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Amplification of histone genes by circular chromosome formation in *Saccharomyces cerevisiae*

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Abstract

Proper histone levels are critical for transcription, chromosome segregation, and other chromatin-mediated processes[1-7]. In *Saccharomyces cerevisiae*, the histones H2A and H2B are encoded by two gene pairs, named *HTA1-HTB1* and *HTA2-HTB2* (ref. 8). Previous studies have demonstrated that when *HTA2-HTB2* is deleted, *HTA1-HTB1* dosage compensates at the transcriptional level[4,9]. Here we show that a different mechanism of dosage compensation, at the level of gene copy number, can occur when *HTA1-HTB1* is deleted. In this case, *HTA2-HTB2* amplifies via creation of a new, small, circular chromosome. This duplication, which contains 39 kb of chromosome II, includes *HTA2-HTB2*, the histone H3-H4 locus *HHT1-HHF1*, a centromere and origins of replication. Formation of the new chromosome occurs by recombination between two Ty1 retrotransposon elements that flank this region. Following meiosis, recombination between these two particular Ty1 elements occurs at a greatly elevated level in *hta1-htb1Δ* mutants, suggesting that a decreased level of histones H2A and H2B specifically stimulates this amplification of histone genes. Our results demonstrate another mechanism by which histone gene dosage is controlled to maintain genomic integrity.

Although early genetic studies of *S. cerevisiae* genes encoding histones H2A and H2B clearly demonstrated that they are essential for growth[10,11], deletion of either of the *H2A-H2B* loci seemed to allow viability[4,6]. In those studies, *hta2-htb2Δ* mutants were shown to grow normally, presumably owing to increased transcription of *HTA1-HTB1*[4,9]. In contrast, *hta1-htb1Δ* mutants were shown to have several mutant phenotypes, including defects in transcription and chromatin structure[3,4,6,12]. Notably, several unresolved mysteries have surrounded *hta1-htb1Δ* mutants. First, it was reported[13] that some *hta1-htb1Δ* strains contain two copies of *HTA2-HTB2*, although this possible duplication was not characterized. Second, the *S. cerevisiae* deletion project reported that, whereas an *hta1Δ* mutant is viable, an *htb1Δ* mutant is inviable[14]. Finally, although *hta1-htb1Δ* mutants have been constructed in an S288C background, such mutants are inviable in W303 background (P. Kaufman and M. A. Osley, personal communication).

As an initial step in further characterizing *hta1-htb1Δ* mutants, we analysed strains previously suspected of containing an *HTA2-HTB2* duplication[13]. In this analysis we included the *hta1Δ* mutant from the *S. cerevisiae* deletion project[14]. To test for possible genomic changes, chromosomes were separated using contour-clamped homogeneous electric field (CHEF) gels and then probed by Southern analysis using an *HTA2-HTB2*

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probe (Fig. 1a). Our results show that for all *hta1-htb1Δ* strains and the *hta1Δ* strain, the *HTA2-HTB2* probe hybridized both to chromosome II, the normal location of *HTA2-HTB2*, and to a second prominent band (Fig. 1b). Thus, in these strains, *HTA2-HTB2* exists as a second copy that is not part of chromosome II.

To determine the size of the *HTA2-HTB2* amplification in *hta1-htb1Δ* mutants, we tested for amplification of flanking sequences. *HTA2-HTB2* is in a 33-kb region, flanked by Ty1 elements, that contains the centromere and 15 genes, including the histone *H3-H4* locus *HHT1-HHF1* (Fig. 1a; Supplementary Fig. 1). Our results show that sequences between the Ty1 elements display the same hybridization pattern as *HTA2-HTB2* in the *hta1-htb1Δ* strains (Fig. 1b, c). In contrast, sequences just outside the Ty1 elements were present in single copy in all strains tested (Fig. 1c). These results establish that the amplified region is delimited by the flanking Ty1 elements.

