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Histone chaperones regulate histone exchange during transcription

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Transcription by RNA polymerase II is accompanied by dynamic changes in chromatin, including the eviction/deposition of nucleosomes or the covalent modification of histone subunits. This study examined the role of the histone H3/H4 chaperones, Asf1 and HIR, in histone mobility during transcription, with particular focus on the histone exchange pathway, using a dual histone expression system. The results showed that the exchange of H3/H4 normally occurs during transcription by the histone chaperones. Both Asf1 and HIR are important for histone deposition but have a different effect on histone exchange. While Asf1 mediated incorporation of external H3/H4 and renewal of pre-existing histones, HIR opposed it. The balance of two opposing activities might be an important mechanism for determining current chromatin states.

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Introduction

The eukaryotic genome is packed in chromatin as nucleosomes. Nucleosome is the repeating unit containing two copies of the histones H2A, H2B, H3, and H4 (Luger *et al*, 1997). DNA functions such as transcription, replication, repair, and recombination are strongly influenced by the packaging state of the DNA in chromatin.

The bulk of the nucleosomes are assembled when the DNA is replicated in the S phase through the replication coupled (RC) pathway that is mediated by the histone H3/H4 chaperone, chromatin assembly factor 1 (CAF-1) (Verreault, 2000; Loyola and Almouzni, 2004). Outside of S phase, the histones are deposited into the nucleosome by HIRA (HIR complex in *Saccharomyces cerevisiae*) via a replication independent (RI) pathway (Henikoff and Ahmad, 2005). Each histone deposition pathway is related to the transcriptionally active or inactive chromatins. HIRA mediates the accumulation of the variant histone H3.3 in the active euchromatic region, while CAF-1 mediates the canonical H3 in the heterochromatic region (Mckittrick *et al*, 2004; Tagami *et al*, 2004). In yeast, HIR and CAF-1 are genetically redundant in the nucleosome assembly, although their precise roles are unclear. Another histone H3/H4 chaperone, Asf1, interacts with both CAF-1 and HIR, and affects both the RC and RI pathways (Krawitz *et al*, 2002; Green *et al*, 2005).

Nucleosomes normally block the progression of RNA polymerase II (pol II). Therefore, transcription must be accompanied by a large change in chromatin. One such change is the eviction and deposition of histones. Histones are evicted from and deposited onto the pol II track during transcription (Bernstein *et al*, 2004; Kristjuhan and Svejstrup, 2004; Lee *et al*, 2004; Schwabish and Struhl, 2004). The histone H2A/H2B chaperone, FACT (*SPT16* and *POB3* in yeast, and their homologs, hSpt16 and SSRP1, in human) complex, travels with pol II, binds the H2A/H2B dimers, and mediates the disassembly and reassembly of the nucleosomes (Formosa *et al*, 2002; Kireeva *et al*, 2002; Beloserkovskaya and Reinberg, 2004). The eviction and deposition process has a potential to provide a chance for histones to be exchanged along the gene. Indeed, H2A/H2B and H3/H4 is actively replaced during transcription, but with different kinetics: H2A/H2B is easily exchanged, but H3/H4 is less frequently exchanged and appears to be more strictly dependent on transcription (Kimura and Cook, 2001; Thiriet and Hayes, 2005; Jamai *et al*, 2007). In addition, the extent of H3 exchange is not even along the gene. H3 exchange is mainly observed around the promoter (Chow *et al*, 2005; Dion *et al*, 2007; Jamai *et al*, 2007; Mito *et al*, 2007). However, it also occurs within an entire region of the actively transcribed gene, with a lesser extent, compared to the promoter region (Choi *et al*, 2005; Schwartz and Ahmad, 2005; Wirbelauer *et al*, 2005; Daury *et al*, 2006). These reports indicate that multiple H3/H4 exchange pathways operate simultaneously. Although many factors are supposed to play a role in chromatin dynamics, the precise features of the changes in chromatin, the mobility of the histone subunits, and the responsible factors are not completely understood.

This study examined the role of the H3/H4 chaperones in chromatin dynamics to determine the changes that occur in chromatin during transcription. The episomal expression of histones H3 or H4 under the *TFA1* promoter enabled histone mobility to be monitored along the DNA during transcription. Here we report that histone H3/H4 exchange occurs preferentially at the transcription sites and is mediated by Asf1 and HIR. Interestingly, Asf1 mediates the deposition of new histones, while HIR mediates the deposition of old histones. Their balanced activity might be important for maintenance or renewal of chromatin during transcription.

