RNA Polymerase II Holoenzyme Contains SWI/SNF Regulators Involved in Chromatin Remodeling

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Summary

The RNA polymerase II holoenzyme contains RNA polymerase II, a subset of general transcription factors and SRB regulatory proteins. We report here that *SWI* and *SNF* gene products, previously identified as global gene regulators whose functions include remodeling chromatin, are also integral components of the yeast RNA polymerase II holoenzyme. The SWI/SNF proteins are components of the SRB complex, also known as the mediator, which is tightly associated with the RNA polymerase II C-terminal repeat domain. The SWI/SNF components provide the holoenzyme with the capacity to disrupt nucleosomal DNA and thus facilitate stable binding of various components of the transcription initiation complex at promoters.

Introduction

Regulation of class II genes involves a complex interplay among gene-specific activators and cofactors, the general transcription apparatus, and chromatin. Gene-specific activators bind to promoters and stimulate transcription, at least in part, by binding and recruiting the general transcription apparatus (Chen et al., 1994; Hengartner et al., 1995; Ingles et al., 1991; Lin et al., 1991; Xiao et al., 1994; reviewed by Tjian and Maniatis, 1994; Sheldon and Reinberg, 1995; Emili and Ingles, 1995; Carey, 1995). Chromatin structure can affect the transcriptional activity of genes by blocking access of the transcription apparatus to promoters (Knezetic and Luse, 1986; Bresnick and Felsenfeld, 1993; Felsenfeld, 1992; Lorch et al., 1988; Workman and Roeder, 1987). The SWI and SNF proteins are global regulators that function by antagonizing repression mediated by nucleosomes, altering chromatin structure to facilitate binding of the transcription apparatus (Côté et al., 1994; Hirschhorn et al., 1992; Imbalzano et al., 1994; Kwon et al., 1994; reviewed by Carlson and Laurent, 1994; Peterson and Tamkun, 1995; Winston and Carlson, 1992). It is not yet clear how the SWI/SNF proteins are

targeted to promoters, although some gene-specific activators may interact directly with these proteins (Yoshinaga et al., 1992).

Genetic and biochemical studies in yeast indicate that the form of the transcription initiation apparatus generally responsible for mRNA synthesis in vivo is an RNA polymerase II holoenzyme (Barberis et al., 1995; Hengartner et al., 1995; Kim et al., 1994; Koleske and Young, 1994; Thompson and Young, 1995; reviewed by Carey, 1995; Emili and Ingles, 1995; Koleske and Young, 1995). This megadalton-sized complex contains RNA polymerase II, general transcription factors, and additional components called suppressor of RNA polymerase B (SRB) regulatory proteins. The SRB proteins are a hallmark of the holoenzyme. The genes encoding the nine known SRB proteins were identified through a selection for factors involved in transcription initiation by RNA polymerase II in vivo, and all are required for normal yeast cell growth. Essentially all of the SRB protein in cells is tightly associated with the holoenzyme, while approximately 80% of RNA polymerase II and general transcription factors are found independent of this complex (Koleske and Young, 1995). Experiments with temperature-sensitive *SRB* mutants indicate that the RNA polymerase II holoenzyme is the form of the transcription initiation apparatus employed at the majority of class II promoters in vivo (Thompson and Young, 1995). Other experiments have shown that recruiting a component of the SRB complex to promoters, presumably in association with the holoenzyme, suffices to obtain activated levels of transcription in vivo (Barberis et al., 1995).

The yeast *SWI* genes were first identified as positive regulators of HO transcription (Stern et al., 1984), and *SWI1*, *SWI2*, and *SWI3* were later shown to be required for the activation of a broad spectrum of inducible genes in vivo (Peterson and Herskowitz, 1992; Yoshinaga et al., 1992). Similarly, the *SNF* genes were originally identified as positive regulators of *SUC2* (Neigeborn and Carlson, 1984), and *SNF2*, *SNF5*, and *SNF6* were subsequently found to be essential for activation of a diverse set of inducible genes (Laurent and Carlson, 1992; Laurent et al., 1991; Peterson and Herskowitz, 1992). Further study revealed that *SWI2* and *SNF2* are the same gene. Genetic evidence indicated that the *SWI* and *SNF* genes are involved in similar processes in gene activation (Carlson and Winston, 1992). Indeed, the discovery that SWI1, SWI2/SNF2, SWI3, SNF5, SNF6, and SNF11 proteins copurify in a large complex confirmed that the *SWI/SNF* gene products function together (Cairns et al., 1994; Côté et al., 1994; Peterson et al., 1994; Treich et al., 1995). Genetic and biochemical evidence implicated the SWI/SNF proteins in chromatin remodeling via nucleosome disruption (Cairns et al., 1994; Côte et al., 1994; Hirschhorn et al., 1992; Peterson et al., 1994).

Several lines of evidence led us to investigate whether SWI and SNF proteins are components of the RNA polymerase II holoenzyme, and furthermore, whether SWI/SNF proteins are components of the SRB-containing protein complex that is tightly associated with the C-terminal repeat domain (CTD) in the holoenzyme.

First, genetic evidence suggests a functional relationship between the *SWI* and *SNF* gene products and the CTD. Strains containing mutations in *SWI* genes exhibit a large number of defects similar to those due to a truncation of the RNA polymerase II CTD (Nonet et al., 1987; Peterson and Herskowitz, 1992; Peterson et al., 1991). In addition, the CTD and the *SWI/SNF* gene products show similar genetic interactions with mutations in *SIN1* and *SIN2*, genes that encode chromatin-associated proteins (Peterson and Herskowitz, 1992; Peterson et al., 1991). Second, the *SRB* gene products have functional and physical interactions with the RNA polymerase II CTD (Koleske and Young, 1995), which has been implicated in the response to activators in yeast and mammalian cells (Allison and Ingles, 1989; Gerber et al., 1995; Scafe et al., 1990). Third, the holoenzyme appears to be responsible for initiating transcription of most, if not all, class II genes in yeast, and the *SWI* and *SNF* gene products are required for transcriptional induction of a large number of genes in vivo (Thompson and Young, 1995; Peterson et al., 1991). Finally, there are perhaps a dozen polypeptides in purified yeast RNA polymerase holoenzyme that have yet to be identified.

We report here that the yeast RNA polymerase II holoenzyme contains SWI2/SNF2, SWI3, SNF5, and SNF11. The SWI/SNF proteins are components of the SRB complex, also known as the mediator, which is tightly associated with the RNA polymerase II CTD. Both the holoenzyme and the SRB/SWI/SNF complex have ATP-dependent nucleosome disruption activities previously ascribed to the SWI/SNF complex. In addition, the holoenzyme facilitates the binding of TATA box–binding protein (TBP) to nucleosomal DNA in an ATP-enhanced manner.

