Regulation of Histone Gene Expression in Budding Yeast

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ABSTRACT We discuss the regulation of the histone genes of the budding yeast *Saccharomyces cerevisiae*. These include genes encoding the major core histones (H3, H4, H2A, and H2B), histone H1 (*HHO1*), *H2AZ* (*HTZ1*), and centromeric H3 (*CSE4*). Histone production is regulated during the cell cycle because the cell must replicate both its DNA during S phase and its chromatin. Consequently, the histone genes are activated in late G1 to provide sufficient core histones to assemble the replicated genome into chromatin. The major core histone genes are subject to both positive and negative regulation. The primary control system is positive, mediated by the histone gene-specific transcription activator, *Spt10*, through the histone upstream activating sequences (UAS) elements, with help from the major G1/S-phase activators, SBF (**S**wi4 cell cycle box **b**inding **f**actor) and perhaps MBF (Mlul cell cycle box binding factor). *Spt10* binds specifically to the histone UAS elements and contains a putative histone acetyltransferase domain. The negative system involves negative regulatory elements in the histone promoters, the RSC chromatin-remodeling complex, various histone chaperones [the histone regulatory (HIR) complex, *Asf1*, and *Rtt106*], and putative sequence-specific factors. The SWI/SNF chromatin-remodeling complex links the positive and negative systems. We propose that the negative system is a damping system that modulates the amount of transcription activated by *Spt10* and SBF. We hypothesize that the negative system mediates negative feedback on the histone genes by histone proteins through the level of saturation of histone chaperones with histone. Thus, the negative system could communicate the degree of nucleosome assembly during DNA replication and the need to shut down the activating system under replication-stress conditions. We also discuss post-transcriptional regulation and dosage compensation of the histone genes.

THE histone genes have been studied intensively for several decades in model organisms and in humans. Initially, they were studied because they encode proteins of major importance and their function in packaging DNA into the nucleus as chromatin was clearly understood and because they are excellent models for cell-cycle-dependent regulation of gene expression. However, the histone genes are atypical in that there are multiple copies of most histone genes in all organisms. In this regard, the yeasts are perhaps the most tractable model organisms. In particular, the budding yeast *Saccharomyces cerevisiae* possesses only two copies each of the major core histone genes. This review focuses on the regulation of the yeast histone genes.

The histones are highly positively charged proteins that package DNA, which is negatively charged, into the nucleus in the form of chromatin, the substance of chromosomes. Packaging was initially thought to be the only function of the histones, but it is now clear that they also participate in gene regulation via both classical and epigenetic mechanisms. Nevertheless, the regulatory mechanisms appear to act primarily by controlling the extent to which DNA is made accessible to regulatory factors, *i.e.*, by controlling packaging.

The basic structural unit of chromatin is the nucleosome core, which is composed of two molecules each of the four core histones—H2A, H2B, H3, and H4—formed into an octamer, around which is wrapped ~147 bp of DNA in 1.75 superhelical turns (Luger *et al.* 1997). The central ~80 bp of the nucleosome is organized by an H3-H4 tetramer containing two molecules each of H3 and H4. H2A-H2B dimers are bound on both sides of the H3-H4 tetramer. More complex eukaryotes possess several variants of the histones H2A and

H3. The only variants found in budding yeast are H2A.Z (a close variant of H2A) and CenH3 (an H3 variant found only in centromeric nucleosomes). The nucleosome is the structural repeat unit of chromatin and includes the linker DNA and the linker histone H1 (Thoma *et al.* 1979). *In vivo*, nucleosomes are regularly spaced along the DNA, with a characteristic repeat length. In budding yeast, the repeat is ~165 bp (Thomas and Furber 1976; Lohr *et al.* 1977). Many regulatory regions coincide with gaps in the nucleosomal array, referred to as nucleosome-depleted regions (Yuan *et al.* 2005).

Typical H1 histones have a central globular domain flanked by a short N-terminal tail and a long, very positively charged, C-terminal tail. The globular domain binds near the dyad axis of the nucleosome core, sealing the two turns of DNA, and the C-terminal tail binds along the linker DNA, helping to fold the nucleosomal array into a higher-order structure. Most cells have about one molecule of H1 per nucleosome, but those cells that space their nucleosomes close together, *i.e.*, with short linker DNA (short repeat length), tend to have significantly less H1. Examples include neuronal cells (Thomas and Thompson 1977) and budding yeast (Thomas and Furber 1976; Lohr *et al.* 1977). The budding yeast H1 is atypical in that it possesses two globular domains (Landsman 1996; Patterson *et al.* 1998; Sanderson *et al.* 2005). In yeast, there is only about one H1 per 37 nucleosomes (Friedkin and Katcoff 2001).

The structure of chromatin provides some clues as to the principles that might be important in regulating the histone genes and histone production. First, the equal stoichiometry of the histones in the nucleosome core suggests that the major core histones (H2A, H2B, H3, and H4) would be produced in approximately equal proportions and that production of H1 would be a certain fraction of this. Second, as every scientist who has attempted to reconstitute chromatin using DNA and histones knows from bitter experience, even a very small excess of histones over DNA is sufficient to aggregate and precipitate the chromatin. This problem occurs when the DNA charge neutralization exceeds a critical value (Clark and Kimura 1990). Thus, we would expect the cell to regulate the relative and absolute amounts of each histone produced and its mode of delivery to the DNA very tightly. These requirements account for the coregulation of histone genes and their complex regulation at the transcriptional, post-transcriptional, and post-translational levels.

Histone production must be regulated during the cell cycle because the cell must replicate not only its DNA during S phase, but also its chromatin. Consequently, the histone genes are activated in late G1 to provide sufficient core histones to assemble the replicated DNA into chromatin. Clearly, enough histone to assemble an entire genome's worth of DNA into nucleosomes must be synthesized in a timely fashion. Inhibition of DNA synthesis results in rapid repression of the histone genes, indicating that their expression is tightly coupled to replication (Osley 1991). In more complex eukaryotes, some histone gene expression is inde-

pendent of replication, providing histones for chromatin assembly occurring outside S phase (Tagami *et al.* 2004), but it is unclear to what extent this is true for yeast.

