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Role of histone tails in nucleosome remodeling by *Drosophila* NURF

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The *Drosophila* nucleosome remodeling factor NURF utilizes the energy of ATP hydrolysis to perturb the structure of nucleosomes and facilitate binding of transcription factors. The ATPase activity of purified NURF is stimulated significantly more by nucleosomes than by naked DNA or histones alone, suggesting that NURF is able to recognize specific features of the nucleosome. Here, we show that the interaction between NURF and nucleosomes is impaired by proteolytic removal of the N-terminal histone tails and by chemical cross-linking of nucleosomal histones. The ATPase activity of NURF is also competitively inhibited by each of the four *Drosophila* histone tails expressed as GST fusion proteins. A similar inhibition is observed for a histone H4 tail substituted with glutamine at four conserved, acetylatable lysines. These findings indicate a novel role for the flexible histone tails in chromatin remodeling by NURF, and this role may, in part, be independent of histone acetylation.

Keywords: *Drosophila*/histone tails/nucleosome remodeling/NURF

Introduction

Biochemical and genetic experiments over the past decade have confirmed the prevailing assumption that the organization of eukaryotic DNA in nucleosomes exerts a general repressive effect on gene activity (Felsenfeld, 1992; Kornberg and Lorch, 1992, 1995; Patterton and Wolffe, 1996). Alleviation of this repression is necessary to allow the initiation of transcription. This derepression involves perturbation of nucleosome structure by multiple cellular mechanisms, including the binding of sequence-specific transcription factors (Adams and Workman, 1993; Tsukiyama *et al*., 1994; Chen and Workman, 1994), histone modification (Lee *et al.*, 1993; Puerta *et al.*, 1995; Taunton *et al.*, 1996; Wolffe and Pruss, 1996) and the action of the energy-dependent protein complexes SWI/SNF and NURF (Carlson and Laurent, 1994; Côté *et al.*, 1994; Tsukiyama and Wu, 1995).

The SWI/SNF and NURF multi-protein complexes hydrolyze ATP through related ATPase subunits SWI2/SNF2 (Laurent *et al.*, 1993; Cairns *et al.*, 1994; Côté *et al.*, 1994) and ISWI (Tsukiyama *et al.*, 1995) to induce changes in nucleosome structure, allowing increased access to nucleosomal DNA for sequence-specific transcription factors. The NURF complex facilitates GAGA transcription factor-mediated nucleosome disruption on nucleosome arrays assembled from crude extracts (Tsukiyama and Wu, 1995). The SWI/SNF complex confers persistent nucleosome reorganization at sites of GAL4 transcription factor binding in a nucleosome array assembled from core histones and tandem repeats of a nucleosome positioning sequence (Owen-Hughes *et al.*, 1996). In the absence of sequence-specific DNA-binding factors, both the SWI/SNF and NURF complexes are also able to perturb the structure of single nucleosomes reconstituted from purified components.

Despite these apparent functional similarities, the size and composition of the SWI/SNF and NURF complexes are substantially different. NURF is a four polypeptide complex of ~500 kDa (Tsukiyama and Wu, 1995), while the SWI/SNF complex contains at least 10 polypeptides with a combined mol. wt of ~2000 kDa (Cairns *et al.*, 1994; Côté *et al.*, 1994; Peterson *et al.*, 1994). The interactions between the two protein complexes and chromatin are also different, as revealed by DNase I footprinting. The SWI/SNF complex disrupts the DNase I digestion pattern of nucleosomal DNA such that it resembles a superimposition of naked DNA and nucleosomal DNA (Côté *et al.*, 1994; Imbalzano *et al.*, 1994), while the pattern conferred by the NURF complex shows protection and enhancements from DNase I cleavage at specific sites on nucleosomal DNA. Of special interest is the means by which the ATPase activity of the SWI/SNF and NURF complexes can be stimulated. The ATPase activity of SWI/SNF is stimulated equally by free DNA and nucleosomal DNA (Laurent *et al.*, 1993), while that of NURF is stimulated by nucleosomes significantly more than it is by free DNA or free histones (Tsukiyama and Wu, 1995). These findings suggest that the substrate in chromatin recognized by the SWI/SNF complex is DNA, while that recognized by NURF is the DNA–histone complex. The distinct substrate requirements for the two protein complexes may reflect distinct pathways of nucleosome remodeling.

