Uncoupling of the Patterns of Chromatin Association of Different Transcription Elongation Factors by a Histone H3 Mutant in *Saccharomyces cerevisiae*  

Amanda Lloyd, Katie Pratt, Erica Siebrasse, Matthew D. Moran, and Andrea A. Duina*  

Biology Department, Hendrix College, Conway, Arkansas  

Received 17 October 2008/Accepted 20 November 2008  

The transcription elongation complexes yFACT, Spt4/Spt5, and Spt6/Iws1 were previously shown to follow similar patterns of association across transcribed genes in *Saccharomyces cerevisiae*. Using a histone H3 mutant, we now provide evidence that the mechanism of association of yFACT across genes is separable from that adopted by Spt4/Spt5 and Spt6/Iws1.  

In recent years, it has become clear that the transcription elongation process in eukaryotic cells is highly dynamic, requiring the contributions of many elongation factors possessing a variety of biochemical activities. Many of these factors have been shown to promote changes, either directly or indirectly, in the structure of chromatin to facilitate passage of RNA polymerase II across transcribed genes and, in certain cases, to prevent the inappropriate initiation of transcription within open reading frames (ORFs) (6, 10, 26). Although a number of studies have defined some of the physical interactions that occur between elongation factors (for some examples, see references 14, 21, and 23, and for a review, see reference 15), little is known about the mechanisms that control how these factors prevent the inappropriate initiation of transcription within gene analyzed. As expected, and consistent with previous reports (4, 13, 18), in wild-type cells we detected strong binding of Spt16 to the 5’ regions of both ORFs and no binding to a region significantly downstream from the end of each ORF, whereas in H3-L61W cells we observed a dramatic shift in Spt16 distribution toward the 3’ end of each gene (Fig. 1B, left panel [F2,30 = 129.9; P < 0.001] and right panel [F2,30 = 54.49; P < 0.001], and Fig. 2B, left panel [F2,30 = 40.18; P < 0.001] and right panel [F2,30 = 24.15; P < 0.001]). We now show that the distribution of Pob3 across both loci is also greatly shifted toward the 3’ regions of both ORFs in the context of H3-L61W (Fig. 1C, left panel [F2,12 = 23.08; P < 0.001] and right panel [F2,12 = 12.13; P = 0.001], and Fig. 2C, left panel [F2,12 = 3.50; P = 0.024] and right panel [F2,12 = 6.60; P = 0.012]). Although the magnitude of the 3’ shift for Pob3 was lower than that seen for Spt16, our data, combined with the fact that Spt16 and Pob3 are components of the yFACT complex (3, 9), support the notion that at least to a large extent, yFACT as a whole is affected in the H3-L61W mutant. Interestingly, we routinely observed increased levels of Spt16 and Pob3 binding to a nontranscribed region on chromosome V (4; this study) as well as increased Spt16 binding to a telomeric region on chromosome VI not associated with transcription in H3-L61W cells compared to the case in wild-type cells (data not shown), suggesting that yFACT might bind abnormally to nontranscribed regions throughout the genome in H3-L61W cells.  

The Spt4, Spt6, and Spt16 elongation factors were originally identified through similar genetic selection experiments and are thought to participate in the transcription elongation process through interactions with chromatin (7, 16, 20, 25).
Whereas genetic and biochemical studies indicate that Spt4, Spt6, and Spt16 share some common functional features with each other (11), these three factors are found in different complexes, namely, Spt4/Spt5 (DSIF in humans), Spt6/Iws1, and yFACT, respectively, with distinct biochemical properties (1, 2, 24). Consistent with the idea that the functions of these complexes are to some degree related with each other, a recent study has shown that Spt4/Spt5, Spt6/Iws1, and yFACT display the same pattern of association across PMA1, ADH1, and several other transcribed genes (13). However, it is not clear whether these complexes utilize the same or different mechanisms to regulate their association with transcribed genes and whether there is an interdependent relationship in their ability to interact with chromatin.

We took advantage of the H3-L61W mutant to obtain some insights into these questions. For these studies, we performed ChIPs to determine whether the patterns of association of Spt6 and yFACT, respectively, with distinct biochemical properties (1, 2, 24). Consistent with the idea that the functions of these complexes are to some degree related with each other, a recent study has shown that Spt4/Spt5, Spt6/Iws1, and yFACT display the same pattern of association across PMA1, ADH1, and several other transcribed genes (13). However, it is not clear whether these complexes utilize the same or different mechanisms to regulate their association with transcribed genes and whether there is an interdependent relationship in their ability to interact with chromatin.