The Yeast Histone Genes

S. cerevisiae possesses two genes encoding each of the four major core histones, plus single genes encoding H2A.Z (*HTZ1*), centromeric H3 (*CSE4*), and H1 (*HHO1*) (Figure 1A). The major core histone genes are organized into four loci, each containing two histone genes divergently transcribed from a central promoter. Two loci, *HHT1-HHF1* and *HHT2-HHF2*, encode identical H3 and H4 proteins (Smith and Murray 1983). Neither of these gene pairs is essential, but the cell requires one *HHT-HHF* locus for survival, indicating that H3 and H4 are essential (Smith and Stirling 1988). The other two loci, *HTA1-HTB1* and *HTA2-HTB2*, encode almost identical H2A and H2B proteins (Hereford *et al.* 1979). Initially, it was thought that *HTA1-HTB1* and *HTA2-HTB2* could also substitute for one another, although it was clear that *HTA1-HTB1* is not equivalent to *HTA2-HTB2* because deletion of the former is associated with a significant growth phenotype, whereas deletion of the latter has no obvious effect (Norris and Osley 1987). However, it is now clear that *hta1-htb1Δ* cells survive only if the *HTA2-HTB2* locus has been duplicated and that this duplication can occur in the S288C strain background but not in the W303 background because the latter lacks one of two Ty1 elements required for the duplication (Libuda and Winston 2006). Thus, in the strictest sense of the term, the *HTA1-HTB1* locus is essential, whereas the *HTA2-HTB2* locus is not. The *CSE4* gene is essential (Wysocki *et al.* 1999) because of its critical role in kinetochore formation. The *htz1* null mutant grows slowly (Jackson and Gorovsky 2000). The *hho1* null mutant has no growth phenotype (Patterson *et al.* 1998). In conclusion, all four major core histones and centromeric H3 are essential for survival, but H2A.Z and H1 are not.

Extensive studies of the regulation of the yeast histone genes have revealed that both positive and negative systems are involved. For an excellent and comprehensive review of the early work in this field, the reader should consult Osley (1991). In our laboratory, the focus is on the *HTA1-HTB1* locus. We will use this locus as a guiding example in what follows.

Cell-Cycle-Dependent Activation of the Histone Genes

Spt10 binds specifically to the histone upstream activating sequence elements

Histone upstream activating sequence (UAS) elements are found in all four of the major core histone promoters (Osley 1991; Eriksson *et al.* 2005), but not in the *HHO1*, *CSE4*, or *HTZ1* promoters (Figure 1, A and B). Each of the four divergent major histone promoters contains four UAS elements located roughly midway between the genes (Figure 1A). When

Figure 1 The yeast histone genes. (A) Organization of the histone genes. The histone UAS elements are shown as numbered open arrowheads, which indicate their orientation. Confirmed high-affinity binding sites for Swi4 (SBF) are shown as blue boxes with arrowheads to indicate orientation; additional low-affinity sites exist but are not shown. An exact match to the Mbp1 consensus (ACGCGT) is indicated as a green box; there might be other high-affinity sites that differ from the consensus. The NEG region in *HTA1-HTB1* is indicated by a red bar. Putative NEG elements are indicated by red boxes and the CCR' element by an orange

transposed to a heterologous promoter, multiple copies of the histone UAS are sufficient for cell-cycle regulation with the correct timing (Osley *et al.* 1986). The histone UAS is specifically recognized by the *Spt10* transcription factor, which has the unusual property of requiring two histone UAS elements for high-affinity binding (Eriksson *et al.* 2005).

SPT10 was first identified as one of a set of *SPT* genes, mutations in which suppress Ty1 insertion mutations (Fassler and Winston 1988). The *SPT* genes encode many proteins important in transcription, including subunits of the SAGA histone-modifying complex (Grant *et al.* 1998), *TBP* itself, and histones (Clark-Adams *et al.* 1988; Winston and Sudarsanam 1998; Yamaguchi *et al.* 2001). *SPT10* is not an essential gene, but the null allele is associated with very slow growth and defects in gene regulation (Denis and Malvar 1990; Natsoulis *et al.* 1991; Prelich and Winston 1993; Yamashita 1993; Dollard *et al.* 1994; Natsoulis *et al.* 1994). *Spt10* contains a histone acetyltransferase (HAT) domain similar to that of *Gcn5* (Neuwald and Landsman 1997), but it has not been possible to demonstrate HAT activity, despite many attempts by our laboratory and others.

Our interest in *Spt10* began when we were searching for the HAT responsible for acetylating nucleosomes at the *CUP1* promoter (Shen *et al.* 2002). Histone acetylation of *CUP1* promoter nucleosomes is low in *spt10Δ* cells, and *CUP1* transcription is significantly reduced (Shen *et al.* 2002; Kuo *et al.* 2005). However, we were unable to show that *Spt10* is present at the *CUP1* promoter by chromatin immunoprecipitation (ChIP). Eventually, we turned our attention to the fact that *Spt10* had been identified as an activator of the histone genes (Sherwood and Osley 1991; Dollard *et al.* 1994; Hess *et al.* 2004) and its presence at the histone promoters had been confirmed by ChIP (Hess *et al.* 2004), indicating that its effects on the histone genes are likely to be direct. Using genome-wide expression microarrays, we showed that the transcription of hundreds of genes is affected in *spt10Δ* cells, most of which showed increased expression in the mutant, implying that *Spt10* acts primarily as a repressor—not the usual activating function often expected of HATs. We confirmed that *Spt10* is present at the histone gene promoters, but we could not detect it at any other genes, including those strongly affected in *spt10Δ* cells (Eriksson *et al.* 2005).

