Potential role of the histone chaperone, CAF-1, in transcription

Hye-Jin Kim, Ja-Hwan Seol & Eun-Jung Cho[*]

College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea

The eukaryotic genome forms a chromatin structure that contains repeating nucleosome structures. Nucleosome packaging is regulated by chromatin remodeling factors such as histone chaperones. The *Saccharomyces cerevisiae* H3/H4 histone chaperones, CAF-1 and Asf1, regulate DNA replication and chromatin assembly. CAF-1 function is largely restricted to non-transcriptional processes in heterochromatin, whereas Asf1 regulates transcription together with another H3/H4 chaperone, HIR. This study examined the role of the yeast H3/H4 histone chaperones, Asf1, HIR, and CAF-1 in chromatin dynamics during transcription. Unexpectedly, CAF-1 was recruited to the actively transcribed region in a similar way to HIR and Asf1. In addition, the three histone chaperones genetically interacted with Set2-dependent H3 K36 methylation. Similar to histone chaperones, Set2 was required for tolerance to excess histone H3 but not to excess H2A, suggesting that CAF-1, Asf1, HIR, and Set2 function in a related pathway and target chromatin during transcription. [BMB reports 2009; 42(4): 227-231]

INTRODUCTION

The eukaryotic genome is packed into chromatin by formation of a nucleosome, a repeating unit containing two copies each of histones H2A, H2B, H3, and H4 (1). Hence, functions such as transcription, replication, repair, and recombination are strongly influenced by the packaging state of DNA into chromatin. The key players in these processes are the histone chaperones that mediate histone assembly and disassembly (2-4).

The H3/H4 histone chaperone, CAF-1, is a complex of Cac1, Cac2, and Cac3 in *Saccharomyces cerevisiae* and involved in chromatin assembly coupled to DNA replication in the S phase (5), whereas HIR (a complex of Hir1, Hir2, Hir3, and Hpc2 in yeast) is involved in H3/H4 deposition in the replication-independent pathway (6-8). Another H3/H4 chaperone, Asf1, interacts with both CAF-1 and HIR, affecting both replication-dependent and independent pathways (6, 9).

*Corresponding author. Tel: 82-31-290-7781; Fax: 82-31-292-8800;
E-mail: echo@skku.edu

The HIR genes, *HIR1, HIR2, HIR3,* and *HPC2,* genetically interact with the transcription-coupled H2A/H2B chaperone, FACT (a complex of Spt16, Spt6, and Pob3) (10). *ASF1* has a similar genetic interaction profile with the *HIR* genes and is directly involved in *PHO* gene activation (11, 12). *ASF1* is also important for repression of internal aberrant transcription and for the release and deposition of histones during transcriptional elongation (13-15), suggesting that HIR and Asf1 are important chromatin regulators during transcription. In contrast, the role of CAF-1 during transcription is unclear, as no CAF-1 subunits have significant genetic interactions with these elongation factors (10). Although Asf1 and CAF-1 work together in heterochromatin function, their role in euchromatin has not been elucidated. This study examined the role of the yeast H3/H4 histone chaperones, Asf1, HIR, and CAF-1 in chromatin dynamics during transcription. Unexpectedly, CAF-1 was recruited to the actively transcribed region, as was HIR and Asf1. In addition, three histone chaperones genetically interacted with Set2-dependent H3 K36 methylation, implying that Set2, Asf1, HIR, and CAF-1 function in a related pathway. Similar to histone chaperones, Set2 was required for tolerance to excess histone H3 but not to excess H2A, suggesting that they all play a role in transcriptional regulation via chromatin.