We took advantage of the H3-L61W mutant to obtain some insights into these questions. For these studies, we performed ChIPs to determine whether the patterns of association of Spt6 and yFACT, respectively, with distinct biochemical properties (1, 2, 24). Consistent with the idea that the functions of these complexes are to some degree related with each other, a recent study has shown that Spt4/Spt5, Spt6/Iws1, and yFACT display the same pattern of association across PMA1, ADH1, and several other transcribed genes (13). However, it is not clear whether these complexes utilize the same or different mechanisms to regulate their association with transcribed genes and whether there is an interdependent relationship in their ability to interact with chromatin.

We took advantage of the H3-L61W mutant to obtain some insights into these questions. For these studies, we performed ChIPs to determine whether the patterns of association of Spt6 and yFACT, respectively, with distinct biochemical properties (1, 2, 24). Consistent with the idea that the functions of these complexes are to some degree related with each other, a recent study has shown that Spt4/Spt5, Spt6/Iws1, and yFACT display the same pattern of association across PMA1, ADH1, and several other transcribed genes (13). However, it is not clear whether these complexes utilize the same or different mechanisms to regulate their association with transcribed genes and whether there is an interdependent relationship in their ability to interact with chromatin.

We took advantage of the H3-L61W mutant to obtain some insights into these questions. For these studies, we performed ChIPs to determine whether the patterns of association of Spt6 and yFACT, respectively, with distinct biochemical properties (1, 2, 24). Consistent with the idea that the functions of these complexes are to some degree related with each other, a recent study has shown that Spt4/Spt5, Spt6/Iws1, and yFACT display the same pattern of association across PMA1, ADH1, and several other transcribed genes (13). However, it is not clear whether these complexes utilize the same or different mechanisms to regulate their association with transcribed genes and whether there is an interdependent relationship in their ability to interact with chromatin.

We took advantage of the H3-L61W mutant to obtain some insights into these questions. For these studies, we performed ChIPs to determine whether the patterns of association of Spt6 and yFACT, respectively, with distinct biochemical properties (1, 2, 24). Consistent with the idea that the functions of these complexes are to some degree related with each other, a recent study has shown that Spt4/Spt5, Spt6/Iws1, and yFACT display the same pattern of association across PMA1, ADH1, and several other transcribed genes (13). However, it is not clear whether these complexes utilize the same or different mechanisms to regulate their association with transcribed genes and whether there is an interdependent relationship in their ability to interact with chromatin.

We took advantage of the H3-L61W mutant to obtain some insights into these questions. For these studies, we performed ChIPs to determine whether the patterns of association of Spt6 and yFACT, respectively, with distinct biochemical properties (1, 2, 24). Consistent with the idea that the functions of these complexes are to some degree related with each other, a recent study has shown that Spt4/Spt5, Spt6/Iws1, and yFACT display the same pattern of association across PMA1, ADH1, and several other transcribed genes (13). However, it is not clear whether these complexes utilize the same or different mechanisms to regulate their association with transcribed genes and whether there is an interdependent relationship in their ability to interact with chromatin.

We took advantage of the H3-L61W mutant to obtain some insights into these questions. For these studies, we performed ChIPs to determine whether the patterns of association of Spt6 and yFACT, respectively, with distinct biochemical properties (1, 2, 24). Consistent with the idea that the functions of these complexes are to some degree related with each other, a recent study has shown that Spt4/Spt5, Spt6/Iws1, and yFACT display the same pattern of association across PMA1, ADH1, and several other transcribed genes (13). However, it is not clear whether these complexes utilize the same or different mechanisms to regulate their association with transcribed genes and whether there is an interdependent relationship in their ability to interact with chromatin.

