Yeast linker histone Hho1p is required for efficient RNA polymerase I processivity and transcriptional silencing at the ribosomal DNA

Anat Levy*, Miri Eyal*, Gitit Hershkovits*, Mali Salmon-Divon*, Michael Klutstein[†], and Don Jay Katcoff*[‡]

*The Mina and Everard Goodman Faculty of Life Sciences, Bar Ilan University, Ramat Gan 52900, Israel; and [†]Department of Molecular Biology, Hebrew University Medical School, Jerusalem 91120, Israel

Nucleosome core particles in eukaryotes are linked by a stretch of DNA that is usually associated with a linker histone. Here, we show in yeast, that the presence of yeast linker histone Hho1p represses expression of a pol II transcribed gene (*MET15*) embedded in the rDNA. *In vivo* deletions of Hho1p sequences showed that the second globular domain is sufficient for that repression, whereas the presence of the N terminus is required for its derepression. In contrast, a run-on assay confirmed by a ChIP experiment showed that Hho1p is required for maximal pol I processivity during rDNA transcription. Psoralen accessibility experiments indicated that Hho1p is necessary for normal rDNA compaction. DNA array expression analysis comparing RNA transcripts in wild-type and *hho1* strains before and after a heat-shock showed that Hho1p is necessary to achieve wild-type mRNA levels of transcripts that encode ribosomal components. Taken together, our results suggest that Hho1p is involved in rDNA compaction, and like core histones, is required for efficient rDNA transcription by pol I.

chromatin | rDNA

The core histones of the nucleosome are among the most phylogenetically conserved proteins known (1). DNA (146 bp) are wrapped around the histone core in nearly two turns. In most eukaryotes, there is a stretch of DNA ranging from <20- to ≈70-nt bp, linking the nucleosome core particles (2). An additional histone, termed linker histone or histone H1, is usually associated with this DNA. When either native or reconstituted chromatin is treated with micrococcal nuclease, limited DNA digestion results in DNA fragments 146 bp long, whereas in the presence of linker histone, an additional 20 bp are protected (3), indicating that the linker histone interacts with DNA that extends beyond the nucleosome core.

Sequence and structural comparisons between known linker histones (4) have shown that most linker histones contain a globular domain having a winged helix-turn-helix motif (5–7). This domain has been shown to asymmetrically bind the DNA as it enters and exits the nucleosome. Specific sites on the globular domain have been identified that are required for the interactions between the histone and the DNA (7, 8). Although it is largely unstructured, the C terminus has been shown to facilitate the formation of a stem structure of the DNA entering and exiting the nucleosome core (9, 10). Neither the N nor the C termini sequences are conserved (11), although both are heavily basic, and their amino acid composition is similar (12).

The temporal transcription of histones, including linker histones, is very tightly cell-cycle-regulated, with transcription occurring during the S phase of the cell cycle (13). As is the case in other organisms, the yeast linker histone gene, *HHO1*, is transcribed during the S phase of the cell cycle (14). Furthermore, in a high-throughput chromatin immunoprecipitation experiment, of all 32 proteins tested (all involved in regulating various stages of gene regulation), none generated a ChIP pattern that was more similar to the four core histones than Hho1p (15). In two immunoprecipitation experiments, Hho1p

was identified in a complex together with the core histones (16, 17). Together, these data argue strongly that yeast *HHO1* encodes a functional homologue to linker histones in other species.

Despite its similarity to other linker histones, sequence comparison of Hho1p with other linker histones showed that it contains an additional globular domain (18), and that both globular domains conform to the canonical winged helix structure (19, 20). The protein sequences flanking the first globular domain resemble the N and C termini of typical linker histones. Fig. 1*A* shows a schematic comparison of human histone H1c and the yeast linker histone Hho1p.

The linker DNA in yeast is unusually short (21–23). Because the globular domain of linker histones has been shown to bind DNA entering and exiting the nucleosome, it was proposed that each globular domain might individually bind an adjacent nucleosome, where the short linker distance is regulated by other factors (18, 20, 23). Alternatively, it was proposed that Hho1p could be responsible for the short distance between nucleosomes by binding adjacent nucleosomes simultaneously (18).

A number of NMR studies have investigated the structure of Hho1p with the goal of understanding the nature of the two domains (19, 20, 24). Each domain has been shown to have the typical winged helix-turn-helix motif. However, whereas the first globular domain is relatively structurally stable, the second one is much less so (19, 20).

