Decreased Expression of Specific Genes in Yeast Cells Lacking Histone H1*

Karen Hellauer‡, Edith Sirard‡, and Bernard Turcotte‡§¶

From the Departments of ‡Medicine, §Biochemistry, and ¶Microbiology and Immunology, Royal Victoria Hospital, McGill University, Montréal, Québec H3A 1A1, Canada

Chromatin plays an important role in regulating eukaryotic gene expression. Chromatin is composed of DNA wrapped around a nucleosome core (consisting of two copies of the well conserved histones H2A, H2B, H3, and H4) and a more variable linker histone H1. Various in vitro and in vivo studies have implicated histone H1 as a repressor of gene expression or as an activator, but its exact role is still unclear. Sequencing of the yeast genome has led to the identification of a putative histone H1 gene. Biochemical studies demonstrated that yeast does indeed possess a bona fide histone H1. However, deletion of the unique yeast H1 gene is not associated with any phenotypes, and it was questioned whether it plays any role. To address this issue, we performed whole-genome microarray analysis to identify genes that are affected by H1 removal. Surprisingly, deletion of the gene encoding histone H1 does not result in increased gene expression but rather in a modest reduction. Northern blot analysis of selected genes confirmed the results obtained with the microarray analysis. A similar effect was observed with an integrated lacZ reporter. Thus, our data demonstrate that removal of yeast histone H1 only results in decreased gene expression.

In recent years, numerous studies have shown a functional role of chromatin in regulating eukaryotic gene expression. Nucleosomes are the basic repeating unit of chromatin. The nucleosome core is composed of DNA wrapped around an octamer composed of two copies of each of the core histones H2A, H2B, H3, and H4 (1, 2). In addition, a linker histone H1 is thought to interact with the DNA located between nucleosome cores (3). Core histones are highly conserved between species ranging from yeast to human. Histones have been shown to repress transcription by preventing access of transcription factors to target DNA sequences. Core histones are subject to various modifications including acetylation. A number of complexes with histone acetyltransferase activity have been characterized (reviewed in Refs. 4–6). How histone acetylation results in increased transcription is not clear. One model states that acetylation of core histone tails loosens the interaction with DNA, resulting in easier access of the transcriptional machinery and leading to increased gene transcription (5).

However, there is also growing evidence that histone acetylation (and other modifications) may serve as signals for interaction with other proteins (reviewed in Ref. 7). Conversely, other complexes have been shown to possess histone deacetylase activity and are involved in repression of gene expression (6). Thus, a relatively clear picture of the role of core histones along with complexes involved in core histone modification has emerged in recent years.

Linker histones are less conserved throughout evolution when compared with core histones. Moreover, the location of linker histone H1 and its role in regulating gene expression are less clear (reviewed in Ref. 8). The addition of H1 has been shown to inhibit transcription in vitro (9), but no effect of H1 was observed in other studies (10, 11). The deletion of Tetrahymena H1 does not result in global alteration of transcription but rather in selective decreased and increased gene expression in vivo (12). Similarly, deletion of the unique H1 gene in Aspergillus nidulans does not result in any apparent phenotype (13). In Xenopus oocytes, overexpression of H1 results in repression of the oocyte 5 S rRNA gene but has no effect on somatic 5 S rRNA (14, 15). Mice lacking the testis-specific histone H1t do not show any defect in spermatogenesis. H1t-deficient germ cells show a normal H1 to nucleosome ratio, perhaps because of compensation by other H1 isoforms (16), a fact that could account for the absence of a phenotype. Deletion of the genes encoding other H1 isoforms does not lead to any apparent phenotype in mice or chicken cells (17–19).

For many years, there has been no evidence for the existence of histone H1 in yeast. This was in agreement with the unusually short nucleosomal repeat length observed in this organism (20). However, sequencing of the yeast genome revealed a unique open reading frame (ORF) encoding a putative H1 gene (21, 22). Recombinant yeast H1 was shown to behave like H1 found in higher eukaryotes because it forms a stable ternary complex with a reconstituted core dinucleosome in vitro (23). Moreover, a fusion of H1 and the green fluorescent protein is localized in the nucleus (22). Deletion of the H1 gene is not lethal in yeast and does not result in any apparent phenotype such as slower growth, alteration in telomeric silencing, mating, sporulation, or induction of gene expression (22–24). In addition, upon removal of histone H1, the nucleosomal repeat length is unchanged in yeast and in A. nidulans (13, 23).

