Cellular and Molecular Life Sciences

Regulation of histone gene transcription in yeast

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Abstract Histones are the primary protein component of chromatin, the mixture of DNA and proteins that packages the genetic material in eukaryotes. Large amounts of histones are required during the S phase of the cell cycle when genome replication occurs. However, ectopic expression of histones during other cell cycle phases is toxic; thus, histone expression is restricted to the S phase and is tightly regulated at multiple levels, including transcriptional, post-transcriptional, translational, and post-translational. In this review, we discuss mechanisms of regulation of histone gene expression with emphasis on the transcriptional regulation of the replication-dependent histone genes in the model yeast *Saccharomyces cerevisiae*.

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Introduction

Chromatin refers to the complex mixture of DNA and proteins that together constitute the eukaryotic chromosome. The nucleosome, the fundamental repeating unit of chromatin, is composed of a histone octamer around which a 147-bp stretch of DNA is wrapped. Each octamer is composed of two H3–H4 histone dimers bridged together as a stable tetramer that is flanked on either side by two separate H2A–H2B dimers [1]. The four core histones (H2A, H2B, H3, and H4) are among the most conserved eukaryotic proteins in primary amino acid sequence and are uniformly small in size (<20 kD), highly enriched in basic amino acids (>20 % lysine/arginine residues), and contain the conserved histone fold domain that interacts non-specifically with the surrounding DNA and mediates histone–histone interactions [1, 2].

Histone proteins are generally divided into two classes. The first class is composed of replication-dependent (RD) histones whose expression is induced right before and during DNA replication (see below). This class includes the core set of histones that compose the nucleosome, H2A, H2B, H3, H4, as well as the linker histones that bind the linker DNA located between nucleosomes. Linker histones are thought to coordinate the packing of nucleosomes into higher order chromatin structure. The second class of histone proteins is composed of histone variants that are generally expressed at a relatively low level through all stages of the cell cycle, and are therefore classified as replication-independent (RI). RI histone variants have specialized, distinct functions. For example, the variants of the core histones H2A and H3, named H2A.Z and H3.3, respectively, function specifically in transcription [3], whereas the H2A variant, H2A.X, is linked to maintenance of genome stability [4]. We will focus on a review of mechanisms that

regulate the expression of the set of RD core histone genes encoding H2A, H2B, H3, and H4.

A detailed understanding of the regulation of RD histone expression is of considerable general interest for several reasons. First, expression of RD core histone gene expression is subject to exquisite regulation in eukaryotes. When a eukaryotic cell replicates its DNA in the synthetic or S phase of the cell cycle, the genome doubles in size, demanding a corresponding doubling of histones to package the newly synthesized genome. Therefore, a burst of RD histone expression is needed to produce histones for quick and efficient packaging of the newly synthesized DNA. In fact, histone expression in yeast starts prior to S phase—during late G1 phase—to ensure that adequate histones are present as DNA is synthesized [5]. Second, DNA replication in the absence of new histone synthesis in the model yeast *Saccharomyces cerevisiae* (*Sc*) is lethal to the cell [6]. Additionally, if RD core histones are present in excess and accumulate as free soluble proteins, cell fitness is compromised and cells become sensitive to DNA-damaging agents [7]. Therefore, RD core histone synthesis must be repressed outside of S phase—during G1, G2 mitosis (M phase)—and when DNA damage forces the cell to abruptly stop replicating its DNA in order to repair it. The achievement of RD histone expression homeostasis requires complex regulation, which occurs at different levels ranging from transcriptional to post-translational. Here, we review the available literature on the mechanisms underlying the regulation of histone expression in *Sc* with emphasis on transcriptional regulation.

RD core histones in Yeast

All known eukaryotes possess more than one gene encoding each RD core histone protein. For example, the human and mouse genomes encode 14 copies of the RD histone H4 gene alone [8]. Consequently, molecular genetic analysis of core histone genes can be technically challenging. The most experimentally manageable eukaryote with respect to genetic analysis of RD histones is *Sc*, which arguably remains the most useful model to understand the mechanisms regulating core histone gene expression. The *Sc* genome contains two copies of each RD histone gene, each arranged in opposite orientation to a gene encoding its partner within the nucleosome: *HHT1–HHF1* and *HHT2–HHF2*, the two gene pairs encoding core histones H3 and H4 [9], and *HTA1–HTB1* and *HTA2–HTB2*, the two gene pairs encoding H2A and H2B [10]. Each pair of genes is regulated by centrally located, divergent promoter elements [11]. All eight genes share a common expression pattern that is cell cycle-dependent (Fig. 1): very low expression occurs outside of S phase except in late G1 (immediately
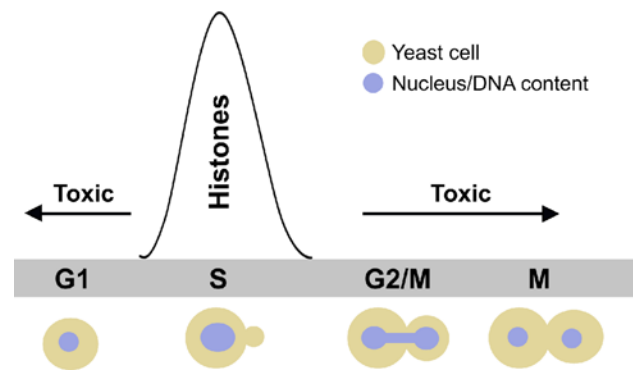

Fig. 1 Core histone expression during the yeast cell cycle. RD core histone genes are expressed exclusively in late G1 and S phase of the cell cycle, in parallel with the replication of DNA. Outside of the S phase, histone expression is toxic; therefore, cells tightly restrict histone production to the S phase. See text for details

prior to initiation of DNA replication) when histone gene transcripts begin to accumulate before peaking in mid-S phase, after which expression levels decline until cells exit S phase and enter into the G2- and M-phases [5].

Intuitively, it makes sense that RD histone expression should occur primarily in S phase, but what is the evidence that restricted histone expression is important for proper cell division and fitness? Several dramatic phenotypes are associated with inappropriate histone gene expression: (1) if levels of core histones are inadequate yet DNA replication proceeds, yeast cells exhibit dramatic genome instability or mitotic arrest [12, 13], (2) a failure to repress histone expression following DNA replication and RD chromatin assembly—for example, in cells with over- or ectopic expression of core histones—is highly toxic to *Sc* and other eukaryotic cells (Fig. 1), due to failure to correctly segregate chromosomes at mitosis [14], and (3) the accumulation of soluble histones results in sensitivity to DNA-damaging chemicals which is exacerbated by deletion of genes whose wild-type (WT) role is to negatively regulate expression of RD histones [7]. For example, a loss of function mutation in the *LSM1* gene, which results in increased histone gene mRNA outside of S phase (see section below on post-transcriptional regulation), causes a genome instability phenotype manifested experimentally by sensitivity to hydroxyurea (HU), a chemical that depletes deoxyribonueotides and causes DNA replication fork disruption, thus triggering a DNA damage checkpoint which arrests cells within S phase, allowing them to repair their damaged DNA. The HU-sensitive phenotype of an *lsm1Δ* strain can be suppressed by lowering histone dosage through deletion of one of the two respective H2A–H2B or H3–H4 encoding gene pairs [15]. The deleterious effects of histone overexpression are not exclusive to yeast/fungi. For example, in *Drosophila melanogaster*, a loss of function mutation in

the abnormal oocyte (*ABO*) gene, which encodes a protein that negatively regulates core histone gene expression during embryogenesis, is maternal-lethal suggesting that WT expression levels of genes encoding core histones is critical for normal embryogenesis [16].

