Linker Histone H1 Regulates Specific Gene Expression but Not Global Transcription In Vivo

Xuetong Shen and Martin A. Gorovsky
Department of Biology
University of Rochester
Rochester, New York 14627

Summary

In a linker histone H1 knockout strain (ΔH1) of Tetrahymena thermophila, the number of mature RNAs produced by genes transcribed by pol I and pol III and of most genes transcribed by pol II remains unchanged. However, H1 is required for the normal basal repression of a gene (*ngoA*) in growing cells but is not required for its activated expression in starved cells. Surprisingly, H1 is required for the activated expression of another gene (*CyP*) in starved cells but not for its repression in growing cells. Thus, H1 does not have a major effect on global transcription but can act as either a positive or negative gene-specific regulator of transcription in vivo.

Introduction

In the eukaryotic nucleus, DNA is highly compacted into a nucleoprotein complex referred to as chromatin. Thus, in vivo, the structure of chromatin must at a minimum passively allow regulated transcription, which requires gene activation as well as repression. Recent evidence strongly indicates that chromatin actually plays active roles in these processes (for review see Wolffe, 1995).

The most fundamental level of chromatin organization is a periodic and particulate unit, the nucleosome, with a repeat length of approximately 200 bp of DNA. In the nucleosome, DNA is wrapped twice (approximately 80 bp/turn) in a left-handed superhelix around an octameric core containing two each of the four conserved core histones H2A, H2B, H3, and H4. In most organisms, nucleosome cores are separated by a variable length of linker DNA that is associated with a single molecule of a fifth, less conserved histone usually referred to as the linker histone or H1.

The structures of the core histone octamer and the core particle have been determined by X-ray crystallography (for review see Pruss et al., 1995). The core octamer has a tripartite structure with a central $(H3–H4)_2$ tetramer that interacts with two H2A–H2B dimers. The core histones have globular highly α-helical C-terminal domains that are involved in histone–histone interactions and DNA binding. They also contain more basic unstructured N-terminal tails thought to interact with DNA at the outside of the superhelical turns, possibly extending into the linker regions. The tails also are subject to extensive secondary modification and can interact with nonhistone proteins.

Histone H1 in most organisms has three distinct domains, a central globular region, a basic N-terminal region, and a C-terminal tail. The precise location of H1 in chromatin is not known (Pruss et al., 1995), although there is general agreement that it interacts at least in part with linker DNA and protects another 20 bp of DNA from nuclease digestion in addition to the 146 bp protected by the core histone octamer.

The core histone octamer can repress transcription from DNA templates in vitro (Owen-Hughes and Workman, 1994; Paranjape et al., 1994). The notion that the core histones also can serve as general repressors of transcription in vivo is strongly supported by many studies (for review see Grunstein, 1990), most notably in the budding yeast. In Saccharomyces cerevisiae, histone depletion results in nucleosome depletion and activates inducible pol II genes, whereas localization of a nucleosome over a promoter represses transcription. In yeast and in mammalian cells, activation of transcription of some genes has been shown to be accompanied by removal or alteration of a nucleosome positioned over a promoter. Thus, core histones form an evolutionarily conserved octameric complex that, both in vivo and in vitro, can compact DNA and block access of transcription factors to their target sequences.

Mutational analyses of histone genes in S. cerevisiae have also demonstrated that, in vivo, core histones affect the expression of specific genes both positively and negatively. H4 and H3 N-termini both repress silent mating loci and telomeric heterochromatin in yeast (Fisher-Adams and Grunstein, 1995). More strikingly, deletion of the H4 N-terminal residues 4–23 or mutagenesis of the acetylatable lysines in this region decreases activation of the GAL1 promoter, while deletion of residues 4–15 of H3 or mutagenesis of acetylatable lysines hyperactivates GAL1. These differences are reflected in different effects of mutations in the two histones on chromatin structure of the GAL1 promoter (Fisher-Adams and Grunstein, 1995). Thus, the histone H4 N-terminal tail functions in both gene activation and repression. Mutations in the N-termini of H3 and H4 also show promoter-specific effects, activating the expression of some genes but not others. The mechanistic basis for these promoter-specific effects is unknown. They likely reflect preexisting differences in chromatin structure at the different genes (e.g., particular relationships between nucleosome position and promoter or upstream activating sequence elements) or differences in the way specific transcription factors or regulatory proteins interact with nucleosomes (Paranjape et al., 1994). These studies in yeast argue strongly that the core histones serve as global repressors of transcription and also interact in specific ways with the transcriptional apparatus of different genes.

In vitro, H1 binding to naked DNA inhibits transcription, and addition of H1 increases the transcriptional repression observed with core particles alone (Owen-Hughes and Workman, 1994; Paranjape et al., 1994). Biochemical studies have also consistently demonstrated a partial depletion or alteration in the association of H1 with active genes in diverse organisms including Drosophila, Xenopus, chickens, mammals, and Tetrahymena (for review see Zlatanova and Van Holde, 1992). An unfortunate limitation on in vivo studies of the function of

Table 1. Dry Weight, Total RNA, and Relative Transcription Rate in Wild-Type and Linker Histone Knockout Strains

	Wild-Type	ΔH1	ΔMicLH
Dry weight of log phase cells (ng per cell)	1.86 ± 0.04 (n = 3)[a]	1.76 ± 0.07 (n = 3)	1.87 ± 0.12 (n = 3)
Total RNA of log phase cells (pg per cell)	179 ± 23 (n = 7)	173 ± 20 (n = 9)	165 ± 14 (n = 3)
Total RNA of starved cells (pg per cell)[b]	91	95	108
Relative transcription rate in log cells[c]	1.00	1.02	NA
Relative transcription rate in starved cells[c]	0.35	0.40	NA

[a] Number of measurements.
[b] Average of two measurements.
[c] Average of two measurements, transcription rate in nuclei isolated from wild-type log cells set as 1.00.

linker histones has been the failure so far to demonstrate their existence in yeast, thus precluding the kinds of gene replacement studies that have furthered our understanding of the role of the core histones in transcription. Expression of a heterologous H1 gene in S. cerevisiae has been shown to have highly deleterious effects on growth and viability and to repress expressed genes (Linder and Thoma, 1994; Miloshev et al., 1994). In Xenopus, increased expression of H1 in embryos specifically repressed transcription of oocyte 5S RNA genes but not of other pol III transcripts (Bouvet et al., 1994). Conversely, partial depletion of H1 by targeted ribozyme treatment (Bouvet et al., 1994; Kandolf, 1994) specifically increased expression of oocyte 5S genes. Aside from those studies, the role of linker histone in transcription in vivo remains largely unexplored.