To determine whether all *hta1-htb1Δ* strains would contain the same amplification, additional *hta1-htb1Δ* strains were constructed by two methods. First, we deleted *HTA1-HTB1* in a diploid, sporulated the *HTA1-HTB1/hta1-htb1Δ* heterozygote, and dissected tetrads. Among 78 tetrads analysed, 149/156 *HTA1-HTB1* spores germinated and grew normally. In contrast, most *hta1-htb1Δ* spores failed to germinate, suggesting that this deletion causes inviability. Eventually, 20/156 *hta1-htb1Δ* spores formed colonies, appearing two days later than the wild-type colonies (Fig. 2a). (In a separate experiment, 70 *hta1-htb1Δ* spores that failed to form colonies were examined by microscopy and all failed to proceed past a single cell division (Supplementary Table 1; Supplementary Discussion).) When the viable *hta1-htb1Δ* strains were restreaked, they had a growth rate similar to that of the wild type (Supplementary Fig. 2a). To test the new viable *hta1-htb1Δ* strains for the amplification, 15 were examined by CHEF gel Southern analyses (Fig. 2b and data not shown) and all were shown to contain the amplification event observed in Fig. 1. This suggests that the amplification is required for the viability of *hta1-htb1Δ* strains and that it occurs at a high frequency in *hta1-htb1Δ* spores (see Supplementary Discussion). Additional experiments show that the *HTA2-HTB2* genes within the amplification are sufficient for viability in *hta1-htb1Δ* strains (Supplementary Fig. 3). Southern hybridizations and real-time polymerase chain reaction (PCR) determined that the amplification copy number is approximately one per cell (data not shown).

In a second approach, we constructed an *hta1-htb1Δ* haploid strain containing an *HTA1-HTB1 URA3* plasmid. Three independent cultures were plated on 5-fluoroorotic acid (5-FOA) medium to select for viable derivatives without the plasmid, thus generating new *hta1-htb1Δ* strains. Of the resistant (5-FOA^R) colonies generated, 6/32 examined contained the amplification (Supplementary Fig. 2b). The frequency of the amplification was determined to be 3×10^{-5} amplification events per cell (Supplementary Table 2; Supplementary Discussion). Of the *hta1-htb1Δ* strains without the amplification, ten were tested and shown to be chromosome II disomes; all ten had a severe growth defect (Supplementary Fig. 2). Altogether, these results show that *hta1-htb1Δ* strains require two copies of *HTA2-HTB2* for viability. The remainder of our studies focused on the amplification event.

Several results suggested that the amplified version of *HTA2-HTB2* is circular and formed by homologous recombination between the Ty1 elements (Supplementary Discussion). To test for a circle, three experiments were performed. First, PCR was performed with primers designed to amplify a product only if the Ty1-Ty1 recombination on chromosome II occurred. Our results show that, in contrast to wild-type strains, all *hta1-htb1Δ* strains tested produced the PCR product expected from the Ty1-Ty1 recombinant (Fig. 3a). Second, Southern analyses of restriction-enzyme-digested genomic DNA were probed for fragments

that would exist in only the recombinants. Our results demonstrate a novel restriction fragment with a size consistent with a 39 kb circular chromosome formed by recombination between the Ty1 elements (Supplementary Fig. 4). Third, homothallic switching (HO) endonuclease was used to cut the amplified DNA *in vivo*. If the amplified DNA is circular, then linearization by HO endonuclease digestion would cause a large shift in its migration in CHEF gels. Therefore, *hta1-htb1Δ* strains were constructed with a HO endonuclease site integrated near the *HTA2-HTB2* locus and with a plasmid containing the *HO* gene under the control of the *GAL1* promoter. Our results show that upon *HO* induction, the migration of the amplified DNA shifts markedly, consistent with the linearization of a 39 kb circular chromosome (Fig. 3b; compare lanes 15 and 16). Collectively, these results provide strong evidence that the amplified *HTA2-HTB2* locus is a novel circular chromosome.

Two experiments were performed to examine the role of Ty1 elements in the amplification. First, to test whether both Ty1 elements on chromosome II are required for the amplification, *HTA1-HTB1/hta1-htb1Δ* diploids that were homozygous for deletion of one of the two Ty1 elements were constructed. For the deletion of *YBLWTy1-1*, 37 tetrads were analysed and only 4/74 *hta1-htb1Δ* spores formed colonies. For the deletion of *YBRWTy1-2*, 46 tetrads were analysed and only 1/92 *hta1-htb1Δ* spores grew. All five of these strains grew extremely slowly, and none of them contained the amplification (Fig. 4a). Genetic analysis suggested that these strains are disomic for chromosome II. The conclusion that the amplification requires both Ty1 elements is also supported by experiments with *S. cerevisiae* W303. Unlike the S288C background, deletion of *HTA1-HTB1* causes inviability in all *hta1-htb1Δ* spores in the W303 background (P. Kaufman and M. A. Osley, personal communication). Our analysis of W303 has shown that only a solo *δ* element exists in place of *YBRWTy1-2* (Supplementary Fig. 5). Taken together, our results strongly suggest that each of the Ty1 elements on chromosome II is required for the amplification event.