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Results

Histones are exchanged during transcription

Transcription by pol II on a chromatin template is accompanied by dynamic changes in the chromatin structure, such as the eviction/deposition or exchange of histones. To understand histone mobility and the role of histone chaperones, a slight modification of a dual histone expression strategy was applied (Schermer *et al*, 2005). HA-H3 or Flag-H4 was produced episomally from the *TFA1* promoter, whose expression is not controlled by the cell cycle (Ferea *et al*, 1999). In this strategy, most of the nontagged endogenous histones are produced in the S phase, and are incorporated into the chromatin mainly through the RC pathway. In contrast, the *TFA1* promoter expresses HA-H3 (or Flag-H4) continuously. Therefore, tagged histones can be incorporated into the chromatin through both the RC and RI pathway because there is a ready supply of soluble histones throughout the cell cycle. Hence, if the chromatin histones are exchanged with histones from the soluble histone pool (a source in *trans*) during transcription, the level of tagged histones should increase comparatively in the transcribed region by replacing pre-existing histones. With this system, the galactose-inducible genes were analyzed by changing the medium from raffinose (off) to galactose (on), and then to glucose (off) to observe dynamic incorporation of tagged histones. The cells were treated with the α-factor to arrest them in the G1 phase before the galactose induction in an attempt to minimize the incorporation of HA-H3 (or Flag-H4) through replication (Figure 1A).

The cross-linking of HA-H3 to the *GAL1*-promoter-linked-*YLR454* (~8 kb gene) increased gradually as the cells were sequentially incubated in raffinose, galactose, and glucose media (Figure 1B, top panel). The expression of HA-H3 under the *TFA1* promoter was not altered by the carbon source or the α factor in the media (Supplementary Figure S1). Nevertheless, the occupancy of HA-H3 across p*GAL1-YLR454*, but not in the upstream or downstream flanking regions, was higher in glucose (off) than in raffinose (off), indicating that HA-H3 from a soluble histone pool was incorporated successfully into the chromatin during transcription via galactose induction (relative value in raffinose equals 1). However, the total level of H3 immunoprecipitated with the anti-H3 antibody was low during transcription, but recovered to the original levels after transcription had been turned off, as reported previously (Figure 1B, the second panel) (Kristjuhan and Svejstrup, 2004; Schwabish and Struhl, 2004). The transcription state of the target gene depending on the carbon source was confirmed by pol II occupancy (Figure 1B, the third panel). This suggests that total histone H3 is rapidly displaced and deposited onto a transcribed region, during which the continuous exchange of H3 also occurs.

We then determined if HA-H3 incorporation was specific to transcription. First, we examined nontranscribed regions such as intergenic and telomeres. The occupancy of HA-H3 in silent regions was not altered by media changes (Figure 1C). Second, constitutively transcribed genes not regulated by galactose, such as *SST2*, *RIF2*, *YLR455*, or *YLR456*, which are located in the flanking region of p*GAL1-YLR454* (Figure 1B), or *PMA1*, did not show occupancy changes of HA-H3, while *GAL7* increased (Figure 1C). The relative fold difference in the HA-H3 occupancy was invisible in these nonactivated genes unless their transcription was induced by galactose. Lastly, we monitored HA-H3 occupancy in *GAL7* after transcription was inhibited by shifting the *rpb1-1* (yeast mutant expressing a temperature-sensitive largest subunit of pol II) to 37°C for 30 min prior to galactose induction (Nonet *et al*, 1987). Without transcription, HA-H3 incorporation was not changed (Figure 1D).

Flag-H4 was expressed under the *TFA1* promoter to determine if H4 was also exchanged during transcription. Flag-H4 was preferentially exchanged at the same sites of transcription as H3 (Figure 1E), with minor differences in the exchange profile. The cross-linking of Flag-H4 during galactose incubation was observed as high as the glucose sample, although H4 appeared to be evicted and deposited normally (data not shown). In addition, H4 exchange tended to be more biased toward the promoter. All together, increased occupancy of HA-H3 and Flag-H4 in the p*GAL1-YLR454* gene upon galactose induction was transcription specific, and certainly due to stable incorporation of H3/H4 from a soluble histone pool.