Tetrahymena thermophila provides a model system to study linker histone function in transcription in vivo. Like most ciliates, Tetrahymena cells contain two nuclei (for review and references see Gorovsky, 1973, 1980). Macronuclei in vegetative Tetrahymena cells are transcriptionally active somatic nuclei. Macronuclear linkers are associated with an H1 whose size, solubility properties, lysine richness, and cell division–associated phosphorylation are typical of that class of histones but that lacks the central globular domain found in the H1s of multicellular eukaryotes (Wu et al., 1986; Hayashi et al., 1987). It is encoded by the *HHO* gene. Micronuclei in vegetative cells are diploid transcriptionally inert germline nuclei. Micronuclear linkers are associated with four proteins (α, β, γ, and δ), collectively referred to as MicLH, that are derived by proteolytic processing from a 70 kDa polyprotein precursor encoded by the micronuclear linker histone (*MLH*) gene (Allis et al., 1984; Wu et al., 1994). Both the *HHO* gene and the *MLH* gene are present as single copies in the T. thermophila (haploid) genome, allowing functional study by gene knockout experiments using recently developed methods for gene replacement (Gaertig et al., 1994). Like other eukaryotes, Tetrahymena cells regulate gene expression mostly at the level of transcription (Stargell et al., 1990). Thus, this system provides a unique opportunity for elucidating linker histone function in transcription in vivo.

We have previously shown that linker histones are not essential for survival by disrupting linker histone genes in the transcriptionally active macronucleus (Shen et al., 1995). In the current study, the effect of linker histone loss on transcription was investigated in vivo. We find that linker histone H1 does not appear to affect global transcription. In cells lacking H1 (ΔH1), the number of mature RNAs produced by genes transcribed by each of the three classes of RNA polymerases remains unchanged. However, basal transcription of at least one repressed gene (*ngoA*) is increased, suggesting that H1 can function as a gene-specific repressor. Surprisingly, in these cells, the activated transcription of at least one gene (*CyP*) is reduced, suggesting an unexpected role of H1 in gene-specific activation. Thus, like core histones, a linker histone can function as either a positive or negative regulator of gene expression in vivo.

Results

RNA Content and Dry Weight Are Unchanged in Cells Lacking Linker Histones

Tetrahymena cells lacking linker histones grow normally (Shen et al., 1995), suggesting that critical functions such as replication, transcription, and protein synthesis in these cells are not grossly altered. We also have shown that ΔH1 and ΔMicLH (MicLH knockout) cells contain normal amounts of DNA in their nuclei (Shen et al., 1995), suggesting that DNA replication is unaffected. To determine the RNA levels in linker histone knockout strains, total RNA was isolated from a known number of wild-type cells, cells lacking H1 (ΔH1), and cells lacking the micronuclear linker histones (ΔMicLH) in log growth phase by the guanidine isothiocyanate–CsCl method (Chirgwin et al., 1979). Total RNA was measured by spectrophotometry. Isolated RNA was of good quality, as indicated by 260 nm/280 nm values greater than 1.8. In wild type, total RNA per cell was about 180 pg (Table 1). Total RNA per cell in ΔH1 and ΔMicLH cells was not significantly different from that of wild-type cells (Table 1), indicating that total RNA transcripts are not changed upon loss of linker histones. In cells starved for 18 hr, total RNA per cell was about half that in growing cells. Again, no large differences were detected among the RNA contents of starved wild-type, ΔH1, and ΔMicLH cells (Table 1).

To investigate the effect of linker histone loss on overall protein contents, the dry weights of wild-type, ΔH1, and ΔMicLH cells were measured. If protein amounts are changed significantly, dry weight should reflect the change, since proteins make up more than half of dry weight (Calzone et al., 1983). Triplet samples of known numbers of cells from log phase were dried and weighed. Dry weight per cell was about 1.8–1.9 ng for wild-type, ΔH1, and ΔMicLH cells (Table 1). Coupled with the fact that ΔH1 and ΔMicLH cells have normal doubling times (Shen et al., 1995), this suggests that

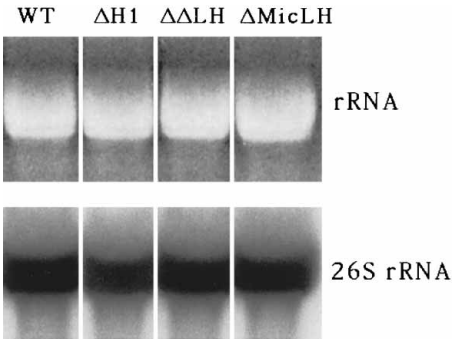

Figure 1. RNA Polymerase I Transcripts Are Unchanged in Linker Histone Knockout Strains

Total RNA from 10,000 cells was separated on a formaldehyde–agarose gel and stained with ethidium bromide (top). The rRNA band consists of 17S rRNA and two nicked halves of 26S rRNA. rRNA remains unchanged in ΔH1, ΔMicLH, and ΔΔLH strains. Using a 26S rRNA-specific probe on a Northern blot (bottom), little difference was detected in the 26S rRNA levels among wild-type, ΔH1, ΔMicLH, and ΔΔLH strains.

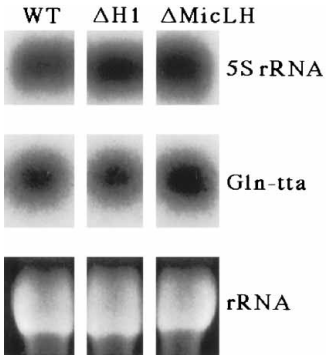

Figure 2. RNA Polymerase III Transcripts Are Unchanged in Linker Histone Knockout Strains

Northern analysis of RNA polymerase III transcripts. Total RNA was isolated from wild-type, ΔH1, ΔMicLH cells at 200,000 cells per ml during log growth. Total RNA (10 μg) was analyzed in each lane. Using a 5S rRNA-specific probe (top), little difference in 5S rRNA levels was detected among wild-type, ΔH1, and ΔMicLH strains. Using a Gln-tta tRNA-specific probe (middle), little difference in Gln-tta tRNA level was detected among wild-type, ΔH1, and ΔMicLH strains. Bottom panel shows ethidium bromide–stained rRNA as loading control.

protein synthesis in linker histone knockout cells is not significantly altered.

RNA Polymerase I Transcripts Are Not Affected in Cells Lacking Linker Histones

In Tetrahymena cells as in other eukaryotes, rDNA is transcribed by an α-amanitin–insensitive RNA polymerase I into precursor rRNA, which is processed into mature 26S, 5.8S, and 17S rRNAs (for review and references see Cech et al., 1982). To determine the effect of linker histone loss on RNA polymerase I transcripts, rRNA was examined by ethidium-bromide staining of total RNA from equal numbers of cells run on an RNA gel. In Tetrahymena cells, the rRNA band consists of 17S rRNA and two halves of the 26S rRNA that is nicked during maturation (Eckert et al., 1978). No changes in rRNA amounts were detected in ΔH1, double knockouts of H1 and MicLH (ΔΔLH; Shen et al., 1995), or in ΔMicLH (Figure 1). To confirm this, a 26S rRNA-specific probe was used to hybridize total RNA from equal numbers of cells on a Northern blot. There were no detectable differences in total hybridization to 26S rRNA among ΔH1, ΔΔLH, ΔMicLH, and wild type (Figure 1), indicating that accumulation of mature RNA polymerase I transcripts is not affected by the loss of linker histones.

