The Biochemical and Phenotypic Characterization of Hho1p, the Putative Linker Histone H1 of Saccharomyces cerevisiae*

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There is currently no published report on the isolation and definitive identification of histone H1 in Saccharomyces cerevisiae. It was, however, recently shown that the yeast HHO1 gene codes for a predicted protein homologous to H1 of higher eukaryotes (Landely et al., 1996) Trends Biochem. Sci. 21, 287–288; Ushinsky et al., 1996 Trends Biochem. Sci. 21, 287–288; Ushinsky, S. C., Bussey, H., Ahmed, A. A., Wang, Y., Friesen, J., Williams, B. A., and Storms, R. K. (1997) Yeast 13, 151–161), although there is no biochemical evidence that shows that Hho1p is, indeed, yeast histone H1. We showed that purified recombinant Hho1p (rHho1p) has electrophoretic and chromatographic properties similar to linker histones. The protein forms a stable ternary complex with a reconstituted core di-nucleosome in vitro at molar rHho1p:core ratios up to 1. Reconstitution of rHho1p with H1-stripped chromatin confers a kinetic pause at ~168 base pairs in the micromcoccal nuclease digestion pattern of the chromatin. These results strongly suggest that Hho1p is a bona fide linker histone. We deleted the HHO1 gene and showed that the strain is viable and has no growth or mating defects. Hho1p is not required for telomeric silencing, basal transcriptional repression, or efficient sporulation. Unlike core histone mutations, a hho1Δ strain does not exhibit a Sin or Spt phenotype. The absence of Hho1p does not lead to a change in the nucleosome repeat length of bulk chromatin nor to differences in the in vivo micromcoccal nuclease cleavage sites in individual genes as detected by primer extension mapping.

The basic structural unit of eukaryotic chromatin is the nucleosome core, composed of an octameric complex of two copies of each of the core histones H2A, H2B, H3, and H4 onto which 146 bp1 of DNA is spooled as 1.75 turns of a left-handed superhelix (1–4), and is continued to adjacent nucleosome cores through a variable length of linker DNA. In a nucleosome the histone H1 is associated with the outside of the core and protects 168 bp or two full superhelical turns of DNA from MNase cleavage (5, 6). Histone H1 was first identified in 1951 as a lysine rich “subsidiary” histone in calf thymus (7) and was subsequently shown to be an extended family of histone iso-variants or variants present in a wide variety of eukaryotes (8). Although the structural role of the nucleosome core and the spatial placement of the core histones within the octamer are well established (1, 3, 4, 9), the function and the precise location of the linker histone has been more elusive (reviewed in Ref. 10). Electron microscopic and hydrodynamic studies have shown that histone H1 is required for the full salt-dependent condensation of chromatin in vitro (11–13). These observations have led to the proposal that the function of the linker histone is the partial neutralization of the negatively charged DNA backbone, allowing the close approach of the internucleosomal linker DNA that would normally columically repel in the fully compacted 30 nm fiber (for a review, see Ref. 14 and references cited therein). This role of H1 is supported by the observed reduction in the size of mitotic chromatids and nuclear volume concurrent with the appearance of histone H1 during the mid-blastula transition of the developing Drosophila embryo (15). It is also consistent with the approximately 2-fold expansion of the Tetrahymena micro- or macronucleus in the absence of the four micronuclear-specific micLH polypeptides or macronuclear-specific H1 protein, respectively (16). This implied role of H1 in chromatin condensation is dynamic and is modulated by the cell cycle-dependent reversible phosphorylation of H1 by p34cdk2 (reviewed in Refs. 17 and 18). This modification presumably allows the interaction of additional factors such as the structural maintenance of chromosomes (SMC) class of proteins with chromatin, affecting full condensation into the mitotic chromosome (see Ref. 19 for a review).

There has been no definitive identification of a linker histone in Saccharomyces cerevisiae, although some studies have reported indirect evidence for the existence of an H1-like protein in yeast (20–23). However, the absence of a direct biochemical identification of the histone led some investigators to suggest that it is absent in yeast (16). This proposed lack is unusual, since eukaryotes that phylogenetically diverge both before (such as Tetrahymena) and after (such as Psammechinus) yeast have been shown to contain linker histones.

The sequencing of chromosome XVI in yeast (24) revealed the presence of a predicted open reading frame (YPL127C) with regions of significant sequence homology to the H1 histones of higher eukaryotes (25, 26). Theoretical model building* sug-

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1 The abbreviations used are: bp, base pair(s); MNase, micrococcal nuclease; PAGE, polyacrylamide gel electrophoresis; 5-FOA, 5-fluoroorotic acid; CSM, complete synthetic medium.