Results

Anti-SRB and Anti-SWI Antibodies Coprecipitate Holoenzyme

SRB regulatory proteins are found tightly and exclusively associated with other components of the RNA polymerase II holoenzyme in cell extracts. If SWI and SNF proteins are subunits of the RNA polymerase II holoenzyme, then antibodies against SRB5 should precipitate both the holoenzyme and SWI/SNF proteins from crude extracts. The results in Figure 1 show that this is indeed the case. SWI2/SNF2, SWI3, and SNF5 proteins coprecipitate with holoenzyme obtained through SRB5 immunoprecipitation. The fraction of SWI and SNF proteins immunoprecipitated from the crude extract appears to be the same as that of the SRB proteins. Control proteins introduced into the crude lysate did not coprecipitate, indicating that the immunoprecipitate was specific for the holoenzyme. When the immunoprecipitation experiment was carried out with antibody against SWI3, essentially identical results were obtained (Figure 1). The SWI/SNF and SRB proteins were immunoprecipitated from the crude extract with similar efficiency whether the immunoprecipitating antibody used was directed against SRB5 or SWI3. A control experiment with antibody against TGFβ failed to precipitate SWI/SNF or SRB proteins. These results indicate that
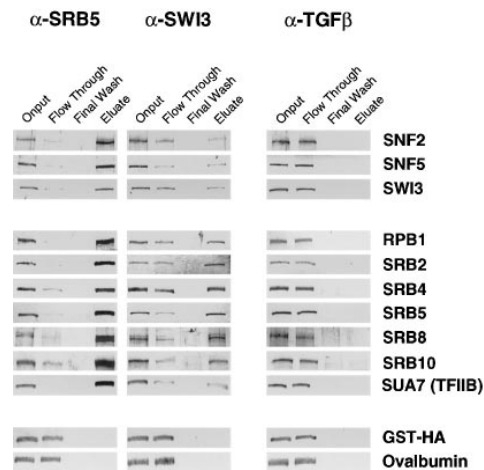

Figure 1. Immunoprecipitation of RNA Polymerase II Holoenzyme from Crude Extracts Using Anti-SRB5 and Anti-SWI3 Antibodies

Immunoprecipitations were from a crude DEAE fraction prepared as described by Hengartner et al. (1995). Immunoprecipitations were carried out with affinity-purified antibodies against SWI3, SRB5, or TGFβ. Ovalbumin and HA-tagged GST were added to each reaction prior to precipitation to serve as controls for specific immunoprecipitation; 1/50 of the onput and flowthrough, and 1/5 of the final wash and eluate were subjected to SDS–PAGE and analyzed by Western blotting using specific antibodies.

SRB and SWI/SNF proteins are tightly associated with one another.

Purified Holoenzyme Contains SWI/SNF Proteins

The immunoprecipitation results led us to investigate whether SWI and SNF proteins are components of purified yeast RNA polymerase II holoenzyme. Antibodies against selected SWI and SNF proteins were used to determine whether these proteins coelute with the RNA polymerase II holoenzyme in the final purification step of the holoenzyme. The data in Figure 2A demonstrate that SNF2/SWI2, SNF5, SWI3, and SNF11 proteins coelute with other known components of the holoenzyme and with transcription activity.

The holoenzyme contains stoichiometric amounts of RNA polymerase II, SRB proteins, and general transcription factors. To ascertain whether the SWI/SNF proteins are stoichiometric components of the holoenzyme, the amounts of SNF2 and SNF5 were estimated by Western blot analysis with various amounts of recombinant proteins as standards (Figure 2B). These data indicate that the purified RNA polymerase II holoenzyme contains approximately equimolar amounts of SNF2, SNF5, and SRB5, the latter being a standard against which other holoenzyme components have previously been compared (Koleske and Young, 1994). Since yeast cells contain between 2000 and 4000 molecules of RNA polymerase II holoenzyme, it appears that there are at least this number of SWI2/SNF2 and SNF5 molecules per cell.

SWI/SNF Proteins Are Components of CTD-Binding SRB Complexes

Genetic evidence indicates that the SRB regulatory proteins and the RNA polymerase II CTD have related functions in transcription initiation and that these involve
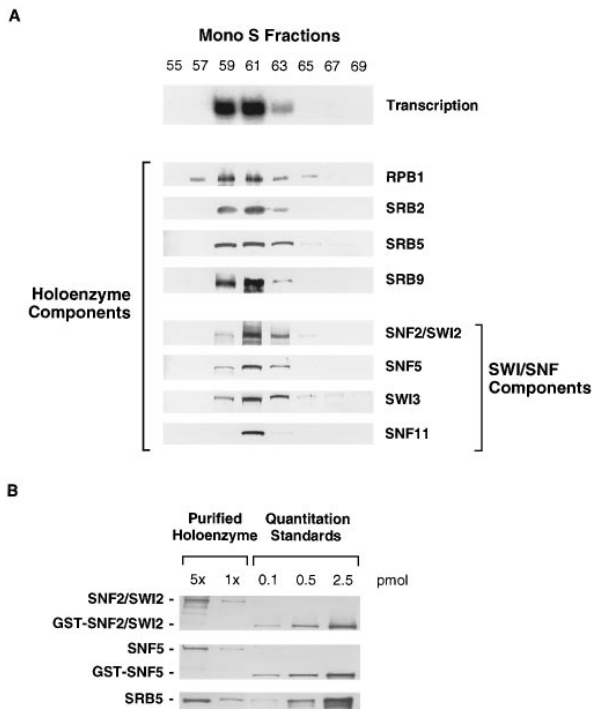
Figure 2. SWI/SNF Proteins Are Components of Purified RNA Polymerase II Holoenzyme

(A) RNA polymerase II holoenzyme eluted from a Mono S column, the last chromatographic step in the purification procedure (Koleske and Young, 1994), was analyzed for transcriptional activity and for the presence of SRB and SWI/SNF proteins by Western blotting.
(B) Quantitative Western blots were used to determine the relative amounts of SRB5 and SWI/SNF proteins in the holoenzyme. Known amounts of recombinant GST–SNF2/SWI2$_{1256-1703}$, GST–SNF5$_{1-193}$, and SRB5 were subject to SDS–PAGE and Western blot analysis along with 2.5 μl and 0.5 μl of purified holoenzyme. There are similar levels of SNF2/SWI2, SNF5, and SRB5 in the purified holoenzyme Previous studies have shown that RPB1 and other SRB proteins are equimolar in purified holoenzyme (Koleske and Young, 1994).
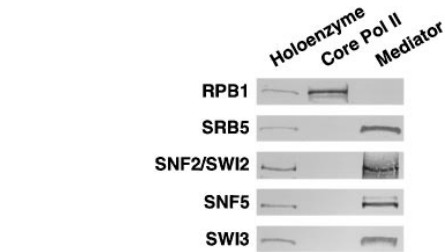