Asf1 and HIR are important for exchange of H3/H4

We next examined the role of Asf1 and HIR (consisting of Hir1, Hir2, Hir3, and Hpc2 in budding yeast) in the eviction/deposition and the exchange of histones in our system. Both *asf1*Δ and *hir1*Δ responded to the α factor as efficiently as wild type and G1 arrest was stably maintained during experiment as indicated by the flow cytometry analysis of DNA content (Supplementary Figure S2). Interestingly, as shown in Figure 2A (top panel), HA-H3 occupancy was significantly reduced in *asf1*Δ compared with that of the wild type (~64% in average, data from wild type in Figure 1B is plotted overlapped). Similarly, the extent of histone deposition (recovery of total H3 occupancy in glucose) was also reduced in *asf1*Δ as reported (~85%) (Figure 2A, bottom panel) (Schwabish and Struhl, 2006). However, the reduced deposition of histones could not account for the lower level of HA-H3 incorporation observed in this mutant. The incorporation efficiency of HA-H3 in *asf1*Δ was still lower than the wild type even after normalization to total H3 levels (Figure 2C). Total level of HA-H3 was unaffected by *asf1*Δ or *hir1*Δ (data not shown). In contrast, *hir1*Δ incorporated HA-H3 more efficiently than the wild type (~137%), even though the deposition of H3 was apparently reduced to a similar extent as *asf1*Δ (Figure 2B). Interestingly, the *hir1*Δ-dependent increase of HA-H3 incorporation was more prominent around the promoter and the 5′ of the coding region (~191% in this region when normalized to the H3 level) than downstream (Figure 2B and C). This suggests that (1) Asf1 and Hir1 are important for the eviction and deposition of histones during transcription, and are particularly important for restoring H3 levels after transcription is turned off, as reported, and (2) Asf1 and Hir1 play an additional role in the exchange and stable incorporation of new H3 from a soluble histone source. Asf1 appears to enhance the stable incorporation of external HA-H3 into chromatin while Hir1 appears to oppose it. This suggests that H3/H4 chaperones might assist pol II to progress by facilitating the eviction/deposition of histones, and at the same time regulate chromatin states by mediating the exchange of histones through the balance of the two opposing

