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Asf1 Mediates Histone Eviction and Deposition during Elongation by RNA Polymerase II

Short Article

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Summary

Histones are rapidly evicted and deposited during transcription by RNA polymerase (Pol) II, but a factor that mediates histone eviction in vivo has not yet been identified. Here, we show that the histone chaperone Asf1 associates with promoters and coding regions of transcriptionally active genes. Asf1 mediates histone H3, but not H2B, eviction and deposition during Pol II elongation, suggesting that nucleosome assembly and disassembly occur in a stepwise fashion. Lastly, Asf1 inhibits internal initiation from cryptic promoters within coding regions. These results strongly suggest that Asf1 functions as an elongation factor to disassemble and reassemble histones during Pol II elongation.

Introduction

Although nucleosomes are extremely stable biochemical entities, histone-DNA interactions are highly dynamic in vivo. First, transcriptional activator proteins can cause unfolding and dissociation of histones from promoter regions in the yeast *Saccharomyces cerevisiae* (Deckert and Struhl, 2001; Boeger et al., 2003; Reinke and Horz, 2003). Second, the yeast Swr1 complex mediates ATP-dependent exchange of the histone variant H2AZ (Korber et al., 2004; Mizuguchi et al., 2004). Third, in flies and mammals, the histone H3.3 variant is deposited into chromatin in a manner linked to transcriptional elongation (Ahmad and Henikoff, 2002; Janicki et al., 2004; Schwartz and Ahmad, 2005). Lastly, during transcriptional elongation by RNA polymerase (Pol) II, nucleosomes are in a highly dynamic equilibrium with all four histones being evicted and deposited upon passage of Pol II through coding regions (Kristjuhan and Svejstrup, 2004; Lee et al., 2004; Schwabish and Struhl, 2004; Zhang et al., 2005; Zhao et al., 2005). The dynamic behavior of histones during transcriptional elongation in vivo is critical, because Pol II itself is extremely inefficient at elongation on nucleosomal templates in vitro (Izban and Luse, 1991).

FACT and Spt6 are elongation factors that travel with Pol II through coding regions (Krogan et al., 2002; Pokholok et al., 2002; Mason and Struhl, 2003; Saunders et al., 2003) and are required for normal levels of histone occupancy in actively transcribed coding regions (Kaplan et al., 2003; Schwabish and Struhl, 2004). As such, FACT and Spt6 are important for histone deposition during Pol II elongation, and yeast cells lacking either FACT or Spt6 inappropriately initiate transcription from cryptic promoters within coding regions (Kaplan et al., 2003; Mason and Struhl, 2003). FACT (Belotserkovskaya et al., 2003) and Spt6 (Bortvin and Winston, 1996) can independently deposit core histones onto DNA in vitro, strongly suggesting that their roles in transcription-coupled histone deposition in vivo are direct. In addition to FACT and Spt6, the Eaf3-containing Rpd3 histone deacetylase complex and Set2 histone methylase are important for maintaining normal chromatin structure after Pol II elongation and for inhibiting initiation within coding regions (Carrozza et al., 2005; Joshi and Struhl, 2005; Keogh et al., 2005).

Very little is known about factors that evict histones during Pol II elongation in vivo. In vitro, FACT can facilitate Pol II elongation on chromatin templates by destabilizing nucleosomes and evicting H2A and H2B from DNA (Orphanides et al., 1998, 1999; Belotserkovskaya et al., 2003). However, there is no evidence that FACT is important for histone eviction in vivo (Schwabish and Struhl, 2004), and loss of FACT does not affect Pol II elongation rate or processivity in yeast (Mason and Struhl, 2005).

In this paper, we address the role of Asf1 in histone dynamics during Pol II elongation. Asf1, a component of the replication-coupling chromatin assembly factor (RCAF), copurifies with H3 and H4 and can assemble core histones into chromatin in vitro and during DNA replication and repair in vivo (Tyler et al., 1999). Asf1 is also important for Hir-dependent assembly of silent chromatin (Sharp et al., 2001; Krawitz et al., 2002), histone deposition at the *PHO5* promoter during chromatin reassembly after activator-mediated eviction of nucleosomes (Schermer et al., 2005), and replication-independent deposition throughout the cell cycle (Robinson and Schultz, 2003; Green et al., 2005). In addition to its multiple roles in histone deposition, Asf1 is important for activator-dependent eviction of H3 and H4 at the *PHO5* and *PHO8* promoters and for subsequent transcriptional activation (Adkins et al., 2004; Korber et al., 2006), and it may play a role in global disassembly of chromatin in vivo (Adkins and Tyler, 2004). Lastly, loss of Asf1 causes selective changes in gene expression that are independent of DNA replication and protein synthesis (Zabaronick and Tyler, 2005).