RESULTS AND DISCUSSION

The histone chaperone, CAF-1, is recruited to the transcriptional region

H3/H4 chaperones, Asf1, and the HIR complex are recruited to the gene during transcription. Here, we analyzed the replication-dependent H3/H4 chaperone, CAF-1, as well as Asf1 and HIR. To monitor protein occupancy, we performed a chromatin immunoprecipitation (ChIP) assay with *S. cerevisiae* expressing each subunit of histone chaperone complex, Cac1-Flag, Asf1-TAP, Hir1-Flag and Hir2-Flag, from their genomic loci. We examined the *PMA1* gene, which is constitutively expressed and widely used for the detection of transcription factors (Fig. 1A). Interestingly, Cac1 as well as Asf1, Hir1, and Hir2, cross-linked at high levels to the promoter, coding, and 3'UTR of *PMA1* (Fig. 1B, upper panel), indicating that CAF-1, Asf1, and HIR were associated with the transcribed region. HIR is a complex consisting of Hir1, Hir2, Hir3, and Hpc2, while CAF-1 is composed of Cac1, Cac2, and Cac3. The cross-linking of other subunits of each complex was also examined to confirm transcription-dependent recruitment.
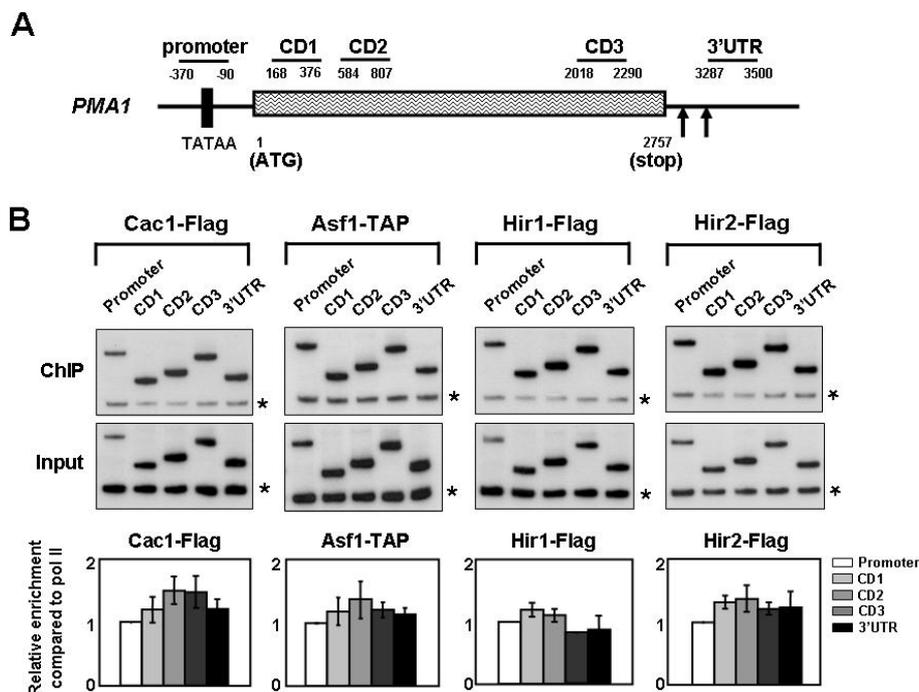
Fig. 1. The histone chaperones are associated with actively transcribed regions. (A) Schematic representation of the *PMA1* locus, including primer locations for ChIP. (B) Histone chaperones are associated with the entire transcribed region of *PMA1*. ChIP was performed with Asf1-TAP (YFC215), Hir1-Flag (YAG102), Hir2-Flag (YAG104), and Cac1-Flag (YAV1) using anti-Flag or IgG antibodies as described in the Materials and Methods. Representative results of at least three independent experiments are shown (Upper panel). The fold enrichment of each ChIP value was compared with that of pol II (8WG16 IP). The value obtained at the promoter region was normalized to 1 (Bottom panel). Asterisks indicate an internal control for background.

They all similarly cross-linked to *PMA1* (Supplementary Fig. 1). Their recruitment profile was further analyzed by normalization with pol II (Fig. 1B, bottom panel), and resembled the association pattern of the elongation factors, Spt2, Spt4, Spt5, Spt6, Spt16, and Elf1, all involved in chromatin dynamics, whose association persists over the 3′UTR (14, 16-18).

