

GAL4 directs nucleosome sliding induced by NURF

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The *Drosophila* nucleosome remodeling factor (NURF) is an imitation switch (ISWI)-containing chromatin remodeling complex that can catalyze nucleosome repositioning at promoter regions to regulate access by the transcription machinery. Mononucleosomes reconstituted *in vitro* by salt dialysis adopt an ensemble of translational positions on DNA templates. NURF induces bi-directional ‘sliding’ of these nucleosomes to a subset of preferred positions. Here we show that mononucleosome sliding catalyzed by NURF bears similarity to nucleosome movement induced by elevated temperature. Moreover, we demonstrate that the GAL4 DNA-binding domain can extend NURF-induced nucleosome movement on a *GAL4-E4* promoter, expanding the stretch of histone-free DNA at GAL4 recognition sites. The direction of NURF-induced nucleosome movement can be significantly modulated by asymmetric placement of tandem GAL4 sites relative to the nucleosome core particle. As such, sequence-specific, transcription factor-directed nucleosome sliding is likely to have substantial influence on promoter activation.

Keywords: chromatin/nucleosome/NURF/remodeling/transcription

Introduction

The genome of eukaryotes is packaged into nucleosomes, which limit the access of enzyme complexes that process DNA, including the transcription machinery. This constraint can be relieved in part by the action of ATP-dependent chromatin remodeling complexes. Four families of complexes containing SWI2–SNF2, imitation switch (ISWI), Mi-2 and Ino80 ATPases have been described (Cairns, 1998; Aalfs and Kingston, 2000; Shen *et al.*, 2000; Vignali *et al.*, 2000). The nucleosome remodeling factor (NURF) complex is one of three ISWI-containing, chromatin remodeling factors purified from *Drosophila* (NURF, ACF and CHRAC) (Tsukiyama *et al.*, 1995; Ito *et al.*, 1997; Varga-Weisz *et al.*, 1997). NURF has a molecular mass of ~500 kDa and contains three subunits (NURF301, NURF55 and NURF38) in addition to the ISWI ATPase (Tsukiyama *et al.*, 1995;

Gdula *et al.*, 1998; Martinez-Balbas *et al.*, 1998; Xiao *et al.*, 2001). NURF is a nucleosome-stimulated ATPase that has been shown to facilitate nucleosome repositioning mediated by sequence-specific DNA-binding factors on the promoters of *hsp70* (Tsukiyama *et al.*, 1994; Tsukiyama and Wu, 1995) and *GAL4-E4* (Mizuguchi *et al.*, 1997, 2001).

Studies using purified ISWI protein or ISWI-containing protein complexes indicate that these enzymes reposition nucleosomes by catalyzing nucleosome sliding, defined as the movement of the histone octamer *in cis*, without irretrievable displacement from DNA (Corona *et al.*, 1999; Hamiche *et al.*, 1999; Längst *et al.*, 1999). However, the direction of enzyme-catalyzed nucleosome sliding can differ significantly, depending on the individual chromatin remodeling complex. For example, NURF slides reconstituted mononucleosomes from central to distal sites whereas ACF or CHRAC mobilizes nucleosomes in the opposite direction, from distal to central locations (Corona *et al.*, 1999; Hamiche *et al.*, 1999; Längst *et al.*, 1999; Eberharter *et al.*, 2001). These differences appear to be determined by the unique properties of the distinct large subunits, NURF301 and ACF1, of the NURF and ACF–CHRAC complexes, respectively (Ito *et al.*, 1999; Eberharter *et al.*, 2001; Xiao *et al.*, 2001). However, it is unclear how these components cooperate with the ISWI engine to mobilize nucleosomes to different positions. Moreover, the relationship between sequence-dependent nucleosome stability and the outcome of ATP-dependent nucleosome sliding has been largely unexplored.

The relative location of transcription factor-binding sites on a positioned nucleosome can substantially affect site accessibility to proteins. In general, the wrapping of DNA in a nucleosome reduces the affinity of transcription factors for their cognate sites, although in at least one instance, a nucleosome can positively affect factor binding. The liver-enriched transcription factor HNF3 binds more stably to nucleosome core particles than to free DNA (Cirillo and Zaret, 1999). In contrast, glucocorticoid receptor, thyroid hormone receptor and Fos/Jun bind to nucleosomal DNA with slightly lower affinity than binding to free DNA (Li and Wrangé, 1993; Wong *et al.*, 1995; Ng *et al.*, 1997). GAL4, c-Myc, heat shock factor (HSF), specificity protein 1 (SP1) and TFIID bind to nucleosomal DNA with an affinity at least one order of magnitude lower than their affinities for free DNA (Taylor *et al.*, 1991; Vettese-Dadey *et al.*, 1994; Wechsler *et al.*, 1994; Cirillo and Zaret, 1999). The affinity of nuclear factor 1 and TATA-binding protein (TBP) for nucleosomal DNA is lower by at least two orders of magnitude (Pina *et al.*, 1990; Imbalzano *et al.*, 1994; Blomquist *et al.*, 1999).

