compared to the average for untreated cultures, shown with the standard error.

Northern hybridization analysis, CHEF electrophoresis, and Southern hybridization analysis: Northern hybridization analysis was performed as previously described, with all probes generated by labeling of PCR products (Ausubel *et al.* 1991). All primers used for PCR are listed in Table S1. The *TPI1* probe has been previously described (Hirschhorn *et al.* 1992). Other probes were as follows: *RNR3*, from 1229 to 1430 bp from the ATG; *HTB1*, from 162 bp 5′ to 61 bp 5′ of the *HTB1* ORF; *HTA2*, from 20 bp 3′ to 134 bp 3′ of the ORF; *HHF1*, from +314 bp from the ATG to 175 bp 3′ of the ORF; *HHT2*, from 91 bp 5′ to 1 bp 5′ of the ORF; *HHF2*, from +301 bp from the ATG to 198 bp 3′ of the ORF; and *ACT1*, from 367 bp to 1015 bp from the ATG. The analysis of intact yeast chromosomes by CHEF gels followed by Southern hybridization analysis was performed as previously described (Libuda and Winston 2006). Probes for the amplified region [*HTA2-HTB2* and 3′*YBLWTy1-1* (amplification probe B)] were also prepared as previously described (Libuda and Winston 2006).

Controlling histone H3-H4 gene-pair transcription: Strains FY2784 and FY4 were grown at 30° to 1–2 $\times 10^7$ cells/ml in YPgal, YP + 2% raffinose (YPraf) + 0.05% galactose, YPraf + 0.01% galactose, or YPraf + 0.005% galactose. Total RNA was prepared from these cultures as described previously (Swanson *et al.* 1991).

RESULTS

A screen for deletions that alter the frequency of *HTA2-HTB2* amplification: Our previous results demonstrated that the histone H2A-H2B gene pair, *HTA2-HTB2*, can amplify by recombination between flanking Ty1 elements to form a small circular chromosome (Libuda and Winston 2006). The frequency of the amplification event becomes significantly elevated in a mutant background in which histone H2A and H2B levels are reduced by deletion of the other H2A-H2B locus, *HTA1-HTB1*. This observation raised the possibility that the *HTA2-HTB2* amplification occurs in wild-type cells when there is a need to increase H2A-H2B levels. To investigate what circumstances or factors may regulate the *HTA2-HTB2* amplification event, we screened for gene deletions that either increase or decrease its frequency. This screen, described in detail in materials and methods, took advantage of our previous finding that *HTA2-HTB2* amplification is required for survival in an *(hta1-htb1)*Δ background. To conduct the screen, we constructed double mutants that contain both *(hta1-htb1)*Δ and a deletion of each of the 4815 nonessential genes from the *S. cerevisiae* deletion set and tested them for their level of survival, an indicator of the frequency of *HTA2-HTB2* amplification.

From this screen, we identified 102 deletions that decrease or eliminate *(hta1-htb1)*Δ viability and 53 deletions that increase *(hta1-htb1)*Δ viability (Table S2). Among the deletions that decrease or eliminate *(hta1-*

TABLE 2

Effects of DNA replication and histone gene deletions on *(hta1-htb1)*Δ viability and *HTA2-HTB2* amplification

Mutation	*(hta1-htb1)*Δ/*HTA1-HTB1* tetrad dissection		CHEF gel Southern analysis: *(hta1-htb1)*Δ with amplification/ total viable *(hta1-htb1)*Δ
	Viable *HTA1-HTB*/Total *HTA1-HTB1*[a]	Viable *(hta1-htb1)*Δ/Total *(hta1-htb1)*Δ[a]	
None	149/156 (96)	20/156 (13)	20/20 (100)
*dpb3*Δ	36/36 (100)	29/36 (82)	8/8 (100)
*dpb4*Δ	27/30 (90)	26/30 (87)	5/6 (83)
*rrm3*Δ	38/38 (100)	18/38 (47)	9/9 (100)
*clb5*Δ[b]	32/29 (110)[b]	23/29 (79)[b]	4/6 (67)
*mec1*Δ *sml1*Δ	62/70 (89)	7/70 (10)	5/7 (71)
*dun1*Δ	37/38 (97)	5/38 (13)	5/5 (100)
*sml1*Δ	60/60 (100)	12/60 (20)	12/12 (100)
*(hht1-hhf1)*Δ	63/66 (95)	58/66 (88)	7/26 (27)
*(hht2-hhf2)*Δ	92/98 (94)	79/98 (81)	21/25 (84)

[a] The numbers in columns 2 and 3 represent the number of viable spores over the total number of spores with that genotype. Percentage viability for each genotype is reported in parentheses.

[b] This *(hta1-htb1)*Δ/*HTA1-HTB1* tetrad dissection was done in a *clb5*Δ/*CLB5* heterozygote, and numbers were calculated on the basis of an estimation of the total number of *clb5*Δ *HTA1-HTB1* and *clb5*Δ *(hta1-htb1)*Δ spores.

*htb1)*Δ viability, we identified genes encoding an RNA polymerase II component (*RPB4*), a protein that interacts with RNA polymerases (*NNF2*), histone-related factors (*UAF30*, *KAP114*, *SPT10*, and *HDA1*), and a component of ribonucleotide reductase (*RNR4*). Notably, no deletions in genes encoding recombination proteins were identified in our screen, which is likely due to redundancy among some recombination mechanisms and because some recombination mutants fail to sporulate, a requirement for our screen. Among the deletions that cause an increase in *(hta1-htb1)*Δ viability, we identified genes encoding Mediator coactivator complex components (*MED1*, *NUT1*, *SRB2*), the CDK submodule of Mediator (*SRB8*, *SRB9*, *SRB10*, *SRB11*), the Lsm1-7 complex (*LSM1*, *LSM6*, *LSM7*), histones H3 and H4 (*HHF1*, *HHT1*, *HHT2*), and the DNA replication components (*DPB3*, *DPB4*, *RRM3*, and *CLB5*). Deletion of the genes identified that encode Mediator components appear to derepress transcription of *HTA2* in a cell-cycle-dependent manner (our unpublished results). Increased expression of *HTA2-HTB2* might suppress the requirement for the *HTA2-HTB2* amplification. Among the deletions identified, we have focused on two sets that increase viability: the genes involved in DNA replication and the genes encoding histones H3 and H4.

Deletion of genes involved in DNA replication increases *HTA2-HTB2* amplification frequency: Our screen for deletions that increase *(hta1-htb1)*Δ viability identified four genes that influence DNA replication: *DPB3*, *DPB4*, *RRM3*, and *CLB5* (Table S2). *DPB3* and *DPB4* encode the two nonessential components of DNA polymerase ε (Pol ε) and have been implicated in replication fork progression (Araki *et al.* 1991; Dua *et al.* 1998; Ohya *et al.* 2000). In addition, previous studies have demonstrated roles for Dpb3 and Dpb4 in telomeric silencing (Tsubota *et al.* 2006) and have shown that Dpb4 is also a component of the Isw2 complex (Iida and Araki 2004; McConnell *et al.* 2004). *RRM3* encodes a helicase that has been shown to be required for the normal progression of DNA replication forks (Ivessa *et al.* 2000, 2002; Azvolinsky *et al.* 2006, 2009). Interestingly, Rrm3 has also been shown to be associated with Pol2, the catalytic subunit of Pol ε (Azvolinsky *et al.* 2006). *CLB5* encodes a cyclin that activates Cdc28 during S phase (Epstein and Cross 1992; Kuhne and Linder 1993; Schwob and Nasmyth 1993). In *clb5*Δ mutants, S phase is extended and approximately half of the origins fail to fire (Epstein and Cross 1992; Kuhne and Linder 1993; Schwob and Nasmyth 1993; Donaldson *et al.* 1998; McCune *et al.* 2008). To confirm the effect of these deletions on *HTA2-HTB2* amplification, *(hta1-htb1)*Δ/*HTA1-HTB1* diploids that also contained the relevant deletion were constructed and analyzed by a tetrad viability assay (Libuda and Winston 2006). Briefly, in this assay, an *(hta1-htb1)*Δ/*HTA1-HTB1* diploid is sporulated, and tetrads are dissected to determine the frequency of viability of *(hta1-htb1)*Δ progeny. Our earlier studies showed that ~13% of the *(hta1-htb1)*Δ progeny are viable due to formation of the *HTA2-HTB2* amplification, which most likely occurs post-meiotically (Libuda and Winston 2006). Our results demonstrate that, when *dpb3*Δ, *dpb4*Δ, *rrm3*Δ, or *clb5*Δ is present, the *(hta1-htb1)*Δ progeny have a significantly higher level of viability than those in a wild-type background (Table 2; Figure 1A). To determine if the viable *(hta1-htb1)*Δ progeny with these gene deletions contained the *HTA2-HTB2* amplification, CHEF gel Southern blots were performed. In this method, the presence of the *HTA2-HTB2* amplification is assayed by the presence of two bands that hybridize to an *HTA2-HTB2* probe: chromosome II, the normal

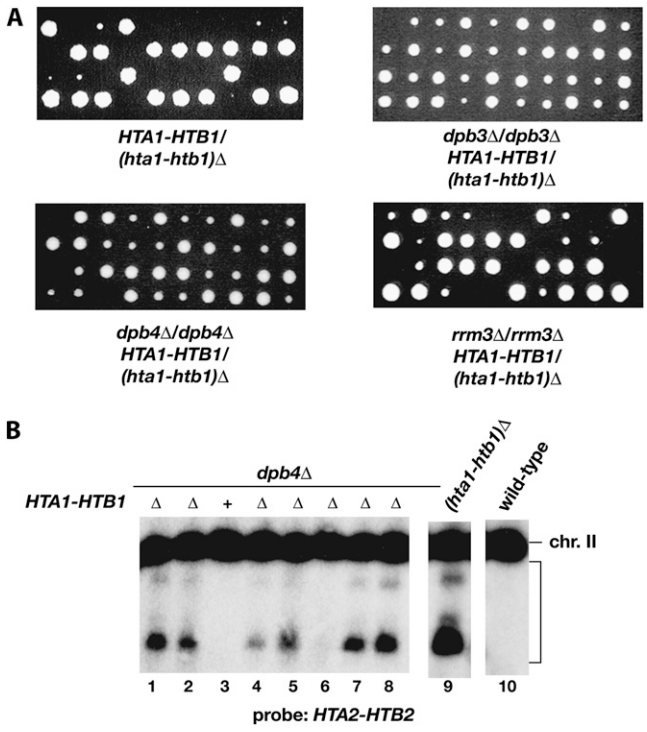

Figure 1.—DNA replication gene deletions increase the frequency of *HTA2-HTB2* amplification in *(hta1-htb1)*Δ strains. (A) Diploids that were *HTA1-HTB1/(hta1-htb1)*Δ and also homozygous for deletion of genes involved in DNA replication (*DPB3*, *DPB4*, *RRM3*) were sporulated and tetrads were dissected. Shown are representative tetrad plates after 3 days of growth at 30° on YPD. Similar results were obtained for *clb5*Δ (Table 2). (B) The double mutants [*dpb3*Δ, *dpb4*Δ, *rrm3*Δ, or *clb5*Δ combined with *(hta1-htb1)*Δ] were analyzed for *HTA2-HTB2* amplification by CHEF gel Southern hybridization analysis. A representative CHEF gel Southern blot is shown for the *dpb4*Δ *(hta1-htb1)*Δ strains. A and B are from the same Southern blot. A summary of the tetrad and CHEF gel Southern analysis results is in Table 2.