Figure 3. SWI/SNF Proteins Are Present in Mediator Purified Using 8WG16 Monoclonal Antibodies

Mediator was Western blotted along with holoenzyme and core polymerase and probed for the presence of SWI/SNF proteins. The mediator preparation was previously assayed (Hengartner et. al., 1995) and shown to have all transcriptional activities previously described (Kim et. al., 1994).

the response to transcriptional regulators (Allison and Ingles, 1989; Gerber et al., 1995; Scafe et al., 1990; Koleske and Young, 1995). Since the SWI and SNF proteins are also involved in activation of a wide variety of genes and since mutations in SWI and SNF genes can produce phenotypes similar to those observed with mutations in SRB genes, we investigated whether SWI and SNF proteins are associated with the SRB complex. The SRB protein complex can be released from the holoenzyme when the latter is treated with monoclonal antibodies against the CTD, and this preparation has been called mediator (Kim et al., 1994). We previously prepared a mediator complex according to the procedure of Kim et al. (1994), confirmed that it has the coactivator activity described by these investigators, and showed that the mediator contains all of the SRB proteins (Hengartner et al., 1995). When this mediator preparation was assayed for the presence of SNF2/SWI2, SNF5, and SWI3 proteins by Western blot, all three SWI/SNF proteins were found (Figure 3).

The SRB complex can also be isolated from crude extracts using a recombinant CTD column (Thomp-

son et al., 1993). An SRB complex was purified extensively by using a recombinant glutathione S-transferase (GST)–CTD column, followed by chromatography with Mono S and Mono Q columns (Figure 4A). The SRB, SWI, and SNF proteins bind to a GST–CTD column, but not to a control GST column, indicating that they bind specifically to the CTD (Figure 4B). Sliver staining and Western blotting confirm that both a multiprotein complex containing SRB proteins and each of the three assayed SWI/SNF proteins coelute from the Mono Q col-
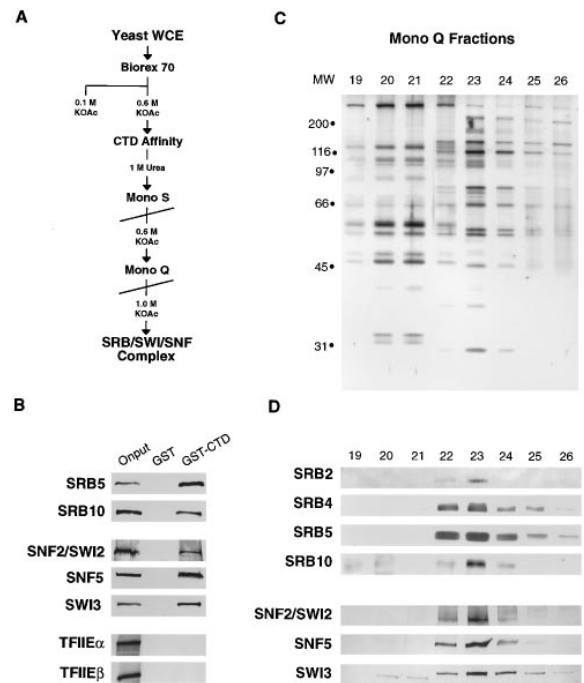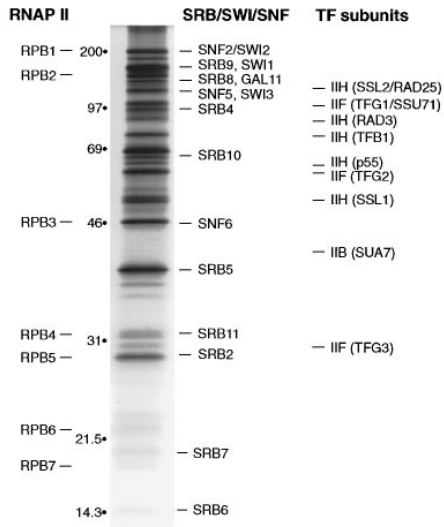

Figure 4. An SRB/SWI/SNF Complex Purified Using CTD Affinity Chromatography

(A) Schematic diagram of the purification.
(B) SRB, SWI, and SNF proteins bind specifically to a GST–CTD column. Western blot analysis of proteins eluted from a GST column and from a GST–CTD column. TFIIE was a negative control for specific retention, as it does not bind GST or GST–CTD.
(C) Silver stain of fractions across the final Mono Q column.
(D) Western blot analysis of SRB and SWI/SNF proteins across the final Mono Q column.

A Holoenzyme

RNAP II

RPB1 — 200•
RPB2 —

97•

69•

RPB3 — 46•

RPB4 — 31•
RPB5 —

RPB6 — 21.5•
RPB7 —

14.3•

SRB/SWI/SNF

— SNF2/SWI2
— SRB9, SWI1
— SRB8, GAL11
— SNF5, SWI3
— SRB4

— SRB10

— SNF6

— SRB5

— SRB11
— SRB2

— SRB7

— SRB6

TF subunits

— IIH (SSL2/RAD25)
— IIF (TFG1/SSU71)
— IIH (RAD3)
— IIH (TFB1)

— IIH (p55)
— IIF (TFG2)

— IIH (SSL1)

— IIB (SUA7)

— IIF (TFG3)

B SRB/SWI/SNF Complex

200•

116•
97•

66•

45•

31•

— SNF2/SWI2
— SRB9, SWI1
— SRB8, GAL11
— SNF5, SWI3
— SRB4

— SRB10

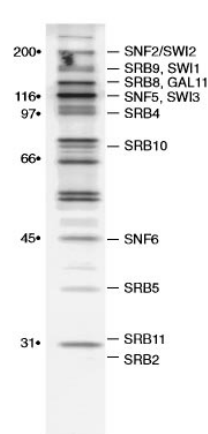— SNF6

— SRB5

— SRB11
— SRB2

Figure 5. Components of the RNA Polymerase Holoenzyme and the SRB/SWI/SNF Complex

(A) Silver stain of purified RNA polymerase II. Bands that correspond in size to RNA polymerase core subunits, SRB, SWI, and SNF proteins, and general transcription factor IIB, IIF, and IIH subunits are indicated.
(B) Silver stain of the SRB/SWI/SNF complex. Bands that correspond in size to SRB, SWI, and SNF proteins are indicated.

umn (Figures 4C and 4D). There are approximately 25 polypeptides in this complex, and several correspond in size to previously identified SRB, SWI, and SNF proteins (Figure 5). No signals were obtained when Western blots containing the SRB/SWI/SNF complex were probed with antibodies against RNA polymerase II, TBP, TFIIB, or the TFB1 subunit of TFIIH (data not shown). These results indicate that the SRB complex is in fact an SRB/SWI/SNF complex and, furthermore, that the SWI and SNF proteins interact with the holoenzyme, at least in part through their association with RNA polymerase II CTD.