The HIR complex associates with a discrete region of the histone genes during the cell cycle, and CAF-1 is involved in replication-coupled chromatin assembly Asf1 is physically and functionally linked to both activities, but recruitment of the CAF-1 complex to the transcribed gene is unexpected, despite a similar role for CAF-1 and Asf1 in protein-protein interactions in heterochromatin. CAF-1, Asf1, and HIR all cross-linked specifically to the entire region of the actively transcribed gene, indicating that they function as general transcription elongation factors as well as transcriptional corepressor (HIR/Asf1) and replication-coupled chromatin assembly factor (CAF-1/Asf1). In fact, none of them are essential and are largely redundant in yeast chromatin function.

Recruitment of histone chaperones does not depend on Rtf1, Ctk1, or Set2

Histone chaperones must be recruited to the chromatin upon transcription. Asf1 recruitment was unaffected by *hir1Δ* or *cac1Δ*, indicating that protein-protein interactions between Asf1 and HIR or CAF-1 are not needed for Asf1 recruitment (Fig. 2), despite their known interactions (9, 19). Similarly, the recruitment of Hir2 and Cac1 was unaffected by *asf1Δ*, which suggests that their recruitment is not dependent on each other
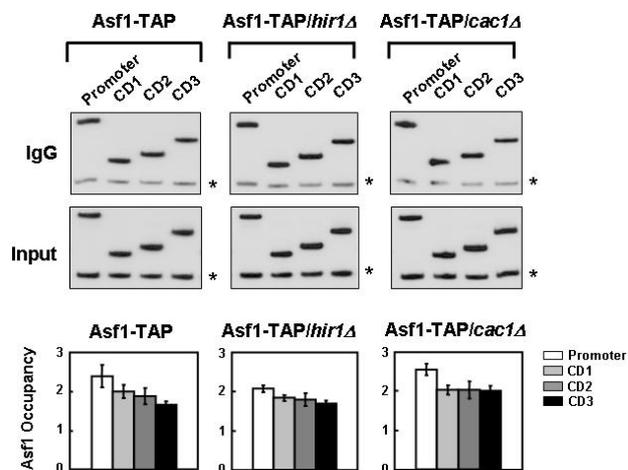

Fig. 2. Asf1 recruitment is independent of Hir1 or Cac1. ChIP was performed with yeasts expressing TAP-tagged Asf1 in the WT (YC215), *hir1Δ* (YC204), or *cac1Δ* (YC205) as described in Fig. 1 (Upper panel). Each PCR signal was normalized to the intergenic control and the input signal (Bottom panel).

(data not shown). To determine whether elongation factors target histone chaperones to the chromatin, we constructed a set of mutants lacking *rtf1Δ* (a component of the PAF1 elongation complex), *ctk1Δ* (a kinase of the CTD of elongating polymerase), or *set2Δ* (a CTD-associated histone H3 methyltransferase). The mutants, *rtf1Δ, ctk1Δ,* or *set2Δ,* did not change Hir1, Hir2,

or Cac1 recruitment (Supplementary Fig. 2). These data suggest that the transcription elongation factors, Rtf1, Ctk1, and Set2, are not required for HIR and CAF-1 recruitment to *PMA1*.

In yeast, the Swi/Snf complex alters nucleosome positioning at transcription sites (20). It travels with elongating Pol II and mediates activator-dependent removal of H3 (21 and references therein). In the absence of Snf2, H3 removal is delayed and Hir2 occupancy is decreased, indicating that ATP-dependent chromatin remodeling factors such as the Swi/Snf complex can initiate chromatin change during transcription. As such, the histone chaperones may be recruited to the transcribed genes by recognizing the initial changes in chromatin structure introduced by other chromatin remodeling factors, such as Swi/Snf or polymerase II itself, and then facilitate the process of chromatin change.

The function of histone chaperones is related to *SET2*-dependent H3 K36 methylation

CAF-1 does not show any significant genetic interactions with elongation factors, unlike Asf1/HIR (10). However, the localization of CAF-1 suggests that it might be related to transcription.

Set2 is a histone H3 methyltransferase and contributes to transcriptional elongation through H3 K36 methylation. The phenotype of *set2Δ* can be reproduced by the mutation of lysine 36 to alanine. To examine the role of CAF-1 in transcription, we constructed yeast strains with each histone chaperone subunit deleted in the histone shuffle background, and then transformed them to introduce the H3 wild type or K36A allele on the *TRP* plasmid. Each transformant was grown in SC-TRP medium and spotted serially on a YPD plate with or without 5-fluoro-orotic acid (FOA). FOA counter-selects for yeast that have lost the original source of wild type H3 carried on the *URA* plasmid.

