

Histone H2A.Z has a conserved function that is distinct from that of the major H2A sequence variants

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Received May 11, 2000; Revised and Accepted August 10, 2000

ABSTRACT

***Saccharomyces cerevisiae* contains three genes that encode members of the histone H2A gene family. The last of these to be discovered, *HTZ1* (also known as *HTA3*), encodes a member of the highly conserved H2A.Z class of histones. Little is known about how its *in vivo* function compares with that of the better studied genes (*HTA1* and *HTA2*) encoding the two major H2As. We show here that, while the *HTZ1* gene encoding H2A.Z is not essential in budding yeast, its disruption results in slow growth and formamide sensitivity. Using plasmid shuffle experiments, we show that the major H2A genes cannot provide the function of *HTZ1* and the *HTZ1* gene cannot provide the essential function of the genes encoding the major H2As. We also demonstrate for the first time that H2A.Z genes are functionally conserved by showing that the gene encoding the H2A.Z variant of the ciliated protozoan *Tetrahymena thermophila* is able to rescue the phenotypes associated with disruption of the yeast *HTZ1* gene. Thus, the functions of H2A.Z are distinct from those of the major H2As and are highly conserved.**

INTRODUCTION

In eukaryotes, many genes, including those encoding histones, occur as members of multigene families. Except for very recent gene duplications, in which inactivating mutations have not yet had time to accumulate, this implies that the multiple copies of a gene have some important function subject to positive evolutionary selection. There are three possible explanations for the existence of multigene families. In the first case, referred to as dosage repetition, genes are repeated because, in some physiological state, an organism needs more gene product than can be provided by a single gene. In the second case, referred to as regulatory repetition, multiple genes are required for regulatory reasons. For example, if a gene expressed in two different physiological or developmental conditions is duplicated, each copy may lose sequences required for expression in one or the other condition or gain new regulatory sequences independently of the other gene. Finally, in the third case, referred to as variant repetition, the coding region of one of a pair of duplicated genes may evolve

a new function which, like that of the original gene, also confers a selectable advantage.

Examples of each type of gene repetition appear to occur among histone genes. The large number of tandemly repeated clusters of histone genes found in many organisms (1,2) almost certainly reflects dosage repetition. A likely example of regulatory repetition has been described in sea urchins in which different H4 genes are expressed at different stages of development, but the switch does not involve any change in the sequence of the H4 proteins (3).

The most interesting type of histone gene repetition is variant repetition, because it implies that there are functional differences between nucleosomes containing different histone isotypes. However, variant repetition is the most difficult to demonstrate. It requires demonstration that the non-identical coding sequences of two members of a histone gene family placed under the control of the same regulatory sequences have biologically significant differences in function (i.e. different phenotypes). This difficulty is best illustrated by a recent study of the H3 gene family in *Tetrahymena* (4). *Tetrahymena* contains two H3 proteins differing at 16 residues, a remarkable difference for copies of such a highly conserved protein in the same organism. Nonetheless, disrupting the gene encoding the divergent, constitutively expressed H3, which is the only H3 expressed in non-growing cells, was without detectable phenotype and resulted in the expression of the other, normally replication-dependent H3 gene. Thus, in spite of the unusually large number of amino acid differences between these two H3s, it appears that it is their expression pattern, not their primary sequence that distinguishes them.