Although linker histones appear to be ubiquitous in all eukaryotes and they have been intensively studied for many years, a clear understanding of the precise function of these proteins remains elusive. This stems largely from the absence of a clear phenotype in cells lacking linker histones. In lower eukaryotes, knockouts of linker histones had little measurable effect on phenotype. In Tetrahymena linker histone, knockouts in the macronucleus had little effect on RNA levels of different transcripts, although the volume of the nucleus increased by about a factor of two (25). In yeast, Hho1p disruption resulted in a small measurable increase in homologous recombination and a small shortening of life span (26). In higher eukaryotes, the presence of linker histones is required for proper embryogenesis (27, 28).

An earlier study in our laboratory showed, using chromatin immunoprecipitation, that Hho1p is preferentially localized to
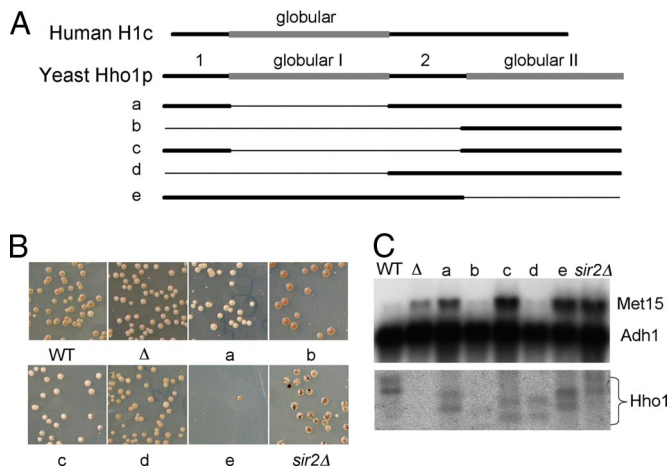
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Fig. 1. An *HHO1* disruption results in increased *MET15* expression. (*A*) Schematic diagram illustrating *hho1* deletions a-e. Human H1c is drawn as a reference (drawn to scale). (*B*) Mutant and wild-type strains were plated on Pb⁺ indicator plates. The lighter the color of the colony, the more *MET15* expression, and the lower the presumed rDNA compaction. Sectors resulting from homologous recombination in the rDNA and loss of the *MET15* gene are most numerous in the *sir2*Δ strain (JS218). Colonies were plated at 30°C for 5 days and then at 4°C for 1 additional week before photography. (*C*) Northern blot monitoring the expression of *MET15* in the different mutants of *Hho1* and *sir2*. Fragments of *MET15* and *ADH1* were used simultaneously as probes. Identical amounts of total cell RNA were loaded in each lane. A parallel gel (shown below) was probed with an *Hho1* fragment. There are consistently two different-sized transcripts for *Hho1*. The differing sizes of the *Hho1* transcripts in the different lanes correspond to the deletions made in the *Hho1* gene by the mutagenesis. The *hho1*Δ deletion strain shows no *hho1* transcript. Quantitation of the transcripts, normalized to the *Adh1* probe, is presented in Table S1).

the rDNA (29). In this article, we describe experiments that functionally analyze yeast linker histone Hho1p, using a model system developed by the Boeke laboratory to monitor rDNA chromatin structure (30). In that system, *MET15* is embedded in one of the rDNA repeats, and its expression is monitored in various genetic backgrounds. We constructed yeast strains that express only portions of Hho1p and tested the expression of the *MET15* transcript embedded in the rDNA in each of these strains. Here, we show evidence that the second globular domain of Hho1p alone is sufficient to repress the expression of the *MET15*, whereas the presence of the N terminus of Hho1p promotes its transcription. By measuring psoralen accessibility to the rDNA we demonstrate that yeast linker histone is required for normal compaction of the chromatin at the rDNA locus. Finally, we show that normal processivity of RNA polymerase I transcription of rDNA requires the presence of *HHO1*.

Results

Hho1p Impedes Expression of a Pol II Transcript Embedded in rDNA.
To address the question of whether we could detect Hho1p function in the rDNA, we used a system developed by the Boeke laboratory to investigate the function of other proteins known to bind the rDNA. In that system, the transcription of a *MET15* gene embedded in the NTS2 of one rDNA repeat is monitored by examining the color of the colonies when plated on Pb⁺ plates (30). Colonies expressing high levels of Met15p are white, whereas low levels of Met15p result in colonies having various shades of brown color that correlate with *MET15* expression. When the *MET15* gene product is completely absent, the colonies are very dark, approaching black. When *MET15* is lost as a result of recombination, colonies form very dark brown sectors.