Second, to test whether the amplification can occur if the Ty1 elements are replaced by a different sequence of similar length, we replaced each Ty1 with linearized plasmid Ylp5 (ref. 15). Ylp5 is 5.5 kb long (compared with 5.9 kb for Ty1) and contains no homology to Ty1 elements. To measure the recombination frequency, *HTA1-HTB1/(hta1-htb1)Δ* diploids homozygous for the Ty1 replacements with Ylp5 were sporulated. From 115 tetrads, 44/230 *hta1-htb1Δ* spores (19.1%) contained the amplification, a frequency comparable to that observed for amplification with the Ty1 elements. Both CHEF gels (Fig. 4b) and PCR (data not shown) confirmed that the amplification was an extrachromosomal circle. Thus, Ty1 elements are not required for the amplification, suggesting that any homologous sequence of adequate length is sufficient.

The amplification event that we have described occurs at a greatly elevated frequency in *hta1-htb1Δ* mutants following meiosis, compared with the frequencies of previously studied Ty-Ty recombination events[16-18]. This high frequency might reflect a genome-wide elevation of recombination in *hta1-htb1Δ* mutants due to reduced levels of histones H2A and H2B. Alternatively, the high frequency of recombination may be specific to this pair of Ty1 elements. To distinguish between these possibilities, we measured the recombination frequency for two additional pairs of adjacent Ty1 elements under the same conditions in which the histone gene amplification occurs. As a control, we first showed that each of these pairs of Ty1 elements has normal levels of mitotic recombination (Supplementary Table 3). Our results (Fig. 4c) show that the level of Ty1-Ty1 recombination that gives rise to the amplification is significantly higher than for either of the other Ty1-Ty1 recombination events following meiosis, demonstrating that there is not a genome-wide elevation of recombination. Rather, the recombination event that forms the amplification appears to be enhanced specifically at this locus in *hta1-htb1Δ* spores.

How can a high level of recombination between two specific Ty elements be explained? Indeed, previous studies have shown that Ty elements are generally cold for meiotic recombination and, in fact, suppress the activity of nearby recombination hotspots[19]. Several factors have been suggested to control meiotic recombination levels, including chromatin structure and histone modifications[20]. In addition, earlier analysis of *hta1-htb1Δ* mutants demonstrated that they have localized, rather than general, effects on chromatin structure[3]. Thus, when H2A and H2B levels are reduced, as in *hta1-htb1Δ* spores, there may be a specific local alteration of chromatin structure to create a hotspot for recombination, resulting in the observed amplification frequency. Conceivably, the recombination frequency could also be affected by transcription levels, as has been suggested for the *S. cerevisiae* recombination enhancer that controls the directionality of mating-type switching[21,22]. However, our results suggest that Ty1 transcription is not a critical factor (see Supplementary Discussion).

Ty elements have been suggested to mediate genomic rearrangements under selective pressure[23]. Our work has identified a previously unknown mechanism by which Ty elements enable histone genes to dosage-compensate in response to reduced histone levels. To our knowledge, this is the first example of a natural role for Ty elements that is dependent upon their specific genomic position. This amplification mechanism would also allow the transient modulation of histone levels in wild-type cells in response to the need for altered histone levels; the amplification could occur when more histones are required, and the circular chromosome could be easily lost when this requirement ends. The amplification described here suggests that similar transposon-related mechanisms may serve in adaptive gene amplification in other organisms, including humans, where 45% of the genome consists of transposons[24,25].

METHODS

Details of strains, plasmids, media, CHEF gel analysis, Southern blot hybridization analysis, PCR across Ty elements, and HO endonuclease experiments can be found in Supplementary Information.