We previously reported the presence of a gene in *Saccharomyces cerevisiae* encoding a third member of the HTA gene family encoding the H2A class of histones (5). This gene (referred to as *HTA3* or *HTZ1*) encodes a member of the H2A.Z (also referred to as H2A.F or H2A.F/Z) class of H2A variants, a highly conserved type of histone found in diverse eukaryotes, including vertebrates, invertebrates, protozoa and fungi. Phylogenetic analyses indicate that the H2AZs diverged from the major H2As early in eukaryotic evolution and are at least as evolutionarily conserved as the major H2As (6). The gene encoding H2A.Z has been shown either to be essential (ciliates and flies) (7,8) or to be required for normal growth (fission yeast) (9). These properties suggest that H2A.Z has an important function that is distinct from that of the major H2As. However, only a single study has actually demonstrated that the H2A.Z protein itself appears to have a function that is distinct from that of the major H2A (10). A small deletion in the *Drosophila* H2AvD gene encoding H2A.Z is lethal and can

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be rescued by transformation with the H2AvD gene. Null mutants in H2AvD fail to develop through pupation. By replacing regions unique to H2AvD with residues in the homologous position from the major H2A, essential elements unique to H2AvD were mapped to histone fold regions involved in docking the H2A/H2B dimer to the H3/H4 tetramer (11). However, because the assay was a developmental defect, these studies leave unanswered the question of whether the two types of H2A protein are each sufficient for cell survival. In addition, since reciprocal replacements were not done, it is not clear if the H2A.Z variant could perform the function of the major H2A. Such a demonstration is difficult in *Drosophila* inasmuch as it would require placing multiple copies of the H2A.Z coding region under the regulatory sequences of the highly repeated, major H2A genes.

We describe here an experimental analysis of the function of H2A.Z in *S.cerevisiae* showing that the *HTZ1* gene is not essential but its disruption results in slow growth and conditional lethality in formamide-containing medium. These functions can be rescued with plasmids containing the gene encoding H2A.Z from either yeast or from the ciliated protozoan *Tetrahymena thermophila*. We also show that the major and minor histone H2A sequence variants are functionally distinct, i.e. the minor H2A.Z variant cannot supply major H2A function and vice versa. Coupled with earlier studies showing that the genes encoding the *Tetrahymena* major H2As can provide the essential function of the yeast major H2A genes (12), these results demonstrate that the functions unique to each type of H2A are highly conserved.

MATERIALS AND METHODS

Yeast strains

The diploid yeast strain used to generate the $\Delta HTZ1$ mutant (JJY1) was EJ72 (*gat⁻ trp1 leu2 ura3-52 his4*) (M.-H.Kuo, PhD thesis, University of Rochester, 1995). The strains used for the plasmid shuffle experiments were XLY1 (MAT α *his3 Δ 200 lys2-801^a ade2-101^o ura3-52 GAL suc2 hta1 hta2 <pJC102>*) and XLY2 (MAT α *his3 Δ 200 tyr1 ade2-101^o ura3-52 GAL suc2 hta1 hta2 <pJC102>*) (12). The *yHTZ1* gene was also knocked out in XLY2 using pJJ2.1r and the resulting strain designated JJY100 (MAT α *his3 Δ 200 tyr1 ade2-101^o ura3-52 GAL suc2 hta1 hta2 hta3 <pJJ90>*).

Plasmid construction

To clone the *yHTZ1* gene, a 2.3 kb *yHTZ1* fragment was amplified with primers yH2A.Z-1 (CGGAATTCTTTCTCCCCTTCTCTTAC) and yH2A.Z-2 (CCCGGAATTCCTTGCTTTAGTCCTCTT), digested with *Nsi*I and *Spe*I and the resulting 1.2 kb fragment was ligated to the *Pst*I and *Spe*I sites of pBSISK⁻ to make pJJ1. A longer 1.6 kb *yHTZ1* fragment was amplified from cosmid pEOA215 (13), which contains a 29 kb fragment from chromosome XV that includes *yHTZ1*, using oligonucleotides yH2A.Z-1 and yH2A.Z-20 (GGTATGCTCGAGTTGCAACGCACAAAGCTT). This PCR product was blunt end ligated to the *Sma*I sites of both pBSISK⁺ and pRS313 (14), a HIS3 CEN6 ARSH4 plasmid, to make pJJ42 and pJJ43, respectively. To make the *yHTZ1* gene disruption construct (pJJ2.1r), pJJ1 was digested at *Bam*HI and *Bgl*III sites located within the *yHTZ1* gene and ligated to a 3.8 kb *Bam*HI