location of this locus, and the amplification (Libuda and Winston 2006). The results of this analysis showed that, indeed, most *(hta1-htb1)*Δ colonies with these deletions do contain the amplification (Table 2; Figure 1B). The small number that do not contain the amplification likely are disomic for chromosome II, another event that allows for *(hta1-htb1)*Δ survival (Libuda and Winston 2006). Taken together, these results suggest that deletion of these four genes increases the frequency of the *HTA2-HTB2* amplification, possibly due to stalled replication forks or extended S phase.

Treatment with hydroxyurea induces amplification formation in wild-type cells: Our finding that the *HTA2-HTB2* amplification frequency is increased by mutations that impair DNA replication suggests that amplification might occur more frequently in wild-type cells when replication is impaired. Therefore, we measured the amplification frequency after treatment with MMS, a DNA-damaging agent, or HU, which slows DNA replication (Feng *et al.* 2006; Alvino *et al.* 2007). To measure the amplification frequency in wild-type cells, we adapted a previously described method that can detect two copies of the amplified region in a wild-type background, the *coq1::HIS3* reporter (Figure 2A; Chan and Botstein 1993; Libuda and Winston 2006; see materials and methods for details). To control for general effects on Ty1-Ty1 recombination, we also measured recombination frequencies for two different Ty1-*URA3*-Ty1 recombination reporters (Libuda and Winston 2006).

After treatment with MMS, the frequency of *HTA2-HTB2* amplification formation increased ~3.5-fold, while Ty-*URA3*-Ty recombination was not significantly increased (Figure 2B; materials and methods). Previous studies (Parket and Kupiec 1992) also showed that MMS treatment does not increase Ty-Ty recombination. To assay the effect of slowed DNA replication, we measured *HTA2-HTB2* amplification frequency after growth in different levels of HU. Our results showed that the amplification frequency increased with increasing HU concentrations, up to an 18-fold stimulation in 200 mm HU (Figure 2B; materials and methods). Moreover, Ty1-*URA3*-Ty1 recombination frequency was at most only modestly affected by HU (Figure 2B; materials and methods). The significant stimulation in amplification frequency upon treatment with HU implicates a role for DNA replication fork progression in *HTA2-HTB2* amplification.

Previous studies have shown that impaired replication or DNA damage results in reduced levels of mRNAs from histone genes and increased levels of mRNAs from replication-stress-induced genes, such as *RNR3* (Lycan *et al.* 1987; Elledge and Davis 1990; Gasch *et al.* 2001; Su *et al.* 2004; Zhao 2004). To test whether these transcriptional changes occur in our amplification assays, we measured both histone gene and *RNR3* mRNA levels from cells treated with 200 mm HU. Our results show that histone gene mRNAs begin to decline after 5 min in 200 mm HU, reaching their minimal level by 15–30 min and then recovering to near-wild-type levels by 240 min (Figure 3). In addition, our results show that *RNR3* mRNA levels increase during the 200 mm HU treatment, peaking at 120 min (Figure 3). As shown later, this increase is similar to the increase in *RNR3* mRNA levels observed in cells with significantly reduced histone H3-H4 gene-pair expression. Overall, these results support previous findings that demonstrated changes in both histone and replication-stress-induced mRNA levels upon HU treatment (Lycan *et al.* 1987; Elledge and Davis 1990; Gasch *et al.* 2001). Moreover, these findings connect conditions that cause *HTA2-HTB2* amplification, altered DNA replication, and decreased histone gene transcription.

Replication checkpoint pathway components are not required for *HTA2-HTB2* amplification formation in *(hta1-htb1)*Δ strains: Replication fork pauses and slowed

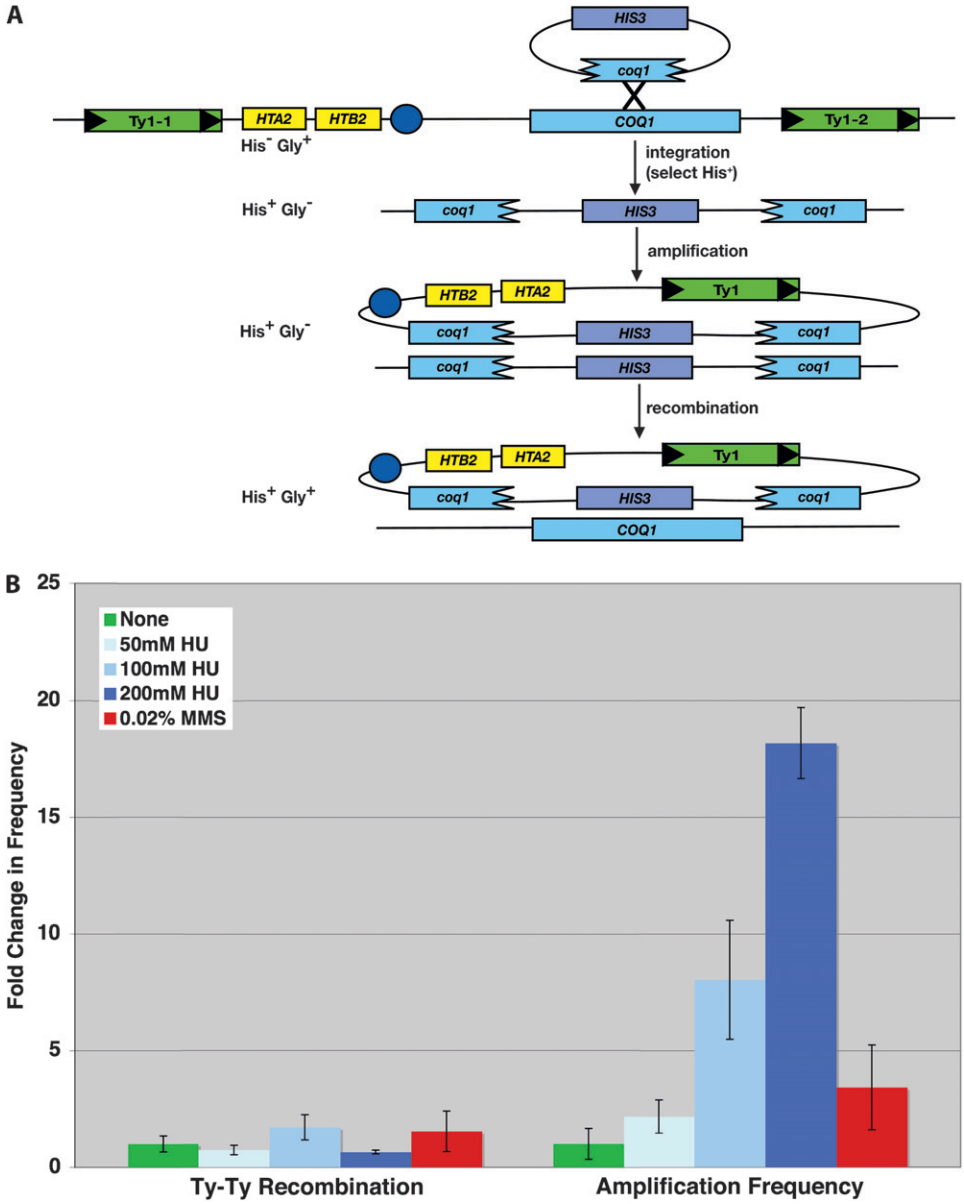

FIGURE 2.—HU treatment increases the frequency of *HTA2-HTB2* amplification in wild-type strains. (A) Diagram of the *coq1::HIS3* ploidy marker for monitoring the *HTA2-HTB2* amplification event. An integrating plasmid containing an internal *COQ1* restriction fragment and a *HIS3* marker was used to transform a *his3*Δ strain to His$^+$. Integration of this plasmid as diagrammed disrupts the *COQ1* gene, thereby conferring the inability to grow on nonfermentable carbon sources, such as glycerol (Gly$^-$ phenotype; ASHBY *et al.* 1992). Strains that have undergone the amplification (or chromosome II disomy) can become His$^+$ Gly$^+$ by recombination between the duplicated region of the *coq1::HIS3* ploidy marker on one of the two copies. (B) Wild-type strains carrying both the *coq1::HIS3* reporter and a Ty1-Ty1 recombination reporter as a control were grown in YPD with MMS or HU for 24 hr. The changes in *HTA2-HTB2* amplification frequency and Ty1-Ty1 recombination frequency were normalized to strains grown without treatment. Each bar represents the average and standard error of the mean from three or more independent cultures.

DNA replication, as is observed with HU treatment, activate the replication checkpoint to assist with recovery from the pauses (ELLEDGE and DAVIS 1990; LAMBERT and CARR 2005). Because we observed increased *HTA2-HTB2* amplification frequency with HU treatment, we wanted to determine if the replication checkpoint pathway is required for this amplification event. Since deletions of genes encoding replication checkpoint pathway components cause HU sensitivity, we were unable to use the *coq1::HIS3* reporter assay to address this question. Instead, we used the tetrad viability assay, measuring the frequency of the survival of *(hta1-htb1)*Δ progeny in homozygous deletions of *MEC1* and *DUN1*, two genes required for the checkpoint pathway. For *mec1*Δ, the diploids were also homozygous for *sml1*Δ, which is required to suppress the lethality of *mec1*Δ (ZHAO *et al.* 1998). Our results show that *mec1*Δ *sml1*Δ, *dun1*Δ, and *sml1*Δ do not significantly alter the frequency of *HTA2-HTB2* amplification (Table 2). On the basis of CHEF gel analysis (Figure S1), most of the viable *(hta1-htb1)*Δ strains in those backgrounds contained the *HTA2-HTB2* amplification. These results suggest that the replication checkpoint pathway is not required for *HTA2-HTB2* amplification.