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suggested that these regions of homology may assume a structural conformation similar to that of the known single-winged helix structure of the chicken H5 (27) and H1.11L (28) globular domains. Ushinsky et al. (26) have shown that the predicted open reading frame is functional and that a fusion of the gene product with fluorescent green protein is located in the nucleus. These results contributed to the assignment of YPL127C as the yeast H1 linker histone. In particular, we investigated sequence similarities to H1 (25).

The recombinant protein was eluted from the column with 300 mM imidazole, 200 mM NaCl, and 20 mM Tris-\(\text{HCl} \quad (\text{pH} 7.9)\) (binding buffer). The column was washed with 25 ml of binding buffer, followed by 15 ml of 40 mM imidazole, 200 mM NaCl, and 20 mM Tris-\(\text{HCl} \quad (\text{pH} 7.9)\). The recombinant protein was eluted from the column with 300 mM imidazole, 200 mM NaCl, and 20 mM Tris-\(\text{HCl} \quad (\text{pH} 7.9)\), and 500-ml fractions were collected. Fractions enriched in recombinant Hho1p were identified by SDS-PAGE electrophoresis, pooled, and loaded onto a CM-Sephadex column (1 ml bed volume) equilibrated in phosphate buffer (10 mM sodium phosphate (pH 7.0), 0.25 mM EDTA, and 0.25 mM phenylmethylsulfonyl fluoride) containing pure recombinant Hho1p were identified by SDS-PAGE, pooled, and concentrated, and the buffer was replaced with 10 mM sodium phosphate (pH 7.0), 0.25 mM EDTA, and 0.25 mM phenylmethylsulfonyl fluoride with a P10 centric enzyme (Amicon). The samples were stored frozen at \(-70^\circ\text{C}\). The rHho1p concentration was determined by the absorbance at 230 nm using an extinction coefficient of 18.5 (34).

**Phenotypic Characterizations**—The 5-fluoroorotic acid (5-FOA) sensitivity of a strain was determined by diluting 1 ml of an overnight culture to an \(A_{600}\) of 1.0, and 10 ml of this culture, serially diluted 10-fold, was applied to a complete synthetic medium (CSM, 6.7 g/liter yeast nitrogen base without amino acids, 20 g/liter glucose, 0.7 g/liter amino acid supplement (Bio 101), and 20 g/liter bacto agar) plate lacking the appropriate amino acid in the absence or presence of 0.1% (w/v) 5-fluoroorotic acid (Jersey Laboratories). The plates were incubated at 30 °C for 2–3 days.

The spore germination efficiency of a strain was determined by inoculating a single spore colony into 10 ml of pre-sporulation medium (0.25% (w/v) glucose, 1% (w/v) potassium acetate, 0.6% (w/v) yeast nitrogen base without amino acids, 0.5% (w/v) yeast extract, and 0.5% (w/v) bacto peptone) and incubating the culture with vigorous shaking at 30 °C to an \(A_{600}\) of 0.5–0.6. The cells were pelleted, washed in sporulation medium (1% (w/v) potassium acetate, all auxotrophic supplements at
0.25 × shown in Ref. 33), resuspended in 5-ml volumes of sporulation medium to an A_{600} of 0.2, and incubated at 22 °C for 5 days. The sporulation efficiency of a culture was quantified by counting the number of tetrad relative to the total number of cells and tetrads in a hemocytometer.

The β-galactosidase assays were performed as described in Ref. 35.

Chromatin Reconstitution and Nuclease Digestions—The 390-bp 32P-labeled fragment (10 ng) containing a tandem dimer of the sea urchin 5 S rRNA gene was incubated with 40 μg of H1-stripped HeLa chromatin (a gift from M. Vignali and T. Owen-Hughes) in a 100-μl volume of reconstitution buffer (10 mM Tris-Cl (pH 8.0), 0.25 mM EDTA and 0.25 mM phenylmethylsulfonyl fluoride) containing 1,000 mM NaCl at 37 °C for 30 min. The ionic strength was reduced to 800 and 600 mM NaCl by the sequential addition of reconstitution buffer at 30-min intervals at 37 °C. Where rhHho1p was added, the sample was split into aliquots following the dilution to 600 mM NaCl, and rhHho1p was added in the amounts indicated in the text. The dilution of fractions in the presence or absence of rhHho1p was continued to 400 mM NaCl after 30 min, followed by a stepwise 2-fold dilution with reconstitution buffer at 37 °C at 30-min intervals to a final concentration of 12.5 mM NaCl. Aliquots (20 μl) were run on a 0.7% (w/v) agarose gel in 0.5× TBE at 100 V, and the gel was dried and autoradiographed. The reconstitution of H1-stripped HeLa chromatin with Hho1p was performed exactly as described, except that the 5 S rRNA gene dimer fragment was omitted, and rhHho1p was added at a molar ratio of 1:1 (H1:rhHho1p). This and a similarly treated sample lacking Hho1p were digested with concentrations of MNase indicated in the text. The digestion products were purified and electrophoresed on an 8% (w/v) polyacrylamide gel in 1× TBE at 150 V. The preparation of nuclei, digestion of chromatin and free DNA, and the primer extension of the recovered DNA template were performed as described previously (30).