To test the effect of Hho1p on *MET15* expression embedded in the rDNA, we created a strain in which the entire *HHO1*

coding sequence was deleted. We then compared the colors of the colonies when plated on Pb⁺ containing medium. As seen in Fig. 1*B*, *hho1*Δ colonies were significantly whiter than wild-type colonies that developed a brown color. This indicated that *MET15* expression was higher in the *hho1*Δ strain than in the wild type. We verified these results by performing a Northern blot using RNA extracted from exponentially growing cells, probing with a *MET15* probe. Deletion of *HHO1* resulted in a significant increase in *MET15* RNA levels, as can be seen in Fig. 1*C*. No significant change was seen in the sectoring of the colonies, indicating that homologous recombination was not drastically changed in the rDNA by deletion of *HHO1*. These data indicate that Hho1p serves to repress the RNA polymerase II transcription of *MET15* when embedded in the rDNA.

The Second Globular Domain of Hho1p Is Sufficient to Partially Repress *MET15* Expression, Whereas the Hho1p N Terminus Relieves This Repression.
As described above, yeast linker histone Hho1p contains two globular domains. Although the two globular domains are similar, they have been shown to have some structural differences (19, 20, 24, 31). The first globular domain (closer to the N terminus) is structurally more stable than the second globular domain, raising the possibility that only the first domain is functional. To address these and other questions, we used the "delitto perfetto" strategy (32) to express only parts of the Hho1p molecule in yeast strains harboring the *MET15* gene embedded in their rDNA. Details of these constructs (mutants a-e) can be seen in Fig. 1*A*. Primers used for their construction are listed in Table S2. In each of these strains, the mutant hho1p was expressed from its native chromosomal location without any additional modifications (e.g., insertion of a selectable marker).

Using the *MET15* assay described above, we monitored the color and sectoring of colonies formed when the mutant strains that lacked one or more Hho1p domains were plated on Pb⁺ indicator plates. In addition, we performed Northern analysis on RNA extracted from each of the cultures grown in rich media, hybridizing with a *MET15* radiolabeled sequence. Our results clearly showed that the level of *MET15* mRNA detected by colony color and Northern analysis depended on the *hho1* mutant, as can be seen in Figs. 1 *B* and *C*.

Strikingly, *hho1* mutants b and d that contain the second globular domain but do not contain the N terminus have low levels of *MET15* expression, similar to wild type. However, like the *sir2*Δ strain, mutants a, c, and e, each of which contain the N terminus, express high levels of *MET15*. Our data also indicate that the second globular domain itself (mutant b) is sufficient to repress *MET15* transcription embedded in the rDNA, whereas when the N terminus is present together with the second globular domain (mutant c), this repression is lost. Levels of *ADH1* RNA expressed from its normal chromosomal location are unaffected by any of the *hho1* mutants (Fig. 1*C*).

Processivity of RNA Polymerase I Depends on Hho1p.
The experiments described above used a model system in which an RNA polymerase II transcribed gene (*MET15*) is used to monitor rDNA structure. Because this system is somewhat artificial and might not reflect native rDNA structure accurately, we asked how RNA polymerase I transcription in native rDNA is affected by the presence of Hho1p. To that end, we used a transcription run-on assay in which actively transcribing cells in the log phase of growth were labeled for a short time (10 min) with radiolabeled UTP. RNA was immediately extracted from wild-type and *hho1*Δ strains and hybridized with set amounts of short fragments of rDNA fixed to nitrocellulose in a slot blot. Each fragment represents a different region of the rDNA repeat. Three DNA fragments were chosen (see Fig. 2*A*) (33): (*i*) 5′ ETS close to the 5′ end of the 35S pre-rRNA transcript; (*ii*) 25S near
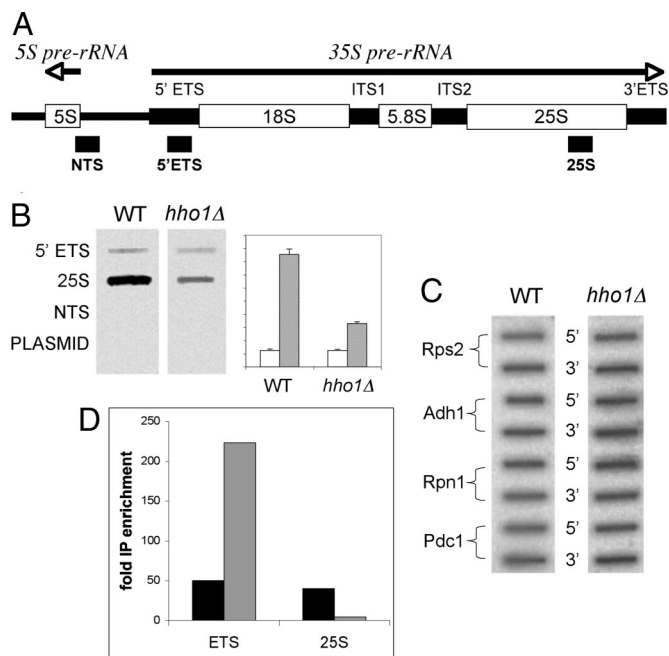
Fig. 2. Pol I processivity is defective in the *hho1Δ* mutant. Run-on transcription was performed as described in *Methods*. (*A*) Schematic diagram of the rDNA transcription unit. RNA polymerase I transcribes the 35S primary rRNA transcript. DNA sequences serving as probes in *B* and corresponding to the indicated segments of the nontranscribed spacer (NTS), the 5′ external transcribed spacer (5′ ETS), and the 25S rRNA are indicated. (*B*) Slot blot hybridization of the probes described in *A* with labeled RNA from wild-type and *hho1Δ* cells. Quantitation of two separate experiments with standard deviation bars on right (arbitrary abscissa units). Open and stippled bars represent ETS and 25S probes, respectively. (*C*) Slot blot hybridization of PCR amplified DNA fragments from 5′ and 3′ ends of genes transcribed by pol II. Primers are listed in Table S3. (*D*) Quantitation of ChIP experiment verifying defective RNA pol I processivity in *hho1Δ*. cross-linked, HA-tagged RNA pol I was immunoprecipitated from wild type and *hho1Δ* cells together with associated DNA. After cross-link reversal, quantitative PCR of ETS and 25S rDNA sequences were performed. Black and stippled bars represent enrichment of the DNA sequences relative to input DNA precipitated from wild-type and *hho1Δ* extracts, respectively.