We took advantage of the H3-L61W mutant to obtain some insights into these questions. For these studies, we performed ChIPs to determine whether the patterns of association of Spt6 and yFACT, respectively, with distinct biochemical properties (1, 2, 24). Consistent with the idea that the functions of these complexes are to some degree related with each other, a recent study has shown that Spt4/Spt5, Spt6/Iws1, and yFACT display the same pattern of association across PMA1, ADH1, and several other transcribed genes (13). However, it is not clear whether these complexes utilize the same or different mechanisms to regulate their association with transcribed genes and whether there is an interdependent relationship in their ability to interact with chromatin.

We took advantage of the H3-L61W mutant to obtain some insights into these questions. For these studies, we performed ChIPs to determine whether the patterns of association of Spt6 and yFACT, respectively, with distinct biochemical properties (1, 2, 24). Consistent with the idea that the functions of these complexes are to some degree related with each other, a recent study has shown that Spt4/Spt5, Spt6/Iws1, and yFACT display the same pattern of association across PMA1, ADH1, and several other transcribed genes (13). However, it is not clear whether these complexes utilize the same or different mechanisms to regulate their association with transcribed genes and whether there is an interdependent relationship in their ability to interact with chromatin.

We took advantage of the H3-L61W mutant to obtain some insights into these questions. For these studies, we performed ChIPs to determine whether the patterns of association of Spt6 and yFACT, respectively, with distinct biochemical properties (1, 2, 24). Consistent with the idea that the functions of these complexes are to some degree related with each other, a recent study has shown that Spt4/Spt5, Spt6/Iws1, and yFACT display the same pattern of association across PMA1, ADH1, and several other transcribed genes (13). However, it is not clear whether these complexes utilize the same or different mechanisms to regulate their association with transcribed genes and whether there is an interdependent relationship in their ability to interact with chromatin.
To test this possibility, we performed ChIP experiments with H3-L61W cells that also express a previously isolated Spt6 mutant, Spt6-790, that moderately suppresses the Spt6 3′-shift phenotype at PMA1 and other genes (4). We found that whereas Spt6-790 significantly suppressed the Spt6 3′-shift phenotype at PMA1 in H3-L61W cells (Fig. 3A) ($F_{2,21} = 16.05; P < 0.001$), the same mutation did not suppress the defect in Spt6 distribution over H3-L61W cells (Fig. 3B) ($F_{2,15} = 0.57; P = 0.58$). These data support the notion that H3-L61W causes its effects on the protein tagged with a TAP tag). We found that Spt6 displayed a minor but statistically significant 3′ shift at both genes in the context of H3-L61W (Fig. 1D [$F_{2,18} = 6.70; P = 0.007$] and 2D [$F_{2,18} = 11.43; P = 0.002$]). However, the pattern of Spt4 association across either gene was not significantly perturbed by the H3-L61W mutant (Fig. 1E [$F_{2,12} = 3.16; P = 0.08$] and 2E [$F_{2,12} = 1.99; P = 0.18$]). The observation that the pattern of chromatin association of yFACT at PMA1 and ADH1 can be uncoupled from that of Spt4 and Spt6 by a mutation (i.e., H3-L61W) provides evidence that (i) there is no strict requirement for the presence of Spt4 or Spt6 for yFACT association with chromatin, since high levels of yFACT are seen at regions of genes (3′ ends) that are not or only poorly occupied by Spt4 and Spt6; and (ii) the mechanisms that regulate yFACT interaction with genes in vivo are distinct from those regulating chromatin association of Spt4 and Spt6.

Spt16 and Spt6 both have histone chaperoning activity, and defects in these factors have been shown to result in cryptic transcription from within ORFs, presumably due to defects in nucleosome assembly in the wake of RNA polymerase II passage (12, 18). Given these shared characteristics, we entertained the possibility that the cause for the minor distribution shift observed for Spt6 across genes in H3-L61W cells could be a secondary effect of abnormal Spt6-histone H3 interactions and that suppressor mutations that improve Spt6-histone H3 interactions might in turn also suppress the Spt6 distribution defects. To test this possibility, we performed ChIP experiments with H3-L61W cells that also express a previously isolated Spt6 mutant, Spt6-790, that moderately suppresses the Spt6 3′-shift phenotype at PMA1 and other genes (4). We found that whereas Spt6-790 significantly suppressed the Spt6 3′-shift phenotype at PMA1 in H3-L61W cells (Fig. 3A) ($F_{2,21} = 16.05; P < 0.001$), the same mutation did not suppress the defect in Spt6 distribution over PMA1 in the context of H3-L61W cells (Fig. 3B) ($F_{2,15} = 0.57; P = 0.58$). These data support the notion that H3-L61W causes its effects on the protein tagged with a TAP tag.