RESULTS

Is Hho1p the Yeast Linker Histone H1?—We investigated the assigned identity of Hho1p as a linker histone in yeast by studying the properties of a recombinant Hho1p (rhHho1p). Histidine-tagged rhHho1p was overexpressed in E. coli cells and isolated from the sonicated cell lysate by passage over a nickel-agarose column (Fig. 1A). The identity of the band indicated as rhHho1p (Fig. 1A) was confirmed by its absence in identically performed mock isolations from E. coli containing only the vector without the HHO1 coding sequence. Although the amino acid sequence of Hho1p predicts a molecular mass of approximately 28 kDa, the protein migrates at a size equivalent to approximately 33 kDa during SDS-PAGE (Fig. 1, A and B). This anomalously slow electrophoretic behavior closely resembles that of linker histones from other species (36) and is likely due to the basicity of the protein, which has a predicted isoelectric point of 10.2. The recovered rhHho1p was next purified from contaminating nickel-agarose binding E. coli proteins by passage over a cation exchange resin, eluted by a linear salt gradient. Recombinant Hho1p eluted off the cation exchange column at approximately 700 mM NaCl (Fig. 1B). This chromatographic trait of rhHho1p is also very similar to that of linker histones from higher eukaryotes (37). These properties, however, reflect only the amino acid composition of the protein and not its proposed function as a linker histone. We therefore tested the ability of the purified recombinant protein to conform to the two common characteristics of a linker histone: (i) to form a stable ternary complex with a reconstituted nucleosome core in vitro, and (ii) to produce a kinetic pause at approximately 168 bp in the MNase digestion pattern of H1-stripped native chromatin.

rhHho1p Forms a Stable Ternary Complex with a Reconstituted Core Di-nucleosome in Vitro—Several investigators have shown that both the full-length and globular domain of recombinant and native linker histones can be reconstituted with nucleosome cores in vitro (13, 38). Since the proposed role of histone H1 is the neutralization of the charge of the internucleosomal linker DNA, we chose to investigate the association of rhHho1p with a reconstituted core di-nucleosome, which contains a short length of linker DNA and is expected to resemble a natural H1 substrate more closely. A 390-bp radiolabeled fragment containing a tandem repeat of sea urchin 5 S DNA (39) was reconstituted into a di-nucleosome in the presence of a range of rhHho1p concentrations, and the reconstitution products were electrophoretically resolved on an agarose gel (Fig. 2). Addition of rhHho1p to the core di-nucleosome resulted in the formation of two slower migrating species. The faster migrating of these two species (N1) appears at lower molar Hho1p input ratios and decreases toward higher input ratios, concurrent with an increase in the slower migrating complex (N2). At a molar input ratio of one molecule of rhHho1p per core, essentially all of the core di-nucleosome exists as the N2 species. At higher molar input ratios or at ionic strengths in excess of approximately 100 mM NaCl, the reconstituted aggregates and does not enter the gel matrix. This result clearly demonstrates that rhHho1p forms a stable ternary complex with a reconstituted core di-nucleosome. The apparent conversion of the N1 to the N2 species as a function of rhHho1p input suggests that at lower rhHho1p ratios a single molecule of rhHho1p binds to the core di-nucleosome forming the N1 complex. At higher rhHho1p: core ratios a second molecule of rhHho1p binds to the N1 complex, resulting in the appearance of the N2 complex.