We proposed that *Spt10* acts *directly and solely* at the major core histone gene promoters, which it activates (Eriksson *et al.* 2005). Thus, in *spt10Δ* cells, a shortage of histones results in a wide range of *indirect* effects on a large number of genes due to a general disruption of chromatin

box. (B) Sequences of the functional histone UAS elements (nucleotides in red form the *Spt10*-binding site) (adapted from Eriksson *et al.* 2005). (C) Sequences of confirmed high-affinity Swi4 sites. (D) Sequence motif for the NEG element (from Mariño-Ramírez *et al.* 2006).

structure. The evidence in support of our hypothesis (Eriksson *et al.* 2005) includes the following:

1. Spt10 contains a DNA-binding domain that binds specifically to the histone UAS element with the consensus sequence (G/A)TTC-N₇-TTC(G/T)C (Figure 1B). Pairs of histone UAS elements are found only in the major histone promoters.
2. There is a general disruption of nucleosome spacing in *spt10Δ* cells, indicated by a smeary nucleosomal ladder after micrococcal nuclease digestion.
3. The endogenous 2-μm plasmid has fewer supercoils in *spt10Δ* cells than in wild-type cells, indicating that fewer nucleosomes are assembled in *spt10Δ* cells.
4. The severe growth defect exhibited by *spt10Δ* cells can be rescued completely by a high copy plasmid carrying *HTA1-HTB1* and *HHT1-HHF1*.
5. Analysis of expression microarray data indicates that there is a good correlation between the genes affected in *spt10Δ* cells and those affected in cells depleted of H4 (Wyrick *et al.* 1999).

These observations amount to strong evidence in favor of our model.

We can account for the *spt* phenotype if it is argued that the Ty1 promoter is activated by depletion of nucleosomes. In addition, in common with mutants in a number of other chromatin-associated genes, *spt10Δ* cells exhibit cryptic initiation by RNA polymerase II within open reading frames, which can be explained by a more general depletion of nucleosomes, which would tend to expose TATA-like elements in coding regions (Kaplan *et al.* 2003).

The most serious difficulty for the model is that the levels of histone mRNA are only modestly decreased in *spt10Δ* cells (Hess *et al.* 2004; Eriksson *et al.* 2005). This might be because these measurements were performed in asynchronous cells; histone mRNA peaks are greatly reduced in synchronized *spt10Δ* cells (Xu *et al.* 2005). In addition, there is weak activation of some histone genes by SBF (Swi4 cell cycle box binding factor), which could be sufficient to prevent lethality (see below). We argue that *spt10Δ* cells grow very slowly partly because they must accumulate sufficient histone mRNA to provide sufficient histones for assembly of the replicated genome into chromatin before S phase can be completed.

The domain structure of Spt10 is shown in Figure 2A. Unlike the intact protein, the DNA-binding domain (DBD) binds specifically and with high affinity to a single UAS when expressed by itself (Figure 2B). The N-terminal domain (NTD) is required for Spt10 homodimer formation and dictates the requirement for recognition of two histone UAS elements, presumably through a conformational change in the Spt10 dimer (Mendiratta *et al.* 2007). The requirement for binding two histone UAS elements should result in a dramatic increase in specificity because pairs of histone UAS elements are present only in the major core histone gene

Figure 2 Structure of the histone gene activator, Spt10. (A) Domain structure of Spt10: NTD, N-terminal domain; HAT, histone acetyltransferase domain; DBD, DNA-binding domain; C1 and C2, arbitrarily defined C-terminal domains. (B) Model for cooperative binding of the Spt10 dimer to a pair of histone UAS elements. The NTD-HAT-DBD portion of Spt10 forms a relatively compact dimer stabilized through interactions between its N-terminal domains (N). The C1 and C2 domains have been omitted for clarity. It is proposed that the DNA-binding domains, D, are oriented such that the DNA-binding site (black crescents) cannot interact fully with a single UAS element. However, if two such elements are present, the combined interactions of each DBD with part of a UAS are sufficient to trigger a conformational change in Spt10, resulting in high-affinity binding. The DBD alone can bind with high affinity to a single UAS. Adapted from Mendiratta *et al.* (2007).

promoters and such pairs occur nowhere else in the yeast genome (Eriksson *et al.* 2005).

The DBD contains an unusual H₂C₂ zinc finger, which is homologous to the N-terminal zinc-finger domain of foamy retrovirus integrase (Mendiratta *et al.* 2006), the enzyme responsible for insertion of retroviral DNA into the host genome. We proposed that the N-terminal domain of integrase might therefore also be a sequence-specific DNA-binding domain (Mendiratta *et al.* 2006). The recent crystal structure of foamy virus integrase complexed with DNA lends support to this proposal (Hare *et al.* 2010) because the N-terminal domain of integrase interacts with the ends of the viral DNA, which include the sequence TTC that is found in the half-sites recognized by Spt10 (Figure 1B).

Although the HAT domain has not been shown to possess HAT activity, the putative catalytic residues are required for the activation of a reporter gene (Hess *et al.* 2004), implying that HAT activity is critical for activation. There is indirect evidence that Spt10 might acetylate H3-K56 at the histone gene promoters (Xu *et al.* 2005), but it has been shown since that Rtt109 is the HAT responsible for most, if not all, H3-K56 acetylation in yeast (Driscoll *et al.* 2007; Han *et al.* 2007).

In conclusion, Spt10 is a very unusual activator in that it contains a sequence-specific DNA-binding domain and a HAT domain. Given that there is no obvious classical

Figure 3 Contributions of Spt10, SBF, and the UAS elements to the activation of *HTA1-HTB1* (idealized). Cells were arrested with α -factor and released. (Top) The total *HTA1-HTB1* mRNA peak (black) is composed of two peaks: a small, Swi4-dependent early peak (green) and a later major peak dependent on Spt10 (blue). Also shown is the effect of mutations on the UAS elements (purple). (Bottom) Binding of Spt10 and Swi4 at the *HTA1-HTB1* promoter, as shown by ChIP. Adapted from Eriksson *et al.* (2011).

activation domain in Spt10, the usual arrangement in which a sequence-specific activator binds at the promoter and recruits a HAT co-activator might have been bypassed in Spt10 by fusing a HAT domain directly to a DNA-binding domain (Figure 2). Perhaps Spt10 must in turn recruit a protein with an activation domain because activation domains have other functions, such as direct or indirect recruitment of RNA polymerase II and general transcription factors. The famous herpes simplex virus VP16 protein provides an example of a recruited activation domain as it has no DNA-binding domain (O'Hare 1993).