RNA Polymerase III Transcripts Are Not Affected in Cells Lacking Linker Histones

RNA polymerase III transcribes small RNAs, such as 5S rRNA and tRNAs. To determine the effect of linker histone loss on RNA polymerase III transcription, the 5S rRNA levels were measured using Northern blotting. There was little difference in the 5S rRNA levels of wild-type, ΔH1, and ΔMicLH cells (Figure 2). To rule out the possibility that RNA isolation or Northern blotting or both were biased against small RNAs, slot blot hybridization was also performed to measure 5S rRNA abundance without RNA isolation (Grimes et al., 1988). Using this method, the 5S rRNA levels in ΔH1 and ΔMicLH cells were also indistinguishable from those in control cells (data not shown).

A Gln-tta tRNA probe specific for the glutamine tRNA recognizing the TAA codon in T. thermophila (Kuchino et al., 1985) was also used to analyze RNA polymerase III transcripts on Northern blots. Little difference in Gln-tta tRNA levels was detected among wild-type, ΔH1, and ΔMicLH cells (Figure 2). Thus, two specific RNA polymerase III transcripts are not affected by the loss of linker histones.

Most RNA Polymerase II Transcripts Are Not Affected in Cells Lacking Linker Histones

RNA polymerase II is responsible for transcription of most genes that encode mRNA. In Tetrahymena cells, gene expression is controlled at the level of transcription. When the relative transcriptional activities of 14 genes were determined in different physiological or developmental states (growth, starvation, and conjugation) in which many of the genes showed striking differences in RNA abundance, in every case except one (Love et al., 1988) changes in transcription accompanied changes in RNA abundance. Thus, differential transcription, not differential RNA degradation, is the major mechanism regulating RNA abundance in Tetrahymena cells (Stargell et al., 1990). Since most mRNAs are polyadenylated in Tetrahymena cells (Calzone et al., 1983), the amount of total polyA$^+$ mRNA should reflect global transcription by RNA polymerase II.

To determine the total polyA$^+$ mRNA level, Northern blot analysis was performed using polyT or polyU as probes. Total RNA from known numbers of cells was blotted and rRNA was used as an internal standard, since its level does not change in cells lacking linker histones (see Figure 1). Using a 30 nt polyT probe, the sizes of mRNAs detected ranged from less than 1 kb to greater than 6 kb. Most mRNAs were between 1–2 kb

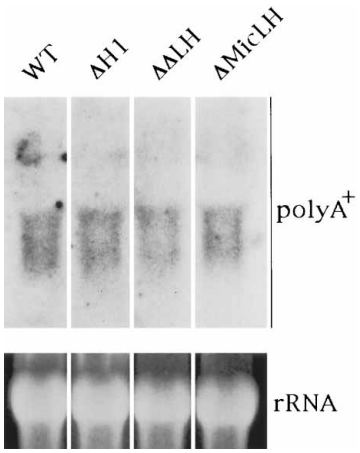

Figure 3. Total PolyA$^+$ Transcripts Are Unchanged in Linker Histone Knockout Strains

Northern analysis of polyA$^+$ transcripts. Total RNA was isolated from wild-type, ΔH1, ΔMicLH, ΔΔLH cells at 200,000 cells per ml during log growth. Total RNA (10 μg) was analyzed in each lane. A 30 nt polyT probe was used to detect polyA$^+$ transcripts (top). The bulk of the hybridization was observed to migrate faster than rRNA. Total polyA$^+$ transcripts remain unchanged in ΔH1, ΔMicLH, and ΔΔLH strains. Bottom panel shows ethidium bromide–stained rRNA as loading control.

(Figure 3). No change in total polyA$^+$ mRNA level was detected in ΔH1, ΔΔLH, or ΔMicLH cells (Figure 3). Likewise, no differences in total polyA$^+$ mRNA levels were observed in starved cells (data not shown). Similar analyses using a polyU probe gave similar results (data not shown). Thus, the steady-state level of polyA$^+$ mRNA is not changed, indicating that most RNA polymerase II transcripts are not affected by loss of linker histones.

To extend these observations to specific mRNAs, transcripts of a number of genes in log cells were examined by Northern blots, including genes encoding histones H3 and hv1, nuclear nonhistone proteins HMG B and HMG C, and the cytoskeletal proteins actin and β-tubulin. No differences in message size or amounts for any of these mRNAs were detected in ΔH1 or ΔMicLH strains (data not shown), consistent with the conclusion that most RNA polymerase II transcripts are not affected by the loss of linker histones.

Global Transcription Is Not Affected in Cells Lacking Linker Histones

While the absence of changes in steady-state levels of RNA is most simply explained by the absence of changes in transcription, it is possible that increased or decreased transcription combined with parallel increased or decreased decay can result in seemingly unchanged steady-state RNA levels. To address this, nuclear run-on experiments were performed to examine total transcriptional activity. Using equal numbers of nuclei isolated from control and ΔH1 cells, transcription activity was determined by ^{32}P-UTP incorporation from a known number of nuclei. The relative transcription rates of each type of cells was determined using nuclei isolated from control log cells as a standard (1.00). In

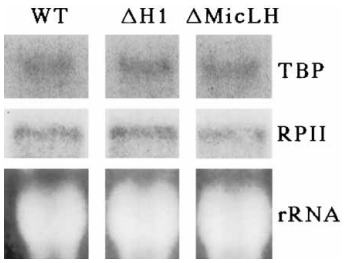

Figure 4. mRNAs for TBP and *RPII* Are Unchanged in Linker Histone Knockout Strains

Northern analysis of mRNAs for TBP and for *RPII*. Total RNA was isolated from wild-type, ΔH1, ΔMicLH cells at 200,000 cells per ml during log growth. Total RNA (10 μg) was analyzed in each lane. Using a TBP gene-specific probe (top), a 1.4 kb band was detected. No change in TBP mRNA size or amount was observed in ΔH1 or ΔMicLH strains. Using a Tetrahymena *RPII*-specific probe (middle), a band of about 6 kb was detected. No change in *RPII* mRNA size or amount was observed in ΔH1 or ΔMicLH strains. Bottom panel shows ethidium bromide–stained rRNA as loading control.

nuclei isolated from ΔH1 log cells, the relative transcription rate was 1.02 (Table 1), indicating that linker histone H1 does not affect general transcription. In starved cells, transcription activity is reduced 2- to 3-fold. Again, little difference is detected in relative transcription rate between wild-type (0.35) and ΔH1 (0.40; Table 1). It should be noted that the majority of run-on transcription in log cell macronuclei is from the 17S and 26S ribosomal RNA genes, while in starved cell nuclei the majority of transcription is nonribosomal (presumably pol II) transcription (K. Shupe and M. A. G., unpublished data). These results strongly indicate that linker histone H1 does not affect global transcription of pol I and pol II genes in vivo.

