Tax-dependent Displacement of Nucleosomes during Transcriptional Activation of Human T-Cell Leukemia Virus Type 1*

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The human T-cell leukemia virus type 1 (HTLV-1) is integrated into the host cell DNA and assembled into nucleosomes. Within the repressive chromatin environment, the virally encoded Tax protein mediates the recruitment of the coactivators CREB-binding protein/p300 to the HTLV-1 promoter, located within the long terminal repeats (LTRs) of the provirus. These proteins carry acetyltransferase activity that is essential for strong transcriptional activation of the virus in the context of chromatin. Consistent with this, the amino-terminal tails of nucleosomal histones at the viral promoter are acetylated in Tax-expressing cells. We have developed a system in which we transfect Tax into cells carrying integrated copies of the HTLV-1 LTR driving the luciferase gene to analyze changes in “activating” histone modifications at the LTR. Unexpectedly, Tax transcriptional activation led to an apparent reduction of these modifications at the HTLV-1 promoter and downstream region that correlates with a similar reduction in histone H3 and linker histone H1. Micrococcal nuclease protection analysis showed that less LTR-luciferase DNA is nucleosomal in Tax-expressing cells. Furthermore, nucleosome depletion correlated with RNA polymerase II recruitment and loss of SWI/SNF. The M47 Tax mutant, deficient in HTLV-1 transcriptional activation, was also defective for nucleosome depletion. Although this mutant formed complexes with CREB and p300 at the HTLV-1 promoter in vivo, it was unable to mediate RNA polymerase II recruitment or SWI/SNF displacement. These results support a model in which nucleosomes are depleted from the LTR and transcribed region during Tax-mediated transcriptional activation and correlate RNA polymerase II recruitment with nucleosome depletion.

The human T-cell leukemia virus type 1 (HTLV-1) is the etiological agent of adult T-cell leukemia and HTLV-1-associated myelopathy/tropical spastic paraparesis (1–4). Although not fully understood, the cellular events leading to the onset of both diseases appear to be initiated by the HTLV-1-encoded Tax protein (5). Tax functions as a potent activator of HTLV-1 transcription in part via the formation of a complex with CREB (or other activating transcription factor/CREB members) and the three CRE enhancer sequences located within the HTLV-1 promoter (6–8). Tax contributes to the stability of the ternary complex by binding directly to the GC-rich sequences flanking the octanucleotide CREs (9–12). These sequences, called viral CREs, are absolutely required for strong Tax transcriptional activation of HTLV-1 (13–15). Once associated with the promoter, Tax and CREB form a complex with the cellular coactivators CBP/p300 (16, 17). These coactivators are believed to participate in pre-initiation complex formation, culminating in strong HTLV-1 transcriptional activation (18).

The HTLV-1 provirus is integrated into the genome of the infected host cell and assembled into nucleosomes. This chromatin packaging renders promoter DNA less accessible to the binding of transcription factors and therefore represses transcription. One mechanism for overcoming this repression is acetylation of the amino-terminal tails of the nucleosomal histones. This modification is proposed to increase the accessibility of nucleosomal DNA for transcription factor binding (19–21). Additionally, this and other histone modifications can serve as a platform for the binding of transcriptional regulatory proteins (22). Previous studies have demonstrated that histone deacetylase inhibitors increase the level of HTLV-1 transcription and that Tax and histone deacetylase complex occupancy are mutually exclusive (23, 24). It has also been shown that an increase in histone tail acetylation on HTLV-1 promoter-associated nucleosomes correlates with an increase in viral RNA in HTLV-1-infected T-cells. Among proteins that carry intrinsic histone acetyltransferase activity, the coactivators CBP/p300 have been shown to have an essential role in HTLV-1 transcriptional activation. Our laboratories, as well as others, detected CBP/p300 at the integrated HTLV-1 promoter in T-cells expressing high levels of Tax (23, 24). This finding is consistent with several previous studies showing that Tax directly facilitates the recruitment of CBP/p300 to the HTLV-1 promoter (16, 17, 25, 26). Furthermore, we and others have shown that the histone acetyltransferase activity of p300 is essential for strong HTLV-1 transcription in a chromatin context (18, 27). Using chromatin assembled with core histones lacking their amino-terminal tails and using specific inhibitors of CBP/p300, we have found that CBP/p300 participate in critical chromatin-specific, histone tail-independent acetylation events during transcriptional activation by Tax (28). These data suggest that, in addition to the core histone tails, another target of acetylation by p300 functions in mediating strong Tax transactivation in a chromatin environment.