These results raise the question of whether histone H1 plays any role in regulating gene expression in yeast. To address this issue, we performed whole-genome microarray analysis of yeast cells carrying a deletion of the H1 gene. Strikingly, our analysis did not provide any evidence for increased expression of any gene because of the removal of histone H1. Unexpect-
edly, we observed rather an overall (but modest) decrease of gene expression with 27 genes affected by a factor of 2 or more.

MATERIALS AND METHODS

Strains, Media, and Growth Conditions—The Saccharomyces cerevisiae strains used in this study are derived from BY4741 or BY4742 (25) and were obtained from H. Bussey (Saccharomyces deletion project, Research Genetics) and were isogenic to strains BY4741 and BY4742 (25). Yeast cells were grown in rich medium (YPD (26)) to an A600 of 0.8–1.0 for DNA isolation.

Microarray Analysis—Total RNA was isolated with the hot phenol procedure (27). RNA was further purified with Qiagen columns according to the manufacturer’s protocol. cDNA labeling and hybridization were performed exactly as described (28). Custom-made yeast whole-genome microarrays (~6200 yeast ORFs) were obtained from the Microarray Center at the Ontario Cancer Institute (Toronto, Canada). Scanning and quantification were performed exactly as described (29).

Briefly, chips A were hybridized with a mixture of wild type cDNA labeled with Cy3 (WT-Cy3) and Cy5 (WT-Cy5); chip B, a mixture of WT-Cy3 and Δhho1-Cy5; chip C, a mixture of Δhho1-Cy3 and WT-Cy5; chip D, a mixture of Δhho1-Cy3 and Δhho1-Cy5. The ratio of Δhho1/WT for each ORF obtained from chips B and C was normalized with the corresponding ratio of the same ORF from chips A and D. Because each ORF is duplicated on the same chip, four ratios obtained from chips B and C were normalized individually with the four ratios obtained from chips A and D; i.e. 16 values were obtained for each ORF. Moreover, the results presented in Table I are an average of two independent experiments performed with independent RNA preparations.

Southern and Northern Blot Analysis—Genomic DNA was isolated according to Ref. 30. Southern and Northern blot analysis were done according to standard procedures (31). Hybridizations were performed at 42°C in 50% formamide, 1 M NaCl, 2.5× Denhardt’s solution, 0.5% SDS, and 10% dextran sulfate. The probe for Southern blot analysis of the deletion of the HHO1 gene was obtained by PCR amplification of the promoter region of the HHO1 gene with genomic DNA and the primers CCGGATCTTCTGCGTTCAGTGAATT and GGAATTCATCAGGTAATTCTGGTGGCG.

The deletions were confirmed by Southern blot analysis of the promoter region of the HHO1 gene using genomic DNA isolated from strain YPH499 (32). Custom-made yeast whole-genome microarrays (~6200 yeast ORFs) were obtained from the Microarray Center at the Ontario Cancer Institute (Toronto, Canada). Scanning and quantification were performed exactly as described (29).

RESULTS

In our study, we used a wild type yeast haploid strain and strains (either MATa or MATα) carrying a deletion of the entire open reading frame of the HHO1 gene encoding histone H1. The deletions were confirmed by Southern blot analysis of the promoter region of the HHO1 gene using genomic DNA isolated from strain YPH499 (32). Custom-made yeast whole-genome microarrays (~6200 yeast ORFs) were obtained from the Microarray Center at the Ontario Cancer Institute (Toronto, Canada). Scanning and quantification were performed exactly as described (29).