Histone H3-H4 gene-pair deletions increase *(hta1-htb1)*Δ viability but have different effects on *HTA2-HTB2* amplification formation: In our screen for deletions that increase *(hta1-htb1)*Δ viability, we also identified the two histone H3 genes, *HHT1* and *HHT2*, and a histone H4 gene, *HHF1*. The *S. cerevisiae* H3-H4 genes are encoded in two divergently transcribed gene pairs, *HHT1-HHF1* and *HHT2-HHF2*. On the basis of the screen results, we tested a deletion of each gene pair by the tetrad viability assay. In these tests, *(hta1-htb1)*Δ/

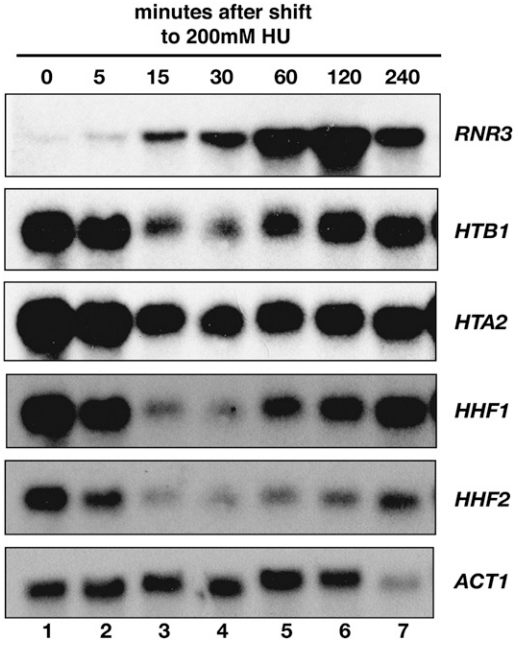

Figure 3.—HU treatment decreases histone transcripts and increases replication-stress-induced transcripts. Wild-type strains carrying the *coq1::HIS3* reporter, which assays for *HTA2-HTB2* amplification formation, were grown in rich medium, and HU was added to a final concentration of 200 mM HU. Samples were taken after HU addition at the times indicated, and RNA was prepared. Northern blots were probed for histone gene transcripts and for the replication-stress-induced transcript, *RNR3*. *ACT1* served as a loading control. All panels are from the same Northern blot that was stripped and reprobed for each of the indicated sequences.

HTA1-HTB1 diploids were also homozygous for either *(hht1-hhf1)*Δ or *(hht2-hhf2)*Δ. Each diploid was sporulated, followed by tetrad dissection and analysis to determine the frequency of viable *(hta1-htb1)*Δ progeny. Our results show that the deletion of each of the H3-H4 gene pairs caused a large increase in the viability of *(hta1-htb1)*Δ progeny—to 89% in the *(hht1-hhf1)*Δ strain background and 79% in the *(hht2-hhf2)*Δ strain background (Table 2, Figure 4A). To determine if the viable *(hta1-htb1)*Δ *(hht1-hhf1)*Δ and *(hta1-htb1)*Δ *(hht2-hhf2)*Δ strains contained the *HTA2-HTB2* amplification, we performed Southern analysis of CHEF gels (materials and methods). Surprisingly, only 27% of *(hht1-hhf1)*Δ *(hta1-htb1)*Δ spores contained the amplification (Table 2, Figure 4B), suggesting that the *(hht1-hhf1)*Δ deletion can suppress the inviability caused by *(hta1-htb1)*Δ in the absence of any amplification. In contrast, 84% of *(hht2-hhf2)*Δ *(hta1-htb1)*Δ spores contained the amplification, strongly suggesting that *(hht2-hhf2)*Δ increases *HTA2-HTB2* amplification frequency.

To confirm these distinct genetic interactions between the deletions of the two different H3-H4 loci and *(hta1-htb1)*Δ, we constructed each class of double mutant by crosses and then tested for viability. In each cross, one parent contained *(hta1-htb1)*Δ and also contained a plasmid with *HTA1-HTB1* and *URA3* (plasmid pSAB6). This strain did not contain any amplification. The second parent contained a deletion of one of the two H3-H4 loci, either *(hht1-hhf1)*Δ or *(hht2-hhf2)*Δ, and also did not contain the amplification. The viability of the histone double mutants was then tested in the progeny by the ability to grow on medium containing 5-FOA, a compound that counterselects for *URA3*. The *(hta1-htb1)*Δ *(hht1-hhf1)*Δ double mutants were 5-FOA resistant (10/10 progeny from 12 complete tetrads), confirming that *(hht1-hhf1)*Δ suppresses *(hta1-htb1)*Δ inviability. In contrast, the *(hta1-htb1)*Δ *(hht2-hhf2)*Δ double mutants were 5-FOA sensitive (7/7 progeny from 11 complete tetrads). This result suggests that *(hht2-hhf2)*Δ does not significantly suppress *(hta1-htb1)*Δ inviability. Taken together, these results suggest that, surprisingly, *(hht1-*

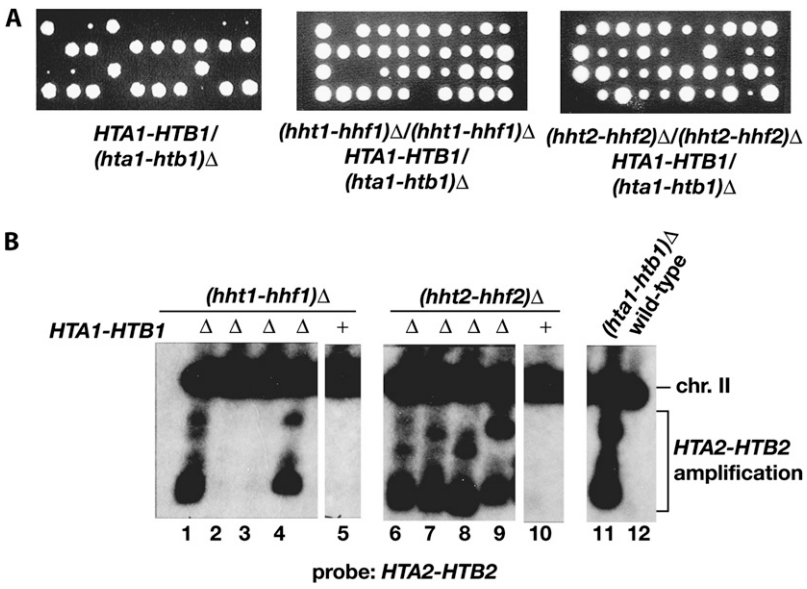

Figure 4.—Histone H3-H4 gene-pair deletions have different effects on *HTA2-HTB2* amplification formation. (A) Tetrad dissections were performed on *HTA1-HTB1/(hta1-htb1)*Δ diploids that were also homozygous for deletion of one of the histone H3-H4 gene pairs (*HHT1-HHF1*, *HHT2-HHF2*). A representative tetrad plate is shown for each strain. (B) Histone H3-H4 gene-pair deletions [*(hht1-hhf1)*Δ, *(hht2-hhf2)*Δ] with *(hta1-htb1)*Δ were analyzed for the amplification by a CHEF gel Southern blot probed for *HTA2-HTB2*. All panels are from the same Southern blot. A summary of the tetrad and CHEF gel Southern analysis results for deletion of the histone H3-H4 gene pairs is in Table 2.

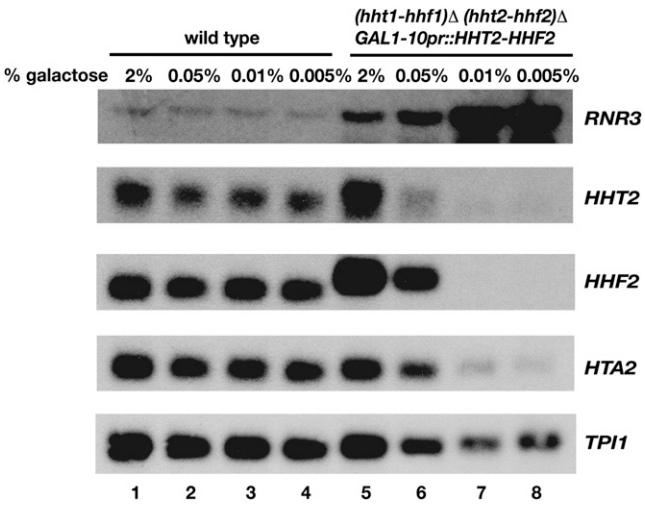

Figure 5.—Reduced histone H3-H4 gene-pair transcript levels increase replication-stress-induced transcripts. A wild-type strain and a strain deleted for both histone H3-H4 loci [*(hht1-hhf1)*Δ *(hht2-hhf2)*Δ] but containing a plasmid with *HHT2-HHF2* under the control of the galactose-regulatable promoter *GAL1-10*pr were grown in rich media with either 2% galactose (YPgal) or 2% raffinose (YPraf) containing one of three different concentrations of galactose (0.05%, 0.01%, and 0.005%) to regulate the amount of *HHT2-HHF2* transcription. Northern blots of these mRNAs were probed for histone gene transcripts (*HHF2*, *HHT2*, and *HTA2*), and the replication-stress-induced gene, *RNR3*. *TPI1* served as the loading control. All panels are from the same Northern blot that was stripped and reprobed for each of the indicated sequences.

*hhf1)*Δ suppresses the need for the *HTA2-HTB2* amplification, whereas *(hhf2-hht2)*Δ enhances its frequency. We note that previous studies showed that *HHT1-HHF1* is expressed at a substantially lower level than *HHT2-HHF2* (Cross and Smith 1988). These differences likely lead to the different phenotypes with respect to *HTA2-HTB2* amplification (see discussion).

