Catalytic activity of the yeast SWI/SNF complex on reconstituted nucleosome arrays

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A novel, quantitative nucleosome array assay has been developed that couples the activity of a nucleosome ‘remodeling’ activity to restriction endonuclease activity. This assay has been used to determine the kinetic parameters of ATP-dependent nucleosome disruption by the yeast SWI/SNF complex. Our results support a catalytic mode of action for SWI/SNF in the absence of nucleosome targeting. In this quantitative assay SWI/SNF and ATP lead to a 100-fold increase in nucleosomal DNA accessibility, and initial rate measurements indicate that the complex can remodel one nucleosome every 4.5 min on an 11mer nucleosome array. In contrast to SWI/SNF action on mononucleosomes, we find that the SWI/SNF remodeling reaction on a nucleosome array is a highly reversible process. This result suggests that recovery from SWI/SNF action involves interactions among nucleosomes. The biophysical properties of model nucleosome arrays, coupled with the ease with which homogeneous arrays can be reconstituted and the DNA accessibility analyzed, makes the described array system generally applicable for functional analysis of other nucleosome remodeling enzymes, including histone acetyltransferases.

Keywords: chromatin/gene regulation/nucleosome arrays/restriction enzyme/SWI–SNF

Introduction

Putative chromatin remodeling complexes, such as the yeast SWI/SNF complex (for a recent review see Burns and Peterson, 1997) and its mammalian homologs (Kwon *et al.*, 1994; Wang *et al.*, 1996), histone acetyltransferases, such as GCN5 (Brownell *et al.*, 1996), CBP (Bannister and Kouzarides, 1996), P300 (Ogryzko *et al.*, 1996), P/CAF (Yang *et al.*, 1996) and TAFII₂₅₀ (Mizzen *et al.*, 1996), and histone deacetylases (Taunton *et al.*, 1996; Alland *et al.*, 1997; Hassig *et al.*, 1997) are emerging as key players in the processes of cellular differentiation and oncogenesis. These factors appear to act as effectors for a large number of sequence-specific transcription factors such as members of the nuclear receptor superfamily (Hanstein *et al.*, 1996; Kamei *et al.*, 1996; Heinzel *et al.*, 1997; Nagy *et al.*, 1997), the *myb* proto-oncogene (Dai *et al.*, 1996), cAMP response element binding protein (Kwok *et al.*, 1994), the proto-oncogenes *c-jun* and *c-fos*

(Bannister and Kouzarides, 1995; Kamei *et al.*, 1996), bHLH proteins (Eckner *et al.*, 1996; Sartorelli *et al.*, 1997), the p53 proto-oncogene (Gu *et al.*, 1997; Lill *et al.*, 1997), the Rel proto-oncogene (Gerritsen *et al.*, 1997), E2F1 (Trousche and Kouzarides, 1996) and a growing number of other transcription factors involved in regulated gene expression (Kasten *et al.*, 1996; Oliner *et al.*, 1996; Horvai *et al.*, 1997; Kadosh and Struhl, 1997). In fact, chromatin remodeling is emerging as a prerequisite for nearly all regulated RNA polymerase II-mediated transcription *in vivo*.

The prototype of nucleosome remodeling complexes is the yeast SWI/SNF complex. Yeast SWI/SNF complex consists of 11 tightly associated subunits (Cairns *et al.*, 1994; Cote *et al.*, 1994; Treich *et al.*, 1995). Extensive genetic studies in *Saccharomyces cerevisiae* indicate that the SWI/SNF complex is required for the induction of a class of genes regulated at the transcriptional level and that it functions by antagonizing chromatin-mediated transcriptional repression (reviewed in Winston and Carlson, 1992; Peterson and Tamkun, 1995; Burns and Peterson, 1997). Previous *in vitro* studies have established that the yeast SWI/SNF complex destabilizes nucleosomes *in vitro* and that ATP hydrolysis is required for this reaction to occur (Cote *et al.*, 1994). This SWI/SNF ‘remodeling’ reaction involves a disruption of DNA–histone interactions but does not by itself result in nucleosome displacement (Cote *et al.*, 1994; Owen-Hughes *et al.*, 1996). This activity can lead to an enhanced affinity of transcription factors for their sites when these sites are incorporated into nucleosomes (Cote *et al.*, 1994; Owen-Hughes *et al.*, 1996; Utley *et al.*, 1997). The yeast SWI/SNF complex displays nanomolar affinity for DNA fragments longer than 200 bp and for synthetic four way DNA junctions; moreover, ATPase activity is stimulated by these DNAs (Quinn *et al.*, 1996). The majority of *in vitro* studies that have focused on the mechanism of SWI/SNF action have utilized mononucleosome substrates; in these studies nearly stoichiometric ratios of SWI/SNF to nucleosomes were required to observe nucleosome disruption (Cote *et al.*, 1994; Imbalzano *et al.*, 1994, 1996; Kwon *et al.*, 1994; Utley *et al.*, 1997).

Nucleosome arrays are more likely to mimic the natural substrate of the SWI/SNF complex, and therefore we decided to study SWI/SNF activity on nucleosome arrays. The DNA template that we have used is composed of 11 head-to-tail repeats of a 5S rRNA gene from *Lytechinus variegatus*. Each repeat can rotationally and translationally position a nucleosome after *in vitro* reconstitution (Simpson and Stafford, 1983; Simpson *et al.*, 1985; Hansen *et al.*, 1989; Dong *et al.*, 1990). In order to quantitate the activity of SWI/SNF complex on such arrays, we exploited an assay described by Polach and Widom (1995). This assay is based on measuring the kinetics of restriction

enzyme digestion of sites located within a reconstituted, positioned mononucleosome. Using this experimental system, Polach and Widom were able to demonstrate that mononucleosomal DNA accessibility can be treated as a dynamic equilibrium between accessible and occluded DNA. They introduced a new thermodynamic constant, K_{conf} , the conformational equilibrium constant of the nucleosome. K_{conf} is defined as the ratio of the rate of digestion of nucleosomal DNA and the rate of digestion of naked DNA per unit of enzyme. K_{conf} ranges from 10^{-1} to 10^{-6} , reflecting the fact that the occluded state of nucleosomal DNA is ten to a million times more prevalent than the accessible state, depending on the restriction enzyme used and the location of the restriction site within the nucleosome (Polach and Widom, 1995).

We introduced one of the 5S nucleosome positioning sequences described by Polach and Widom (1995), which bears a unique *SaII* site that is close to the dyad axis of a reconstituted nucleosome, into the middle of an array of ten 5S nucleosome positioning sequences. We show here that the rate of *SaII* cleavage of this nucleosome in the context of an array is decreased 2.5 orders of magnitude when naked and nucleosomal DNAs are compared, in agreement with the value obtained with a mononucleosome (Polach and Widom, 1995). Addition of purified yeast SWI/SNF complex increased K_{conf} 2 orders of magnitude, reflecting an increase in the accessibility of nucleosomal DNA to *SaII* by a factor of ~100. Furthermore, SWI/SNF activity on these array templates is readily observed when the concentration of nucleosomes is in large excess over SWI/SNF. We have taken advantage of the quantitative nature of this remodeling assay to estimate a turnover rate for SWI/SNF activity and also to investigate the rate at which SWI/SNF-remodeled nucleosomes convert back to the inaccessible state.

Results

Characterization of reconstituted nucleosome arrays

A diagram of the DNA template used in this study is shown in Figure 1A. It consists of 11 head-to-tail 208 bp 5S rRNA nucleosome positioning sequences from *Lytechinus variegatus*. The sixth repeat bears a unique *SaII* endonuclease restriction site which upon nucleosome reconstitution is located close to the dyad axis of symmetry of the nucleosome (Polach and Widom, 1995). This DNA template was labeled on each terminus with [α - ^{32}P]dCTP and nucleosomes were reconstituted by salt gradient dialysis starting with a ratio of purified chicken erythrocyte histone octamers to nucleosome positioning sequences of 1.

