Cell, Vol. 106, 685–696, September 21, 2001, Copyright ©2001 by Cell Press

Nucleosome Sliding via TBP DNA Binding In Vivo

Stavros Lomvardas and Dimitris Thanos[1]
Department of Biochemistry
and Molecular Biophysics
Columbia University
630 West 168th Street
New York, New York 10032

Summary

Here, we show that a nucleosome obstructing transcription from the IFN-β promoter slides in vivo in response to virus infection, thus exposing the previously masked TATA box and the initiation site, a requirement for transcriptional activation. Our experiments also revealed that this mode of chromatin remodeling is a two-step reaction. First, the enhanceosome recruits the SWI/SNF chromatin-remodeling complex that modifies the nucleosome to allow binding of TBP. Second, DNA bending is induced by TBP binding, and the nucleosome slides to a new position. Experiments with other DNA binding proteins demonstrated a strong correlation between the ability to bend DNA and nucleosome sliding, suggesting that the sliding is induced by the bend.

Introduction

Eukaryotic genes are contained within a higher order complex of DNA and histones called chromatin. Although packaging of DNA into chromatin provides the means for compaction of the entire genome to fit in the nucleus, it restricts the access of the many regulatory proteins required for essential biological processes such as DNA replication, transcription, and recombination (Kornberg and Lorch, 1999). The chromatin, however, is not a static structure, but rather a dynamic assembly that condenses and decondenses (remodeling) in response to specific signals during cell life (Wolffe, 1995). Chromatin remodeling requires a specific set of enzymes that modify the nucleosome, the building block of chromatin (Kornberg, 1974). These enzymes fall into two classes: the first includes ATP-dependent chromatin remodeling activities that use energy derived from ATP hydrolysis to alter nucleosomal structure and/or arrangement, whereas the second class includes enzymes that add acetyl groups to the histone N termini (Kingston and Narlikar, 1999; Strahl and Allis, 2000; Vignali et al., 2000; Fry and Peterson, 2001). Therefore, the question of how cells maintain and control expression of their genes necessitates a clear understanding of how the chromatin is altered in order to allow access of the transcriptional machinery to promoters. It appears that one of the roles of transcription factors is recruiting these nucleosome modifying activities, which function in concert to alter the chromatin structure of promoters in a way that exposes previously masked critical regulatory DNA sequences (Lemon and Tjian, 2000). Recent genetic and biochemical experiments suggest that, in general, histone acetylation and ATP-dependent chromatin remodeling are coupled (Fry and Peterson, 2001). Thus, in the case of mitotically expressed yeast genes, histone acetylation requires prior ATP-dependent chromatin remodeling (Cosma et al., 1999; Krebs et al., 1999, 2000), whereas in the case of non-cell cycle regulated genes in yeast or mammals, histone acetylation precedes chromatin remodeling, and it is required for an efficient recruitment of the ATP-dependent chromatin remodelers (Agalioti et al., 2000; Dilworth et al., 2000; Syntichaki et al., 2000; Hassan et al., 2001, Reinke et al., 2001).

The SWI/SNF chromatin remodeling activity, together with the related RSC, NURD, NURF, Mi-2, and CHRAC complexes, utilizes ATP to alter nucleosomal structure. (Aalfs and Kingston, 2000; Peterson, 2000; Vignali et al., 2000). Our current view postulates that this is accomplished either by nucleosome sliding along the DNA (Langst et al., 1999; Whitehouse et al., 1999; Hamiche et al., 1999; Jaskelliof et al., 2000; Lorch et al., 2001), or by inducing a continuous ATP-dependent DNA twist that provides the force for creating accessible DNA sites, even in the absence of histone movement (Havas et al., 2001; Gavin et al., 2001). It is possible, however, that both mechanisms are linked in such a way that changes of the DNA structure might facilitate subsequent nucleosome sliding. Importantly, the nature of chromatin alterations during the course of transcriptional switches is not well understood. Although previous studies have indicated that nucleosomes obstructing the access of transcription factors or the basal transcriptional machinery are generally modified, the nature of these modifications still remains largely unknown (Almer et al., 1986; Verdin et al., 1993; Verdone et al., 1996; Lohr, 1997; Rubbi et al., 1997; Sewack and Hansen, 1997; Li et al., 1998; Weinmann et al., 1999; Reeves et al., 2000; Shen et al., 2001; Weinmann et al., 2001). We do not know how these nucleosomes are modified in vivo and why these modified nucleosomes allow transcription to occur. On the other hand, transcriptional repression has been linked with ISWI2-induced nucleosome sliding which masks the binding site for an activator (Goldmark et al., 2000), or with RSC-dependent nucleosome positioning at the TATA box (Moreira and Holmberg, 1999). Here, we describe the mechanisms and the nature of chromatin remodeling during activation of the IFN-β gene upon virus infection.

The IFN-β enhancer is recognized by three distinct sets of coordinately activated transcription factors (NF-κB, IRFs and ATF-2/c-Jun), which, with the help of the architectural factor HMG I(Y), bind cooperatively to the enhancer to form an enhanceosome (Maniatis et al., 1998; Munshi et al., 1999; Merika and Thanos, 2001). HMG I(Y) organizes the enhanceosome into a structure that optimally interacts with chromatin-modifying activities and general transcription factors. The accurate execution of the IFN-β transcriptional switch depends on the ordered recruitment of GCN5 and CBP that acetylate

[1]Correspondence: dt73@columbia.edu

HMG I(Y) at distinct lysine residues, inducing opposite effects on enhanceosome stability (Munshi et al., 1998, 2001). The enhanceosome assembles at the nucleosome-free promoter region of the IFN-β gene and activates transcription by targeting the two precisely positioned nucleosomes that flank the enhancer (Agalioti et al., 2000). One of these nucleosomes lies adjacent to the TATA box and masks the start site of transcription, whereas the other lies immediately upstream of the enhancer. First, the GCN5/PCAF complex is recruited, which acetylates the nucleosomes, and this is followed by recruitment of the CBP-PolII holoenzyme complex. Nucleosome acetylation, in turn, facilitates SWI/SNF recruitment by CBP, resulting in chromatin remodeling. This recruitment program culminates with the binding of TFIID to the promoter and initiation of transcription (Agalioti et al., 2000). The nature of the remodeled chromatin and the mechanism by which SWI/SNF generates a transcriptionally permissive environment have remained unknown. We demonstrate here that virus infection induces sliding of the nucleosome that is adjacent to the TATA box to a new position, thus fully exposing the TATA box and the start site of transcription. Our experiments revealed that this mode of chromatin remodeling is a two-step reaction. First, the nucleosome is modified by SWI/SNF. Second, DNA bending is induced by TBP binding, and the nucleosome slides to a new position. Experiments with other DNA binding proteins demonstrated a strong correlation between the ability to bend DNA and nucleosome sliding, suggesting that the sliding is induced by the bend.

Results

Virus Infection Induces Nucleosome Sliding at the IFN-β Promoter In Vivo

We had previously shown that the nucleosome masking the IFN-β core promoter is remodeled during transcriptional activation, as judged by restriction site accessibility experiments of isolated nuclei (Agalioti et al., 2000). However, these experiments did not address the question of whether remodeling in this case is coupled with changes in the histone-DNA contacts or nucleosome mobilization on the DNA. To address this question, we mapped the nucleosome borders in HeLa cells that were either mock or virus infected for 8 hr. The histone-DNA contacts were fixed by formaldehyde crosslinking, followed by micrococcal nuclease treatment of isolated nuclei. DNA extracted from the resulting mononucleosomes (Figure 1A) was annealed with radiolabeled primers α, β, and γ, spanning nucleosomes I and II (Figure 1E; Agalioti et al., 2000), followed by primer extension. Figure 1B shows that in uninfected cells, primers α and β produce a 73 bp fragment (Figure 1B, lanes 1 and 2) that marks the borders of nucleosome II, whereas primer γ produces a 70 bp (Figure 1B, lane 5) fragment that defines the position of nucleosome I (Agalioti et al., 2000). Remarkably, when primer α was annealed with DNA extracted from mononucleosomes prepared from virus-infected cells, an additional extended product of 37 bp was revealed (lane 3), whereas primer β revealed an additional product of 109 bp (lane 4). These results indicate that in a fraction of promoters, nucleosome II slides 36 bp downstream in response to virus infection. Furthermore, quantitation of the signal in the bands corresponding to the mobilized nucleosome revealed that only 15% of the total nucleosome II population corresponds to the new position. This number is in agreement with the fact that only 10%–20% of the cells are induced to transcribe the endogenous IFN-β gene in response to virus infection (Senger et al., 2000). In contrast to nucleosome II sliding, nucleosome I did not change its position upon virus infection (Figure 1B, compare lanes 5 and 6). Taken together, these experiments demonstrate that virus infection induces sliding of nucleosome II, but not of nucleosome I. The former attains a new stable position 36 bp downstream of its original place, thus fully exposing the TATA box and the start site of transcription.