Ty1-Ty1 recombination assay

Ty1-Ty1 recombination markers were constructed to determine the level of Ty1-Ty1 recombination in wild-type and *hta1-htb1Δ* strains. To do this, plasmid B155, which contains a complete Ty1 and *URA3*, was used to transform FY26. Integrants resulting from recombination between the Ty1 element on the plasmid and a Ty1 element in the genome result in two Ty1 elements flanking 17 kb of plasmid sequences including *URA3*. The integration location was mapped using CHEF gels, Southern blots and PCR analysis. Two different integrants were chosen for further analysis: in FY2526, B155 integrated at *YPLWTy1-1* on chromosome XVI, and in FY2523, B155 integrated at *YDRWTy1-5* on chromosome IV. By a series of crosses (FY2526 × FY2525 and FY2523 × FY2524) and selection on 5-fluoroanthranilic acid (5-FAA) media to lose the pDL2 plasmid, *HTA1-HTB1/hta1-htb1Δ* diploid strains, homozygous for either of the Ty1-Ty1 recombination markers, were constructed. For Fig. 4c, the resultant diploids were then sporulated and tetrads dissected to assess the stability of the Ty1-Ty1 recombination marker in both wild-type and *hta1-htb1Δ* spores. If the frequency of Ty1-Ty1 recombination throughout the genome is elevated to the same frequency of the Ty1-Ty1 recombination event that generates the amplification (14–18%), then 14–18% of *hta1-htb1Δ* strains with the amplification would be expected to be Ura^{-}.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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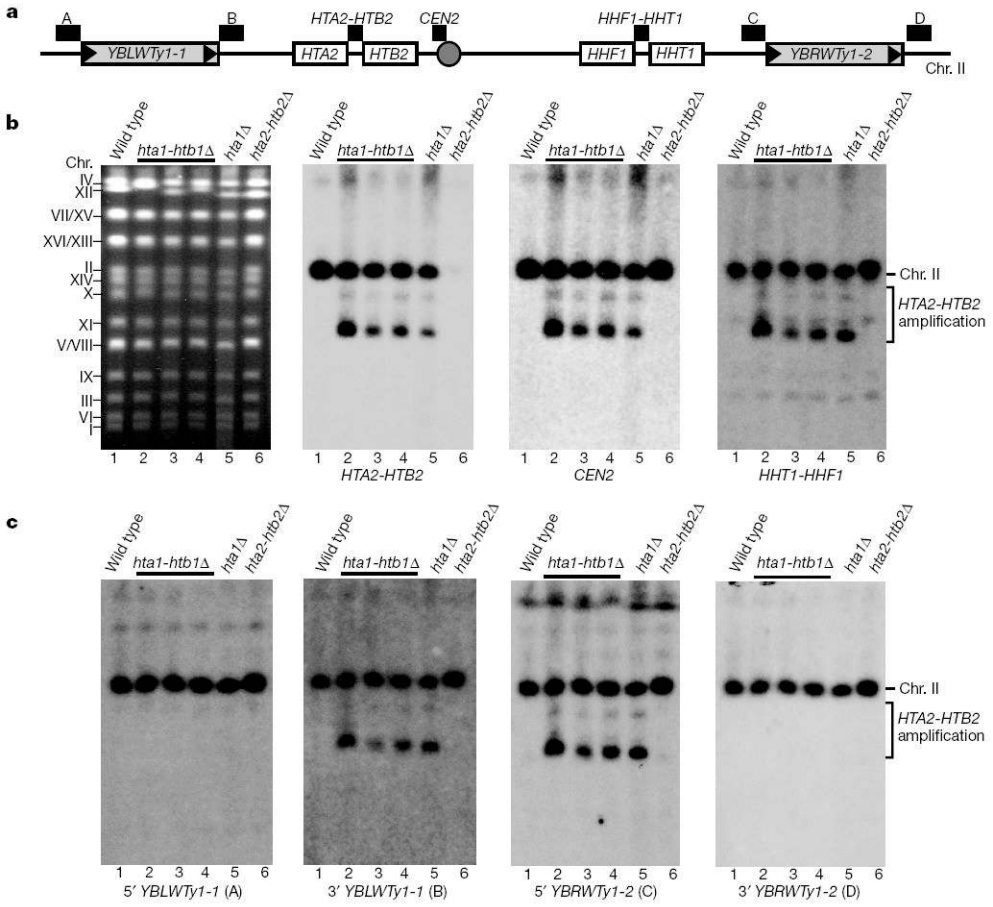