We also examined the levels of two mRNAs encoding components of the basal transcription apparatus (for review see Lewin, 1994): the universal transcription factor, TATA-binding protein (TBP; Cormack and Struhl, 1992), and the conserved large subunit of RNA polymerase II (*RPII*; Allison et al., 1985). Using a Tetrahymena TBP probe (Stargell and Gorovsky, 1994) and a probe made from a partial cDNA of Tetrahymena *RPII*, a single-copy gene that shares high sequence homology to *RPII* genes from other organisms (data not shown), no differences in message size or amounts for either of these two mRNAs were detected in ΔH1 or ΔMicLH strains on Northern blots (Figure 4), consistent with an unchanged basal transcription machinery in linker histone knockouts.

Linker Histone H1 Represses Basal Transcription of a Specific Gene (*ngoA*)

We have shown that linker histone H1 has little or no effect on general transcription or on the expression of a number of constitutively expressed genes. Next, we wished to determine whether it had an effect on the expression of inducible genes. Certain genes in Tetrahymena cells are not expressed in one physiological stage but are induced in another. Such genes offer an opportunity to investigate the role of linker histone H1 in regulated transcription.

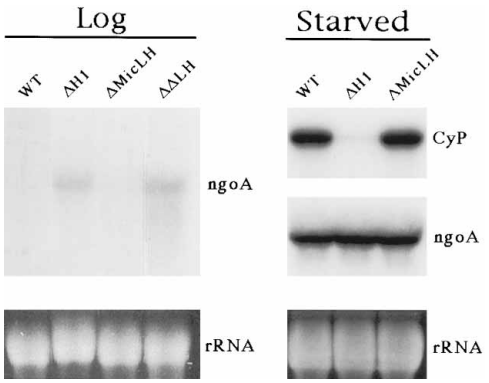

Figure 5. Expression of mRNAs for *ngoA* and *CyP* Genes in Linker Histone Knockout Strains

Northern analysis of *ngoA* and *CyP* expression. Total RNA was isolated from wild-type, ΔH1, ΔMicLH, ΔΔLH cells at 180,000 cells per ml during log growth. Total RNA was also isolated from 18 hr starved wild-type, ΔH1, and ΔMicLH cells at 300,000 cells per ml in starvation medium. Total RNA (10 μg) was analyzed in each lane. Using a *ngoA*-specific probe, no *ngoA* mRNA was detectable in wild-type or ΔMicLH in log cells, while a weak but clear *ngoA* mRNA expression was observed in both ΔH1 and ΔΔLH log cells (top left). In starved cells, *ngoA* mRNA levels were high and similar in wild-type, ΔH1, and ΔMicLH strains (middle right). Using a *CyP*-specific probe, no *CyP* mRNA was detectable in wild-type, ΔH1, ΔΔLH, or ΔMicLH log cells (data not shown). In starved cells, *CyP* mRNA levels were about 10-fold higher in both wild-type and ΔMicLH than in ΔH1 strains (top right). Bottom panel shows ethidium bromide–stained rRNA as loading control.

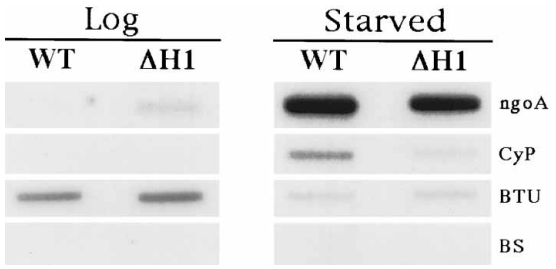

Figure 6. Transcription of *ngoA* and *CyP* Genes in Linker Histone Knockout Strains

Transcriptional analysis by nuclear run-on experiments. Nuclei were isolated from wild-type and ΔH1 cells at 160,000 cells per ml during log growth (left). Nuclei were also isolated from 20 hr starved wild-type and ΔH1 cells at 240,000 cells per ml in starvation medium (right). Probes from top to bottom were *ngoA*, *CyP*, *BTU* (β-tubulin genes), and BS (Bluescript vector control). *ngoA* transcription was not detectable in wild-type log cells, but basal transcription was detected in ΔH1 log cells. Similar and high *ngoA* transcription was detected in wild-type and ΔH1 starved cells (top). *CyP* transcription was not detected in wild-type or ΔH1 log cells, but in starved cells *CyP* transcription was about 3- to 5-fold higher in wild-type than in ΔH1 (second panel). Similar high levels of transcription of *BTU* were detected in wild-type and ΔH1 log cells, while similar low levels of *BTU* transcription were detected in wild-type and ΔH1 starved cells (third panel). BS vector control showed no hybridizations (bottom).

Two major histone H3 genes, *HHT1* and *HHT2*, are expressed in growing cells but not in starved cells (Bannon et al., 1983). When their mRNA levels were examined by Northern blotting in cells lacking linker histones, normal levels of both mRNAs were observed in log cells. No accumulation of either mRNA was detected in starved cells (data not shown), indicating that neither *HHT1* nor *HHT2* expression is affected by the loss of linker histones.

ngoA is a nongrowth-specific gene of unknown function (Martindale and Bruns, 1983). Its mRNA can be detected in stationary or starved cells but not in growing cells. Nuclear run-on experiments showed that transcription of *ngoA* correlates with its mRNA expression pattern; i.e., *ngoA* transcription can be detected in starved cells but not in log cells (Stargell et al., 1990). When *ngoA* mRNA was examined by Northern blotting, a weak but clear basal expression in vegetative growing cells was detected in both ΔH1 and ΔΔLH but not in control or ΔMicLH cells (Figure 5), suggesting that linker histone H1 is involved in the basal repression of the *ngoA* gene during vegetative growth. This low level of induction was detected in three of four experiments. However, during starvation, the activated *ngoA* mRNA level was not changed in ΔH1 cells (Figure 5) or in ΔΔLH cells (data not shown). This is consistent with a gene-specific role of linker histone H1 in repression of basal transcription and the absence of a role in activated transcription.