In the deletion of the HHO1 gene was obtained by PCR amplification of the promoter region of the HHO1 gene with genomic DNA and the primers CCGGATCTTCTGCGTTCAGTGAATT and GGAATTCATCAGGTAATTCTGGTGGCG. The PCR product was cut with EcoRI and BamHI and subcloned into Bluescript KS+ (Stratagene) cut with the same enzymes. A HpaI-HpaI fragment of the lacZ gene was used as a probe to verify proper integration of the EFT1 reporter (see below). Probes for Northern blot analysis were obtained by PCR amplification of specific open reading frames using genomic DNA and the following oligos: SSE1 gene, CCGGATCTTCTGCGTTCAGTGAATT and GGAATTCATCAGGTAATTCTGGTGGCG.

The PCR products were cut with EcoRI and BamHI and subcloned into Bluescript KS+ (Stratagene) cut with the same enzymes. Fragments were gel-purified and randomly labeled (31) for Northern blot analysis.

β-Galactosidase Assays—An EFT1 reporter was constructed by amplifying its promoter using genomic DNA isolated from strain YPH499 (32) and the following oligos: ATCGAATCTTGGAATCCGCTGCTGAAGTTATGAC and CCGGATCTTCTGCGTTCAGTGAATT and GGAATTCATCAGGTAATTCTGGTGGCG. The PCR product was cut with Xhol and BamHI and subcloned into plasmid p178MB (a high copy plasmid with a URA3 selection marker (33)) cut with BamHI and Xhol to give EFT1-lacZ Z-μ. The reporter contains about 700 base pairs of promoter sequences relative to the ATG. The ATG of the promoter is used to initiate translation of the lacZ gene. For correct chromosomal integration the reporter was obtained by deleting 2-μm sequences by cutting with HindIII and religating the backbone to give EFT1-lacZ-2P. The resulting plasmid was linearized by cutting at the unique Apal site located in the URA3 marker. DNA was transformed into BY4741 or Δhho1a and colonies selected on plates lacking uracil. Proper integration of the reporter was verified by Southern blot analysis.

Strains BY4741 and Δhho1a were also transformed with EFT1-lacZ. Colonies were grown overnight in YPD and diluted 2000-fold in minimal medium (SD, Ref. 26) supplemented with the appropriate amino acids and adenine. β-Galactosidase assays were performed with permeabilized cells (34). Values are the average of at least three independent experiments performed at least in duplicate.

Fig. 1. Southern blot analysis of the HHO1 gene deletions. Top, schematic map of the region encompassing the HHO1 gene. The numbering in base pairs (bp) is relative to the initiator codon. The black rectangle corresponds to the ORF of the HHO1 gene; striped rectangle, probe used for Southern blot analysis; open rectangle, G418 resistance gene, which was used for selection of the deletion mutants (see “Materials and Methods”). Bottom, Southern blot of the WT and the deletion strains Δhho1a and Δhho1a, which are isogenic to strains BY4741 and BY4742 (25), respectively. The relevant sizes of DNA fragments are shown in kilobases (kb) on the left.
FIG. 2. Effect of histone H1 removal on overall mRNA levels. A summary of the microarray analysis performed with strains BY4741 and Δhho1a (see “Materials and Methods”). The number of genes is given on the left relative to their expression in the Δhho1a strain as compared with the wild type strain (mRNA levels Δhho1a/WT strain).

In this study, we have performed whole-genome analysis of yeast cells carrying a deletion of the HHO1 gene that encodes histone H1. Results show a slight decrease of expression for the majority of the yeast genes. However, only a small fraction of the genes (27 of 6216) had mRNA levels reduced by 2-fold or more. This subtle (but significant) effect of histone H1 likely explains why previous analyses have failed to detect any phenotype (22–24). Similarly, no variation in the expression of a limited number of tested genes was observed with the exception of CYC1 (see below) (22–24). Importantly, we observe only a slight increase (less than 50%) in expression for a small number of genes. Thus, in yeast, H1 is not a general repressor of gene expression.

Studies performed in Tetrahymena thermophila have shown previously that deletion of the gene encoding histone H1 is not lethal and does result in global changes of transcription as assayed by measuring the overall RNA content. However, both increases in basal expression (but not activated transcription) and decreases in gene expression were observed in cells lacking H1 (12). Similarly, no phenotype is observed in A. nidulans lacking H1 (13). In contrast, H1 in Ascomobulus immersus is essential for long life span but not early vegetative growth (36).