We used four complementary approaches to characterize our reconstituted arrays with respect to the efficiency of the reconstitution, nucleosome positioning and saturation of the templates with nucleosomes. First, we sedimented the unassembled and reconstituted DNAs on 13–35% linear glycerol gradients (Figure 1B). This analysis indicated that no free DNA template is present after reconstitution. SDS–PAGE analysis of the purified reconstitutes also indicates that the arrays contained an equi-molar stoichiometry of the four core histones. Second, we labeled only one end of the template DNA with [α - ^{32}P]dATP and exposed the reconstituted arrays to micrococcal nuclease.

Partial micrococcal nuclease digestion showed that the arrays consist of the predicted 11 evenly spaced nucleosomes (Figure 1C). Third, to assess the degree of nucleosome saturation and positioning, the arrays were exposed to *EcoRI* restriction endonuclease. Each 5S positioning sequence is flanked by two *EcoRI* sites (Figure 1A). Therefore, *EcoRI* digestion of the nucleosome arrays will release either naked, 195 bp DNA fragments (232 bp in the case of the repeat that contains the *SaII* site), or mononucleosomes which migrate on a native acrylamide gel with an apparent mobility of 400 bp (Hansen *et al.*, 1989). When this analysis was performed using arrays that were reconstituted at a histone octamer to 5S repeat ratio of 1.0, <10% of the 5S repeats migrated as naked DNA, 60% migrated as mononucleosomes, 30% migrated as nucleosome multimers and <2% of the DNA remained in the well of the gel (Figure 1D). The nucleosome multimers are indicative of alternative nucleosome positions on the 5S repeats that result in the occlusion of one or more *EcoRI* sites (Hansen *et al.*, 1989; Dong *et al.*, 1990; Pennings *et al.*, 1991; Hansen and Lohr, 1993). The absence of a 232 bp fragment in lane 2 shows that the *SaII* site-bearing 5S repeat is efficiently incorporated into a nucleosome. The appearance of 10% free 5S repeats is consistent with the majority of arrays being fully saturated with 11 nucleosomes (Hansen *et al.*, 1989). Fourth, as a rigorous means to obtain an estimate of the level of nucleosome occupancy of the array templates, reconstituted nucleosome arrays were subjected to a sedimentation velocity analysis in the analytical ultracentrifuge. The integral distribution of sedimentation coefficients of a reconstituted array obtained from the analysis of a typical sedimentation velocity experiment by the method of van Holde and Weischet (1978) is shown in Figure 1E. In this case, ~80% of the arrays sedimented at 28–29S which is consistent with the value expected for a saturated array of 11 nucleosomes (Hansen *et al.*, 1989). In addition, 10% of the arrays appear to sediment as a subsaturated species that may lack one octamer, and 10% sedimented as arrays that either contain 11 octamers with a small amount of additional non-specifically bound histones or as arrays that were slightly folded due to residual salt in the sample.

SaII digestion conditions and kinetics

The SWI/SNF assay we describe in this report consists of a coupled reaction whereby substrate nucleosome arrays are remodeled by the SWI/SNF complex to enhance the access of *SaII* restriction enzyme to its DNA target site. Hence, reaction conditions had to be sought where both the SWI/SNF and the *SaII* reactions could take place. The optimal cleavage of naked DNA by *SaII* requires 150 mM NaCl and 10 mM MgCl_2 . These cation concentrations, however, are known to favor compaction and self-association of saturated nucleosome arrays (Hansen *et al.*, 1989; Schwarz and Hansen, 1994; Schwarz *et al.*, 1996). Conditions were therefore sought where *SaII* would efficiently cleave naked DNA but where the arrays would be in the extended ‘beads on a string’ conformation (Schwarz *et al.*, 1996). Concomitant titration of MgCl_2 and NaCl revealed that *SaII* had near maximal activity in the presence of 2.5 mM MgCl_2 and 125 mM NaCl (data not shown). Under these conditions we observe no self-association of the arrays (data not shown), and, based on previous studies

Fig. 1. Characterization of reconstituted nucleosome arrays.

(A) Schematic representation of the template DNA used to reconstitute nucleosome arrays; five head-to-tail 208 bp 5S rRNA repeats flank both sides of a modified 5S repeat that bears a unique *SalI* site. Wild-type 5S repeats are flanked by *EcoRI* sites that are 195 bp apart, the *SalI*-modified 5S repeat is located on a 232 bp *EcoRI* fragment. (B) Glycerol gradient sedimentation of ³²P-labeled naked template DNA (■) or labeled reconstituted arrays (○). Fractions (0.4 ml) were counted by scintillation and are numbered from the top to bottom of the gradients. Inset shows free and reconstituted histone octamers electrophoresed on an 18% SDS-PAGE that was stained with Coomassie. (C) Time course of micrococcal nuclease digestion of array and naked DNA visualized on a 2% agarose gel. The predicted position of nucleosomes is indicated to the right by ovals. The *SalI* site-containing nucleosome is shaded. (D) *EcoRI* digestion of naked template or the nucleosome arrays followed by electrophoresis of the digestion products on a 4% native polyacrylamide gel. The *SalI*-modified 5S sequence is located on a 232 bp fragment (A) that is visible in the left lane; it represents 9% of the total DNA. All the other 5S sequences consist of 195 bp *EcoRI* fragments (left lane). The slower migrating species in the right hand lane are formed at the expense of the naked DNA species, these are mononucleosomes (1N) and oligonucleosomes (arrowheads). Note that the absence of the 232 bp fragment in the array lane indicates that it is incorporated into nucleosomes. (E) Sedimentation velocity experiment. Reconstituted arrays were sedimented in 10 mM Tris pH 7.5, 1 mM EDTA in an XL-A (Beckman) analytical ultracentrifuge equipped with scanner optics. The integral distribution of sedimentation coefficients is shown. Note that the predicted sedimentation of a saturated array that contains 11 nucleosomes is 28–29S.

where high concentrations of monovalent cations were found to eliminate the Mg^{+2} -dependent stimulation of array self-association (Schwarz *et al.*, 1996; J.Hansen, personal communication), we predict that the arrays are either fully extended or in an intermediate state of compac-

tion. Nucleosome disruption by SWI/SNF complex is optimal in 50–125 mM NaCl and 3–7 mM $MgCl_2$ (Cote *et al.*, 1994; A.Imbalzano, personal communication). Thus, all of the experiments reported in this study were conducted in 125 mM NaCl and 2.5 mM $MgCl_2$.

The first order rate of *SalI* digestion of mononucleosomes bearing the *SalI* target sequence was reported to be ~2-fold lower than the rate on naked DNA (Polach and Widom, 1995). In the context of a nucleosome array we find that the digestion kinetics are biphasic (Figure 2A). In the first phase, 30–50% of the template DNA is digested at a rate similar to that of naked DNA. This is followed by a second phase where the template DNA is cleaved 300 times more slowly (Figure 2A). Increasing the ratio of octamers to 5S repeats during reconstitution did not have a great influence on the fraction of template cleaved during the first phase, indicating that the biphasic kinetics is not due to sub-saturation of the arrays (see also Figure 1). Furthermore, the biphasic kinetics were observed at a higher concentration of array (40 nM), indicating that these kinetics are not due to dilution (van Holde, 1988). In contrast, we did observe a large increase in the fraction cleaved in the first phase when sub-saturated arrays were analyzed (sub-saturated arrays were generated by lowering the ratio of histone octamers to 5S repeats; data not shown). To verify that the biphasic nature of the cleavage kinetics is not a unique feature of the *SalI* site-bearing 5S repeat, we used another restriction enzyme, *MspI*, which cleaves each 5S repeat ~20 bp from the predicted dyad axis of a reconstituted 5S nucleosome (Hansen *et al.*, 1989). A time course of *MspI* digestion revealed that each *MspI* site in the array is also cleaved with biphasic kinetics (Figure 2B). Similar results were obtained for *MspI* digestion of saturated 12mer arrays by Hansen and Lohr (1993). We conclude that the biphasic nature of the kinetics of restriction enzyme cleavage of 5S repeats after reconstitution into nucleosome arrays is not a unique feature of the central, *SalI* site-bearing 5S repeat, but that it is a feature shared by all 5S repeats in the array. One possibility is that this reaction is a measure of the adoption of alternative positions of the histone octamers onto the 5S repeats as suggested earlier (Hansen and Lohr, 1993; see also Figure 1D).