Nucleosome Disruption Activity in Holoenzyme and SRB/SWI/SNF Complex

Previous evidence that SWI1, SWI2, SWI3, SNF5, SNF6, and SNF11 gene products can be isolated as a large multisubunit complex capable of altering nucleosome structure led us to investigate whether the purified RNA polymerase II holoenzyme and the SRB/SWI/SNF complex were able to alter nucleosome structure. Mononucleosome particles were reconstituted from purified histone octamers and a DNA fragment containing two copies of an artificial phasing sequence (Shrader and Crothers, 1989). Digestion of the mononucleosomes with DNase I showed a 10 bp cleavage ladder typical of a rotationally phased nucleosome (Figure 6). Fractions in the last chromatographic step in the purification of the holoenzyme were mixed with mononucleosomes and assayed for the ability to alter nucleosome structure, which can be visualized by changes in the accessibility of the nucleosome to DNase I cleavage. Figure 6A demonstrates that a nucleosome disruption activity coeluted with the RNA polymerase holoenzyme. The ability of the SRB/SWI/SNF complex to alter nucleosome structure was assayed in a similar experiment using fractions from the last step in the SRB/SWI/SNF purification (Figure 6C). The results show that nucleosome disruption activity coeluted with the SRB/SWI/SNF complex. Further analysis of the RNA polymerase II holoenzyme and SRB/

SWI/SNF complex showed that the nucleosome disruption activity was ATP dependent (Figures 6B and 6D), as was previously shown for purified SWI/SNF complexes (Côté et al., 1994; Imbalzano et al., 1994; Kwon et al., 1994). In addition, purified core RNA polymerase II showed no nucleosome alteration capability (data not shown). These data indicate that the SRB/SWI/SNF complex contributes chromatin remodeling activity to the RNA polymerase II holoenzyme.

Purified Holoenzyme and the SRB/SWI/SNF Complex Disrupts Plasmid Chromatin

To characterize further the nucleosome disruption capabilities of the holoenzyme and the SRB/SWI/SNF complex, we employed a supercoiling reduction assay (Figure 7). In this assay, chromatin is assembled onto a relaxed closed-circular plasmid that is subsequently purified by glycerol gradient centrifugation. Each assembled nucleosome introduces approximately one negative supercoil to the plasmid, which can be resolved by agarose gel electrophoresis after the removal of histones. When no protein is added to the nucleosome-assembled plasmid, it is highly supercoiled. Fractions from the last column of the holoenzyme purification (see Figure 2A) were tested for their ability to disrupt nucleosome structure and thereby reduce supercoiling in the presence of added topoisomerase I. As can be seen in Figure 7A, this activity coelutes with holoenzyme transcription activity, with the SRB and SWI/SNF proteins (see Figure 2A), and with nucleosome-core disruption activity (see Figure 6A). The supercoiling reduction activity was dependent on ATP (Figure 7A, compare fraction 61 plus and minus ATP), as has been shown for the human SWI/SNF complex (Kwon et al., 1994). Repeating the experiment using fractions from the last column of the SRB/SWI/SNF complex shows that this complex also has an ATP-dependent supercoiling reduction activity (Figure 7B).

A

**Holoenzyme
Mono S Fractions**

+ ATP

N − 55 57 59 61 63 65 67 69 −

B

**Holoenzyme
Mono S Fraction 60**

− ATP | + ATP

Fraction 60 | Fraction 60

C

**SRB/SWI/SNF Complex
Mono Q Fractions**

+ ATP

N − 19 20 21 22 23 24 25 −

D

**SRB/SWI/SNF Complex
Mono Q Fraction 24**
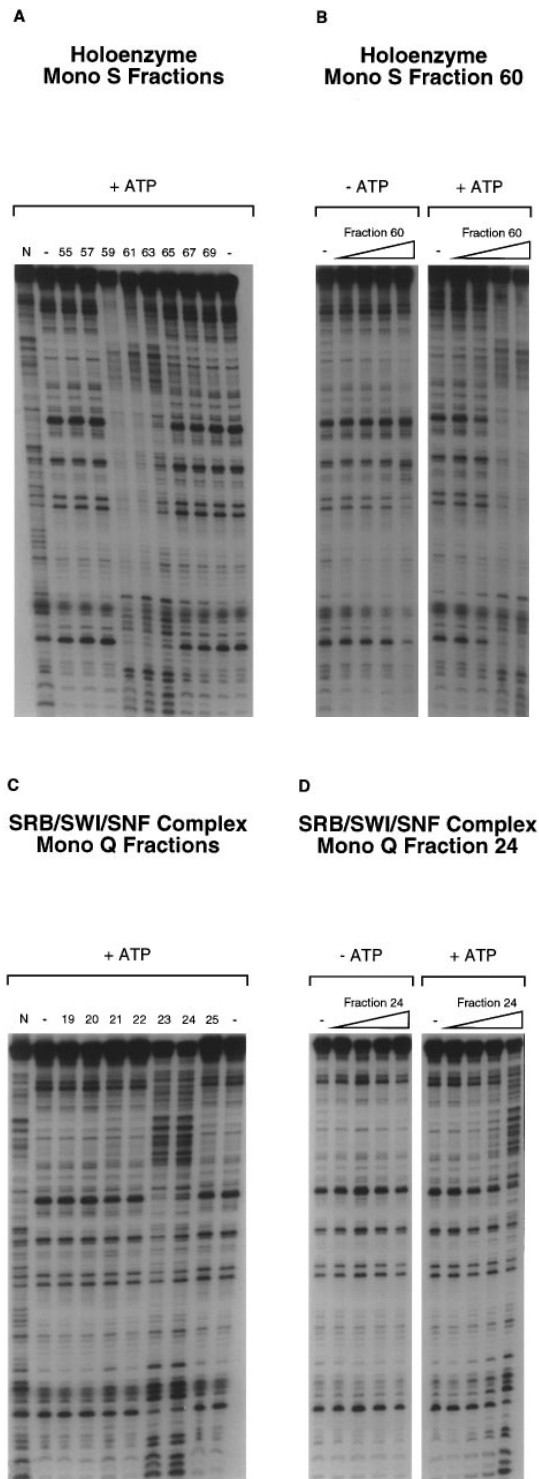
− ATP | + ATP

Fraction 24 | Fraction 24

Figure 6. An ATP-Dependent Nucleosomal Disruption Activity Coelutes with the Holoenzyme and the SRB/SWI/SNF Complex

(A) Fractions from the last column of holoenzyme purification (Figure 2A) were assayed for nucleosomal disruption. The peak of nucleosomal disruption activity is in fractions 59–63, coincident with the peak of transcriptional activity.