Here, we show that Asf1 functions as a Pol II elongation factor that both disassembles and reassembles histones during Pol II elongation. Furthermore, Asf1 inhibits transcriptional initiation from cryptic promoters within coding regions and hence contributes to transcriptional fidelity.

Results

Asf1 Associates with Promoters and Coding Regions of Transcriptionally Active Genes

Using a strain expressing $(Myc)_{18}$-tagged Asf1 from its normal chromosomal location, we examined the relationship of Asf1 occupancy with transcription (Figure 1; see Figures S1 and S2 in the Supplemental Data available with this article online). To examine *GAL*

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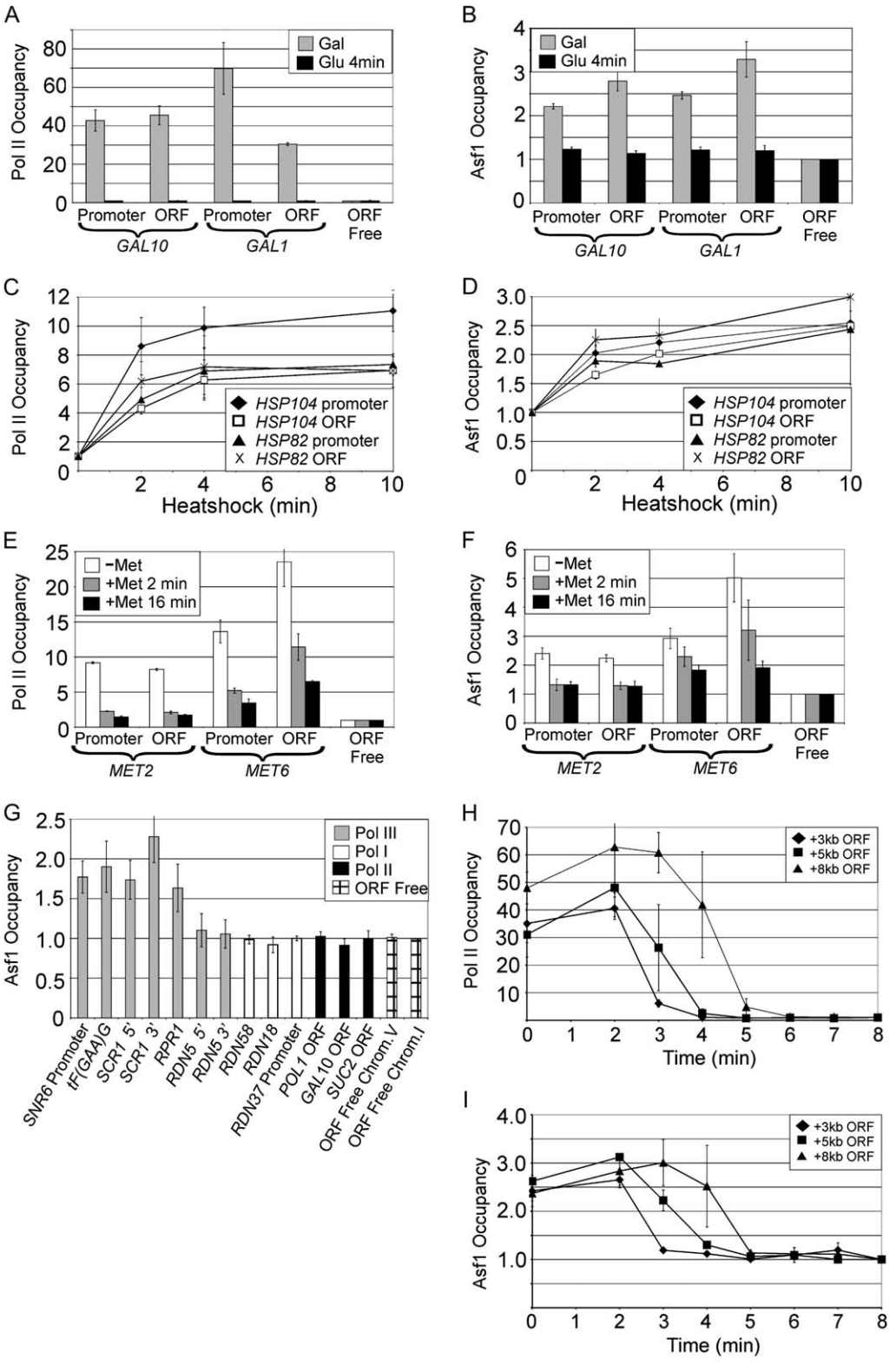

Figure 1. Asf1 Associates with Transcriptionally Active Promoters and Travels with Elongating Pol II

(A and B) (A) Pol II and (B) Asf1occupancy at the indicated regions of *GAL1,10* in cells expressing $(Myc)_{18}$-Asf1 grown in galactose (Gal) medium and then shifted into glucose (Glu) medium for 4 min.