Finding a role for H1 in higher eukaryotes may be complicated by the presence of many isoforms. For example, knockout of the testis-specific H1t does not result in an H1 imbalance, because of the presence of other H1 subtypes, and does not result in any obvious phenotype (16). In contrast, the nonessential histones H1 in yeast and Tetrahymena are more divergent than their mammalian counterparts. For example, Tetrahymena has an unusually small H1 without a globular domain. In contrast, yeast H1 has two putative globular domains unlike mammalian H1 (21). Nonetheless, although A. nidulans has a typical H1, its absence does not result in any apparent phenotype (13). Thus, H1 may perform more specialized functions in these organisms. For example, a majority of yeast genes are expressed under normal growth conditions. This may explain the differences in the role and the primary structure of H1 in yeast as compared with higher eukaryotes.

Our studies point to an effect of yeast H1 on a rather limited number of genes. We do not observe any obvious common

0.3 molecules/cell (35). Moreover, there was no apparent clustering of the genes with reduced mRNA levels in the Δhho1 strain. For example, there was no clustering for these genes near the telomeres or the centromeres of the chromosomes (data not shown). In summary, our whole-genome microarray analysis showed that removal of histone H1 has modest effects on steady-state mRNA levels with about 27 genes being affected by a factor of 2 or more.

To confirm these results, we performed Northern blot analysis on a few selected genes. RNA was isolated from a wild type strain and a strain carrying a deletion of the HHO1 gene. As expected from the microarray analysis, decreased mRNA levels for the genes EFT1, SSE1, and PMA1 were observed in the Δhho1 strain (Fig. 3). The EFT1 and the SSE1 probes cross-hybridized with ribosomal RNA, thus providing an internal control for equal loading and transfer of RNA isolated for the wild type and knockout strains (Fig. 3). PhosphorImager analysis showed that mRNA levels are reduced about 2-fold for SSE1, EFT1, and PMA1 in cells lacking H1. These values are in good agreement with those obtained by microarray analysis (Table I).

The reduced mRNA levels of these specific genes could be because of mRNA destabilization or decreased transcription. To test whether the effect is at the transcriptional level, we constructed a reporter plasmid containing about 700 base pairs of promoter sequences of the EFT1 gene (including the natural ATG) fused to the lacZ gene. The activity of the integrated EFT1 reporter was reduced about 3-fold in the Δhho1 strain (Table II), in agreement with the microarray analysis (Table I).

Even though the lacZ fusion contains the 5′-untranslated region of the EFT1 mRNA, it is very likely that the reduced activity is because of decreased transcription and not mRNA destabilization. Moreover, the data show that the effect of histone H1 on activity of the EFT1 promoter does not depend on a specific chromosomal location.

Interestingly, no significant difference of the EFT1 promoter activity could be detected between the wild type and Δhho1 strains when assayed with a high copy episomal reporter (Table II). This difference may be due to a variation in the copy number of the 2-μm plasmid between wild type and Δhho1 strains or a difference in the chromatin structure of the integrated and episomal EFT1 reporters.

DISCUSSION

In this study, we have performed whole-genome analysis of yeast cells carrying a deletion of the HHO1 gene that encodes histone H1. Results show a slight decrease of expression for the majority of the yeast genes. However, only a small fraction of the genes (27 of 6216) had mRNA levels reduced by 2-fold or more. This subtle (but significant) effect of histone H1 likely explains why previous analyses have failed to detect any phenotype (22–24). Similarly, no variation in the expression of a limited number of tested genes was observed with the exception of CYC1 (see below) (22–24). Importantly, we observe only a slight increase (less than 50%) in expression for a small number of genes. Thus, in yeast, H1 is not a general repressor of gene expression.