**Fig. 1. Purification of recombinant Hho1p.** A, nickel-agarose purification of the histidine-tagged Hho1p. Aliquots (10 μl) of collected fractions were electrophoresed on an SDS-PAGE gel, and the protein was visualized by Coomassie stain. The material that does not bind to the resin is shown in lane 2, and the eluted fractions are shown in lanes 3–7. A molecular mass standard is shown in lane 1. The position of the 28-kDa rhHho1p, which migrates at a position equivalent to approximately 33 kDa, is indicated. The abundant protein that migrates at approximately 25 kDa is a bacterial protein that is also present in a mock isolate. B, fractions enriched in rhHho1p obtained from the nickel-agarose column were pooled and applied to a CM-Sephadex cation exchange column. The Coomassie-stained SDS-PAGE gel of aliquots of the collected fractions is shown. Contaminating bacterial proteins are present in the column flow-through (lane 2). Resin-associated protein was eluted with a linear 200–1,000 mM NaCl gradient (lanes 3–10). The purified rhHho1p elutes from the column as a single peak centered at approximately 700 mM NaCl (lanes 7 and 8).
fragment with a length of approximately 168 bp is clearly visible (indicated by the arrowhead in Fig. 4) apart from the fragment resolving at approximately 146 bp. This result clearly shows that rHho1p extends the protection of nucleosomal DNA to two full superhelical turns, conferring a structural stability to a nucleosome core analogous to that caused by a linker histone.

Yeast Lacking Hho1p Are Viable and Have Normal Growth and Mating Properties—We constructed a haploid yeast strain (YHPG101) in which the single HHO1 coding sequence was partially replaced with the HIS3 selectable marker. The absence of the poly(A) HHO1 mRNA transcript, and therefore the Hho1p protein, was confirmed in the YHPG101 strain by Northern analysis (data not shown). There was no detectable difference in the growth rate of the hho1Δ strain compared with the WT strain. Similarly, yeast cells lacking Hho1p mated as efficiently as WT cells, suggesting that both silent mating type loci and the appropriate cell type-specific genes are properly repressed in the absence of Hho1p.

Represion of Basal Transcription—The repression of basal transcription by nucleosomes is well established (reviewed in Ref. 43). Several studies have also shown that the basal transcription of chromatin templates in vitro is reduced in an H1-dependent manner (44–46). We investigated the possible involvement of Hho1p in basal transcriptional repression in situ using a reporter gene that consists of a minimal PHO5 promoter fused to the URA3 coding sequence (47). Cells that harbor this plasmid do not express URA3 and are resistant to the drug 5-FOA which kills cells that express the URA3 gene product. If basal transcription is elevated, however, the cells become sensitive to 5-FOA. The transcriptional activity of this episomal reporter gene was tested in the WT and hho1Δ strains by measuring the growth of transformants in the presence of 5-FOA (Fig. 5A). At equivalent dilution of cells, comparable numbers of WT and hho1Δ cells survive in the presence of 5-FOA. This result demonstrates that in contrast to the core histones (43), Hho1p does not appear to be involved in the general repression of basal polymerase II transcription in situ. This finding does not, however, exclude the possible involvement of Hho1p in specialized regulatory mechanisms at specific genes.

Hho1p Is Not Required for Telomeric Repression—Several studies have demonstrated the transcriptionally repressive nature of heterochromatin-like structures at yeast telomeres and at the silent mating-type loci (Ref. 48 and references cited therein). The involvement of chromatin in the establishment of these repressive structures was clearly shown by the requirement for the N terminus of histone H4 for full repression at both loci (49) and histone H3 for repression at the telomeric ends and at a partially crippled silent mating-type locus (50). Since Bedoyan et al. (51) have also shown that telomeric chromatin fragments isolated from rat liver nuclei contain H1, we investigated whether Hho1p was required for telomeric silencing in yeast. We constructed an isogenic pair of WT and hho1Δ strains that contained a URAS3 gene integrated at a telomeric locus. In these strains, telomeric repression of the URAS3 gene allows the cells to grow on 5-FOA medium, whereas defective repression results in an increased sensitivity to 5-FOA. As shown in Fig. 5B, the URAS3 gene was strongly repressed in both the WT and hho1Δ cells. In contrast, a strain bearing a sir3Δ deletion, previously implicated in telomeric silencing (49), was highly sensitive to 5-FOA, indicating a loss of repression of the telomeric URAS3 gene. These results indicate that Hho1p does not play a detectable role in telomeric silencing.

SIN Phenotype—SWI/SNF, which is believed to function as a chromatin remodeling complex, is required for the activated
transcription of a set of yeast genes (35). Previous studies have shown that the reduced transcription of the HO gene in swi/snf mutants is partially relieved by SIN mutations (52). This class of mutation appears to function at the level of chromatin, since the SIN2 gene was subsequently shown to be identical to HHT1, one of two copies of the gene coding for histone H3. Mutations in either of the two histone H3 genes or substitution of two highly conserved amino acid residues in the histone fold domain of histone H4 was shown to result in a Sin phenotype (53). We therefore asked whether the absence of Hho1p would similarly result in a Sin phenotype.