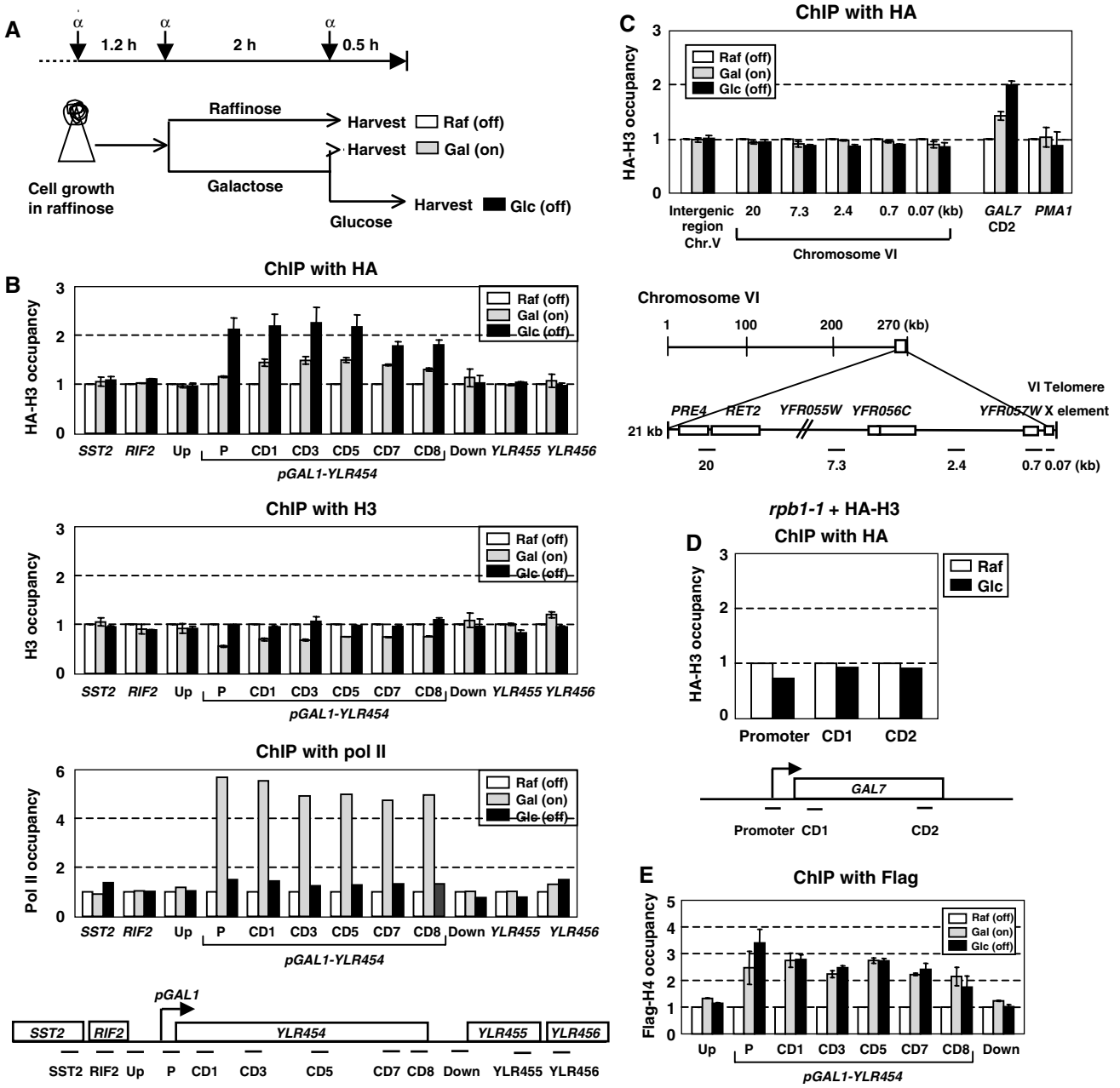

Figure 1 Histone H3/H4 is exchanged during transcription. (**A**) Experimental design. Yeast cells carrying plasmids expressing HA-H3 or Flag-H4 were grown in raffinose medium and treated with the α factor as shown during incubation. (**B**) Histone H3 is actively exchanged during transcription. ChIP with 12CA5 (HA-H3), H3 (total H3), or 8WG16 (pol II) antibodies was performed as described in the Materials and methods with the indicated primer sets at the bottom. Each PCR signal was quantified and normalized to the intergenic control and the input DNA. In order to show the relative fold difference between the samples, the ChIP value obtained in raffinose was arbitrarily set to 1. The data are reported as the mean ± s.d. from at least six independent experiments. (**C**) ChIP to measure the occupancy of HA-H3 in the nontranscribed regions (telomere and intergenic region), nonactivated gene, *PMA1*, and the galactose-activated gene (the coding region of *GAL7*). Bottom, the schematic diagram of the PCR primer pairs for the telomeric region from the right end of chromosome VI. (**D**) The HA-H3 occupancy in *GAL7* regions was monitored in *rpb1-1* after shifting to 37°C. (**E**) The Flag-H4 occupancy in yeast carrying the pRS316-*TFA1*-Flag-H4 was analyzed as described in (A). The data points were obtained from three independent experiments.

activities. Next, yeast with a double deletion of *ASF1* and *HIR1* was examined for the H3 exchange to see whether they function equivalently in the same pathway. Figure 2D shows that HA-H3 occupancy in *asf1*Δ*/hir1*Δ remained lower than wild type. Thus, Asf1 and HIR might not function independently or equivalently. Histone exchanging pathway could be more dependent on Asf1, such that Asf1 activity is a prerequisite for HIR.

Histone mutations on the Asf1-binding surface lead to an aberrant pattern of histone exchange

The behavior of the histone H3 mutants with a specific defect in chaperone interaction was examined to confirm the role of histone chaperones in histone exchange. The interaction between H3 and Asf1 has been studied based on a well-defined structure (Antczak *et al*, 2006; English *et al*, 2006; Agez *et al*, 2007; Natsume *et al*, 2007). The C-terminal α3

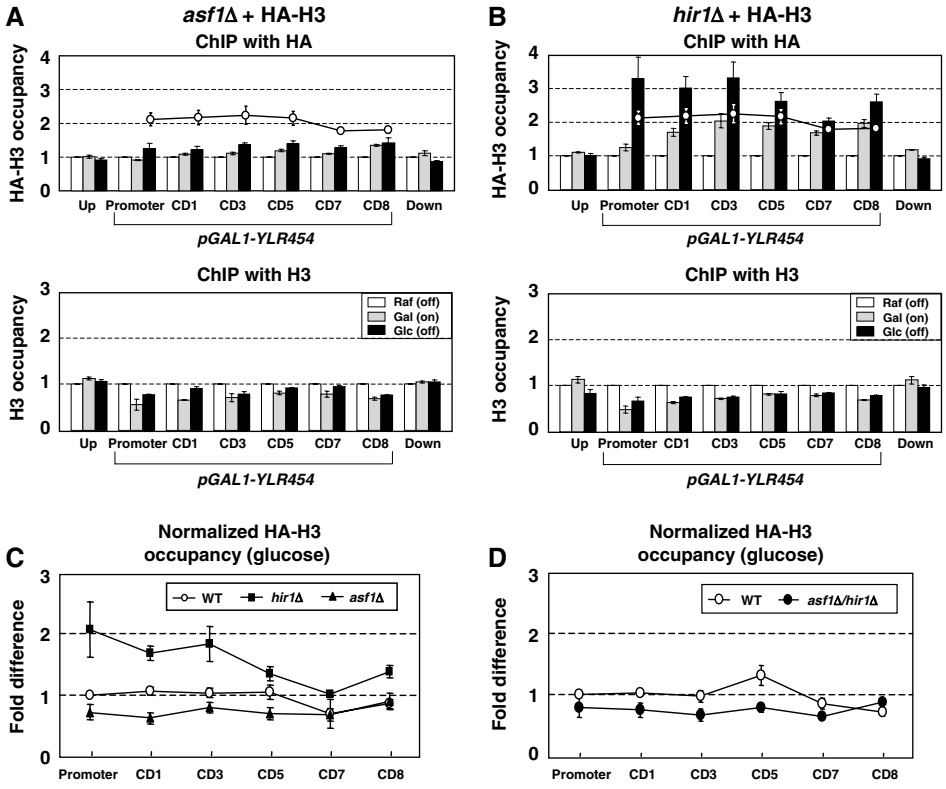

Figure 2 Asf1- and Hir1-dependent exchange of HA-H3. The exchange and stable incorporation of HA-H3 was decreased in *asf1*Δ (YC207) (**A**), while it was increased in *hir1*Δ (YC199) (**B**). ChIP was performed and the data were analyzed as described in Figure 1. The experiment was repeated at least five times. The data points of the HA-H3 occupancy obtained in the wild type (glucose sample) was depicted as open circles. The occupancy of total H3 was performed as described in Figure 1. (**C**, **D**) The relative occupancy of the HA-H3 obtained from each genetic background (glucose sample) is shown as the fold difference (the value obtained from promoter region of the wild type was arbitrarily set to 1). Each value was obtained after normalization by the level of total H3.