fragment from pDG82 (12) containing the *yURA3* gene flanked by 1.1 kb direct repeats of the bacterial *hisG* sequence (15; Fig. 1). To express the 1.2 kb *yHTZ1* fragment in XLY1 and XLY2 yeast strains, the insert from pJJ1 was excised using *Eco*RI and *Spe*I and ligated to the *Eco*RI and *Spe*I sites of pRS313, to make pJJ3. To express the 1.2 kb *yHTZ1* fragment in yeast strain EJ72 and its $\Delta HTZ1$ derivative (JJY1), the insert was excised from pJJ1 at *Kpn*I and *Xba*I sites and ligated to the *Kpn*I and *Xba*I sites of YCplac33 (16), a URA3, CEN4, ARS1 plasmid, to make pJJ11. To express the 1.6 kb *yHTZ1* fragment in JJY1, the insert from pJJ42 was excised at *Kpn*I and *Sac*I sites and ligated to the *Kpn*I and *Sac*I sites of YCplac33, to make pJJ44. To place the *yHTZ1* gene under *yHTA1* regulation, pJJ90, a derivative of pXL90 (12) lacking the *Clal* site within the polylinker, was initially isolated from a *dam⁻ Escherichia coli* strain, digested with *Clal* to eliminate the *yHTA1* coding region and religated to make pJJ91. The *yHTZ1* coding region was then amplified from pJJ1 with primers yH2A.Z-6 (GGGA-GAATCGATGGAAATGGGAAAGAAAA), flanking the 3' coding region, and yH2A.Z-7 (GCCGCACGTATCGATAAC-TTAACATAATG, initiation codon in bold), flanking the 5' coding region. This PCR product was digested with *Clal* (site underlined above) and ligated to the *Clal* site of pJJ91, to make pJJ4. To express this *yHTA1-yHTZ1* gene fusion in JJY1, the 2.2 kb insert from pJJ4 was excised at *Kpn*I and *Spe*I sites and ligated to the *Kpn*I and *Spe*I sites of YCplac33, to make pJJ10. To place the *yHTZ1* gene under *yGAL1* regulation, the *yHTZ1* coding and 3' flanking sequences were amplified with primers yH2A.Z-5 (CGGGGTACCCTCAGGAAAAGCT-CATGGAGG), flanking the 5' coding region, and yH2A.Z-1, flanking the 3' coding region. This PCR product was digested with *Kpn*I and *Eco*RI and ligated to the *Kpn*I and *Eco*RI sites of pYES2, a yeast *URA3* 2 μ plasmid containing the *yGAL1* promoter, to make pJJ15. To express this *GAL1-HTZ1* fusion in yeast XLY1 and XLY2 strains, the insert from pJJ15 was excised with *Afl*III and *Nae*I, filled in with Klenow and ligated to the *Sma*I site of pRS313, to make pJJ6. To place the *yHTA1* gene under *yHTZ1* regulation, 775 bp of the *yHTZ1* promoter was initially amplified using primers yH2A.Z-18 (TGTTAA-GTTATCGATACGTGCGGCTAT) and yH2A.Z-20 (GGTATGCTCGAGTTGCAACGCACAAAGCTT). This PCR product was digested with *Clal* and *Xho*I and cloned into the *Clal* and *Xho*I sites of pRS313, to make pJJ39. A 780 bp fragment containing the *yHTA1* coding region and 3' flank was excised from pJJ90 at *Clal* and *Sac*II sites and then ligated to the *Clal* and *Sac*II sites of pJJ39, to make pJJ40. To express this *yHTZ1-yHTA1* fusion in JJY1, the insert from pJJ40 was excised at *Kpn*I and *Sac*II sites and ligated to YCplac33 at the *Kpn*I and *Sac*II sites to make pJJ41. To express the *yHTZ1-yHTA1* gene fusion from a high copy plasmid, the insert from pJJ40 was excised at *Xho*I and *Sac*II sites and ligated to pRS423, a HIS3 2 μ plasmid, at the *Xho*I and *Sac*II sites, to make pJJ47. To express the three genes encoding the two *Tetrahymena* major H2As and H2A.Z in XLY1 and XLY2 yeast strains, *tHTA1*, *tHTA2* and *tHTZ1* were placed under *yHTA1* regulation to make pXL87, pXL88 and pXL89, respectively. To express these gene fusions in yeast strain JJY1, the inserts from pXL87, pXL88 and pXL89 were excised with *Kpn*I and *Xba*I and ligated to the *Kpn*I and *Xba*I sites of YCplac33 to make pJJ87, pJJ88 and pJJ89, respectively. To express the *yHTA1* gene under its own regulation in JJY1, a 1.2 kb fragment