Replication-dependent (RD) histone gene expression must also be repressed sometime within, in addition to outside, S phase. Upon genotoxic stress, DNA replication may be abruptly blocked or disrupted. If the replication fork encounters DNA damage, a DNA damage checkpoint is triggered, and soluble RD histones may accumulate to toxic levels if expression continued unabated. To prevent this, eukaryotic cells possess mechanisms that tightly link histone expression to DNA replication progression so that histone transcription may be repressed quickly within S phase once a delay or blockage in DNA replication has occurred. In *Sc,* this phenomenon was initially observed when histone expression was assessed in the DNA replication (temperature-sensitive) mutant *cdc8* [5], which encodes thymidylate kinase, an enzyme important for S phase progression [17]. When DNA synthesis is arrested by shifting *cdc8^{ts}* cells to the non-permissive temperature, RD histone expression is quickly halted as well [5]. Further analysis revealed that a rapid arrest of RD histone gene expression also occurs in *Sc* in response to HU [18].

As outlined above, expression of RD histone genes is regulated in a cell cycle-specific manner and in response to DNA damage. In many eukaryotic cells, regulation of histone levels involves transcriptional, post-transcriptional, translational, and post-translational mechanisms. Although in *Sc* regulation of core histone genes is best understood at the transcriptional level, increasing evidence points to regulation at other levels as well. Below, we focus mainly of the regulation of core histone gene transcription in *Sc* with reference to other regulatory mechanisms and models where appropriate.

Cis- and *Trans*-acting factors regulating transcription of RD core histone genes in Sc

Cis-*acting sequences regulating histone gene transcription in Sc*

The *Sc HTA1–HTB1* locus has provided an excellent model for understanding mechanisms involved in the transcriptional regulation of RD histone genes. The first model to explain the mechanism of S phase-specific expression of histone genes proposed a direct coupling of transcription to the DNA replication apparatus, and was based on physical linkage of the *HTB1* gene to an autonomously replicating sequence (ARS) [11]. However, subsequent experiments revealed no effect of the ARS on transcription, but instead uncovered the existence of two separate and important classes of *cis*-acting sequences located in the ~800-bp intergenic regulatory region between *HTA1* and *HTB1* [11]. The first *cis*-acting sequence is referred to as a histone upstream activating sequence (UAS) and is responsible for S phase-specific activation of transcription. In *HTA1–HTB1*, several copies of the 16-bp UAS sequence (GCGAAAAANTNNGAAC) are situated ~200–300 bp upstream of each transcriptional start site and each copy has a positive and additive effect on the S phase-specific periodicity of *HTA1* and *HTB1* mRNA levels [11]. The UASs are found in the intergenic regions of each of the four sets of RD core histone genes in *Sc* [19], and are sufficient to confer cell cycle-regulated expression of a constitutively expressed reporter gene [11].

The second class of *cis*-acting sequence is the cell cycle control region (CCR), which was identified through analysis of a *LacZ* reporter gene linked to the *HTA1–HTB1* regulatory region. Deletion of the CCR in this context causes constitutive expression reporter gene expression, suggesting that CCR negatively influences transcription of RD histone genes [11]. In addition, deletion of the CCR region (also referred to as NEG) causes a loss of cell cycle control of expression of *HTA1–LacZ* despite the continued presence of the UAS that confers cell cycle-dependent expression in the presence of NEG. Importantly, NEG is also required for the rapid arrest of RD histone expression when *Sc* is treated with HU which triggers the intra-S checkpoint as described above [18]. A single copy of the NEG sequence is found in the intergenic regions of three of the four divergent histone gene pairs with the exception being the *HTA2–HTB2* regulatory region. Thus, there are similarities (copies of histone UASs upstream of all eight RD histone genes) and differences (presence of NEG in three of the four histone gene pairs) in transcriptional regulation of the four *Sc* histone gene pairs. A major focus in the field has been to elucidate how these two classes of *cis*-acting sequences collaborate to coordinate the periodic transcription of the core histone genes.

Trans-acting factors with roles in repression of histone gene transcription

HIR complex

HIR1, *HIR2*, and *HIR3* (histone regulation) were the first genes identified as regulators of RD histone transcription, in a screen for mutants with defective *HTA1–lacZ* reporter gene expression [20]. The so-called Hir⁻ phenotype of the three mutants results from a failure to repress expression of both the *lacZ* reporter, and the actual *HTA1* locus is de-repressed as well. Failure to repress reporter/*HTA1*

expression in any of the *hir* mutants is also observed when an intra-S DNA damage checkpoint is triggered with HU [20]. If Hir2 is artificially tethered to a *HTA1–lacZ* reporter gene, it represses transcription in a manner requiring both Hir1 and Hir3 [21], consistent with a role for the Hir proteins as histone gene transcriptional repressors. A fourth gene showing a mutant Hir⁻ phenotype, histone promoter control (*HPC2*), was identified in a separate genetic screen [22]. Interestingly, the Hir⁻ phenotype of the Hir/HPC mutant strains phenocopies NEG deletion, and repression of a NEG-containing *HTA1–LACZ* reporter is lost in a *hir* mutant background [20], suggesting that *HIR/HPC2* gene products act through NEG. Also, deletions of each of the *HIR/HPC* genes affects expression of only three of the four histone gene pairs, the exception being *HTA2–HTB2*, which lacks NEG. Biochemical experiments support a direct regulatory relationship between the Hir/HPC proteins: all four of these gene products co-purify as a protein complex [23, 24] from yeast extracts and individual deletion of any of the four genes compromises integrity of the complex [23]. In addition, all four Hir/Hpc subunits specifically localize to chromatin at the *HTA1–HTB1* region that contains NEG [23, 25] as assessed using chromatin immuno-precipitation (ChIP). Insight into the mechanism of HIR-mediated repression at NEG-dependent histone loci derived from the demonstration that the HIR complex functions as a RI histone H3–H4 chaperone [23, 24]. Correspondingly, individual deletion mutants of members of the HIR complex exhibit a significant decrease in histone density in the regulatory region of NEG/HIR-regulated histone gene pairs [25]. Thus, an intact HIR complex localizes to chromatin at the CCR/NEG region and is necessary for repression of three of the four histone loci (*HTA1–HTB1*, *HTT1–HHF1*, and *HTT2–HFF2*). HIR-mediated histone gene repression is a consequence of its activity as a histone chaperone, likely because the Hir complex helps assemble histones that occlude RNA polymerase II (RNAPII) recruitment onto promoter sequences (see below).