To investigate whether nucleosome II slides in vitro, we performed a variation of the nucleosome mapping experiment described above. A DNA fragment (−143/+183) bearing the IFN-β promoter was reconstituted into nucleosome core particles followed by enhanceosome assembly. Next, the templates were incubated with HeLa nuclear extracts (to recruit chromatin remodeling activities; Agalioti et al., 2000) in the presence of ATP, and after washing, the template was digested with micrococcal nuclease followed by DNA extraction and primer extension. Figure 1C (lanes 1 and 2) shows the expected size (∼145 bp) of the purified and in vitro generated mononucleosomes after micrococcal nuclease digestion. DNA extracted from similarly generated mononucleosomes was annealed with radioactive primer α, followed by primer extension and PAGE. Figure 1D shows that in the absence of the enhanceosome, primer α produces a fragment of 73 bp, consistent with the in vivo mapping experiments (Figure 1B). However, in the presence of the enhanceosome, the same primer produces a 36 and a 38 bp fragment, indicating that the nucleosome slid ∼36 nucleotides downstream from its original position. Thus, both in vivo and in vitro, the chromatin remodeling activities recruited by the enhanceosome induce sliding of nucleosome II to almost the exact same position.

TFIID DNA Binding and SWI/SNF Recruitment Are Required for Nucleosome Sliding

To investigate in detail the biochemical mechanism of enhanceosome-induced nucleosome sliding, we used restriction site accessibility assays to monitor the state of the remodeled nucleosome. In these experiments, a series of radiolabeled and biotinylated IFN-β promoter fragments bearing nucleosome II with or without the enhanceosome were incubated with HNEs in the presence of ATP, followed by washing and incubation with a restriction enzyme. The beads were concentrated and the released fragments were detected by PAGE and autoradiography (Agalioti et al., 2000). All DNA fragments used contain a naturally occurring NcoI site at −10, which in uninduced cells is inaccessible due to the presence of nucleosome II, but becomes fully accessible in response to virus infection (Agalioti et al., 2000). We introduced two additional NcoI sites, the first within nucleosome II at +128 (5 bp from the 3′ edge of the nucleosome, thus simulating the −10 NcoI site on the other side of the nucleosome), and the second 15 bp

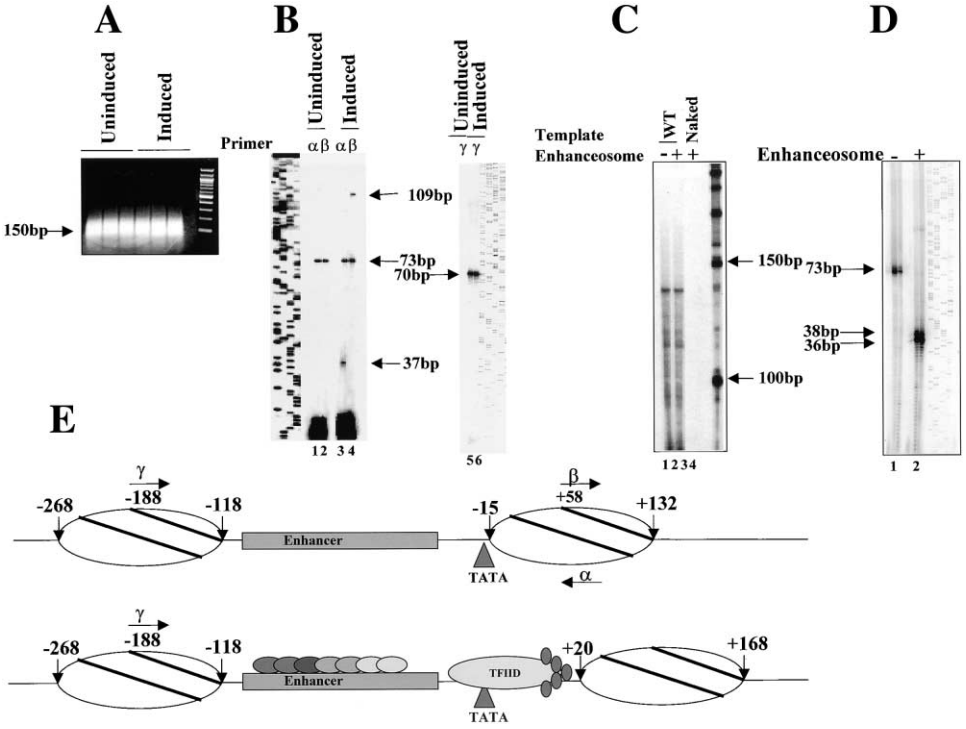

Figure 1. Nucleosome Sliding at the IFN-β Promoter In Vivo and In Vitro

(A) Shown is an ethidium bromide-stained agarose gel containing DNA isolated from mononucleosomes prepared from mock and virus infected HeLa cells.

(B) The DNA from (A) was purified and annealed with radioactive primers (α, β, and γ), followed by primer extension. Shown is a sequencing gel containing the extended products run side by side with sequencing reactions serving as size markers.

(C) A homogeneously labeled IFN-β promoter fragment (−143 to +183) with or without the enhanceosome was reconstituted into a nucleosome and incubated with HNEs in the presence of ATP. The templates were digested with micrococcal nuclease and the resistant DNA fragments were detected after PAGE and autoradiography.

(D) Same as in (C) except that the DNA used was not labeled, and after micrococcal nuclease treatment, the resistant DNA fragments were annealed with radioactive primer α followed by primer extension and PAGE.

(E) Shown is a diagrammatic illustration of the nucleosome structure at the IFN-β promoter before and after virus infection.

away from the nucleosome at +147 (Figure 2, templates A and B, respectively). Templates A and B were radiolabeled at the 5′ end, biotinylated at the 3′ end, attached to paramagnetic beads, and incubated with the HNE. Figure 2A (lane 1) shows that in the absence of the enhanceosome, both NcoI sites in template A are weakly accessible, thus verifying again the position of nucleosome II. However, when the templates bore the enhanceosome, accessibility at the −10 site was increased dramatically, whereas the +128 site remained inaccessible (lane 2), after incubation with HNEs in the presence of ATP. Importantly, the inability to detect cleavage at the +128 site is not due to complete cleavage at the −10 site, since by switching the biotin and radioactivity labels on template A we still obtained the same result (data not shown). When the above experiment was repeated using template B, we found that, as expected, the −10 and +147 sites were inaccessible and accessible, respectively (lane 3). However, the accessibility of these sites was switched on templates bearing the enhanceosome (lane 4). The same conclusion was derived when the radioactive and biotin labels were switched (template C, lanes 15 and 16). That masking of the NcoI site at +147 is indeed due to true nucleosome sliding and not to detachment and reassociation of the histone core to the new position was verified by repeating the sliding experiment in the presence of a vast excess of competitor DNA (Figure 2A, lanes 19–22). These experiments suggest that nucleosome II slides downstream to a new position, thus exposing the −10 site and masking the +147 site.

Next, we used HNEs in which TFIID has been immunodepleted and/or templates that bear mutations at the TATA box (TGTA). Figure 2A (compare lane 5 with 6) shows that both NcoI sites in template A (TGTA) become accessible, whereas in template B (TGTA), the −10 site becomes accessible without the simultaneous masking of the +147 site which remains accessible to the restriction enzyme (compare lanes 7 and 8). A similar result was obtained when wild-type templates B and C were incubated with HNEs lacking TFIID (lanes 13, 14 and 17, 18, respectively). The fact that the −10 NcoI site becomes accessible without simultaneous masking of the +147 site (templates B and C) indicates that the recruited SWI/SNF complex (Agalioti et al., 2000) can alter the DNA path around the histone core in a way that permits restriction enzyme accessibility. However, this modification does not suffice for nucleosome sliding. These experiments imply that chromatin remodeling at the IFN-β promoter is a two-step pathway: first, SWI/