(B) Purified RNA polymerase II holoenzyme (fraction 60) was titrated for activity with and without 4 mM ATP, as indicated.

(C) Fractions from the final column of the SRB/SWI/SNF complex purification (Figure 4) were assayed for nucleosomal disruption. The

peak of nucleosomal disruption activity is in fractions 23 and 24, which is also where the bulk of SRB and SWI/SNF proteins elute.

(D) The SRB/SWI/SNF complex (fraction 24) was titrated for activity with and without 4 mM ATP, as indicated.

Holoenzyme Facilitates the Binding of TBP to Nucleosomes

Previous work has shown that both yeast and human SWI/SNF complexes can facilitate transcription factor binding to nucleosomal DNA containing the relevant factor-binding site (Côté et al, 1994; Imbalzano et al, 1994; Kwon et al, 1994). We tested whether the holoenzyme could increase the binding of TBP to a mononucleosome containing a TBP-binding site. With holoenzyme and ATP present, TBP and TFIIA bound to the mononucleosome at TBP concentrations of 4×10^{-6} M (Figure 8A, lane 7), while no TBP/TFIIA binding was observed in the absence of holoenzyme (Figure 8A, lane 6).

This holoenzyme-facilitated TBP binding might be caused by the stabilizing effects of the additional protein–protein and protein–DNA interactions that occur in the presence of RNA polymerase and general transcription factors, by the ATP dependent nucleosome disruption effects of SWI/SNF, or by a combination of both effects. To address this issue, we tested whether facilitated TBP binding was ATP dependent and observed partial protection of the TATA region on the mononucleosome when ATP is withheld or when ATPγS is used instead of ATP (Figure 8B, lanes 4 and 6). However, addition of ATP enhanced the TBP binding as indicated by the increased protection from DNase I cleavage over the TATA box, the extension of the footprint in the 5′ direction, and the appearance of a hypersensitive band in the 3′ direction (Figure 8B, lane 5). Thus, it appears that the holoenzyme can partially stabilize binding of TBP and TFIIA to a mononucleosome in the absence of ATP. However, the full effect of holoenzyme-facilitated TBP binding requires ATP, presumably because it involves the ATP-dependent nucleosome disruption activity of the SWI/SNF proteins.

Discussion

The RNA polymerase II holoenzyme contains *SWI* and *SNF* gene products, previously identified as global gene coactivators. The SWI and SNF proteins are components of an SRB/SWI/SNF complex, also known as the mediator, which is tightly associated with the RNA polymerase II CTD. Both the holoenzyme and the SRB/SWI/SNF complex have nucleosome disruption activities previously ascribed to the SWI/SNF complex. In addition, the holoenzyme facilitates the binding of TBP to nucleosomal DNA in an ATP-enhanced manner.

Diverse Transcriptional Activators Require SWI/SNF Function In Vivo

Mutations in *SWI1*, *SWI2/SNF2*, *SWI3*, *SNF5*, and *SNF6* cause a substantial reduction in the ability to activate transcription of a wide variety of well-studied genes in yeast cells, including *HO* (Stern et al., 1984), *SUC2* (Neigeborn and Carlson, 1984), *Ty* (Happel et al., 1991),

A

Holoenzyme
Mono S Fractions

B

SRB/SWI/SNF Complex
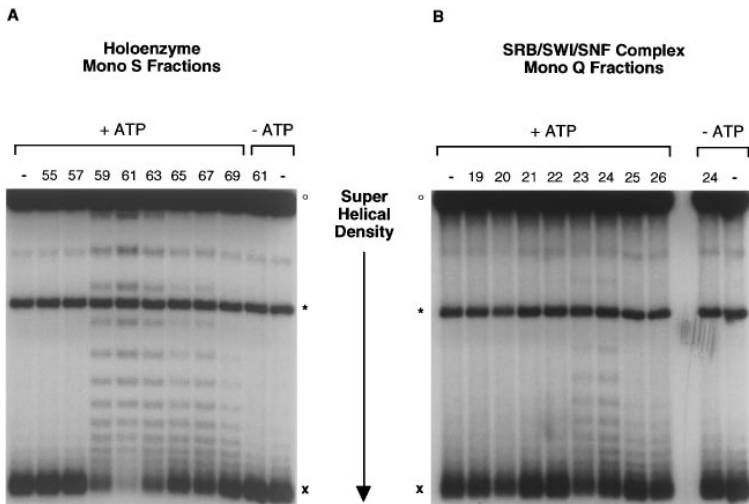Mono Q Fractions

Super
Helical
Density

Figure 7. The Holoenzyme and the SRB/SWI/SNF Complex Reduce the Superhelical Density of Chromatin-Assembled Plasmids in an ATP-Dependent Manner

Fractions from the last column of holoenzyme purification (A) and from the last column of SRB/SWI/SNF complex purification (B) were assayed in the presence of 4 mM ATP. Peak fractions of purified holoenzyme and SRB/SWI/SNF complex were assayed with and without 4 mM ATP present as described in Experimental Procedures. The symbols o, *, and x indicate nicked circular plasmid DNA, linear DNA, and highly supercoiled circular DNA, respectively.

INO1 (Peterson et al., 1991), and *ADH1* and *ADH2* (Peterson and Herskowitz, 1992; Taguchi and Young, 1987). For example, *ADH1* and *SUC2* gene expression is reduced by about an order of magnitude in strains in which *SWI1*, *SWI2*, or *SWI3* has been deleted (Peterson and Herskowitz, 1992). Experiments with reporter constructs have revealed that the *SWI* and *SNF* gene products are required for normal responses to a variety of gene-specific activators in yeast such as GAL4, Drosophila *fushi tarazu*, mammalian glucocorticoid and estrogen receptors, and LexA–GAL4 and LexA–Bicoid fusion proteins (Peterson and Herskowitz, 1992; Laurent and Carlson, 1992; Yoshinaga et al., 1992).