The allele at this locus is either his3Δ200 or his3Δ1.

The allele at this locus is either leu2Δ1 or leu2Δ0.

The original strains yAAD2220 and yAAD2224 also contain a URA3-marked plasmid harboring the wild-type HHT2-HHFI locus. The strains used in these studies were selected for loss of this plasmid and therefore contain only genomic HHT2 or hht2-11 (as indicated) as the sole source of H3 protein.

### Table 1. Saccharomyces cerevisiae strains used in this study

| Strain | Genotype |
|--------|----------|
| yAAD1048 | MATa his3Δ200 leu2Δ1 ura3-52 lys2-128Δ (hht1-hhf1)Δ::LEU2 hht2-11 |
| yAAD1049 | MATa his3Δ200 leu2Δ1 ura3-52 lys2-128Δ (hht1-hhf1)Δ::LEU2 hht2-11 |
| yAAD1052 | MATa his3Δ200 leu2Δ1 ura3-52 lys2-128Δ (hht1-hhf1)Δ::LEU2 |
| yAAD1053 | MATa his3Δ200 leu2Δ1 ura3-52 lys2-128Δ (hht1-hhf1)Δ::LEU2 |
| yAAD1128 | MATa his3Δ200 leu2Δ1 ura3-52 lys2-128Δ (hht1-hhf1)Δ::LEU2 hht2-11 SPT16-790 |
| yAAD2214 | MATa hisΔ3 leu2Δ1 ura3-52 lys2-128Δ (hht1-hhf1)Δ::LEU2 LEU2 SPT16-790 |
| yAAD2215 | MATa hisΔ3 leu2Δ1 ura3-52 lys2-128Δ (hht1-hhf1)Δ::LEU2 hht2-11 POB3-TAP::HIS3MX6 |
| yAAD2220 | MATa hisΔ3 leu2Δ1 ura3-52 lys2-128Δ (hht1-hhf1)Δ::LEU2 hht2-11 SPT16-790 |
| yAAD2223 | MATa hisΔ3 leu2Δ1 ura3-52 lys2-128Δ (hht1-hhf1)Δ::LEU2 hht2-11 SPT16-790 |
| yAAD2224 | MATa hisΔ3 leu2Δ1 ura3-52 lys2-128Δ (hht1-hhf1)Δ::LEU2 hht2-11 SPT16-790 |
| yAAD2226 | MATa hisΔ3 leu2Δ1 ura3-52 lys2-128Δ (hht1-hhf1)Δ::LEU2 hht2-11 SPT16-790 |
| yAAD2241 | MATa hisΔ3 leu2Δ1 ura3-52 lys2-128Δ (hht1-hhf1)Δ::LEU2 hht2-11 SPT16-790 |
| yAAD2242 | MATa hisΔ3 leu2Δ1 ura3-52 lys2-128Δ (hht1-hhf1)Δ::LEU2 hht2-11 SPT16-790 |
| yAAD2243 | MATa hisΔ3 leu2Δ1 ura3-52 lys2-128Δ (hht1-hhf1)Δ::LEU2 hht2-11 SPT16-790 |
| yAAD2244 | MATa hisΔ3 leu2Δ1 ura3-52 lys2-128Δ (hht1-hhf1)Δ::LEU2 hht2-11 SPT16-790 |

All strains were derived from the S288C background. The generation of the hht2-11 and (hht1-hhf1)Δ::LEU2 mutations has been described previously (5). Strains containing the SPT4-TAP::HIS3MX6 and POB3-TAP::HIS3MX6 alleles were generated by crossing commercially available strains harboring the TAP::HIS3MX6 fusions (Open Biosystems) with strains with the appropriate genotypes.

The allele at this locus is either his3Δ200 or his3Δ1.

The allele at this locus is either leu2Δ1 or leu2Δ0.

The original strains yAAD2220 and yAAD2224 also contain a URA3-marked plasmid harboring the wild-type HHT2-HHFI locus. The strains used in these studies were selected for loss of this plasmid and therefore contain only genomic HHT2 or hht2-11 (as indicated) as the sole source of H3 protein.
patterns of chromatin association of Spt16 and Spt6 across genes through two distinct, possibly unrelated mechanisms.