Evidence that reduced histone H3-H4 levels cause paused DNA replication forks: Previous studies demonstrated that repressing histone H4 gene transcription results in the elevation of replication-stress-induced transcripts (Wyrick *et al.* 1999). To determine if a reduction of both H3-H4 gene transcripts can also confer a replication stress signal, we constructed a strain in which the only copies of genes encoding histones H3 and H4 are under the control of the *GAL1-10* promoter (materials and methods). Then we measured the level of *RNR3* mRNA under conditions where *HHT2-HHF2* is expressed at low levels. Our results show that *RNR3* mRNA levels are significantly induced when *HHT2-HHF2* transcripts are reduced (Figure 5). These results are consistent with previous studies (Wyrick *et al.* 1999) that suggest that an alteration in histone stoichiometry can increase replication-pause-induced transcripts. The increase in *RNR3* mRNA levels is similar to that observed upon HU treatment (compare Figure 5 to Figure 3). Interestingly, we also observed that decreasing *HHT2-HHF2* mRNA levels caused a decreased level of *HTA2* mRNA. Taken together, these findings indicate a potential relationship between decreased histone gene transcription, increased replication-stress-induced transcription, and *HTA2-HTB2* amplification.

DISCUSSION

Our previous studies showed that when one histone H2A-H2B locus, *HTA1-HTB1*, is deleted, the second H2A-H2B locus, *HTA2-HTB2*, amplifies as part of a small circular chromosome. In this article, we have presented new results that suggest that this amplification event is stimulated in response to either of two mutually dependent changes: paused/slowed DNA replication forks or reduced histone gene transcription. First, as part of a genetic screen to identify mutations that alter the frequency of *HTA2-HTB2* amplification formation, we identified several genes that encode factors involved in DNA replication. A common phenotype caused by mutations in these genes is the increased pausing of replication forks, suggesting that stalled forks induce *HTA2-HTB2* amplification. Second, we showed that treatment with HU, a chemical that slows DNA replication, also stimulates *HTA2-HTB2* amplification, thereby strongly supporting the idea that alterations in DNA replication induce this event. Third, our mutant screen also led to the demonstration that deletion of the histone H3-H4 locus, *HHT2-HHF2*, increases *HTA2-HTB2* amplification. Finally, we have confirmed and extended previous results from other labs that have shown that slowing DNA replication by HU treatment results in reduced histone mRNA levels and, conversely, that reduced histone gene transcription induces stalled or slowed replication forks. Taken together, our findings suggest that amplification of *HTA2-HTB2* is part of a cellular response that occurs when environmental changes perturb the replication of chromatin and the proper stoichiometry of histones.

Previous studies have shown that recombination proteins are recruited to sites of stalled replication forks (Lambert *et al.* 2005; Bailis *et al.* 2008). Those findings, taken together with our results, suggest that stalled or slowed replication forks within the amplified region are part of the mechanism that forms the *HTA2-HTB2* amplification. Our results also demonstrate that the possible use of stalled or slowed replication forks as part of the signal to form the *HTA2-HTB2* amplification is independent of the replication checkpoint. Current results do not yet explain why the Ty1-Ty1 recombination event that forms the *HTA2-HTB2* amplification occurs at a much greater frequency than other Ty1-Ty1 recombination events. One obvious possibility is that either the mutants that we tested or HU treatment causes a more severe impairment of replication fork

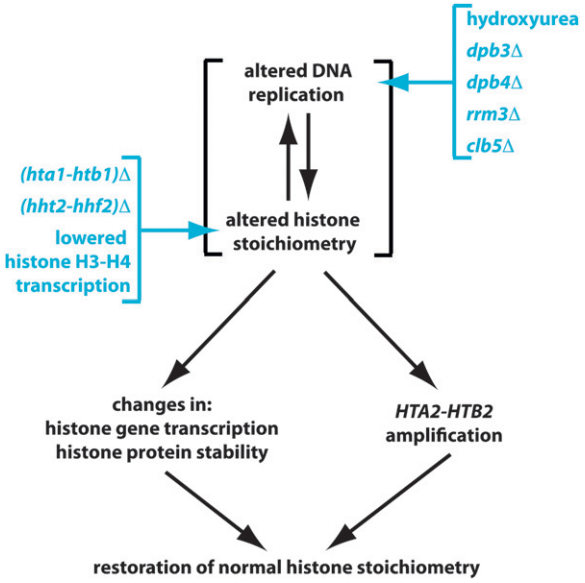

Figure 6.—Model for a response to altered histone levels in a population of wild-type cells. After encountering an environmental condition that alters histone levels, a population of cells can attempt to restore normal histone stoichiometry by stimulating the *HTA2-HTB2* amplification, changing histone gene transcription, or altering histone protein stability. In addition, an environmental stress that directly causes slowed or stalled replication forks may also stimulate the amplification event. The brackets indicate two steps that have not been delineated in this model. The blue type indicates conditions found from this work to stimulate the *HTA2-HTB2* amplification.

progression in the region of the amplification than in other genomic regions. However, previous genomewide studies of HU-treated cells (Feng *et al.* 2006; Alvino *et al.* 2007), of an *rrm3*Δ mutant (Azvolinsky *et al.* 2009), and of a *clb5*Δ mutant (McCune *et al.* 2008) do not support this idea. Thus, some aspect of impaired DNA replication that is yet to be elucidated likely accounts for the greatly enhanced frequency of Ty1-Ty1 recombination that forms the *HTA2-HTB2* amplification.

The data presented here also suggest that histone H3-H4 levels play a key role in determining the response of cells to the reduced H2A-H2B levels caused by *(hta1-htb1)*Δ. Deletion of one H3-H4 gene pair, *(hht2-hhf2)*Δ, stimulates *HTA2-HTB2* amplification in *(hta1-htb1)*Δ strains; conversely, deletion of the other H3-H4 gene pair, *(hht1-hhf1)*Δ, alleviates the requirement for *HTA2-HTB2* amplification in *(hta1-htb1)*Δ strains. These differences are likely caused by the distinct effects that the two deletions have on H3-H4 levels because previous studies have shown that the two H3-H4 loci are transcribed at different levels, with *HHT2-HHF2* producing five- to sevenfold more mRNA than *HHT1-HHF1* (Cross and Smith 1988). Thus, the viability of the *(hht1-hhf1)*Δ *(hta1-htb1)*Δ double mutant suggests that the H3-H4 level in this mutant allows approximately normal stoichiometry between H2A-H2B and H3-H4, although the absolute histone levels may be reduced in the *(hht1-hhf1)*Δ *(hta1-htb1)*Δ double mutant compared to wild type. In contrast, in the *(hht1-hhf2)*Δ *(hta1-htb2)*Δ double mutant, where H3-H4 levels would be lower, histone stoichiometry would be greatly skewed; this acute alteration in histone stoichiometry may signal for amplification of *HTA2-HTB2*, thereby accounting for the increase in *HTA2-HTB2* amplification frequency observed in these double mutants. Since amplification of *HTA2-HTB2* clearly does not restore normal histone stoichiometry when H3-H4 levels are reduced, why would amplification of *HTA2-HTB2* be a response to reduced H3-H4 levels? The *HTA2-HTB2* amplification may represent one of several responses to an altered histone stoichiometry.

Many studies have now shown that *S. cerevisiae* carefully controls histone levels by several distinct mechanisms, including both transcriptional control (Osley 1991) and protein stability (Gunjan and Verreault 2003; Gunjan *et al.* 2005; Singh *et al.* 2009). Our studies demonstrate that *HTA2-HTB2* amplification is yet another method of dosage compensation for altered histone levels. Why are there so many mechanisms to control histone levels? Clearly, either decreased or increased histone levels have a profound effect on growth. At the extreme, altered levels result in inviability (Rykowski *et al.* 1981; Kolodrubetz *et al.* 1982; Smith and Stirling 1988), whereas more modest effects can greatly impair transcription, chromosome stability, and DNA replication (Meeks-Wagner and Hartwell 1986; Clark-Adams *et al.* 1988; Wyrick *et al.* 1999; Prado and Aguilera 2005). Given these threats, and that histone levels can likely be perturbed by a multitude of mechanisms, it seems prudent to have several possible responses to conditions that alter proper histone levels.

Our model suggests than an alteration in histone stoichiometry causes the induction of several response mechanisms, one of which is the formation of the *HTA2-HTB2* amplification (Figure 6). In this model, either altered histone stoichiometry [*(hta1-htb1)*Δ or *(hht2-hhf2)*Δ] or alterations in DNA replication (*dpb3*Δ, *dpb4*Δ, *rrm3*Δ, *clb5*Δ, or HU treatment) stimulate the *HTA2-HTB2* amplification event. Since lowered histone H3-H4 gene transcription induces transcripts characteristic of stalled or slowed replication forks, we propose that altered histone stoichiometry may promote a signaling cascade in which stalled replication forks induce the Ty1-Ty1 recombination event that forms the *HTA2-HTB2* amplification. Alternatively, as observed with the HU treatment, slowed DNA replication may reduce histone levels and, in turn, stimulate the specific Ty1-Ty1 recombination event. In addition to *HTA2-HTB2* amplification, altered histone stoichiometry can induce other pathways known to restore normal histone stoichiometry, such as changes in histone gene transcription or histone

protein stability. Overall, this model illustrates how a population of cells utilizes several mechanisms as a general response to altered histone levels.

The *HTA2-HTB2* amplification, which is the product of a directed Ty1-Ty1 recombination event, represents part of a cellular response for adapting to environmental changes that alter histone stoichiometry. Several studies have demonstrated that Ty elements, in particular their recombination, can provide a rich source for both genetic variation and adaptive genomic changes in response to specific environmental stresses (Rosenberg 2001; Infante *et al.* 2003; Zeyl 2004; Mieczkowski *et al.* 2006; Roth *et al.* 2006). To the best of our knowledge, our results provide some the first evidence that cells can stimulate a specific Ty1-Ty1 recombination event in response to an environmental condition or stress. How a cell can enhance a particular Ty1-Ty1 recombination event is currently unknown. Future studies identifying other directed Ty1-Ty1 recombination events will reveal how the cell can direct manipulation of the genome via transposable elements for adaptation to specific environmental changes.

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LITERATURE CITED

Albertson, D. G., 2006 Gene amplification in cancer. Trends Genet. **22:** 447–455.