helix of H3 (amino acids 122–134) makes direct contact with the N-terminal β strands of Asf1. Consistent with this, H3 K122 is essential for Asf1 binding and develops an *asf1*Δ-like phenotype (Zeocin sensitivity) when mutated (English *et al*, 2006). In particular, K122A is defective in *PHO5* induction, where Asf1-mediated chromatin disassembly is essential (English *et al*, 2006). On the other hand, L109 is located on the secondary interaction surface of H3, which is expected to be less important for Asf1 binding (Figure 3A) (Munakata *et al*, 2000; Mousson *et al*, 2005; Antczak *et al*, 2006; English *et al*, 2006; Agez *et al*, 2007; Natsume *et al*, 2007). Unlike *asf1*Δ, L109A showed no sensitivity to the DNA-damaging agent (MMS; methyl methane sulfonate) or the DNA replication-blocking agents (hydroxyurea and Camptothecine) (Supplementary Figure S3).

The histone exchange pattern of the K122 (K122Q, K122A) and L109A mutants of H3 was examined. Protein level of the H3 mutants under the *TFA1* promoter was measured in the wild-type background in cultures grown without the α factor. All H3 mutants were expressed at similar levels, indicating that protein stability was not significantly affected and they can normally be incorporated into chromatin (Figure 3B). The α factor was then added to examine the transcription-coupled incorporation of the H3 mutants through histone exchange, as described in Figure 1A. Chromatin immunoprecipitation (ChIP) from raffinose and glucose were compared. Interestingly, K122A and K122Q, which were predicted to have a defect in the Asf1 interaction, were not normally incorporated into the chromatin, while L109A was able to incorporate as successfully as wild type (Figure 3C). Remarkably, the incorporation of K122A and K122Q showed an aberrant histone exchange profile within the gene. Histone exchange was mainly affected in the promoter and the 5′ coding region, and it was gradually restored toward the 3′ of the gene, which is opposite to what *hir1*Δ showed in Figure 2B. Although *asf1*Δ developed a defect in histone exchange within the entire gene region, the H3 mutants defective in Asf1 binding lacked H3 exchange proximal of the promoter region. These data suggest the importance of the Asf1–H3 interaction, especially around the promoter region. The broader defect of *asf1*Δ than H3 K122 may suggest that Asf1 might adopt multiple ways to mediate histone exchange during different phases of transcription, or that there are alternative histone exchanging pathways that bypass direct interaction between Asf1 and H3. Taken together, Asf1 and HIR are important for the exchange of H3/H4 along the transcription track, particularly during early transcription, by playing opposite roles.

Direct interaction between Asf1–H3/H4 is important for histone exchange

Detailed analysis of Asf1–HIRA and Asf1–H3/H4 structures predicts that Asf1 is able to interact with HIRA and H3/H4 simultaneously to form a ternary complex by bridging them (Antczak *et al*, 2006; Mousson *et al*, 2005, Tang *et al*, 2006). Based on this structural configuration, it was suggested that