Interestingly, deletion of CAF-1 subunits, HIR subunits, or *ASF1* slowed growth on FOA medium when combined with H3 K36A (Fig. 3). This growth phenotype was specific to K36A

because there was no growth defect with K4A or K79A except *asf1Δ*, which was sensitive to K4A in addition to K36A (Supplementary Fig. 3). Thus, yeast with the histone chaperone mutation prefers to retain wild type H3 rather than K36A. In contrast, a combination of *set2Δ* and H3 K36A did not develop further growth defects, indicating that K36A is not intrinsically defective in growth on FOA (Fig. 3). In addition, *SPT10*, a histone acetyltransferase that regulates the expression of histone genes and affects global transcription (22, 23), did not slow growth on FOA with K36A, indicating that not all histone-related factors have a growth phenotype in combination with K36A. These results suggest that CAF-1, Asf1, HIR, and Set2 might function in a similar pathway in transcriptional elongation but do not overlap completely.

Set2 is required for tolerance to excess histones

Transcription by pol II on the chromatin template is accompanied by dynamic changes in chromatin structure. Therefore, this process can be affected by excess free histones, because the chromatin histones and free histones compete for chaperone binding. To understand the basis of the genetic interaction between H3 K36A and H3/H4 chaperones, we tested whether Set2 is implicated in histone chaperone function by measuring the growth of yeast over-expressing H3 or H2A under the *GAL1* promoter. H3 or H2A overexpression on galactose medium did not affect the growth of the wild type (Fig. 4). However, as a control, *asf1Δ* was sensitive to excess H3 but not H2A (Fig. 4A and B). Interestingly, *SET2* deletion also rendered the cells sensitive to excess H3, but not H2A, which was not the case for the other H3 methyltransferase mutants, *set1Δ* or *dot1Δ* (Fig. 4A). This indicates that all histone methyltransferases are not sensitive to excess H3, even with similar H3 expression levels (Fig. 4B). Set2 was not needed for the recruitment of Hir1 or Asf1 (Supplementary Fig. 2 and data not shown), but was required for tolerating excess histones, specifically H3. Our data indicate that Set2 or K36 methylation and
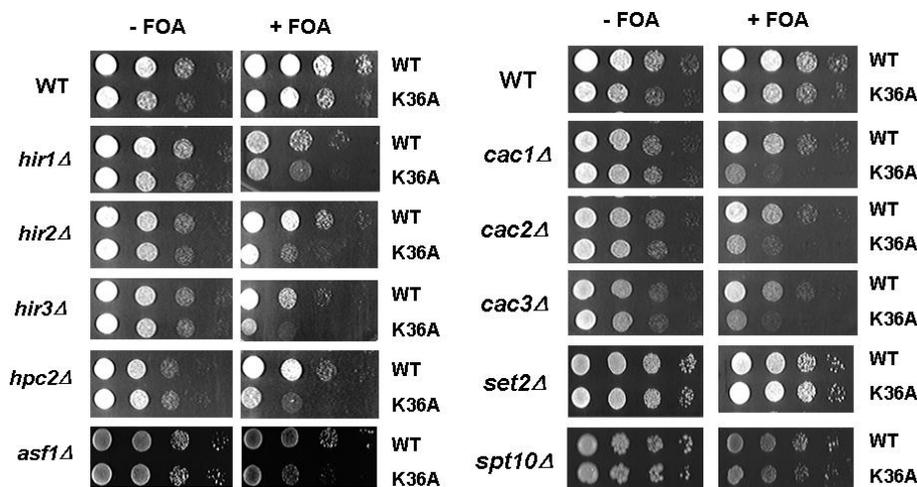