(C and D) (C) Pol II and (D) Asf1 occupancy at the indicated regions of *HSP104* and *HSP82* in cells expressing $(Myc)_{18}$-Asf1 grown in YPD medium and heat shocked at 39ºC for the indicated times.

(E and F) (E) Pol II and (F) Asf1 occupancy at the indicated regions of *MET2* and *MET6* in cells expressing $(Myc)_{18}$-Asf1 grown in medium lacking methionine or treated by the addition of methionine for the indicated times.

transcription, cells were grown in galactose medium (activating conditions) and then shifted into glucose medium (repressing conditions) for 4 min. As expected, Pol II association at the *GAL* promoters and coding regions is nearly eliminated after this brief shift to repressing conditions (Figure 1A). Interestingly, Asf1 associates with the *GAL1/10* promoters and coding regions during transcriptional activation, and it rapidly dissociates from these regions upon transcriptional repression (Figure 1B). Similarly, in response to heat shock, Asf1 association with the *HSP104* and *HSP82* promoters and coding regions increases with similar kinetics as Pol II (Figures 1C and 1D). Lastly, Asf1 association with the *MET2* and *MET6* promoters and coding regions decreases in a manner that strongly correlates with Pol II dissociation when cells are treated with methionine (Figures 1E and 1F). Thus, Asf1 associates with both promoters and coding regions in a manner that correlates with Pol II association and the transcriptional status of the gene.

Asf1 also associates with all Pol III genes tested, except for the gene encoding the 5S RNA (Figure 1G). The 5S gene shows considerably lower levels of Pol III factors than typical Pol III genes (Moqtaderi and Struhl, 2004) and hence is presumably less transcriptionally active. This suggests that Asf1 association at Pol III genes is related to transcriptional activity. The 5S gene is located within the rDNA locus, which is otherwise transcribed by Pol I and is subject to Sir2-dependent silencing such that most copies of the rDNA repeat are transcriptionally inactive (Dammann et al., 1993). All locations tested within the rDNA locus do not show detectable levels of Asf1 association (Figure 1G). Thus, Asf1 associates with highly active Pol III genes, but it does not detectably associate with the Pol I- or Pol III-transcribed regions of the rDNA locus, presumably because only a minority of the rDNA repeats are transcriptionally active at any given time.

Asf1 Travels with Elongating Pol II

We examined the relationship between Asf1 association and Pol II elongation using a gene containing the *GAL1* promoter region upstream of the large (8 kb) *YLR454* coding region. Galactose-grown cells containing this gene were shifted to glucose medium, and the levels of Asf1 and Pol II association at various positions within the *YLR454* coding region were examined at various times after the shift. This approach kinetically monitors the last wave of Pol II transcription, and it has been used to assess traveling of putative elongation factors with Pol II (Strasser et al., 2002; Mason and Struhl, 2003), transcription-coupled histone deposition (Schwabish and Struhl, 2004), and Pol II elongation rates (Mason and Struhl, 2005). The patterns of Asf1 and Pol II occupancy are indistinguishable during the last wave of Pol II transcription (Figures 1H and 1I), indicating that Asf1 travels with elongating Pol II.

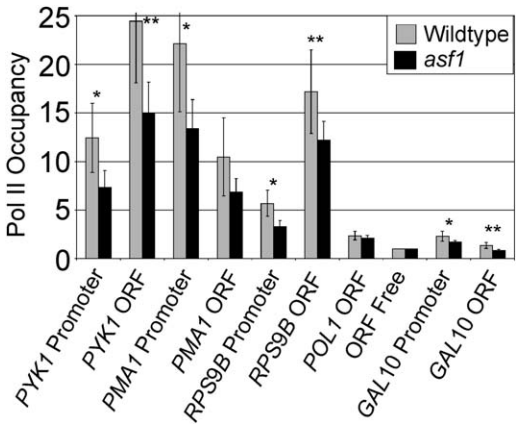

Figure 2. Asf1 Is Important for Normal Levels of Pol II Occupancy at Promoters and Coding Regions

Pol II occupancy in wild-type or *asf1* mutant cells that were grown in YPD medium. Data are expressed as fold over an ORF-free region on chromosome I defined as 1 and represent the average of five independent experiments with standard deviations shown. A t test was used to determine statistical significance, with * denoting $p < 0.025$ and ** denoting $p < 0.01$. Similar results were obtained when Pol II occupancy was calculated as percent immunoprecipitation efficiency.