By limiting our analysis to the second phase of the restriction enzyme reactions, we determined that the first order rate of *SalI* cleavage per unit of *SalI* of nucleosomal DNA is $9.19 \pm 6.4 \times 10^{-7}$ (Figure 2B and data not shown). By comparison, the rate of cleavage of the naked DNA template is $2.86 \pm 0.03 \times 10^{-4}$. These values yield a conformational equilibrium constant of the central nucleosome bearing the *SalI* site of 3.2×10^{-3} which is in good agreement with the mononucleosome study of Polach and Widom (1995). Furthermore, the difference in cleavage rates between naked and array DNA holds over the range of *SalI* concentrations used (Figure 2C), providing support for the view that, in the context of nucleosome arrays, the rate-limiting step in restriction enzyme accessibility to nucleosomal DNA is a dynamic equilibrium between the occluded and exposed state of nucleosomal DNA (Polach and Widom, 1995).

SWI/SNF complex enhances the rate of *SalI* digestion of nucleosome arrays

To assess the capacity of SWI/SNF complex to increase the accessibility of the *SalI* site in the central nucleosome of the array, 2 nM of array was exposed to 5000 U/ml of *SalI*, either in the presence or in the absence of 4 nM SWI/SNF complex and 1 mM ATP (Figure 3A). The

Fig. 2. Kinetics of *SalI* digestion of nucleosome arrays. **(A)** Time course of *SalI* digestion of arrays that contain a unique *SalI* site. Arrays (2 nM) were exposed to 5000 U/ml of *SalI*. The fraction of uncut nucleosome array DNA templates is plotted against time. Note that the reaction proceeds through two phases, a rapid and a slow phase. **(B)** *MspI* digestion of the 11mer *SalI* array. Naked array template DNA (lane 2) was digested with *MspI* for 2 min to generate a ladder of partial digestion products (lane 3) and reconstituted arrays were digested with *MspI* for 10, 30 and 60 min (lanes 4, 5 and 6). The products of a 30 min digestion of the nucleosome array with *SalI* are shown in lane 7. The positions of the 0.6 and 1.5 kb markers are shown to the left of the marker lane (lane 1). Note that the kinetics of *MspI* digestion of the arrays is also biphasic. **(C)** Dependence of the rate of cleavage of naked DNA (○) or nucleosome array (●) on the concentration of *SalI* enzyme. Nucleosome arrays (2 nM) were pre-treated with 250 U/ml of *SalI* for 3 h before addition of *SalI* to final concentrations ranging from 400 to 5000 U/ml. Naked DNA (2 nM) was digested with 50–5000 U/ml *SalI*. In both cases the first order rates of cleavage are proportional to the amount of *SalI* enzyme and differ by a factor of ~300 between naked and nucleosomal DNA.

Fig. 3. SWI/SNF stimulates *SalI* cleavage of nucleosome arrays. (A) Time course of digestion of 2 nM nucleosome array DNA by 5000 U/ml *SalI* in the presence or absence of 4 nM SWI/SNF complex and in the presence or absence of 1 mM ATP (lanes 1–6, 7–12, 13–18 and 19–24 represent time points taken at 5, 15, 30, 60, 90 and 120 min for the four parallel reactions). After phenol purification, the DNA substrate and products were resolved on a 1% agarose gel. The upper band is uncut template DNA and the doublet below (compare with Figure 2B, lane 7) is the product of *SalI* cleavage. (B) Quantitation of a time course similar to that shown in (A), where 5000 U/ml *SalI* were mixed with ± 1 mM ATP and ± 4 nM SWI/SNF complex. The reactions were started by addition of 2 nM arrays.

presence of SWI/SNF and ATP did not affect the rate of cleavage of the initial pseudo-naked DNA (Figure 3, data not shown), but cleavage of the array DNA in the second, slow phase, was greatly enhanced (Figure 3A and B). Under these conditions, *SalI* digestion of the array DNA was essentially complete after 60 min. The enhancement of *SalI* cleavage required both SWI/SNF and ATP, and ATP could not be substituted with the non-hydrolyzable ATP analog, ATP γ S (data not shown).

Initial reaction velocity measurements

In order to obtain an apparent rate of SWI/SNF-mediated nucleosome remodeling in the context of the 11mer array, we measured initial *SalI* digestion rates under limiting SWI/SNF conditions and with an excess of *SalI* enzyme. Labeled array (8 nM) was exposed to 5000 U/ml *SalI* for 1 h to remove the pseudo-naked DNA from the reaction. After 1 h, SWI/SNF complex was added to a final concentration of either 2.0, 1.0, 0.5 or 0.25 nM. The extent of *SalI* cleavage was measured at 2 min intervals for 10 min and longer intervals subsequently (Figure 4A). Initial velocities of SWI/SNF-mediated nucleosome remodeling were obtained by subtracting the SWI/SNF-independent cleavage events from the observed cleavage events and are expressed as pmol of array cleaved per min (Figure 4A). The initial velocities calculated from the data obtained in the first 10 min following SWI/SNF

Fig. 4. Estimation of a turnover rate for nucleosome remodeling by SWI/SNF. (A) Initial rate measurements. An 8 nM solution of nucleosome array was incubated in the presence of 1 mM ATP with 5000 U/ml *SalI* for 1 h. SWI/SNF complex was then added to reach final concentrations of either 2 nM ($\circ$), 1 nM ($\square$), 0.5 nM ($\triangle$) or 0.25 nM (∇). As control, SWI/SNF buffer was added ($\bullet$). The fraction cut array was determined for samples taken at 2 min $\pm$ 5 s intervals for 10 min and then as indicated. (B) Plot of the increase in cleavage rates of nucleosome arrays by *SalI* versus concentration of SWI/SNF. The data are derived from the experiment shown in (A).

addition are directly proportional to the amount of SWI/SNF added (Figure 4B). In this SWI/SNF–*SalI*-coupled reaction, 0.2 pmol of SWI/SNF led to the cleavage of 4 fmoles of array per min by *SalI*. Hence, under conditions where *SalI* and nucleosomes are in excess, it appears to take one SWI/SNF complex 50 min to process one 11mer array. Therefore, if we assume that all nucleosomes on the array are equivalent to the central *SalI* nucleosome, and that SWI/SNF disrupts only one nucleosome of each array per remodeling event, then one SWI/SNF complex remodels one nucleosome every 4.5 min ($k_{\text{cat}} = 0.22/\text{min}$). This value is similar to the turnover numbers that have been obtained for restriction enzymes: 4.0 per min, 0.12 per min and 1.6 per min for *EcoRI* (Modrich and Zabel, 1976), *SalI* (Maxwell and Halford, 1982) and *EcoRV* (Halford and Goodall, 1988) respectively.

Fig. 5. DNA accessibility of SWI/SNF-remodeled nucleosomes. Reactions containing 2 nM array, 100 U/ml *SalI* (squares) and ATP buffer (■) or 1 mM ATP (□), were started by addition of SWI/SNF complex to a final concentration of 4 nM. To determine the fraction of arrays bearing occluded *SalI* sites, 5000 U/ml of *SalI* was added to a reaction lacking ATP (●). The fraction uncut array was determined at the indicated time points after addition of SWI/SNF complex. See text for details.

Evaluation of DNA accessibility in a SWI/SNF-remodeled nucleosome

Having determined a rate for SWI/SNF remodeling, we were in a position to obtain an estimate of the conformational equilibrium constant of a SWI/SNF-remodeled nucleosome. Conditions were chosen where the rate of *SalI* cleavage of naked DNA was 8-fold slower than the rate of SWI/SNF-mediated nucleosome remodeling. Under these conditions, the likelihood of *SalI* encountering an occluded site is low compared with that of encountering an accessible site, and the restriction kinetics therefore reflect the accessibility of the remodeled nucleosome's DNA.