the 3′ end of the transcript; and (*iii*) NTS, a sequence that is not transcribed. Each of the sequences is cloned in a plasmid vector.

As can be seen in Fig. 2*B*, the labeled RNA from both *hho1Δ* and wild-type strains hybridized to the 5′ ETS and the 25S rDNA fragments, whereas hybridization to the untranscribed DNA fragment and to the plasmid vector DNA was undetectable. Strikingly, however, significantly less labeled RNA isolated from the *hho1Δ* mutant hybridized to the 25S rDNA. Quantitation indicated there is about a 3-fold reduction in the hybridization to the 25S rDNA of the RNA isolated from the *hho1Δ* mutant relative to wild type.

Similar experiments with DNA fragments from the 5′ and 3′ of four different pol II transcribed genes (Fig. 2*C*) showed no difference in hybridization to the labeled RNA from either the wild-type or *hho1Δ* strains.

To verify these experiments a ChIP experiment was performed by using an HA-tagged RNA polymerase I, which replaced the native pol I. Probes from within the ETS and 25S were used to measure pol I occupancy during logarithmic growth. Although little difference was seen between pol I occupancy in the wild type, a 10-fold reduction was seen in the downstream 25S probe in the *hho1Δ* strain, confirming the transcription run-on experiment (Fig. 2*D*).

Rate of rRNA Synthesis Is Unaffected by the Presence of Hho1p. To test the rate of rRNA synthesis, we pulse-labeled RNA in wild-type and
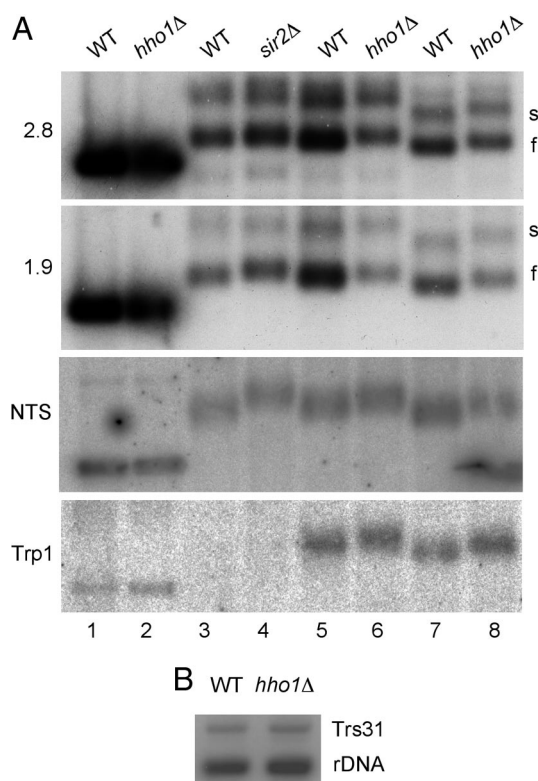