To investigate whether linker histone H1 represses *ngoA* basal expression at the transcriptional level, nuclear run-on experiments were performed. Nuclei were isolated from control and ΔH1 cells at early log phase as well as from starved cells. During early log phase, *ngoA* transcription was not detectable above background level in control cells. However, in ΔH1 cells, weak but clear and reproducible transcription of *ngoA* was detected (Figure 6), strongly suggesting that linker histone H1 represses *ngoA* basal transcription. This weak induction was observed in two of two experiments. Both control and ΔH1 starved cells showed similar and highly induced levels of *ngoA* transcription (Figure 6), consistent with the expression pattern detected by Northern blotting (see Figure 5). Note that transcription of the genes encoding β-tubulin, though differing significantly between growing and starved cells, is indistinguishable in the presence or absence of H1 (Figure 6). These results indicate that linker histone H1, although not a global repressor of transcription, can function as a gene-specific repressor of transcription in vivo.

Linker Histone H1 Activates *CyP* Transcription

To investigate further the role of linker histone H1 in specific gene regulation, we studied another gene exhibiting similar regulated transcription. *CyP* (formerly known as BC11) encodes a cysteine protease (Karrer et al., 1993). It is regulated like *ngoA* in wild-type cells; both its message and its transcription are undetectable in growing cells and are strongly induced by starvation (Stargell et al., 1990).

When *CyP* mRNA was examined in linker histone H1 knockout strains, a surprisingly different pattern emerged. Unlike *ngoA*, *CyP* did not exhibit leaky basal expression in ΔH1 cells at log phase (data not shown), suggesting that it is maintained in the repressed state in the absence of linker histone H1. However, in starved cells, while *CyP* mRNA in both control and ΔMicLH cells was highly induced, it was only slightly induced in ΔH1

cells (Figure 5). Quantitation of the Northern blots indicated that the reduction in the induced level of *CyP* mRNA in ΔH1 cells was about 10-fold, suggesting that linker histone H1 is required for proper activation of *CyP* expression. A similar effect was observed in ΔΔLH cells (data not shown).

To investigate whether linker histone H1 affects *CyP* expression at the transcriptional level, we performed nuclear run-on experiments. *CyP* transcription was not detectable in nuclei isolated from either wild-type or ΔH1 log phase cells (Figure 6), consistent with the expression pattern detected by Northern blotting (see Figure 5). Thus, linker histone H1 does not appear to be involved in *CyP* repression. However, in starved cells, *CyP* transcription was 3- to 5-fold higher in control than in ΔH1 cells (Figure 6), indicating that linker histone H1 was required for the activated transcription of the *CyP* gene. These results strongly argue that linker histone H1, surprisingly, can function as a gene-specific activator of transcription in vivo.

Discussion

Linker histone H1 has long been implicated in general repression of transcription (Zlatanova and Van Holde, 1992). However, our results suggest that linker histone H1 is not likely to be the sole or the major general repressor of transcription in vivo. We have previously shown that linker histones are not essential and that linker histone knockout strains grow normally (Shen et al., 1995). More directly, in this study we have shown that linker histone H1 does not appear to repress transcription of most genes in vivo. Levels of mature transcripts produced by three classes of RNA polymerases were not affected by the loss of linker histones. We have previously ruled out the possibility that the micronuclear linker histone substitutes for H1 by showing that MicLH is not present in the macronuclei of the ΔH1 cells and that both linker histones can be eliminated in the same cells without affecting growth (Shen et al., 1995). Another possibility, that other nonlinker histone proteins can replace linker histone function, is also unlikely, since we could not detect any major differences in the nuclear protein profiles in linker histone knockout strains other than the loss of the linker histones (data not shown). Also, our study is based on complete disruption of naturally existing linker histones in vivo, making it more likely to reflect the physiological role of linker histones than studies using an in vitro system or introducing heterologous linker histones in vivo.

One mechanism whereby H1 could repress general transcription is by condensing chromatin structures, thus limiting the access of the transcription machinery to promoters and enhancers. We have previously shown that linker histones are involved in chromatin condensation in vivo (Shen et al., 1995). ΔH1 cells have enlarged diamidophenylindole-stained macronuclei and normal-sized micronuclei, while ΔMicLH cells have enlarged micronuclei and normal-sized macronuclei. Thin section electron microscopy directly demonstrates that the increase in diamidophenylindole-staining area results, at least in part, from chromatin decondensation (our unpublished data). Thus, in ΔH1 cells, macronuclear chromatin decondensation occurs but general transcription does not increase, suggesting that chromatin decondensation, per se, does not lead to general derepression of transcription. We have shown that in ΔH1 cells, the mRNA levels for the universal transcription factor TBP and for *RPII* were not changed (Figure 4), consistent with unchanged basal transcription machinery in linker histone knockout strains. These observations argue that some part of the transcription machinery other than chromatin decondensation is the limiting factor in general transcription.

Although linker histone H1 does not affect transcription globally, H1 does repress the basal transcription of at least one gene, *ngoA* (a gene of unknown function). In growing cells, there was a small but distinct accumulation of *ngoA* message in cells lacking H1 but not in control cells (Figure 5). The levels of *ngoA* mRNA in growing cells of the H1 knockout strains were far below the levels induced by starvation (Figure 5), suggesting a derepression of basal transcription rather than a true induction. We also showed that *ngoA* repression by H1 occurred at the level of transcription (Figure 6), providing strong evidence that H1 represses basal gene expression in vivo. In Xenopus, Bouvet et al. (1994) demonstrated that overexpression of H1 during early development selectively inhibits the expression of oocyte 5S rRNA gene without affecting expression of somatic 5S rRNA (or U1/U2 or tRNA) genes. Conversely, depletion of H1 specifically increases oocyte 5S rRNA gene transcription. These results, while quite different from ours in detail, provided the first indication that H1 could affect transcription in a gene-specific manner.

Unexpectedly, in Tetrahymena cells, linker histone is required for the activated expression of at least one gene, *CyP*. Unlike *ngoA*, basal expression of the *CyP* message was not derepressed in growing cells; however, the induced level of mRNA in starved cells lacking H1 was reduced over control cells (Figure 5). We also showed that H1 activated *CyP* expression at the level of transcription (Figure 6), providing strong evidence for a novel function of H1 as an activator of transcription in vivo.

Several lines of evidence suggest that linker histone H1 affects basal repression of *ngoA* and activated transcription of *CyP* directly rather than indirectly. First, we have shown that expression of many genes, including some required for basal and activated transcription (TBP and *RPII*), is not affected in the absence of H1. Secondly, if loss of H1 simply derepressed the specific transcription factors required for *ngoA* expression, we might have expected fully induced expression of *ngoA* in growing cells. We observed only low levels of expression. Thirdly, if the incomplete activation of *CyP* transcription during starvation was caused by improper expression of hypothetical starvation-specific activators due to the loss of linker histone H1, we should have observed similar effects on other starvation-specific genes. However, activated transcription of *ngoA* during starvation was not affected by linker histone loss (Figures 5 and 6). Taken together, these considerations argue that linker histone H1 is likely to be directly involved in both the positive and negative regulation of specific genes. Even if the effects of loss of H1 on expression of *ngoA* and *CyP* are indirect, it is important to emphasize that these knockout

studies likely reveal important physiologically relevant functions of H1 in vivo.