Here, we have investigated the specific features of the nucleosome that are necessary for interaction with NURF. We have employed limited trypsin digestion of nucleosomes to remove the unstructured N-terminal histone tails (van Holde, 1989; Richmond *et al.*, 1984, 1993; Arents *et al.*, 1991; Moudrianakis and Arents, 1993), and dimethyl suberimidate (DMS) cross-linking of core histones within the histone octamer to prevent histone rearrangement (Thomas and Kornberg, 1978). Such techniques have been deployed effectively to analyze the role of histone architecture in the accessibility of nucleosomal DNA to transcription factors (Laurent *et al.*, 1993; Lee *et al.*, 1993; Côté *et al.*, 1994; Juan *et al.*, 1994; Vettese-Dadey *et al.*,

1994). We have also investigated the ability of unacetylated, bacterially expressed GST–histone tail fusion proteins to act as competitive inhibitors of the nucleosomal interaction with NURF. Our results indicate a role for each of the four histone tails in the mechanism of chromatin remodeling by NURF, and further reveal, for histone H4, an involvement of tail sequences that is distinct from the four highly conserved, acetylatable lysines at positions 5, 8, 12 and 16.

Results

Effect of histone tail removal on NURF activities

In order to analyze the specific role of the histone tails in the interaction with NURF, reconstituted mononucleosomes were digested with trypsin according to established procedures to remove the flexible tails from all four core histones (Ausio *et al.*, 1989; Thomas, 1989). The extent of trypsinization was monitored by SDS–PAGE, and the complete removal of the histone tails after 30 min of digestion was gauged by the quantitative reduction in size of the histone polypeptides to characteristic subfragments that correspond to the residual globular histone regions (Ausio *et al.*, 1989) (Figure 1A). When trypsinized nucleosomes were incubated with NURF and [γ-^{32}P]ATP, stimulation of the ATPase activity of NURF was decreased upon partial cleavage of the histone tails (10 min), and activity was decreased further upon complete removal of the histone tails (30 min) (Figure 1B). No decrease of ATPase activity was observed when trypsin inhibitors were introduced prior to the reaction. These results indicate an important role for the histone tails in nucleosome interactions with NURF.

Diminished interactions between NURF and trypsinized nucleosomes were also observed by DNase I footprinting analysis. As noted previously, mononucleosomes reconstituted on a *hsp70* promoter fragment are located at multiple translational and rotational positions, leading to an irregular 10 bp repeat in the pattern of DNase I cleavage (Tsukiyama and Wu, 1995). The interactions between NURF and such nucleosomes are characterized by reduced DNase I cleavage at many sites along the 161 bp promoter fragment and typically enhanced cleavage at positions –125 and –126 (Tsukiyama and Wu, 1995) (Figure 1C, lanes 3 and 4). When nucleosomes partially trypsinized for 10 min were incubated with NURF and ATP, the enhanced DNase I cleavages at positions –125 and –126 were no longer detectable, although significant DNase I protection was still observed. When nucleosomes fully trypsinized for 30 min were analyzed, a substantial loss of DNase I footprinting occurred over the 161 bp fragment in addition to the disappearance of the enhanced cleavages at –125 and –126 (Figure 1C, lanes 5–8).