We have proposed that the RNA polymerase II holoenzyme is recruited to promoters by activators in vivo

(Koleske and Young, 1994). Ptashne and colleagues have shown that recruiting a component of the SRB complex to promoters, presumably in association with the holoenzyme, suffices to obtain activated levels of transcription in vivo (Barberis et al., 1995; M. Ptashne, personal communication). Thus, evidence that LexA fusions with SWI2/SNF2, SNF5, SNF6, and SNF11 proteins are sufficient to activate transcription of a target gene in vivo (Laurent et al., 1990, 1991; Treich et al., 1995) might now be interpreted in terms of holoenzyme recruitment to the target promoter.

We propose that recruitment of the holoenzyme to a specific promoter in vivo provides a means to facilitate TBP binding, regardless of the nucleosome structure at that promoter. The holoenzyme can enhance binding of TBP and TFIIA to a mononucleosome in vitro in the absence of ATP (Figure 8), a result compatible with evidence that the polymerase and general transcription factor components of the holoenzyme provide additional protein–protein and protein–DNA interactions that should stabilize TBP binding (Buratowski, 1994). Holoenzyme-facilitated TBP binding to a mononucleosome is greater in the presence of ATP, which presumably reflects the ATP-dependent nucleosome disruption activity of the SWI/SNF proteins. These observations are consistent with the idea that SWI/SNF protein function is necessary at the subset of promoters whose chromatin structure is particularly restrictive for TBP binding.
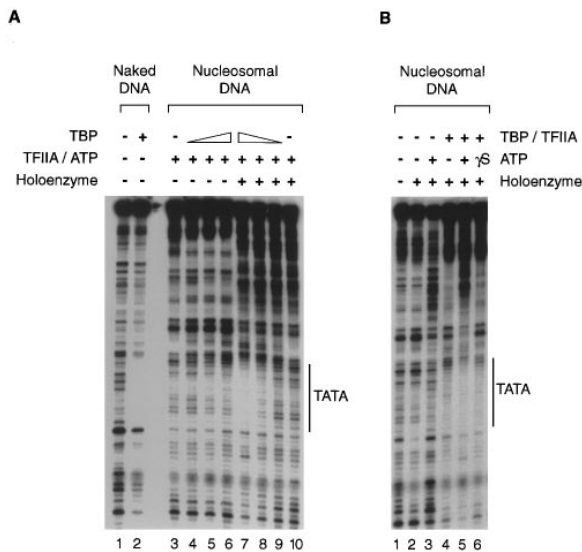
SWI/SNF in the Holoenzyme Accounts for Previous Genetic Observations

The presence of SWI/SNF proteins in the RNA polymerase II holoenzyme and the observation that these proteins are components of a subcomplex that interacts with the RNA polymerase II CTD explain several previous observations. SWI/SNF proteins are necessary for transcription activation of many genes in yeast cells (reviewed by Winston and Carlson, 1992; Carlson and Laurent, 1994; Peterson and Tamkun, 1995); CTD truncation adversely affects the response to activators in yeast and mammalian cells (Allison and Ingles, 1989; Scafe et al., 1990; Gerber et al., 1995). Cells with RNA polymerase II CTD truncation mutations, cells with certain *SRB* mutations, and cells with *SWI1*, *SWI2*, or *SWI3*

A

B

Figure 8. Holoenzyme Facilitates Binding of yTBP and yTFIIA to a Nucleosome Containing a TATA Box in an ATP-Enhanced Manner

(A) Increasing amounts of yTBP in the presence of yTFIIA and 4 mM ATP were tested for the ability to bind to a TATA box containing nucleosome with and without holoenzyme present.

(B) Nucleosomes were incubated with and without holoenzyme, 4 mM ATP or 4 mM ATPγS, yTBP and yTFIIA, as indicated.

mutations exhibit remarkably similar phenotypes (Peterson and Herskowitz, 1992; Thompson et al., 1993; Hengartner et al., 1995). The association of the SRB/SWI/SNF complex with the CTD accounts for the observation that cellular defects due to CTD mutations and SWI mutations can be alleviated by mutations in SIN1 and SIN2, which encode an HMG1-related protein and histone H3, respectively (Kruger and Herskowitz, 1991; Peterson et al., 1991; Peterson and Herskowitz, 1992).

SRB/SWI/SNF Complex Is Associated with the RNA Polymerase II CTD

The SRB/SWI/SNF complex is tightly associated with the RNA polymerase II CTD. Independent attempts to purify various SRB proteins by column chromatography have always led us to purify the same multiprotein complex: the RNA polymerase II holoenzyme (Koleske and Young, 1994; Koleske et al., 1996; Hengartner et al., 1995; Liao et al., 1995; reviewed by Koleske and Young, 1995). Only very small amounts of SRB protein can be detected that are not associated with the holoenzyme. Two different methods have been described that permit partial purification of an SRB subcomplex. An SRB complex can be isolated using a CTD affinity column (Thompson et al., 1993) or by releasing it from a holoenzyme preparation by using monoclonal anti-CTD antibodies (Kim et al., 1994). Because neither of these preparations is homogeneous, we further purified the SRB complex obtained by CTD affinity chromatography (Figure 4). The SRB and SWI/SNF proteins coelute in the final step of the purification.

We also found that the SRB complex isolated by anti-CTD antibody release contains SWI and SNF proteins. Kim et al. (1994) demonstrated that reconstitution of the response of the holoenzyme to activators required the presence of a subcomplex that could be isolated from holoenzyme with anti-CTD antibodies, which contained SRB2, SRB4, SRB5, and SRB6, and was called the mediator of activation. Our own studies with the mediator, which was purified precisely as described by Kim et al. (1994) and has chromatographic and transcriptional properties identical to those originally described for this subcomplex, revealed that it contained all nine of the known SRB proteins (Hengartner et al., 1995). Thus, the mediator preparation and the SRB complex obtained by CTD affinity chromatography contain very similar, if not identical, complexes.

We have shown that the RNA polymerase II holoenzyme, and its SRB/SWI/SNF subcomplex, contain SWI2/SNF2, SWI3, SNF5, and SNF11. Although we do not have direct biochemical evidence that SWI1 and SNF6 are present in the holoenzyme, other genetic and biochemical data indicate that it is highly likely that SWI1 and SNF6 are also subunits of these complexes (Cairns et al., 1994; Côté et al., 1994; Laurent and Carlson, 1992; Laurent et al., 1991; Peterson and Herskowitz, 1992).

Are There Multiple SWI/SNF Complexes?