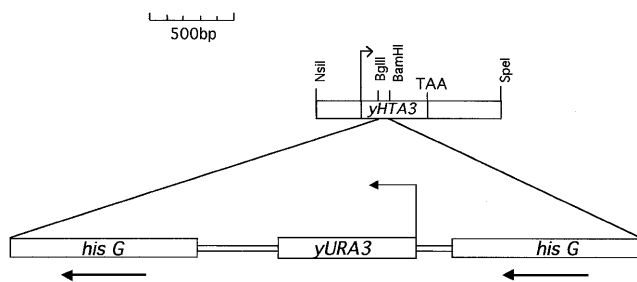


Figure 1. *yHTZ1* gene disruption construct. *yHTZ1* was disrupted by inserting a gene blaster cassette into the body of the *yHTZ1* gene at *Bam*HI and *Bgl*II sites.

containing the *yHTA1* gene was initially excised from pJJ90 at *Eco*RI and *Sac*II sites and ligated to the *Eco*RI and *Sac*II sites of pBSIISK, to make pJJ21. The insert from pJJ21 was then excised at *Bss*HIII sites, filled in with Klenow and ligated to the *Sma*I site of YCplac33, to make pJJ46.

Other procedures

All DNA cloning, sequencing, PCR amplification and site-directed mutagenesis were done essentially as described previously (17). Yeast transformations were done by the lithium acetate method as described (18,19).

RESULTS

To elucidate the function of histone H2A.Z, we disrupted the *HTZ1* gene of *S.cerevisiae* and assayed for its effect. A 'gene blaster' cassette (Fig. 1) containing the *URA3* gene (15) was used to disrupt one of the chromosomal *HTZ1* genes in diploid yeast strain EJ72 (kindly provided by Dr Min-Hao Kuo), which is auxotrophic for uracil. Four heterozygous *HTZ1* knockout transformants were obtained and plated onto medium containing potassium acetate to induce the formation of tetrads containing haploid spores (20). Sixty-three tetrads were dissected and plated onto YPD medium. All spores from each tetrad grew on YPD at 30°C with a 2:2 segregation pattern of small (Δ *HTZ1*) versus large (wild-type) colonies, indicating that while the *HTZ1* gene is not essential, its disruption confers a slow growth phenotype. This slow growth effect was more pronounced at 20°C, at which temperature the Δ *HTZ1* mutant grew 4- to 5-fold slower than its isogenic wild-type strain (Table 1). At 37°C the Δ *HTZ1* and wild-type strains grew similarly. Slow growth was rescued at both 20 and 30°C by a plasmid (pJJ44) containing *yHTZ1* and 700 bp of upstream sequences, demonstrating that this phenotype was due to the absence of H2A.Z (Table 1). A plasmid (pJJ3) containing *yHTZ1* and 300 bp of 5' sequence failed to rescue, suggesting the presence of an upstream activating sequence (UAS) 300–700 bp upstream of the coding region (Table 2, constructs 2 and 3).