There are several mechanisms by which linker histone H1 might affect specific gene expression. One possibility is that H1 plays a role in determining the accessibility of genes to the transcription machinery by compaction of higher order chromatin structure or by altering the attachment of chromatin to the nuclear matrix. However, these mechanisms might be expected to affect most genes similarly, resulting in a more global effect of the removal of H1 on transcription. Another possibility is that releasing H1 from linker-associated sequences modifies local chromatin structures around specific genes by enhancing nucleosome mobility (sliding) or altering nucleosome position, thereby exposing or shielding regulatory regions (Meersseman et al., 1992; Simpson et al., 1993; Wolffe, 1994). These possible mechanisms are not mutually exclusive, and different ones could apply to different genes. They are currently being investigated in our in vivo system.

Like the H3 and H4 core histones in S. cerevisiae (Fisher-Adams and Grunstein, 1995), we have shown that linker histone can also affect the expression of specific genes both positively and negatively. In Tetrahymena cells, H1 repressed basal expression of *ngoA* and was required for fully activated expression of *CyP*. Similarly, depletion of H4 in yeast (Kim et al., 1988), like depletion of H1 in Tetrahymena, did not affect transcription globally; changes in gene expression were highly gene-specific. These similarities can be viewed in two ways. In one view, the chromatin structure of each gene could be unique, established by a combination of factors including sequence-specific placement of nucleosomes, sequence-specific placement of nonhistone DNA binding factors, or particular structural features of the underlying DNA itself. In this view, any global change in histones is translated into different changes in the chromatin architecture of each gene, which results in a gene-specific alteration (either positively or negatively) in the binding of (positive or negative) transcription factors. An alternative view is that much of the specificity lies with the transcription factors themselves. In this view, the constant features of chromatin structure such as the nucleosome core, the N-terminal tails of core histones, or the linker-associated histones can (but need not) be used differently by gene-specific transcription activators or repressors either to facilitate or inhibit their binding, explaining the apparent gene specificity of mutating or deleting these histones. In either case, the recent studies on core histones in S. cerevisiae and linker histones in T. thermophila necessitate changing our view of both classes of histones from nonspecific repressors of transcription to coparticipants with transcription factors in the specificity of transcription in vivo. The recent findings that core histones and some TBP–associated factors show remarkable structural similarities in their histone fold motifs (Xie et al., 1996) and that the structure of the globular region of linker histone H5 resembles that of the transcription factor (HNF-3γ; Ramakrishnan et al., 1993; Clark et al., 1993) suggest that perhaps histones are ancestral transcription factors which later evolved a more structural role in chromatin, while other factors evolved more specialized roles in transcription.

Experimental Procedures

Strains and Culture Conditions

T. thermophila wild-type control strain CU428 was provided by P. J. Bruns (Cornell University). Linker histone knockout strains ΔH1, ΔMicLH, and ΔΔLH were obtained by gene disruption and were described previously (Shen et al., 1995).

Cells were grown in 1 × SPP (Gorovsky et al., 1975) at 30°C. Cell number was measured using a Coulter Counter (Coulter Electronics, Incorporated). For starvation, cells in the logarithmic phase of growth were washed twice in 10 mM Tris–HCl (pH 7.4), then starved for 18–20 hr in 10 mM Tris–HCl (pH 7.4) at 30°C.

Dry Weight Measurement

Logarithmically growing wild-type, ΔH1, or ΔMicLH cells (1 l) were harvested, washed once in water, then resuspended in 50 ml of water and cell numbers counted. Cells were centrifuged and transferred onto preweighed 47 mm filter paper (Nuclepore Corporation). Filters were dried for 6 hr at 50°C, then weighed.

RNA Isolation and Quantitation

Total RNA was isolated according to published procedures (Chirgwin et al., 1979). Growing cells at different cell densities were used for isolating log phase total RNA. Total RNA from starved cells was isolated from cells that had been starved for 18 hr at the density of 300,000 cells/ml.

Total RNA was resuspended in diethyl pyrocarbonate (Sigma Chemical Company)–treated water and then quantitated by measuring absorbance at 260 nm using a Spectronic 1201 spectrophotometer (Milton Roy Company), assuming absorbance of one OD_{260} unit equal to 40 μg/ml RNA.

Cloning a Partial cDNA Encoding Tetrahymena RNA Polymerase II Large Subunit Gene

A partial cDNA encoding the T. thermophila *RPII* was cloned using the polymerase chain reaction. Two primers were constructed based on conserved regions of the same gene in other organisms (Azuma et al., 1991; Li et al., 1989). Primer 1, 5′-TTGGATCCAGAGGTAATTTA ATGGGTAAAAGAGT-3′, corresponds to the conserved amino acid sequence RGNLMGKRV. Primer 2, 5′-TTGGATCCATTCATTTCATC ACCATCGAAATCAGCATTATA-3′, corresponds to the conserved amino acid sequence YNADFDGDEMNL. A 460 bp product was amplified from random primer–generated cDNA from T. thermophila as template. pXS0.0 was constructed by inserting the blunt-ended polymerase chain reaction product into the SmaI site of Bluescript KS vector (Stratagene). The insert was sequenced, and its homology to other eukaryotic polymerases was determined by using Genetics Computer Group sequence analysis software (Devereux et al., 1984).

Northern Analysis

Total RNA (10 μg) isolated from growing or starved Tetrahymena cells was electrophoresed on a 2.2 M formaldehyde–1.5% agarose gel. The gel was stained with ethidium bromide (Sigma Chemical Company) and photographed; then RNA was blotted onto Magnagraph nylon membranes (Micron Separations, Incorporated). ^{32}P-labeled randomly primed probes were made as follows: the 26S rRNA probe was a HindIII fragment from pBS26S that had a 2 kb HindIII fragment containing the Tetrahymena 26S rDNA-transcribed region from pRP9 (Engberg et al., 1980) inserted into the HindIII site of Bluescript vector (Stratagene); the 5S rRNA probe was an EcoRI fragment from pDP6 (Pederson et al., 1984); the TBP probe was an AflII fragment from pTBP.GEN (Stargell and Gorovsky, 1994); the Tetrahymena *RPII* probe was a BamHI–PstI fragment from pXS0.0; the *ngoA* probe was a PstI fragment from pC5.5 (Martindale and Bruns, 1983); the *CyP* probe was two PstI fragments from pCyP (Karrer and Stein-Gavens, 1990). For the above probes, hybridizations were carried out at 42°C in 50% formamide, 5 × SSC, 1 × SPED, 1% SDS, and 200 μg/ml degraded sperm DNA. Final washes were in 0.1 × SSC, 1% SDS at 50°C. A 30 nt polyT probe was synthesized and ^{32}P–end-labeled with T4 polynucleotide kinase (New England Biolabs) for detecting polyA$^+$ mRNAs. The Gln-tta tRNA (Kuchino et al., 1985) probe was synthesized (5′-CAGGTCAAG GGATTTAAAGTCCCCAGTACT-3′) and end-labeled. Hybridizations

for these two probes were carried out at 42°C in 5 × SSC, 1 × SPED, 1% SDS, and 200 μg/ml degraded sperm DNA. Final washes were in 0.1 × SSC, 1% SDS at 42°C. Hybridization was quantitated by densitometry using NIH Image (NIH) software or by using a PhosphorImager (Molecular Dynamics), or both.