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## Table I

| Systematic name | Gene | Gene product | Expression ($\Delta$hhol/WT) |
|-----------------|------|--------------|-------------------------------|
| Secretory pathway | | | |
| YDR238C | SEC26 | Vesicle coat component | 0.47 |
| YGL022W | SST3 | Integral endoplasmic reticulum membrane protein | 0.47 |
| YFL031W | HAC1 | Transcription factor that is required for the unfolded protein response pathway | 0.48 |
| YPR181C | SEC23 | GTPase-activating protein | 0.51 |
| YGL137W | SEC27 | Yeast coat portion $\beta$ subunit (involved in endoplasmic-to-Golgi protein trafficking) | 0.52 |
| YGR432W | NPL3 | Involved as a protein carrier in mRNA export; involved in mitochondrial protein targeting | 0.53 |
| YLR378C | SEC61 | Membrane component of ER protein translocation apparatus | 0.53 |
| Translation | | | |
| YOR133W | EFT1 | Translation elongation factor 1 | 0.41 |
| YPL237W | SUI3 | Translation initiation factor eIF-2 $\beta$ subunit | 0.44 |
| YDR385W | EFT2 | Translation elongation factor 2 | 0.46 |
| YLR249W | YEF3 | Translation elongation factor 3 | 0.50 |
| YOR361C | PRT1 | Translation initiation factor eIF3 subunit | 0.51 |
| Heat shock response | | | |
| YPL240C | HSP82 | Heat shock protein 90; mammalian Hsp90 homolog | 0.47 |
| YPL106C | SSE1 | Hsp70 family member | 0.53 |
| Vacuole | | | |
| YMR297W | PRC1 | Carboxypeptidase Y (proteinase C) | 0.43 |
| YBR286W | APE3 | Vacuolar aminopeptidase yscIII | 0.49 |
| YKL103C | LAP4 | Vacuolar aminopeptidase yscI | 0.53 |
| Metabolism | | | |
| YMR205C | PFK2 | Phosphofructokinase (glycolysis) | 0.33 |
| YPR159W | KRE6 | $\beta$-glucan synthase (putative) | 0.35 |
| YBL099W | ATP1 | Mitochondrial F$_1$F$_0$-ATPase $\alpha$ subunit | 0.41 |
| YMR105C | PGM2 | Phosphoglucomutase | 0.43 |
| YMR250W | GAD1 | Glutamate dehydrogenase | 0.45 |
| YLR304C | ACO1 | Aconitase (mitochondrial) | 0.45 |
| YPR160W | GH1 | Glycogen phosphorylase | 0.45 |
| YGR240C | PFK1 | Phosphofructokinase $\alpha$ subunit (glycolysis) | 0.46 |
| YEL011W | GLC3 | 1,4-Glucan-6-1,4-glucanotransferase | 0.46 |
| YOR371W | ALD4 | Mitochondrial aldehyde dehydrogenase | 0.51 |
| YLR255W | GSY2 | Glycogen synthase (UDP-glucose-starch glucosyltransferase) | 0.51 |
| YPL262W | FUM1 | Fumarase (converts 1-malate to fumarate as part of the trichloroacetic acid cycle) | 0.52 |
| YOL059W | GP2 | Glycerol-3-phosphate dehydrogenase | 0.53 |
| YDL171C | GLT1 | Glutamate synthase (NADPH) | 0.53 |
| YMR189W | GCC2 | Glycine dehydrogenase (decarboxylating) | 0.54 |
| Miscellaneous | | | |
| YGL008C | PMA1 | Plasma membrane H$^+$-ATPase | 0.31 |
| YFL032W | Unknown | | 0.36 |
| YLR379W | Unknown | | 0.43 |
| YHR027C | RPN1 | 26 S proteasome PA700 subunit | 0.43 |
| YMR092C | AIP1 | Actin cortical patch component | 0.47 |
| YGR279C | SCW4 | Soluble cell wall protein | 0.48 |
| YJL157C | FAR1 | Cdc28p kinase inhibitor | 0.50 |
| YLR232W | Unknown | | 0.50 |
| YPL098C | Unknown | | 0.52 |
| YBL100C | Unknown | | 0.52 |
| YPR019W | CDC54 | Essential for initiation of DNA replication; homolog of *Saccharomyces pombe* CDC21 | 0.52 |
| YMR229C | RRP5 | Required for processing of pre-rRNA | 0.52 |
| YPL105C | Unknown | | 0.52 |
| YDR508C | GNP1 | High affinity glutamine permease | 0.53 |
| YML072C | Unknown | | 0.53 |
| YOR086C | Unknown | | 0.53 |
| YPL036W | PMA2 | Plasma membrane ATPase | 0.53 |
| YBR237W | Unknown | | 0.53 |
| YGR281W | YOR1 | ABC transporter | 0.54 |
feature for these genes. For example, they perform different functions such as translation (e.g. EFT1, EFT2, SUI3, YEF3), metabolism (PFK1, PFK2, GPH1, GAD1), and heat shock response (HSP82, SSE1). Reduced mRNA levels are observed for the gene products of the secretory pathway like SEC26, HAC1 (a transcription factor required for the unfolded protein response pathway), SEC23, SEC27, and SEC61. However, we did not observe increased sensitivity of a Δhho1 strain to stress induced by the addition of dithiothreitol to plates or to the glycosylation inhibitor tunicamycin. Similarly, reduced mRNA levels of heat shock genes HSP82 and SSE1 at normal growth temperature (30 °C) does not result in sensitivity of the Δhho1 strain to high temperatures as assayed at 37 °C and 45 °C. Moreover, the expression levels of genes affected by H1 vary over a wide range (0.3–43 molecules/cell) (35). Thus, H1 is not specifically implicated in the activation of genes with, for example, a low transcription rate. In addition, there is no correlation between dependence on the chromatin remodeling complexes SAGA (Spt-Ada-Gcn5-acetyltransferase) or SWI/SNF (35) and H1. Finally, there is no specific location of these genes near the telomeres or the centromeres, unlike what is observed for H4 (37). This finding is in agreement with our observation that a lacZ reporter driven by the EFT1 promoter shows decreased β-galactosidase activity in Δhho1 cells when integrated at the URA3 locus.