To determine if the 2:2 pattern of slow growth reflected segregation of the disrupted *HTZ1* gene, the gene was amplified from the genomic DNA of eight spores (four from each of two dissected tetrads). PCR products from amplifications using primers that flanked the site of the disruption were sized by agarose gel electrophoresis. The spores that exhibited slow growth also contained the disrupted *yHTZ1* gene (Fig. 2). A haploid strain from one of these Δ *HTZ1* spores (JJY1) was used in subsequent experiments.

Table 1. Doubling times of the wild-type and Δ *HTZ1* strains in liquid culture at various temperatures

| Strain | Plasmid | Doubling time (min) | | |
|------------------|-----------|---------------------|--------------|------|
| | | 20°C | 30°C | 37°C |
| Δ H2A.F/Z | None | 1666 \pm 10 | 226 \pm 11 | 278 |
| Wild-type | None | 459 \pm 13 | 143 \pm 16 | 280 |
| Δ H2A.F/Z | YCP-yHTA3 | 447 \pm 9 | 144 \pm 11 | 275 |

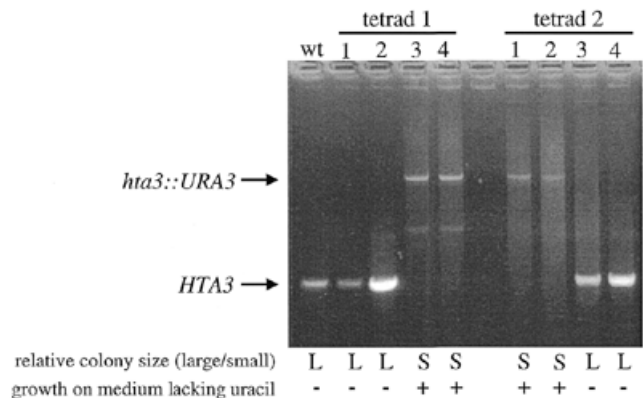
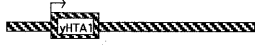
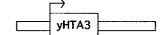
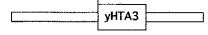
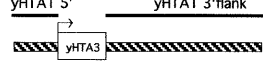
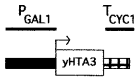

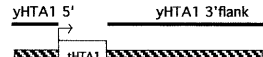
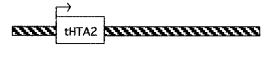
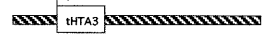


Figure 2. The 2:2 segregation pattern of the *yHTZ1* disruption correlates with the 2:2 segregation pattern of slow growth in haploid spores. L, large colony with wild-type growth; S, small colony with slow growth.

To assay for other phenotypes associated with the knockout, the Δ *HTZ1* mutant (JJY1) was tested for its ability to grow on medium supplemented with a number of different chemicals shown to affect the growth of yeast mutants (17). Both mutant and wild-type strains were tested for growth at 15, 30 and 37°C on medium in which glucose was replaced with either galactose, maltose, glycerol, ethanol, potassium acetate or sucrose. Little or no difference was observed between mutant and wild-type strains. Mutant and wild-type strains were also tested for growth on medium supplemented with chloramphenicol, caffeine, sorbitol, cycloheximide or formamide, as well as acidic medium. Only formamide (18) selectively inhibited growth of the Δ *HTZ1* mutant compared to its isogenic wild-type strain. To characterize this formamide sensitivity further, mutant and wild-type strains were tested on YPD plates containing from 1.5 to 4% formamide. The growth difference between the Δ *HTZ1* mutant and wild-type strains increased with increasing amounts of formamide. At a concentration of 4% the Δ *HTZ1* mutant exhibited little or no growth. This formamide sensitivity was rescued by an *HTZ1*-containing plasmid, indicating that the phenotype was indeed due to the absence of histone H2A.Z (Table 2). Two independently derived *HTZ1* knockout strains (derivatives of W303 and S288C, kindly provided by Drs M. Mitchell Smith and Maria S. Santisteban, University of Virginia) were also formamide sensitive. Thus, this phenotype is likely to be a consistent feature of the absence of H2A.Z in *S.cerevisiae*.