Run-On Transcription

Nuclei were isolated as described (White and Gorovsky, 1988), with the following modifications: first, all operations were carried out on ice; second, spermidine was omitted from medium A and medium B; and third, to isolate nuclei from starved cells, we changed medium A from 3% to 17.1% sucrose and medium B from 1% to 0.3% octanol.

Logarithmically growing CU428 or ΔH1 cells (1 l), at a density of 160,000 cells/ml, were used to obtain nuclei from log cells. CU428 or ΔH1 cells (1 l), which were starved at a density of 240,000 cells/ml for 20 hr, were used to obtain nuclei from starved cells. Isolated nuclei were resuspended in 50% glycerol/50% medium A at 4 × 10^7 nuclei/ml and stored at −20°C.

Run-on transcription was performed as described (Stargell et al., 1990) with the following modifications: first, spermine and spermidine were omitted from all transcription buffers; second, for slot blot hybridization, gene-specific restriction fragments were used. (The *ngoA* probe was a PstI fragment from pC5.5 [Martindale and Bruns, 1983]; the *CyP* probe was two PstI fragments from pCyP [Karrer and Stein-Gavens, 1990]; the BTU probe was a HindIII fragment from pBTU2 [Gaertig et al., 1993]; Bluescript SK [Stratagene] was linearized and used as control [BS]). Third, neutralization solutions (1 M Tris–HCl [pH 7.5] and 20 × SSC) were kept on ice before use; fourth, ^{32}P-labeled RNA was purified using G-25 Sephadex RNA Quick Spin columns (Boehringer Mannheim); and fifth, hybridizations were quantitated densitometrically using NIH Image software and a PhosphorImager.

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References

Allis, C.D., Allen, R.L., Wiggins, J.C., Chicoine, L.C., and Richman, R. (1984). Proteolytic processing of H1-like histones in chromatin: a physiologically and developmentally regulated event in *Tetrahymena* micronuclei. J. Cell Biol. *99*, 1669–1677.

Allison, L.A., Moyle, M., Shales, M., and Ingles, C.J. (1985). Extensive homology amongst the large subunits of eukaryotic and prokaryotic RNA polymerases. Cell *42*, 599–610.

Azuma, Y., Yamagishi, M., Ueshima, R., and Ishihama, A. (1991). Cloning and sequence determination of the *Schizosaccharomyces pombe rpb1* gene encoding the largest subunit of RNA polymerase II. Nucl. Acids Res. *19*, 461–468.

Bannon, G.A., Calzone, F.J., Bowen, J.K., Allis, C.D., and Gorovsky, M. A. (1983). Multiple, independently regulated polyadenylated messages for histone H3 and H4 in *Tetrahymena*. Nucl. Acids Res. *11*, 3903–3917.

Bouvet, P., Dimitrov, S., and Wolffe, A.P. (1994). Specific regulation of *Xenopus* chromosomal 5S rRNA gene transcription in vivo by histone H1. Genes. Dev. *8*, 1147–1159.

Calzone, F.J., Angerer, R.C., and Gorovsky, M.A. (1983). Regulation of protein synthesis in *Tetrahymena*: quantitative estimates of the parameters determining the rates of protein synthesis in growing, starved, and starved-deciliated cells. J. Biol. Chem. *258*, 6887–6897.

Cech, T.R., Zaug, A.J., Grabowski, P.J., and Brehm, S.L. (1982). Transcription and splicing of the ribosomal RNA precursor of *Tetrahymena*. In The Cell Nucleus, H. Busch and L. Rothblum, eds. (New York: Academic Press), pp. 171–204.

Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J., and Rutter, W.J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry *18*, 5294–5299.

Clark, K.L., Halay, E.D., Lai, E., and Burley, S.K. (1993). Cocrystal structure of the HNF-3/fork head DNA–recognition motif resembles histone H5. Nature *364*, 412–420.

Cormack, B.P., and Struhl, K. (1992). The TATA-binding protein is required for transcription by all three nuclear RNA polymerases in yeast cells. Cell *69*, 685–696.

Devereux, J., Haeberli, P., and Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. Nucl. Acids Res. *12*, 387–395.

Eckert, W.A., Kaffenberger, W., Krohne, G., and Franke, W.W. (1978). Indroduction of hidden breaks during rRNA maturation and aging in *Tetrahymena pyriformis*. Eur. J. Biochem. *87*, 607–616.

Engberg, J., Din, N., Eckert, W.A., Kaffenberger, W., and Pearlman, R.E. (1980). Detailed transcription map of the extrachromosomal ribosomal RNA genes in *Tetrahymena thermophila*. J. Mol. Biol. *142*, 289–313.

Fisher-Adams, G., and Grunstein, M. (1995). Yeast histone H4 and H3 N-termini have different effects on the chromatin structure of the GAL1 promoter. EMBO J. *14*, 1468–1477.

Gaertig, J., Thatcher, T.H., McGrath, K.E., Callahan, R.C., and Gorovsky, M.A. (1993). Perspectives on tubulin isotype function and evolution based on the observations that *Tetrahymena thermophila* microtubules contain a single α- and β-tubulin. Cell Motil. Cytoskel. *25*, 243–253.

Gaertig, J., Gu, L., Hai, B., and Gorovsky, M.A. (1994). High frequency vector–mediated transformation and gene replacement in *Tetrahymena*. Nucl. Acids Res. *22*, 5391–5398.

Gorovsky, M.A. (1973). Macro- and micronuclei of *Tetrahymena pyriformis*: a model system for studying the structure and function of eukaryotic nuclei. J. Protozool. *20*, 19–25.

Gorovsky, M.A. (1980). Genome organization and reorganization in *Tetrahymena*. Annu. Rev. Genet. *14*, 203–239.

Gorovsky, M.A., Yao, M.-C., Keevert, J.B., and Pleger, G.L. (1975). Isolation of micro- and macronuclei of *Tetrahymena pyriformis*. Meth. Cell Biol. *9*, 311–327.