In previous studies we have examined transcription factor binding and histone modifications at the viral promoter in Tax-expressing, HTLV-1-infected cells (23, 29). In this study, we sought to better define the epigenetic changes that occur during Tax-dependent activation of HTLV-1 LTR-directed transcription in vivo. These analyses were performed in cells carrying chromosomally integrated HTLV-1 LTR-lucif-

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8 The abbreviations used are: HTLV-1, human T-cell leukemia virus type 1; CRE, cyclic AMP-response element; CREB, cyclic AMP-response element-binding protein; CBP, CREB-binding protein; RNAP II, RNA polymerase II; CHO, Chinese hamster ovary; Luc, luciferase; ChIP, chromatin immunoprecipitation; PCAF, p300/CBP-associated factor; LTR, long terminal repeat.

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erase constructs in the absence or presence of Tax. This approach enabled direct correlation of histone modifications and transcription factor occupancy with or without Tax expression. We characterized histone H3 acetylation associated with the HTLV-1 promoter and within the downstream coding region. This histone tail modification is typically associated with active genes. Unexpectedly, we found that the level of modified histones was reduced at the promoter and within the luciferase coding region in the presence of Tax. We determined that this reduction was a result of a Tax-dependent decrease in nucleosome density.

To begin to investigate the mechanism of Tax-mediated nucleosome depletion, we used the activation-deficient M47 Tax mutant (30). We found that this mutant is also deficient in nucleosome depletion. However, it is fully competent for the formation of a complex with CREB and p300 on the chromosomally integrated viral promoter. Promoted by the Tax-dependent loss of nucleosomes from the coding region of the construct, we compared RNA polymerase II (RNAP II) binding at the viral promoter in cells expressing wild type or M47 Tax. Significantly, we found that M47 Tax is disabled for recruitment of RNAP II. Interestingly, we also found that the SWI/SNF chromatin remodeling complexes are displaced by wild type Tax and that M47 Tax is defective for their displacement. These data correlate Tax-mediated SWI/SNF displacement and RNAP II recruitment with nucleosome depletion. We propose that Tax, SWI/SNF, and RNAP II each plays a role in nucleosome eviction and transcriptional activation of HTLV-1 transcription.

MATERIALS AND METHODS

Cell Culture and Transient Transfection Assays—CHOK1-Luc hamster ovary cells (27) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 2 mM l-glutamine, penicillin-streptomycin, and 50 μg of G418/ml (Geneticin; Invitrogen). For chromatin immunoprecipitation (ChIP) assays, cells were electroporated as described previously (31). Briefly, 2 x 10^5 cells were electroporated with a GenePulser Xcell electroporation device from Bio-Rad in the presence of 20...
obtained for the β-globin promoter. We confirmed that β-globin DNA was represented in the mononucleosomal DNA population by Southern blot analysis of DNA from MNase-treated CHOK1-Luc cells (data not shown).

RESULTS

Tax Expression Reduces Histone Tail Acetylation Associated with the HTLV-1 Promoter and Transcribed Region—There are several lines of evidence that link Tax-mediated HTLV-1 transcriptional activation with amino-terminal tail acetylation on core histones occupying the viral promoter (18, 23, 24). However, the precise role that Tax plays in mediating these changes has not been examined. Therefore, we were interested in directly comparing histone acetylation and methylation patterns on HTLV-1 promoter nucleosomes in the absence and presence of Tax.

To perform these studies, we optimized a system in which we transfected a Tax expression plasmid into CHOK1-Luc cells that carried two to four integrated copies of the HTLV-1 LTR driving expression of the firefly luciferase gene (Fig. 1A) (27). Histone modifications at the viral promoter and within the luciferase gene were analyzed using the ChIP assay. Changes in acetylation were subsequently determined using real-time PCR with a primer set that specifically amplified a region of the HTLV-1 promoter surrounding the three viral CREs and primer sets that amplified a proximal and distal segment of the transcribed luciferase coding region (Fig. 1A). Data was quantified by comparing the signal from the co-immunoprecipitated DNA to that of the input DNA in each experiment. The CHOK1-Luc cells were initially developed to compare activation from a transiently transfected versus an integrated HTLV-1 promoter, which demonstrated the importance of using chromosomally integrated promoter constructs (27). The integrated HTLV-1 promoter is the true physiological substrate for transcription factor binding and Tax transactivation, as the provirus is packaged into nucleosomes following integration into the host cell chromosome. We have used these cells in a previous study to demonstrate mutually exclusive binding of Tax and histone deacetylase complexes at the HTLV-1 promoter (29).