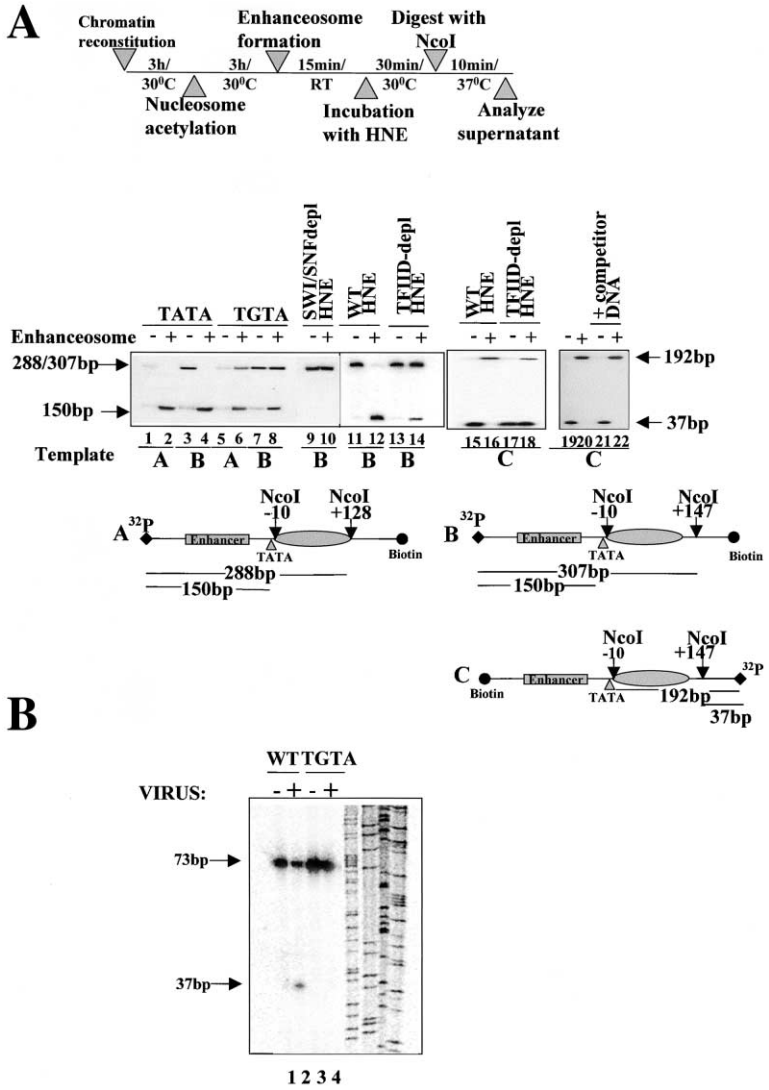

Figure 2. TFIID Binding at the TATA Box Is Required for Nucleosome Sliding

(A) The DNA templates shown at the bottom of the figure were reconstituted to nucleosomes, acetylated by GCN5, and incubated with complete or TFIID-depleted HeLa nuclear extracts, followed by NcoI digestion according to the scheme shown at the top. In lanes 21 and 22, competitor salmon sperm DNA (50 μg/ml) was added during the remodeling reaction. The released radioactive DNA fragments were analyzed by PAGE and detected by autoradiography.

(B) HeLa cells were transfected with the −110 IFN-β CAT (lanes 1 and 2) or with the −110 IFN-β CAT (TGTA) reporter plasmids, followed by mock or virus infection for 8 hr. Mononucleosomes were prepared as in Figure 1, and the DNA was annealed with a radioactive CAT primer followed by primer extension, PAGE, and autoradiography.

SNF-dependent modification of the DNA-histone contacts, and second, nucleosome sliding induced upon TFIID DNA binding.

To investigate whether TFIID DNA binding is also required for nucleosome sliding in vivo, we carried out nucleosome mapping experiments in HeLa cells that were transiently transfected with either the wild-type IFN-β promoter or with the TGTA promoter linked to the CAT gene. Figure 2B shows that the nucleosomal organization of the transiently transfected promoters is similar to that of the endogenous gene. Thus, primer extension, using as a template mononucleosomal DNA prepared from uninduced cells and a CAT primer, produced an extended fragment of 73 bp, which marks the 5′ border of nucleosome II at −15; that is, at the same position with the endogenous gene (Agalioti et al., 2000). In agreement with our findings for the endogenous gene (Figure 1), the same primer produced an additional product of 37 bp (lane 2), thus indicating a 36 bp nucleosome sliding (lane 2). However, nucleosome sliding was not detected when the TGTA template was used (lane 4). Thus, both in vivo and in vitro, TFIID binding to the TATA box is required for nucleosome sliding.

TBP-Induced DNA Bending Is Required for Nucleosome Sliding

The requirement for TFIID DNA binding could be due either to TBP and/or to TAFs. To distinguish between these possibilities, we carried out nucleosome sliding assays on template C (Figure 2A) using TFIID-depleted extracts that were supplemented either with recombinant TBP or with epitope-tagged purified TFIID. Figure 3A (lanes 1 and 2) shows that the enhanceosome induces nucleosome sliding, as judged from the masking of the NcoI site at +147. Nucleosome sliding was abolished either by using the TGTA template or using extracts lacking TFIID (lanes 3–6), consistent with the results of Figure 2. However, addition of increasing amounts of equal DNA binding units of either TBP or TFIID fully restored nucleosome sliding in a dose-dependent manner (lanes 7–22). Thus, TBP DNA binding suffices to induce nucleosome sliding.

TBP could function either by "pushing" the SWI/SNF-altered nucleosome away via steric interference or by facilitating nucleosome sliding through its ability to bend DNA (Kim et al., 1993a, 1993b). To test these possibilities, we generated three additional templates. In the first

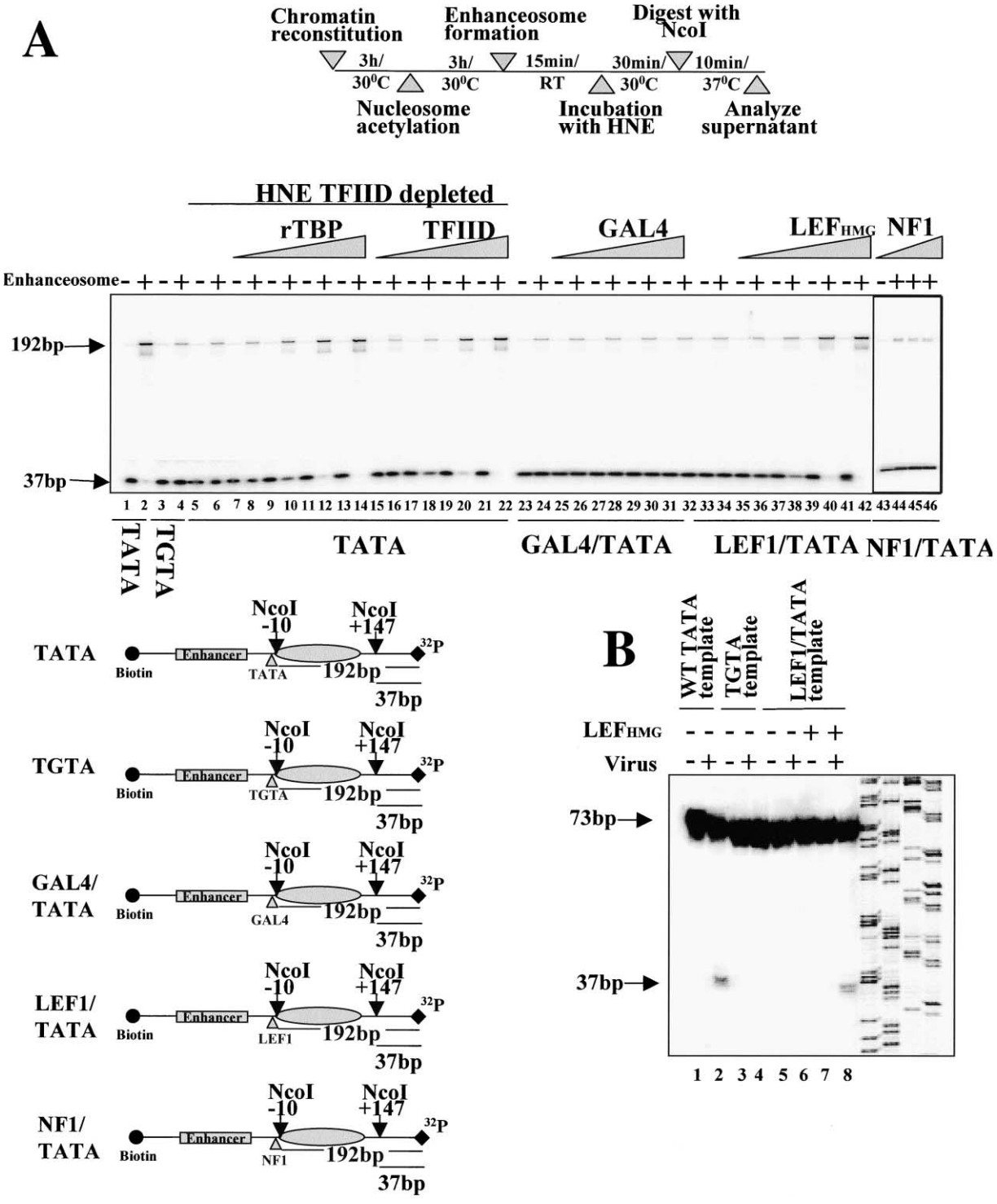

Figure 3. TBP-Induced DNA Bending Is Required for Nucleosome Sliding

(A) The templates shown at the bottom of the figure were used in nucleosome reconstitution experiments followed by acetylation, enhanceosome assembly, and incubation with complete or TFIID-depleted HNEs as indicated. After washing, the templates were reacted with NcoI, concentrated, and the radioactive supernatant was analyzed by PAGE. Increasing amounts of equivalent DNA binding units of TBP, TFIID, GAL4 (1–147), LEF_{HMG}, or NF1 were added as indicated during incubation with the HNEs.