Spt21 plays an activating role in histone gene regulation

Another *SPT* gene, *SPT21*, has been implicated as a histone gene activator (Sherwood and Osley 1991; Dollard *et al.* 1994; Hess *et al.* 2004; Hess and Winston 2005). *SPT21* expression is cell cycle regulated, peaking in early S phase (Cho *et al.* 1998; Spellman *et al.* 1998). ChIP experiments show that Spt21 is present at all four major core histone promoters and that its binding peaks in S phase (Hess *et al.* 2004). The expression of *HTA2-HTB2* is strongly reduced in *spt21Δ* cells and that of *HHT2-HHF2* is weakly reduced, but *HHT1-HHF1* and *HTA1-HTB1* are only slightly

affected (Dollard *et al.* 1994; Hess and Winston 2005). Thus, Spt21 activates *HTA2-HTB2* and *HHT2-HHF2*. However, to complicate matters, *SPT21* was identified as a repressor of an *HTA1* reporter in a screen for regulators of the histone genes (Fillingham *et al.* 2009).

There is evidence that Spt21 stimulates Spt10 binding at all four major histone promoters during S phase, particularly at *HTA2-HTB2* (Hess *et al.* 2004). However, in cells arrested with α -factor, Spt10 binds to the histone promoters independently of Spt21 (Hess *et al.* 2004), indicating that Spt21 is important only for Spt10 binding in S phase. There might be a physical interaction between Spt10 and Spt21, as they co-immunoprecipitate when co-expressed in *Escherichia coli* (Hess *et al.* 2004), although we have found that both proteins can form inclusion bodies when expressed separately in *E. coli* (N. M. McLaughlin, unpublished data), which might compromise the interpretation of this experiment. However, a physical interaction between Spt10 and Spt21 is detected when Spt21 is overexpressed in yeast (Hess *et al.* 2004).

Clearly, Spt21 is an important player in histone gene regulation, but not a critical one, since the null mutant has no growth phenotype, unlike *spt10Δ*. Unfortunately, Spt21 has no identifiable domains to offer clues as to its mode of action. Genetic evidence implicates both Spt21 and Spt10 in transcriptional silencing (Oki *et al.* 2004; Chang and Winston 2011), but this is probably an indirect effect, since neither protein could be detected at telomeres by ChIP. Intriguingly, Spt21 inhibits the formation of “T-bodies,” cytoplasmic granules containing Ty1 RNA (Malagon and Jensen 2008). This observation implies that Spt21 might have an additional function that is distinct from histone gene regulation.

SBF and the timing of histone gene activation

The major G1/S cell-cycle-dependent transcription factors are MBF (MluI cell cycle box binding factor) and SBF (Koch and Nasmyth 1994). MBF and SBF are heterodimers containing Swi6 (a regulatory factor) and a sequence-specific DNA-binding factor: Mbp1 in MBF and Swi4 in SBF (Primig *et al.* 1992; Koch *et al.* 1993). Nucleo-cytoplasmic shuttling of Swi6 is important for its regulatory function (Queralt and Igual 2003). Genome-wide ChIP studies show that SBF and MBF are present at some of the histone gene promoters, but there is some disagreement over which: Iyer *et al.* (2001) report that MBF binds at *HTA2-HTB2* and *HHT2-HHF2* and that SBF binds at *HHT2-HHF2*, and Simon *et al.* (2001) report that SBF binds at *HTA1-HTB1*, *HTA2-HTB2*, and *HHT1-HHF1*. There are modest reductions in the expression of all of the major histone genes in *swi4Δ* cells and very small but significant reductions in the expression of some histone genes in *mbp1Δ* cells (Hess and Winston 2005).

There are two high-affinity SBF-binding sites in the *HTA1-HTB1* promoter, within UAS3 and UAS4, where they overlap the Spt10 sites (Eriksson *et al.* 2011; Figure 1C). Spt10 and SBF binding at UAS3 and UAS4 are mutually

exclusive *in vitro*, presumably due to steric hindrance. Reporter studies using the *HTA1-HTB1* promoter and cells synchronized using α -factor indicate that both *Swi4* and its binding sites in UAS3 and UAS4 are required to drive a small, early peak of expression (Figure 3). SBF and *Spt10* are bound at the *HTA1-HTB1* promoter but inactive in arrested cells, as measured by ChIP (Eriksson *et al.* 2011). After removal of the α -factor, SBF binding peaks in early S phase, coincident with the peak of SBF-mediated expression, and then declines precipitously to low levels by the end of S phase. *Spt10* binding decreases throughout the same period. Both SBF and *Spt10* dissociate from the promoter by the end of S phase (Eriksson *et al.* 2011).

SBF-driven transcription is relatively minor and mutation of the SBF-binding sites within the native *HTA1-HTB1* locus to prevent *Swi4* binding does not confer a growth phenotype. *Spt10* and the UAS elements are required to drive the major peak of expression, which appears significantly later than the *Swi4*-dependent peak. Importantly, mutations that prevent *Spt10* binding at the native *HTA1-HTB1* locus confer a strong growth phenotype (Eriksson *et al.* 2011).

HTZ1, *HHO1*, and *CSE4* all show cell-cycle-dependent expression (Pramila *et al.* 2006). SBF might play an important role in their regulation because all three genes possess a single predicted high-affinity SBF-binding site (Figure 1, A and C). We have confirmed the high-affinity *Swi4* sites in the *HHO1* and *CSE4* promoters, as well as a site in the *HHT2-HHF2* promoter. There are also some low-affinity sites in the histone promoters that might, or might not, be biologically significant (D. J. Clark, unpublished data).

Little is known about the role of MBF in histone gene regulation; there is only one exact match to the consensus site, located in the *HTA2-HTB2* promoter (Figure 1A).

Primary importance of the *Spt10*, SBF, and UAS systems

The primary importance of the UAS system in histone gene regulation is indicated by the fact that mutation of all four UAS elements to prevent the binding of both *Spt10* and SBF almost completely eliminates *HTA1-HTB1* transcription (Eriksson *et al.* 2011). It may be concluded that activation of the *HTA1-HTB1* promoter is primarily due to *Spt10*. SBF plays a supporting role at *HTA1-HTB1*, where it activates a small early peak of transcription. Thus, SBF and *Spt10* together control the timing of *HTA1-HTB1* expression (Eriksson *et al.* 2011). This conclusion is consistent with the fact that *swi4 Δ* is synthetically lethal with *spt10 Δ* (Hess and Winston 2005).