Effect of histone cross-linking on NURF activities

We analyzed the effect of histone cross-linking on NURF activity by incubating DMS-cross-linked mononucleosomes with NURF and [γ-^{32}P]ATP (Figure 2A). DMS reacts with histones primarily through lysine residues, and its long linker arm (~1 nm) can covalently tether virtually any pair of histones (Thomas, 1989). The sites of cross-linking are located in both the structured core histone domains and in the lysine-rich, unstructured N-terminal tails which emerge from the nucleosome core (Suda and Iwai, 1979). As shown in Figure 2B, stimulation of the ATPase activity of NURF was decreased when the nucleosomal histones were partially cross-linked (15 min), and was decreased further to near basal levels when the cross-linking of histones was essentially complete (60 min). These results indicate that constraints imposed on histone rearrangement in the globular or tail regions, or on the availability of histone lysines subjected to monofunctional reaction with DMS (Thomas, 1989), can also affect the ability of the nucleosome to interact with NURF. Consistent with these findings, we failed to observe changes in the DNase I footprinting pattern when NURF was incubated with fully cross-linked mononucleosomes (Figure 2C).

GST–histone tail fusions inhibit nucleosome-stimulated ATPase activity

To investigate further the role of the positively charged, flexible histone tails in nucleosomal interactions with NURF, we made constructs containing sequences corresponding to the *Drosophila* histone tails joined to the C-terminal end of the GST gene. The *Drosophila* GST–histone tail fusion proteins, as well as their conserved yeast counterparts (Hecht *et al.*, 1995), were expressed in *Escherichia coli* and purified by affinity chromatography (Figure 3A). As may be expected of bacterially expressed proteins, the GST–histone tail fusions were not subject to acetylation, as shown by Western blot analysis (data not shown). We then tested the ability of the GST–histone tails to stimulate the intrinsic ATPase activity of NURF. Consistent with previous experiments using purified core histones (Tsukiyama and Wu, 1995), the GST–histone tail fusions, individually or in combination, could not stimulate the ATPase activity of NURF to the level observed for intact nucleosomes (Figure 3B). However, because stimulation of the ATPase activity by nucleosomes is likely to result from a multi-step process involving obligatory interactions with several nucleosomal components, we tested the ability of the histone tail fusions to act as competitive inhibitors of the nucleosome-stimulated ATPase activity. When the GST–*Drosophila* histone tails (referred to as GST–dH3, GST–dH4, GST–dH2A and GST–dH2B) were introduced individually into reactions containing nucleosomes and NURF at a 1:1 molar ratio of fusion protein:NURF, the nucleosome-stimulated ATPase activity of NURF was decreased significantly, with GST–dH3 and GST–dH4 giving a somewhat greater inhibition than GST–dH2A and GST–dH2B (Figure 3C). Control experiments using GST–MyoD and GST failed to show any inhibition. The observed competitive inhibition of the nucleosome-stimulated ATPase activity of NURF indicates that each of the unacetylated histone tails may constitute an important part of the NURF–nucleosome interaction.

The sequences of the yeast histone H3 and H4 tails are nearly identical with those of *Drosophila*, with 44 and 32 identities respectively, and only two conservative substitutions for each histone tail. Hence, it is not surprising that the yeast H3 and H4 tail fusions (GST–yH3 and GST–yH4) also efficiently inhibited the ATPase activity of NURF (Figure 3D). Interestingly, GST–yH2B failed to show inhibition when added to the reaction at comparable molar ratios. Because the amino acid sequences of the