Fig. 3. The slow growth phenotype of the histone chaperone mutants in combination with the H3 K36A allele. The growth of the yeasts with deletions at the indicated gene was analyzed in combination with the H3 wild type or K36A.
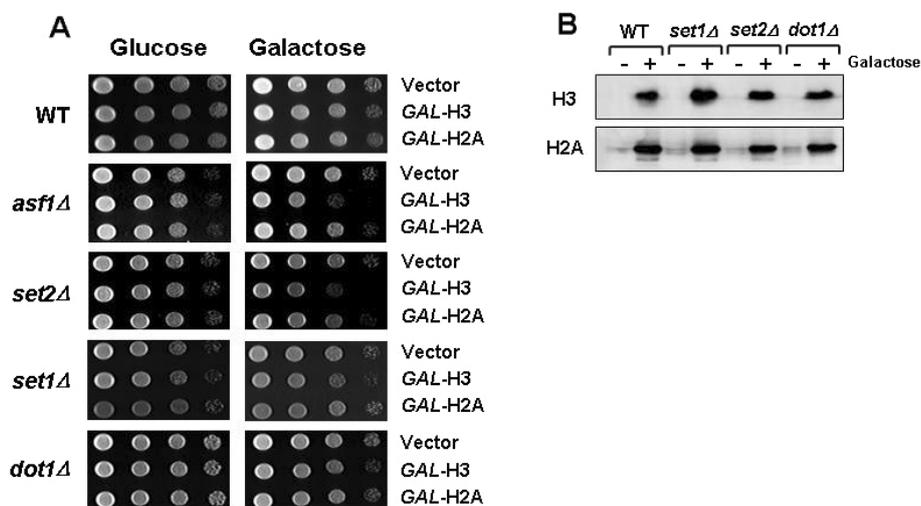
Fig. 4. The *ASF1* and *SET2* mutants are sensitive to over-expression of histone H3. (A) The growth of WT (BY4741), and *asf1Δ, set1Δ, set2Δ,* or *dot1Δ* mutants during overexpression of histone H2A or H3. (B) H3 or H2A were over-expressed in each strain at a similar level. The whole cell extracts prepared from the same strains in (A) were used for immunoblotting with HA-antibody.

H3/H4 histone chaperones play a common role in the pathway affected by excess H3.

Asf1 and HIR play a role in transcription, but CAF-1 does not. Mutations in HIR and *ASF1* subunits produce synthetic growth defects with components of the FACT or PAF1 complex, and *hir1Δ* is lethal when combined with some *SPT4, SPT5,* and *SPT6* alleles (10, 24). Asf1 is a central factor in the activation of *PHO* genes. However, the role of CAF-1 in transcription is indirect, such that it contributes to gene silencing by maintaining heterochromatin. Although CAF-1 has contact with Asf1, it seems to be dispensable for Asf1-dependent transcriptional activation in *PHO* genes. However, CAF-1 is recruited to a transcriptionally active region and functionally required when H3 K36 is mutated, suggesting that CAF-1 might be also involved in transcription-coupled chromatin dynamics. H3 K36 is selectively methylated in actively transcribed regions and is required to keep transcribed chromatin in a repressed state to inhibit internal cryptic transcription (25, 26). The ability of Set2 to repress aberrant chromatin opening may relate to its ability to cope with excess H3, as does CAF-1. However, more work will be needed to determine the precise role of CAF-1 and Set2 in chromatin dynamics and transcription.

MATERIALS AND METHODS

Yeast strain and plasmid construction
The yeast strains used in this study are summarized in Supplementary Table 1. Deletion mutants were constructed by PCR-mediated KanMX4 cassette mutagenesis. pWZ414-F13, pRS314-H3(K4A)-H4, pRS314-H3(K36A)-H4, and pWZ414-F13-H3(K79A)-H4 were previously described (27). The HTH-*HHT2*/pYES2 plasmid (HA tagged *HHT2* under the control of an inducible *GAL1* promoter) was provided by Dr. A. Verreault. The pGALHA-H2A encoding HA-H2A under the control of the *GAL1* promoter was constructed by PCR and confirmed by sequencing.