To address whether the yeast H2A.Z variant could serve as the sole H2A variant in *S.cerevisiae*, we attempted to replace the *yHTA1* gene (encoding yH2A.1) with *yHTZ1* in a plasmid

Table 2. Summary of the plasmid shuffle and mutant phenotype rescue experiments

| Test Constructs | H2A Rescue | | | H2A.Z Rescue | | | | |
|--|----------------------------------|----------------|------------------|------------------|-------------|-----------------------|----------|--------|
| | Growth | Plasmid | Strain | | Slow Growth | Formamide sensitivity | Plasmid | Strain |
| | | | XLY1 | XLY2 | | | | |
| 1) yHTA1  | YES | pJJ90 | JJY101 | JJY102 | NO | NO | pJJ46 | JJY10 |
| 2) yHTA3  | NO | pJJ11 | JJY103 | JJY104 | NO | NO | pJJ3 | JJY11 |
| 3) yHTA3  | NO | pJJ43 | JJY105 | JJY106 | YES | YES | pJJ44 | JJY12 |
| 4) yHTA1::yHTA3  | NO | pJJ4 | JJY107 | JJY108 | YES | YES | pJJ10 | JJY13 |
| 5) yGAL1::yHTA3  | NO | pJJ6 | JJY109 | JJY110 | YES | Not Tested | pJJ6 | JJY109 |
| 6) vector (YCP) | NO | pRS313 | JJY111 | JJY112 | NO | NO | YCplac33 | JJY14 |
| 7) yHTA3::yHTA1  | (Low Copy) NO (High Copy) YES | pJJ40 pJJ47 | JJY113 JJY115 | JJY114 JJY116 | NO | NO | pJJ41 | JJY15 |
| 8) yHTA1::tHTA1  | YES | pXL87 | XLY4 | XLY14 | NO | NO | pJJ87 | JJY87 |
| 9) yHTA1::tHTA2  | YES | pXL88 | XLY5 | XLY15 | NO | NO | pJJ88 | JJY88 |
| 10) yHTA1::tHTA3  | NO | pXL89 | XLY6 | XLY16 | YES | YES | pJJ89 | JJY89 |

Each construct was tested for its ability to replace *yHTA1* and to rescue the mutant phenotypes of the $\Delta HTZ1$ mutant.

shuffle experiment. The major H2A is essential in yeast and cells can survive with either one of the genes (*HTA1* or *HTA2*) encoding a major H2A (19). The yeast strains used in this experiment, XLY1 and XLY2 (12), contain disrupted chromosomal *HTA1* and *HTA2* genes, with *HTA1* function supplied on the URA3, ARS1, CEN3, pJC102 plasmid (21). We initially tested the *yHTZ1* gene regulated by its own promoter on a *HIS3* test plasmid (pJJ43) using a plasmid (pJJ90) containing the wild-type *yHTA1* gene as a positive control and the plasmid vector (pRS313) as a negative control. The XLY1 and XLY2 strains transformed with either the minimal or the extended promoter *yHTZ1* construct (pJJ11 or pJJ43) were plated without histidine and uracil to yield transformants with two plasmids (the test plasmid and the *yHTA1* plasmid). To obtain cells without the *yHTA1* plasmid, colonies were streaked onto medium containing the drug 5-FOA, which kills cells expressing *URA3*. We obtained colonies on the positive control plate but not on either of the experimental plates, suggesting that *yHTZ1*, regulated by its own flanking sequences, is unable to replace *yHTA1* (Table 2, constructs 2 and 3).