How is HIR recruited to NEG? Consistent with specific localization of Hir proteins to chromatin, gel shift assays showed that purified HIR complex binds both nucleosomes and DNA [24]. However, the DNA-binding activity of the HIR complex appears non-specific since HIR binds equally well to NEG and non-NEG containing double-stranded DNA oligonucleotides [24]. Although HIR does not appear to directly bind NEG, the site seems necessary for recruitment of HIR to chromatin in the region. Thus, the precise function of NEG remains enigmatic. Is NEG sufficient or is an additional *trans*-acting factor required to recruit HIR to CCR/NEG region? It has been speculated that there is likely an as yet unidentified sequence-specific DNA-binding factor(s) whose function is to recruit HIR to the CCR/NEG region. The identification of a short peptide

sequence within Hpc2, that when deleted prevents HIR recruitment to histone gene promoters but does not disrupt the integrity of the complex suggests, that one function of Hpc2 within HIR may be to physically interact with this hypothetical *trans*-acting factor [26]. Because the histone UASs flank CCR/NEG, it will be interesting to determine the effect of deleting *SPT10,* a UAS-binding histone gene activator (see below), on HIR recruitment to the CCR/NEG region.

The universality of HIR function in the regulation of histone gene expression remains to be determined. The genes encoding all four HIR components of *Sc* are conserved within the crown eukaryotes (plants, fungi, animal) with the caveat that most have a single protein, HIRA, that is the equivalent of a fusion between the N-terminus of *Sc* Hir1 and the C-terminus of *Sc* Hir2 [27]. In support of a universal role in the regulation of histone gene transcription, ectopic expression of HIRA in human U2OS cells represses transcription of histone genes leading to a block in DNA synthesis [28]. Recently, it was demonstrated that phosphorylation by the WEE1 kinase of H2B at tyrosine 37 upstream of the *Hist1* gene cluster of RD histone genes [8] in human cells is required to recruit HIRA in order to repress transcription of these genes [29]. Mutation of the corresponding tyrosine on H2B in *Sc* (H2BY40A) increases expression of all four histone gene pairs [29] and is dependent on the WEE1-related cell cycle kinase, Swe1. It is not yet known whether H2B phosphorylation disrupts HIR binding in *Sc*, although other mechanisms must also be in play since both NEG-regulated and non-NEG-regulated histone gene loci are affected in the H2B phosphorylation mutant [29]. Besides HIRA, the roles of other putative HIR members in the regulation of histone gene expression (UBN1 similar to Hpc2 [30], CABIN1 similar to Hir3 [31]) have not been tested outside of *Sc*. Nonetheless, in human cells, HIRA, UBN1, and Asf1a localize preferentially to nucleosome-free regions in transcription start sites in gene promoters [32]. Taken together, these data suggest that important aspects of HIR repression of histone gene expression are conserved throughout eukaryote evolution.

Asf1 and Rtt106

HIR-mediated repression of RD histone transcription also involves two additional H3-H4 histone chaperones, Asf1 and Rtt106. Several lines of evidence, including yeast two hybrid assays and affinity purification, have demonstrated that Asf1 interacts with the HIR complex via direct physical interaction with Hir1 [23, 33], an interaction that appears evolutionarily conserved. Consistent with their physical association, deletion of *ASF1* results in a Hir⁻ phenotype [33] with respect to RD histone expression and Asf1 localizes to NEG-containing chromatin [34]. Deletion

of *ASF1* does not disrupt integrity of the HIR complex or localization of HIR subunits to CCR/NEG-containing chromatin [23, 25], but it does cause a decrease in histone density at the CCR/NEG region, similar to that observed for individual deletion of HIR subunits [25]. Together, these results suggest a role for Asf1 in RD histone gene regulation downstream of HIR.

Regulator of Ty1 Transposition (*RTT106*) was originally identified as a gene whose deletion causes an increase in transposition of the Ty1 retroelement [35]. A later large-scale screen revealed that deletion of *RTT106* also enhances the defective gene silencing of a *pol30-879* strain which is mutated for PCNA, an essential DNA replication factor [36]. Subsequent genetic analysis revealed that the *RTT106* function in silencing is linked to *ASF1* and *HIR1*, but independent of CAF-1, another chromatin assembly factor involved with histone assembly on replicating DNA [36]. More recently, a role for Rtt106 in RD histone gene repression was discovered using a novel dual reporter-based synthetic genetic array (R-SGA) screen [25]. In the R-SGA, array-based yeast genetics was used to engineer yeast cells containing a plasmid with the *HTA1* upstream regulatory sequences directing cell cycle-specific synthesis of a green fluorescent protein (GFP) reporter, and also expressing an integrated control reporter composed with a constitutive promoter driving expression of *tdTomato* [37] (RFP). The two reporters were introduced into WT cells and the so-called non-essential deletion array, composed of strains individually deleted for each of the ~5,000 non-essential genes in the yeast genome [38]. As its name implies, the R-SGA method exploits SGA technology [39] that automates yeast genetics, enabling rapid introduction of marked genetic elements of interest into the yeast deletion mutant and other arrayed collections through several replica-pinning steps [40]. In R-SGA, the deletion strains carrying the two reporter genes are monitored for relative GFP:RFP fluorescence levels and compared to that of WT *Sc*. In the *HTA1*–GFP screen, decreased GFP:RFP identified potential *HTA1* activators, while higher GFP:RFP identified putative repressors. As expected for a repressor of *HTA1*, deletion of *ASF1* or any one of the four *HIR* genes resulted in increased GFP:RFP [25]. Interestingly, increased fluorescence was also observed in the strain containing a deletion of *RTT106*, implicating Rtt106 in repression of *HTA1* [25]. Subsequent experiments showed that Rtt106 specifically cross-links to CCR/NEG-containing chromatin in an *ASF1*- and *HIR*-dependent fashion [25]. Also, deletion of *RTT106*, like that of *ASF1* and *HIR1*, causes a decrease in histone density in the regulatory region of the NEG-dependent gene pairs [25]. Rtt106 functions by binding histones H3 and H4 and has in vitro nucleosome assembly activity, consistent with a histone

chaperone role [36]. Rtt106 directly recruits the RSC chromatin remodeling complex (see below) to NEG-containing chromatin outside of S phase [41, 42].

Yta7

The Yeast tat-binding analog 7 (Yta7) protein also functions in HIR/Asf1/Rtt106-mediated repression of the NEG-regulated histone gene pairs in *Sc*. *YTA7* encodes a protein that possesses an AAA-ATPase domain and a bromodomain (BD)-like region [43]. BDs are well characterized acetyl-lysine binding domains, but elucidating the function of Yta7's non-canonical BD has not been straightforward. Deletion studies indicate that, although the Yta7 BD might not bind acetylated histones as do canonical BDs, it is required for specific Yta7 distribution along chromosomes [44]. Yta7 functions as a boundary protein, acting as a physical barrier between regions of silent and active chromatin around the *HMR* locus, a role that requires the presence of the Yta7 BD [43–45]. Deletion of *YTA7* results in the spreading of silent chromatin from the *HMR* locus causing repression of a flanking reporter gene [43].