(B) HeLa cells were transfected with IFN-β CAT (WT) (lanes 1 and 2), IFN-β CAT (TGTA) (lanes 3 and 4), or IFN-β CAT (LEF/TATA) reporters, along with an expression vector encoding LEF_{HMG} (lanes 5–8). The position of the nucleosome was determined as in Figure 2B.

template, we substituted the TATA box with a GAL4 binding site; in the second template, with a binding site for the lymphoid enhancer factor LEF-1; and in the third template, with an NF1 binding site taken from the MMTV promoter. GAL4 binds DNA from the major groove without inducing DNA bending (Marmorstein et al., 1992), whereas LEF-1 binds its site from the minor groove, inducing a dramatic DNA bend similar to the bend induced by TBP (Giese et al., 1992; Love et al., 1995). NF1 cannot bind nucleosomal DNA unless the nucleosome is remodeled, and it is not thought to induce DNA bending (Archer et al., 1991; Kim and Shapiro, 1996). Figure 3A (lanes 23–32) shows that GAL4 binding did not induce nucleosome sliding. However, addition of LEF-1 to the extracts induced nucleosome sliding in a dose-dependent manner and as efficiently as TBP did. Finally, the observation that NF1 did not induce nucleosome sliding (lanes 43–46) excluded the possibility that the difference in promoting nucleosome sliding between the DNA-bending proteins TBP and LEF-1 and the non-DNA-bending proteins GAL4 and NF1 is due to the inability of the former to occupy their binding sites when present in nucleosomal DNA. Thus, nucleosome sliding and DNA binding to nucleosomal templates are not mutually exclusive. Therefore, we conclude that the DNA bending induced by TBP at the TATA box is responsible for sliding of the nucleosome. To test whether a similar pathway operates in vivo, we performed nucleosome-mapping experiments in HeLa cells transiently transfected with the LEF-1 site-containing IFN-β reporter with or without the LEF-1 expression vector. Figure 3B illustrates that nucleosome II on the wild-type template slid upon virus infection (lanes 1 and 2). However, no nucleosome sliding was detected on the template bearing the LEF-1 site or the TGTA template (lanes 3–6). By contrast, coexpression of LEF-1 restored nucleosome sliding in a virus-dependent manner (lanes 7 and 8). Taken together, these experiments suggest that, both in vivo and in vitro, a DNA bend induced at the edge of the nucleosome is required for sliding.

Reconstitution of Nucleosome Sliding Using Purified Components

Next, we sought to reconstitute nucleosome remodeling at the IFN-β promoter using purified components and template C (Figure 2A). Figure 4A (lanes 1 and 2) shows that assembly of the enhanceosome per se did not cause sliding of the nucleosome. Although incubation of the template with highly purified flag-tagged SWI/SNF complex (Schnitzler et al., 1998) induced accessibility at the −10 NcoI site, it did not cause nucleosome sliding (lanes 3 and 4). However, addition of TBP together with SWI/SNF induced nucleosome sliding in an enhanceosome-dependent manner (lanes 5 and 6). In addition, nucleosome sliding, but not hypersensitivity at the −10 NcoI site, requires prior nucleosome acetylation (lanes 7 and 8), thus further expanding the role of histone acetylation in chromatin remodeling. Addition of apyrase after incubation of the nucleosome with SWI/SNF and before addition of TBP did not affect sliding (lanes 9 and 10), indicating that the catalytic activity of SWI/SNF is not required for nucleosome sliding after modification of this nucleosome by SWI/SNF. Remarkably, HMG I(Y) on its own, that is, without the activators, suffices together with TBP and SWI/SNF to induce nucleosome sliding (lanes 11–14). This HMG I(Y)-mediated effect requires its intact binding sites on the enhancer, since it is abolished when a template bearing mutations in all four binding sites was used (lane 15).

Since nucleosome sliding at the IFN-β promoter can be reconstituted in vitro using purified SWI/SNF and TBP, we investigated the stability of the SWI/SNF-modified nucleosome before and after sliding. The nucleosomal template C bearing the enhanceosome was incubated either with SWI/SNF plus TBP or with SWI/SNF alone, followed by washing and treatment with apyrase to inhibit SWI/SNF's catalytic activity for different amounts of time. Next, the templates were reacted with NcoI, concentrated, and the radioactive supernatant was analyzed by PAGE. Figure 4B (lane 1) shows that addition of apyrase 1 min before SWI/SNF and TBP blocked nucleosome sliding. By contrast, addition of apyrase after SWI/SNF and TBP binding did not affect nucleosome sliding (lanes 2–8). When TBP was omitted from the reaction, SWI/SNF induced the −10 NcoI accessibility (compare lanes 1 and 9) in the absence of nucleosome sliding, but the SWI/SNF-modified nucleosome reverts to its original configuration rapidly after inhibiting SWI/SNF's catalytic activity by adding apyrase (lanes 9–15). These experiments suggest that when nucleosome sliding is inhibited, there is a continuous requirement for SWI/SNF to preserve the remodeled nucleosome state.

To investigate the stability of the SWI/SNF-remodeled nucleosome in the absence of sliding in vivo, we transfected HeLa cells with the −110 IFN-β CAT or −110 IFN-β CAT (TGTA) reporters and measured −10 NcoI accessibility throughout the time course of virus infection. Figure 4C shows that the pattern of NcoI accessibility of the transiently transfected IFN-β promoter template mimics that of the endogenous gene (lanes 1–11, Agalioti et al., 2000). That is, the NcoI site remains accessible throughout the time course of virus infection, consistent with the observation that the nucleosome slides in vivo. Remarkably, NcoI accessibility appeared only transiently on the TGTA templates (lanes 12–22), thus perfectly correlating with recruitment of SWI/SNF to the promoter (Agalioti et al., 2000). Since SWI/SNF arrives at the promoter at 6 hr postinfection and has departed by 12 hr (Agalioti et al., 2000), we conclude that the continuous presence of SWI/SNF is required for maintaining the NcoI site accessible in the absence of nucleosome sliding on the TGTA template, consistent with the in vitro data of Figure 4B. When SWI/SNF leaves, this nucleosome reverts to its original configuration. By contrast, in the case of the wild-type template, the NcoI site continues to be accessible because the nucleosome slides.

In summary, the experiments described above showed that (1) SWI/SNF alters the histone-DNA contacts, thus allowing restriction enzyme accessibility; (2) this enzymatic modification does not require histone acetylation, although recruitment of SWI/SNF is enhanced by histone acetylation; (3) TBP binding induces sliding only on nucleosomes that are acetylated and modified by SWI/SNF; (4) changes in the enhancer DNA conformation induced by HMG I(Y) binding are critical for nucleosome