Next, we addressed whether differences in the way the HTA genes are regulated might be responsible for the failure of *yHTZ1* to replace *yHTA1*. Because H2A.Z is expressed at a much lower level than the major H2As in yeast (M.M.Smith and M.S.Santisteban, personal communication), we tested whether the highly inducible *yGAL1* promoter could elevate *yHTZ1* expression to a level sufficient to replace *yHTA1*. The

yHTZ1 coding region fused to the *yGAL1* promoter was unable to replace *yHTA1* in XLY1 and XLY2 strains grown in galactose (Table 2, construct 5), suggesting that the failure of *yHTZ1* to replace *yHTA1* was not due to differences in expression levels between the two genes.

In *S.cerevisiae*, as in other eukaryotes, the difference in the level of expression of the H2A.Z variant between S phase and other phases of the cell cycle is less than that of the major H2As, suggesting that expression of this conserved variant is only partially replication dependent (9,22–24). To rule out the possibility that differences in the pattern of gene expression during the cell cycle might be responsible for the inability of *yHTZ1* to replace *yHTA1*, we tested whether the H2A.Z variant could supply the major H2A function when regulated under control of the *yHTA1* flanking sequences. A plasmid, pJJ4, was constructed in which the 5' and 3' flanking sequences of *yHTZ1* were replaced with those of *yHTA1*. Again, *yHTZ1* failed to replace *yHTA1* (Table 2, construct 4). The *yHTA1* coding region flanked by these same sequences rescued the major HTA deficiency (Table 2, construct 1). These results suggest that structural differences between H2A.1 and H2A.Z rather than differences in their expression are responsible for the inability of *yHTZ1* to supply *yHTA1* function.

To ensure that the chimeric constructs containing *yHTZ1* were functional, each was tested for its ability to rescue phenotypes associated with the *yHTZ1* knockout. The gene fusion

containing the *yHTA1* flanking sequences and the *yHTZ1* coding sequences was tested in the *yHTZ1* knockout strain described above and found to rescue both the slow growth and formamide-sensitive phenotypes (Table 2, construct 4). Testing the *GAL1-yHTZ1* fusion required knocking out the *yHTZ1* gene in another yeast strain, as JJY1 does not grow on galactose. The *yHTZ1* gene was knocked out in XLY2 to make JJY100, lacking all three chromosomal HTA genes, with *yHTA1* supplied on a plasmid (pJJ90). The *GAL1-yHTZ1* fusion rescued the slow growth of colonies on plates, demonstrating that this construct also expresses a functional *yHTZ1* (Table 2, construct 5).

Although *yHTZ1* was unable to supply *yHTA1* function, the possibility remained that *yHTA1* could supply *yHTZ1* function. To address this, *yHTA1*, under the control of its own flanking sequences or those of *yHTZ1*, was tested for its ability to rescue the slow growth and formamide-sensitive phenotypes in JJY1 (Table 2, constructs 1 and 7). Neither construct was able to rescue the mutant phenotypes associated with the *yHTZ1* disruption, supporting the conclusion that these genes have distinct functions. To confirm that the gene fusion was expressing functional *yHTA1* using the *yHTZ1* flanking sequences, this construct was tested for its ability to replace *yHTA1* in XLY1 and XLY2. Although it failed to replace *yHTA1* when expressed from a low copy yeast centromeric plasmid (YCP) vector, it successfully replaced *yHTA1* when expressed from a high copy episomal plasmid (YEP) vector (Table 2, construct 7). In addition to demonstrating that *yHTA1* can be expressed by the *yHTZ1* flanking sequences, this result is also consistent with a lower level of expression of the variant gene than the major gene.