Large multisubunit complexes containing yeast SWI and SNF proteins have been purified to varying extents (Cairns et al., 1994; Côté et al., 1994; Peterson et al., 1994). Characterization of two of these preparations by Western blot analysis did not reveal the presence of SRB proteins (Peterson et al., 1994; Cairns et al., 1994). This suggests that the purification procedures employed in these studies separated the SRB and SWI/SNF proteins or that SWI/SNF complexes can exist independent of the holoenzyme.

Since SWI2/SNF2 and SNF5 are stoichiometric components of the holoenzyme and since yeast cells contain 2000–4000 molecules of RNA polymerase II holoenzyme, there are at least 2000 molecules of SWI2/SNF2 and SNF5 molecules per cell. Based on their SWI/SNF complex purification, Côté et al. (1994) estimated that there are between 50 and 150 copies of the SWI/SNF complex in yeast cells. One interpretation of these results is that most SWI/SNF protein resides in the RNA polymerase II holoenzyme, and the form of SWI/SNF complex purified by Côté et al. (1994) is the small amount of SWI/SNF protein that is in the process of assembly into holoenzyme or, alternatively, it represents a subcomplex that can be dissociated from the holoenzyme.

The ability to immunoprecipitate very similar holoenzyme complexes from crude yeast fractions using anti-SRB and anti-SWI antibodies suggests that most of the SWI/SNF protein in these fractions is associated with the holoenzyme. If the SRB and SWI/SNF proteins were in separate complexes, then the relative ratios of SRB and SWI/SNF proteins would differ in the anti-SRB and anti-SWI immunoprecipitates. However, the similar relative ratios of SRB and SWI/SNF proteins found in immunoprecipitates obtained with anti-SRB and anti-SWI antibodies (Figure 1) indicate that the SRB and SWI/SNF proteins are components of the same complex in the crude extract.

SWI/SNF Function Is Highly Conserved in Eukaryotes

SWI/SNF proteins and their functions appear to be highly conserved in eukaryotes. Putative homologs of SNF2/SWI2 include Drosophila brahma and human hbrm and hBRG1, which have been cloned and implicated in transcriptional regulation (Tamkun et al., 1992; Khavari et al., 1993; Muchardt and Yaniv, 1993). A mammalian homolog of SNF5, called INI1, has also been cloned (Kalpana et al., 1994). A human SWI/SNF complex has been partially purified that has nucleosome disruption activities similar to those of the yeast SWI/SNF complex (Imbalzano et al., 1994; Kwon et al., 1994). The human SWI/SNF complex contains both hBRG1 and INI1 proteins (Kalpana et al., 1994; G. R. S., unpublished data), as would be expected based on the yeast results. Like the yeast SWI/SNF complex, the human SWI/SNF complex facilitates the binding of activators to nucleosomal DNA.

Implications for Mechanisms Involved in Transcriptional Activation

Our evidence indicates that the RNA polymerase II holoenzyme consists of core RNA polymerase II, all the general transcription factors other than TBP and TFIIA, and a CTD-associated SRB/SWI/SNF subcomplex. The presence of the SRB/SWI/SNF subcomplex in the RNA polymerase II holoenzyme has implications for the

mechanisms involved in transcription activation in vivo. Dynamic competition between chromatin proteins and an activator for a specific DNA site could be resolved in favor of the activator once the SWI/SNF-containing holoenzyme was recruited to the promoter. In this model, the activator and the holoenzyme both contribute to stable transcription initiation complex formation; the activator recruits the holoenzyme by binding to a subset of its components, and the SWI/SNF components of the holoenzyme enhance the stability of the activator–DNA interaction by destabilizing nucleosomes. This model is attractive because it provides a simple solution to the question of how SWI/SNF proteins are brought to promoters and it accounts for the coactivating and nucleosome disruption activities observed in vivo and in vitro for the SWI and SNF proteins.

Experimental Procedures

Immunoprecipitations
All immunoprecipitations were done as described (Hengartner et al., 1995). In brief, 50 μl of the DEAE 400 fraction was diluted 1:4 with modified transcription buffer (MTB) (50 mM HEPES–KOH [pH 7.3], 100 mM potassium acetate, 25 mM MgAc, 5 mM EGTA, 1 μM DTT, 10% glycerol, 0.01% NP-40, 1 mM PMSF, 2 mM benzamidine, 2 μM pepstatin A, 0.6 μM leupeptin, and 2 μg/ml chymostatin) minus the postassium acetate. We added 4 μg of ovalbumin, 4 μg HA-GST, and 2 μg of BSA to each reaction prior to the addition of antibody and then added 0.4 μg of affinity-purified α-SRB5, 0.15 μg of affinity-purified α-SWI3, or 1.5 μg of affinity-purified α-TGFβ to the respective reactions and allowed them to incubate 2 hr at 4°C; 15 μl of goat anti-rabbit covalently linked to magnetic beads (Dynal) were then added and incubated for 1 hr at 4°C with constant agitation. Beads were precipitated with a magnet and washed three times in 200 μl of MTB buffer. The final wash contained no NP-40. Proteins were eluted off the magnetic beads by boiling in 20 μl of sample buffer.

Western Blotting
All Western blots were performed as described (Koleske and Young, 1994). Proteins were detected with the following antibodies: SRB2, SRB4, SRB5, SRB6 (Thompson et al., 1993), SRB8, SRB9 (Hengartner et al., 1995), SRB10, SRB11 (Liao et al., 1995), SWI2/SNF2, SNF5 (gift of B. Laurent), SWI3 (gift of C. Peterson), SNF11 (gift of I. Treich and M. Carlson), TFIIEα, and TFIIEβ (C. J. W. and R. A. Y., unpublished data). Quantitative Western blots were performed as described (Koleske and Young, 1994). Recombinant standards were SRB5 (Thompson and Young, 1995), GST–SNF2/SWI2$_{1256-1703}$, and GST–SNF5$_{1-193}$ (gifts of B. Laurent). GST proteins were purified as described (Smith and Johnson, 1988). Concentrations of recombinant proteins were determined using a colorimetric assay (Bio-Rad) with bovine serum albumin as a standard.

Purification of Holoenzyme and Mediator
Holoenzyme was purified as described (Koleske and Young, 1994). Transcription assays for holoenzyme were done as described (Koleske and Young, 1994). Mediator was purified as described (Hengartner et al., 1995).