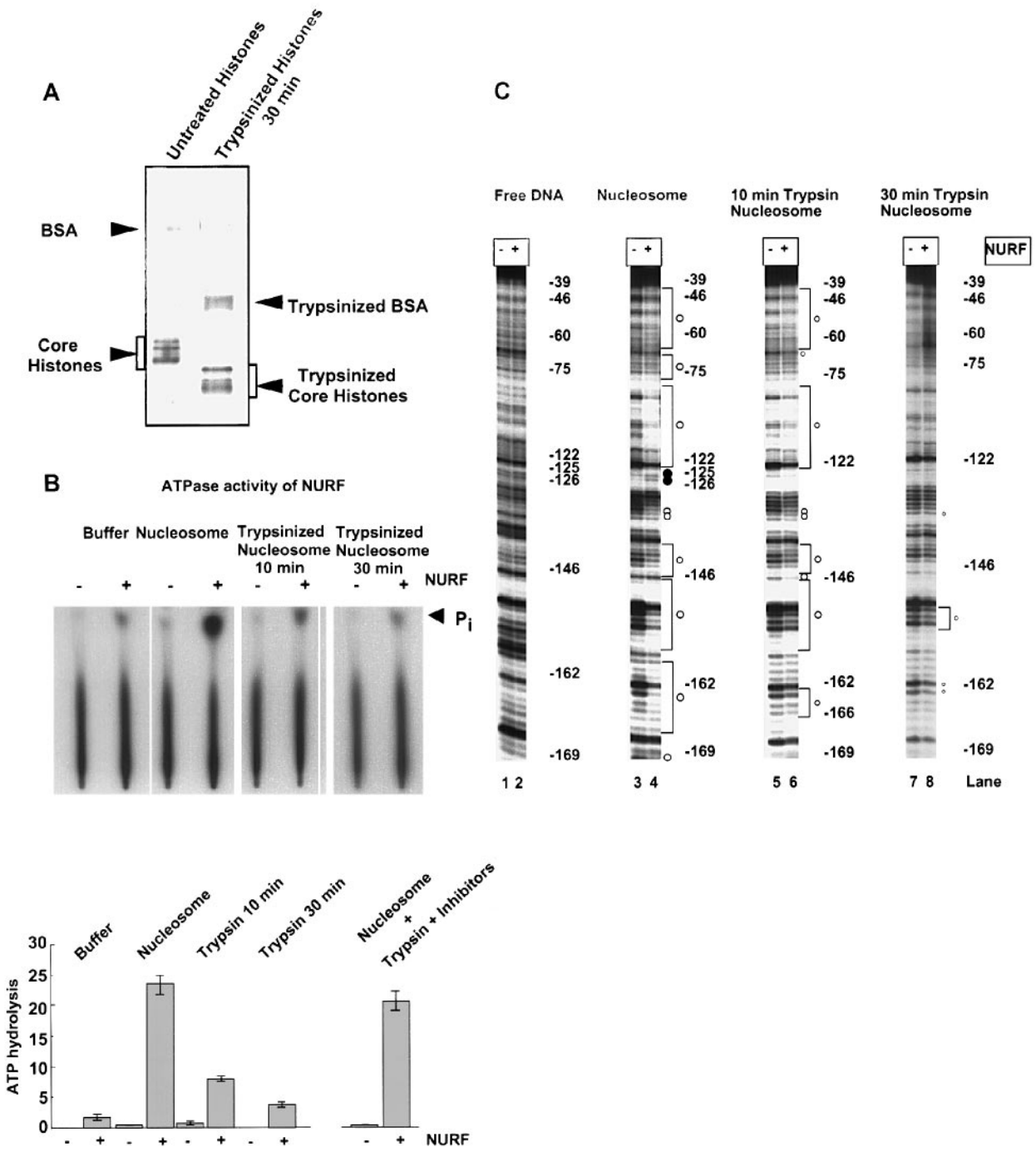

Fig. 1. Effects of histone tail cleavage on NURF activity. (**A**) Reconstituted mononucleosomes (50 µl) were treated with 1 µl of 0.066 ng/µl trypsin solution for 0–60 min at room temperature. The reaction was stopped by addition of 2 µl of a 100 mM AEBSF solution plus 1.85 µl of TLCK solution. Aliquots were taken after 10 (not shown) and 30 min and analyzed by 18% SDS–PAGE. The extent of trypsinization of the tails was monitored by the loss of the full-length core histones and the appearance of shorter peptides corresponding to the trimmed core histones (Ausio *et al.*, 1989). (**B**) Nucleosome-stimulated ATPase activity of NURF is impaired by digestion of histone tails with trypsin for 10 (partial cleavage) and 30 min (complete cleavage). The molar ratio of NURF to nucleosome is ~1:1.8. Thin-layer chromatogram shows ATP hydrolysis to P_i for NURF incubated with buffer and with nucleosomes untreated or treated with trypsin for 10 and 30 min. Experiments were performed in triplicate, and the averaged results are presented with error bars. The presence of trypsin plus inhibitors does not decrease the ATPase activity (this experiment was done in duplicate). (**C**) DNase I footprinting shows the decrease of NURF interactions with trypsinized nucleosomes. The filled circles represent enhanced DNase I cleavages at positions –125 and –126 in the presence of NURF; open circles represent sites of DNase I protection. Small open circles indicate sites of residual protection after 30 min of trypsinization. There is a gel artifact in the –65 region of lane 8 which leads to radioactive trailing and apparent hypersensitivity which is absent in other experiments. We note that the pattern of DNase I cleavage observed in the presence of NURF differs slightly from that previously reported (Tsukiyama and Wu, 1995). The locations of the DNase I-protected regions are very similar, but the sites of enhancement are restricted to positions –125 and –126. The reason for this decrease in the number of enhanced cleavage sites is unclear at present.