Asf1 Is Necessary for Normal Pol II Occupancy

To further test the role of Asf1 in transcription, we examined Pol II occupancy in a wild-type or *asf1* null mutant. We observe a statistically significant decrease in Pol II occupancy in the *asf1* mutant compared to the wild-type (Figure 2). This observation is not inconsistent with the selective positive and negative effects on gene expression observed previously (Zabaronick and Tyler, 2005), because we analyze a small number of genes. In addition, a small general decrease in Pol II transcription might have been missed in the previous study, either due to normalizing the samples to total RNA, effects on RNA stability, or difficulty in measuring such subtle effects on microarrays.

Asf1 Is Important for H3, but Not H2B, Eviction during Pol II Elongation

Using a strain expressing a FLAG-tagged histone H2B as the only source of H2B, we examined H2B and H3 density at the *GAL1,10* locus in wild-type and *asf1* mutant strains. As expected (Schwabish and Struhl, 2004), wild-type cells grown in galactose medium show significantly reduced H2B and H3 density at the *GAL* promoter and coding regions compared to an ORF-free region (Figure 3A). Under these conditions of transcriptional activation, wild-type and *asf1* mutant strains show comparable levels of H2B density, but the *asf1* strain shows approximately 2-fold higher levels of H3 density than the wild-type strain (Figure 3A). In contrast, in glucose-grown cells, levels of H2B and H3

(G) Asf1 occupancy at the indicated Pol III genes and regions of the rDNA locus in cells expressing $(Myc)_{18}$-Asf1 grown in YPD medium. Data in (A)–(G) are expressed as fold over an ORF-free region on chromosome I defined as 1 and represent the average of three to four independent experiments with standard deviations shown.

(H and I) (H) Pol II and (I) Asf1 occupancy at the indicated regions of *GAL1-YLR454* in cells expressing $(Myc)_{18}$-Asf1 grown in galactose (Gal) medium and then shifted into glucose (Glu) for the indicated times. Data represent the average of two independent experiments and are expressed as fold over the control ORF-free region, with the 8 min time point set equal to 1. Standard deviations are shown.

