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

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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 formaldehyde 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 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
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 left 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 are highly repeated, major H2A genes.

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 are highly repeated, major H2A genes.
containing the yHTA1 gene was initially excised from pJJ90 at EcoRI and SalI sites and ligated to the EcoRI and SalI sites of pBSIIK, to make pJJ21. The insert from pJJ21 was then excised at BssHII sites, filled in with Klenow and ligated to the SalI site of YCplac33, to make pJJ46.

Other procedures
All DNA cloning, sequencing, PCR amplification and site-directed mutagenesis were done 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 4- to 5-fold slower than its isogenic wild-type strain (JJY1) when 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 build on these results, we attempted to replace the HTZ1 gene with YCP-yHTA3, indicating that the phenotype was indeed due to the absence of 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 1. Doubling times of the wild-type and ΔHTZ1 strains in liquid culture at various temperatures

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>Doubling time (min)</th>
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</thead>
<tbody>
<tr>
<td>ΔH2A.F/Z</td>
<td>None</td>
<td>1666 ± 10</td>
</tr>
<tr>
<td>Wild-type</td>
<td>None</td>
<td>459 ± 13</td>
</tr>
<tr>
<td>ΔH2A.F/Z</td>
<td>YCP-yHTA3</td>
<td>447 ± 9</td>
</tr>
</tbody>
</table>

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.

Table 2. Strain Doubling time (min) at various temperatures

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Figure 1. yHTZ1 gene disruption construct. yHTZ1 was disrupted by inserting a gene blaster cassette into the body of the yHTZ1 gene at BamHI and BglII sites.
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, pH4, 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

<table>
<thead>
<tr>
<th>Test Constructs</th>
<th>H2A Rescue</th>
<th>H2A.Z Rescue</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Growth</td>
<td>Plasmid</td>
</tr>
<tr>
<td>1) yHTA1</td>
<td>YES</td>
<td>pJJ90</td>
</tr>
<tr>
<td>2) yHTA3</td>
<td>NO</td>
<td>pJJ11</td>
</tr>
<tr>
<td>3) yHTA3</td>
<td>NO</td>
<td>pJJ43</td>
</tr>
<tr>
<td>4) yHTA1::yHTA3</td>
<td>NO</td>
<td>pJJ4</td>
</tr>
<tr>
<td>5) yGAL1::yHTA3</td>
<td>NO</td>
<td>pJJ4</td>
</tr>
<tr>
<td>6) vector (YCP)</td>
<td>NO</td>
<td>pRS313</td>
</tr>
<tr>
<td>7) yHTA3::yHTA1</td>
<td>NO</td>
<td>pJJ4</td>
</tr>
<tr>
<td>8) yHTA1::yHTA1</td>
<td>YES</td>
<td>pRL87</td>
</tr>
<tr>
<td>9) yHTA1::yHTA2</td>
<td>NO</td>
<td>pRL88</td>
</tr>
<tr>
<td>10) yHTA1::yHTA3</td>
<td>NO</td>
<td>pRL89</td>
</tr>
</tbody>
</table>

Each construct was tested for its ability to replace yHTA1 and to rescue the mutant phenotypes of the ∆HTZ1 mutant.

Table 2. Summary of the plasmid shuffle and mutant phenotype rescue experiments
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 JJJ1 does not grow on galactose. The yHTZ1 gene was knocked out in XLY2 to make JJJ100, 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 JJJ1 (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, (HTA1 and HTA2) but not the gene encoding Tetrahymena H2A.Z (HTZ1), 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 ΔHTZ1 mutant. The coding regions of the three Tetrahymena H2A genes, flanked by yHTA1 sequences, were tested in JJJ1 for their ability to rescue slow growth and formamide sensitivity. Tetrahymena HTZ1 rescued both phenotypes while Tetra-
hydromena HTA1 and HTA2 failed to rescue either phenotype (Table 2, constructs 8–10). These results indicate that H2A.Z performs similar functions in Thermophila and S.cerevisiae and that these functions are distinct from those of the major H2A.

Therefore, the conclusion seems inescapable that these two types of H2A.Z 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 Tetra-
hydromena 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 micro-
nucleus 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

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REFERENCES