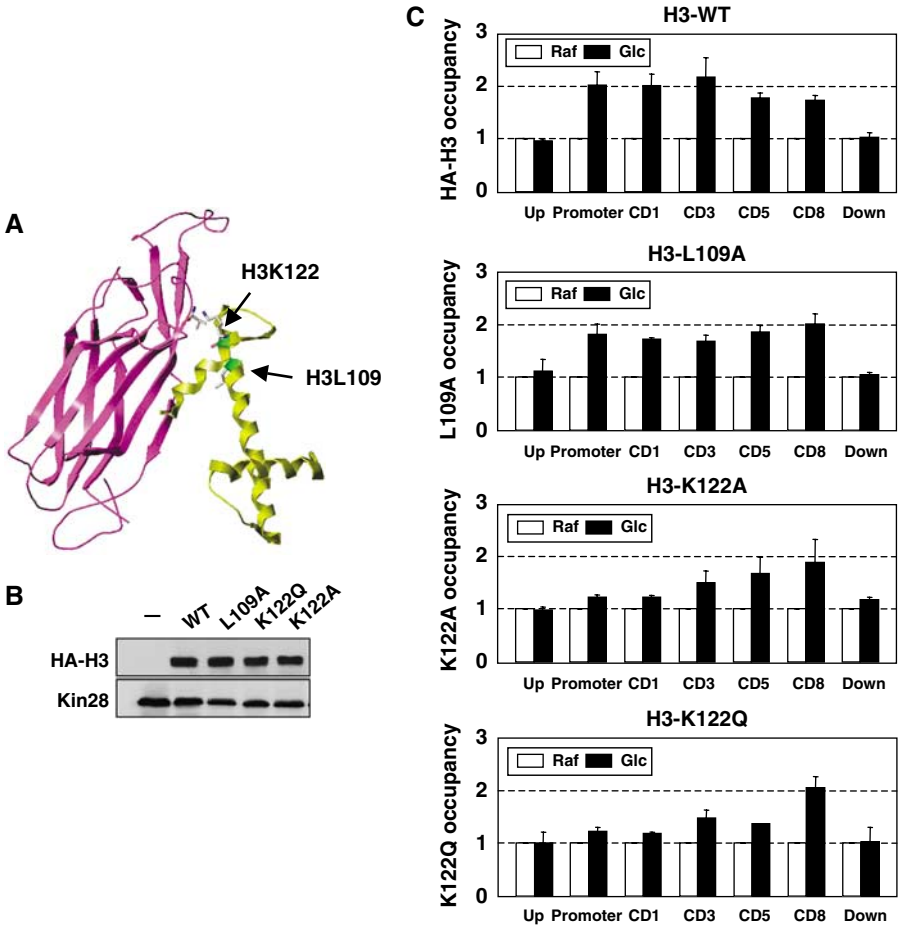

Figure 3 H3 mutation defective in Asf1 interaction leads to the aberrant histone exchange. K122 mutants showed an aberrant pattern of histone exchange. (**A**) The location of each H3 mutation in the structure of the yAsf1 N (1–164) and *Xenopus laevis* H3 (60–135) complex (English *et al*, 2006). Asf1 and H3 are colored in purple and yellow, respectively. The locations of K122 and L109 are colored in green. The residues of L109 (H3), K122 (H3), and V92 (Asf1) are shown as sticks. The diagram was generated using SYBYL 7.3 (Tripos, USA) on the basis of Linux Redllat 4.0. (**B**) The expression level of the H3 mutants under the *TFA1* promoter was similar. The immunoblotting analysis was performed with the whole-cell extract prepared from the wild type (YC73) that harbors the pRS315-*TFA1*-HA-H3, L109A, K122Q, or K122A. (**C**) The incorporation of the H3 mutants was analyzed as described in Figure 1. The experiment was repeated at least two times.

Asf1 has a potential to disassemble the nucleosome to present H3/H4 to HIR. Next, we further analyzed Asf1–H3 interaction during histone exchange and also asked whether direct interaction between Asf1 and HIR is absolutely required. To do this, two Asf1 mutants were examined; V94R, a mutant defective in H3 binding, and D37R/E39R, a mutant defective in HIR binding (Mousson *et al*, 2005). V94R carries *asf1*Δ phenotypes such as DNA damage sensitivity, thermosensitive growth, and a defect in gene silencing, whereas D37R/E39R shows a defect only in gene silencing. The experimental difference using H3-K122 and Asf1 V94R is that H3-K122 verifies Asf1's interaction with external H3, whereas V94R verifies its interaction with both internal and external H3. Wild-type and two Asf1 mutants were expressed at similar levels in yeast (Figure 4A). *asf1*Δ, supplemented with wild-type Asf1, recovered the HA-H3 level via subunit exchange, although the overall efficiency was lower than *ASF1* wild-type yeast (Figure 4B, top panel). However, interestingly, *asf1*Δ supplemented with V94R remained defective (Figure 4B, middle panel). Its pattern was more like *asf1*Δ rather than H3-K122 (Figure 3C), suggesting that Asf1–H3 interaction is a major step to promote nucleosome disassembly along the gene and this disassembly step of pre-existing nucleosome is necessary prior to subunit exchange. Asf1–Hir1 interaction is important for gene silencing but does not have a role in Asf1-dependent DNA damage repair pathway (Mousson *et al*, 2005). Interestingly, H3 incorporation in D37R/E39R was as normal as wild type in our assay. It suggests that direct interaction between Asf1 and Hir1 may not be necessary for determining the extent of histone exchange.

Discussion

Chromatin undergoes a dynamic change as pol II transcribes genes through it. Our results show that histone H3/H4 is evicted, deposited, and actively exchanged. This suggests an intermediate step involving the complete or partial unfolding of nucleosomes into subunits. These results are consistent with recent ideas about transcription-dependent exchange of H3/H4 or potential disruption of H3/H4 tetramer by Asf1 (English *et al*, 2006; Morillon, 2006; Workman, 2006; Kulaeva *et al*, 2007; Natsume *et al*, 2007).