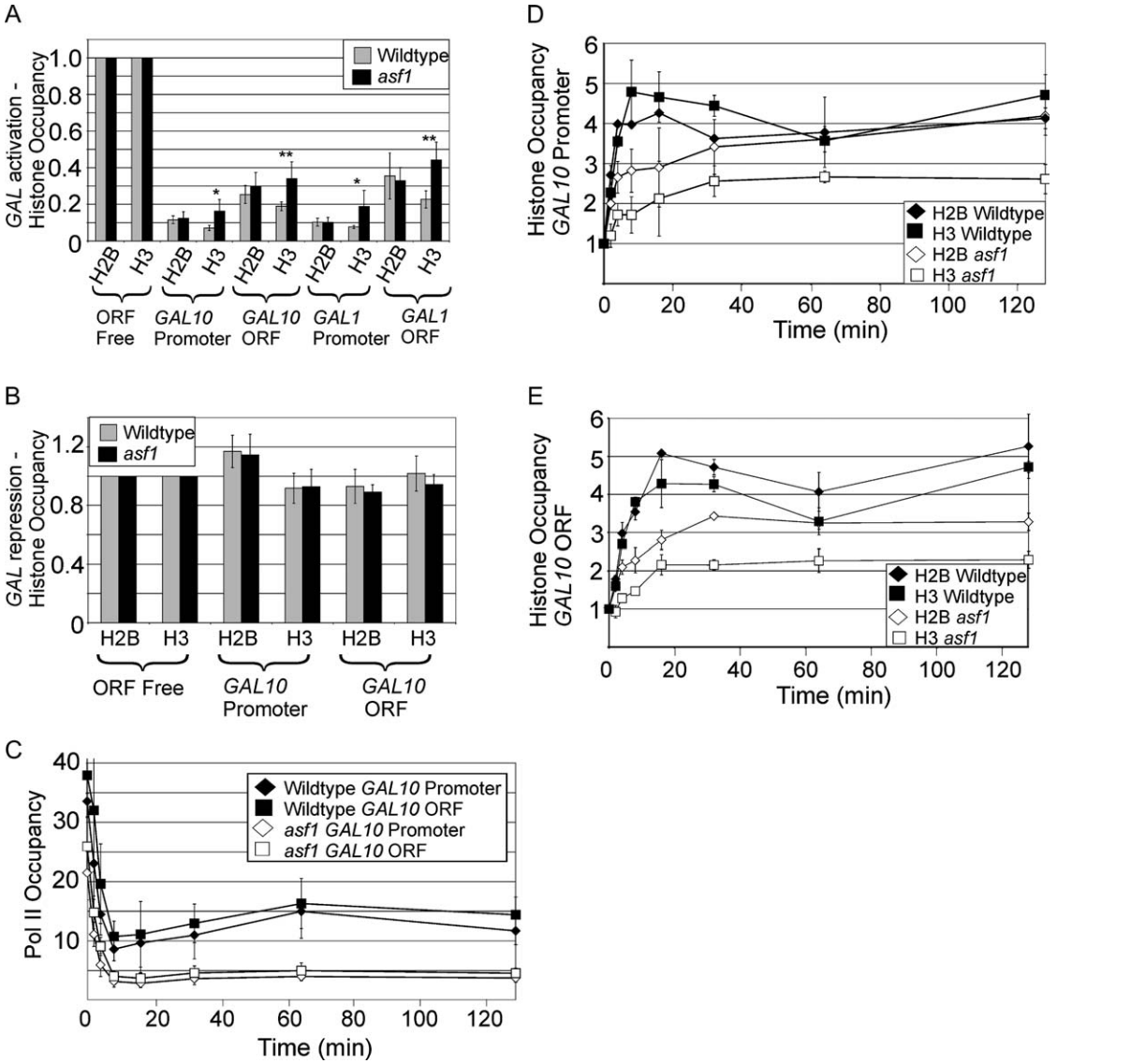

Figure 3. Asf1 Is Important for Eviction and Deposition of H3, but Not H2B, during Pol II Elongation

(A) H3 and H2B occupancy in wild-type or *asf1* cells grown in galactose medium.

(B) H3 and H2B occupancy in wild-type or *asf1* cells grown in glucose medium.

(C) Pol II association at the *GAL10* promoter and coding regions in wild-type or *asf1* cells grown in galactose medium and after addition of glucose for the indicated times.

(D) H3 and H2B occupancy at the *GAL10* promoter in samples from (C).

(E) H3 and H2B occupancy at the *GAL10* coding region in samples from (C).

Values are normalized to an ORF-free region defined as 1, and the 0 min samples in (D) and (E) were also set to 1. Data represent the average of four to five independent (A and B) or two independent (C–E) experiments, with standard deviation bars shown. A t test was used to determine statistical significance, with * denoting $p < 0.025$ and ** denoting $p < 0.01$.

at the *GAL* promoters and coding regions are comparable to histone levels at the ORF-free region in both wild-type and *asf1* mutant strains (Figure 3B).

These observations are consistent with histone eviction at the transcriptionally activated *PHO5* and *PHO8* promoters in vivo (Adkins et al., 2004; Korber et al., 2006), and with the fact that Asf1 interacts with and specifically deposits H3 and H4 on DNA in vitro (Tyler et al., 1999). More importantly, the observations at mRNA coding regions suggest that Asf1 is important for eviction of H3 (and presumably H4), but not histone H2B (and presumably not H2A), during Pol II elongation. Furthermore, the separation of H2A/H2B eviction and H3/H4 eviction in the *asf1* mutant strain suggests that histone eviction coupled to Pol II elongation occurs in a stepwise fashion, as opposed to eviction of the entire histone octamer at once.

Asf1 Is Important for Histone Deposition Coupled to Pol II Elongation

We examined the kinetics of histone deposition during Pol II elongation at the *GAL1-YLR454* gene in wild-type or *asf1* mutant cells. Pol II dissociates from the *GAL10* gene 8 min after glucose addition in both the wild-type

and *asf1* mutant strains (Figure 3C). Also, as expected from Figure 2, Pol II occupancy in the *asf1* mutant is reduced compared to the wild-type strain. In the wild-type strain, H2B and H3 occupancy both increase 4- to 5-fold at the *GAL10* promoter and coding region (Figures 3D and 3E). In the *asf1* mutant, H2B occupancy increases 3- to 4-fold, while H3 occupancy increases only 2- to 3-fold (Figures 3D and 3E). This difference is expected due to the lower occupancy of H2B relative to H3 during transcriptional activation in galactose (Figure 3A), but equal occupancy of histones H2B and H3 during transcriptional repression (Figure 3B).