Alvino, G. M., D. Collingwood, J. M. Murphy, J. Delrow, B. J. Brewer *et al.*, 2007 Replication in hydroxyurea: it's a matter of time. Mol. Cell. Biol. **27:** 6396–6406.

Araki, H., R. K. Hamatake, A. Morrison, A. L. Johnson, L. H. Johnston *et al.*, 1991 Cloning DPB3, the gene encoding the third subunit of DNA polymerase II of *Saccharomyces cerevisiae*. Nucleic Acids Res. **19:** 4867–4872.

Argueso, J. L., J. Westmoreland, P. A. Mieczkowski, M. Gawel, T. D. Petes *et al.*, 2008 Double-strand breaks associated with repetitive DNA can reshape the genome. Proc. Natl. Acad. Sci. USA **105:** 11845–11850.

Ashby, M. N., S. Y. Kutsunai, S. Ackerman, A. Tzagoloff and P. A. Edwards, 1992 COQ2 is a candidate for the structural gene encoding para-hydroxybenzoate:polyprenyltransferase. J. Biol. Chem. **267:** 4128–4136.

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman *et al.*, 1991 *Current Protocols in Molecular Biology*. Greene Publishing Associates and Wiley-Interscience, New York.

Azvolinsky, A., S. Dunaway, J. Z. Torres, J. B. Bessler and V. A. Zakian, 2006 The *S. cerevisiae* Rrm3p DNA helicase moves with the replication fork and affects replication of all yeast chromosomes. Genes Dev. **20:** 3104–3116.

Azvolinsky, A., P. G. Giresi, J. D. Lieb and V. A. Zakian, 2009 Highly transcribed RNA polymerase II genes are impediments to replication fork progression in *Saccharomyces cerevisiae*. Mol. Cell **34:** 722–734.

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.

Brachmann, C. B., A. Davies, G. J. Cost, E. Caputo, J. Li *et al.*, 1998 Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast **14:** 115–132.

Brown, C. J., K. M. Todd and R. F. Rosenzweig, 1998 Multiple duplications of yeast hexose transport genes in response to selection in a glucose-limited environment. Mol. Biol. Evol. **15:** 931–942.

Cha, R. S., and N. Kleckner, 2002 ATR homolog Mec1 promotes fork progression, thus averting breaks in replication slow zones. Science **297:** 602–606.

Chan, C. S., and D. Botstein, 1993 Isolation and characterization of chromosome-gain and increase-in-ploidy mutants in yeast. Genetics **135:** 677–691.

Clark-Adams, C. D., D. Norris, M. A. Osley, J. S. Fassler and F. Winston, 1988 Changes in histone gene dosage alter transcription in yeast. Genes Dev. **2:** 150–159.

Claycomb, J. M., M. Benasutti, G. Bosco, D. D. Fenger and T. L. Orr-Weaver, 2004 Gene amplification as a developmental strategy: isolation of two developmental amplicons in Drosophila. Dev. Cell **6:** 145–155.

Cross, S. L., and M. M. Smith, 1988 Comparison of the structure and cell cycle expression of mRNAs encoded by two histone H3–H4 loci in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:** 945–954.

Donaldson, A. D., M. K. Raghuraman, K. L. Friedman, F. R. Cross, B. J. Brewer *et al.*, 1998 CLB5-dependent activation of late replication origins in *S. cerevisiae*. Mol. Cell **2:** 173–182.

Dua, R., D. L. Levy and J. L. Campbell, 1998 Role of the putative zinc finger domain of *Saccharomyces cerevisiae* DNA polymerase epsilon in DNA replication and the S/M checkpoint pathway. J. Biol. Chem. **273:** 30046–30055.

Dunham, M. J., H. Badrane, T. Ferea, J. Adams, P. O. Brown *et al.*, 2002 Characteristic genome rearrangements in experimental evolution of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **99:** 16144–16149.

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.

Epstein, C. B., and F. R. Cross, 1992 CLB5: a novel B cyclin from budding yeast with a role in S phase. Genes Dev. **6:** 1695–1706.

Feng, W., D. Collingwood, M. E. Boeck, L. A. Fox, G. M. Alvino *et al.*, 2006 Genomic mapping of single-stranded DNA in hydroxyurea-challenged yeasts identifies origins of replication. Nat. Cell Biol. **8:** 148–155.

Fridlyand, J., A. M. Snijders, B. Ylstra, H. Li, A. Olshen *et al.*, 2006 Breast tumor copy number aberration phenotypes and genomic instability. BMC Cancer **6:** 96.

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.

Giaever, G., A. M. Chu, L. Ni, C. Connelly, L. Riles *et al.*, 2002 Functional profiling of the *Saccharomyces cerevisiae* genome. Nature **418:** 387–391.

Graux, C., J. Cools, C. Melotte, H. Quentmeier, A. Ferrando *et al.*, 2004 Fusion of NUP214 to ABL1 on amplified episomes in T-cell acute lymphoblastic leukemia. Nat. Genet. **36:** 1084–1089.

Gresham, D., M. M. Desai, C. M. Tucker, H. T. Jenq, D. A. Pai *et al.*, 2008 The repertoire and dynamics of evolutionary adaptations to controlled nutrient-limited environments in yeast. PLoS Genet. **4:** e1000303.

Gunjan, A., and A. Verreault, 2003 A Rad53 kinase-dependent surveillance mechanism that regulates histone protein levels in *S. cerevisiae*. Cell **115:** 537–549.

Gunjan, A., J. Paik and A. Verreault, 2005 Regulation of histone synthesis and nucleosome assembly. Biochimie **87:** 625–635.

Hahn, P. J., 1993 Molecular biology of double-minute chromosomes. Bioessays **15:** 477–484.

Hastings, P. J., A. Slack, J. F. Petrosino and S. M. Rosenberg, 2004 Adaptive amplification and point mutation are independent mechanisms: evidence for various stress-inducible mutation mechanisms. PLoS Biol. **2:** e399.

Hereford, L. M., M. A. Osley, T. R. Ludwig, II and C. S. McLaughlin, 1981 Cell-cycle regulation of yeast histone mRNA. Cell **24:** 367–375.

Hirschhorn, J. N., S. A. Brown, C. D. Clark and F. Winston, 1992 Evidence that SNF2/SWI2 and SNF5 activate transcrip-

tion in yeast by altering chromatin structure. Genes Dev. **6:** 2288–2298.

Hirschhorn, J. N., A. L. Bortvin, S. L. Ricupero-Hovasse and F. Winston, 1995 A new class of histone H2A mutations in *Saccharomyces cerevisiae* causes specific transcriptional defects in vivo. Mol. Cell. Biol. **15:** 1999–2009.

Hughes, T. R., C. J. Roberts, H. Dai, A. R. Jones, M. R. Meyer *et al.*, 2000 Widespread aneuploidy revealed by DNA microarray expression profiling. Nat. Genet. **25:** 333–337.

Iida, T., and H. Araki, 2004 Noncompetitive counteractions of DNA polymerase epsilon and ISW2/yCHRAC for epigenetic inheritance of telomere position effect in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **24:** 217–227.

Infante, J. J., K. M. Dombek, L. Rebordinos, J. M. Cantoral and E. T. Young, 2003 Genome-wide amplifications caused by chromosomal rearrangements play a major role in the adaptive evolution of natural yeast. Genetics **165:** 1745–1759.

Ivessa, A. S., J. Q. Zhou and V. A. Zakian, 2000 The Saccharomyces Pif1p DNA helicase and the highly related Rrm3p have opposite effects on replication fork progression in ribosomal DNA. Cell **100:** 479–489.

Ivessa, A. S., J. Q. Zhou, V. P. Schulz, E. K. Monson and V. A. Zakian, 2002 Saccharomyces Rrm3p, a 5′ to 3′ DNA helicase that promotes replication fork progression through telomeric and subtelomeric DNA. Genes Dev. **16:** 1383–1396.

Kolodrubetz, D., M. C. Rykowski and M. Grunstein, 1982 Histone H2A subtypes associate interchangeably in vivo with histone H2B subtypes. Proc. Natl. Acad. Sci. USA **79:** 7814–7818.

Koszul, R., S. Caburet, B. Dujon and G. Fischer, 2004 Eucaryotic genome evolution through the spontaneous duplication of large chromosomal segments. EMBO J. **23:** 234–243.

Kugelberg, E., E. Kofoid, A. B. Reams, D. I. Andersson and J. R. Roth, 2006 Multiple pathways of selected gene amplification during adaptive mutation. Proc. Natl. Acad. Sci. USA **103:** 17319–17324.

Kuhne, C., and P. Linder, 1993 A new pair of B-type cyclins from *Saccharomyces cerevisiae* that function early in the cell cycle. EMBO J. **12:** 3437–3447.

Lambert, S., and A. M. Carr, 2005 Checkpoint responses to replication fork barriers. Biochimie **87:** 591–602.

Lambert, S., A. Watson, D. M. Sheedy, B. Martin and A. M. Carr, 2005 Gross chromosomal rearrangements and elevated recombination at an inducible site-specific replication fork barrier. Cell **121:** 689–702.

Lemoine, F. J., N. P. Degtyareva, K. Lobachev and T. D. Petes, 2005 Chromosomal translocations in yeast induced by low levels of DNA polymerase: a model for chromosome fragile sites. Cell **120:** 587–598.

Libuda, D. E., and F. Winston, 2006 Amplification of histone genes by circular chromosome formation in *Saccharomyces cerevisiae*. Nature **443:** 1003–1007.

Liebman, S., P. Shalit and S. Picologlou, 1981 Ty elements are involved in the formation of deletions in DEL1 strains of *Saccharomyces cerevisiae*. Cell **26:** 401–409.

Lycan, D. E., M. A. Osley and L. M. Hereford, 1987 Role of transcriptional and posttranscriptional regulation in expression of histone genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:** 614–621.

Mann, R. K., and M. Grunstein, 1992 Histone H3 N-terminal mutations allow hyperactivation of the yeast GAL1 gene in vivo. EMBO J. **11:** 3297–3306.

McCarroll, S. A., and D. M. Altshuler, 2007 Copy-number variation and association studies of human disease. Nat. Genet. **39:** S37–S42.

McConnell, A. D., M. E. Gelbart and T. Tsukiyama, 2004 Histone fold protein Dls1p is required for Isw2-dependent chromatin remodeling in vivo. Mol. Cell. Biol. **24:** 2605–2613.