Using the quantitative ChIP assay, we measured a histone modification typically associated with active genes (acetylation at H3 Lys-9/14) on nucleosomes present on the LTR-luciferase construct in CHOK1-Luc cells. Many “activating” histone modifications were previously identified on nucleosomes associated with the provirus in Tax-expressing HTLV-1 infected cells (23). CHOK1-Luc cells were transfected with a Tax expression plasmid or a control plasmid, and samples were analyzed 20 h following transfection. Fig. 1B shows the results of this study. Unexpectedly, we found that Tax expression led to a >50% decrease in the detection of H3 Lys-9/14 acetylation at the HTLV-1 promoter and within the luciferase gene.

A similar decrease was observed in a time course experiment examining H3 acetylation levels following transfection of Tax (data not shown). These results indicate that Tax recruitment to the HTLV-1 promoter directly correlates with the loss of histone acetylation at the promoter and within the luciferase gene. We also looked at other activating modifications of H3 and H4 and obtained similar results (data not shown). Fig. 1C shows that Tax expression did not produce a global reduction in the H3 acetylation level in the transfected CHOK-Luc cells.

Loss of Chromatin Modifications during Tax-mediated Activation Correlates with the Loss of Histone H3 and Linker Histone H1—The decrease in histone acetylation at the HTLV-1 promoter upon Tax transactivation was unexpected and is antithetical to widely accepted models of chromatin modifications associated with transcriptional activation. Therefore, we hypothesized that the observed decline in histone tail acetylation upon transcriptional activation by Tax was due to a loss of histones from the integrated LTR-luciferase construct. To test this hypothesis, we used the ChIP assay to analyze histone H3 binding in the absence and presence of Tax. The antibody used in these experiments recognizes the carboxyl-terminal region of histone H3, which lacks sites of post-translational modification and should provide a constitutively available epitope. Using this antibody, we showed that the level of histone H3 associated with the HTLV-1 promoter was reduced by nearly 50% during Tax transactivation (Fig. 2A). Furthermore, the level of histone H3 detected within the proximal region of the luciferase gene was reduced in the presence of Tax. We also examined the binding of linker histone H1 in parallel experiments. Similar to our observations with H3, histone H1 binding at the HTLV-1 LTR and within the luciferase gene decreased following transfection of Tax into the CHOK1-Luc cells (Fig. 2B). These data suggest that the observed Tax-dependent reduction in activating histone modifications is because of a loss of core histones and H1 and thus intact nucleosomes from the LTR-luciferase construct. Our data do not support the replacement of H3 with the transcription-associated variant histone H3.3, as the H3 antibody recognizes both of these histones. In these experiments, the data were standardized against histones H3 and H1 measured at the transcriptionally silent β-globin promoter. We found that Tax expression had no effect on the level of H3 and H1 at the β-globin promoter (Fig. 2C). This control was not feasible in our examination of histone acetylation described in Fig. 1, as activat-
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FIGURE 2. Tax expression correlates with a decrease in histone H3 and linker histone H1 associated with the HTLV-1 LTR and the luciferase gene. Quantification of relative histone levels at the integrated HTLV-1 LTR and the proximal luciferase gene (Luc). ChIP analyses were performed as described in the legend to Fig. 1. A, quantification of relative histone H3 present at the integrated HTLV-1 LTR promoter and the proximal luciferase gene. The anti-H3 antibody was directed against the carboxyl-terminal domain of H3. B, quantification of relative histone H1 present at the integrated HTLV-1 LTR promoter and the proximal luciferase gene. The graphs in A and B are each representative of three independent ChIP experiments. Values obtained for the LTR and luciferase gene were standardized to the value obtained for the β-globin promoter. Standardized values of histone levels in the presence of Tax were normalized to respective standardized values of histone levels in the absence of Tax (set to 1). C, quantification of relative histone H3 and H1 levels at the transcriptionally silent β-globin promoter.