Negative Regulation of the Histone Genes

The NEG region

Deletion of 54 bp located between UAS2 and UAS3 in the *HTA1-HTB1* promoter results in derepression (Osley *et al.* 1986). We refer to this 54-bp region as the NEG region (Figure 1A). A search for sequence motifs within the NEG region revealed a 19-bp imperfect palindrome, named the

CCR' element. When inserted into a heterologous promoter, CCR' confers cell-cycle-dependent repression on a reporter driven by a UAS from *CYC1*, but the window of expression is significantly earlier than that of the histone genes (Osley *et al.* 1986).

A search for motifs common to the histone promoters identified, in addition to the UAS, a motif found in the *HTA1-HTB1*, *HHT1-HHF1*, and *HHT2-HHF2* promoters but not in that of *HTA2-HTB2*, referred to as the NEG element (Osley 1991; Marino-Ramirez *et al.* 2006). The NEG element in *HTA1-HTB1* is located within the NEG region (Figure 1, A and D). The evidence for its role in repression is circumstantial, based on the fact that *HTA2-HTB2* has no NEG element and is not subject to the same negative control system.

It has been proposed that the NEG region limits expression of the histone genes solely to S phase by repressing expression at other stages of the cell cycle (Osley *et al.* 1986). However, this model cannot account for the cell-cycle-dependent expression of *HTA2-HTB2*, which lacks the NEG region. Furthermore, the negative regulatory region in *HHT1-HHF1* is not required for cell-cycle-dependent expression, whereas the UAS elements are (Freeman *et al.* 1992). Although much is known about the properties of the NEG region in *HTA1-HTB1*, crucially, the transcription factors that presumably recognize the CCR' and NEG elements have not yet been identified.

The histone regulatory complex: a negative regulator of the histone genes

Two independent genetic screens for factors that negatively regulate the expression of the histone genes identified the histone regulatory genes (*HIR1*, *HIR2*, and *HIR3*) and the histone periodic control gene (*HPC2*) (Osley and Lycan 1987; Xu *et al.* 1992). The *Hir1*, *Hir2*, *Hir3*, and *Hpc2* proteins together form the HIR complex, which acts as a histone chaperone (Green *et al.* 2005; Prochasson *et al.* 2005). Another histone chaperone, *Asf1*, is associated with the HIR complex (Green *et al.* 2005). Null mutants in components of the HIR complex do not have a growth phenotype (Sherwood *et al.* 1993), but *asf1 Δ* cells grow poorly (Le *et al.* 1997).

Mutations in *HIR* genes derepress all the major histone genes except *HTA2-HTB2*, as shown by significant expression of the histone genes outside S phase (Osley and Lycan 1987; Xu *et al.* 1992). Although the levels of histone mRNA are increased in *hir* mutants relative to wild type, expression is still cell cycle dependent in that a peak is observed in S phase (Osley and Lycan 1987; Xu *et al.* 1992). In contrast, depletion of *Asf1* from cells has little effect on the levels of histone transcripts during the cell cycle (Zabaronick and Tyler 2005), suggesting that the HIR complex and associated *Asf1* do not have redundant functions at the histone promoters. Deletion of the NEG region also derepresses *HTA1-HTB1*, implying that HIR-mediated repression works through the NEG region (Osley and Lycan 1987). The HIR complex is present at all of the histone promoters except

HTA2-HTB2, as determined by ChIP (Fillingham *et al.* 2009). Although the HIR complex binds DNA with high affinity, it does not bind specifically to the histone promoters (Prochasson *et al.* 2005). The HIR complex is presumably recruited to the histone promoter through interaction with an unidentified sequence-specific transcription factor, probably involving the N-terminal domain of *Hpc2* (Vishnoi *et al.* 2011).

The HIR complex has a role in the tight coupling of histone gene expression to DNA replication. When DNA synthesis is inhibited by hydroxyurea, histone message levels are drastically reduced. This reduction is transient; the message levels are restored as cells adapt to replication stress (Libuda and Winston 2010). Histone gene expression is rendered immune to hydroxyurea-mediated repression by deletion of the NEG region, by *hir* mutations, and by *asf1* mutations, again with the exception of *HTA2-HTB2* (Hereford *et al.* 1981, Lycan *et al.* 1987; Osley and Lycan 1987; Xu *et al.* 1992; Spector and Osley 1993; Spector *et al.* 1997; Sutton *et al.* 2001; Ng *et al.* 2002).

Rather confusingly, the HIR complex is involved in diverse processes in addition to histone gene regulation, apparently acting as a histone chaperone in replication-independent nucleosome assembly (Green *et al.* 2005; Prochasson *et al.* 2005), *GAL* transcript elongation by RNA polymerase II (Nourani *et al.* 2006), heterochromatin silencing (Sharp *et al.* 2001), and kinetochore function (Sharp *et al.* 2002). Thus, the HIR complex is not a specific repressor for the histone genes. The reader is referred to Amin *et al.* (2012b) for an in-depth review of the HIR complex.

Mechanisms of Histone Gene Regulation

Replication-dependent histone gene expression is regulated by both positive (UAS) and negative (NEG) elements in the major core histone gene promoters. The sequence-specific factors *Spt10*, SBF, and perhaps MBF bind to UAS elements and act as transcriptional activators (Eriksson *et al.* 2005, 2011). Unfortunately, sequence-specific DNA-binding transcription factors that recognize elements in the NEG region, such as NEG and CCR', have not yet been identified. However, a surprisingly large number of NEG-region-dependent transcription cofactors have been identified, including ATP-dependent chromatin remodelers [RSC (remodels structure of chromatin), SWI/SNF, and *Yta7*] and histone chaperones (HIR complex, *Asf1*, and *Rtt106*). None of these factors appears to be specific to the histone genes, but they play important roles in their activation and repression. We now discuss how these cofactors might fit into the mechanism by which the sequence-specific factors choreograph events during the cell cycle.