Fig. 3. Deletion of *Hho1* leads to increased chromatin accessibility to psoralen. (*A*) Psoralen cross-linking of rDNA. Wild-type, *hho1Δ*, and *sir2Δ* logarithmic cell cultures were exposed to 10 μg/ml psoralen and 365-nm UV light for 40 min. DNA was extracted, digested with EcoR1, electrophoresed on a 1.3% agarose gel, the cross-linking reversed and blotted onto a nitrocellulose membrane. The blot was probed with probes that recognize 1.9 and 2.8 kb fragments of the 35S rRNA transcript, the rDNA NTS, and the *TRP1* gene. Strains used for the *SIR2* control were deleted for *TRP1*. Blots were exposed to a phosphorimager screen. Cultures in lanes 1–6 were harvested at 0.3 OD$_{600}$, lanes 7 and 8 at 0.9 OD$_{600}$. (*B*) Copy number comparison of wild-type and *hho1Δ* strains. Extracted DNA was digested with EcoR1 and BamH1, electrophoresed, and blotted as above. The blot was probed with a mixture of two probes recognizing the 1.9-kb rDNA fragment and single-copy *TRS31* DNA. Quantitation of the bands showed an rDNA/*TRS31* ratio of 4.31 in the wild-type and 3.74 in the *hho1Δ* strain.

hho1Δ cultures in midlog phase with ^{3}H-uridine for 5 min. We then extracted the RNA and counted cpm/μg of RNA in a scintillation counter. All counts were TCA-precipitable, indicating that the measurement reflected incorporated ^{3}H-uridine into RNA. No significant difference was observed between the strains in three parallel repeated experiments.

Complete rDNA Compaction Depends on Hho1p. Accessibility to psoralen has been successfully used to distinguish between actively transcribing ("slow") and quiescent ("fast") copies of rDNA (34) and to evaluate the degree of chromatin compaction (30). As can be seen in Fig. 3*A*, deletion of Hho1p [like Sir2p (30)] results in increased accessibility of rDNA to psoralen that is detected by its reduced mobility in an agarose gel at all three loci tested (NTS, ETS, and 25S), indicating that Hho1p contributes to chromatin compaction. Interestingly, a similar result was obtained at *TRP1*. This is not surprising, because Hho1p is scattered throughout the chromatin (15). No significant difference in rDNA copy number was detected between wild-type and *hho1Δ* strains (Fig. 3*B*).

Hho1p Affects RNA Polymerase II Transcription at Sites Outside the rDNA Locus. A recent article described changes in binding of a large number of proteins to yeast chromatin after heat shock

using a "ChIP-on-chip" approach (15). Looking at the occupancy levels of quiescent and active RNA polymerase II promoters relative to non-promoter regions, the authors (15) showed that promoter occupancy by Hho1p is significantly lower at active promoters than at non-promoter regions, as is the case for core histones. Furthermore, upon heat shock, Hho1p is redistributed on the chromatin in a similar manner as core histones.

Against this background, we decided to revisit the question of polymerase II gene expression across the genome as a function of Hho1p presence and heat shock. We used a DNA array produced by Agilent on which 60-mer oligonucleotides are synthesized directly on the slides. This process yields DNA spots of very similar size, excellent uniformity, and small molar variability between spots. We expected that our results using this DNA array would be more reliable than results published earlier using a spotted DNA array (35). We extracted RNA from logarithmically growing wild-type and *hho1*Δ cells at both 25°C and 37°C after a 15-min heat shock. cRNA was synthesized from these RNA samples, labeling with Cy5 and Cy3, as described in supporting information (SI) *Text*.

When comparing the wild type with the *hho1*Δ mutant grown at 25°C, examination of all genes except those with very low expression (4,285 genes with raw scores >500) showed that only 147 genes had RNA levels >1.3-fold higher in the wild type than in the *hho1*Δ mutant, whereas 163 genes had RNA levels >1.3-fold higher in the *hho1*Δ mutant than in the wild type. We found no correlation between the genes reported to be differentially expressed in the earlier publication (35) and our results. After a heat shock from 25°C to 37°C, 568 genes showed >1.3-fold lower expression levels in the *hho1*Δ mutant than in the wild type, whereas 413 genes showed >1.3-fold higher expression levels in the mutant than in wild type.