Yta7 was initially implicated in regulation of histone gene expression because deletion of *yta7* causes a genetic interaction with deletion of *HIR1* [46]. Subsequent ChIP analysis revealed that Yta7 localizes specifically to regulatory regions of the HIR-regulated histone loci [25, 34, 46]. Consistent with these experiments, the R-SGA screen discussed above identified *YTA7* as a putative activator of *HTA1*, since a *yta7Δ* strain had reduced GFP:RFP fluorescence relative to a WT strain [25]. Also, *HTA1* mRNA levels are reduced in yeast cells deleted for *YTA7* [25, 34]. ChIP experiments from a *yta7Δ* strain showed significant mis-localization of Rtt106, but not Hir1 or Asf1, across the *HTA1* locus, with spreading of Rtt106 from the NEG region through the *HTA1* ORF where it is not normally found [25, 34]. Additionally, the Yta7 AAA-ATPase domain is required to prevent Rtt106 spreading, since a *yta7-K460A* point mutation which is predicted to disrupt AAA-ATPase activity does not stop Yta7 localization to chromatin but is unable to prevent Rtt106 spreading [47]. Thus, one function of Yta7 in histone gene repression appears to be the restriction of HIR- and Asf1-recruited Rtt106 to the regulatory region of NEG-regulated histone gene pairs. Rtt106 spreading into the *HTA1* coding region is therefore postulated to result in repression of transcription, presumably by inhibiting transcriptional elongation by RNAPII, explaining the identification of Yta7 as an activator of *HTA1*–*GFP* in an R-SGA assay [25]. Like HIRA and Asf1, Yta7 is conserved in higher order eukaryotes [48]. The ATAD2 protein of humans is highly similar to Yta7 and its expression has been correlated with the proliferative growth of several aggressive cancers; however, its precise function

and any relationship with RD core histone gene expression remains to be determined [49–51]. Although unrelated at the sequence level, Yta7 appears to function similarly to mammalian CCCTC-binding factor (CTCF), which binds insulator elements to act as a barrier protein in vertebrates, and also regulates gene expression by binding to the regulatory region of imprinted genes [52].

Model for histone gene repression

How do the HIR, Asf1, and Rtt106 histone H3–H4 chaperones collaborate with boundary protein Yta7 to repress transcription of *HTA1–HTB1*, *HHT1–HHF1*, and *HHT2–HHF2*? Although HIR and Asf1 interact directly, Rtt106 interaction with Asf1 (or HIR) appears to be indirect and mediated through histones H3–H4. Mutations that disrupt/reduce the histone-binding ability of Asf1 and/or Rtt106 result in reduced or abolished recuitment of Rtt106 to the histone loci as well as increased transcription of *HTA1* [34, 53, 54]. Rtt106's function in repression appears linked to another protein complex previously implicated in repression of CCR/NEG-regulated histone loci, the ATP-dependent chromatin remodeling complex remodels structure of chromatin (RSC). A genome-wide analysis of RSC localization revealed HIR-dependent RSC association with CCR/NEG-containing chromatin regions [42]. RSC recruitment to histone loci coincides with periods of histone gene repression [42], and is dependent on Asf1 and Rtt106 [41]. Consistent with a functional linkage to Rtt106, the RSC component Rsc8 spreads along with Rtt106 in a *yta7* mutant background [47]. Although the presence of RSC at histone regulatory regions is tightly linked to that of Rtt106, its exact contribution to repression remains unclear. Whether NEG-regulated histone gene repression depends on expression of any RSC complex components remains to be tested. A simple model explaining repression of NEG-dependent RD core histone genes outside of S phase is diagrammed in Fig. 2. In this model, Rtt106 collaborates with RSC to assemble H3–H4 onto chromatin. Chromatin assembly occludes promoter sequences, which prevents the recruitment of the general transcription apparatus and RNAPII [47].

What is the signal for repression of histone transcription as the S phase draws to a close? The negative feedback model [34, 55, 56] proposes that soluble histone levels are monitored by HIR/Asf1/Rtt106. According to this hypothesis, when DNA replication is almost finished there are fewer available genomic locations for assembly of newly synthesized histones. Therefore, at this time, all histone chaperones including HIR, Asf1, and Rtt106 become fully charged with their histone substrate, which in turn facilitates their assembly onto NEG-containing chromatin with concomitant promoter occlusion and reduced recruitment
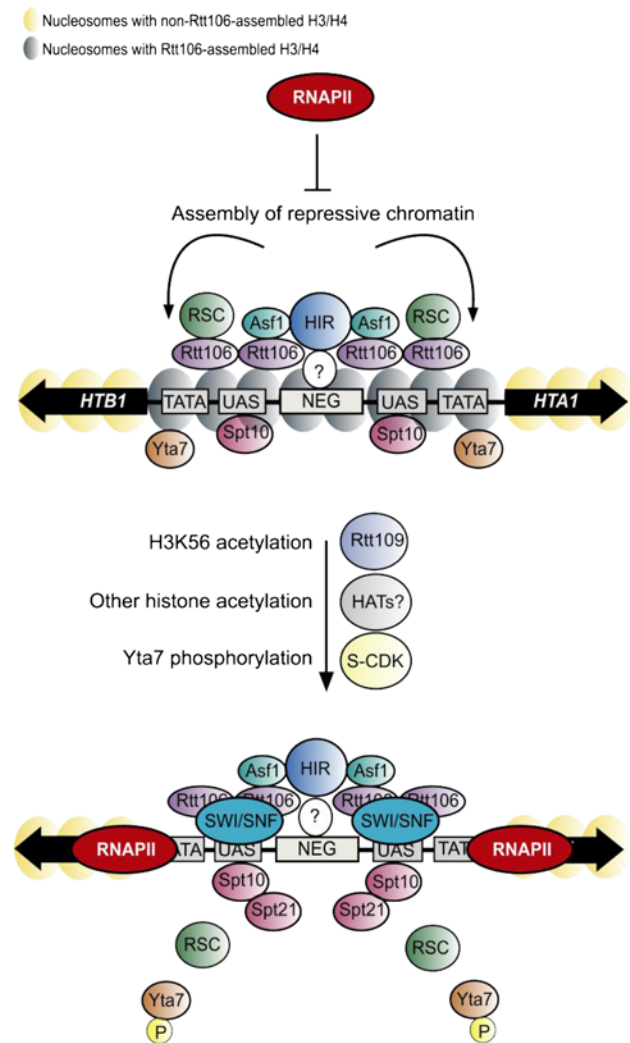