McCune, H. J., L. S. Danielson, G. M. Alvino, D. Collingwood, J. J. Delrow *et al.*, 2008 The temporal program of chromosome replication: genomewide replication in *clb5*Δ *Saccharomyces cerevisiae*. Genetics **180:** 1833–1847.

Meeks-Wagner, D., and L. H. Hartwell, 1986 Normal stoichiometry of histone dimer sets is necessary for high fidelity of mitotic chromosome transmission. Cell **44:** 43–52.

Mieczkowski, P. A., F. J. Lemoine and T. D. Petes, 2006 Recombination between retrotransposons as a source of chromosome rearrangements in the yeast *Saccharomyces cerevisiae*. DNA Repair (Amst.) **5:** 1010–1020.

Norris, D., and M. A. Osley, 1987 The two gene pairs encoding H2A and H2B play different roles in the *Saccharomyces cerevisiae* life cycle. Mol. Cell. Biol. **7:** 3473–3481.

Norris, D., B. Dunn and M. A. Osley, 1988 The effect of histone gene deletions on chromatin structure in *Saccharomyces cerevisiae*. Science **242:** 759–761.

Ohya, T., S. Maki, Y. Kawasaki and A. Sugino, 2000 Structure and function of the fourth subunit (Dpb4p) of DNA polymerase epsilon in *Saccharomyces cerevisiae*. Nucleic Acids Res. **28:** 3846–3852.

Orr, W., K. Komitopoulou and F. C. Kafatos, 1984 Mutants suppressing in trans chorion gene amplification in Drosophila. Proc. Natl. Acad. Sci. USA **81:** 3773–3777.

Osley, M. A., 1991 The regulation of histone synthesis in the cell cycle. Annu. Rev. Biochem. **60:** 827–861.

Osley, M. A., and L. Hereford, 1982 Identification of a sequence responsible for periodic synthesis of yeast histone 2A mRNA. Proc. Natl. Acad. Sci. USA **79:** 7689–7693.

Parket, A., and M. Kupiec, 1992 Ectopic recombination between Ty elements in *Saccharomyces cerevisiae* is not induced by DNA damage. Mol. Cell. Biol. **12:** 4441–4448.

Payen, C., R. Koszul, B. Dujon and G. Fischer, 2008 Segmental duplications arise from Pol32-dependent repair of broken forks through two alternative replication-based mechanisms. PLoS Genet. **4:** e1000175.

Prado, F., and A. Aguilera, 2005 Partial depletion of histone H4 increases homologous recombination-mediated genetic instability. Mol. Cell. Biol. **25:** 1526–1536.

Rachidi, N., P. Barre and B. Blondin, 1999 Multiple Ty-mediated chromosomal translocations lead to karyotype changes in a wine strain of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **261:** 841–850.

Rose, M. D., F. Winston and P. Hieter, 1990 *Methods in Yeast Genetics: A Laboratory Course Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Rosenberg, S. M., 2001 Evolving responsively: adaptive mutation. Nat. Rev. Genet. **2:** 504–515.

Roth, J. R., E. Kugelberg, A. B. Reams, E. Kofoid and D. I. Andersson, 2006 Origin of mutations under selection: the adaptive mutation controversy. Annu. Rev. Microbiol. **60:** 477–501.

Rykowski, M. C., J. W. Wallis, J. Choe and M. Grunstein, 1981 Histone H2B subtypes are dispensable during the yeast cell cycle. Cell **25:** 477–487.

Schacherer, J., Y. Tourrette, J. L. Souciet, S. Potier and J. De Montigny, 2004 Recovery of a function involving gene duplication by retroposition in *Saccharomyces cerevisiae*. Genome Res. **14:** 1291–1297.

Schwob, E., and K. Nasmyth, 1993 CLB5 and CLB6, a new pair of B cyclins involved in DNA replication in *Saccharomyces cerevisiae*. Genes Dev. **7:** 1160–1175.

Selmecki, A., A. Forche and J. Berman, 2006 Aneuploidy and isochromosome formation in drug-resistant Candida albicans. Science **313:** 367–370.

Singh, R. K., M. H. Kabbaj, J. Paik and A. Gunjan, 2009 Histone levels are regulated by phosphorylation and ubiquitylation-dependent proteolysis. Nat. Cell Biol. **11:** 925–933.

Smith, M. M., and V. B. Stirling, 1988 Histone H3 and H4 gene deletions in *Saccharomyces cerevisiae*. J. Cell Biol. **106:** 557–566.

Spradling, A. C., 1981 The organization and amplification of two chromosomal domains containing Drosophila chorion genes. Cell **27:** 193–201.

Stark, G. R., and G. M. Wahl, 1984 Gene amplification. Annu. Rev. Biochem. **53:** 447–491.

Su, C., G. Gao, S. Schneider, C. Helt, C. Weiss *et al.*, 2004 DNA damage induces downregulation of histone gene expression through the G1 checkpoint pathway. EMBO J. **23:** 1133–1143.

Swanson, M. S., E. A. Malone and F. Winston, 1991 SPT5, an essential gene important for normal transcription in *Saccharomyces cerevisiae*, encodes an acidic nuclear protein with a carboxy-terminal repeat. Mol. Cell. Biol. **11:** 4286.

Tower, J., 2004 Developmental gene amplification and origin regulation. Annu. Rev. Genet. **38:** 273–304.

Tsubota, T., R. Tajima, K. Ode, H. Kubota, N. Fukuhara *et al.*, 2006 Double-stranded DNA binding, an unusual property of

DNA polymerase epsilon, promotes epigenetic silencing in *Saccharomyces cerevisiae*. J. Biol. Chem. **281:** 32898–32908.

Vernon, M., K. Lobachev and T. D. Petes, 2008 High rates of "unselected" aneuploidy and chromosome rearrangements in tel1 mec1 haploid yeast strains. Genetics **179:** 237–247.

Vogt, N., S. H. Lefevre, F. Apiou, A. M. Dutrillaux, A. Cor *et al.*, 2004 Molecular structure of double-minute chromosomes bearing amplified copies of the epidermal growth factor receptor gene in gliomas. Proc. Natl. Acad. Sci. USA **101:** 11368–11373.

Winston, F., C. Dollard and S. L. Ricupero-Hovasse, 1995 Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S288C. Yeast **11:** 53–55.

Wyrick, J. J., F. C. Holstege, E. G. Jennings, H. C. Causton, D. Shore *et al.*, 1999 Chromosomal landscape of nucleosome-dependent gene expression and silencing in yeast. Nature **402:** 418–421.

Zeyl, C., 2004 Capturing the adaptive mutation in yeast. Res. Microbiol. **155:** 217–223.

Zhao, J., 2004 Coordination of DNA synthesis and histone gene expression during normal cell cycle progression and after DNA damage. Cell Cycle **3:** 695–697.

Zhao, X., E. G. Muller and R. Rothstein, 1998 A suppressor of two essential checkpoint genes identifies a novel protein that negatively affects dNTP pools. Mol. Cell **2:** 329–340.

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Alterations in DNA Replication and Histone Levels Promote Histone Gene Amplification in *Saccharomyces cerevisiae*

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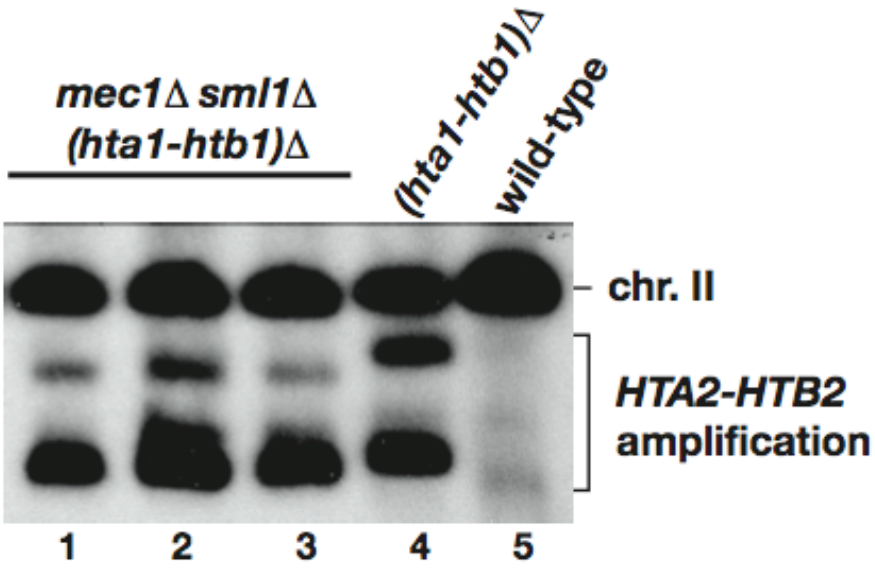

FIGURE S1.—*HTA2-HTB2* amplification is not dependent upon the DNA replication checkpoint. DNA replication checkpoint deletions (*mec1*Δ *sml1*Δ, *dun1*Δ, *sml1*Δ) with *(hta1-htb1)*Δ were analyzed for the *HTA2-HTB2* amplification by CHEF gel Southern blot using a probe for the amplified region on chromosome II (amplification probe B; (Libuda and Winston 2006)). A representative CHEF gel Southern blot is shown for *mec1*Δ *sml1*Δ *(hta1-htb1)*Δ strains. A summary of the tetrad and CHEF gel Southern analysis results for DNA replication checkpoint genes is in Table 2.