SRB/SWI/SNF Complex Purification
Whole-cell extract was prepared from Red Star yeast as described (Thompson et al., 1993). We centrifuged 1.2 liters of the ammonium sulfate pellet for 30 min at 5,000 rpm in an RC3B centrifuge (Sorvall). The pellet was resuspended in 900 ml of buffer A (20 mM K-HEPES [pH 7.6], 1 mM EDTA, 1 mM DTT, 20% glycerol, and protease inhibitors [Thompson et al., 1993]). The suspension was centrifuged again for 30 min at 5,000 rpm in an RC3B centrifuge (Sorvall). The supernatant was mixed with 200 g (dry) of BioRex 70 and stirred for 20 min. The suspension was packed into a column with a 5 cm diameter and washed with 1.5 liters of buffer A plus 100 mM KOAc. Bound proteins were eluted with buffer A plus 600 mM KOAc. Fractions containing protein were pooled, frozen in liquid nitrogen, and stored at −70°C until use. Eluates from two BioRex columns (320 ml, 1.0 g of protein) were thawed and pooled; 320 ml of buffer A plus 2% Triton X-100 were added, and the mixture was centrifuged for 30 min at 12,000 rpm in a GSA rotor (Sorvall). The supernatant was loaded onto a 15 ml CTD affinity column prepared as described (Thompson et al., 1993) at a flow rate of 200 ml/hr. The column was washed with 100 ml of buffer A plus 300 mM KOAc plus 1% Triton X-100, or 100 ml of buffer A plus 300 mM KOAc. Bound proteins were eluted with buffer A plus 300 mM KOAc plus 1 M urea at a flow rate of 25 ml/hr. Fractions containing protein (3.7 mg) were pooled, frozen in liquid nitrogen, and stored at −70°C. The CTD column was equilibrated with buffer A plus 300 mM KOAc plus 1% Triton X-100, and the flowthrough was loaded again. The column was washed and eluted as before. Fractions containing protein (1.8 mg) were pooled, frozen in liquid nitrogen, and stored at −70°C. The CTD eluates were pooled, diluted with 1.5 vol of buffer A plus 0.01% NP-40, and centrifuged for 10 min at 17,000 rpm in an SS-34 rotor (Sorvall). The supernatant was loaded onto a Mono S HR 5/5 (Pharmacia) at a flow rate of 0.3 ml/min. The column was washed with 3 ml of buffer A plus 120 mM KOAc plus 0.01% NP-40. Bound proteins were eluted with a 20 ml gradient of buffer A plus 0.01% NP-40 from 120 mM to 1000 mM KOAc. Fractions were frozen in liquid nitrogen and stored at −70°C until use. Fractions containing SRB4 and SRB5 as assayed by Western blotting were pooled and diluted with 2 vol of buffer B (20 mM Tris OAc [pH 7.6] plus 20% glycerol plus 1 mM DTT plus 0.01% NP-40 plus protease inhibitors). The mixture was centrifuged for 5 min in a microcentrifuge. The supernatant was loaded onto a Mono Q HRR 5/5 column (Pharmacia) at a flow rate of 0.3 ml/min. The column was washed with 1 ml of buffer B plus 200 mM KOAc. Bound proteins were eluted with a 40 ml gradient of buffer B from 200 mM to 2000 mM KOAc. The yield of SRB complex was approximately 100 μg. We analyzed 1 μl of each fraction by silver staining and 7.5–10 μl of each fraction by Western blotting.

Nucleosomal Disruption and Facilitated Transcription Factor Binding Assays
The PH MLT (Figure 6) or PH MLT($+$3) (Figure 8) restriction fragments were assembled into rotationally phased mononucleosome particles, purified by glycerol gradient centrifugation, and assayed as described (Imbalzano et al., 1994). At the nucleosome concentrations and reaction conditions employed in this and previous studies, nucleosomes were determined to be stable on the basis of resistance to micrococcal nuclease, the appearance of a 10 bp repeat pattern upon DNase I digestion, and exhibition of reduced mobility upon electrophoresis in native polyacrylamide gels. We have not observed the appearance of free DNA due to nucleosome dissociation in any of our experiments.

In Figure 6, holoenzyme fractions were the same as those used in Figure 1A. In Figure 6A, 0.3 μl of each fraction was assayed in the presence of 4 mM ATP. For the titration of holoenzyme, 0 μl, 0.015 μl, 0.05 μl, 0.15 μl, and 0.5 μl of fraction 60 was used, respectively, with and without 4 mM ATP as indicated. SRB/SWI/SNF fractions were the same as those used in Figures 3C and 3D. In Figure 6C, 1.7 μl of each fraction was assayed in the presence of 4 mM ATP. For the titration in Figure 6D, 0 μl, 0.07 μl, 0.2 μl, 0.7 μl, and 2.0 μl of fraction 24 was used, respectively, with and without 4 mM ATP as indicated.

For Figure 8, binding of yeast TBP (yTBP) and yTFIIA to nucleosomes containing the PH MLT($+$3) restriction fragment was performed as previously described (Imbalzano et al., 1994). In Figure 8A, all reactions contained 4 mM ATP. Following a 30 min incubation at 30°C in the presence or absence of holoenzyme (as indicated), increasing amounts of yTBP in the presence of yTFIIA were added. TBP concentrations were 0 (lanes 1, 3, and 10), 0.04 μM (lanes 2, 4, and 9), 0.4 μM (lanes 5 and 8), and 4 μM (lanes 6, 7). yTFIIA (1.5 μM) was also added to all reactions. In Figure 8B, reactions were treated with holoenzyme, alone (lanes 2 and 4), in the presence of 4 mM ATP (lanes 3 and 5), or in the presence of 4 mM ATPγS (lane 6) for 30 min at 30°C, followed by addition of 4 μM yTBP in the presence of 1.5 μM yTFIIA.

Recombinant yTBP was purified as described (Hoey et al., 1990), except that the heparin peak was further purified on a Mono S HR5/5 FPLC column (Pharmacia). Recombinant yTFIIA was purified as described (Ranish et al., 1992).

Supercoiling Reduction Assay

Plasmid chromatin was assembled and purified as described (Kwon et al., 1994). Reactions, total volume 12.5 μl, contained chromatin (2 ng of DNA), 1 U of topoisomerase I (Promega), 2.5 μl of 30% glycerol gradient buffer, 7 μl of buffer A minus KCl, 7 mM MgCl$_2$, 50–100 mM KOAc (final), 4 mM ATP where indicated, and 2 μl of holoenzyme Mono S fractions or 1 μl of SRB/SWI/SNF complex Mono Q fractions. Reactions were stopped after 90 min at 30°C by addition of 6 μl of stop buffer (3% SDS, 100 mM EDTA, 50 mM Tris–HCl [pH 8.0], 25% glycerol, 2 mg/ml proteinase K). Reactions were incubated for 90 min at 37°C and resolved on a 2% agarose gel (50 mM Tris–phosphate [pH 7.3], 1mM EDTA) for 40 hr, at 40 V. Gels were dried and exposed to film.

Acknowledgments

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