Figure 1. Characterization of *HTA2-HTB2* amplification in *hta1-htb1Δ*

a, A simplified genome map of the *HTA2-HTB2* region on chromosome II. The white boxes represent the *HTA2-HTB2* and *HHT1-HHF1* histone loci. The grey boxes represent the Ty1 elements, *YBLWTy1-1* and *YBLWTy1-2*, with the long terminal repeats (known as LTRs or *δ* elements) shown as black triangles. The grey circle represents the centromere. The probes used in **b** and **c** are represented as black bars above the map in **a**; the probe used for each Southern analysis is indicated below the blots in **b** and **c**. A complete map of the region is shown in Supplementary Fig. 1. **b**, Separated chromosomes from *S. cerevisiae* strains were analysed on a CHEF gel. Each panel shows a wild-type strain (lane 1), three *hta1-htb1Δ* strains (lanes 2–4), the *hta1Δ* strain from the deletion project (lane 5), and an *hta2-htb2Δ* strain (lane 6). The left panel is an ethidium-bromide-stained gel showing the positions of the chromosomes. The altered migration of chromosome XII is a consequence of the rDNA repeats, which are known to vary in number[26]. The other three panels show analysis of the same gel by Southern hybridization analysis. **c**, Chromosomes from the same strains were examined as in **b** using probes just outside (probes A and D) and within (probes B and C) the region flanked by *YBLWTy1-1* and *YBLWTy1-2*. The *hta1Δ* strain from the deletion set has the identical pattern to our *hta1-htb1Δ* strains. We assume that during construction of the deletion set, the *hta1Δ* strain acquired the amplification and the *htb1Δ* strain did not. Chr., chromosome.

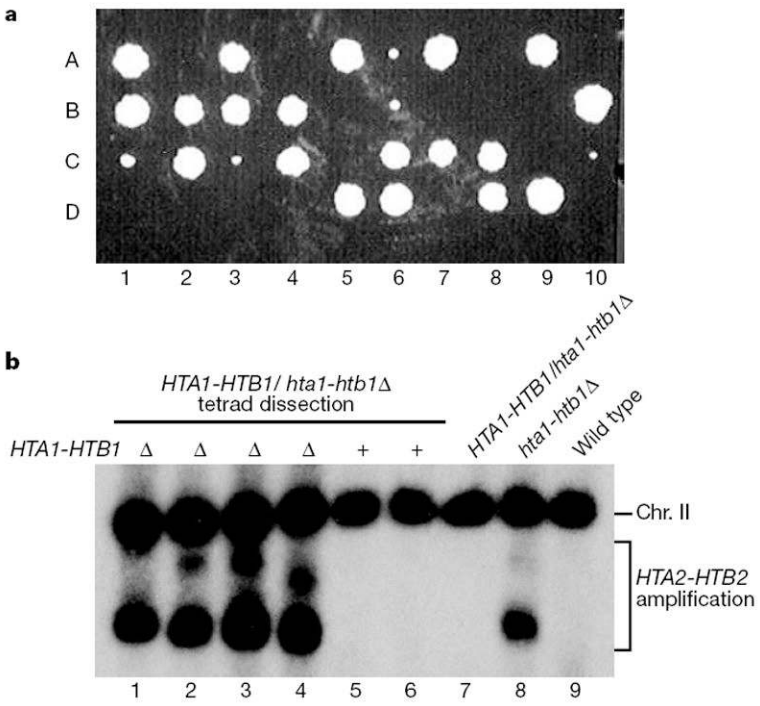


Figure 2. Analysis of newly constructed *hta1-htb1Δ* strains

a, Strains from *HTA1-HTB1/hta1-htb1Δ* tetrad dissection. Shown is a representative tetrad dissection plate. Each column shows growth of the four progeny from a single tetrad. The large colonies are wild type and the small colonies are *hta1-htb1Δ*. The apparent slow growth of the *hta1-htb1Δ* colonies is caused by slow germination (see Supplementary Discussion). Most spores that failed to germinate, for example, tetrad 1 spore D, were also *hta1-htb1Δ*. **b**, Four *hta1-htb1Δ* progeny (lanes 1–4) and two wild-type progeny (lanes 5 and 6) generated from the *HTA1-HTB1/(hta1-htb1)Δ* tetrad dissection were analysed by a CHEF gel Southern analysis probed with *HTA2-HTB2*. Controls include the *HTA1-HTB1/hta1-htb1Δ* parent diploid (lane 7), an *hta1-htb1Δ* haploid (lane 8) and a wild-type strain (lane 9). All strains were tested with the same probes as Fig. 1 and shown to contain the same amplified region (data not shown).