To characterize the gene sets that were differentially expressed (Datasets S1 and S2), we used Gene Set Enrichment software (36) (http://genie.weizmann.ac.il/genomica_web/gene_sets.jsp) that compares input gene sets with published gene sets derived from published DNA array experiments and from Gene Ontology (GO) annotations. When we compared the list of genes whose activity was higher in the *hho1*Δ mutant than in the wild type, either when cells were grown at 25°C or heat shocked to 37°C, we found no clear molecular function categories that were significantly enriched, although there was enrichment of genes that encode membrane bound proteins (Fig. 4). In contrast, when we compared the list of genes whose activity was lower in the *hho1*Δ mutant than in the wild type, we found that a large proportion of the genes whose products are essential for translation were significantly down-regulated. In particular, the RNA encoding most of the structural components of the ribosome was found in significantly lower amounts in the *hho1*Δ mutant than in the wild type, both at 25°C and after heat shock (see Fig. 4). These results complemented the results presented above that showed that rDNA is transcribed less efficiently in the *hho1*Δ mutant.

Discussion

Linker histones are less evolutionarily conserved than core histones. Furthermore, it is clear that although linker histones have an important role in the dynamics of chromatin structure, they function in subtle ways (37). Functional studies have focused on reconstituted chromatin with mutant linker histones and on *in vivo* systems lacking one or more isoforms of the histone. The *in vivo* studies were limited by the lack of a convenient system in which mutant linker histone could be expressed from its own promoter at its native position in the chromosome without any additional modifications. Furthermore, there was not a convenient assay system to measure linker histone function.
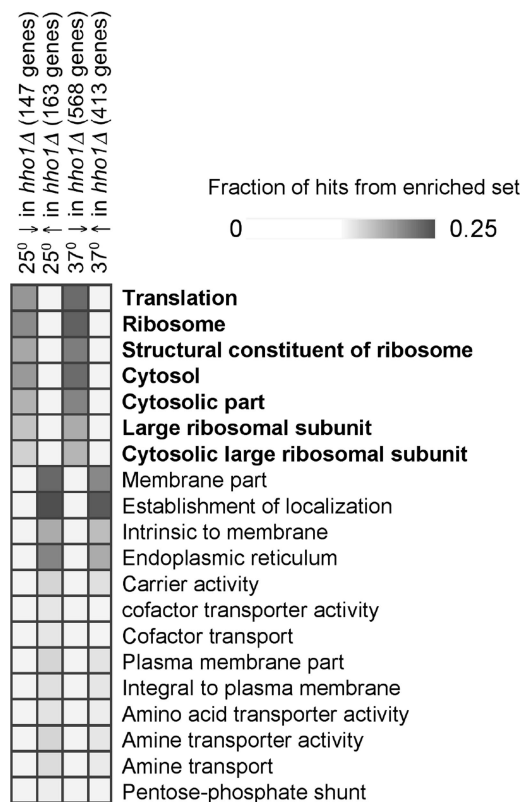

Fig. 4. GO ontology of gene lists generated by expression microarray analysis. The fraction of hits from sets that are found in different GO ontology sets are displayed graphically (36). Here, we show only 20 of the categories. The analysis shown here was performed on gene sets after removal of genes expressed at a very low level (<500 units in the raw data). Above each column, the title relates to whether the gene set was higher (↑) or lower (↓) in the *hho1*Δ mutant than the wild type, and how many genes were in each set (in parentheses). GO ontologies that relate to translation are bolded. The complete figure and quantitative results can be found in Fig. S1.

Here, we address both these limitations of previous studies and show that they can be overcome. Using the "delitto perfetto" method (32), we constructed yeast strains whose mutant linker histone Hho1p was expressed from its natural promoter at its normal chromosomal location with no other modifications.

Because we had shown earlier that Hho1p concentrates in the rDNA (29), we sought an assay that would monitor Hho1p function in that context. Smith and Boeke (30) showed that the expression of *MET15*, which is transcribed by RNA polymerase II, is influenced by the local rDNA structure. Here, we showed (Fig. 1) that, as was the case in *sir2* mutants (30), the partial silencing of *MET15* embedded in the rDNA in the wild type strains was decreased in *hho1*Δ colonies, as seen by their losing their brown color on the Pb$^+$ indicator plates. In addition, varying degrees of brown color were seen in *hho1* mutants expressing only portions of the molecule. These data show that this experimental system can be used effectively to monitor yeast linker histone function *in vivo*. Previous experiments (30) using this system indicated that *MET15* expression levels were a consequence of chromatin compaction at the rDNA locus. Our psoralen accessibility results confirm that Hho1p facilitates chromatin compaction of the rDNA, and at least one other locus (*TRP1*).

Linker histones typically contain a conserved globular domain that is preceded and followed by basic domains whose composition but not sequence is conserved. Hho1p has a similar structure, except that an additional globular domain is found at the N terminus. Sequence comparisons of Hho1p with other

linker histones have shown that the first globular domain of Hho1p is more similar to other linker histones with respect to the presence of specific conserved lysine residues in putative DNA binding site II (K59 and K61) than the second domain, which lacks these lysines at homologous positions (N192 and G194). Structurally, this first globular domain is also more reminiscent of linker histones from other organisms than the second globular domain. The second domain has a less stable structure at physiological salt concentrations (19, 24). We decided, therefore, to test these domains individually in our *MET15* assay system.