Nucleosome Loss Is Localized to the LTR and Transcribed Luciferase Gene—To analyze the extent of the nucleosome loss, we conducted ChIP and MNase/dot blot analysis at regions within the plasmid that immediately flank the integrated LTR-luciferase construct (Fig. 4A, Regions 1 and 3). We used the ChIP assay to measure histone H3 binding at these flanking sites and did not detect a significant change in H3 association in the absence or presence of Tax (Fig. 4B). MNase treatment followed by dot blot analysis corroborated these observations (Fig. 4, C and D). We also did not detect a change in accessibility at the β-globin promoter in the absence or presence of Tax (Fig. 4C). These data indicate that the Tax-induced reduction in nucleosomes within the LTR-luciferase construct was limited to this region and did not extend upstream or downstream of these relevant sequences.

The Activation Domain of Tax Is Required for Nucleosome Depletion—To begin to characterize the mechanism of nucleosome depletion, we used the quantitative ChIP assay to compare wild type Tax with a transcriptionally defective Tax mutant. Tax M47 carries a double point mutation (L319R/L320S) in the activation domain of the protein and has been shown to be defective for transcriptional activation of HTLV-1 but not transcription via the NF-κB pathway (30). As expected, we found that transfection of CHOK1-Luc cells with the M47 Tax expression plasmid produced a negligible increase in luciferase activity when compared with wild type Tax (Fig. 5A). We were next interested in determining whether M47 Tax promotes nucleosome depletion from the LTR-luciferase construct. We used the quantitative ChIP assay to compare the levels of histone H3 and linker histone H1 at the HTLV-1 LTR promoter in the presence of wild type or M47 Tax. Fig. 5B shows that, in contrast to wild type Tax, M47 Tax produced no significant reduction in total histone H3 or H1. The level of histone H3 and H1 within the luciferase gene was similarly unaffected by M47 Tax (data not shown). Paradoxically, M47 Tax produced a reduction in H3 Lys-9/14 acetylation levels, similarly unaffected by M47 Tax (data not shown). Paradoxically, M47 Tax produced a reduction in H3 Lys-9/14 acetylation levels, however, to a lesser degree than that observed with wild type Tax (data not shown). Together, these data indicate that the M47 Tax transcriptional activation defect correlates with a defect in nucleosome depletion.

Because such widespread nucleosome depletion from a mammalian gene is unprecedented, it was important to ensure that the nucleosome reduction at the HTLV-1 promoter was not due to a Tax-mediated global reduction of histone proteins in the cell. Fig. 5C shows that neither wild type nor M47 Tax affected cellular histone H3 levels at the time point following transfection, when cells were harvested for the ChIP assay.

Nucleosome Depletion Correlates with Displacement of SWI/SNF and Recruitment of RNA Polymerase II—Interestingly, quantitative ChIP analyses showed that the binding of M47 Tax to the HTLV-1 LTR is comparable with that observed with wild type Tax (Fig. 6A). Furthermore, CREB and p300 binding were also enhanced in the presence of wild type and M47 Tax (Fig. 6A). These data show that M47 Tax is not defective for complex formation with CREB and p300 on the HTLV-1 promoter in a chromatin environment. The fact that wild type and M47 Tax similarly and strongly recruit p300 to the HTLV-1 promoter indicates that the transcriptional and nucleosomal mobilization defect in M47 Tax occurs in post-CREB and post-coactivator recruitment, consistent with in vitro binding data (26). We therefore conclude that nucleosome depletion over the LTR-luciferase gene region is directly coupled with the binding of transcriptionally competent Tax at the HTLV-1 promoter.

Because a subset of chromatin remodeling complexes has been shown to unfold nucleosomes, we were interested in determining if it...
was involved in nucleosome depletion from the HTLV-1 promoter. Brg1 and Brm are the catalytic subunits of the ATP-dependent chromatin remodeling complexes SWI/SNF. Brg1 has been shown to interact with Tax and function in HTLV-1 transcriptional activation (36). Therefore, we used the ChIP assay to analyze SWI/SNF (Brg1 and Brm) interactions with the LTR-luciferase construct in the absence or presence of Tax (Fig. 6B). In the absence of Tax, we were able to detect both Brg1 and Brm at the promoter, but unexpectedly we found that Tax
expression resulted in the displacement of both chromatin remodeling proteins from the LTR. In contrast, expression of M47 Tax increased Brg1 and Brm occupancy at the viral promoter (Fig. 6B). These results suggest that the function of Brg1 and Brm in HTLV-1 transcription is related to maintaining the HTLV-1 proviral DNA assembled into chromatin in the absence of Tax. It is possible that SWI/SNF participate in nucleosomal depletion upon Tax-mediated transcriptional activation and are displaced in concert with the nucleosomes.