Fig. 2 Repression/activation model for the regulation of RD histone gene transcription in yeast. *Upper panel* a repressed *HTA1–HTB1* gene promoter. Here, HIR recruits Rtt106, RSC, Asf1, and Yta7 in order to generate a repressive chromatin structure (nucleosomes with Rtt106-assembled H3/H4) at promoters. *NEG* also CCR (cell cycle control region), *UAS* upstream activating sequence and TATA (TATA-box). A repressive chromatin structure prevents the recruitment of the basal transcription machinery and RNAPII. See text for details. *Lower panel* the *HTA1–HTB1* promoter in an active state. Rtt109-dependent incorporation of H3 acetylated at K56 (H3K56ac) enables recruitment of SWI/SNF, which generates a nucleosome-depleted promoter, enabling RNAPII recruitment. Other unidentified HATs are very likely involved in this process as well (see text for details). S phase forms of CDK1 (S-CDK) then phosphorylate Yta7 causing its eviction from promoters (which is tracked by the RSC), which is important for efficient promoter escape and transcript-elongation by RNAPII. Spt10 and Spt21 have important roles in these processes as well, but their molecular functions remain to be revealed

of RNAPII. This model is supported by analysis of yeast strains deleted for genes encoding the chromatin assembly factors/histone chaperones Spt4, Spt5, and Spt6, which exhibit reduced transcription of *HTA1–lacZ* in a manner

dependent on NEG [57]; in several cases, the defective activation phenotype is suppressed by H3–H4 over-expression [57]. These observations are consistent with the negative feedback model: deletion of genes encoding other histone chaperones like Spt4, Spt5, and Spt6 may decrease the histone-binding 'capacity' in the cell, promoting loading/charging of other chaperones like HIR/Asf1/Rtt106 earlier than normal; this may cause premature repression of histone genes transcription and thus less histone mRNA. In this scenario, NEG-dependence is a simple consequence of HIR/Asf1/Rtt106-mediated assembly or repressive chromatin—without NEG, there can be no HIR-mediated repression.

Trans-acting factors with roles in activation of histone gene transcription

SWI/SNF

The first activator identified to function in histone gene expression was the SWI/SNF ATP-dependent chromatin remodeling complex [58]. SWI/SNF subunits Swi2 and Snf5 specifically cross-link to NEG region chromatin in a cell cycle-regulated fashion, with peak association during late G1/S when histone genes are transcribed [41, 58]. Deletion of *SNF5* strongly reduces histone gene expression but only in the presence of intact NEG or HIR, suggesting that NEG-dependent, HIR-mediated repression (also including Asf1 and Rtt106, see above) creates a transcriptional block that must be overcome by ATP-dependent chromatin remodeling [58]. Snf5 ChIP assays revealed that recruitment of SWI/SNF to the *HTA1* locus requires the putative lysine acetyltransferase *SPT10* (see below) and is abolished in a H3K56R mutant [59]. Specific dependence of SWI/SNF recruitment on the H3K56-specific histone acetyl-transferase (HAT) *RTT109* (see below) remains to be established.

In addition, deletion of genes encoding any of the four HIR subunits, Asf1, or Rtt106 prevents recruitment of SWI/SNF subunits to histone genes [15, 58]. Rtt106 is likely to directly recruit SWI/SNF through a physical interaction that has demonstrated both in vivo and in vitro [41].

Spt10 and *Spt21*

SPT10 (suppressor of Ty) and *SPT21* were originally identified in a genetic screen for mutations that suppress a transcriptional defect linked to insertion of a Ty1 transposon, permitting transcription of a flanking reporter to initiate from the 3′ long terminal repeat (LTR) of Ty1 elements [60, 61]. *SPT10* is required for transcription of all core histone genes including the non-NEG-containing *HTA2–HTB2* loci [59, 62]. *SPT10* encodes a DNA-binding protein

that binds with high affinity, and in a cooperative manner, to pairs of the histone UAS elements [63], four of which are found in the regulatory regions of the four histone gene pairs (see above). In addition to its DNA-binding domain, Spt10 possesses a histone acetyl-transferase (HAT) domain of the Gcn5-related N-acetyltransferases (GNAT) family [64]. Consistent with a role in histone acetylation, *SPT10* is required for the S phase-dependent accumulation of H3K9ac, H3K18ac, and H3K56ac in the regulatory region of all four histone gene loci [59]. Mutation of a putative catalytic residue in the HAT domain of Spt10 [62] results in a loss-of-function phenotype but HAT activity for Spt10 has never been directly demonstrated. Also, the catalytic-site mutant of Spt10 is very unstable in vivo [64], making it difficult to link the phenotype to a loss of putative HAT activity.

The Spt21 protein is functionally linked to Spt10 [65]. Spt21 is required for S phase binding of Spt10 to the regulatory region of all four histone gene pairs [66]. Deletion of *SPT21* results in significant reduction of transcription of the *HTA2–HTB2* [67] gene pair that does not contain a CCR/NEG region. In addition, Spt10 and Spt21 physically interact in vitro and, like Spt10, Spt21 cross-links to the regulatory region of all four histone gene pairs [66]. Spt10 and Spt21 are also functionally linked to transcriptional silencing [68] and affect repression of the three NEG-dependent histone loci [69]. The precise roles of Spt10 and Spt21 in regulating histone gene transcription and whether HAT activity is involved remain to be elucidated.

MBF and SBF

MBF and SBF are activators of G1/S-expressed genes [70] and were functionally linked to the histone gene activation using global ChIP analyses [71, 72]. SBF and MBF share a common regulatory subunit called Swi6, and have dedicated DNA binding subunits, Swi4 and Mbp1, in SBF and MBF, respectively [73]. Consistent with a role in activation of histone gene expression, deletion of either *SWI4* or *MBP1* reduces levels of histone mRNA, and either mutant is lethal when combined with a mutation of *SPT10* [66]. This synthetic lethality makes sense in that consensus binding sites for Swi4 and Mbp1 overlap with the histone UAS, suggesting that SBF/MBP1 and Spt10 function in separate activation pathways [74]. In fact, Spt10 and SBF together can recapitulate the WT cell-cycle dependence of *HTA1–HTB1* transcription with SBF responsible for a small early peak of transcription (late G1) and Spt10 for the remainder (S phase) [74]. The mechanism by which SBF and MBP1 activate histone gene transcription remains to be determined. Given what is known about SBF/MBF activation of G1-S genes, possible mechanisms include antagonism of

HIR/Rtt106-assembled chromatin or recruitment of classic co-activators such as SAGA or Mediator.

Rtt109–Vps75

As well as identifying new repressors of histone genes (see above), R-SGA analysis of *HTA1–GFP* also identified *RTT109* and *VPS75* as genes encoding potential activators of histone gene expression [25]. Expression of *HTA1* is decreased in cells deleted for *RTT109* consistent with a role in activation [25]. *RTT109* encodes a HAT that, in conjunction with Asf1 [75], catalyzes H3K56ac [76–78], a modification associated with RD chromatin assembly [79]. During S phase, Rtt109–Asf1-catalyzed H3K56ac enhances RD chromatin assembly by increasing the interaction between H3–H4 dimers and Rtt106 and CAF-1 histone chaperones [80]. Additionally, H3K56ac leads to chromatin disassembly during transcription activation of *PHO5* [81]. During this process, Rtt109-acetylated H3K56 on Asf1-bound H3–H4 is exchanged with unacetylated histones on chromatin leading to disassembly in conjunction with ATP-dependent chromatin remodeling. R-SGA also implicated *VPS75* in regulation of *HTA1* [25]. In vivo, Rtt109 forms a stable protein complex with Vps75 which is also an H3–H4 histone chaperone [82, 83]. Rtt109–Vps75 acetylates H3K9ac in vivo in a pathway that requires *ASF1* [84].