Transcription cofactors: chromatin remodelers and histone chaperones

RSC and SWI/SNF are similar ATP-dependent chromatin-remodeling complexes capable of sliding nucleosomes along

DNA, driving conformational changes in nucleosomes, and removing histones from DNA (reviewed by Cairns 2009). However, RSC binding correlates with repression of the histone genes (Ng *et al.* 2002), whereas SWI/SNF binding correlates with their activation (Dimova *et al.* 1999; Ferreira *et al.* 2011) (Figure 4). Much evidence indicates that these complexes are recruited to promoters by sequence-specific transcription factors, but this paradigm is challenged by the fact that RSC contains two sequence-specific DNA-binding factors, *Rsc3* and *Rsc30* (Badis *et al.* 2008). Indeed, there are several predicted sites for *Rsc3* and *Rsc30* in the histone promoters, although it is not yet clear how reliable these predictions are. Therefore, RSC could be one of the sequence-specific factors binding in the NEG region, although it is also detected at *HTA2-HTB2* (Ng *et al.* 2002), which has no NEG region.

RSC associates in a cell-cycle-dependent fashion with the histone promoters, as shown by ChIP (Ng *et al.* 2002) (Figure 4). RSC binding at *HTA1-HTB1* is dependent on the HIR complex, but binding at *HTA2-HTB2* is independent of HIR (Ng *et al.* 2002), suggesting that the link between the HIR complex and RSC binding is not straightforward. In contrast, the SWI/SNF complex activates histone gene transcription, but, surprisingly, its recruitment to the promoter also depends on the HIR complex (Dimova *et al.* 1999), indicating a mechanistic link between the activating and repressing systems. Intriguingly, the binding of both SWI/SNF and RSC is dependent on the histone chaperone *Rtt106* (Ferreira *et al.* 2011). Thus, the two ATP-dependent remodeling complexes, RSC and SWI/SNF, have antagonistic roles at the histone promoters, presumably by mediating changes in chromatin structure during switches between the activated and repressed states. These antagonistic roles are reflected by their cell-cycle-dependent binding profiles, which are out of phase with one another, such that the SWI/SNF peak coincides with activation and the RSC peak coincides with repression (Figure 4).

Histone chaperones bind histones and facilitate nucleosome assembly and disassembly (reviewed by Ransom *et al.* 2010). *Rtt106* is a chaperone for histones H3 and H4 (Huang *et al.* 2005); it binds preferentially to H3 acetylated on K56 (Li *et al.* 2008; Zunder *et al.* 2012). *Rtt106* was identified as a repressor of *HTA1* expression in a genetic screen, which also identified two other histone chaperones as repressors: the HIR complex and *Asf1* (Fillingham *et al.* 2009). Thus, three different histone chaperones are involved, although physical interactions between all of them have been reported *in vitro* and *in vivo* (Sutton *et al.* 2001; Green *et al.* 2005; Fillingham *et al.* 2009; Ferreira *et al.* 2011), suggesting that they might act together, with apparently redundant functions. The *HTA1-HTB1* promoter is more depleted of nucleosomes in *rtt106Δ* and *hir1Δ* cells than in wild-type cells, suggesting that these chaperones might facilitate assembly of nucleosomes on the histone promoters, which then repress transcription (Fillingham *et al.* 2009).

Figure 4 Cell-cycle-dependent binding of the RSC and SWI/SNF ATP-dependent chromatin-remodeling complexes at the *HTA1-HTB1* promoter. The binding of TFIIB, a general transcription factor present at active promoters, coincides with that of SWI/SNF, but is out of phase with that of RSC. Thus, SWI/SNF binding correlates with activation, and RSC binding correlates with repression. ChIP data for TFIIB and RSC were adapted from Ng *et al.* (2002); data for SWI/SNF and *HTA1* expression were adapted from Ferreira *et al.* (2011). Note that, although these experiments were done in two different laboratories, the times between the first and second cell-cycle peaks are very similar, and so a direct comparison is reasonable.

In the same screen, Fillingham *et al.* (2009) identified some activators and co-activators of *HTA1*, including *Swi4*, *Rtt109-Vps75* (the HAT complex responsible for acetylating H3-K56), and *Yta7*. However, Gradolatto *et al.* (2008) identified *Yta7* as a repressor of the histone genes, and so there is some disagreement here. *Yta7* is a putative ATP-dependent remodeling protein containing a bromodomain that binds preferentially to the tail domain of H3 (Gradolatto *et al.*

2009). However, *Yta7* has little effect on histone mRNA levels, and its activities are not confined to the histone genes: it has direct effects on many inducible genes (Lombardi *et al.* 2011). It has been proposed that *Yta7* acts post-translationally, probably by regulating the amount of H3 in chromatin (Lombardi *et al.* 2011).

Mechanism of histone gene regulation during the cell cycle

The factors described above act together to regulate histone gene transcription during the cell cycle. Although we do not understand many of the molecular mechanisms underlying the events that occur during the cycle, the process of switching between activated and repressed states can be outlined.

In α -factor-arrested cells, the *HTA1-HTB1* promoter is already bound by two sequence-specific transcriptional activators, *Spt10* and SBF, but they are inactive. In the case of SBF, this is probably due to *Whi5*-mediated inactivation (Costanzo *et al.* 2004; De Bruin *et al.* 2004), which involves recruitment of the Rpd3L histone deacetylase (Takahata *et al.* 2009). SBF is subsequently activated through phosphorylation of *Whi5* by the *Cln3-Cdc28* kinase (Costanzo *et al.* 2004; De Bruin *et al.* 2004). A similar mechanism may be employed to maintain *Spt10* in an inactive state.

After release from α -factor arrest, SBF activates an initial small peak of *HTA1* and *HTB1* transcription as the cells enter S phase. Shortly after, *Spt10* activates a much larger peak of transcription (Eriksson *et al.* 2011). The mechanisms by which *Spt10* and SBF activate transcription are not understood. SBF activity has been generally correlated with dimethylation of H3-K79 by *Dot1* (Mellor 2009; Schulze *et al.* 2009), but how this mark facilitates activation by SBF is unclear. The HAT domain of *Spt10* is important for activation (Hess *et al.* 2004), but what it acetylates, if anything, is unknown. *Spt10* is required for the appearance of the S-phase-specific H3-K56ac mark at the *HTA1-HTB1* promoter, and both *Spt10* and H3-K56ac are required for the binding of SWI/SNF prior to transcription (Xu *et al.* 2005). The *Rtt109-Vps75* HAT complex is probably responsible for all H3-K56 acetylation (Han *et al.* 2007), and *rtt109Δ* cells show reduced *HTA1* transcription, suggesting that *Rtt109* is indeed an important co-activator (Fillingham *et al.* 2009). This leads to a simple model in which *Spt10* mediates recruitment of *Rtt109*, which then acetylates H3-K56, facilitating SWI/SNF binding (Figure 5). However, evidence that *Spt10* interacts with *Rtt109* is lacking. At the end of S phase, *Spt10* and SBF binding at the *HTA1-HTB1* promoter are reduced to low levels, accounting for the observed decline in *HTA1-HTB1* transcription (Eriksson *et al.* 2011).