Growth conditions and analysis
Yeasts were grown at 30°C in either YPD media or in synthetic minimal media lacking the nutritional supplements required for plasmid maintenance. For histone expression, yeasts were transformed with the plasmids HTH-*HHT2*/pYES2, pGALHA-H2A, or the empty vector (pYES2). Yeasts were grown overnight in SC-URA containing 2% raffinose, serially diluted 10-fold, and spotted on SC-Uracil with either glucose or galactose as the carbon source. The plates were incubated for 3-4 days at 30°C before analysis. For the genetic interaction assay between histone chaperones and H3 alleles, each histone shuffle strain carrying the histone chaperone mutation was transformed with the plasmids individually and then tested for the ability to grow on YPD with 5-fluoroorotic acid (FOA).

Immunoblotting
To analyze the overexpression of histones, yeast cultures were harvested 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 after 1 min of vortexing. The resulting lysate was clarified by centrifugation and separated on a 15% denaturing polyacrylamide gel and analyzed by immunoblotting with HA-antibody (Roche).

Chromatin Immunoprecipitation (ChIP)
The ChIPs were performed essentially as described (13). The PCR signals were quantified using a Phosphoimager (Fujix BAS 2040) and normalized to the input DNA and the intergenic control. The PCR primers were described previously (28).

REFERENCES

1. Luger, K., Mader, A. W., Richmond, R., Sargent, D. F. and Richmond, T. J. (1997) Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **389**, 251-260.

2. Adams, C. R. and Kamakaka, R. T. (1999). Chromatin assembly: biochemical identities and genetic redundancy. *Curr. Opi. Genet. Dev.* **9**, 185-190.

3. Mellor, J. (2006) Dynamic nucleosomes and gene transcription. *Trends Genet.* **22**, 320-329.

4. Workman, J. L. (2006) Nucleosome displacement in transcription. *Genes Dev.* **20**, 2009-2017.

5. Smith, S.M. and Stillman, B. (1989) Purification and characterization of DAF-1 a human cell facor required for chromatin assembly during DNA replication *in vitro. Cell* **58**, 15-25.

6. Green, E. M., Antczak, A. J., Bailey, A. O., Franco, A. A., Wu, K. J., Yates, J. R. and Kaufman, P. D. (2005) Replication-independent histone deposition by the HIR complex and Asf1. *Curr. Biol.* **15**, 2044-2049.

7. Prochasson, P., Florens, L., Swanson, S. K., Washburn, M. P. and Workman, J. L. (2005) The HIR corepressor complex binds to nucleosomes generating a distinct protein/DNA complex resistant to remodeling by SWI/SNF. *Genes Dev.* **19**, 2534-2439.

8. Tagami, H., Ray-Gallet, D., Almouzni, G. and Nakatani, Y. (2004) Histone H3.1 and H3.3 complex mediate nucleosome assembly pathway dependent or independent of DNA synthesis. *Cell* **116**, 51-61.

9. Sharp, J. A., Fouts, E. T., Krawitz, D. C. and Kaufman, P. D. (2001) Yeast histone deposition protein Asf1p requires Hir proteins and PCNA for heterochromatic silencing. *Curr. Biol.* **11**, 463-473.

10. Formosa, T., Ruone, S., Adams, M. D., Osen, A. E., Eriksson, P., Yu, Y., Rhoades, A. R., Kaufman, P. D. and Stillman, D.J. (2002) Defects in SPT16 or POB3(yFACT) in saccharomyces cerevisiae cause dependence on the Hir/Hpc pathway: polymerase passage may degrade chromatin structure. *Genetics* **162**, 1557-1571.

11. Adkins, M. W. and Tyler, J. K. (2004) The histone chaperone Asf1p mediates global chromatin disassembly *in vivo. J. Biol. Chem.* **279**, 52069-52074.

12. Korber, P., Barbaric, S., Luckenbach, T., Schmid, A., Schermer, U. J., Blaschke, D. and Hörz, W. (2006) The histone chaperone Asf1 increases the rate of histone eviction at the yeast PHO5 and PHO8 promoters. *J. Biol. Chem.* **281**, 5539-5545.

13. Kim, H. J., Seol, J. H., Han, J. W., Youn, H. D. and Cho, E. J. (2007) Histone chaperones regulate histone exchange during transcription. *EMBO J.* **26**, 4467-4474.