The SWII-dependent activity of the HO promoter was investigated in strains containing an HO-lacZ gene fusion integrated at the ho locus (54). In the presence of the β-galactosidase substrate, 5-bromo-4-chloro-3-indolyl β-D-galactosynoside, WT SWII strains produce blue colonies, swi1 colonies remain white, and mutations allowing activation of the HO-lacZ gene in swi1 strains result in blue colonies, defined to confer a Sin phenotype. Analysis of three hho1 sin1 HO-lacZ segregants showed no restoration of lacZ expression (data not shown). Also, the hho1Δ mutant did not alleviate the slow growth defect of a swi1 mutant (data not shown). Together these results show that deletion of the HHOI gene does not result in a Sin phenotype.

Analysis of a hho1Δ sin1 Double Mutant—The SIN1 gene was identified as a mutation that alleviated transcriptional defects due to inactivation of the SWI-SNF complex. The predicted Sin1p protein has an amino acid composition similar to the chromatin-associated mammalian non-histone HMG1 protein, and it has been suggested to be involved in the creation of a proper chromatin context for transcription (55). Strains containing a deletion of the SIN1 gene are viable and show no detectable growth or transcriptional defects. We asked whether the absence of Hho1p causes a synthetic phenotype in conjunction with a deletion of SIN1. To this end, basal expression from the episomal CYC1-lacZ fusion gene that contains only a minimal promoter (56) was measured in a WT, hho1Δ, sin1, and a hho1Δ sin1 strain. The hho1Δ sin1 strain was viable and showed no detectable growth defect (data not shown). Consistent with the basal transcription results presented above, neither the hho1Δ, sin1, nor the hho1Δ sin1 strain exhibited any elevation in the basal transcription levels of the reporter gene as assayed by β-galactosidase activity (Table II). The repressed state of a URA3 gene integrated at a telomeric locus was also unaffected in the hho1Δ sin1 strain (data not shown).
SPT Phenotype—The SPT genes (suppressor of Ty) were identified by selection for extragenic suppressors of the transcriptional defects caused by δ and Ty insertions in the 5’ regions of the HIS4 and LYS2 genes (57, 58). Distinct classes of SPT genes have been identified, one of which includes SPT11/HTA1 and SPT12/HTB1 encoding histones H2A and H2B, respectively (59). To determine whether loss of Hho1p causes Spt phenotype-like mutations of these core histones, we investigated the effect of the hho1Δ mutation on transcription of the his4-912Δ and lys2-1286 alleles. In an Sptδ his4-912Δ strain, the predominant HIS4 transcript is initiated in the solo δ insertion, producing an abnormally long HIS4 transcript in which the normal HIS4 translation start is not used, resulting in a Hisδ phenotype. In an Sptδ his4-912Δ strain, a wild-type HIS4 transcript is present in addition to the solo δ-mediated transcript, resulting in histidine prototrophy (Hisδ). Similarly, for Sptδ lys2-1286Δ, LYS2 transcription initiation at the δ sequence within the 5’-exon of the LYS2 coding region, resulting in a nonproductive short transcript and lysine auxotrophy. The presence of an Spt mutation results in transcription initiation at the wild-type start site, resulting in lysine prototrophy. The ability of an SPT mutation to modulate the transcriptional activation of the solo δ insertion and adjacent gene is not currently understood at a mechanistic level.

To determine if deletion of HHO1 causes an Sptδ phenotype, a hho1Δ strain was crossed to a strain carrying the his4-912Δ and lys2-1286 alleles, sporulated, and 88 spores from 22 four-spored tetrads were scored for both His and Lys phenotypes. Since both of the parental strains are Lys+, the ability of the hho1Δ mutation to act as an Spt allele should be exhibited as a deviation from a 0:4 Lys+:Lys− segregation pattern. Of the 88 spores analyzed, none were Lys+. Analysis of the Spt phenotype for the his4-912 Δ allele was consistent with the results observed for lys2-1286Δ (data not shown). Together these results indicate that a deletion of HHO1 does not result in an Spt phenotype.