ATP-dependent chromatin remodeling complexes can alter the structure of nucleosomes and thereby facilitate the binding of transcription factors (Cote *et al.*, 1994; Imbalzano *et al.*, 1994; Kwon *et al.*, 1994; Cairns *et al.*,

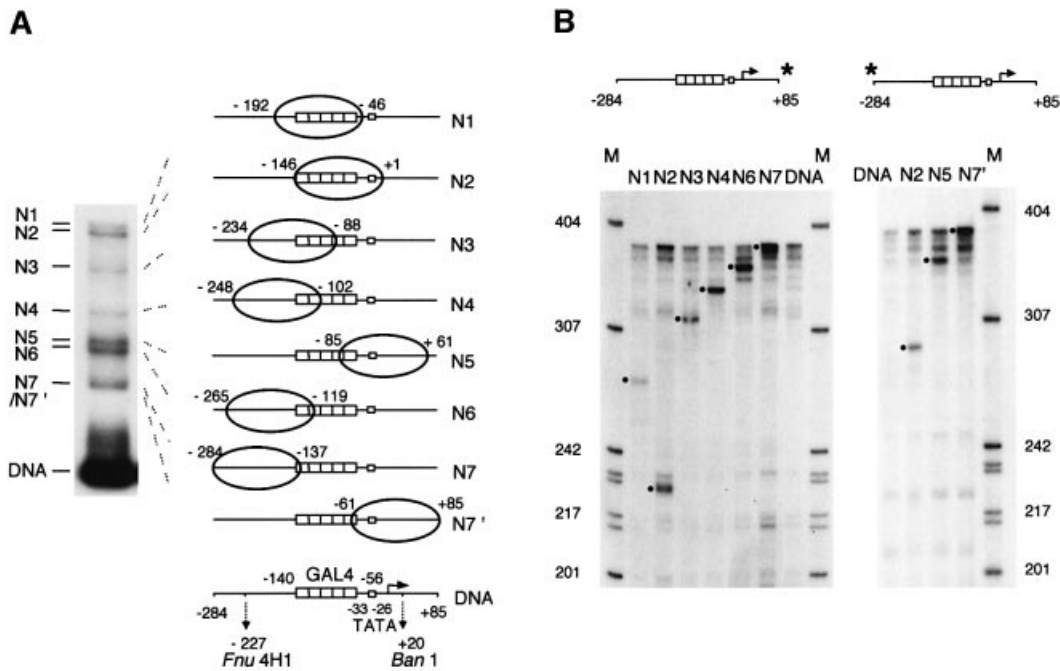


Fig. 1. Reconstituted nucleosomes occupy multiple positions on the *GAL4-E4* promoter. (A) Native PAGE of mononucleosomes reconstituted on a 369 bp fragment carrying the *GAL4-E4* promoter. Major nucleosome species are indicated as N1–N7'. Nucleosome positions and GAL4-binding sites determined by Exo III footprinting (precise to ± 2 bp) are illustrated on the right. The TATA box and restriction enzyme sites are indicated. (B) Exo III footprinting. Gel bands corresponding to individual N1–N7' nucleosome species (DNA was radiolabeled at either end of *GAL4-E4* promoter) were excised, nucleosomes were eluted and digested with Exo III (400 U/ml) for 2 min at 37°C. DNAs were analyzed by electrophoresis in a 6% polyacrylamide gel containing 8 M urea. M indicates pBR322 *Hpa*II fragments as markers. Dots indicate major Exo III pauses. The Exo III footprint of GAL4 (–153 to –44) extends beyond the recognition sites (–140 to –56) (Carey *et al.*, 1989; Carey and Smale, 2000).

1996; Wang *et al.*, 1996; Burns and Peterson, 1997). In the presence of remodeling enzymes, sequence-specific DNA-binding transcription factors such as GAGA factor, HSF, nuclear factor E2, GAL4 derivatives and nuclear factor κ B induce the rearrangement of nucleosome arrays in the vicinity of their DNA-binding sites (Pazin *et al.*, 1994, 1997; Tsukiyama *et al.*, 1994; Wall *et al.*, 1995; Armstrong and Emerson, 1996). In this study, we have investigated the contributions of transcription factor binding to NURF-induced nucleosome sliding in a highly purified system. We show how NURF and the DNA-binding domain (DBD) of GAL4 cooperate in the mobilization of a single nucleosome. Binding of GAL4-DBD in the presence of NURF extends nucleosome sliding to generate a nucleosome-free region at GAL4 recognition sites. Conversely, GAL4-DBD can also create a boundary that prevents nucleosome re-entry, thus constraining the direction of nucleosome sliding. We also compared the thermal induction of nucleosome mobility with NURF-induced sliding, and found that the two processes share interesting similarities.

Results

Nucleosomes adopt multiple positions on the *GAL4-E4* promoter

The positioning of a histone octamer with respect to DNA sequence is a function of the intrinsic, sequence-dependent bendability of DNA over the histone octamer (Luger *et al.*, 1997; Travers and Drew, 1997; Richmond and Widom, 2000). We analyzed the distribution of mononucleosomes

on a 369 bp DNA fragment containing five tandem GAL4-binding sites upstream of the adenovirus E4 TATA box and minimal core promoter (Pazin *et al.*, 1994; Mizuguchi *et al.*, 1997). Mononucleosomes were reconstituted by salt dialysis using purified histone octamers, and fractionated by native PAGE. The electrophoretic migration of nucleosomes is influenced by the length and spatial orientation of the linker DNA extending from both ends of the core particle—migration is slow for centrally located nucleosomes and increases linearly as nucleosome placement approaches either fragment end (Duband-Goulet *et al.*, 1992; Meersseman *et al.*, 1992). Using this assay, at least seven nucleosome species (N1–N7) could be observed on the 369 bp *GAL4-E4* promoter fragment (Figure 1A).