Model for histone gene activation

We previously proposed that activation of S phase-specific transcription of NEG-regulated histone genes occurs by overcoming constitutive HIR/Rtt106 repressive chromatin [25]. Specifically, we suggest that Rtt109 enhances transcription of the *HTA1* gene (and likely other histone genes) by encouraging chromatin disassembly at the locus through deposition of H3K56ac–H4 dimers, promoting a more "open" nucleosome conformation [79, 85]. When incorporated into chromatin at the NEG-region, H3K56ac promotes SWI/SNF-dependent chromatin disassembly and subsequent activation of transcription. SWI/SNF is possibly recruited initially to the NEG-regulated histone loci via its demonstrated physical interaction with Rtt106 (Fig. 2). Evidence for this model includes the observation that SWI/SNF recruitment to *HTA1* is abolished in a H3K56R strain [59], and the fact that histone promoters are nucleosome-free in *asf1∆*, *hir1∆*, and *rtt106∆* strains [25], suggesting that proper NEG-based repression is a function of chromatin assembly. Further tests of the model ought to include assessment of SWI/SNF recruitment in RTT109 (and VPS75) mutants, and analysis of the interplay between SWI/SNF, Rtt106, and H3K56ac in promoting proper histone gene expression.

A seeming paradox exists in this model in that we propose Rtt109 activates histone gene expression but Asf1, its cellular collaborator in H3 acetylation, is a repressor. This paradox is potentially resolvable in a scenario in which Asf1 both activates and represses core histone transcription, an idea that has been previously suggested by Sutton et al. [33]. Thus, we propose that, like Rtt106, HIR and Asf1 are also tethered to histone promoters through the cell cycle [25]. Continuous HIR/Asf1-based recruitment of Rtt106 would ensure repressive chromatin is always assembled outside of S phase. However, in late G1 and S phase when core histone genes are transcribed, Rtt109 works via the tethered Asf1 to acetylate H3 which is then deposited into chromatin via Rtt106, leading to activation.

The model for histone gene activation we summarize above raises many questions that remain to be addressed:

(1) What directs Rtt109's acetylation activity to histone promoters? We suggest that UAS-recruited Spt10, either by itself or with Spt21, directs the assembly of Rtt109-acetylated H3–H4 (H3K56ac) into chromatin at histone promoters in late G1/S. Vps75 may also have a role since *SPT10* is also required for accumulation of H3K9ac (59), which is catalyzed by Rtt109–Vps75 in an Asf1-dependent pathway [84].

(2) Is there redundancy in histone acetylation at histone gene promoters during transcriptional activation? To date, Rtt109 has only been shown to activate *HTA1*. Interestingly, Gcn5, the other H3K9 HAT in yeast, acetylates H3K18 and has been implicated in RD chromatin assembly of H3 and H4. In vivo, *SPT10* is required for accumulation of H3K9ac and H3K18ac in addition to H3K56ac in the coding region of *HTA2* [59]. It will be interesting to determine if Gcn5 functions in a partially redundant pathway with Rtt109 in histone gene expression downstream of Spt10. Interestingly, H3K56ac is also enriched in chromatin at histone promoters in human cells [86]. The specific HAT(s) responsible for H3K56ac at histone promoters and identity of any downstream proteins recruited following histone modification remain to be determined.

(3) How are Spt10/21 activated in S phase? In the related ascomycete yeast *S. pombe*, S phase activation of core histone transcription is directed by the Ams2 protein [87], a member of the GATA-type transcription factor family [88]. Activation by Ams2 appears to be restricted to S phase by different E3 ligases that promote its ubiquitination and degradation pre- (G1) and post-S phase (G2/M) [89, 90]. Although any evolutionary relationship between Ams2 and the Spt10 or Spt21 is unclear, it will be interesting to determine if they are subject to similar post-translational regulation.

Regulation of transcriptional elongation at RD histone loci

Cell cycle control of histone gene expression is not restricted to regulation of RNAPII recruitment. Transcriptional elongation at the RD histone loci is also subject to cell cycle control. Outside of the S phase, the AAA-ATPase domain of Yta7 is important for boundary function, as defined by correct positioning of RSC and Rtt106 [47]. During S phase, the N-terminus of Yta7 (where the AAA-ATPase domain is located) is hyper-phosphorylated by both the S phase-specific forms of cyclin-dependent kinase 1 (Cdk1) and casein kinase 2 (CK2). Cdk1 is the major cell cycle kinase in yeast [91] while CK2 has general roles in regulating transcription [92]. Phosphorylation of Yta7 promotes its dissociation from *HTA1* chromatin during S phase, which in turn is essential for promoter escape and elongation by RNAPII [47], probably through histone eviction [93]. Notably, Yta7 regulation by Cdk1 represents the first concrete mechanistic link between the cell cycle machinery and to histone gene transcription. In human cells, Cdk colocalizes with the NPAT proteins to histone gene clusters in subnuclear Cajal bodies [94, 95] and phosphorylation of NPAT by cyclin E-CDK2 activates histone gene transcription [94–96]. The underlying molecular mechanisms of NPAT regulation, however, remain elusive.

Spt4, Spt5, Spt6, and the FACT complex, composed of Pob3 and Spt16, are regulators of transcriptional elongation, and all three can be cross-linked specifically to the coding region of the constitutively transcribed *PMA1* gene [25]. Spt16 also associates with *HTA1* chromatin during the time in the cell cycle when *HTA1* is repressed [47], implicating FACT in repression of histone gene transcription. Spt6 and Rtt106 are known to function in parallel to suppress cryptic initiation at an internal promoter within the *FLO8* gene and both associate with the coding region of constitutively transcribed genes ([25], see above, [97]). However, at the *HTA1–HTB1* locus there exists a physical separation of Rtt106 (and HIR/Asf1), which crosslinks to the regulatory region, from Spt4, Spt5, Spt6, and FACT, which cross-link to the coding region [25]. Thus, although one function of Yta7 may be to restrict Rtt106 to the regulatory region, an additional function may be to ensure exclusive regulation by HIR/Asf1/Rtt106 by preventing FACT and/or Spt4, Spt5, and Spt6 from accessing the region.