The experiment displayed in Figure 2C indicates that 100 U/ml of *SalI* cleaves ~10 pmol of naked template DNA per min when the DNA concentration is 2 nM. Extrapolation from the initial velocity data shown in Figure 4B indicates that a 4 nM solution of SWI/SNF complex can remodel 80 pmol of reconstituted array per min. We therefore set up reactions containing $\pm$ ATP, 4 nM SWI/SNF complex, 2 nM array and 100 U/ml of *SalI*. In the presence of SWI/SNF but in the absence of ATP, 55% of the arrays were cleaved after 210 min (Figure 5). At such low *SalI* concentrations the digestion kinetics are no longer biphasic, suggesting that only the pseudo-naked *SalI* sites are cleaved in this time course (Figure 5). Consistent with this view, a similar fraction of array template is cleaved at high *SalI* concentrations early in the time course (Figure 5, closed circles). In the presence of SWI/SNF and ATP, cleavage proceeded with first-order kinetics, and 89% of the arrays were cleaved by 210 min (Figure 5). Quantitation of this SWI/SNF reaction yielded a first order rate of cleavage per unit of *SalI* of $9.3 \pm 4.8 \times 10^{-5} \text{ U}^{-1} \text{ min}^{-1}$. The rate of cleavage of naked DNA by *SalI* is $2.86 \pm 0.03 \times 10^{-4} \text{ U}^{-1} \text{ min}^{-1}$ (Figure 2). Thus, in the presence of SWI/SNF and ATP, the *SalI* site in the central nucleosome is cleaved only 3-fold slower than naked DNA and about 100 times faster than the unremodeled nucleosome. Expressed in terms of K_{conf} , the remodeled *SalI* nucleosome has a K_{conf} of 3×10^{-1} , 2 orders of magnitude higher than a control *SalI*-bearing nucleosome.

Re-addition of substrate experiment

To determine whether each SWI/SNF complex can remodel more than one nucleosome in our assay, a classic

Fig. 6. SWI/SNF functions catalytically to remodel nucleosome arrays. Substrate re-addition experiment. A reaction containing 5000 U/ml *SalI*, 1 mM ATP and 2 nM SWI/SNF complex was started by addition of 2 nM arrays. Aliquots were taken for analysis after 5, 15, 45 and 59 min. An additional 2 nM of arrays was added three times at 1 h intervals (●) and aliquots taken as in the preceding hour. Also shown is a parallel reaction where ATP was omitted and no array substrate was added back (○).

substrate re-addition experiment was performed. Two nM of *SalI* nucleosome array (corresponding to 22 nM of nucleosomes) was added back three times at 60 min intervals to a reaction that initially contained 2 nM SWI/SNF complex, 5000 U/ml *SalI* and 2 nM *SalI* array. In a control experiment, unlabeled nucleosome arrays were used in the initial incubation and 2 nM of naked labeled DNA was added back three times. In these control add-backs, >95% of the naked DNA was cleaved within 5 min, indicating that *SalI* activity did not decrease over time (data not shown). As a second control, ATP was omitted from the reaction, no array was added back, and the fraction uncut array was monitored. In this case, after 15 min, 47% of the arrays were uncut; after 4 h, 32% of the arrays were still uncut, indicating that in this particular experiment the first order rate of cleavage per unit of *SalI* of nucleosomal DNA was $3.34 \times 10^{-7} \text{ U}^{-1} \text{ min}^{-1}$ (Figure 6). In a reaction that contained SWI/SNF, the rate of *SalI* cleavage of the initial aliquot of labeled array was dramatically enhanced (Figure 6). Furthermore, after each re-addition of substrate, SWI/SNF was able to stimulate *SalI* digestion of the arrays. Subtracting the SWI/SNF-independent *SalI* cleavage of nucleosomal DNA from the SWI/SNF-stimulated *SalI* cleavage events indicates that 0.5 pmol of array, corresponding to 5.5 pmol of nucleosomes, were remodeled by 0.4 pmol of SWI/SNF complex during the 4 h time course. This demonstrates multiple rounds of nucleosome remodeling events per SWI/SNF complex.

Remodeling of nucleosome arrays by yeast SWI/SNF complex requires continuous ATP hydrolysis

Two groups have reported that continuous ATP hydrolysis is not required for human SWI/SNF- (Imbalzano *et al.*, 1996) or yeast SWI/SNF- (J.Cote, C.L.Peterson and J.L.Workman, submitted) dependent disruption of a rotationally positioned mononucleosome. In one case, ATP-dependent disruption of a mononucleosome was stable for extended periods of time even after removal of ATP and SWI/SNF (J.Cote, C.L.Peterson and J.L.Workman,

Fig. 7. SWI/SNF remodeling of nucleosome arrays requires continuous ATP hydrolysis. (A) Chicken erythrocyte histone octamer nucleosome arrays (4 nM) and SWI/SNF complex (4 nM) were incubated with 1 mM ATP for 1 h in the presence (□) or absence (■ and ●) of 1 U of apyrase. At $t = 60$ min, 5000 U/ml *SalI* was added with (■) or without (□ and ●) 1 U of apyrase. (B) Identical to (A), but nucleosome arrays were reconstituted with HeLa cell histone octamers.

submitted). To test whether SWI/SNF remodeling of nucleosome arrays is also irreversible, we used apyrase to remove ATP at different times during the SWI/SNF–*SalI*-coupled assay. In the reactions shown in Figure 7, 2 nM of nucleosome array, 2 nM SWI/SNF and 1 mM ATP was incubated at 37°C in the absence of *SalI*; after 1 h, 5000 U/ml *SalI* was added. If apyrase was added at the start of the reaction, then the rate of *SalI* cleavage was identical to the rate in the absence of SWI/SNF (open squares). As shown in Figure 7A, when apyrase was added concomitantly with *SalI* (at time 60 min), the digestion kinetics were identical to the reaction that received apyrase at time zero. At this concentration of apyrase, all detectable ATP is removed in <2 min (data not shown). Thus, although nearly 90% of the arrays potentially could have been in the remodeled state after this first 60 min incubation (see Figure 3B and Figure 7A, closed circles), the disrupted state was not stable and was efficiently reversed before addition of *SalI* and apyrase. Thus, in the context of a nucleosome array the remodeling reaction is rapidly reversible and ATP is continuously required for SWI/SNF-mediated enhancement of *SalI* cleavage of nucleosomal DNA. Besides the use of nucleosome arrays versus mononucleosomes, the sole difference between our experiments and a previous study (J.Cote, C.L.Peterson and J.L.Workman, submitted) is the source of histone octamers; chicken erythrocytes versus HeLa cells. However, we observed the same continuous requirement for ATP when HeLa cell octamers were used to generate nucleosome arrays (Figure 7B).

Fig. 8. *SalI* does not target SWI/SNF to nucleosomes that contain a *SalI* site. Competition studies. A reaction containing 2 nM of labeled nucleosome array (equivalent to 22 nM nucleosomes), 2 nM SWI/SNF complex and 1 mM ATP (○) was incubated with 5000 U/ml *SalI* either in the absence (○) or in the presence of a 10-fold excess of nucleosomes located on the 11mer *SalI* array itself (●), a 9mer array that lacks *SalI* sites (■), or a 5mer array that lacks *SalI* sites (▲). The fraction of the uncut, labeled *SalI* array template was measured after 25, 45, 90 and 186 min. Note that the 9mer array that lacks a *SalI* site competes for SWI/SNF activity with nearly the same efficiency as the 11mer array that contains a *SalI* site.

Does *SalI* target SWI/SNF activity?

One potential concern in this study is that *SalI* might preferentially target the SWI/SNF complex to the nucleosome bearing a *SalI* site and that we therefore cannot extrapolate our results to the 10 other nucleosomes of the array. To test this possibility, we carried out competition experiments using unlabeled arrays devoid of *SalI* sites. If *SalI* does not target SWI/SNF, then arrays that lack *SalI* sites should compete well for SWI/SNF. On the other hand, if *SalI* efficiently targets SWI/SNF to the nucleosomes that bear the *SalI* restriction site, then increasing the concentration of arrays devoid of *SalI* sites should not affect SWI/SNF stimulation of *SalI* activity.