We mapped the positions of reconstituted nucleosomes by exonuclease III (Exo III) digestion after elution of each species from the corresponding gel band. The upstream boundary of each nucleosome was determined using the site of a prominent pause in the pattern of Exo III digestion; this pause is absent from the digestion pattern of free DNA (Figure 1B). Downstream boundaries were also determined by Exo III digestion (Figure 1B), or calculated using the location of the upstream nucleosome boundary. Accordingly, N1 and N2 nucleosomes were mapped centrally over the tandem GAL4-binding sites; N3, N4 and N6 nucleosomes were located further upstream; the N5 nucleosome was located over the TATA box and downstream sequences, and N7/N7', the fastest migrating nucleosomes, mapped to either end of the *GAL4-E4* fragment (Figure 1).

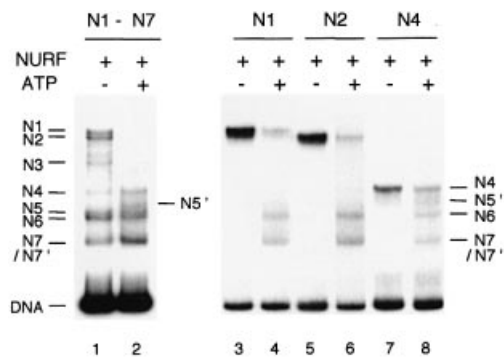


Fig. 2. NURF mediates nucleosome sliding on the *GAL4-E4* promoter. 369 bp *GAL4-E4* mononucleosomes (40 nM) were incubated with NURF (0.4 nM) in the absence or presence of ATP for 30 min at 26°C (lanes 1 and 2), followed by native 4.5% PAGE in 0.5× TBE. N1, N2 and N4 nucleosomes were eluted from the gel slice and incubated with NURF and ATP as indicated, before electrophoresis on a second native gel (lanes 3–8).

NURF catalyzes nucleosome sliding on the *GAL4-E4* promoter

To assess the influence of NURF on the distribution of nucleosomes on the *GAL4-E4* fragment, we incubated 369 bp mononucleosomes with NURF and ATP, and analyzed nucleosome positions by native gel electrophoresis. A change in nucleosome distribution was clearly apparent: N1, N2, N3 and N5 nucleosomes were depleted, while N6 and N7/N7' nucleosomes became more prevalent (Figure 2, lane 2). In addition, the N4 nucleosome was weakly detected, and a novel position located between N4 and N5 (N5') was sometimes observed. This distribution of nucleosomes did not change after incubation with higher levels of NURF or for a longer period (data not shown), indicating that nucleosome distribution had reached equilibrium.

To examine further the movement of nucleosomes from a single location, we purified N1, N2 and N4 nucleosomes by native gel electrophoresis. After incubation with NURF and ATP, all three individual nucleosome species showed movement primarily to the N6 and N7/N7' positions, confirming these as the favored locations when nucleosomes are mobilized by NURF (Figure 2, lanes 4, 6 and 8). The results indicate that NURF catalyzes nucleosome movement from central to peripheral positions on the 369 bp *GAL4-E4* promoter, leading to partial exposure of GAL4-binding sites (see Figure 1A).

Comparison of NURF- and heat-induced nucleosome sliding

The standard protocol for reconstituting nucleosomes by dialysis of a mixture of core histones and DNA from high salt (Rhodes and Laskey, 1989) apparently traps some nucleosomes in non-equilibrium positions (Drew, 1991; Widom, 1999). Such reconstituted mononucleosomes have been shown to undergo temperature-induced mobility and redistribution (Pennings *et al.*, 1991; Flaus and Richmond, 1998). We examined how heat affects the distribution of mononucleosomes reconstituted on the 369 bp *GAL4-E4* promoter. Analysis of nucleosome positions showed that as 369 bp nucleosomes were heated to temperatures up to 60°C before cooling and native gel

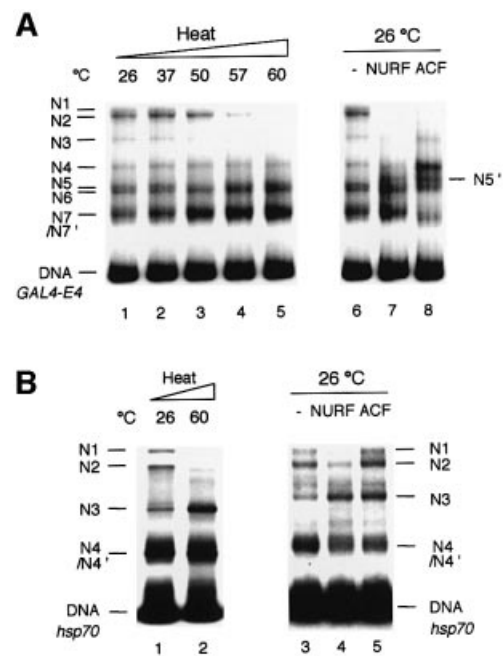


Fig. 3. Comparison of heat- and NURF-induced nucleosome sliding. Mononucleosomes reconstituted on *GAL4-E4* (A) or *hsp70* (B) radiolabeled DNAs were incubated for 30 min at the indicated temperatures in TE buffer and 1 mg/ml BSA. Samples were cooled on ice, and analyzed by native PAGE as in Figure 2 legend. Mononucleosomes were incubated with NURF (0.4 nM), [(A) lane 7; (B) lane 4] or ACF (1.6 nM), [(A) lane 8; (B) lane 5] in nucleosome sliding buffer at 26°C. Samples were electrophoresed as in Figure 2 legend.

electrophoresis (at room temperature), a gradual loss of N1, N2 and N3 nucleosomes was observed. Upon heating to 60°C, N5/N6 and N7/N7' were the preferred nucleosome positions, with N7/N7' being dominant; the N4 position remained as a weakly detected band (Figure 3A, lanes 1–5). Heat- and NURF-induced relocation of nucleosomes bear interesting similarities, although there are noticeable differences—heating resulted in greater abundance of the N7/N7' positions and failed to reveal the novel N5' position.