TABLE S1

Sequences of oligonucleotides used in these studies

name	sequence	use	position (5' end of oligo)
FO5008	CCTGCCCCAACAGATAAAAACAAGCAAGGGTCAACCG TGAGATTGTACTGAGAGTGCAC	*DPB3* deletion	12 bp 5' of *DPB3* ORF
FO5009	TTCTCATTGCGTGTATGTATATGTATAACATATTGCA TCGCTGTGCGGTATTTCACACCG	*DPB3* deletion	34 bp 3' of *DPB3* ORF
FO5010	GCGGCACAATAGCAGAAGCCACAGAATATTACCTAGA GGAGATTGTACTGAGAGTGCAC	*DPB4* deletion	102 bp 5' of *DBP4* ORF
FO5011	AGTGGTGGCAAGCACTACTAGACAGTTTCCATAGCGG GGCTGTGCGGTATTTCACACCG	*DPB4* deletion	1 bp 3' of *DPB4* ORF
FO5326	CGAGAGATTTGTTCTTATAAGACATCCCGATGTTCAG GTCGCAGATTGTACTGAGAGTGCAC	*RRM3* deletion	12 bp 5' of *RRM3* ORF
FO5327	CGCCGCATACGTAGGCTTAGCTAAATAGATGGCTTCT AGACTGTGCGGTATTTCACACCG	*RRM3* deletion	152 bp 3' of *RRM3* ORF
FO5006	GTTCACGCGCTTTGCCCACATTGGGATAGCGCCCACA GCAGATTGTACTGAGAGTGCAC	*CLB5* deletion	305 bp 5' of *CLB5* ORF
FO5007	CAGTTCAACCGTTTCCGGATGCATGTAGCGAGGGTA GACCTGTGCGGTATTTCACACCG	*CLB5* deletion	418 bp 3' of *CLB5* ORF
FO727	GGCGCGTGTAAGTTACAGACAAGCGATCCCTATTCCA TGAGATTGTACTGAGAGTGCAC	*HHT1-HHF1* deletion	100 bp 3' of *HHT1* ORF

FO730	TAACCACATGGAAAGCCATAAATCTTGCCTCCTACAA GGCTGTGCGGTATTTCACACCG	*HHT1-HHF1* deletion	250 bp 3' of *HHF1* ORF
FO4596	GAGATGTTTGTATGATGTCCCCCCAGTCTAAATGCAT AGCTGTGCGGTATTTCACACCG	*HHT2-HHF2* deletion	26 bp 3' of *HHT2* ORF
FO4597	CACCGATTGTTTAACCACCGATTGTTTAACCACCGAA ACAGATTGTACTGAGAGTGCAC	*HHT2-HHF2* deletion	18 bp 3' of *HHF2* ORF
FO4363	AGGGGCTTAACATACAGTAAAAAAGGCAATTATAGTG AAGCTGTGCGGTATTTCACACCG	*DUN1* deletion	40 bp 5' of *DUN1* ORF
FO4364	CCAGATTCAAACAATGTTTTTGAAATAATGCTTCTCA TGTAGATTGTACTGAGAGTGCAC	*DUN1* deletion	40 bp 3' of *DUN1* ORF
FO4693	GTATCTAAGAGAAGAAAAGAACAGAACTAGTGGGAAA TGAGATTGTACTGAGAGTGCAC	*SML1* deletion	39 bp 5' of *SML1* ORF
FO4694	CTCACTAACCTCTCTTCAACTGCTCAATAATTTCCCG CTCTGTGCGGTATTTCACACCG	*SML1* deletion	71 bp 3' of *SML1* ORF
FO1075	AAGAACGACATACACCGCGTAAAGGCCCACAAGACTG CCTGTGCGGTATTTCACACCG	*MEC1* deletion	1 bp 5' of *MEC1* ORF
FO1076	ACATAATTGTTTCGATCACATTCATCAACGCTACTTC AAGATTGTACTGAGAGTGCAC	*MEC1* deletion	6884 bp 3' of *MEC1* ORF
FO6051	CCGTCTCAGAATTGGATCGT	*RNR3* Northern probe	+1229 bp from *RNR3* ATG
FO6052	ATTGTTTCCGTTGGAACTGC	*RNR3* Northern probe	+1430 bp from *RNR3* ATG

FO844	TGTATGTGTGTATGGTTTATTTGTG	*HTB1* Northern probe	162 bp 5' of *HTB1* ORF
FO907	GAGAACGATGGATTTAAAATCAAGAG	*HTB1* Northern probe	61 bp 5' of *HTB1* ORF
FO5415	CAAAGCAAATCAAACATTCTTGTC	*HTA2* Northern probe	20 bp 3' of *HTA2* ORF
FO5416	CACTGTCTTTAATAAAACGACGCTAT	*HTA2* Northern probe	134 bp 3' of *HTA2* ORF
FO5269	ACGGTTTCGGTGGTTAATTT	*HHF1* Northern probe	+314 bp from *HHF1* ATG
FO5270	TCAAACTGTTTAAACCCAATTAAAA	*HHF1* Northern probe	175 bp 3' of *HHF1* ORF
FO6182	TTTTATATAGGACCACTGTTTTGTGA	*HHT2* Northern probe	91 bp 5' of *HHT2* ORF
FO6183	TGTGGAGTGTTTGCTTGGAT	*HHT2* Northern probe	1 bp 5' of *HHT2* ORF
FO5329	CAATCGGTGGTTAAACAATCGG	*HHF2* Northern probe	+301 bp from *HHF2* ATG
FO5330	GGGAAGACTATCTAAGACAGTTCG	*HHF2* Northern probe	198 bp 3' of *HHF2* ORF
FO1408	TACCCGCCACGCGTTTTTTTCTTT	*ACT1* Northern probe	-367 bp from *ACT1* ATG
FO1409	ATTGAAGAAGATTGAGCAGCGGTTTG	*ACT1* Northern probe	+1015 bp from *ACT1* ATG

TABLE S2

Results from synthetic genetic array analysis for regulators of *(hta1-htb1)Δ* viability

candidate gene[d]	alternative gene names[d]	systematic name[d]	*(hta1-htb1)Δ* growth[e] (SC-his-leu-arg+can[b])	*(hta1-htb1)Δ yfg1Δ*[a] growth[e] (SC-his -leu -arg +can+G418[c])	gene ontology category[d]
increases *(hta1-htb1)*Δ viability					
ERG3	*SYR1, PSO6*	YLR056W	++++	+	ergosterol biosynthesis; endocytosis; ER
IRA2	*CCS1, GLC4*	YOL081W	++	+	mitochondria
PRM4		YPL156C	++++	+	Mating
SSE1	*LPG3, MSI3*	YPL106C	++++	+	HSP90 chaperone complex; telomere maintenance
MSI1	*CAC3*	YBR195C	++	+	CAF-1 Complex; chromatin, DNA repair, transcription
DPB4		YDR121W	+++	+	DNA pol epsilon; ISW2 chromatin complex
YDR431W		YDR431W	++	+	Dubious
CIN8	*KSL2, SDS15*	YEL061C	++	+	MT kinesin motor; Mitotic sister chromatid segregation
RRM3	*RTT104*	YHR031C	+++	+	DNA helicase (rDNA replication and Ty1 transposition)
KKQ8		YKL168C	++++	+	putative kinase
PAC10	*GIM2, PFD3, RKS2*	YGR078C	+++	+	tubulin; prefoldin complex
YJL131C		YJL131C	++++	+	putative protein; mitochondria localized
BUD26		YDR241W	+++	+	dubious
RPS6A		YPL090C	++++	+	40S ribosomal
CLB5		YPR120C	++++	+	CDK in DNA replication
HSV2		YGR223C	+++	+	phosphoinositide binding
RPL19B		YBL027W	++++	+	60S ribosomal
YBL081W		YBL081W	++++	+	hypothetical protein
AVT5		YBL089W	++	+	putative transporter
DST1	*PPR2*	YGL043W	++	+	TFIIS; transcription elongation; meiotic recombination

[a]*yfg1Δ* indicates a gene deletion from the nonessential gene deletion set. Most of the deletion strains listed in this table have not been confirmed to have the correct deletion.

[b]SC-his-leu-arg+can indicates selection for growth of *(hta1-htb1)Δ* strains with or without *yfg1Δ* (note: typical growth for *(hta1-htb1)Δ* without *yfg1Δ* on this medium is +/-).

[c]SC-his-leu-arg+can+G418 indicates selection for *(hta1-htb1)Δ yfg1Δ*.

[d]Information regarding gene names, systematic names, and gene ontology categories were obtained from the *Saccharomyces* Genome Database (www.yeastgenome.org)

[e]Growth phenotype scores: ++, +++, and ++++ = a range of very strong growth; + = good growth; -/+ and +/- = intermediate growth; and - = no growth

KEX2	*SRB1, QDS1, VMA45*	YNL238W	++	+	proprotein convertase
GCR2		YNL199C	++	+	transcriptional activator (glycolysis)
YNL198C		YNL198C	++	+	Dubious
LSM1	*SPB8*	YJL124C	++++	+	degradation of cytoplasmic mRNAs; RNA binding and catabolism
FYV1		YDR024W	++	+	Dubious
YBR277C		YBR277C	+++	+	Dubious
NRG1		YDR043C	++++	+	Transcriptional Repressor; glucose repression
RPS14A	*CRY1, RPL59*	YCR031C	+++	+	40S ribosomal; telomere maintenance
PAT1	*MRT1*	YCR077C	+++	+	Topoisomerase II; chromosome segregation; mRNA processing
HHF1		YBR009C	++++	+	Histone H4
HHT1		YBR010W	++++	+	Histone H3
MBP1		YDL056W	+++	+	Transcription Factor; Repressor; DNA replication (complex with Swi6); Cell cycle
RPP1A	*RPLA1*	YDL081C	+++	+	p1 alpha ribosome
SRB9	*SSN2, NUT8, SCA1, UME2, RYE3, MED13*	YDR443C	+++	+	Mediator complex; SRB8-11 complex; transcription; telomere maintenance
SWI4	*ART1*	YER111C	++++	+	SBF complex (Swi4-6); DNA binding; transcription factor; cell cycle
NRP1		YDL167C	+++	+	Unknown
LSM7		YNL147W	++++	+	mRNA catabolism; telomere maintenance
MED1		YPR070W	+++	+	Mediator complex; transcription; telomere maintenance
NUT1	*MED5*	YGL151W	++	+	Mediator complex; transcription
LSM6		YDR378C	+++	+	mRNA catabolism
YGL177W		YGL177W	++	+	Dubious
PPZ1		YML016C	++	+/-	Phosphatase; cell cycle progression
YPL260W		YPL260W	++	+/-	Unknown
YBR225W		YBR225W	++	+/-	Unknown
MUP3		YHL036W	++	+/-	Metionine permease
VMR1		YHL035C	++	+/-	potential Cdc28 substrate; vaculolar?
SBP1	*SSB1; SSBR1*	YHL034C	++	+/-	snoRNA binding; 35S transcript processing
RPS25A	*RPS31A*	YGR027C	++	+/-	40S ribosomal
YLL007C		YLL007C	++	+/-	Unknown
YOL150C		YOL150C	++	+/-	Dubious