We carried out coupled SWI/SNF–*SalI* reactions using 2 nM SWI/SNF complex, 5000 U/ml *SalI*, 2 nM labeled *SalI* array and a 10-fold molar excess of nucleosomes incorporated into either (i) an 11mer (the *SalI* array), (ii) a 9mer array lacking a *SalI* site or (iii) a 5mer array (the *SalI* array template that had been pre-cleaved with *SalI* prior to reconstitution). *EcoRI* digestion followed by native gel electrophoresis indicated that the competitor and labeled arrays were equivalently saturated (>80%, data not shown). The extent of *SalI* cleavage of the labeled array was measured at 25, 45, 90 and 186 min. As can be seen in Figure 8, the 9mer array that lacked a *SalI* site was able to compete for SWI/SNF almost as well as the nucleosomes positioned on the 11mer array that contained a *SalI* site. The 5mer arrays were also able to compete, but were less effective than the larger arrays. Similar results were obtained when competitor arrays were present at only a 2-fold molar excess of nucleosomes (data not shown). These results indicate that *SalI* does not efficiently target SWI/SNF activity, and furthermore that there is a positive correlation between array size and the capacity of arrays to compete for SWI/SNF complex.

Discussion

We have described a quantitative assay to measure nucleosomal DNA accessibility *in vitro* in a context that mimics nucleosome arrays on chromosomes *in vivo*. It consists of a two enzyme, coupled reaction where nucleosomal DNA in the middle of an array of 11 nucleosomes must first be

disrupted by a chromatin remodeling enzyme to serve as an efficient substrate for the *SalI* restriction endonuclease. This assay is simple, rapid and easily quantitated. In contrast, many other nucleosome disruption assays are either technically difficult (limited DNase I digestion; Cote *et al.*, 1994), time consuming (Southern blotting of limited MNase digestion ladders; Tsukiyama and Wu, 1995) or provide only an indirect readout of nucleosome disruption (gel retardation analysis of factor binding; Cote *et al.*, 1994). Using the restriction enzyme assay to probe DNA accessibility in the context of an array, we have analyzed the kinetic properties of the yeast SWI/SNF complex. Our data provide the first evidence that SWI/SNF is truly an enzyme that facilitates accessibility of nucleosomal DNA in a catalytic fashion.

Catalytic activity of SWI/SNF

A classical demonstration of catalysis involves the demonstration that the catalyst can convert more substrate than there is of itself, indicating multiple rounds of reaction per catalyst molecule. We have shown that 2 nM of SWI/SNF can enhance the *SalI* cleavage rate on 2.5 nM of *SalI* nucleosomes embedded in arrays consisting of 11 nucleosomes. The *SalI* site-bearing nucleosome is essentially equivalent to the other 10 nucleosomes on the array, thus 0.5 pmol of SWI/SNF complex potentially was able to remodel 5.5 pmol of nucleosomes. Our estimate for the level of catalytic activity for SWI/SNF is probably an under-estimate since the remodeled arrays that are cleaved by *SalI* (the 5mer array products) are not removed as substrates for additional SWI/SNF action. These remodeling events, however, can no longer be scored. A second complication in the quantitation of catalysis, and in the determination of a turnover number, is that we do not know how many nucleosomes of each array are remodeled per molecule of SWI/SNF. For example, the interaction of SWI/SNF with one nucleosome at the end of the array might be sufficient to disrupt all 11 nucleosomes. Alternatively SWI/SNF may only remodel one nucleosome at a time. Consistent with this latter possibility, we have recently shown by electron spectroscopic imaging (ESI) that the binding of each SWI/SNF complex to an array leads to the disruption of only a few nucleosomes per array (disruptions were visually detected by calculating ATP-dependent alterations in the histone:DNA ratios of individual nucleosomes; D.Basett-Jones, J.Cote, C.Landel, C.Peterson and J.L.Workman, submitted). Thus, for each remodeling event that disrupts a nucleosome containing a *SalI* site, a large excess of nucleosomes that do not have *SalI* sites must also have been disrupted.

In every previous study of SWI/SNF mechanism, near stoichiometric ratios of SWI/SNF to nucleosomes were required to observe efficient nucleosome disruption. Why is our assay more sensitive? One likely explanation lies in the composition of reconstituted nucleosome substrates in the previous studies. For example, in the studies of Cote *et al.* (1994) and Utley *et al.* (1997), mononucleosomes were reconstituted by the transfer of octamers from short arrays of HeLa nucleosomes. In the subsequent SWI/SNF reactions only the fate of the labeled mononucleosomes was followed, even though the reaction contained a large excess of short nucleosome arrays. Our results

suggest that there is a positive correlation between the apparent affinity of SWI/SNF for arrays and the number of nucleosomes on an array (Figure 8). Thus, in the former case, SWI/SNF may preferentially occupy the short donor arrays during the reaction, and near stoichiometric levels of SWI/SNF may be required to observe disruption of the labeled mononucleosome. In contrast, our assay uses a nucleosome array template that has an almost homogeneous composition (Figure 1E).

In the course of this study we found that ATP was continuously required for the coupled SWI/SNF-*SalI* reaction to proceed efficiently. The continued requirement for ATP indicates that SWI/SNF nucleosome remodeling is reversible. This result is consistent with a previous analysis of SWI/SNF action on a nucleosome array template (Owen-Hughes *et al.*, 1996). The coupled SWI/SNF-*SalI* reaction may therefore be described more accurately as a superimposed reaction where the first step depends on an equilibrium between remodeled and un-remodeled nucleosomes followed by the irreversible, restriction enzyme-mediated cleavage of array DNA. Reversal from the SWI/SNF-mediated remodeled state has not been observed when mononucleosomes were used as substrates for SWI/SNF (Imbalzano *et al.*, 1996; Cote *et al.*, 1997). One possibility is that the rate at which remodeled nucleosomes revert back to an inaccessible state may be influenced by the locations of the histone tails which are known to undergo major rearrangements when arrays are converted to mononucleosomes (reviewed in Fletcher and Hansen, 1996; Hansen, 1997). Alternatively, reversal may require an interaction between nucleosome particles located on the same DNA segment. In either model, tail function or nucleosome-nucleosome interactions might be the subject of regulation, such as the acetylation/deacetylation of the histone N-termini (Pennisi, 1997) and/or the binding of nuclear factors to favor or counteract the maintenance of the remodeled state induced by SWI/SNF.

SWI/SNF targeting in vivo

SWI/SNF complex is required for the expression of only a small number of yeast genes. Recently we have shown that at least one role of SWI/SNF complex is to facilitate the binding of a transcriptional activator to a nucleosomal site (Burns and Peterson, 1997). SWI/SNF complex is not an abundant nuclear protein; independent measurements by two groups estimate that it is present at only 100–200 copies per yeast nucleus (Cote *et al.*, 1994; B.Cairns and R.Kornberg, personal communication). Thus, how is SWI/SNF targeted to nucleosomes where it must act? One possibility is that SWI/SNF is not targeted *per se*, but that it randomly disrupts nucleosomes and the remodeling event is only fixed when a transcriptional activator binds to the disrupted nucleosome. Consistent with this model, we have shown here that SWI/SNF remodeling is rapidly reversed in the absence of transcription factor binding. In addition, Owen-Hughes *et al.* (1996) have shown that only a combination of SWI/SNF and a bound activator were able to induce a persistent change in the structure of a reconstituted nucleosome array. This random 'targeting' model, however, requires a rapid turnover rate for SWI/SNF remodeling. We have estimated a remodeling turnover rate of 4.5 min per nucleosome, and thus the 200 molecules

of SWI/SNF in a yeast nucleus would require over 35 h to randomly remodel the $\sim 10^5$ nucleosomes in a haploid yeast nucleus. In contrast, SWI/SNF-dependent remodeling *in vivo* of four nucleosomes in the *SUC2* upstream regulatory region requires <60 min (Hirschhorn *et al.*, 1992; I.Gavin and R.Simpson, personal communication). It is possible, of course, that the turnover rate of SWI/SNF *in vivo* is several orders of magnitude faster than we have estimated *in vitro*, or that a large percentage of nucleosomes in the nucleus are excluded from interaction with SWI/SNF. For instance, in mammalian cells SWI/SNF does not appear to associate with heterochromatic regions (Reyes *et al.*, 1997), and in yeast, the TUP1/SSN6 complex appears to antagonize the ability of SWI/SNF to function at the glucose-repressed *SUC2* locus (I.Gavin and R.Simpson, personal communication).