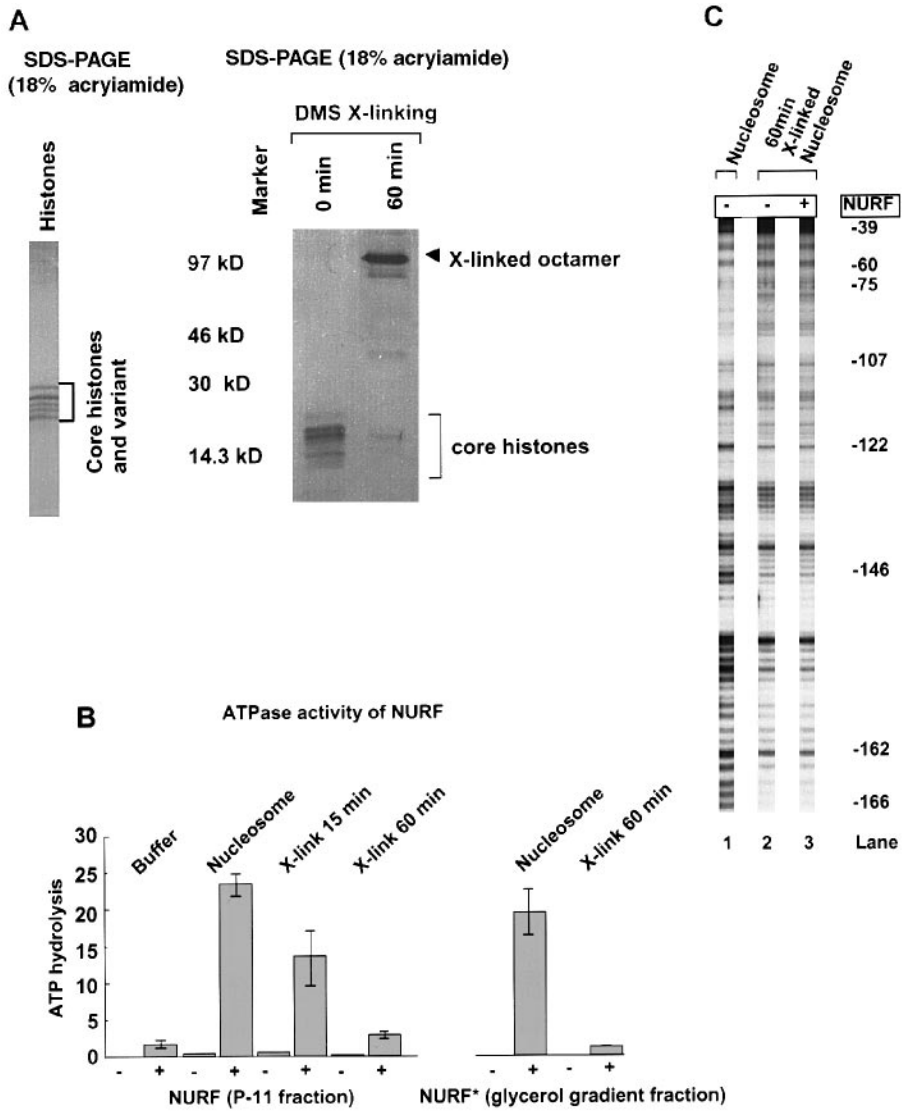

Fig. 2. Effects of histone–histone cross-linking on NURF activity. (**A**) Left panel: starting material: purified histones (Simon and Felsenfeld, 1979) verified by SDS–PAGE (18% acrylamide). As reported previously (Becker and Wu, 1992), there is an undefined histone variant or modified histone present in the preparation from 0–20 h embryos. Right panel: aliquots of nucleosomes were taken after 15, 30 (not shown) and 60 min of DMS treatment and analyzed by SDS–PAGE (18% gel), followed by silver staining. The extent of cross-linking was verified by the disappearance of the individual histones and the appearance of a new protein band migrating at ~90 kDa. This band (X-linked octamer) was absent when DMS was omitted from the reaction. (**B**) Nucleosome-stimulated ATPase activity of NURF (P-11 fraction) (Tsukiyama and Wu, 1995) is impaired by DMS cross-linking of the histone octamer. The bar graph displays the percentage ATP hydrolysis after subtraction of the endogenous P_i present in the [γ-^{32}P]ATP sample (average results of three experiments). Similar results are obtained with NURF* (glycerol gradient fraction, the purest available sample) (Tsukiyama and Wu, 1995). (**C**) DNase I footprinting shows the absence of NURF interactions with cross-linked nucleosomes. The radiolabeled fragment used for nucleosome reconstitution spans the *hsp70* promoter from position –185 to –30 plus 6 bp from the pBlueScript vector. As observed in other work (Vettese-Dadey *et al.*, 1994), the process of DMS cross-linking introduces changes in the pattern of DNase I cleavage of nucleosomal DNA.