SRB8	*SSN5; MED12; NUT6; GIG1; YCR080W*	YCR081W	+++	+/-	Mediator Complex; transcription; telomere maintenance
HHT2		YNL031C	+++	+/-	Histone H3
BMH1	*APR6*	YER177W	+++	+/-	DNA binding; DNA damage checkpoint; signaling; vesical transport
GIM3	*PFD4*	YNL153C	+++	+/-	prefoldin complex; chaperone complex
CAN1		YEL063C	+	+	arginine permease; deletion = can resistance
MDM20	*DEC1*	YOLO076W	+	+/-	NatB acetyl transferase complex; mitochondrial inheritance
HAT2		YEL056W	+	+/-	Hat1-Hat2 Acetyltransferase complex (Histone H3/H4); telomere silencing
SRB10	*SSN3, GIG2, NUT&, UME3, RYE5, CDK8*	YPL042C	+	+/-	CDK of Mediator complex; transcription; meiosis; telomere maintenance
ATP5	*OSC1*	YDR298C	+	+/-	ATP synthesis
YDR336W		YDR336W	+	+/-	Unknown
SRB2	*MED20; HRS2*	YHR041C	+	+/-	Mediator complex; transcription factor; telomere maintenance

decreases (hta1-htb1)Δ viability

RPS13A	*RPS9B; SUP36*	YBR189W	+	-	40S ribosome
YMR293C		YMR293C	+	-	Unknown; mitochondria
YMR304C		YMR304C-A	+	-	Dubious
CLA4	*ERC10*	YNL298W	+	-	CDC42 activated kinase; cytokinesis; exit from mitosis; cell polarity
VAM10		YOR068C	+	-	Vaculoe fusion
UAF30		YOR295W	+	-	Upstream Activator Factor Complex (RNA pol I specific; contains Histone H3 and H4); transcription; rDNA binding
CPA1		YOR303W	+	-	Citrulline biosynthesis (arginine precursor)
SPE2		YOL052C	+	-	spremidine/spermine biosynthesis
RTG1		YOL067C	+	-	transcriptional coactivator (bHLH); mitochondria
YPL205C		YPL205C	+	-	Dubious
POS5		YPL188W	+	-	NADH kinase; mitochondria
TGS1		YPL157W	+	-	RNA methlytransferase; snRNA capping; ribosome biogenesis
YPL144W		YPL144W	+	-	Unknown; telomere maintenance

UME1	*WTM3*	YPL139C	+	-	transcription corepression; meiosis regulation
MRP51		YPL118W	+	-	mitochondria
HIS7		YBR248C	+	-	histidine biosynthesis
DPB3		YBR278W	+	-	DNA replication; DNA pol II; telomeric silencing
TIM13		YGR181W	+	-	mitochondria
PDX1		YGR193C	+	-	E3BP; mitochondria
VAM3	*PTH1*	YOR106W	+	-	Vacuole
MCT1		YOR221C	+	-	mitochondria
YDR246C		YDR246C	+	-	Unknown; oxidoreductase
MNN11		YER001W	+	-	Golgi
VPS35	*GRD9; VPT7*	YJL154C	+	-	Endosome; Golgi
VPS36	*GRD12; VAC3; VPL11*	YLR417W	+	-	ESCRT-II complex; telomere maintenance
YLR235C		YLR235C	+	-	Dubious
KAP114		YGL241W	+	-	Imports Spt15, Histone H2A and H2B, and NAP1
SNF8	*VPS22*	YPL002C	+	-	ESCRT-II complex; transcription regulated by glucose; telomere maintenance
VPS66		YPR139C	+	-	Vacuole
SEM1	*DSS1; HOD1*	YDR363W-A	+	-	26S proteasome; telomere maintenance
YNL171C		YNL171C	+	-	Dubious
DOA1	*UFD3; ZZZ4*	YKL213C	+	-	promotes NHEJ in stationary phase; DSB DNA repair; Ubiquitin
BSC2		YDR275W	+	-	Unknown
RPS7A	*RPS30*	YOR096W	+	-	40S ribosome
COG8	*DOR1*	YML071C	+	-	Golgi complex
YML090W		YML090W	+	-	Dubious
VPS9	*VPL31; VPT9*	YML097C	+	-	vacuole; telomere maintenance
MYO5		YMR109W	+	-	Myosin
PKR1		YMR123W	+	-	V-ATPase assembly factor in ER
BRR1		YPR057W	+	-	snRNP protein; RNA binding
SPT10	*CRE1; SUD1*	YJL127C	+	-	Putative histone acetyltransferase; Activates Histone genes; transcription; chromatin
ARG3		YJL088W	+	-	Arginine biosynthesis
THR1		YHR025W	+	-	threonine metabolism
PPA1	*VMA16*	YHR026W	+	-	Vacuole
VPS25	*VPT25*	YJR102C	+	-	ESRT-II complex; Ubiquitin; Telomere maintenance
KCS1		YDR017C	+	-	Vacuole
PMP1		YCR024C-A	+	-	enzyme regulator
VPS53		YJL029C	+	-	Golgi
HIT1		YJR055W	+	-	Unknown; telomere maintenance
ERI1	*RIN1*	YPL096C-A	+	-	Er protein

HDA1		YNL021W	+	-	Histone deacetylase complex; deletion increases H2B, H3, and H4 acetylation; chromatin; transcription
COG6	*COD2; SEC37*	YNL041C	+	-	Golgi
VPS27	*GRD11; SSV17; VPL23; VPL27; VPT27; DID7*	YNR006W	+	-	ER protein
UBP1		YDL122W	+	-	Ubiquitin; ER
FAR11		YNL127W	+	-	pheromone cell cycle arrest
HIS5		YIL116W	+	-	histidine biosynthesis
FLX1		YIL134W	+	-	FAD transport; mitochondria
RRB1		YMR131C	+	-	ribosome
LAT1	*ODP2, PDA2*	YNL071W	+	-	pyruvate dehydrogenase; mitochondria
MSK1		YNL073W	+	-	lysine-tRNA ligase; mitochondria
NNF2		YGR089W	+	-	chromosome segregation; genetic and physical interactions w/ Rpb8 (RNA pol I, II, III)
YDL062W		YDR062W	+	-	Dubious
YDR455C		YDR455C	+	-	Dubious
TOM1		YDR457W	+	-	E3 ubiquitin ligase; mRNA export; regulation of transcriptional coactivators
VPS60	*MOS10; CHM5*	YDR486C	+	-	vacuole
QRI8	*UBC7*	YMR022W	+	-/+	ubiguitin; ER; chromatin assembly/disassembly
HSC82	*HSP90*	YMR186W	+	-/+	chaperone of Hsp90; protein folding; telomere maintenance
MDJ2		YNL328C	+	-/+	Mitochondria
RPS6B	*LPG18; RPS101; RPS102*	YBR181C	+	-/+	40S ribosome
KIN1		YDR122W	+	-/+	serine/threonine kinase
LEU3		YLR451W	+	-/+	leucine biosynthesis
HXK2	*HEX1; HKB; SCI2*	YGL253W	+	-/+	glucose metabolism
AEP3		YPL005W	+	-/+	mRNA metabolism; mitochondria
YNL190W		YNL190W	+	-/+	Unknown
PEX1	*PAS1*	YKL197C	+	-/+	ATPase; peroxisome
HIS6		YIL020C	+	-/+	histidine biosynthesis
RPL34B		YIL052C	+	-/+	60S ribosome

VPS20	*CHM6; VPT20; VPL10*	YMR077C	+	-/+	ESCRT-III complex; ubiquitin; vacuole
IES3		YLR052W	+	-/+	INO80 chromatin remodeling complex
NAB6		YML117W	+	-/+	putative RNA binding protein
SLM5		YCR024C	+	-/+	Mitochondria
YCR049C		YCR049C	+	-/+	Dubious
CSM2		YIL132C	+	-/+	meiotic chromosome segregation; DNA repair
RPL16A	*RPL13*	YIL133C	+	-/+	60S ribosome
VPS3	*PEP6; VPL3; VPT17*	YDR495C	+	-/+	Vacuole
PUF6		YDR496C	+	-/+	mRNA binding; transcription; mating; ribosome biogenesis
YGL149W		YGL149W	+	-/+	Dubious
CIK1		YMR198W	-	-	microtubule motor; spindle; meiosis; sister chromatid segregation
GSG1	*TRS85; MUM1*	YDR108W	-	-	ER; Golgi; meiosis; TRAPP complex
BFR1		YOR198C	-	-	mRNP complex; mRNA metabolism; meiosis; mitosis regulation
RPB4	*CTF15*	YJL140W	-	-	RNA pol II; transcription; telomere maintenance; mRNA export
VPS66		YPR139C	-	-	Vacuole
RPS8A		YBL072C	-	-	40S ribosome
PER1	*COS16*	YCR044C	-	-	ER
STP22	*VPS23*	YCL008C	-/+	-	ESCRT-1 complex; ubiquitin; telomere maintenance
SAT4	*HAL4*	YCR008W	-/+	-	mitotic cell cycle; kinase
YGL218W		YGL218W	-/+	-	Dubious
RTG2		YGL252C	-/+	-	mitochondria; SLIK complex; transcription
VMA7		YGR020C	-/+	-	Vacuole
RPL34B		YIL052C	-/+	-	60S ribosome; telomere maintenance
RIM13	*CPL1*	YMR154C	-/+	-	protease
SLM5		YCR024C	-/+	-	Mitochondria
FEN2		YCR028C	-/+	-	plasma membrane
FKH2		YNL068C	-/+	-	transcription factor; cell cycle; silencing
RPS21B		YJL136C	-/+	-	40S ribosome
SEL1	*UBX2*	YML013W	+/-	-	ubiquitin; ER
YML013C-A	*YML012C-A*	YML013C-A	+/-	-	Dubious (overlaps with SEL1)
YAP1	*PAR1; SNQ3*	YML007W	+/-	-	bZIP transcription factor
MRPL3		YMR024W	+/-	-	mitochondria

ADA1	*HFI1; SUP110; SRM12; GAN1*	YPL254W	+/-	-	SAGA; transcription; chromatin; histone acetylation; telomere maintenance
HTA2	*H2A2*	YBL003C	+/-	-	Histone H2A