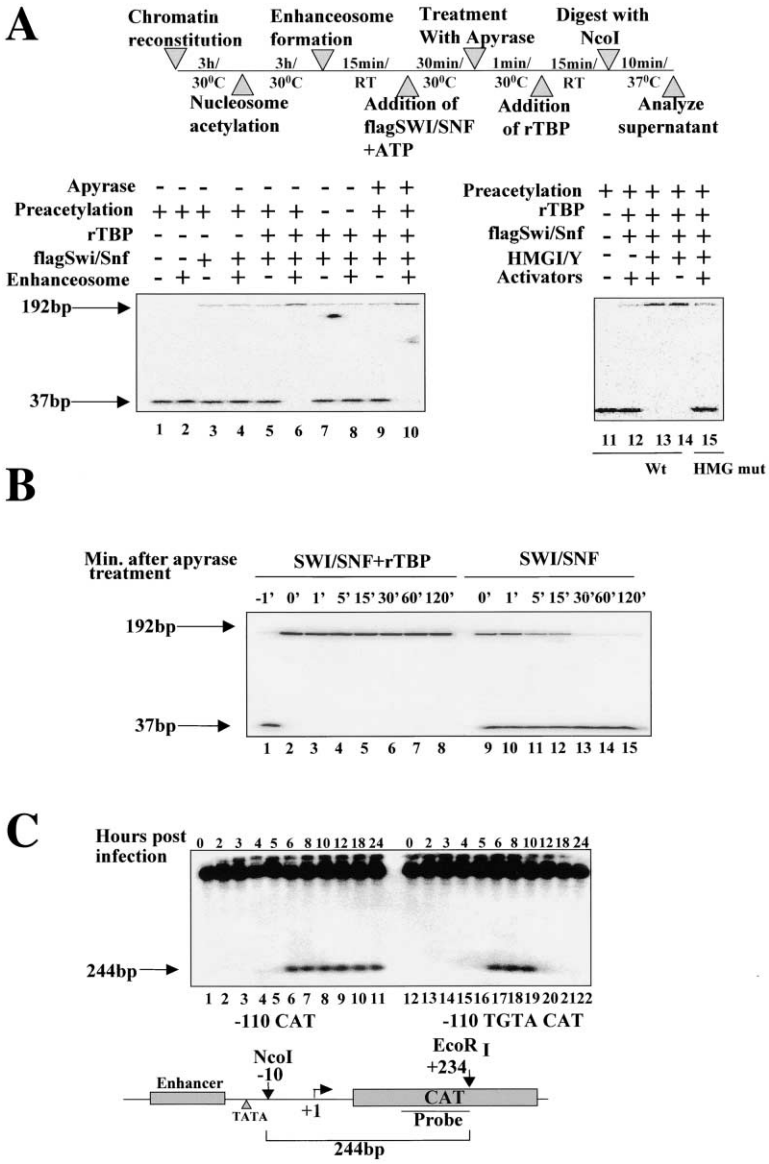

Figure 4. Reconstitution of Nucleosome Sliding In Vitro using Purified Components

(A) The WT template of Figure 3A was used in nucleosome reconstitution experiments. Next, the templates were treated according to the scheme shown at the top of the figure. After digestion with NcoI, the beads were concentrated, and the radioactive supernatant was analyzed by PAGE and detected by autoradiography.

(B) Same as in (A) except that apyrase was added as indicated for increasing amounts of time before NcoI digestion.

(C) HeLa cells were transfected as in Figure 2B and nuclei were prepared and digested with NcoI. The DNA was isolated and cleaved with EcoRI before agarose gel electrophoresis and Southern blotting. The lower part of the figure, which is not drawn to scale, shows the restriction map of the template and the probe used.

sliding; (5) the catalytic activity of SWI/SNF is not required for sliding of an already modified nucleosome; and (6) when nucleosome sliding is blocked, there is a continuous requirement for SWI/SNF's catalytic activity to maintain the modified state.

Nucleosome Sliding Is Required for Activation of Transcription

Our experiments raised the following question: is nucleosome sliding required for activation of transcription, or is it the consequence of preinitiation complex assembly due to TBP binding? To address this question, we designed templates that bear different lengths of DNA downstream of the nucleosome's 3′ border (Figure 5, templates α, β, γ, δ, ϵ, and ζ), thus either preventing nucleosome sliding or permitting sliding at defined positions. Therefore, the nucleosome in template α cannot slide, whereas templates β, γ, and δ can slide 16, 26, and 36 bp, respectively, acquiring a position at the end of the DNA fragment. By contrast, in templates ϵ and ζ, the nucleosome would slide to its natural position (36 bp downstream). These templates were used in nucleosome reconstitution experiments. Figure 5A shows that the boundaries of the nucleosome reconstituted in each template are identical (–15 to +131), as judged from exonuclease III digestion. Next, the templates with or without the enhanceosome were incubated with HNEs in the presence of ATP, and the position of the nucleosome was determined by a combination of restriction site accessibility assays and micrococcal nuclease digestion experiments (data not shown). As expected, we found that the nucleosome did not slide on template α. By contrast, the nucleosome slid 36 bp on templates δ, ϵ, and ζ and 16 and 26 bp on templates β and γ, respectively (data not shown), thus verifying our predictions. The in vitro transcription experiment of Figure 5B, using the same set of templates, demonstrates that prevention of nucleosome sliding abolishes activated transcription (lanes 1 and 2), despite the fact that TFIID is recruited

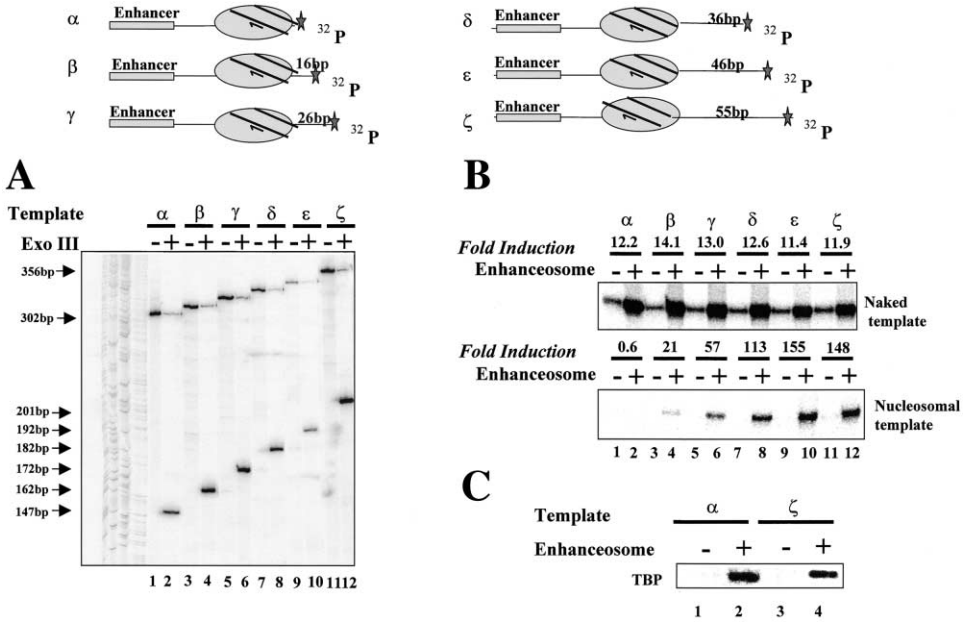

Figure 5. Nucleosome Sliding Is Required for Activation of Transcription

(A) The templates shown at the top of the figure were labeled, reconstituted to nucleosomes, and incubated with ExoIII. PAGE was used to determine the size of the ExoII resistant fragments.

(B) Shown is an in vitro transcription experiment using either naked (top panel) or nucleosome-containing templates.

(C) Templates α and ζ were reconstituted into nucleosomes, attached to paramagnetic beads, and were incubated with HNEs. The beads were washed, and the recruited TBP protein was detected by Western blotting.

to this template (Figure 5C). However, transcription activated from templates β and γ is gradually restored (Figure 5B, lanes 3–6), thus indicating that exposure of the transcription start site (template β) and/or the downstream sequence (template γ) due to nucleosome sliding is a prerequisite for transcriptional activation. When the nucleosome acquired its natural "slid" position, transcription reached maximal levels (Figure 5B, lanes 8–12). As a control, we showed that the levels of transcription obtained from the same templates but without chromatin are similar (Figure 5B). We conclude that nucleosome sliding is not a consequence of the transcriptional activation process, but instead is required for critical regulatory promoter elements, such as the start site of transcription and downstream elements, to become accessible to the basal transcriptional apparatus.

Discussion

Existence of nucleosomes over the TATA box and the start site of transcription, presumably due to the high affinity of TATA-like sequences for core histones (Roychoudhury et al., 2000), appears to be a common theme of promoter structure, thus underscoring the role of architectural specificity of chromatin structure in regulation of gene transcription. An inherent property of the transcriptional machinery is that it cannot assemble on promoters containing a nucleosome, thus ensuring that genes are not aberrantly expressed (Imbalzano et al., 1994). Therefore, a requirement for nucleosome remodeling is critical mainly for the assembly of the basal machinery, since in general, the polymerase can transcribe DNA through nucleosomes (Lorch et al., 1987). We imagine three ways by which the transcriptional machinery could get access on gene promoters bearing nucleosomes. First, the nucleosome is displaced (nucleosome loss); second, the nucleosome is altered in a way that permits preinitiation complex assembly; and third, the nucleosome changes its position on the DNA (nucleosome sliding). Previous in vitro experiments using purified chromatin-remodeling factors and nucleosomal templates have indicated that almost all the chromatin remodeling complexes under certain conditions can carry out at least one of these modifications (reviewed in Vignali et al., 2000). However, the mechanism by which these chromatin remodelers function in vivo on target genes was largely unknown.