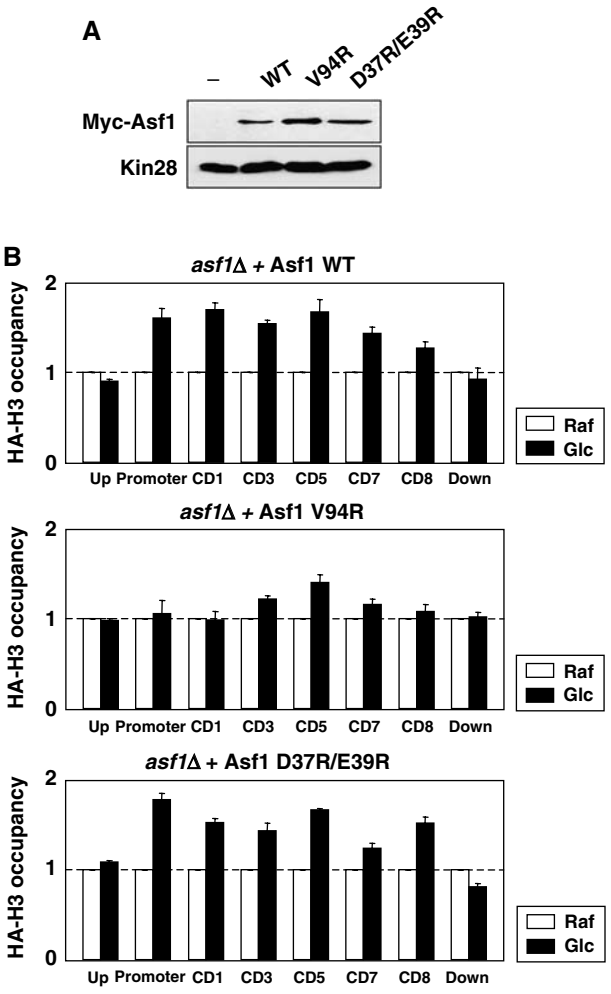

Figure 4 Asf1 in direct contact with H3 is important for histone exchange. (**A**) Wild type and Asf1 mutants, V94R and D37R/E39R, were expressed in *asf1*Δ. All Asf1 construct provide 13 Myc tag. (**B**) HA-H3 occupancy within a target gene was diminished in V94R mutant, but not in D37R/E39R. The incorporation of HA-H3 in Asf1 mutants was analyzed as described in Figure 1. The experiment was repeated at least four times.

Asf1 and HIR mediate dynamic histone exchange while transcription is ongoing. Interestingly, the exchange of pre-existing histones with external histones was decreased in *asf1*Δ, but increased in *hir1*Δ. While both Asf1 and Hir1 deposit histones into chromatin, the histone source they utilize might be different. Asf1 catalyzes the incorporation of histones from a source in *trans* (external free histones), while Hir1 catalyzes the incorporation of histones from a source in *cis* (original chromatin histones). It resembles the two opposing activities of Spt16 and Pob3 within the FACT complex for the concerted disassembly and reassembly of the nucleosomes during transcription (Formosa *et al*, 2002; Beloserkovskaya and Reinberg, 2004).

The role of histone chaperones in histone eviction and deposition has been observed mostly in the *PHO5* and *PHO8* promoters. Asf1 is important for the activation-dependent eviction of the nucleosomes from the promoters of the *PHO* genes. Moreover, upon repression, Asf1 and HIR are responsible for the deposition of nucleosomes to the promoters (Adkins *et al*, 2004; Schermer *et al*, 2005; Korber *et al*, 2006). We assumed that histone eviction/deposition and subunit exchange must take place simultaneously, such that subunits could be exchanged while nucleosomes are deposited. According to our data, the increase in H3 exchange led by *hir1*Δ and the decrease in H3 exchange led by *asf1*Δ are more prominent around the promoter region, indicating that Asf1 and HIR are major histone exchanging partners that operate together in this region. The predominant exchange of H3 at the promoter has been reported by other groups (Chow *et al*, 2005; Jamai *et al*, 2007). Our results suggest Asf1 and HIR to be excellent devices that modulate the chromatin states during early transcription. In addition to Asf1-HIR pair, we assume that there must be other pathways that allow continuous histone exchange throughout the coding region. As *asf1*Δ and V94R diminish overall histone exchange along the gene, Asf1 might play an overlapping role downstream, such as by providing split nucleosomes to other proteins.

Although the role of HIR in the histone exchange pathway is not completely understood, there is some concern as to what the general consequence of their opposing activities is. One possibility is that the renewal of chromatin can be regulated by the extent of histone exchange through the balance between the two activities. If so, histone exchange might allow modification of nucleosome composition or pre-existing histone modification. In this regard, it is interesting that Asf1 is particularly essential for H3 exchange around the promoter and the early-transcribed region through a direct interaction with H3.