Interestingly, H2B and H3 reach full occupancy by minute 16 (and often by minute 4 or 8) in the wild-type strain, but full occupancy may take up to 32 min in the *asf1* mutant strain (Figures 3D and 3E; data not shown for the *GAL1* gene). This difference between the kinetics of histone deposition in wild-type and *asf1* mutant strains cannot be explained by a reduced rate of transcriptional repression, because Pol II dissociation from the *GAL10* promoter and coding region occurs at indistinguishable rates in the two strains (Figure 3C). In addition, histone deposition is essentially complete by 32 min, and as the doubling time of *asf1* mutants is approximately 4.5 hr in galactose medium (and not significantly affected during the initial 128 min after glucose addition; data not shown), replication-dependent deposition of histones is unlikely to significantly affect the results. Our observations at the *GAL10* promoter are consistent with the previous observation that Asf1 is important for rapid histone deposition at the *PHO5* promoter upon inactivation of the gene-specific activator (Schermer et al., 2005). More importantly, our results suggest that Asf1 is important for rapid histone deposition during Pol II elongation.

Asf1 Inhibits Transcription Initiation from Cryptic Promoters within Coding Regions

To test whether Asf1 affected the Pol II elongation rate, we examined the last wave of Pol II transcription across the *GAL1-YLR454* gene in a wild-type and *asf1* strain (Mason and Struhl, 2005). As observed with all other putative elongation factors tested (Mason and Struhl, 2005), the Pol II elongation rate in the *asf1* mutant strain is comparable to that in the wild-type strain (Figures 4A and 4B). Interestingly, under conditions in which the *GAL1* promoter is repressed, the *asf1* mutant strain shows higher levels of Pol II occupancy in the *YLR454* ORF toward the 3′ end of the gene when compared to the wild-type strain (Figures 4A and 4B). This phenotype resembles that of strains depleted for FACT, Spt6, Eaf3, or Set2 histone methylase (Kaplan et al., 2003; Mason and Struhl, 2003; Carrozza et al., 2005; Joshi and Struhl, 2005), and it suggests that loss of Asf1 leads to inappropriate transcriptional initiation within coding regions.

We confirmed this suggestion by determining RNA levels in wild-type and *asf1* mutant strains at the 5′ and 3′ portions of several genes that initiate transcription from cryptic promoters within coding regions under other circumstances. Specifically, the 3′:5′ RNA ratios of the *GAL1-YLR454*, *SPB4*, and *VPS72* genes are elevated in the *asf1* deletion strain as compared to the wild-type strain (Figure 4C). As expected (Kaplan et al., 2003; Mason and Struhl, 2003), this effect of Asf1 on internal initiation is gene specific, as it is not observed at the *RAD18* or *STE11* genes. Thus, Asf1 plays a role in restoring repressive chromatin structure after Pol II elongation that inhibits inappropriate initiation from cryptic promoters within coding regions.