SWI/SNF may also be directly targeted to specific nucleosomes by directly interacting with transcriptional activators or components of the transcription machinery. SWI/SNF has been reported to associate with the rat glucocorticoid receptor (GR; Yoshinaga *et al.*, 1992), estrogen receptor (Ichinose *et al.*, 1997) and the RNA polymerase II holoenzyme (Wilson *et al.*, 1996), and a limited amount of targeting of SWI/SNF function to a mononucleosome by GR has been reported (Ostland Farrants *et al.*, 1997). The quantitative assay that we have described here is ideal for investigating functional targeting of SWI/SNF. For instance, we have engineered modified versions of the *SalI* site-bearing 11mer array that also contain one or more GAL4-binding sites in the linker region between the fifth and sixth nucleosomes of the array. Addition of GAL4 or GAL4-protein fusions to a remodeling reaction that contains limiting concentrations of SWI/SNF should provide a quantitative assay for the detection of targeting events.

Applicability of the coupled array assay to analysis of histone acetyltransferases/deacetylases

One advantage of model 5S nucleosome arrays is that they possess all of the fundamental properties required to achieve chromosomal level DNA compaction (i.e. 30 nM fibers; reviewed in Fletcher and Hansen, 1996). When such model arrays are incubated in low salt buffers (≤ 5 mM NaCl), arrays assume an extended 'beads on a string' structure. Addition of monovalent cations alone can induce folding of the arrays to an 'intermediate level', and addition of divalent cations alone results in structures that are compacted to the same degree as native 30 nM fibers. Importantly, folding of arrays, even in the presence of salts, is absolutely dependent upon the histone N-termini (Schwarz *et al.*, 1996) and is sensitive to their acetylation state (Garcia-Ramirez *et al.*, 1995). The histone N-termini are also required for chromatin folding *in vivo* (Allan *et al.*, 1982), and histone acetylation is correlated with chromatin decondensation and transcription *in vivo* (reviewed in Grunstein, 1990). Thus the biophysical properties of model nucleosome arrays make them ideal substrates for analyzing the properties of histone acetyltransferases and deacetylases. Since compaction of nucleosome arrays is predicted further to occlude factor binding sites, the quantitative restriction endonuclease assay that we have described here may prove invaluable for the functional dissection of histone acetyltransferases and

histone deacetylases, and for the development of reagents that influence the biochemical activities of effectors of chromatin remodeling.

Materials and methods

Plasmid constructions

A 2.3 kb DNA fragment encompassing 10 *L.varieagatus* 5S repeats and five Gal4-binding sites was released from plasmid pG5-208-10 (Owen-Hughes *et al.*, 1996) by *MluI*-*PvuII* digestion. Blunt ends were generated with Klenow polymerase and the DNA fragment was inserted into the *SmaI* site of a pBS-SK+ (Stratagene) derivative lacking the *SpeI* site to yield pCL3. Construct 'c' of Polach *et al.* (Polach and Widom, 1995), was amplified by PCR using the following primers; GCGTACCAAGTTAGCATAActgaaggattata and GCGTCTAGTCTAGACCATGG-AACCGAGCCCTATGCTTCGCGAGTCCAGTG and subcloned into the *SmaI* site of pBS-SK+ to yield plasmid pCL6. The 240 bp *XbaI* DNA fragment from pCL6 bearing the modified 5S positioning sequence was inserted into plasmid pCL3 which had been cleaved with *NheI* and *SpeI* to release the central five Gal4-binding sites and the flanking *SalI* sites to yield plasmid pCL7c. Plasmid pCL7b consists of nine wild-type head-to-tail 208-5S sequences and was obtained in the screen for pCL7c.

Reagent preparation

SWI/SNF purification was as described (Cote *et al.*, 1994) but the last step, a Superose 6 gel filtration step, was replaced by sedimentation for 16 h on a 13–30% glycerol gradient in E buffer [20 mM HEPES (pH 8.0), 350 mM NaCl, 10% glycerol, 0.1% Tween 20] at 33 000 g in a SW-28 rotor (Beckman). The concentration of complex was determined to be 200 nM by comparative Western blot analysis using an antibody against the SNF5 subunit, seven different concentrations of recombinant SNF5 protein (Quinn *et al.*, 1996) and five different concentrations of purified SWI/SNF complex. The approximate concentration of SWI/SNF complex determined by absorbance at 280 nM was in rough agreement with the Western analysis. Histone octamers were purified from chicken erythrocytes (Pel-Freez) as described by Hansen *et al.* (1989). HeLa cell histone octamers devoid of histone H1 were a kind gift of Dr T.Imbalzano (Imbalzano *et al.*, 1996). Octamer concentrations were determined by measurements of A_{230} (Stein, 1979). Large amounts of array DNA templates were obtained by a modification of the method of Hansen and Rickett (1989), except that *HhaI*, or *NorI* and *HindIII* digestions of 1–2 mg of plasmids pCL7c or pCL7b were followed by FPLC purification on a Biorad 150A exclusion matrix. The templates were labeled by the Klenow polymerase fill-in reaction using [α - 32 P]dCTP (6000 μ Ci/mmol, Amersham). Nucleosome arrays were reconstituted on the DNA templates in a Spectra/Por microdialyzer (Spectrum, Houston, TX), using the salt dialysis protocol of Hansen and Lohr (1993), except that 100 mM NaCl was used as the final dialysis step. Apyrase was from Sigma (A-6410) and was diluted to a concentration of 1 U/ μ l as described (Imbalzano *et al.*, 1996). When appropriate, *SalI* restriction enzyme (10^5 U/ml, New England Biolabs) was diluted in the recommended dilution buffer. SDS-PAGE analysis of the commercial *SalI* enzyme stock indicated that 5000 U/ml of *SalI* is equivalent to a 10 nM solution (data not shown).

Characterization of reconstituted arrays

Glycerol gradient sedimentation of arrays was carried out on 10–30% linear glycerol gradients in 10 mM Tris pH 7.5, 125 mM NaCl, 2.5 mM $MgCl_2$, 1 mM DTT. Sedimentation was for 16 h at 33 000 g in a SW-28 rotor (Beckman). Micrococcal nuclease digests were performed in 3 mM $CaCl_2$, 25 mM Tris-HCl (pH 7.4) using 0.01 U of Worthington (Freehold, NJ) micrococcal nuclease. Digestions were performed with a concentration of either 4 or 40 nM array; the patterns of digestion were identical. The *EcoRI* array saturation assay was carried out with a concentration of 40 nM of array as described by Hansen *et al.* (1989) except that the digestions were performed in the *SalI* digestion buffer (see below). Gel photographs were scanned and quantitated using NIH Image software. Sedimentation velocity studies were performed in a Beckman XL-A analytical ultracentrifuge equipped with scanner optics as described (Schwartz and Hansen, 1994). The A_{260} of the samples was 0.6–0.8.