Post-transcriptional regulation of histone mRNAs

Clear mechanisms exist to regulate histone mRNAs post-transcriptionally. In metazoans, RD core histone genes are not polyadenylated, but contain a unique 3′ end structure which consists of an unusual highly conserved stem loop structure that forms in the 3′-untranslated region (UTR) of the mRNA. The conserved stem-loop binding protein (SLBP) interacts with this structure and regulates all downstream events including proper termination, mRNA export, translation, and regulated mRNA degradation post-S phase [98]. Most lower eukaryotes do not share this unique form of regulation although some protozoans appear to encode proteins with similarity to SLBP [99]. In yeast, histone mRNAs are polyadenylated, albeit possessing shorter polyA tails than most yeast genes [100, 101]. Despite the absences in *Sc* of both a stem loop in 3′-UTR of RD core histone mRNA and the SLBP protein, 3′-UTR-dependent post-transcriptional regulation is a significant contributor to their characteristic cell cycle expression pattern [18]. Fusion of the 3′-UTR of *HTB1* to a reporter gene under the control of the *GAL1* promoter is sufficient to cause S phase-specific transcript accumulation in the presence of galactose [102]. Thus, it could be argued that mechanisms exist throughout the cell cycle to degrade histone mRNAs, and that this mechanism is antagonized specifically in S phase. Consistent with this hypothesis, a protein that specifically binds the *HTB1* 3′-UTR has been seen using a gel shift assay with S phase-specific whole cell extracts [103]. The identity of this protein remains unknown.

What are the mechanisms in *Sc* that monitor histone mRNA accumulation? Regulated mRNA degradation generally occurs in either the 3′–5′ or 5′–3′ direction. Generally, the first step in degradation of a polyadenylated mRNA is deadenylation, which produces an oligoadenylated mRNA. Degradation of oligoadenylated mRNA then proceeds via either of two riboexonucleolytic pathways: the exosome complex degrades mRNA from the 3′–5′ direction, while Xrn1 degrades messages from the 5′–3′ direction. For the Xrn1 exoribonuclease to function, de-capping of the mRNA must first occur (in *Sc* by Dcp1/Dcp2). The Lsm1-7–Pat1 complex is composed of seven Sm-like proteins (Lsm1 through Lsm7 as well Pat1) and functions to stimulate de-capping by binding to oligoadenylated mRNAs. Binding of Lsm1-7–Pat1 to deadenylated mRNA is enhanced on mRNAs that also carry U-rich sequences at their 3′ end [104]. There is evidence that RD core histone accumulation is monitored post-transcriptionally by both the exosome and Xrn1. [15]. For example, deletion of exosome component *RRP6* results in continued accumulation of *HTB1* mRNA after S phase [105], while deletion of *LSM1* causes a G1-specific increase in histone mRNA levels and a synthetic growth defect in combination with a *HIR1* deletion [15]. Consistent with an important interplay of transcriptional post-transcriptional regulatory mechanisms for ensuring the appropriate histone, gene deletion of both *HIR1* and *LSM1* causes a dramatic fitness defect. Also, combined deletion of *LSM1* and the exosome component

SKI2 causes greater accumulation of all histone mRNAs than seen in either single deletion strain, indicating that RD histone mRNA abundance is regulated by both 3′–5′ or 5′–3′ degradation pathways [15].

What targets Lsm1-7-Pat1 to histone mRNA? Post-S phase, the metazoan Lsm1-7-Pat1 complex is recruited to histone mRNA as a consequence of SLBP-stimulated olig-ouridylation of histone mRNA, due to recruitment of terminal uridylyl transferases (TUTases) [106]. The core histone genes do not appear to contain an obvious U-rich sequence in their 3′-UTR and no TUTase has been characterized in yeast. One candidate worth testing are the non-canonical poly A-polymerase Trf4 and Trf5, members of the TRAMP complex previously implicated in the regulation of histone mRNA abundance in Sc [107]. Deletion of *TRF5* is strongly synthetic sick/lethal with *rad53∆* and *hir1∆*, and *trf5∆* mutants have elevated levels of histone mRNA [107]. It has been suggested that Trf4/5 may function upstream of the exosome [107], but whether it functions upstream of Lsm1-7–Pat1 and has TUTase activity remains to be tested.

The data we summarize above shows that core histone mRNA accumulation is monitored by the major exoribonucleic activities of the cell. An important regulatory question is how S phase histone mRNAs avoid the action of exoribonucleic pathways to enable S phase-specific transcript accumulation. The answer probably lies in the 3′ UTR of RD core histone mRNA. As mentioned above, a previous study found an unidentified S phase protein that can specifically associate with *HTB1* mRNA in vitro [103]. This protein—or another regulator—may function to prevent association of the Lsm1-7–Pat1 complex and encourage guidance of histone mRNAs to the ribosome for productive translation. In addition, histone mRNAs are subject to regulation at the level of polyA tail length. As noted above, histone mRNAs have very short polyA tails during S phase, and relatively longer polyA tails in G1 phase [101]. SEN1 encodes an essential helicase-like protein that binds Nab3 and Nrd1, which together function in transcription termination, and interacts with the exosome via Nrd1 [108]. One model proposes that Sen1 coordinates transcription termination with the addition of a longer polyA tail to histone mRNAs outside of S phase, which ultimately results in exosome-mediated transcript degradation [101]. An untested prediction of this model is that Sen1 should cross-link to RD core histone genes outside of S phase. A more speculative extension to this model proposes that Yta7, which does not interact with the NEG region during S phase, coordinates Sen1-based termination of transcription so that longer polyA tails are produced, which are then shunted to the nuclear exosome for degradation. A genetic study suggest the alternate possibility that Yta7 functions in the same pathway as Mex67, a poly(A) RNA binding protein involved in regulated nuclear mRNA export, and

a component of the nuclear pore complex [109]. A derivative of Mex67 encoded by the *mex67-5* conditional allele remains in the cytoplasm at the restrictive temperature, a phenotype that is suppressed by deletion of *YTA7* [109]. Since Yta7 localizes to chromatin when histone expression is not required, an additional function of Yta7 may be to antagonize the nuclear export of histone mRNA. As noted earlier, a Yta7-13A phospho-mutant remains on chromatin through S phase and has a severe defect in S phase histone expression [47], and *HTB1* mRNA is retained in the nucleus during G1 [101]. It will be interesting to determine if the *yta7-13A* mutant shows nuclear retention of *HTB1* mRNA in S phase.

Translational and post-translational regulation of histone mRNAs

In the slime mold *Physarum polycephalum* and protist *Leishmania infantum,* regulation of RD core histone synthesis occurs at least in part at the level of translation [110, 111]. In metazoans, SLBP coordinates translation by interacting with SLIP1, which interacts with translation initiation factor eIF4G [112]. In human cells, the La protein, which contains the RNA binding La motif, has been reported to stabilize S phase histone mRNA, promoting their translation [113]. Whether or not RD core histone translation is subject to cell cycle regulation in *Sc* remains unclear, but the La-related proteins Sro9 and Slf1 co-purify with RD core histone mRNA [114]. Sro9 shuttles between the nucleus and cytoplasm and specifically cross-links to actively transcribed genes [115]. Further work is required to determine whether Sro9 and/or Slf1 function to escort S phase histone mRNA to the ribosome, antagonizing mRNA degradation pathways.