As can be seen in Fig. 1, mutants a–d lack the first globular domain. In addition, each contains one or both of the domains denoted as 1, at the N terminus, and 2, between the globular domains, in the figure. Strikingly, mutants a and c have considerably higher *MET15* RNA levels than the wild type or than mutants b and d. An isogenic *sir2* strain had high *MET15* RNA levels (30), which were similar to those in our a and c *hho1* mutants.

The globular domain of linker histones has been shown to be asymmetrically positioned at the entrance and exit of the DNA from the nucleosome core, presumably facilitating higher-order compaction of the chromatin (10, 38). *In vitro* studies showed that the C terminus is entwined with up to 30 bp of DNA entering and exiting the core particle, producing a stem structure (9, 10). In yeast, this domain may not be available for this function, because it is relatively short and is followed by a second globular domain. It is tempting to speculate that, during evolutionary time, the duplication of the globular domain in yeast might have facilitated the ability of yeast to deal with reduced linker DNA length by preventing the DNA stem formation, thereby permitting a shorter stretch of DNA between the nucleosome cores. The poor growth phenotype of mutant e, which contains only the first globular domain was reminiscent of reports that sea urchin histone H1 expressed in yeast is toxic (39, 40). Because the overall structure of mutant e (globular domain with flanking sequences) is similar to linker histones carrying only one globular domain, it may be that the C terminus of the mutant prevents normal yeast nucleosome spacing.

In vitro studies suggest that the linker histone N terminus might somehow be able to interfere with the binding of the globular domain, thus decreasing the wrapping of DNA around the core histones (9). If the N terminus of Hho1p functions similarly, it might assist pol II in displacing nucleosomes during transcription. This idea is supported by our data that show that the N terminus (domain 1 in Fig. 1*A*) is required for efficient *MET15* transcription. Mutants b and d each lack that domain and thus have reduced *MET15* transcription, whereas mutants a, c, and e each have the N-terminal domain, and each efficiently transcribes the *MET15* gene. Although the Hho1p wild type contains the N terminus, it also has two globular domains, presumably making it more difficult for the polymerase to disrupt nucleosomes, thereby resulting in low *MET15* transcription.

Schäfer *et al.* (41) showed that both globular domains of Hho1p can bind four-way junction DNA simultaneously, implying that one molecule of Hho1p is capable of binding two nucleosomes. We propose that the globular domains each bind linker DNA and thus repress pol II transcription, whereas the N terminus of Hho1p is used by components of the transcription and/or chromatin remodeling machinery to disrupt those interactions. Such a model might explain why the wild-type yeast that contain two globular domains in Hho1p expressed *MET15* at a relatively low level.

The *MET15* expression assay monitors transcription of a pol II transcript embedded in the rDNA. Recent data have indicated that, whereas a nucleosomal context impedes pol II transcription, pol I transcribes more efficiently in the presence of core histones (42). We asked, therefore, how transcription of the 35S pre-RNA would be affected by the lack of Hho1p. As described above, a transcription run-on experiment showed that, as in the case of core histones, yeast linker histone is required for efficient

rDNA transcription. In our assay, we used two probes within the 35S pre-RNA template, one near the start of transcription and one near its 3′ terminus. After a 10-min labeling of wild-type cells with ^{32}P UTP, the hybridization signal using the 3′ probe was about three times more intense than the hybridization signal using the 5′ probe. However, when using an RNA probe made from the *hho1Δ* mutant, the intensity of the bands was the same. We interpret these data as evidence that the processivity of pol I transcription in the *hho1Δ* mutant is substantially lower than in the wild type. This interpretation is supported by our ChIP experiment in which RNA pol I occupancy is ≈10-fold greater at the start of the 35S pre-RNA template than near its end.

The rates of transcription appear to be unaffected by Hho1p, because a 5-min pulse of ^{3}H-uridine labeled RNA to the same specific activity in wild-type and *hho1* strains. These results support the notion that nucleosomes serve to maintain a high transcription rate of the pol I complex rather than to inhibit its progress as they do for pol II transcription. They may also be a reflection of data that indicate the pol I complex transcribes through dynamic nucleosomal chromatin (37, 43). In our cultures, lowered pol I processivity in *hho1* mutants is not a limiting factor of yeast growth rate or a determinant of rRNA levels, because neither is reduced in an *hho1Δ* strain. Curiously, our ChIP experiment shows an ≈4-fold greater RNA pol I occupancy at the start of the 35S pre-RNA template in the *hho1Δ* strain than in the wild type. This might indicate a stall in the pol I downstream of the ETS probe in the *hho1Δ* mutant.