We had previously shown that the genes encoding the *Tetrahymena* major H2As, (*tHTA1* and *tHTA2*) but not the gene encoding *Tetrahymena* H2A.Z (*tHTZ1*), could replace *yHTA1* when tested in a plasmid shuffle experiment in the XLY1 and XLY2 strains (12). However, at the time these experiments were done it was not known that yeast had a *HTZ1* gene. Therefore, we tested whether the *Tetrahymena* HTA genes could rescue the phenotypes associated with the Δ *HTZ1* mutant. The coding regions of the three *Tetrahymena* H2A genes, flanked by *yHTA1* sequences, were tested in JJY1 for their ability to rescue slow growth and formamide sensitivity. *Tetrahymena HTZ1* rescued both phenotypes while *Tetrahymena HTA1* and *HTA2* failed to rescue either phenotype (Table 2, constructs 8–10). These results indicate that H2A.Z performs similar functions in *T.thermophila* and *S.cerevisiae* and that these functions are distinct from those of the major H2A.

DISCUSSION

Formal demonstration that two protein encoding sequences in the same multigene family have distinct functions requires rigorous elimination of the possibility that any functional differences between the two genes are due to qualitative or quantitative differences in gene expression. Such criteria are rarely met. In the studies described here we have demonstrated that the sequences encoding the major H2A of yeast or the H2A.Z variant cannot replace one another's function. This is the case even when they are overexpressed or expressed under the control of the regulatory sequences of the other gene.

Therefore, the conclusion seems inescapable that these two types of H2As have reciprocally distinct functions in yeast. We have also shown that these distinct functions are remarkably conserved. Thus, a gene encoding a major H2A of a ciliated protozoan can rescue the function of the major H2A in yeast, but cannot rescue the function of H2A.Z. Conversely, the gene encoding the *Tetrahymena* H2A.Z can rescue the function of H2A.Z in yeast, but not the function of the major H2A. These results confirm and extend phylogenetic analyses (6) that indicated that these two distinct and conservatively evolving H2As had diverged very early in eukaryotic evolution.

It is common to think of the function of the major H2As in terms of nucleosome structure and to seek specialized functions for conserved histone H2A.Z variants. However, the studies described here argue that (at least) two distinct, highly conserved functions must be sought for the H2A multigene family, in addition to the obvious structural function of participating in a nucleosome. The overall conservation of the structure of the major and the variant H2As argues that both are likely to participate in most of the protein–protein interactions required to form the protein core of the nucleosome (11,25). As shown here, and in *Drosophila* (10), the major H2A cannot perform all of the functions of the H2A.Z variant. In addition, we have shown here, for the first time, that the H2A.Z variant cannot perform all of the functions of the major H2A.

Despite having been studied in a number of different organisms, the function of histone H2A.Z is unknown. Studies in *Tetrahymena* strongly suggest a role for H2A.Z in transcription. In *Tetrahymena*, H2A.Z (formerly called hv1) is localized only in the transcriptionally active macronucleus and not in the transcriptionally inert micronucleus of vegetative cells. However, it does appear in micronuclei during conjugation when the micronucleus becomes transcriptionally active (26). In *S.cerevisiae*, expression of the *HTZ1* gene was disrupted as part of a large-scale gene disruption screen involving 2000 individual genes and found to result in reduced growth in a variety of media (27). Studies have suggested a role for H2A.Z in chromosome segregation during mitosis in fission yeast (9) and during development in *Drosophila* (10). All of these diverse effects could be secondary manifestations of an effect on transcription.

In summary, we have shown that despite their sequence similarities, the major and minor H2A genes of *S.cerevisiae* each have at least one important, conserved function that cannot be supplied by the other, even when their regulatory sequences are swapped. Thus, the differences between these two genes must lie within their coding regions and it should now be possible to apply the powerful techniques of yeast genetics to determine these functions.

ACKNOWLEDGEMENTS

We thank Min-Hao Kuo for yeast strains and advice on yeast genetics and Lynn Spitz for help with the tetrad dissections. This research was supported by Public Health Service grant GM-21793 from the National Institutes of Health.

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