In the case of the IFN-β gene, the nucleosome that obstructs transcription begins 5 bp downstream of the TATA box and extends over the start site of transcription (Figure 6). The enhanceosome, which forms upon virus infection, counteracts this repressive effect by instructing a recruitment program beginning with histone acetyl-transferases acetylating this nucleosome, followed by recruitment of SWI/SNF via CBP (Agalioti et al., 2000). SWI/SNF's recruitment is stabilized by the acetylated histone N termini, presumably via its interaction with the bromodomains in BRG1 or BRM (Figure 6). Then, SWI/SNF modifies the histone-DNA contacts, causing changes in the writhe and/or the superhelicity of the DNA around the histone core, without changing the relative position of the histone core relative to DNA. Apparently, however, these changes suffice for binding of TBP to the nearby TATA box, thus completing the assembly of the basal machinery. Simultaneously, the

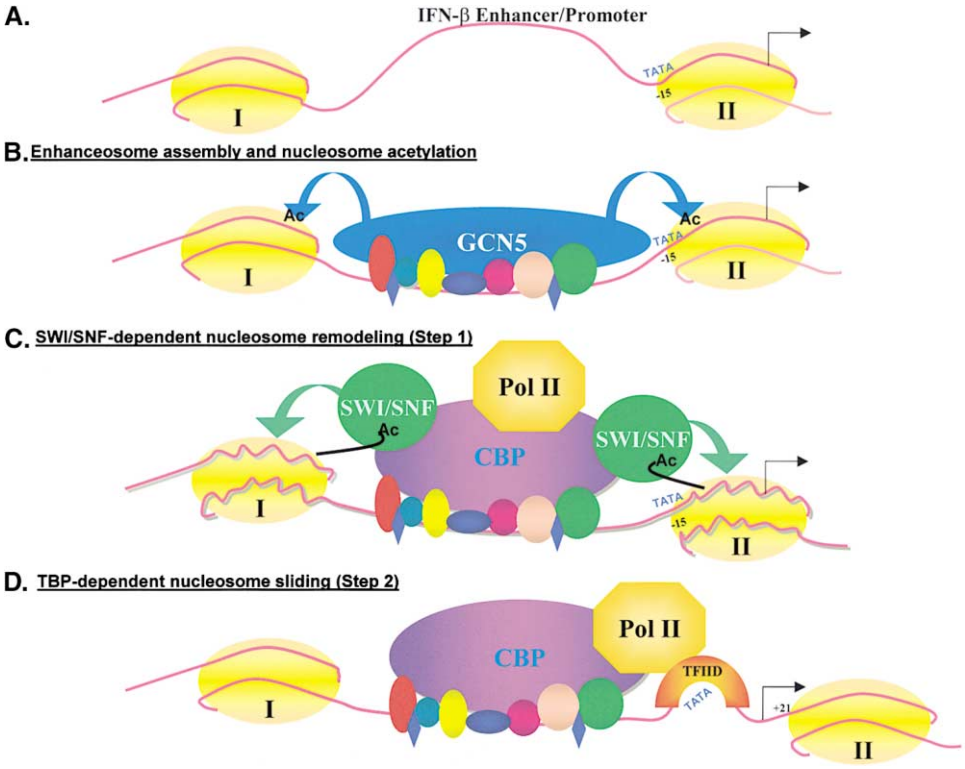

Figure 6. Model Depicting Chromatin Remodeling at the IFN-β Promoter

(A) The IFN-β enhancer/promoter is flanked by two nucleosomes (I and II). Nucleosome II masks the TATA box and the start site of transcription (shown by an arrow).

(B) Virus infection induces enhanceosome assembly and recruitment of the GCN5 complex that acetylates both nucleosomes

(C) Next, the enhanceosome recruits the CBP/PolII holoenzyme complex and SWI/SNF whose recruitment is stabilized by the acetylated nucleosome. SWI/SNF acts on the nucleosome by modifying the histone-DNA contacts (DNA shown as ruffled lines).

(D) Nucleosome modification by SWI/SNF allows recruitment of TFIID to the promoter by the enhanceosome. The radical DNA bend induced by TBP binding promotes nucleosome sliding to a new position 36 bp downstream, thus fully exposing the core promoter and allowing initiation of transcription.

radical DNA bend induced by TBP on DNA causes repositioning of the nucleosome to a new location 36 bp downstream, thus fully exposing the start site of transcription (Figure 6). The observation that a heterologous DNA-bending protein, such as LEF-1, can substitute for TBP in nucleosome sliding implies that any DNA bend induced at this position could induce sliding of the SWI/SNF-modified nucleosome. Nevertheless, these experiments provide a biological role for the ability of TBP to induce DNA bending. Interestingly, TBP binding is also required for SWI/SNF-induced nucleosome remodeling on templates bearing a GCN4 binding placed between the two positioned nucleosomes of the PHO5 promoter (I. Topalidou and G. Thireos, personal communication).

Our experiments showed that although SWI/SNF is capable of modifying the path of DNA around the histone core, this modification does not suffice for transcriptional activation in the absence of nucleosome sliding. Using a series of DNA templates that permit nucleosome sliding at defined positions, we found that maximal transcriptional activation occurs only when the start site of transcription and the downstream 21 bp are exposed. This result implies that downstream promoter elements are required for transcriptional activation, perhaps by serving as docking sites for several of the components of the transcriptional apparatus (Burke and Kadonaga, 1997). Whether these sequences are recognized by specific proteins in the basal apparatus or serve as nonspecific anchoring sites is not known. Nevertheless, nucleosome sliding is a prerequisite for transcriptional activation of the IFN-β gene.

How does TBP-induced DNA bending promote nucleosome sliding of the SWI/SNF-remodeled nucleosome? The TBP-DNA crystal structure revealed that the DNA shape should contribute to a positive writhe that counteracts the negative twist from unwinding (Kim et al., 1993a, 1993b). Interestingly, SWI/SNF and other chromatin remodeling machines generate an accessible nucleosome by driving changes in the writhe and twist of the DNA (Gavin et al., 2001; Havas et al., 2001). Furthermore, the observation that HMG I(Y) binding to the enhancer is required for TBP-induced nucleosome sliding further underscores the critical role of alterations in DNA conformation in nucleosome sliding. This is because binding of HMG I(Y) to the enhancer from the minor groove bends the DNA toward the major groove (Falvo et al., 1995), that is, similar to the TBP-induced DNA bending. Consistent with this is the observation that deletion of the HMG I(Y)-like domains present in the NURF301 subunit of the NURF complex impair

nucleosome sliding (Xiao et al., 2001 [September issue of *Molecular Cell*]). Taken together, we propose that the changes on DNA structure induced by HMG I(Y) and TBP binding are diffused to the nearby SWI/SNF-altered nucleosome, "forcing" it to adopt a new thermodynamically stable state by sliding 36 bp downstream from its original position. There are several predictions of this model verified by our experiments. First, by inhibiting TBP binding in vivo or in vitro, the SWI/SNF-altered nucleosome is unstable, thus requiring continuous ATP hydrolysis in order to be preserved at this state, consistent with previous in vivo and in vitro studies (Biggar and Crabtree, 1999; Sudarsanam et al., 1999; Jaskelliof et al., 2000). More specifically, we showed that in vivo, the nucleosome reverts to its previous state when SWI/SNF departs from the promoter, and in vitro, when ATP is removed from the reaction. Second, an LEF-1 induced DNA bend fully complements TBP's role in nucleosome sliding, thus indicating that most likely it is the DNA bend induced by TBP, and not any other function of the protein, that promotes nucleosome mobilization. Unexpectedly, we found that histone acetylation is also required for nucleosome sliding, at a step following the recruitment of SWI/SNF. Thus, although ordinarily histone acetylation is required for SWI/SNF recruitment (Agalioti et al., 2000), it is also critical for mobilization of an already remodeled nucleosome. We speculate that unacetylated histone tails interact with the DNA/and or protein, and therefore could inhibit nucleosome sliding by functioning as "brakes." This inhibitory effect is partially neutralized by acetylation, which is thought to weaken these interactions (Hong et al., 1993; Mutskov et al., 1998), thus permitting nucleosome movement.