Although Hho1p is more abundant at the rDNA locus than at other loci (29), it is distributed throughout the genome (15). Although it was shown earlier that expression levels of only a minority of genes was affected by the disruption of *HHO1* (35), we revisited this question using higher-quality DNA arrays (Agilent) and using RNA that was extracted from isogenic wild-type and *hho1Δ* yeast before and after heat shock. As can be seen in Fig. 4, a significant number of genes were affected by the disruption of *HHO1*. Our results at 25°C were in agreement with previous Northern blot results of a small number of transcripts (29). Classification of genes whose expression changed >1.3-fold according to GO annotations showed that a large proportion of the genes whose products are involved in translation are down regulated in the *hho1* mutant. Although it is possible that the down-regulation is a direct consequence of the absence of Hho1p at these genes, it is more likely that it is a result of general down-regulation of rRNA transcription. It is well known that synthesis of all of the ribosomal components is highly regulated, and it has been recently shown that RNA levels encoding ribosome components depends on rRNA transcription (44, 45).

When our datasets were compared with datasets from other DNA array experiments, we found a significant correlation with ChIP-on-chip experiments that used RAP1 (46) and RPD3 (47). Rap1p targets the promoters of genes that encode ribosomal proteins, thus it is not surprising that the datasets overlap. Rpd3p (a histone deacetylase) is part of a complex that contains Sin3p and Sap30p and is involved in transcriptional silencing. It has been shown to be required for the silencing of rDNA genes entering stationary phase (48) and for maintenance of rDNA chromatin structure (49). The overlap of these datasets with ours suggests that Hho1p may functionally interact with Rap1p and Rpd3p at the rDNA to modulate its chromatin structure.

The assays demonstrated in this article now afford the opportunity to use the power of yeast genetics to functionally dissect Hho1p and to study its interactions with other proteins in the context of rDNA. As was found previously for H3 and H4 core histones, yeast linker histone Hho1p acts to inhibit transcription of a pol II transcript (*MET15*), whereas it is necessary for efficient transcription of a pol I transcript. It will be interesting in the future to continue dissection of the Hho1p molecule to better understand how linker histone functions in different chromatin contexts.

Methods

Yeast Strains and Growth Conditions. The parent yeast strain for mutant constructs in this article was JS237 (*MATα, his3Δ200, met15Δ1, ura3-167, leu2Δ1, RDN1::Ty1-met15*) (30). This strain carries *MET15* in the rDNA. JS218 is *sir2::His-3*. Experiments comparing wild-type and *hho1Δ* strains used BY4741 (*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0*) and its derivative, Y02125 (*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, YPL127C::kanMX4*), respectively, obtained from Euroscarf (http://web.uni-frankfurt.de/fb15/mikro/euroscarf/col_index.html). The parent strain carrying 6X HA-tagged RNA polymerase I was Y312 (*MATa, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, trp1::hisG, can1::hisG, ade2::hisG, RPA42::HA-TRP1*). A strain deleted in *Hho1* was made by transformation with a PCR fragment carrying *YPL127C::kanMX4*.

Mutant strain construction is described in *SI Text*. Pb⁺ indicator plates were as described (30), except they contained 40 mg of adenine hemisulfate per l.

Transcription Run-On Assay. The transcription run-on assay was performed essentially as described (33, 50). Details can be found in *SI Text*.

RNA Pulse–Chase. To pulse-label with ³H-uridine, the cells were transformed with a plasmid containing *URA3* (pUG35) and grown to midlog phase in ura⁻ media. Cells were labeled with 5 μCi/ml uridine (41 Ci/mmol) for 5 min.

ChIP. Wild-type and *hho1Δ* strains harboring an HA-tagged version of RNA pol I were grown to 0.3 OD₆₀₀ in yeast extract/peptone/dextrose. Chromatin was cross-linked by formaldehyde, and ChIP was performed (51). Products were purified by the PCR Clean-Up System (Promega) and analyzed by quantitative radioactive PCR. Products of PCR were run on a polyacrylamide gel and quantified using a phophoimager and TINA software (Fuji).

Psoralen Cross-Linking. Psoralen cross-linking and analysis were performed for 40 min essentially as described (52).

DNA Array Hybridization. DNA array hybridizations were carried out on Agilent whole genome arrays, as described in *SI Text*.

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