Histone expression is also regulated at post-translational level in *Sc*. For example, the negative feedback model described above proposes that histone protein levels are monitored and a feedback mechanism induces repression of transcription. In addition, a Rad53-dependent mechanism exists to degrade excess soluble histone proteins in *Sc* [7, 116]. The Rad53 checkpoint kinase phosphorylates soluble histones not bound to a histone chaperone leading to their ubiquitination and subsequent degradation by the proteasome [117].

Summary/future perspectives

In this review, we have summarized work exploring many facets of histone gene regulation, many of which impinge in some way on the chromatin at histone loci regulatory regions. Nucleosome occupancy at histone loci has been

assessed in two ways. First, cross-linking of histone H3 or H2B to the *HTA1–HTB1* promoter revealed that H3 and H2B levels are significantly reduced in chromatin isolated from *asf1Δ*, *hir1Δ*, or *rtt106Δ* mutant backgrounds [25]. Second, nucleosome occupancy at the NEG-regulated histone gene pairs was assessed using MNase to specifically purify nucleosome-bound DNA which was then hybridized to tiling microarrays with 4-bp resolution [25, 118]. This experiment revealed that the NEG-containing regulatory region including the promoter sequence is nucleosome-free in *hir1Δ* and *rtt106Δ* strains [25]. These experiments were performed with chromatin isolated from asynchronously growing cells. It will be useful to assay nucleosome occupancy at histone loci using yeast chromatin harvested from cells proceeding synchronously through the cell cycle. The model in Fig. 2 predicts that histone promoter regions will be nucleosome-free during time cell cycle periods corresponding to histone gene expression (late G1 and during S phase). In contrast, at all other times of the cell cycle when histone expression is repressed, nucleosomes should be positioned over the promoters as a result of recruitment of the HIR/Asf1/Rtt106 histone chaperones, which in turn assemble histones into a repressive chromatin structure that spreads towards the promoter, inhibiting transcription. The same repressive nucleosome pattern should be seen along the histone gene regulatory regions upon DNA damage, in a manner dependent on *HIR1* and *RTT106*. These types of experiments will provide a view of the dynamics of nucleosome assembly at histone gene regulatory regions in response to genomic stress and cell cycle regulatory signals.

Several interesting questions concerning HIR-, Asf1-, and Rtt106-mediated chromatin assembly remain to be addressed. Asf1, HIR, and Rtt106 all appear to be individually capable of depositing histones so why do they function in an apparent linear pathway? Which of these three histone H3–H4 chaperones deposit histones onto DNA and what are their roles as chaperones of parental versus newly synthesized histones [119, 120]? Does HIR function at NEG-regulated histone gene pairs simply to recruit Rtt106 via Asf1, or is HIR involved in a more complicated histone transfer pathway? Genetic evidence supports a more complex role for the HIR complex: the defect in *HTA1* transcription in an *rtt106Δ* strain is not as severe as that seen in a *HIR1* deletion mutant [25]. This phenotype may reflect dual roles for Rtt106 in both activation (via SWI/SNF recruitment) and repression (via chromatin assembly and recruitment of RSC), or may point to an additional as yet uncharacterized histone chaperone pathway leading to HIR-dependent repression. The mechanism of Rtt106 spreading in the absence of the chromatin boundary protein Yta7 [25] deserves further investigation. In particular, it will be important to assess whether Rtt106 spreading is

dependent on Rtt106 homo-oligomerization [121], or its preference for H3K56ac–H4 dimers. Interestingly, despite the absence of Yta7 in S phase, Rtt106 remains restricted to the CCR/NEG region of chromatin at *HTA1* throughout the entire cell cycle [25]. This and other observations hint at the existence of a previously unknown and potentially novel mechanism of chromatin remodeling and transcriptional regulation.

Although it is clear that the H3K56 HAT Rtt109 and the putative HAT Spt10 are involved in histone gene regulation, their mechanisms of action remain obscure. It will be useful to ask whether H3K56ac levels at histone gene promoters are abolished in cells deleted for *RTT109*, consistent with a role upstream of Rtt109 in H3K56ac at histone gene promoters. Also, the role of Rtt109-binding partner and H3–H4 histone chaperone Vps75 needs be clarified. Possible Vps75 functions include stabilization of Rtt109 or Rtt109–Vps75-based acetylation of H3K9 or K56 [84, 122]. Furthermore, the precise relationship between H3K56ac, Rtt109, and Spt10 remains unclear, and acetylation targets of Spt10 are unknown.

Many other questions remain concerning the activation of RD core histone expression in *Sc*. For example, it is unclear how efficient DNA replication through the histone gene loci is coordinated with the high levels of S phase transcription of RD core histone genes. R-SGA of *HTA1–GFP* in an *RRM3* deletion strain revealed a phenotype consistent with a role in *HTA1* activation [25]. *RRM3* (also called *RTT104* [35]) encodes a DNA helicase that promotes progression of the replication fork through obstacles in the DNA, as might occur during active transcription. Further analysis will be required to understand how Rrm3 may coordinate these two processes to promote RD core histone transcription.

Although it is clear that histone transcription is linked to CDKs (see discussion of Yta7 above), very few direct targets of CDKs are known to regulate histone transcription. For example, mitotic B-type cyclins are involved in the alleviation of HIR-mediated repression at the G1 to S transition [26], but the target of this regulation and the underlying mechanisms are unknown. Kinase targets involved in the repression of histone genes following genotoxic stress also remain elusive. Checkpoint kinases directly regulate the inhibition of DNA replication by phosphorylation of key factors [123]. As DNA-replication and histone transcription are tightly linked, it is very likely that checkpoint kinases also act on histone regulators to repress histone gene transcription in DNA damaging conditions.

Much of what is known about the regulation of histone gene expression in *Sc* applies to the well-studied CCR/NEG containing histone gene pairs. By contrast, little is known concerning mechanisms underlying the regulation of the *HTA2–HTB2* gene pair that contains a histone UAS but not

NEG in its regulatory region. Why one of the core histone gene pairs, *HTA2–HTB2*, is regulated by a HIR-independent mechanism remains unclear, and this apparently distinct pathway deserves further exploration. We do know that all four of the histone pairs are subject to histone UAS-mediated cell cycle activation by Spt10/Spt21 and SBF. However, what is the basis of activation? Are a different set of proteins involved in overcoming repressive chromatin at *HTA2–HTB2*? Functional genomic approaches such as R-SGA using an *HTA2–GFP* reporter may reveal specific regulators that act at the *HTA2–HTB2* locus. In addition, the R-SGA approach [25] can be extended to explore other arrayed mutant collections, such as the collection of strains carrying temperature-sensitive alleles of essential genes and arrays of strains expressing over-expression alleles [124, 125]. Finally, R-SGA of a GFP reporter linked not to the promoter but to the 3′-UTR of *HTA1* has the potential to yield new information about post-transcriptional regulation of RD histone expression.

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