Grimes, A., McArdle, H.J., and Mercer, J.F.B. (1988). A total extract dot blot hybridization procedure for mRNA quantitation in small samples of tissues or cultured cells. Anal. Biochem. *172*, 436–443.

Grunstein, M. (1990). Histone function in transcription. Annu. Rev. Cell Biol. *6*, 643–678.

Hayashi, T., Hayashi, H., and Iwai, K. (1987). *Tetrahymena* histone H1: isolation and amino acid sequence lacking the central hydrophobic domain conserved in other H1 histones. J. Biochem. *102*, 369–376.

Kandolf, H. (1994). The H1A histone variant is an in vivo repressor of oocyte-type 5S gene transcription in *Xenopus laevis* embryos. Proc. Natl. Acad. Sci. USA *91*, 7257–7261.

Karrer, K.M., and Stein-Gavens, S. (1990). Constancy of adenine methylation in *Tetrahymena* macronuclear DNA. J. Protozool. *37*, 409–414.

Karrer, K.M., Peiffer, S.L., and DiTomas, M.E. (1993). Two distinct gene subfamilies within the family of cysteine protease genes. Proc. Natl. Acad. Sci. USA *90*, 3063–3067.

Kim, U.-J., Han, M., Kayne, P., and Grunstein, M. (1988). Effects of histone H4 depletion on the cell cycle and transcription of *Saccharomyces cerevisiae*. EMBO J. *7*, 2211–2219.

Kuchino, Y., Hanyu, N., Tashiro, G., and Nishimura, S. (1985). *Tetrahymena thermophila* glutamine tRNA and its gene that corresponds to UAA termination codon. Proc. Natl. Acad. Sci. USA *82*, 4758–4762.

Lewin, B. (1994). Eukaryotic transcription and RNA processing. In Gene V, B. Lewin, ed. (New York: Oxford University Press), pp. 847–877.

Li, W.-B., Bzik, D.J., Gu, H., Tanaka, M., Fox, B.A., and Inselberg, J. (1989). An enlarged largest subunit of *Plasmodium falciparum*

RNA polymerase II defines conserved and variable RNA polymerase domains. Nucl. Acids Res. *17*, 9621–9636.

Linder, C., and Thoma, F. (1994). Histone H1 expressed in *Saccharomyces cerevisiae* binds to chromatin and affects survival, growth, transcription, and plasmid stability but does not change nucleosomal spacing. Mol. Cell. Biol. *14*, 2822–2835.

Love, H.D., Jr., Allen-Nash, A., Zhao, Q., and Bannon, G.A. (1988). mRNA stability plays a major role in regulating the temperature-specific expression of a *Tetrahymena thermophila* surface protein. Mol. Cell. Biol. *8*, 427–432.

Martindale, D.W., and Bruns, P.J. (1983). Cloning of abundant mRNA species present during conjugation of *Tetrahymena thermophila*: identification of mRNA species present exclusively during meiosis. Mol. Cell. Biol. *3*, 1857–1865.

Meersseman, G., Pennings, S., and Bradbury, E.M. (1992). Mobile nucleosomes—a general behavior. EMBO J. *11*, 2951–2959.

Miloshev, G., Venkov, P., Van Holde, K., and Zlatanova, J. (1994). Low levels of exogenous histone H1 in yeast cause cell death. Proc. Natl. Acad. Sci. USA *91*, 11567–11570.

Owen-Hughes, T., and Workman, J.L. (1994). Experimental analysis of chromatin function in transcription control. Crit. Rev. Eukaryot. Gene Expr. *4*, 403–441.

Paranjape, S.M., Kamakaka, R.T., and Kadonaga, J.T. (1994). Role of chromatin structure in the regulation of transcription by RNA polymerase II. Annu. Rev. Biochem. *63*, 265–297.

Pederson, D.S., Yao, M.-C., Kimmel, A.R., and Gorovsky, M.A. (1984). Sequence organization within and flanking clusters of 5S ribosomal RNA genes in *Tetrahymena*. Nucl. Acids Res. *12*, 3003–3021.

Pruss, D., Hayes, J.J., and Wolffe, A.P. (1995). Nucleosomal anatomy—where are the histones? Bioessays *17*, 161–170.

Ramakrishnan, V., Finch, J.T., Graziano, V., Lee, P.L., and Sweet, R.M. (1993). Crystal structure of globular domain of histone H5 and its implications for nucleosome binding. Nature *362*, 219–223.

Shen, X., Yu, L., Weir, J.W., and Gorovsky, M.A. (1995). Linker histones are not essential and affect chromatin condensation in vivo. Cell *82*, 47–56.

Simpson, R.T., Roth, S.Y., Morse, R.H., Patterton, H.-G., Cooper, J.P., Murphy, M., Kladde, M.P., and Shimizu, M. (1993). Nucleosome positioning and transcription. Cold Spring Harbor Symp. Quant. Biol. *58*, 237–245.

Stargell, L.A., and Gorovsky, M.A. (1994). TATA-binding protein and nuclear differentiation in *Tetrahymena thermophila*. Mol. Cell Biol. *14*, 723–734.

Stargell, L.A., Karrer, K.M., and Gorovsky, M.A. (1990). Transcriptional regulation of gene expression in *Tetrahymena thermophila*. Nucl. Acids Res. *18*, 6637–6639.

White, E.M., and Gorovsky, M.A. (1988). Localization and expression of mRNA for a macronucleus-specific histone H2A variant (hv1) during the cell cycle and conjugation in *Tetrahymena*. Mol. Cell. Biol. *8*, 4780–4786.

Wolffe, A.P. (1994). Nucleosome positioning and modification: chromatin structures that potentiate transcription. Trends Biochem. Sci. *19*, 240–244.

Wolffe, A. (1995). Chromatin Structure and Function (London: Academic Press).

Wu, M., Allis, C.D., Richman, R., Cook, R.G., and Gorovsky, M.A. (1986). An intervening sequence in an unusual histone H1 gene of *Tetrahymena thermophila*. Proc. Natl. Acad. Sci. USA *83*, 8674–8678.

Wu, M., Allis, C.D., Sweet, M.T., Cook, R.G., Thatcher, T.H., and Gorovsky, M.A. (1994). Four distinct and unusual linker proteins in a mitotically dividing nucleus are derived from a 71 kilodalton polyprotein, lack $p34^{cdc2}$ sites, and contain protein kinase A sites. Mol. Cell. Biol. *14*, 10–20.

Xie, X., Kokubo, T., Cohen, S.L., Mirza, U.A., Hoffmann, A., Chait, B.T., Roeder, R.G., Nakatani, Y., and Burley, S.K. (1996). Structural similarity between TAFs and the heterotetrameric core of the histone octamer. Nature *380*, 316–322.

Zlatanova, J., and Van Holde, K. (1992). Histone H1 and transcription: still an enigma? J. Cell Sci. *103*, 889–895.