Hho1p Is Not Required for Efficient Sporulation—Since we have shown above that the rate of growth of a mitotically dividing yeast cell is unaffected by the absence of Hho1p, we asked whether Hho1p may be required for meiosis. This was investigated by determining the sporulation efficiency of diploid strains. The sporulation efficiencies, expressed as the percentage of tetrads relative to the total number of cells, is shown in Table III. Referring to Table III, it is seen that approximately 83% of wild-type diploid cells sporulated. In the case of the homozygous hho1Δ/hho1Δ strain, approximately 60% of the diploids sporulated over the same period. To investigate whether this decrease was due to the absence of Hho1p or due to a genetic difference between the two congenic diploid strains, a wild-type copy of the HHO1 gene was reintroduced at the URA3 locus in the hho1Δ/hho1Δ diploid strain. This strain sporulated at approximately 72% efficiency (see Table III). Although these results suggest a minor involvement of Hho1p in sporulation, a WT/hho1Δ strain sporulated at approximately 37% efficiency. Thus, we cannot exclude the contribution of genotypic differences between the compared congenic strains. A similar result was obtained with a sin1/sin1 hho1Δ/hho1Δ double mutant strain (see Table III). In all cases examined, the spores germinated and grew normally (data not shown). These data suggest that although Hho1p may make a minor contribution to the efficiency of sporulation, it is not absolutely required for meiosis or spore germination.

There Is No Detectable Change in the Chromatin Structure of a hho1Δ Strain—The association of H1 with chromatin, in addition to changing the degree of compaction, has also been shown to change the spacing between adjacent nucleosomes (60). We therefore compared the nucleosome repeat length of bulk chromatin in the isogenic WT and hho1Δ strains. No differences were detected (data not shown). To ensure that minor structural differences are not overlooked, we investigated the chromatin structure of selected regions at single nucleotide resolution. In Fig. 6 we show the primer extension mapping of the micrococcal nuclease scissors in the STE6 gene and the centromeric region of chromosome III in vivo. A comparison of the MNase cleavage sites in chromatin and free DNA at the STE6 locus (Fig. 6A) shows a clear repetitive, nucleosomal pattern in both the WT and the hho1Δ strain. There is no readily detectable change in the MNase accessibility at the pseudo-dyad axis or within the short internucleosomal linker in the absence of Hho1p. Nor is there evidence of a change in the extent of the nucleosome footprint or the nucleosome repeat length throughout the STE6 gene. The primer extension footprinting of the centromeric region of chromosome III also shows a nucleosomal organization abutting the centromeric region which remains relatively nucleosome-resistant in the hho1Δ strain. To address the possibility that Hho1p is degraded dur-

### Table II

| Strain                  | Miller units | n  |
|-------------------------|--------------|----|
| WT                      | 0.26 (+0.11) | 4  |
| sin1                    | 0.24 (+0.07) | 4  |
| hho1Δ                   | 0.27 (+0.05) | 4  |
| hho1Δ sin1              | 0.29 (+0.04) | 4  |

Basal polymerase II transcription in a hho1Δ sin1 double mutant

The expression of β-galactosidase from a fusion gene driven by a minimal CYC1 promoter was determined in the strains indicated and is reported in Miller units.
The diploid strains indicated were allowed to sporulate for 5 days. The average ratio of the number of tetrads to total cells, counted in a hemocytometer, is indicated as a percentage. The standard deviation of at least four independent determinations for each strain is shown in parentheses.

| Strain       | Sporulation efficiency |
|--------------|------------------------|
| WT/WT (CY246) | 82.9% (±4.8)          |
| hho1Δ/hho1Δ (CY703) | 59.5% (±4.2)          |
| hho1Δ/hho1Δ_uwa3::HHO1 (CY704) | 71.8% (±3.4)          |
| sin1/sin1 (CY705) | 79.5% (±2.0)          |
| hho1Δ/hho1Δsin1/sin1 (CY706) | 68.6% (±3.3)          |
| hho1Δ/WT       | 37.0% (±6.2)          |

**Fig. 6. Chromatin structure of strain YHGPG101.** A, MNase cleavage sites in the STE6 gene. Chromatin in nuclei from WT (YPH500, lanes 5–7) and hho1Δ (YHGPG101, lanes 8–10) cells was digested with 10 units/ml (lanes 5 and 8), 5 units/ml (lanes 6 and 9), and 2.5 units/ml (lanes 7 and 10) MNase. DNA, purified from the WT nuclei, was digested with 0.1 unit/ml (lane 11), 0.05 unit/ml (lane 12), or 0.025 unit/ml (lane 13) MNase. Dideoxy terminated sequencing standards are shown in lanes 1–4. The location of relevant sequence features and positioned nucleosomes are indicated to the left and right of the panel, respectively. B, MNase cleavage sites in the centromeric region of chromosome III. Chromatin in nuclei from WT (YPH500, lanes 2–4) and hho1Δ (YHGPG101, lanes 5–7) cells and purified DNA was digested as described for A. The location of the four conserved sequence elements and previously identified hypersensitive site (86) is indicated to the left of the panel. The distribution of the MNase cleavage sites was visualized by extension of a 32P-labeled primer that anneals downstream of the a2 operator in STE6 (A) or downstream of box 1 (86) in the centromeric region of chromosome III (B), and the extension products were resolved on a 6% (w/v) 8 M urea polyacrylamide gel. Autoradiographs of the gels are shown.