Experimental Procedures

In Vivo and In Vitro Nucleosome Mapping

HeLa cell monolayers (2.5×10^7) were fixed with 1.1% formaldehyde for 45 min at room temperature. The cells were harvested and disrupted in a Dounce homogenizer in 0.3 M Sucrose, 2 mM Magnesium Acetate, 3 mM Calcium Chloride, 1% Triton X-100, and 10 mP Hepes [pH 7.9]. The lysate was spun through a pad of 25% glycerol, 5 mM Magnesium Acetate, 0.1 mM EDTA, and 10 mM Hepes [pH 7.4] at 1000 g for 15 min, and the nuclei were resuspended at an OD_{260} = 200 in the above buffer. The OD_{260} corresponding to DNA absorbance was calculated in a small aliquot after reversing the crosslink, proteinase K treatment and phenol-chloroform extraction, and ethanol precipitation. Next, nuclei at an $OD_{260(DNA)}$ = 100 were incubated with micrococcal nuclease (500 units/ml) in a buffer containing 25 mM KCl, 4 mM $MgCl_2$, 1 mM $CaCl_2$, 50 mM Tris [pH 7.4], and 12.5% glycerol at 37°C for 10 min. The reaction was stopped by adding an equal volume of 2% SDS, 0.2 M NaCl, 10 mM EDTA, 10 mM EGTA, 50 mM Tris [pH 8.0], and proteinase K (100 μg/ml) for 2 hr at 37°C. The crosslink was reversed by heating at 65°C overnight. The DNA was extracted and the samples were incubated with RNase A (100 μg/ml), followed by phenol-chloroform extraction and ethanol precipitation. The DNA was separated on 1.7% agarose gels, and fragments of an average size of 150 bp were purified, denatured, and hybridized with 20 ng of endlabeled primer. Primer extension was performed at 37°C using 13 units of Sequenase in 1× sequenase buffer containing 0.01 M DTT and 0.1 mM dNTPs. The product was analyzed in 8% sequencing gels. Nuclesome mapping of the transfected templates was carried out after transient transfection of HeLa cells in 10 cm tissue culture plates using 10 μg of −110 IFN-β CAT or −110 IFN-β CAT (TGTA) plasmids.

In vitro nucleosome mapping was performed as follows. Nucleosomes were reconstituted as previously described (Agalioti et al., 2000) on immobilized homogeneously radiolabeled DNA templates. The nucleosomes were acetylated with GCN5 HAT domain protein, and the templates were incubated with 10 pmol of NFκB, ATF2/cJun, and IRF1, and 5 pmol of HMGI(Y). Unbound proteins were removed, and 60 μg of HNE was added in the presence of 4 mM ATP. The Dynabeads were washed, and Micrococcal Nuclease was added at a final concentration of 0.1 u/ml. The supernatant after precipitation was collected and separated on denaturing gels. The band corresponding to 146 bp was eluted, and the DNA was used for primer extension as described above.

The restriction enzyme accessibility, the recruitment reactions, and the ExoIII assays were done as previously described (Agalioti et al., 2000).

In vitro transcription reactions were performed using 75 ng of immobilized PCR fragments as previously described (Agalioti et al., 2000).

TFIID and SWI/SNF depletions were carried out by incubating nuclear extract with TBP, TAFII250, and BRG1, BRM specific antibodies, respectively. Western blot analysis revealed that in the case of TFIID, the extracts were depleted more than 95%, whereas in the case of SWI/SNF, the depletion was more than 80% successful.

Acknowledgments

We thank Ruddy Grosschedl for the LEF expression vectors, Gordon Hager for purified NF1, Cheng-Ming Chiang for epitope-tagged TFIID, Paul Lieberman and Jim Goodrich for TBP expression vectors, and Athina Antonaki for help with the transfections. We also thank Tom Maniatis, Richard Mann, Richard Axel, Craig Peterson, Nikhil Munshi, and Theodora Agalioti for comments and critical reading of the manuscript, and George Thireos and Carl Wu for communicating unpublished results. Finally, we thank Jane Kawaoka for artwork. This work was supported from NIH (GM-54605), the Pew Scholars Program in Biomedical Sciences, and the March of Dimes.

Received May 11, 2001; revised July 24, 2001.

References

Aalfs, J.D., and Kingston, R.E. (2000). What does chromatin remodeling mean? Trends Biochem. Sci. *25*, 548–555.

Agalioti, T., Lomvardas, S., Bhavin, P., Yie, J., Maniatis, T., and Thanos, D. (2000). Ordered recruitment of chromatin modifying and general transcription factors to the IFNβ promoter. Cell *103*, 667–678.

Almer, A., Rudolph, H., Hinnen, A., and Horz, W. (1986). Removal of positioned nucleosomes from the yeast PHO5 promoter upon PHO5 induction releases additional upstream activating DNA elements. EMBO J. *5*, 2689–2696.

Archer, T.K., Cordingley, M.G., Wolford, R.G., and Hager, G.L. (1991). Transcription factor access is mediated by accurately positioned nucleosomes on the mouse mammary tumor virus promoter. Mol. Cel. Biol. *11*, 688–698.

Biggar, S.R., and Crabtree, G.R. (1999). Continuous and widespread roles for the Swi-Snf complex in transcription. EMBO J. *18*, 2254–2264.

Burke, T.W., and Kadonaga, J.T. (1997). The downstream core promoter element, DPE, is conserved from Drosophila to humans and is recognized by $TAF_{II}60$ of Drosophila. Genes Dev. *11*, 3020–3031.

Cosma, M.P., Tanaka, T., and Nasmyth, K. (1999). Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle and developmentally regulated promoter. Cell *97*, 299–311.

Dilworth, F.J., Fromental-Ramain, C., Yamamoto, K., and Chambon, P. (2000). ATP driven chromatin remodeling activity and histone acetyltransferases act sequentially during transactivation by RAR/RXR in vitro. Mol. Cell *7*, 1049–1058.

Falvo, J.V., Thanos, D., and Maniatis, T. (1995). Reversal of intrinsic DNA bends in the IFNb gene enhancer by transcription factors and HMG I(Y). Cell *83*, 1101–1111.

Fry, C.J., and Peterson, C.L. (2001). Chromatin remodeling enzymes: who's on first? Curr. Biol. *11*, R185–R197.

Gavin, I., Horn, P.J., and Peterson, C.L. (2001). SWI/SNF chromatin remodeling requires changes in DNA topology. Mol. Cell *7*, 97–104.

Giese, K., Cox, J., and Grosschedl, R. (1992). The HMG domain of lymphoid enhancer factor bends DNA and facilitates assembly of functional nucleoprotein structures. Cell *69*, 185–195.

Goldmark, J.P., Fazzio, T.G., Estep, P.W., Church, M.G., and Tsukiyama, T. (2000). The Isw2 chromatin remodeling complex represses early meiotic genes upon recruitment by Ume6p. Cell *103*, 423–433.

Hamiche, A., Sandaltzopoulos, R., Gdula, D., and Wu, C. (1999). ATP dependent histone octamer sliding mediated by the chromatin remodeling complex NURF. Cell *97*, 833–842.

Hassan, A.H., Neely, K.E., and Workman, J.L. (2001). Histone acetyltransferase complexes stabilize SWI/SNF binding to promoter nucleosomes. Cell *104*, 817–827.

Havas, K., Flaus, A., Phelan, M., Kingston, R., Wade, P., Lilley, D., and Owen Hughes, T. (2001). Generation of superhelical torsion by ATP dependent chromatin remodeling complexes. Cell *103*, 1133–1142.

Hong, L., Schroth, G.P., Metthews, H.R., Yau, P., and Bradbury, E.M. (1993). Studies of the DNA binding properties of histone H4 amino terminus. Thermal denaturation studies reveal that acetylation markedly reduces the binding constant of the H4 tail to DNA. J. Biol. Chem. *268*, 305–314.

Imbalzano, A.N., Kwon, H., Green, M.R., and Kingston, R.E. (1994). Facilitated binding of TATA−binding protein to nucleosomal DNA. Nature *370*, 481–485.

Jaskelliof, M., Gavin, I., Peterson, C.L., and Logie, C. (2000). SWI/SNF-mediated nucleosome remodeling: Role of histone octamer mobility in the persistance of the remodeled state. Mol. Cell. Biol. *20*, 3058–3068.

Kim, J., and Shapiro, D.J. (1996). In simple synthetic promoters YY-1 induced DNA bending is important in transcription activation and repression. Nucleic Acids Res. *24*, 4341–4348.

Kim, J.L., Nikolov, D.B., and Burley, S.K. (1993a). Co-Crystal structure of TBP recognizing the minor groove of a TATA element. Nature *365*, 520–527.