Finally, we were interested in determining whether RNAP II plays a role in nucleosome depletion, because this process is associated with strong transcriptional activation by Tax and occurs throughout the LTR-luciferase region. An antibody directed against a non-carboxyl-terminal domain portion of the largest subunit of the polymerase was used in the quantitative ChIP assay to measure binding. We found that RNAP II binding increased 4-fold at the HTLV-1 promoter upon wild type Tax expression, whereas M47 Tax did not affect RNAP II enrichment at the promoter (Fig. 7A). These data indicate that the complex formed with M47 Tax, CREB, p300, and possibly SWI/SNF at the HTLV-1 promoter is not sufficient for RNAP II recruitment. Furthermore, RNAP II binding at the viral promoter correlates with nucleosome depletion. As a control, we measured the effect of Tax expression on RNAP II occupancy at the β-globin promoter. As expected, Tax had no effect on RNAP II recruitment to the transcriptionally inactive gene (Fig. 7B).

DISCUSSION

In this study we have used the ChIP assay to characterize changes in histone tail modifications on nucleosomes at chromosomally integrated copies of the HTLV-1 promoter during Tax-mediated transcriptional activation. Based on many studies showing an increase in histone acetylation on other promoters during transcriptional activation, we expected to observe an increase in activating histone modifications at the HTLV-1 promoter upon Tax expression. Thus, we were surprised to find that Tax expression correlated with a decline in the levels of histone H3 acetylation at the HTLV-1 promoter and within the downstream luciferase gene. We found that the basis for this Tax-mediated reduction was a general decrease in histone density within these regions. In addition, we have found that less LTR-luciferase DNA is nucleosomal when Tax is expressed in the cells. Importantly, we have also found that linker histone H1 is associated with the LTR-luciferase construct in the absence of Tax and is displaced from the DNA upon transcriptional activation by Tax. Further, this displacement is localized to the LTR-luciferase region and does not extend upstream or downstream of these sequences. Together, these data indicate that Tax mediates a reduction in nucleosome occupancy at the HTLV-1 promoter and coding region.

Interestingly, previous studies demonstrated the presence of acetylated histones at the HTLV-1 promoter in the presence of Tax (18, 23, 24). Both Georges et al. (18) and Lu et al. (24) observed an increase in p300-dependent histone acetylation at the HTLV-1 promoter in vitro using histone acetyltransferase assays. However, these studies were carried out using purified, recombinant components; therefore RNAP II, and possibly other regulatory factors that participate in chromatin remodeling and nucleosome displacement, were not present in the assays. In the work done by Lemasson et al. (23), histone modifications have been examined at the LTR and coding region in the presence of constitutively expressed Tax in HTLV-1 infected cells. Thus, histone acetylation levels in the absence and presence of Tax have not been compared. In the studies presented herein, we found that, relative to the β-globin promoter, activating histone modifications were readily detectable at the LTR and downstream coding region following Tax expression. These data indicate that the nucleosomes that remain following partial nucleosome displacement contain acetylated histones. Interestingly, our data also show that the nucleosomes that reside in the LTR-luciferase region contain acetylated histones prior to Tax transcriptional activation.

Nucleosome depletion has been shown to occur on a number of genes in yeast and on two genes in Drosophila (37–41). However, it has only been conclusively demonstrated to occur in mammalian cells at the
functions in nucleosome depletion from the LTR-somes from these genes (41, 48, 49). We propose that RNAP II also histone H3 associated with the LTR and moter binding by M47 Tax does not lead to a reduction in the level of CREB, p300, and the viral CRE (8, 26). In contrast, we found that pro-CREB and p300 to the HTLV-1 LTR in living cells. Our results extend wild type Tax. Furthermore, wild type and M47 Tax similarly recruit somally integrated HTLV-1 promoter is comparable with the binding of (30). We demonstrated that the binding of M47 Tax to the chromo-some integration from the LTR and transcribed region, we performed ChIP assays for nucleosome eviction and reformation (38). This mechanism may account for the partial nucleosome depletion over the LTR-luciferase construct. Alternatively, incomplete nucleosome loss from the LTR-luciferase construct may reflect the fact that we have examined an asynchronous population of Tax-expressing cells, and nucleosome eviction from the integrated construct may occur at a specific stage of the cell cycle that is permissive to Tax-mediated transcriptional activation. Alternatively, or in addition, some of the integrated LTR-Luc copies may be intractable to full activation by Tax.