So how does the negative regulatory system fit into this picture? The CCR' element within the NEG region confers cell-cycle dependence on a reporter driven by a constitutive UAS element, but the timing is early relative to histone gene expression (Osley *et al.* 1986). Thus, the reporter is repressed *except* during an early window. We speculate that

Figure 5 Model for the activated and repressed states of the *HTA1-HTB1* promoter. In late G1 and S phase, the histone genes are transcriptionally active. Activation occurs through SBF and Spt10 binding at the UAS elements (open triangles). Activation proceeds through an unknown mechanism involving the SWI/SNF-remodeling complex, the binding of which is dependent on the NEG region (red box) and perhaps on a putative sequence-specific NEG transcription factor (red circle). The promoter is shown depleted of nucleosomes (gray ovals), perhaps due to SWI/SNF, but direct evidence for this chromatin structure is lacking. Note that there is likely to be some nucleosome formation on the promoter because there is an enrichment for acetylated H3-K56. Cell-cycle kinases might be involved in activating Spt10; its HAT domain is required for its activation function. Outside S phase, Spt10 and SBF are no longer bound; whether they are displaced or degraded is not known. Establishment of the repressed state depends on histone chaperones (HIR, Rtt106, and Asf1), the putative NEG factor, and the RSC remodeling complex, which are proposed to facilitate nucleosome assembly on the promoter.

Spt10 and SBF act maximally during this window of opportunity and are then progressively dampened by the NEG system. In the absence of the NEG system, transcription activated by Spt10 and SBF is at a higher level and maintained longer, such that the expression peak merges into that of the next cell cycle. An additional, potentially vital, role for the NEG system is its ability to mediate a rapid shutdown of histone gene transcription if cells are subjected to replication stress.

The involvement of so many chromatin-remodeling factors suggests that these events are probably accompanied by major changes in the chromatin structure of the histone genes during the cell cycle, but this remains to be confirmed.

By analogy with other inducible promoters in yeast, it might be expected that the activated histone promoters are heavily depleted of nucleosomes and that the repressed promoters are packaged into nucleosomes (Figure 5). A reasonable model is that the histone chaperones (the HIR complex, Asf1, and Rtt106) assemble nucleosomes on the histone promoters toward the end of S phase (Fillingham *et al.* 2009) and that these nucleosomes are then positioned appropriately for repression by the RSC complex, perhaps targeted by Rsc3 or Rsc30. It has been hypothesized that, in late G1, the HIR complex is converted from a corepressor into a co-activator (Dimova *et al.* 1999) and that then it cooperates with Asf1 and Rtt106 to recruit the SWI/SNF complex (Dimova *et al.* 1999; Ferreira *et al.* 2011). This might involve the Rtt109-Vps75 HAT complex because Rtt109 interacts with Asf1 (Tsubota *et al.* 2007). In addition, HIR-mediated repression is alleviated by the activities of the mitotic Clb1 and Clb2 cyclins, probably through direct phosphorylation of the HIR complex (Amin *et al.* 2012a). We speculate that SWI/SNF is involved in repositioning and disassembly of repressive nucleosomes to facilitate activation by Spt10 and SBF. Clearly, a detailed description of the changes in chromatin structure that occur at the histone loci is needed to test this model.

It has been proposed that the maintenance of histone chaperone-mediated chromatin domains is the primary mechanism of cell-cycle regulation of the histone promoters (Fillingham *et al.* 2009). Thus, repression is mediated by Rtt106, the HIR complex, and Asf1, which cooperate to create a repressive chromatin structure at the promoter, the boundaries of which might be limited by the putative ATP-dependent chromatin-remodeling complex Yta7. Repression is alleviated through Rtt109, a HAT for H3-K56. This is a reasonable model that describes how the expected changes in chromatin structure might be effected as the histone genes cycle between on and off states. However, the additional hypothesis of Fillingham *et al.* (2009)—that sequence-specific activators (*i.e.*, Spt10 and SBF) are not required for cell-cycle-dependent regulation of the histone genes—is untenable. The chromatin factors involved are all general cofactors known to be present at many diverse promoters: Where would the specificity for the histone genes come from?

Instead, we believe that the activating system is the primary source of specificity for the following reasons: (1) Only the histone UAS element confers cell-cycle-dependent regulation with the correct timing (Osley *et al.* 1986); (2) mutations in the negative regulatory sequence of *HHT1-HHF1* do not affect cycling, whereas mutations in the UAS elements do (Freeman *et al.* 1992); (3) *HTA2-HTB2* cycles similarly to the other major core histone loci but has no NEG system (Osley and Lycan 1987; Xu *et al.* 1992); (4) Spt10 is the only known sequence-specific factor dedicated to histone gene expression (Eriksson *et al.* 2005)—none of the known NEG system components are specific for the histone genes; (5) mutations in the UAS elements in *HTA1-HTB1*

that specifically prevent *Spt10* binding result in poor growth (Eriksson *et al.* 2011); (6) there is no expression from an *HTA1-HTB1* reporter carrying UAS mutations that prevent the binding of both *Spt10* and SBF (Eriksson *et al.* 2011); and (7) the phenotypes of null mutants of NEG system components, with the exception of *rsc* mutants, are minor relative to that of *spt10Δ*, which results in very slow growth.