14. Nourani, A., Robert, F. and Winston, F. (2006) Evidence that Spt2/Sin1, an HMG-like factor, plays roles in transcription elongation, chromatin structure, and genome stability in Saccharomyces cerevisiae. *Mol. Cell Biol.* **26**, 1496-1509.

15. Schwabish, M. A. and Struhl, K. (2004) Evidence for eviction and rapid deposition of histones upon transcriptional elongation by RNA polymerase II. *Mol. Cell Biol.* **24**, 10111-10117.

16. Kaplan, C. D., Holland, M. J. and Winston, F. (2005) Interaction between transcription elongation factors and mRNA 3'-end formation at the Saccharomyces cerevisiae GAL10-GAL7 Locus. *J. Biol. Chem.* **280**, 913-922.

17. Kim, M., Ahn, S. H., Krogan, N. J., Greenblatt, J. F. and Buratowski, S. (2004) Transitions in RNA polymerase II elongation complexes at the 3' ends of genes. *EMBO J.* **23**, 354-364.

18. Prather, D., Krogan, N. J., Emili, A., Greenblatt, J. F. and Winston, F. (2005) Identification and characterization of Elf1, a conserved transcription elongation factor in Saccharomyecs cerevisiae. *Mol. Cell Biol.* **25**, 10122-10135.

19. Krawitz, D. C., Kama, T. and Kaufman, P. D. (2002) Chromatin assembly factor I mutants defective for PCNA binding require Asf1/Hir proteins for silencing. *Mol. Cell Biol.* **22**, 614-625.

20. Hirschhorn, J. N., Brown, S. A., Clark, C. D. and Winston, F. (1992) Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. *Genes Dev.* **6**, 2288-2298.

21. Schwabish, M. A. and Struhl, K. (2007) The Swi/Snf complex is important for histone eviction during transcriptional activation and RNA polymerase II elongation *in vivo. Mol. Cell Biol.* **27**, 6987-6995.

22. Eriksson, P. R., Mendiratta, G., Mclaughlin, N. B., Wolfsberg, T. G., Marino-Ramirez, L., Pompa, T. A., Jainerin, M., Landsman, D., Shen, C. H. and Clark, D. J. (2005) Global regulation by the yeast spt10 protein is mediated through chromatin structure and the histone upstream activating sequence elements. *Mol. Cell Biol.* **25**, 9127-9137.

23. Xu, F., Zhang, K. and Grunstein, M. (2005) Acetylation in histone H3 globular domain regulates gene expression in yeast. *Cell* **121**, 375-385.

24. DeSilva, H., Lee, K. and Osley, M. A. (1998) Functional dissection of yeast Hir1p, a WD repeat-containing transcriptional corepressor. *Genetics* **148**, 657-667.

25. Carrozza, M. J., Li, B., Florens, L., Suganuma, T., Swanson, S. K., Lee, K. K., Shia, W. J., Anderson, S., Yates, J., Washburn, M. P. and Workman, J. L. (2005) Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. *Cell* **123**, 581-592.

26. Keogh, M. C., Kurdistani, S. K., Morris, S. A., Ahn, S. H., Podolny, V., Collins, S. R., Schuldiner, M., Chin, K., Punna, T., Thompson, N. J., Boone, C., Emili, A., Weissman, J. S., Hughes, T. R., Strahl, B. D., Grunstein, M., Greenblatt, J. F., Buratowski, S. and Krogan, N. J. (2005) Cotranscriptional Set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. *Cell* **123**, 593-605.

27. Seol, J. H., Kim, H. J., Yang, Y. J., Kim, S. T., Youn, H. D., Han, J. W., Lee, H. W. and Cho, E. J. (2006) Different roles of histone H3 lysine 4 methylation in chromatin maintenance. *Bio. Biophy. Res. Comm.* **349**, 463-470.

28. Cho, E. J., Kobor, M. S., Kim, M., Greenblatt, J. and Buratowski, S. (2001) Opposing effects of Ctk1 kinase and Fcp1 phosphatase at serine 2 of the RNA polymerase II C-terminal domain. *Genes Dev.* **24**, 3319-3329.