A similarity between NURF- and heat-induced nucleosome sliding was also observed using the 359 bp *hsp70* promoter fragment. Both heat treatment and the action of NURF relocated nucleosomes to the preferred N3 position (Figure 3B, lanes 2 and 4). In contrast, ACF, the ISWI-containing nucleosome assembly and spacing factor (Ito *et al.*, 1997, 1999; Eberharter *et al.*, 2001) mobilized nucleosomes to the N1 and N2 positions as much as the N3 position (Figure 3B, lane 5). On the *GAL4-E4* promoter, ACF induced nucleosome movement preferably to N4 more than the N6 and N7 positions (Figure 3A, lane 8).

GAL4-DBD clears nucleosomes from cognate sites

In the absence of other factors, the repositioning of nucleosomes by NURF uncovers some, but not all, of the five GAL4-binding sites on the *GAL4-E4* promoter. To evaluate the influence of the GAL4-DBD on nucleosome remodeling, we incubated purified N1 or N4 nucleosomes with NURF and saturating amounts of GAL4-DBD (GAL4 residues 1–147) (Lin *et al.*, 1988; Carey *et al.*, 1990). The resulting complexes of GAL4-DBD and nucleosomes

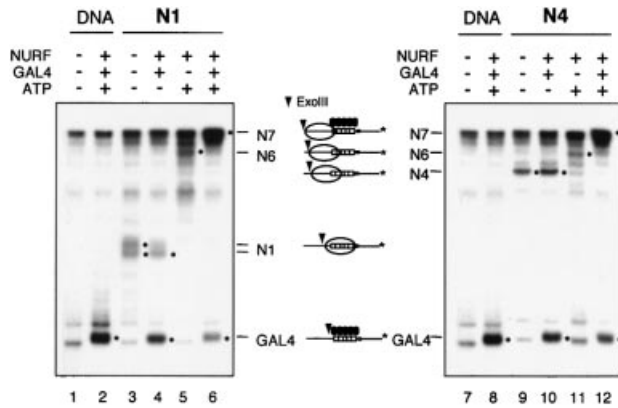


Fig. 4. GAL4-DBD and NURF-induced extension of nucleosome sliding—Exo III footprinting analysis. Mononucleosomes were reconstituted on the end-labeled 369 bp *GAL4-E4* promoter. Gel-purified nucleosomes (0.025 nM) and carrier DNA (20 μ g/ml), N1 (lanes 1–6), N4 (lanes 7 and 8) were incubated with NURF (0.2 nM) and GAL4-DBD (0.3 nM) in the presence or absence of ATP as indicated. Reaction mixtures were digested by Exo III (400 U/ml) and analyzed on an 8 M urea–6% polyacrylamide gel. Dots indicate major Exo III pauses. Diagrams show the position of nucleosomes (open circles), GAL4 (filled ovals) and Exo III pauses (arrowheads).

were analyzed by Exo III protection (the 369 bp fragment was 5' end-labeled at the downstream end). When the N1 nucleosome was mobilized in the presence of both GAL4-DBD and NURF, we observed strong protection from digestion by Exo III at the upstream end of the 369 bp fragment (Figure 4, lane 6). Such enhanced protection from digestion was not detected in the absence of ATP or GAL4-DBD (Figure 4, lanes 4 and 5). Similarly, the N4 nucleosome displayed enhanced protection from Exo III digestion at the upstream fragment end (Figure 4, lanes 10–12). The results indicate that nucleosomes are relocated to the upstream end (or the downstream end; see below) of the DNA fragment.

To confirm these findings, we analyzed the positioning of nucleosomes by restriction enzyme analysis after micrococcal nuclease (MNase) digestion and DNA purification (Dong *et al.*, 1990; Studitsky *et al.*, 1994; Davey *et al.*, 1998; Studitsky, 1999). Nucleosomes reconstituted with uniformly labeled, 369 bp *GAL4-E4* DNA were purified by glycerol gradient centrifugation to remove free DNA and dinucleosome particles. Analysis of the size of core particle DNA upon MNase digestion showed the expected \sim 150 bp DNA (Figure 5A, left panel and B, lane 1). When GAL4-DBD was incubated with 369 bp nucleosomes, MNase digestion and DNA purification revealed fragments of \sim 100 bp and from \sim 145 to 240 bp (Figure 5B, lane 3). We attribute the \sim 100 and \sim 145 bp fragments to protection from cleavage by tandemly bound GAL4-DBDs and the nucleosome core particle, respectively, and the fragment series between \sim 145 and 240 bp to combined protection by N1–N7 positioned core particles and GAL4-DBDs (Figure 5A, middle panel). When NURF and ATP were included in the incubation of GAL4-DBD and the N1–N7 nucleosome population, the MNase digestion pattern strikingly resolved into a major DNA species of \sim 240 bp (Figure 5B, lane 4 and illustration), which is attributed to combined protection by tandemly bound GAL4-DBDs and a nucleosome relocated to either

end of the 369 bp fragment. In such experiments, we also observed a faint, \sim 145 bp fragment, and increased abundance of the \sim 100 bp fragment. This increase may result from MNase cleavage at a more accessible junction between the GAL4-DBDs and the relocated nucleosome core particle, or reflect some loss of histone octamers during nucleosome repositioning.