Post-Transcriptional Regulation

Post-transcriptional regulation of the histone genes is very important in more complex organisms (Marzluff *et al.* 2008), and this is also true of yeast. The major core histone genes are regulated post-transcriptionally (Hereford *et al.* 1982). The peak in histone transcript levels occurs later than that of mRNA synthesis, as measured in pulse-chase experiments. This effect is attributed to an increase in histone mRNA stability as the cells enter S phase. In contrast, inhibition of DNA replication results in a major reduction in the half-lives of the *HTA1* and *HTB1* mRNAs (Hereford *et al.* 1981), as well as shutdown of transcription through the NEG system (see above).

The mechanism by which mRNA stability is regulated is unclear, but it might involve the 3'-untranslated regions (3'-UTR) of the histone mRNAs. The 3'-UTR of *HTB1* can confer cell-cycle regulation on a heterologous mRNA, although the peak of expression is late relative to native *HTB1* expression (Xu *et al.* 1990; Campbell *et al.* 2002). A distal downstream element (DDE) in the 3'-UTR of *HTB1* influences both the cycling of the transcript and the site of cleavage prior to polyadenylation; the DDE may bind an unidentified S-phase-specific protein believed to affect mRNA stability (Campbell *et al.* 2002; some of the data in this article have been retracted). It seems likely that the 3'-UTR affects mRNA stability, but it is possible that it acts at the transcriptional level through the DNA rather than the mRNA.

The TRAMP complexes play a role in histone mRNA degradation. TRAMP4 and TRAMP5, containing the poly(A) polymerases *Trf4* and *Trf5*, recognize various mRNAs and deliver them to the nuclear exosome (Reis and Campbell 2007), where they stimulate the activity of the *Rrp6* exoribonuclease (Callahan and Butler 2010). Elevated levels of core histone transcripts are present in *trf4Δ*, *trf5Δ*, and *rrp6Δ* cells. In addition, *trf4* is synthetically lethal with *rad53*, *asf1*, and *hir1* (Reis and Campbell 2007), all of which are involved in the negative regulation of histone gene expression.

Histone genes and dosage compensation

An unusual feature of *HTA1-HTB1* regulation is that the mRNA level is sensitive to gene copy number: introduction of an extra copy of the *HTA1-HTB1* locus results in a doubling of both the total amount of transcript and the transcript turnover rate. Consequently, the levels of *HTA1* and *HTB1* mRNA are unaltered (Osley and Hereford 1981). Thus, a post-transcriptional dosage compensation mechanism

is active, acting through changes in mRNA turnover rates. The *HTA2-HTB2* locus does not show dosage compensation: deletion of *HTA1-HTB1* leads to a severe growth phenotype, but deletion of *HTA2-HTB2* is compensated for by increased *HTA1-HTB1* transcription (Norris and Osley 1987). *HHT1-HHF1* and *HHT2-HHF2* do not compensate for one another (Cross and Smith 1988; Smith and Stirling 1988).

Dosage compensation of the *HTA1-HTB1* locus also involves the NEG system (Moran *et al.* 1990). Transcription of an *HTA1* reporter gene can be repressed or activated, depending on the number of *HTA* and *HTB* genes in the cell, whereas an *HTA2* reporter gene is unaffected. Furthermore, dosage compensation of *HTA1-HTB1* requires histone production because a frameshift mutation introduced into *HTB1* eliminates dosage compensation. This very interesting observation suggests that a feedback system might simultaneously involve the NEG system, the 3'-UTR, and histones (Moran *et al.* 1990).

Regulation of histone degradation

Cellular histone levels are monitored by *Rad53*, a major checkpoint kinase involved in the DNA damage response and required for stabilization of stalled replication forks (Gunjan and Verreault 2003; Singh *et al.* 2009). In *rad53Δ* cells, histone overexpression is lethal because excess histones are not degraded, presumably resulting in aggregation of chromatin (Gunjan and Verreault 2003). Although *Rad53* can phosphorylate all four core histones *in vitro*, it is unclear whether it does this *in vivo* (Singh *et al.* 2010). Histones associated with *Rad53* are both phosphorylated and polyubiquitinated prior to degradation by the proteasome (Singh *et al.* 2010). The reader is referred to the review by Gunjan *et al.* (2006) for further details.

Hypothesis: Negative Feedback Links the UAS and NEG Systems

The involvement of so many histone chaperones in histone gene regulation is intriguing and might have a special significance for the histone genes because histones are the end product. The NEG system could be part of a negative feedback loop that communicates the degree to which genomic nucleosome assembly is complete, and therefore when the histone genes should be shut off (Figure 6).

When nucleosome assembly is complete at the end of S phase, the chaperones responsible for delivering histones to newly replicated DNA would be expected to become fully charged with histones because there is no DNA left for nucleosome assembly. Thus, the signal to the NEG system for shutdown of the UAS system could be the extent to which chaperones bound at the histone promoters are charged with histones. Prior to S phase, the chaperones will be charged with histones from the previous cycle. When S phase is initiated, the chaperones will deposit their histones on newly replicated DNA as it becomes available. Eventually, the chaperones will be depleted of histones, thereby relieving

Figure 6 Hypothesis: Negative feedback by histones links the positive and negative regulatory systems. The histone genes are activated by Spt10 and SBF bound to the histone UAS elements (open triangles), followed by transcription and translation to produce histones. The NEG system inhibits activation through a putative sequence-specific NEG factor (open red circle) bound to the NEG region (red box), which recruits various histone chaperones (HIR, Rtt106, and Asf1: red oval). Inhibition occurs only if histones are bound to the chaperones. The chaperones will be fully charged with histone and maximally inhibitory when there is no replicated DNA available in the cell for nucleosome assembly—when replication is complete (outside S phase) or if replication forks are stalled (e.g., in the presence of hydroxyurea). Outside S phase, the activators are displaced or degraded (arrows). Our hypothesis is based particularly on our own work and that of Moran *et al.* (1990) and Fillingham *et al.* (2009). There are additional levels of control at the levels of mRNA stability and Rad53-mediated histone degradation (not shown).

inhibition of histone gene transcription. The UAS system would then work maximally because the chaperones are mostly histone-free. Transcription of the histone genes would eventually be shut down as the chaperones become recharged with new histones and S phase is completed. This hypothesis also accounts for the histone gene shutdown that occurs when replication forks are stalled because there would again be no DNA available for nucleosome assembly, and histone-bound chaperones would accumulate. We are currently testing this hypothesis.

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