Kim, Y., Geiger, J.H., Hahn, S., and Singler, P.B. (1993b). Crystal structure of a yeast TBP/TATA−box complex. Nature *365*, 512–520.

Kingston, R.E., and Narlikar, G.J. (1999). ATP-dependent remodeling and acetylation as regulators of chromatin fluidity. Genes Dev. *13*, 2339–2352.

Kornberg, R.D. (1974). Chromatin structure: a repeating unit of histones and DNA. Science *184*, 868–871.

Kornberg, R.D., and Lorch, Y. (1999). Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. Cell *98*, 285–294.

Krebs, J.E., Kuo, M.H., Allis, C.D., and Peterson, C.L. (1999). Cell cycle regulated histone acetylation required for the expression of the yeast HO gene. Genes Dev. *13*, 1412–1421.

Krebs, J.E., Fry, C.J., Samuels, M.L., and Peterson, C.L. (2000). Global role for chromatin remodeling enzymes in mitotic gene expression. Cell *102*, 587–598.

Langst, G., Bonte, E.J., Corona, D.F., and Becker, P.B. (1999). Nucleosome movement by CHRAC and ISWI without disruption or trans-displacement of the histone octamer. Cell *97*, 843–852.

Lemon, B., and Tjian, R. (2000). Orchestrated response: a symphony of transcription factors for gene control. Genes Dev. *14*, 2551–2569.

Li, G., Chandler, P.S., Wolffe, P.A., and Hall, T.C. (1998). Architectural specificity in chromatin structure at the TATA box *in vivo*: Nucleosome displacement upon β-phaseolin gene activation. Proc. Natl. Acad. Sci. USA *95*, 4772–4777.

Lohr, D. (1997). Nucleosome transactions on the promoters of the yeast GAL and PHO genes. J. Biol. Chem. *272*, 26795–26798.

Lorch, Y., LaPointe, J.W., and Kornberg, R.D. (1987). Nucleosomes inhibit the initiation of transcription but allow chain elongation with the displacement of histones. Cell *49*, 203–210.

Lorch, Y., Zhang, M., and Kornberg, R. (2001). RSC unravels the nucleosome. Mol. Cell *7*, 89–95.

Love, J.J., Li, X., Case, D.A., Giese, K., Grosschedl, R., and Wright, P.E. (1995). Structural basis for DNA bending by the architectural transcription factor LEF-1. Nature *376*, 791–795.

Maniatis, T., Falvo, J.V., Kim, T.H., Kim, T.K., Lin, C.H., Parekh, B.S., and Wathelet, M.G. (1998). Structure and function of the IFN beta enhanceosome. Cold Spring Harb. Symp. Quant. Biol. *63*, 609–620.

Marmorstein, R., Carey, M., Pthashne, M., and Harrison, S.C. (1992). DNA recognition by GAL4: structure of a protein-DNA complex. Nature *356*, 408–414.

Merika, M., and Thanos, D. (2001). Enhanceosomes. Curr. Opin. Gen. Dev. *11*, 205–208.

Moreira, J.M., and Holmberg, S. (1999). Transcriptional repression of the yeast CHA1 gene requires the chromatin remodeling complex RSC. EMBO J. *18*, 2836–2844.

Munshi, N., Merika, M., Yie, J., Senger, K., Chen, G., and Thanos, D. (1998). Acetylation of HMGI(Y) by CBP turns off IFNβ expression by disrupting the enhanceosome. Mol. Cell *2*, 457–467.

Munshi, N., Yie, J., Merika, M., Senger, K., Lomvardas, S., Agalioti, T., and Thanos, D. (1999). The IFN-β enhancer: A paradigm for understanding activation and repression of inducible gene expression. Cold Spring Harb. Symp. Quant. Biol. *64*, 149–159.

Munshi, N., Agalioti, T., Lomvardas, S., Merika, M., Chen, G., and Thanos, D. (2001). Coordination of a transcriptional switch by HMG I(Y) acetylation. Science, *293*, 1133–1136.

Mutskov, V., Gerber, D., Angelov, D., Ausio, J., Workmann, J., and Dimitrov, S. (1998). Persistent interactions of core histone tails with nucleosomal DNA following acetylation and transcription factor binding. Mol. Cell. Biol. *18*, 6293–6304.

Peterson, C.L. (2000). ATP chromatin remodeling: Going mobile. FEBS Lett. *476* (1-2), 68–72.

Reeves, R., Leonard, W.J., and Nissen, M.S. (2000). Binding of HMG I(Y) imparts architectural specificity to a positioned nucleosome on the promoter of the human interleukin-2 receptor α gene. Mol. Cell. Biol. *20*, 4666–4679.

Reinke, H., Gregory, P.D., and Horz, W. (2001). A transient histone hyperacetylation signal marks nucleosomes for remodeling at the PHO8 promoter in vivo. Mol. Cell *7*, 529–538.

Roychoudhury, M., Sitlani, A., Lapham, J., and Crothers, D.M. (2000). Global structure and mechanical properties of a 10-bp nucleosome positioning motif. Proc. Natl. Acad. Sci. USA *97*, 13608–13613.

Rubbi, L., Camilloni, G., Caserta, M., Di Mauro, E., and Venditti, S. (1997). Chromatin structure of the Saccharomyces cerevisiae DNA topoisomerase I promoter in different growth phases. Biochem. J. *328*, 401–407.

Schnitzler, G., Sif, S., and Kingston, R.E. (1998). Human SWI/SNF interconverts a nucleosome between its base state and a stable remodeled state. Cell *94*, 17–27.

Senger, K., Merika, M., Agalioti, T., Yie, J., Escalante, C.R., Chen, G., Aggarwal, A.K., and Thanos, D. (2000). Gene repression by coactivator repulsion. Mol. Cell *6*, 931–937.

Sewack, G.F., and Hansen, U. (1997). Nucleosome positioning and transcription-associated chromatin alterations on the human estrogen-responsive pS2 promoter. J. Biol. Chem. *272*, 31118–31129.

Shen, C.S., Leblanc, B.P., Alfier, J.A., and Clark, D.J. (2001). Remodeling of yeast CUP1 chromatin involves activator dependent repositioning of nucleosomes over the entire gene and flanking sequences. Mol. Cell. Biol. *21*, 534–547.

Strahl, B.D., and Allis, C.D. (2000). The language of covalent histone modifications. Nature *403*, 41–45.

Sudarsanam, P., Cao, Y., Wu, L., Laurent, B.C., and Winston, E. (1999). The nucleosome remodeling complex Snf/Swi, is required for the maintenance of transcription in vivo and is partially redundant with the histone acetyltransferase GCN5. EMBO J. *18*, 3101–3106.

Syntichaki, P., Topalidou, I., and Thireos, G. (2000). The GCN5 bromodomain coordinates nucleosome remodeling. Nature *404*, 414–417.

Verdin, E., Paras, P.J., and van Lint, C. (1993). Chromatin disruption in the promoter of human immunodeficiency virus type I during transcriptional activation. EMBO J. *12*, 3249–3259.

Verdone, L., Camillioni, G., Di Mauro, E., and Caserta, M. (1996). Chromatin remodeling during Saccharomyces cerevisiae ADH2 gene activation. Mol. Cell. Biol. *16*, 1978–1988.

Vignali, M., Hassan, A.H., Neely, E.K., and Workman, J. (2000). ATP dependent chromatin remodeling complexes. Mol. Cell. Biol. *20*, 1899–1910.

Weinmann, A.S., Plevy, S.E., and Smale, S.T. (1999). Rapid and selective remodeling of a positioned nucleosome during the induction of IL-12 p40 transcription. Immunity *11*, 665–675.

Weinmann, A.S., Mitchell, D.M., Sanjabi, S., Bradley, M.N., Hoffmann, A., Liou, H., and Smale, S.T. (2001). Nucleosome remodeling at the IL-12 p40 promoter is a TLR-dependent, Rel independent event. Nat. Immunol. *2*, 51–57.

Whitehouse, I., Flaus, A., Caims, B.R., White, M.F., Workman, J.L., and Owen Hughes, T. (1999). Nucleosome mobilization catalyzed by the yeast SWI/SNF complex. Nature *400*, 784–787.

Wolffe, A. (1995). Chromatin Structure and Function (London: Academic Press).

Xiao, H., Sandaltzopoulos, R., Wang, H.-M., Hamiche, A., Ranallo, R., Lee, K.-M., Fu, D., and Wu, C. (2001). Dual functions of largest NURF subunit NURF301 in nucleosome sliding and transcription factor interactions. Mol. Cell, in press.