We confirmed the positional assignments of the relocated nucleosomes by restriction enzyme analysis, using a *BanI* site at downstream position +20 and a *Fnu4H1* site at -227 upstream of the *GAL4-E4* promoter (Figure 5A, right). *BanI* digested a fraction (33%) of the 240 bp DNA, generating 173 and 65 bp fragments. This fraction represents nucleosomes that moved to the downstream end (N7') of the *GAL4-E4* fragment (Figure 5B, lane 6 and illustration). *Fnu4H1* digested 68% of the 240 bp DNA, generating fragments of 183 and 57 bp; this fraction represents nucleosomes that moved to the upstream end (N7) (Figure 5B, lane 8 and illustration). Collectively, our results indicate that the combined actions of GAL4-DBD and NURF mobilize nucleosomes to either one or the other end of the 369 bp fragment, away from the tandem GAL4-binding sites.

We also studied the effects on nucleosome positioning of GAL4(1–94), which lacks the cryptic activation region of GAL4(1–147) (Lin *et al.*, 1988; Carey *et al.*, 1989, 1990; Workman *et al.*, 1991). GAL4(1–94) also directs NURF-induced movement of nucleosomes to either end of the 369 bp fragment (Figure 5C, lanes 2 and 4). The results indicate that the minimal DNA binding and dimerization domains of GAL4 are sufficient to direct nucleosome repositioning.

Relative placement of GAL4 modulates direction of nucleosome movement

To explore further how placement of GAL4 sites in relation to the nucleosome core particle might direct nucleosome movements, we analyzed the repositioning of purified nucleosomes in which the GAL4 sites are located asymmetrically. In the N2 nucleosome, GAL4 sites are placed inside the core particle, bordering the upstream edge, whereas the N4 nucleosome has GAL4 sites placed in the linker and overlapping the downstream edge of the core particle (Figure 6). Control experiments were performed in which purified N2 or N4 nucleosomes were treated with NURF in the absence of GAL4-DBD, followed by MNase digestion, DNA purification and *BanI* or *Fnu4H1* cleavage to map nucleosome positions. The repositioning of nucleosomes by NURF (alone) shows a moderate preference of N2 or N4 nucleosomes for upstream locations. The N2 species moves upstream to N7, N6, N4 positions (55% *Fnu4H1*-sensitive; \sim 13% N7), or moves downstream to N7', N5' positions (36% *BanI*-sensitive; \sim 6% N7'). The N4 species also moves upstream to N7, N6, N4 positions (63% *Fnu4H1*-sensitive; \sim 15% N7), or downstream to N7', N5' positions (43% *BanI*-sensitive; \sim 3% N7') (Figure 6A).

The inclusion of GAL4-DBD in the reaction significantly alters the outcome of NURF-induced nucleosome mobility. As indicated by the presence of the 240 bp fragment protected from MNase digestion, the combination of NURF and GAL4-DBD drives the N2 nucleosome to one or the other end of the 369 bp fragment (Figure 6B,