Chromatin can be remodeled by substituting conventional histones with variants by their cognate histone chaperones together with chromatin remodeling complexes. HIRA in higher eukaryotes is a histone chaperone specific to H3.3 and is purified in association with Asf1 (Tagami *et al*, 2004; Green *et al*, 2005). HIRA and Asf1 cooperate to contribute to nucleosome formation *in vitro* (Green *et al*, 2005). Given the activities of the yeast chaperones, Asf1 (in this case, Asf1a, as HIRA preferentially binds Asf1a rather than Asf1b) might participate more actively in the HIRA-dependent RI pathway than has been generally understood. This predicts that the concerted activity of HIRA and Asf1 is essential for both introducing H3.3 into and retaining H3.3 within the chromatin (Mito *et al*, 2005). Hence, any epigenetic information, either histone modifications or nucleosome composition, can be preserved, while it has a continuous opportunity to change. However, more study will be needed to determine the precise roles of Asf1 and HIR in the dynamic regulation of the chromatin states and gene expression.

Materials and methods

Yeast strain and plasmid construction

Yeast strains used in this study: YC73 (*MAT***a**, *ura3-1*, *leu2-3,112*, *trp1-1*, *his3-11,15*, *ade2-1*, *can1-100*, *TRP1*::pGAL1-*YLR454w*), YC199 (as YC73, *hir1*Δ::*KanMX*), YC207 (as YC73, *asf1*Δ::*KanMX*), YC252 (as YC73, *asf1*Δ::*HIS3*, *hir1*Δ::*KanMX*), or Y262 (*MAT***a**, *ura3-52*, *rpb1-1*; provided by DK Lee).

The deletion mutants were constructed using one-step PCR-mediated disruption, which replaces the entire gene with the KanMX4 cassette. The *GAL1-YLR454* strains were constructed by the one-step integration of the *GAL1* promoter fragment upstream of the *YLR454w* coding region. The pRS315-*TFA1*-HA-H3 or pRS316-*TFA1*-Flag-H4 (*TFA1* promoter driven HA-H3 or Flag-H4) was generated by PCR using pRS315-*CEG1* as a starting material, which

was provided by S Buratowski. The pRS315-*TFA1*-HA-H3 K122Q, K122A, or L109A (*TFA1* promoter driven HA-H3 mutants) were generated by site-directed mutagenesis. pRS316-*ASF1*, V94R, or D37R/E39R (13Myc-tagged Asf1 or mutants under the endogenous promoter) was constructed using pRS315-Asf1-13myc, V94R-13myc, or D37R/E39R-13myc as a starting material (provided by F Ochsenbein), respectively. All constructs were confirmed by sequencing.

Growth conditions and analysis

Yeasts were grown at 30°C in either YPD media or in synthetic minimal media that lacked the nutritional supplements required for maintaining plasmids. For the galactose induction experiment, cells were grown to an $A_{600} = 0.4$ in SC-LEU or URA plus 2% raffinose, treated with the α factor (5 μg/ml), and incubated for 1.2 h. The synchronized cells were divided into two parts and incubated in the medium containing 2% raffinose or 2% galactose in the presence of α factor. After 2 h, half of the 2% galactose culture was quickly switched to a medium containing 2% glucose plus the α factor to repress *GAL1* and incubated for another 0.5 h. In *rpb1-1*, yeast cells were grown overnight at 24°C. Before α arrest, the temperature of the culture was shifted to 37°C to inactivate Rpb1.

Immunoblotting

To analyze the expression of tagged histones, yeasts were harvested by centrifugation and boiled in a cracking buffer (8 M urea, 40 mM Tris–HCl, pH 8.0, 5% SDS, 0.1 M EDTA, 0.01% β-mercaptoethanol, 0.001% bromophenol blue supplemented with complete protease inhibitors) for 10 min with occasional vortexing. The resulting lysate was clarified by centrifugation and separated onto a 15% denaturing polyacrylamide gel and analyzed by immunoblotting with 12CA5 (Roche), Myc (Roche), or Kin28 (Covance) antibodies.

ChIP

ChIPs were performed as described previously (Cho *et al*, 2001; Kim *et al*, 2005). The PCR signals were quantified using a Phosphoimager (Fujix BAS 2040) and normalized to the input DNA and the intergenic control. PCR primers were (the coordinates are defined relative to the translation initiation site): *PMA1* CD2 (+584 to +807), *GAL1-YLR454* promoter (−271 to +69), CD1: 1 kb (+951 to +1149), CD3: 3 kb (+2954 to +3150), CD5: 5 kb (+5091 to +5283), CD7: 7 kb (+7096 to +7278), CD8: 8 kb (+7536 to +7866), *SST2* (+28 to +196), *RIF2* (+561 to +786), upstream region of *YLR454* (−755 to −525), downstream (+8741 to +8873), *YLR455* (+374 to +546), *GAL7* promoter (−296 to −80), CD1 (+59 to +390), CD2 (+570 to +907), intergenic region Chromosome V (position on chromosome, 9716–9863).

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (http://www.embojournal.org).

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