**DISCUSSION**

**Is Hho1p the Yeast Linker Histone H1?**—We have shown above that recombinant Hho1p has electrophoretic and chromatographic properties similar to the linker histones of higher eukaryotes. Although there are several other lysine-rich proteins in the yeast genome with expected biochemical properties similar to Hho1p, we have also shown that rHho1p forms a stable ternary complex with a core di-nucleosome in vitro. This association appears to be specific, since the titration of the core di-nucleosome with rHho1p leads to the stepwise appearance of two well-defined supershifted complexes, most likely a core di-nucleosome containing one and two rHho1p molecules, respectively. These complexes are formed at rHho1p/core ratios similar to those found for the linker histones of higher eukaryotes (38). Although we have not shown direct chromatin association in vivo, Ushinsky et al. (26) have shown that a fusion of Hho1p with the fluorescent green protein is located in the nucleus, strongly suggesting that the nucleosome core binding Hho1p is chromatin-associated.

The predicted secondary structure of the assigned globular domain of Hho1p suggests a single-winged helix protein fold similar to that of the chicken H5 (27) and H1.11L (28) globular domains. Several other DNA-binding proteins such as HNF-3γ (62) and CAP (63) exhibit a similar winged helix fold. Although these proteins do bind to DNA, they differ in an important aspect from linker histones. Virtually all linker histone isoforms from a wide variety of organisms and tissue types have three conserved basic amino acid residues at positions corresponding to Lys-40, Arg-42, and Lys-52 in chicken histone H5. These three amino acid residues, which form a predicted secondary DNA-binding site (27), was shown to be essential for the proper binding of H1 to a nucleosome core, protecting ~168 bp of nucleosomal DNA from MNase digestion (40). We have shown above that yeast Hho1p has perfectly conserved amino acid residues at each of these three positions. Furthermore, the reconstitution of H1-striped HeLa chromatins with rHho1p caused the protection of two full superhelical turns of nucleosomal DNA from exonucleolytic cleavage by MNase. Taken together, these data show that Hho1p acts like a true linker histone.

**What Is the Role of Histone H1?**—A substantial body of evidence exists that implicates histone H1 in chromatin condensation (reviewed in Ref. 14). We have systematically investigated an extensive list of possible phenotypes that may reflect the aberrant organization or improper condensation of chromatin in the hho1Δ strain and have shown that a yeast cell lacking Hho1p appears to function as efficiently as a WT cell. This result is not unexpected, since mice, homozygous for an H10 gene disruption, were found to grow and reproduce normally with no anatomical or histological abnormality, although other H1 variants may have compensated for the absence of H10 (64). Similarly, Gorovsky and colleagues (16, 65) have shown in Tetrahymena that vegetative growth, general polymerase I, II, and III transcription, protein synthesis, and general nucleosome repeat length are all unaffected by the absence of the four micronucleus-specific miCLH peptides or macronucleus-specific H1. Linker histone-dependent changes were, however, observed in the nuclear volume, the transcriptional regulation of specific genes, and the efficiency of meiotic division (65). Although we did observe a difference in the sporulation efficiency of a WT versus a hho1Δ yeast strain, we could not exclude the possible contribution of minor genetic background differences to this observation. Thus, although Hho1p may have an effect, it is not required for sporulation and spore formation.