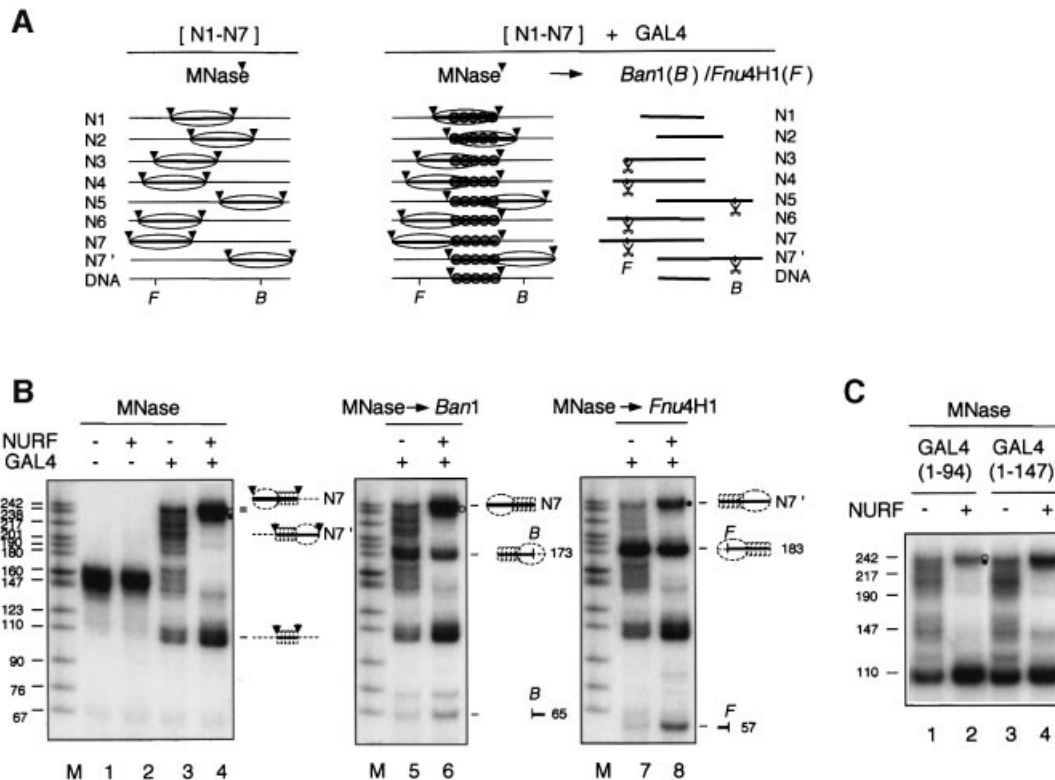


Fig. 5. GAL4-DBD and NURF-induced extension of nucleosome sliding—MNase and restriction enzyme analysis. Nucleosomes were reconstituted on ³²P uniformly labeled 369 bp *GAL4-E4* fragment and incubated with GAL4 and/or NURF prior to MNase digestion. To map boundaries of MNase protection, DNA was purified and digested with *BanI* (B) or *Fnu4H1* (F). (A) Schematic illustration showing mononucleosomes and GAL4-DBD binding on 369 bp *GAL4-E4* promoter. Arrowheads mark the boundaries of MNase protection. (B) Mononucleosome fractions (0.7 nM) purified by glycerol gradient centrifugation were incubated with NURF (0.2 nM) and/or GAL4 (30 nM monomer) for 30 min at 26°C, followed by MNase (25 U/ml) digestion (lanes 1–4). DNA was purified and further digested with *BanI* (1300 U/ml, lanes 5 and 6) or *Fnu4H1* (333 U/ml, lanes 7 and 8) and analyzed by 8% PAGE in TBE and autoradiography. Dots (filled and unfilled) indicate fragments protected by complexes of GAL4-DBD and N7 or N7' nucleosomes, respectively. Binding of GAL4-DBD to free DNA or nucleosomes generates 109 and 145–240 bp MNase resistant fragments, respectively. In the absence of GAL4-DBD, MNase digestion of N1–N7 nucleosomes yields 145–155 bp fragments from core particles. (C) Mononucleosomes were incubated with GAL4(1–94) and GAL4(1–147) (20 nM monomer) in the presence or absence of NURF, and analyzed by MNase digestion and DNA gel electrophoresis. MNase cleavage at sites flanking the tandemly bound GAL4-DBDs appears to be more efficient for GAL4(1–94).

lane 2). Digestion of the 240 bp fragment with *BanI* (Figure 6B, lane 3) and *Fnu4H1* (Figure 6B, lane 4), indicates that N7 (36%; *Fnu4H1*-sensitive) and N7' (64%; *BanI*-sensitive) are the only major nucleosome positions adopted. The N4 nucleosome shows highly preferential movement to the upstream N7 nucleosome position. Eighty-nine percent of N4 nucleosomes were driven to the N7 (*Fnu4H1*-sensitive) position, while 11% moved to the N7' (*BanI*-sensitive) position (Figure 6B, lanes 7 and 8). Hence, the relative placement of the GAL4-binding sites on the nucleosome core particle can have a significant influence on the direction of nucleosome movement. Given the bi-directional nature of NURF-induced nucleosome mobility (Hamiche *et al.*, 1999), the results also imply that a DNA-binding protein can impose a substantial barrier to re-entry of nucleosomes that were moved away from its cognate sites.

Discussion

Recent studies have demonstrated that several ATP-dependent chromatin remodeling complexes have the ability to generate nucleosome movement *in cis* on a specific DNA fragment (Hamiche *et al.*, 1999; Längst

et al., 1999; Whitehouse *et al.*, 1999; Brehm *et al.*, 2000; Guschin *et al.*, 2000). For the ISWI complex NURF, sliding of mononucleosomes has been so far demonstrated on the hsp70 promoter and the sea urchin 5S RNA gene (Hamiche *et al.*, 1999). In this report, we extended the analysis of nucleosome sliding by NURF to the *GAL4-E4* model promoter, and characterized the impact of the GAL4 DNA-binding motif on the outcome of nucleosome remodeling. We also compared features of NURF-induced nucleosome sliding and nucleosome mobility induced by heat treatment.

The stability of nucleosomes is influenced by the intrinsic, sequence-dependent bendability of the double helix (Luger *et al.*, 1997; Travers and Drew, 1997; Richmond and Widom, 2000). We observed at least six different translational positions of the nucleosome reconstituted by salt dialysis on the 369 bp *GAL4-E4* promoter fragment. Upon heating, the N1, N2 and N3 nucleosomes are induced to slide, generating a distribution that bears some similarity to nucleosomes mobilized by the ATP-dependent activity of NURF. These findings suggest that the mechanism of NURF action has a component that may mimic the thermal alterations of nucleosomes that lead to nucleosome mobility.