The factors involved in transcription-coupled nucleosome eviction have begun to be elucidated in yeast. Genome-wide analyses have revealed that nucleosomes are underrepresented in DNA regions with multiple conserved transcription factor binding sites (37, 39). Together, these results support a general model in which transcription factor binding can preclude nucleosome assembly at promoter regions. However, some promoters may require additional or alternative processes for nucleosome removal, as has been shown for the PHOS and PHO8 promoters, which require the histone chaperone Asf1p for nucleosome eviction (47). The level of activation from certain yeast promoters has been correlated with the extent of histone loss from these promoters. For the GAL genes and the HSP82 gene in yeast and for HSP70 gene in Drosophila, RNAP II has been implicated in the reduction of nucleosomes from these genes (41, 48, 49). We propose that RNAP II also functions in nucleosome depletion from the LTR-luciferase construct, because similar to the above genes, nucleosomes are also removed from the coding region.

To investigate the mechanism of Tax-mediated nucleosome deple-tion from the LTR and transcribed region, we performed ChIP assays with cells expressing the transcriptionally impaired M47 Tax mutant (30). We demonstrated that the binding of M47 Tax to the chromo-somally integrated HTLV-1 promoter is comparable with the binding of wild type Tax. Furthermore, wild type and M47 Tax similarly recruit CREB and p300 to the HTLV-1 LTR in living cells. Our results extend previous in vitro studies showing that M47 Tax forms a complex with CREB, p300, and the viral CRE (8, 26). In contrast, we found that promoter binding by M47 Tax does not lead to a reduction in the level of histone H3 associated with the LTR and luciferase gene. These results indicate that M47 Tax does not mediate activator-dependent loss of nucleosomes from the promoter. We found that the deficiency of M47

Tax in nucleosome depletion is correlated with the inability of this mutant to recruit RNAP II. These results suggest that RNAP II is involved, at least in part, in reducing the nucleosome density over the LTR-luciferase construct. A model illustrating the results of our studies is shown in Fig. 8.

Because p300 is recruited to the HTLV-1 promoter by both wild type and M47 Tax, the mechanism of nucleosome depletion is unlikely to directly involve p300-mediated histone tail acetylation. Further, previous data on Tax transactivation from chromatin templates in vitro (28) reveal that histone tail acetylation is not the principal function of p300 in HTLV-1 transcription. Although a previous study has shown that M47 Tax is defective for binding to p300/CBP-associated factor (PCAF) (50), we were unable to analyze PCAF binding in this study. PCAF, which carries histone acetyltransferase activity, interacts with Tax and augments transcription from a transiently transfected HTLV-1 promoter (27, 51). However, we have been unable to detect PCAF binding at the HTLV-1 promoter using the ChIP assay with various antibodies (23). Therefore, it remains unclear as to whether PCAF plays a role in nucleo-some depletion from the HTLV-1 LTR and coding region.

The inability of transcriptionally defective M47 Tax to recruit RNAP II points to a role for the elongating enzyme in nucleosome reduction. This idea is supported by the large increase in RNAP II on the LTR promoter upon activation by Tax. However, we cannot rule out the involvement of other factors in this process, because nucleosomes are depleted upstream of the transcription start site as well as within the coding region. Two models could explain how polymerase displaces nucleosomes, each based on the ability of the elongating enzyme complex to traverse and reform nucleosomes in its wake. In the first model, the considerable transcriptional activation by Tax could be linked to a high density of elongating polymerase complexes on the DNA-inhibiting nucleosome reformation. This model has been proposed to account for the loss of nucleosomes from the coding regions of the yeast GAL genes (48). In the second model, Tax may be disrupting one or more of the components in the polymerase complex that functions in nucleo-some reformation. Both Spt6 and FACT (facilitates chromatin tran-
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complex have been shown to be involved in this process (49, 52) and may be directly or indirectly targeted by Tax. We are currently investigating which of these models best describes nucleosome depletion during Tax transactivation.

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