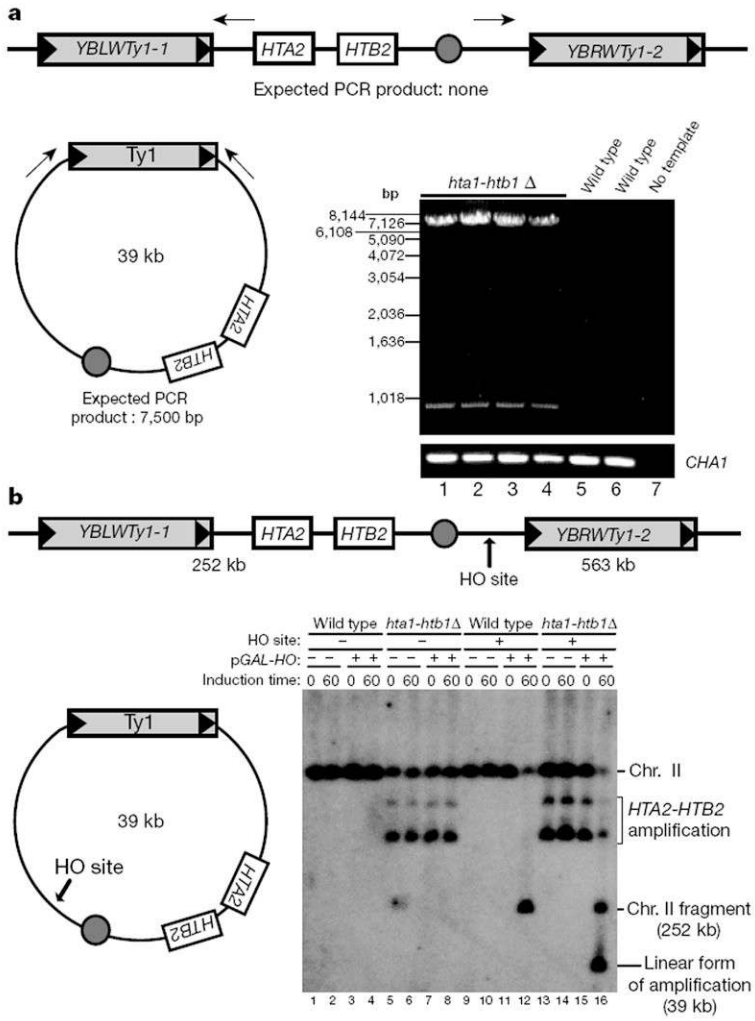
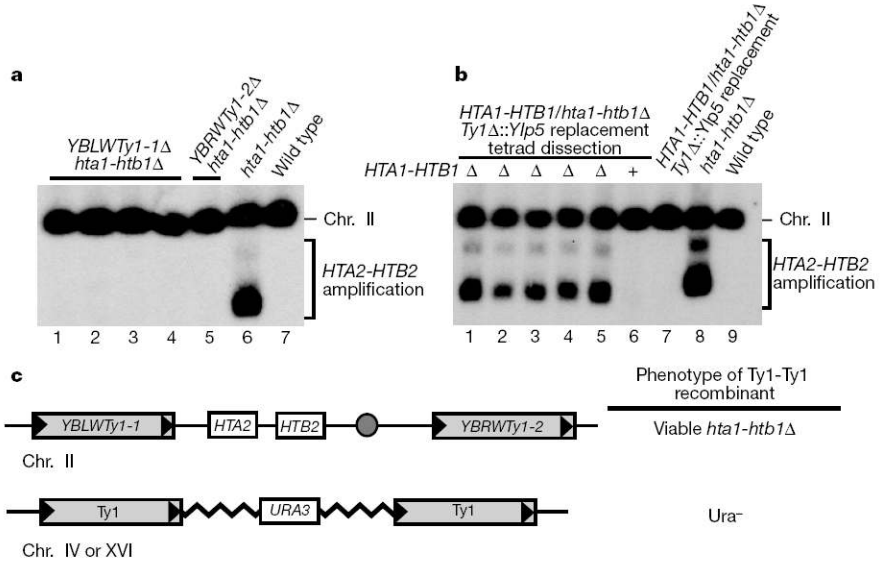