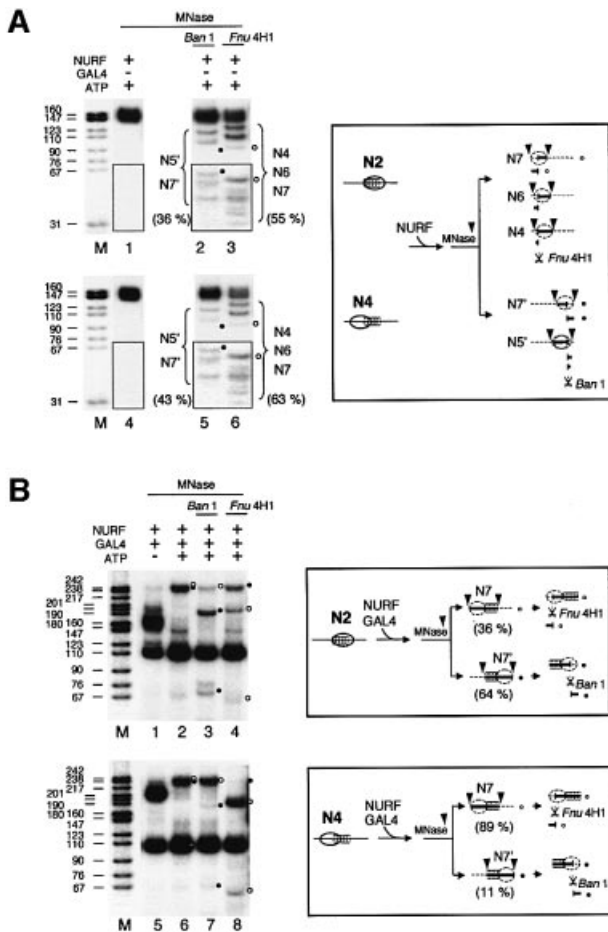


Fig. 6. Placement of GAL4 sites modulates direction of nucleosome sliding. (A) Gel-purified 369 bp *GAL4-E4* mononucleosomes N2 (upper panel) and N4 (lower panel) were incubated with NURF in the presence of ATP and digested with MNase; DNA was purified, further digested with *BanI* and *Fnu4H1*, and analyzed by PAGE and autoradiography as in the legend to Figure 5. The lower part of the gel (indicated by the box) was exposed to film ~3 times longer than the upper part and contrast enhanced to visually reveal small DNA fragments. Quantitation of radioactive band intensity was performed by phosphorimager analysis. The percentage restriction enzyme cleavage was calculated by subtraction of the residual ~150 bp nucleosome core particle DNA from an equivalent, uncleaved sample (lanes 1 and 4). Restriction fragments derived from N7 and N7' nucleosomes are indicated by unfilled and filled dots, respectively. The 81 bp fragment derived from *BanI* cleavage of N7' core particle DNA is not visible at this exposure; note that the N7 and N7' percentage values given in the text are approximate. (B) Gel-purified 369 bp *GAL4-E4* mononucleosomes N2 (upper panel) and N4 (lower panel) were incubated with NURF and GAL4-DBD, in the presence or absence of ATP, and analyzed as above. Dots (unfilled and filled) indicate fragments corresponding to, or derived from, N7 and N7' nucleosomes, respectively. The distribution of N7 and N7' nucleosomes was calculated by phosphorimager analysis of radioactive fragments, and averaged from three sets of experiments.

NURF-induced nucleosome movement on the *GAL4-E4* promoter renders *GAL4* sites only partially free of nucleosomes. The distribution of nucleosome positions can be further perturbed by introduction of GAL4-DBD into the purified remodeling system. In accordance with previous observations, we found that binding of GAL4-DBD to nucleosomal DNA in the absence of remodeling enzymes does not lead to the repositioning of nucleosomes (Pazin *et al.*, 1994; Owen-Hughes *et al.*, 1996; Mizuguchi

et al., 1997). However, the combined activities of GAL4-DBD and NURF proteins result in extended sliding of nucleosomes to clear the GAL4-binding sites, leaving an expanded stretch of nucleosome-free DNA. We suggest that a similar mechanism of extended nucleosome sliding could account for the documented repositioning of nucleosomes by GAGA factor, HSF and NURF on the *hsp70* and *hsp26* promoters (Tsukiyama *et al.*, 1994; Tsukiyama and Wu, 1995; Wall *et al.*, 1995). Such generation of chromatin accessibility is likely to be required to facilitate other steps in promoter activation, e.g. binding of TFIID and recruitment of RNA polymerase II. In this context, it is of interest that a recent report demonstrates contributions to nucleosome sliding as well by the TATA-binding protein and other proteins that bend promoter DNA (Lomvardas and Thanos, 2001).

The relative placement of GAL4 sites on the nucleosome can also have a strong influence on the direction of nucleosome movement. When tandem GAL4 sites are placed asymmetrically on the nucleosome core particle (e.g. overlapping a linker and one edge of the core particle), GAL4-DBD binding in the presence of NURF activity mobilizes nucleosomes preferentially in the direction of the other, unbound linker DNA. It has been previously shown that GAL4-DBD binds with greater affinity to free DNA than to nucleosomes (Taylor *et al.*, 1991), and proceeds cooperatively from one end of the core particle until all sites are occupied (Vettese-Dadey *et al.*, 1994). Hence, GAL4-DBD binding initiating in the linker DNA and spreading into the core particle should constrain the direction of nucleosome sliding by providing a dominant boundary against back-sliding due to the bi-directional nature of NURF-induced nucleosome mobility (Hamiche *et al.*, 1999). Taken together, these findings indicate that a DNA-binding factor should be continuously required for the maintenance of NURF-induced nucleosome positioning, a conclusion consistent with the results of an earlier study using a crude chromatin assembly system containing ISWI complexes (Pazin *et al.*, 1997).

Under our assay conditions, binding of GAL4-DBD extended NURF-induced sliding largely *in cis*. However, it has been reported that binding of transcription factors such as GAL4 or glucocorticoid receptor to nucleosomal DNA stimulates SWI-SNF-induced displacement *in trans* (Owen-Hughes and Workman, 1996; Owen-Hughes *et al.*, 1996; Ostlund Farrants *et al.*, 1997). These findings underscore differences in the remodeling mechanism between NURF and other ATP-dependent chromatin remodeling complexes. NURF-induced nucleosome sliding may be functionally effective only when the intrinsic DNA structure of specific promoter sequences allows nucleosome movement away from elements for recognition by the transcription machinery. In situations where promoter sequences dictate inherently stable nucleosome positions, or where immobile neighbors block NURF-induced nucleosome sliding, it may be necessary to bring on additional remodeling proteins like SWI-SNF with capacity for enhanced sliding or nucleosome eviction. Our present studies should provide a stimulus to additional investigations on the relative nucleosome sliding strengths of the family of ATP-dependent chromatin remodeling complexes.