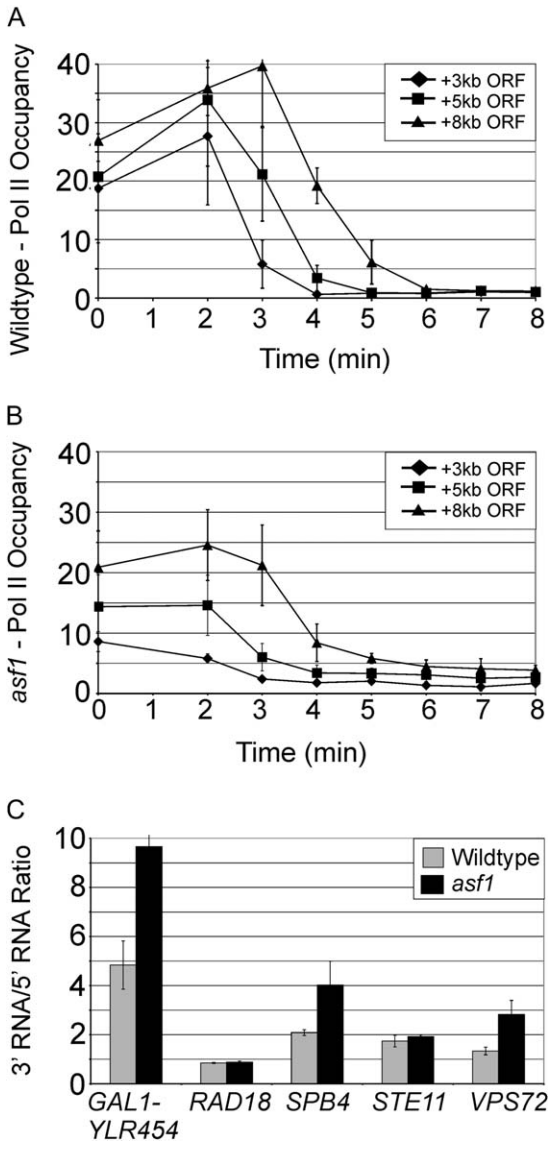

Figure 4. Asf1 Does Not Affect Pol II Elongation Rate, but It Inhibits Transcription Initiation from Cryptic Sites within Coding Regions

(A) Pol II occupancy at the indicated regions of *GAL1-YLR454* in wild-type cells grown in galactose medium and then shifted into glucose for the indicated times. Data represent the average of three independent experiments and are expressed as fold over the control ORF-free region, with the 8 min time point set equal to 1. Standard deviations are shown.

(B) Same experiment as (A), except that *asf1* cells were analyzed.

(C) Ratio of 3′ to 5′ RNA levels of the indicated genes in wild-type and *asf1* cells. RNA levels were normalized to an internal *POL1* control and represent the average of three independent experiments, with standard deviations shown.

Discussion

Asf1, a histone H3/H4 chaperone, is important for nucleosome assembly during DNA replication and repair

in vivo (Tyler et al., 1999), assembly of silent chromatin (Sharp et al., 2001; Krawitz et al., 2002), and replication-independent deposition throughout the cell cycle (Robinson and Schultz, 2003; Green et al., 2005). Here, we show that Asf1 associates with promoters and coding regions of DNA in a manner linked to transcriptional activity. The presence of Asf1 at promoters is consistent with previous observations that Asf1 is important for activator-mediated eviction of H3 and H4 at the *PHO5* and *PHO8* promoters (Adkins et al., 2004; Korber et al., 2006) and for reassembly of H3 and H4 upon loss of activation (Schermer et al., 2005). Further, it suggests that Asf1 is directly involved in activator-dependent histone exchange at promoters. Asf1-mediated effects on histone exchange at the *GAL* promoters involve H3 (and presumably H4), but not H2B (and presumably not H2A). This suggests that activator-dependent assembly and disassembly of nucleosomes at promoters occur in a stepwise fashion, with distinct activities being responsible for H3/H4 exchange and for H2A/H2B exchange.

More importantly, our results uncover a role of Asf1 during Pol II elongation. Asf1 associates with the coding regions of transcriptionally active genes, and it travels with elongating Pol II (Figure 1). Furthermore, Asf1 is important both for eviction and deposition of H3, but not H2B, during Pol II elongation (Figure 3). Loss of Asf1 also causes a 2-fold decrease in Pol II association at all transcriptionally active coding regions tested, suggesting that it has a general effect on the efficiency of Pol II transcription. Lastly, Asf1 is involved in reassembling repressive chromatin structure in the wake of Pol II transcription, such that transcriptional initiation from cryptic promoters within coding regions is inhibited (Figure 4). Taken together, these observations suggest that Asf1 functions as a Pol II elongation factor.

The dynamic eviction and deposition of histones during Pol II elongation in vivo is critical, because Pol II is inherently inefficient at elongation on nucleosomal templates (Izban and Luse, 1991). Our results indicate that Asf1 is part of the process that mediates histone deposition during Pol II elongation, a process that also involves the FACT (Mason and Struhl, 2003; Schwabish and Struhl, 2004) and Spt6 (Kaplan et al., 2003) chromatin-modifying complexes. Asf1, FACT, and Spt6 have distinct and important roles, because cells depleted of any one of these factors show decreased histone density and increased initiation within coding regions. We suggest that Asf1 functions as the H3/H4 chaperone in the context of FACT- and Spt6-mediated alteration of chromatin. In addition, Asf1-mediated deposition of H3 (and presumably H4) during Pol II elongation is involved in the restriction of Pol II initiation to promoter regions, and hence is likely to be linked to Set2 methylation of H3-K36 and deacetylation by the Eaf3-containing histone deacetylase complex (Carrozza et al., 2005; Joshi and Struhl, 2005; Keogh et al., 2005). The fact that Asf1 associates with transcriptionally active coding regions suggests that this inhibitory effect on internal initiation is direct, although an indirect effect of Asf1 remains formally possible. Lastly, Asf1-mediated deposition of histones during Pol II elongation likely accounts for much, and perhaps all, of the replication-independent deposition of histones that occurs through the yeast cell cycle (Green et al., 2005).