We previously hypothesized that the presence of H3-L61W may prevent a 3'-untranslated region dissociation signal from reaching Spt6 at the end of the transcription process, thereby resulting in a higher abundance of Spt6 at 3' versus 5' regions of genes (4). Given recent data showing that Spt6 directly interacts with the globular regions of histones H3 and H4 (22), this putative signal is likely to involve direct interactions between Spt6 and these histones. The observation that the H3-L61W mutant has only a modest effect on Spt6 and no effect on Spt4 distribution across the genes assayed here suggests that these elongation factors rely on 3'-untranslated region dissociation signals that are, at least to some degree, separable from those used by yFACT (i.e., signals that are mostly H3-L61W insensitive). Future work will focus on elucidating the nature of the mechanisms that regulate the dynamic association of yFACT with chromatin during the transcription process. Of particular interest in this regard will be determining the role of posttranslational modifications of histones, such as H2B ubiquitylation, which was recently shown to affect Spt6 function and chromatin association (8, 19), in regulating the proper departure of yFACT following transcription. In addition, the recent finding that conditions that promote transcriptional stress cause increased levels of histone occupancy and a Chd1-dependent increase in histone H3-K4 methylation at 3' ends of genes (27) will be investigated in the context of our model, particularly since Chd1 and yFACT have been shown to interact with each other (14, 21).

We are grateful to Joseph Martens, Richard Murray, and Reine Protacio for helpful comments on the manuscript and to Kacey Swin-dle and Landon Reeves for technical assistance. We thank Tim For-mosa for antibodies against the Spt6p and Spt6p proteins. We also thank Aminie Nourani, Mary Bryk, and Varsha Kaushal for assistance on real-time PCR analysis.

This material is based upon work supported by the National Science Foundation under grant 0543412, by NIH grant P20 RR16460-03 from the IDeA Networks of Biomedical Research Excellence (INBRE) Program of the National Center for Research Resources, and by start-up funds from Hendrix College to A.A.D. K.P. was supported by a Student Undergraduate Research Fellowship (SURF) grant from the Arkansas Department of Higher Education.