SWI/SNF-SalI-coupled enzyme reaction conditions

For the coupled SWI/SNF-*SalI* reactions, reconstituted arrays were mixed with SWI/SNF complex in a buffer containing final concentrations

of 125 mM NaCl, 2.5 mM MgCl₂, 1 mM DTT, 10 mM Tris-HCl (pH 7.9), 100 µg/ml BSA and 2.6% glycerol. In experiments where the *SalI* concentration was varied, the glycerol concentration was kept constant. Where indicated, 1 U of apyrase was added per 50 µl of reaction mixture. Where indicated, ATP was added to a final concentration of 1 mM. The reactions were incubated, at 37°C. At the indicated time points, an aliquot of the reaction was vigorously mixed for 10 s with 25 µl water and 50 µl 1:1 solution of phenol/chloroform. The purified DNA fragments were resolved by non-denaturing agarose gel electrophoresis in the presence of 50 µg/ml ethidium bromide. The gels were then dried onto 3 MM Whatman paper. The fraction of uncut array DNA template was obtained by phosphorimager analysis using the ImageQuant software and taking the ratio of uncut signal to the sum of cut and uncut signal present in the lane.

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References

- Allan, J., Harborne, N., Rau, D.C. and Gould, H. (1982) Participation of core histone 'tails' in the stabilization of the chromatin solenoid. *J. Cell Biol.*, **93**, 285–297.
- Alland, L., Muhle, R., Hou, H., Jr, Potes, J., Chin, L., Schreiber-Agus, N. and DePinho, R.A. (1997) Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression [see comments]. *Nature*, **387**, 49–55.
- Bannister, A.J. and Kouzarides, T. (1995) CBP-induced stimulation of c-Fos activity is abrogated by E1A. *EMBO J.*, **14**, 4758–4762.
- Bannister, A.J. and Kouzarides, T. (1996) The CBP co-activator is a histone acetyltransferase. *Nature*, **384**, 641–643.
- Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R. and Edmondson, C.D. (1996) *Tetrahymena* histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. *Cell*, **84**, 843–851.
- Burns, L.G. and Peterson, C.L. (1997) Protein complexes for remodeling chromatin. *Biochim. Biophys. Acta*, **1350**, 159–168.
- Cairns, B.R., Kim, Y.-J., Sayes, M.H., Laurent, B.C. and Kornberg, R.D. (1994) A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products isolated from yeast. *Proc. Natl Acad. Sci. USA*, **91**, 1950–1954.
- Cote, J., Quinn, J., Workman, J.L. and Peterson, C.L. (1994) Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science*, **265**, 53–60.
- Dai, P., Akimaru, H., Tanaka, Y., Hou, D.X., Yasukawa, T., Kanei-Ishii, C., Takahashi, T. and Ishii, S. (1996) CBP as a transcriptional coactivator of c-Myb. *Genes Dev.*, **10**, 528–540.
- Dong, F., Hansen, J.C. and van Holde, K.E. (1990) DNA and protein determinants of nucleosome positioning on sea urchin 5S rRNA gene sequences *in vitro*. *Proc. Natl Acad. Sci. USA*, **87**, 5724–5728.
- Eckner, R., Yao, T.P.K., Oldread, E. and Livingston, D.M. (1996) Interaction and functional collaboration of p300/CBP and bHLH proteins in muscle and B-cell differentiation. *Genes Dev.*, **10**, 2478–2490.
- Fletcher, T.M. and Hansen, J.C. (1995) Core histone tail domains mediate oligonucleosome folding and nucleosomal DNA organization through distinct molecular mechanisms. *J. Biol. Chem.*, **270**, 25359–25362.
- Fletcher, T.M. and Hansen, J.C. (1996) The nucleosomal array: structure/function relationships. *Crit. Rev. Eukaryot. Gene Expr.*, **6**, 149–188.
- Garcia-Ramirez, M., Rocchini, C. and Ausio, J. (1995) Modulation of chromatin folding by histone acetylation. *J. Biol. Chem.*, **270**, 17923–17928.
- Gerritsen, M.E., Williams, A.J., Neish, A.S., Moore, S., Shi, Y. and Collins, T. (1997) CREB-binding protein/p300 are transcriptional coactivators of p65. *Proc. Natl Acad. Sci. USA*, **94**, 2927–2932.
- Grunstein, M. (1990) Nucleosomes: regulators of transcription. *Trends Genet.*, **6**, 395–399.
- Gu, W., Shi, X.-L. and Roeder, R.G. (1997) Synergistic activation by CBP and p53. *Nature*, **387**, 819–823.
- Halford, S.E. and Goodall, A.J. (1988) Modes of DNA cleavage by the *EcoRV* restriction endonuclease. *Biochemistry*, **27**, 1771–1777.
- Hansen, J.C. (1997) The core histone amino termini: Combinatorial interaction domains link chromatin structure with function. *Chemtracts: Biochem. Mol. Biol.*, in press.
- Hansen, J.C. and Lohr, D. (1993) Assembly and structural properties of subsaturated chromatin arrays. *J. Biol. Chem.*, **268**, 5840–5848.
- Hansen, J.C. and Rickett, H. (1989) Large-scale purification of plasmid insert DNA sequences using low-percentage agarose exclusion chromatography. *Anal. Biochem.*, **179**, 167–170.
- Hansen, J.C. and Wolffe, A.P. (1994) A role for histones H2A/H2B in chromatin folding and transcriptional repression. *Proc. Natl Acad. Sci. USA*, **91**, 2339–2343.
- Hansen, J.C., Ausio, J., Stanik, V.H. and van Holde, K.E. (1989) Homogeneous reconstituted oligonucleosomes, evidence for salt-dependent folding in the absence of histone H1. *Biochemistry*, **28**, 9129–9136.
- Hansen, J.C., van Holde, K.E. and Lohr, D. (1991) The mechanism of nucleosome assembly onto oligomers of the sea urchin 5S DNA positioning sequence. *J. Biol. Chem.*, **266**, 4276–4282.
- Hanstein, B., Eckner, R., DiRenzo, J., Halachmi, S., Liu, H., Searcy, B., Kurokawa, R. and Brown, M. (1996) p300 is a component of an estrogen receptor coactivator complex. *Proc. Natl Acad. Sci. USA*, **93**, 11540–11545.
- Hassig, C.A., Fleischer, T.C., Billin, A.N., Schreiber, S.L. and Ayer, D.E. (1997) Histone deacetylase activity is required for full transcriptional repression by mSin3A. *Cell*, **89**, 341–347.
- Heinzel, T. et al. (1997) A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. *Nature*, **387**, 43–48.
- Hirschhorn, J.N., Brown, S.A., Clark, C.D. and Winston, F. (1992) Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. *Genes Dev.*, **6**, 2288–2298.
- Horvai, A.E., Xu, L., Kozus, E., Brard, G., Kalafus, D., Mullen, T.M., Rose, D.W., Rosenfeld, M.G. and Glass, C.K. (1997) Nuclear integration of JAK/STAT and Ras/AP-1 signaling by CBP and p300. *Proc. Natl Acad. Sci. USA*, **94**, 1074–1079.
- Ichinose, H., Garnier, J.-M., Chambon, P. and Losson, R. (1997) Ligand-dependent interaction between the estrogen receptor and the human homologues of SWI2/SNF2. *Gene*, **188**, 95–100.
- 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.
- Imbalzano, A.N., Schnitzler, G.R. and Kingston, R.E. (1996) Nucleosome disruption by human SWI/SNF is maintained in the absence of continued ATP hydrolysis. *J. Biol. Chem.*, **271**, 20726–20733.
- Kadosh, D. and Struhl, K. (1997) Repression by Ume6 involves recruitment of a complex containing Sin3 corepressor and Rpd3 histone deacetylase to target promoters. *Cell*, **89**, 365–371.
- Kamei, Y. et al. (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell*, **85**, 403–414.
- Kasten, M.M., Ayer, D.E. and Stillman, D.J. (1996) Sin3-dependent transcriptional repression by interaction with the Mad1 DNA-binding protein. *Mol. Cell Biol.*, **16**, 4215–4221.
- Kwok, R.P., Lundblad, J.R., Chivria, J.C., Richards, J.P., Bachinger, H.P., Brennan, R.G., Roberts, S.G., Green, M.R. and Goodman, R.H. (1994) Nuclear protein CBP is a coactivator for the transcription factor CREB [see comments]. *Nature*, **370**, 223–226.
- Kwon, H., Imbalzano, A.N., Khuvvari, P.A., Kingston, R.E. and Green, M.R. (1994) Nucleosome disruption and enhancement of activator binding by a human SWI/SNF complex. *Nature*, **370**, 477–481.
- Lill, N.L., Grossman, S.R., Ginsberg, D., DeCaprio, J. and Livingston, D.M. (1997) Binding and modulation of p53 by p300/CBP coactivators. *Nature*, **387**, 823–827.
- Maxwell, A. and Halford, S.E. (1982) The *SalGI* restriction endonuclease. Enzyme specificity. *Biochem. J.*, **203**, 93–98.
- Mizzen, C.A. et al. (1996) The TAF(II)250 subunit of TFIID has histone acetyltransferase activity. *Cell*, **87**, 1261–1270.
- Modrich, P. and Zabel, D. (1976) *EcoRI* endonuclease. Physical and catalytic properties of the homogenous enzyme. *J. Biol. Chem.*, **251**, 5866–5874.