Although histone eviction is necessary for efficient Pol II elongation, factors that mediate such eviction in vivo have not been identified. Here, we demonstrate that Asf1 is directly involved in H3 (and presumably H4) eviction that occurs during Pol II elongation. Strains lacking Asf1 have increased H3 density at transcriptionally active coding regions in comparison to wild-type strains. Importantly, Asf1 does not affect eviction of H2B (and presumably H2A) during Pol II elongation. This suggests that another factor, such as FACT (Belotserkovskaya et al., 2003), may function to remove the H2A/H2B dimers, before Asf1 can evict histones H3 and H4. Lastly, loss of Asf1 does not completely block eviction of H3 at coding regions of active genes, indicating that another factor(s) also contributes to H3 eviction in wild-type strains. The existence of additional factors for H3 eviction explains why Pol II elongation can occur to a significant extent in the *asf1* deletion strain.

How does Asf1 travel with elongating Pol II and mediate both eviction and deposition of histones? One possibility is that Asf1 physically interacts with Pol II or an associated elongation factor (e.g., FACT, Spt6, Paf1, TREX complexes). We do not favor this possibility because such physical interactions have never been observed in extensive biochemical and two-hybrid analyses. Alternatively, Asf1 might recognize disrupted chromatin structure in which histone-DNA contacts are weakened, thereby allowing exchange of H3 and H4 between DNA and Asf1. An attractive feature of this model is that it provides a common mechanism by which Asf1 can be "recruited" to sites of Pol II elongation, DNA replication, enhancer-bound proteins, and highly active Pol III genes.

We therefore suggest the limited amount of Pol II elongation that occurs on nucleosomal templates causes sufficient disruption of histone-DNA contacts to permit association of Asf1. Once associated, Asf1 interacts with histones H3 and H4, thereby further destabilizing histone-DNA contacts, with the resulting eviction of these histones from the DNA and facilitation of Pol II elongation through the region. After passage of Pol II, Asf1 deposits H3 and H4 back on the DNA, and in the absence of subsequent rounds of transcription, Asf1 dissociates from the chromatin. As mentioned above, eviction (and presumably deposition) of H2A/H2B dimers occurs in a step distinct from Asf1-dependent exchange of histones H3 and H4. Thus, in this view, Asf1 is linked to the basic mechanism of Pol II elongation, which involves both the disruption and restoration of normal chromatin structure upon passage of Pol II. Furthermore, Asf1 both recognizes and influences chromatin structure in a processive manner as Pol II travels through an mRNA coding region.

Experimental Procedures

Yeast Strains

The Asf1-18xMyc strain was generated from JDY51 (Deckert and Struhl, 2002), and H2B occupancy was monitored with a strain expressing FLAG-tagged H2B (Ng et al., 2002; Schwabish and Struhl, 2004). The *asf1* null mutant was made via one-step gene replacement with a KanMX cassette, was verified by PCR, and was shown to grow slowly (the expected phenotype). *GAL1-YLR454* strains (Mason and Struhl, 2003) were constructed via one-step gene replacement. Growth media and conditions are described in

Supplemental Data. Asf1 protein levels are unaffected by any of the growth conditions tested (Figure S1).

Chromatin Immunoprecipitation

Chromatin immunoprecipitation was carried out as described previously (Schwabish and Struhl, 2004) using monoclonal antibodies to Myc (9E10; Upstate) epitope, Rbp1 (8WG16; Covance), FLAG epitope (anti-FLAG M2; Sigma), and polyclonal antibodies to histone H3 (Abcam). Percent immunoprecipitation efficiency was determined by dividing the amount of PCR product in the immunoprecipitated sample by the amount of PCR product in the input sample. Relative occupancy values were calculated by dividing the percent immunoprecipitation efficiencies of the target DNA by an intergenic region of chromosome I, which was usually defined as 1.0. Primer pair locations and other technical details are provided in Supplemental Data, and a control experiment involving an untagged strain is provided in Figure S2.

RNA Analysis

DNase I-treated RNA was purified with the RNeasy Qiagen kit, and cDNA was generated with a poly-dT primer and Superscript II reverse transcriptase (Invitrogen). Real-time PCR was used to quantify cDNA levels using the level of *POL1* RNA as an internal control.

Supplemental Data

Supplemental Data include two figures and can be found with this article online at http://www.molecule.org/cgi/content/full/22/3/415/DC1/.

Acknowledgments

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