Figure 3. Tests for the presence of a 39 kb circular chromosome

a, PCR test for recombination between *YBLWTy1-1* and *YBLWTy1-2*. The left side shows an abbreviated view of the *HTA2-HTB2* region of chromosome II, before and after recombination between the two Ty1 elements. The arrows indicate the PCR primers designed to amplify a product only if the recombination event occurred. The right side shows an ethidium-bromide-stained gel of the PCR products. Lanes 1–4 are four independently derived *hta1-htb1Δ* strains containing the amplification. The presence of the 7,500 bp PCR product is consistent with the predicted recombination event. The less intense 930 bp band is probably due to crossover PCR between partly synthesized products. *CHA1* is a positive control for the PCR. **b**, Cleavage of the amplification by HO endonuclease. The left diagram indicates the location of the HO endonuclease site on chromosome II and the amplification. The right side shows an *HTA2-HTB2*-probed CHEF gel Southern analysis of wild-type and *hta1-htb1Δ* strains, with and without the HO site, before and after *HO* induction. The 252 kb band in lanes 12 and 16 is a fragment of chromosome II produced by HO cleavage. The 39 kb band in lane 16 indicates a linearized form of the amplification.



Ty1-Ty1 location (Chr.)	Relevant genotype of progeny	Spore viability	Recombination frequency to form amplification	Ura⁻ spores/total viable spores	Recombination frequency to form Ura⁻
YDRWTy1-5 (Chr. IV)	*HTA1-HTB1*	381/402		1/381	0.3%
	hta1-htb1Δ	58/402	14.4%	0/58	<1.7%
YPLWTy1-1 (Chr. XVI)	*HTA1-HTB1*	370/392		2/368	0.5%
	hta1-htb1Δ	72/392	18.4%	0/72	<1.4%

Figure 4. Characterization of the recombination event to amplify histone genes

a, Analysis of Ty1 deletion mutants. *HTA1-HTB1/hta1-htb1Δ* diploids that are homozygous for deletion of either *YBLWTy1-1* or *YBLWTy1-2* were sporulated and *hta1-htb1Δ* survivors were analysed for the amplification. Shown is a CHEF gel Southern analysis probed with *HTA2-HTB2. YBLWTy1-1Δ hta1-htb1Δ* strains (lanes 1–4) and the *YBLWTy1-2Δ hta1-htb1Δ* strain (lane 5) do not contain the *HTA2-HTB2* amplification. Controls are an *hta1-htb1Δ* mutant with normal Ty1 elements (lane 6) and a wild-type strain (lane 7). **b**, Replacement of the Ty1 elements. *HTA1-HTB1/hta1-htb1Δ* diploids in which the Ty1 elements *YBLWTy1-1* and *YBRWTy1-2* were each replaced with Ylp5, were sporulated and dissected. Surviving *hta1-htb1Δ* strains (lanes 1–5) and wild-type progeny (lane 6) were analysed as described for **a**. Controls include the parent diploid (lane 7), an *hta1-htb1Δ* haploid (lane 8), and a wild-type strain (lane 9). By PCR, the *HTA2-HTB2* amplification in the Ty1 replacement *hta1-htb1Δ* strains was confirmed to be a circular chromosome (data not shown). Note that owing to its slightly smaller size, the band for the Ylp5-containing amplification migrates faster than the Ty1-containing amplification (compare lanes 1–5 with lane 8). **c**, Frequency of Ty1-Ty1 recombination in wild-type and *hta1-htb1Δ* strains. The diagram depicts the Ty1 flanked region of chromosome II with the *HTA2-HTB2* locus and the Ty1-*URA3*-Ty1 configuration on chromosome IV or XVI. In the Ty1-*URA3*-Ty1 constructs, the Ty1 elements are 17 kb apart, compared with 33 kb for the Ty1 elements on chromosome II. The expected phenotypes of recombinants at both classes of loci are indicated. *HTA1-HTB1/hta1-htb1Δ* diploid strains were constructed that are homozygous for either Ty1-*URA3*-Ty1 configuration. The diploids were sporulated and analysed for the phenotypes of recombinants at either locus. The table presents the results of the Ty1-Ty1 recombination assay. The amplification frequency was found to be 14–18% in *hta1-htb1Δ*. In contrast, none of the viable *hta1-htb1Δ* progeny lost the *URA3* marker,

demonstrating that the *HTA2-HTB2* amplification event occurs at a significantly higher frequency than the Ty1-*URA3*-Ty1 recombination event. All three pairs of Ty1 elements are capable of normal levels of recombination during mitotic growth (Supplementary Table 3). In a separate experiment, the *HTA2-HTB2* amplification frequency in wild-type strains following meiosis was found to be close to 0% (see Supplementary Methods).