REFERENCES

1. Belotserkovskaya, R., S. Oh, V. A. Bondarenko, G. Orphanides, V. M. Stu-ditsky, and D. Reinberg. 2003. FACT facilitates transcription-dependent nucleosome alteration. Science 301:1090-1093.
2. Bortvin, A., and F. Winston. 1996. Evidence that Spt6p controls chromatin structure by a direct interaction with histone. Science 272:1473-1476.
3. Brewster, N. K., G. C. Johnston, and R. A. Singer. 1998. Characterization of the CP complex, an abundant dimer of Cdc68 and Pol3 proteins that reg-ulates yeast transcriptional activation and chromatin repression. J. Biol. Chem. 273:21972-21979.
4. Duina, A. A., A. Rufiange, J. Bracey, J. Hall, A. Nourani, and F. Winston. 2007. Evidence that the localization of the elongation factor Spt16 across transcribed genes is dependent upon histone H3 integrity in Saccharomyces cerevisiae. Genetics 177:101-112.
5. Duina, A. A., and F. Winston. 2004. Analysis of a mutant histone H3 that perturbs the association of Swi/Snf with chromatin. Mol. Cell. Biol. 24:561- 570.
6. Eisenberg, J. C., and A. Shilatifard. 2006. Leaving a mark: the many footprints of the elongating RNA polymerase II. Curr. Opin. Genet. Dev. 16:184-190.
7. Fassler, J. S., and F. Winston. 1988. Isolation and analysis of a novel class of suppressor of Ty insertion mutations in Saccharomyces cerevisiae. Genetics 118:203-212.
8. Fleming, A. B., K. Cheng-Fu, C. Hillyer, M. Pikaart, and M. A. Osley. 2008. H2B ubiquitylation plays a role in nucleosome dynamics during transcription elongation. Mol. Cell 31:57-66.
9. Formosa, T., P. Eriksson, J. Wittmeyer, J. Ginn, Y. Yu, and D. J. Stillman. 2001. Spt6p-Pob3p and the HMG protein Nhp6p combine to form the nucleo-some remodeling factor NudF. Mol. Cell 7:1451-1457.
10. Hartzog, G. A. 2003. Transcription elongation by RNA polymerase II. Curr. Opin. Genet. Dev. 13:119-126.
11. Hartzog, G. A., J. L. Speer, and D. L. Lindstrom. 2002. Transcription elongation and chromatin remodeling. Annu. Rev. Biochem. 71:793-830.
12. Kaplan, C. D., L. Laprade, and F. Winston. 2003. Transcription elongation factors repress transcription initiation from cryptic sites. Science 301:1096-1099.
13. Krogan, N. J., S. H. Ahn, N. J. Krogan, J. F. Greenblatt, and S. Buratowski. 2004. Transitions in RNA polymerase II elongation complexes at the 3' ends of genes. EMBO J. 23:354-364.
14. Krogan, N. J., M. Kim, S. H. Ahn, G. Zhong, M. S. Kobor, G. Cagney, A. Emili, A. Shilatifard, S. Buratowski, and J. F. Greenblatt. 2002. RNA poly-merase II elongation factors of Saccharomyces cerevisiae: a targeted pro-teomics approach. Mol. Cell. Biol. 22:6979-6992.
15. Li, B., M. Carey, and J. L. Workman. 2007. The role of chromatin during transcription. Cell 128:707-719.
16. Malone, E. A., C. D. Clark, A. Chiang, and F. Winston. 1991. Mutations in SPT16/CDC68 suppress cis- and trans-acting mutations that affect promoter function in Saccharomyces cerevisiae. Mol. Cell. Biol. 11:5710-5717.
17. Martens, J. A., and F. Winston. 2002. Evidence that Swi/Snf directly represses transcription in S. cerevisiae. Genes Dev. 16:2231-2236.
18. Mason, P. B., and K. Struhl. 2003. The FACT complex travels with elongat-ing RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo. Mol. Cell. Biol. 23:8323-8333.
19. Pavri, R., R. Zhu, G. Li, P. Trojer, S. Mandal, A. Shilatifard, and D. Reinberg. 2006. Histone H2B monoubiquitylation functions cooperatively with FACT to regulate elongation by RNA polymerase II. Cell 125:703-717.
20. Rowley, A. R., A. Singer, and G. C. Johnston. 1991. CDC68, a yeast gene that affects regulation of cell proliferation and transcription, encodes a protein with a highly acidic carboxyl terminus. Mol. Cell. Biol. 11:5718-5726.
21. Simic, R., D. L. Lindstrom, H. G. Tran, K. L. Roinick, P. J. Costa, A. D. Johnson, G. A. Hartzog, and K. M. Arndt. 2003. Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. EMBO J. 22:1846-1856.
22. Stuwe, T., M. Hothorn, E. Lejeune, V. Rybin, M. Borfeld, K. Scheffzek, and A. G. Ladurner. 2008. The FACT Spt16p “peptidase” domain is a histone H3-H4 binding module. Proc. Natl. Acad. Sci. USA 105:8884-8889.
23. Tardiff, D. F., K. C. Abruzzi, and M. Rosbash. 2007. Protein characterization of Saccharomyces cerevisiae RNA polymerase II after in vivo cross-linking. Proc. Natl. Acad. Sci. USA 104:19948-19953.
24. Wada, T., T. Takegi, Y. Yamaguchi, A. Ferdous, T. Imai, S. Hirose, S. Sugimoto, K. Yano, G. A. Hartzog, F. Winston, S. Buratowski, and H. Handa. 1998. DSIF, a novel transcription elongation factor that regulates RNA polymerase II processivity, is composed of human Spt4 and Spt5 homologs. Genes Dev. 12:343-356.
25. Winston, F., D. T. Chaleff, B. Valent, and G. R. Fink. 1984. Mutations affecting Ty-mediated expression of the HIS4 gene of Saccharomyces cerevi-siae. Genetics 107:179-197.
26. Workman, J. L. 2008. Nucleosome displacement in transcription. Genes Dev. 22:2009-2017.
27. Zhang, L., S. Schroeder, N. Fong, and D. L. Bentley. 2005. Altered nucleo-some occupancy and histone H3-K4 methylation in response to ‘transcriptional stress.’ EMBO J. 24:2379-2390.