Materials and methods

Preparation of DNA fragments

A 369 bp DNA fragment containing five GAL4-binding sites and the TATA box from the adenovirus *E4* promoter was generated by PCR from pGIE-0 (Pazin *et al.*, 1994) with the primers 5'-GCGAATTCAGATCTC-CAGATGCTACACAATTAG-3' and 5'-GCGAATTCCTCGAGCTTAC-CAGTAAAAAGAAAACCTA-3' using *Pfu* DNA polymerase (Stratagene). DNA fragments for Exo III footprinting were prepared by labeling one of the primers at the 5'-end with [γ - 32 P]ATP and performing PCR with the other primer unlabeled. For MNase analysis, the DNA fragment was uniformly labeled by PCR synthesis using [α - 32 P]dNTPs. The double-stranded PCR products were gel-purified and concentrated by ethanol precipitation. The radiolabeled 359 bp *hsp70* DNA fragment was prepared according to Hamiche *et al.* (1999).

Nucleosome reconstitution

Nucleosomes were assembled from a 32 P-labeled 369 bp *GAL4-E4* DNA fragment and *Drosophila* histone octamers by the salt jump method as described previously (Hamiche *et al.*, 1999). Two micrograms of carrier plasmid DNA and 50–200 ng of 32 P-labeled linear DNA fragment were mixed with 0.8–2.4 μ g of core histones in 2 M NaCl, 10 mM Tris–HCl pH 7.5, 0.1 mg/ml bovine serum albumin (BSA), in a total volume of 10 μ l. This mixture was diluted to 0.5 M NaCl, and then dialyzed against TE buffer (10 mM Tris pH 7.5, 1 mM EDTA). Reconstituted nucleosomes were analyzed on a 4.5% polyacrylamide gel (acrylamide:bisacrylamide, 29:1) containing 0.5 \times TBE or TE buffer. Nucleosome species were excised and eluted from the gel in buffer containing carrier DNA (50 μ g/ml) as described previously (Hamiche *et al.*, 1999). For the experiments in Figures 5 and 6, mononucleosomes were separated from excess free DNA and dinucleosomes by glycerol gradient sedimentation. Glycerol gradient (5–20%) in TE was centrifuged in an SW40 Ti rotor at 36 000 r.p.m. for 16 h at 4°C. Mononucleosome peak fractions were directly used in reactions with NURF and GAL4-DBD, followed by MNase digestion.

Purification of GAL4 transcription factors

GAL4(1–147) is described in Mizuguchi *et al.* (1997). GAL4-DBD(1–94) was cloned into the *Nco*I and *Bam*HI sites of the pET 28a expression vector. GAL4-DBD proteins were expressed in *Escherichia coli* and purified with heparin–Sepharose CL6B (Amersham Pharmacia Biotech) and GAL4-binding affinity resin as described previously (Mizuguchi *et al.*, 1997). The amount of each GAL4 protein was normalized based on DNA-binding activity.

Exo III protection

Native gel bands corresponding to each nucleosome species (radiolabeled at either end of *GAL4-E4* promoter) were excised from 4.5% polyacrylamide gel containing TE as described previously (Hamiche *et al.*, 1999). Nucleosomes were eluted and digested with Exo III (400 U/ml) for 2 min at 37°C, followed by electrophoresis in a 6% polyacrylamide gel containing 8 M urea.

Nucleosome remodeling

NURF was purified to the final glycerol gradient step from 0 to 12 h *Drosophila* embryos as described previously (Tsukiyama and Wu, 1995; Sandaltzopoulos *et al.*, 1999) except that Mono-Q and Mono-S columns (Amersham Pharmacia Biotech) were substituted for Q–Sepharose and phosphocellulose resin. Mononucleosomes reconstituted on *GAL4-E4* promoter fragment (40 nM), or each nucleosome species eluted from the gel, were incubated with NURF (0.2–0.4 nM) or purified recombinant ACF (0.8–1.6 nM; a kind gift from J.Kadonaga's laboratory) in sliding buffer (10 mM Tris–HCl pH 7.5, 50 mM NaCl, 3 mM MgCl₂, 1 mM β -mercaptoethanol, 1 mg/ml BSA) containing 1 mM ATP for 30 min at 26°C. Nucleosome sliding was analyzed by 4.5% PAGE in 0.5 \times TBE.

Micrococcal nuclease and restriction enzyme digestion

Nucleosomes were digested with MNase (25 U/ml) in the presence of 20 μ g/ml sheared sperm salmon DNA and 1 mM CaCl₂ for 30 min on ice and 3 min at 37°C as recommended by Davey *et al.* (1998). The digestions were stopped with the addition of 100 μ l of stop solution (20 mM EDTA, 0.2 M NaCl, 1% SDS, 0.25 mg/ml glycogen, 0.2 mg/ml proteinase K) and incubated for 30 min at 37°C. MNase digests were purified by phenol–chloroform extraction and ethanol precipitation. Purified DNAs were further digested with *Bam*I (1300 U/ml) or *Fnu*4HI

(333 U/ml) at 37°C for 30 min, analyzed by TBE–8% PAGE at 250 V for 2 h and visualized by autoradiography.

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