It was previously shown that histone H1 represses basal transcription in vitro (44–46). We could not detect any dere-
pression in basal polymerase II transcription of a reporter gene driven by either a PHO5 or a CYC1 promoter in the hho1Δ strain. It is possible that in vivo the regulation of only selected genes is affected, as was found for the ngoA and Cyp genes in Tetrahymena lacking a linker histone (65). The regulatory effect on these two starvation-specific genes is intriguing, since Roth et al. (66) have shown that starvation of Tetrahymena is accompanied by dephosphorylation of histone H1 and presumably changes in the condensation state of chromatin. It is not clear whether the differential transcriptional effect is mechanistically direct or indirect. In the former case it may be related to a requirement for a compact structure placing transcription regulatory components within a required spatial proximity. Alternatively, structural features of the compacted fiber or a region of chromatin-associated histone H1 itself may be directly recognized by components involved in transcription of select genes. In the latter case, improperly condensed chromatin may disrupt the general nuclear architecture, influencing compartmentalization of specialized structures. Interestingly, a differential effect on transcription was also observed by Linder and Thoma (67) who reported that the overexpression of the sea urchin H1α protein in yeast repressed polymerase I-transcribed RNA genes and the polymerase II-transcribed ACT1 and URA3 genes but not the polymerase II-transcribed Ty gene.

Two previous studies have shown that expression of an exogenous H1 in yeast at a stoichiometry well below that of the core histones resulted in a marked decrease in cell viability (23, 67). The evident interpretation of this lethality is that the overexpression of H1 at moderate levels results in an excess of linker histones in yeast that already contains H1p. However, it was shown that the overexpression of the mouse H1c and H1e variants in 3T3 cells had little effect on the growth properties and viability of the cells in culture (68). It is not entirely clear why overexpression of sea urchin H1 in yeast and mouse H1 in 3T3 cells should differ so drastically in their effects. One possibility is that the different states of differentiation of cultured cells and vegetatively growing yeast result in differences in the general chromatin organization. Alternatively, the contrasting results in yeast and 3T3 cells may be due to the specific histone variants. The expression of H1 variants is tissue-specific and developmentally regulated (69) and has been shown to differ in both efficacy of chromatin condensation (70) and modulation of gene expression (71). It is also possible that the sea urchin H1 overexpressed in yeast does not localize properly to appropriate regions of chromatin or interferes with the localization or post-translational modification of the endogenous H1p.

Absence of H1p did not result in any bulk translational reorganization of nucleosomes or a change in the chromatin organization of specific regions. The only major structural change H1 was previously shown to confer on chromatin in vitro, apart from condensation, was an increase in the nucleosomal repeat length (60). Since the bulk nucleosome repeat length of yeast in the presence of H1p is approximately 160 bp, adjacent nucleosomes are closely stacked and joined by a linker of negligible length. The absence of a linker histone is therefore not expected to result in a further reduction of the nucleosome repeat length. It is also possible that the H1p protein is present at very low levels in mitotically cycling cells or is only associated with specific regions of chromosomes, in which case the absence of H1p is not expected to cause a detectable change in the structure of bulk chromatin.

Given that the absence of H1p in yeast or micH1/H1 in Tetrahymena (16) does not appear to affect cell viability and growth rate, one may ask why the linker histones are evolutionarily conserved? We note that all measurements were performed under optimal growth conditions in the laboratory. It is possible that undetected effects may become much more pronounced under sub-optimal conditions of a natural environment. Such effects, even if minor, may play a significant role over evolutionary periods, thus maintaining selective pressure on H1 and H1p.

Acknowledgments—We thank Marissa Vignali and Tom Owen-Hughes for a generous supply of H1-stripped HeLa chromatin; Michael Cronan for the PHO5-URA3 plasmid; Virginia Zakian for the URA3:ADH4 and sir3URA3:ADH4 strains; Fred Winston for the SPT15/CYPI strains; E. N. (1997) Trends Biochem. Sci. 22, 75–77. The regulatory effect on these two starvation-specific genes is intriguing, since Roth et al. (66) have shown that starvation of Tetrahymena is accompanied by dephosphorylation of histone H1 and presumably changes in the condensation state of chromatin. It is not clear whether the differential transcriptional effect is mechanistically direct or indirect. In the former case it may be related to a requirement for a compact structure placing transcription regulatory components within a required spatial proximity. Alternatively, structural features of the compacted fiber or a region of chromatin-associated histone H1 itself may be directly recognized by components involved in transcription of select genes. In the latter case, improperly condensed chromatin may disrupt the general nuclear architecture, influencing compartmentalization of specialized structures. Interestingly, a differential effect on transcription was also observed by Linder and Thoma (67) who reported that the overexpression of the sea urchin H1α protein in yeast repressed polymerase I-transcribed RNA genes and the polymerase II-transcribed ACT1 and URA3 genes but not the polymerase II-transcribed Ty gene.

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The Biochemical and Phenotypic Characterization of Hho1p, the Putative Linker Histone H1 of *Saccharomyces cerevisiae*
Hugh G. Patterton, Carolyn Church Landel, David Landsman, Craig L. Peterson and Robert T. Simpson

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