- Nagy,L., Kao,H.-Y., Chakravarti,D., Lin,R.J., Hassig,C.A., Ayer,D.E., Schreiber,S.L. and Evans,R.M. (1997) Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. *Cell*, **89**, 373–380.
- Ogryzko,V.V., Schiltz,R.L., Russanova,V., Howard,B.H. and Nakatani,Y. (1996) The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell*, **87**, 953–959.
- Oliner,J.D., Andresen,J.M., Hansen,S.K., Zhou,S. and Tjian,R. (1996) SREBP transcriptional activity is mediated through an interaction with the CREB-binding protein. *Genes Dev.*, **10**, 2903–2911.
- Ostland Farrants,A.K., Blomquist,P., Kwon,H. and Wrangé,O. (1997) Glucocorticoid receptor–glucocorticoid response element binding stimulates nucleosome disruption by the SWI/SNF complex. *Mol. Cell. Biol.*, **17**, 895–905.
- Owen-Hughes,T., Utley,R.T., Cote,J., Peterson,C.L. and Workman,J.L. (1996) Persistent site-specific remodeling of a nucleosome array by transient action of the SWI/SNF complex. *Science*, **273**, 513–516.
- Pazin,M.J. and Kadonaga,J.T. (1997) SWI2/SNF2 and related proteins: ATP-driven motors that disrupt protein–DNA interactions? *Cell*, **88**, 737–740.
- Pennings,S., Meersseman,G. and Bradbury,E.M. (1991) Mobility of positioned nucleosomes on 5 S rDNA. *J. Mol. Biol.*, **220**, 101–110.
- Pennisi,E. (1997) Opening the way to gene activity [news]. *Science*, **275**, 155–157.
- Peterson,C.L. (1996) Multiple SWIItches to turn on chromatin? *Curr. Opin. Genet. Dev.*, **6**, 171–175.
- Peterson,C.L. and Tamkun,J.W. (1995) The SWI/SNF complex: a chromatin remodeling machine? *Trends Biochem. Sci.*, **20**, 143–146.
- Peterson,C.L., Dingwall,A. and Scott,M.P. (1994) Five SWI/SNF gene products are components of a large multisubunit complex required for transcriptional enhancement. *Proc. Natl Acad. Sci. USA*, **91**, 2905–2908.
- Polach,K.J. and Widom,J. (1995) Mechanism of protein access to specific DNA sequences in chromatin: A dynamic model for gene regulation. *J. Mol. Biol.*, **254**, 130–149.
- Quinn,J., Fyrberg,A.M., Ganster,R.W., Schmidt,M.C. and Peterson,C.L. (1996) DNA-binding properties of the yeast SWI/SNF complex. *Nature*, **379**, 844–847.
- Reyes,J.C., Muchardt,C. and Yaniv,M. (1997) Components of the human SWI/SNF complex are enriched in active chromatin and are associated with the nuclear matrix. *J. Cell Biol.*, **137**, 263–274.
- Sartorelli,V., Huang,J., Hamamori,Y. and Kedes,L. (1997) Molecular mechanisms of myogenic coactivation by p300: direct interaction with the activation domain of MyoD and with the MADS box of MEF2C. *Mol. Cell. Biol.*, **17**, 1010–1026.
- Schwarz,P. and Hansen,J.C. (1994) Formation and stability of higher order chromatin structures. *J. Biol. Chem.*, **269**, 16284–16289.
- Schwarz,P.M., Felthauer,A., Fletcher,T.M. and Hansen,J.C. (1996) Reversible oligonucleosome self-association: dependence on divalent cations and core histone tail domains. *Biochemistry*, **35**, 4009–4015.
- Simpson,R.T. and Stafford,D.W. (1983) Structural features of a phased nucleosome particle. *Proc. Natl Acad. Sci. USA*, **80**, 51–55.
- Simpson,R.T., Thoma,F. and Brubaker,J.M. (1985) Chromatin reconstituted from tandemly repeated cloned DNA fragments and core histones: a model system for study of higher order structure. *Cell*, **42**, 799–808.
- Stein,A. (1979) DNA folding by histones: the kinetics of chromatin core particle reassembly and the interaction of nucleosomes with histones. *J. Mol. Biol.*, **130**, 103–134.
- Taunton,J., Hassig,C.A. and Shreiber,S.L. (1996) A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science*, **272**, 408–411.
- Treich,I., Cairns,B.R., De Los Santos,T., Brewster,E. and Carlson,M. (1995) SNF11, a new component of the yeast SNF–SWI complex that interacts with a conserved region of SWI2. *Mol. Cell. Biol.*, **15**, 4240–4248.
- Trouche,D. and Kouzarides,T. (1996) E2F1 and E1A_{12S} have a homologous activation domain regulated by RB and CBP. *Proc. Natl Acad. Sci. USA*, **93**, 1439–1442.
- Tsukiyama,T. and Wu,C. (1995) Purification and properties of an ATP-dependent nucleosome remodeling factor. *Cell*, **83**, 1011–1020.
- Tsukiyama,T., Daniel,C., Tamkun,J. and Wu,C. (1995) ISWI, a member of the SWI2/SNF2 ATPase family, encodes the 140 kDa subunit of the Nucleosome Remodeling Factor. *Cell*, **83**, 1021–1026.
- Utley,R.T., Cote,J., Owen-Hughes,T. and Workman,J.L. (1997) SWI/SNF stimulates the formation of disparate activator–nucleosome complexes but is partially redundant with cooperative binding. *J. Biol. Chem.*, **272**, 12642–12649.
- van Holde,K.E. (1988) *Chromatin*. Springer-Verlag, New York.
- van Holde,K.E. and Weisheit,W.O. (1978) Boundary analysis of sedimentation-velocity experiments with monodisperse and paucidisperse solutes. *Biopolymers*, **17**, 1387–1403.
- VargaWeisz,P.D., Blank,T.A. and Becker,P.B. (1995) Energy-dependent chromatin accessibility and nucleosome mobility in a cell-free system. *EMBO J.*, **14**, 2209–2216.
- Wang,W., Xue,Y., Zhou,S., Kuo,A., Cairns,B.R. and Crabtree,G.R. (1996) Diversity and specialization of mammalian SWI/SNF complexes. *Genes Dev.*, **10**, 2117–2130.
- Wilson,C.J., Chao,D.M., Imbalzano,A.N., Schnitzler,G.R., Kingston,R.E. and Young,R.A. (1996) RNA polymerase II holoenzyme contains SWI/SNF regulators involved in chromatin remodeling. *Cell*, **84**, 235–244.
- Winston,F. and Carlson,M. (1992) Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. *Trends Genet.*, **8**, 387–391.
- Yang,X.-J., Ogryzko,V.V., Nishikawa,J., Howard,B.H. and Nakatani,Y. (1996) A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. *Nature*, **382**, 319–324.
- Yoshinaga,S.K., Peterson,C.L., Herskowitz,I. and Yamamoto,K. (1992) Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. *Science*, **258**, 1598–1604.

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