What is the mechanism of action of histone H1? Our study did not include genes transcribed by RNA polymerases I and III. However, given the absence of growth differences in minimal media (24), it is unlikely that altered transcription by RNA polymerase I or III could account for the decreased expression of the genes observed in a ΔHHO1 knockout strain. Yeast studies have shown that H1 has no effect on nucleosome positioning on the POT1 and the YIL161W genes (38), which are not affected by H1 removal according to our microarray analysis. Similarly, nucleosome positioning is independent of histone H1 in Tetrahymena (39). We observed decreased expression of EFT1 irrespective of its chromosomal location (Table II). However, the effect of H1 deletion was not observed with an EFT1-lacZ reporter present on a 2-μm plasmid (Table II). This finding is somewhat surprising because high copy reporters generally behave like endogenous genes (2). We do not know the basis for the difference between the results obtained with integrated and episomal reporters. No difference in expression of the CYC1 gene was observed with our microarray analysis. However, using a low copy episomal CYC1-lacZ reporter, increased β-galactosidase activity (2.5-fold) was observed in a Δhho1 strain (22). Thus, the CYC1 promoter present on an episomal plasmid may not behave like the endogenous gene, as observed for EFT1. Decreased activity with the EFT1 gene is not due to ORF sequences because the EFT1 promoter linked to the lacZ gene and integrated at the URA3 locus (or episomal reporters) behaved like the natural gene.

It is possible that the affected genes have an unusual nucleosomal ladder in their promoter regions making them sensitive to H1 removal. Deletion of the H1 gene in Tetrahymena results in selectively decreased and increased gene expression (12). It was proposed that the removal of H1 increases the accessibility of trans-acting protein complexes that act as activators or repressors of transcription (40). In yeast, a high level of acetylation of core histones is observed (41). Thus, the removal of histone H1 may affect the acetylation state of core histones of some genes. For instance, yeast genes affected by the removal of histone H1 may be more accessible to histone deacetylases resulting in decreased gene expression. This would explain the overall decrease of gene expression observed in yeast cells lacking histone H1 (Fig. 2). In conclusion, our studies provide additional evidence that histone H1 has a more specialized function than the core histones. Importantly, yeast H1 is involved in the general activation of genes, with a limited number of genes being more affected by its removal. Thus, in yeast, H1 is not involved in the repression of gene expression. Our studies were performed with cells grown under rich medium. Testing the effect of H1 removal under more stringent conditions may identify a larger number of genes that are affected by histone H1.

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