Drosophila and yeast H2B tails are substantially less conserved (41% identity) when compared with the sequences of *Drosophila* and yeast H2A, H3 and H4 (H2A, 65% identity; H3, 93% identity; H4, 94% identity), this result suggests that the interaction between NURF and the *Drosophila* histone H2B tail may be species specific.

Histone acetylation and NURF ATPase activity

We next analyzed the inhibitory effect of a yeast H4 tail mutant in which four conserved lysines at positions 5, 8, 12 and 16 were substituted with glutamine [GST–yH4 (Q5,8,12,16); Hecht *et al.*, 1995]. Mutation of all four acetylatable lysines affects cell division, blocks sporulation, decreases transcriptional activation of *GAL1* and *PHO5* and affects genome integrity (Megee *et al.*, 1990, 1995; Park and Szostak, 1990; Durrin *et al.*, 1991). A yeast H4 K16Q mutant also affects silencing of the mating type locus *in vivo*, and this substitution at Lys16 in the context of the same substitution at lysines 5, 8 and 12 abolishes binding to the silencing information regulator SIR3 (Hecht *et al.*, 1995). As shown in Figure 3D, the GST–yH4 (Q5,8,12,16) mutant protein was still able to inhibit the ATPase activity of NURF substantially, although not to the same extent as wild-type GST–yH4. As the substitution of four positively charged lysines with glutamine in the GST–yH4 (Q5,8,12,16) protein mimics the

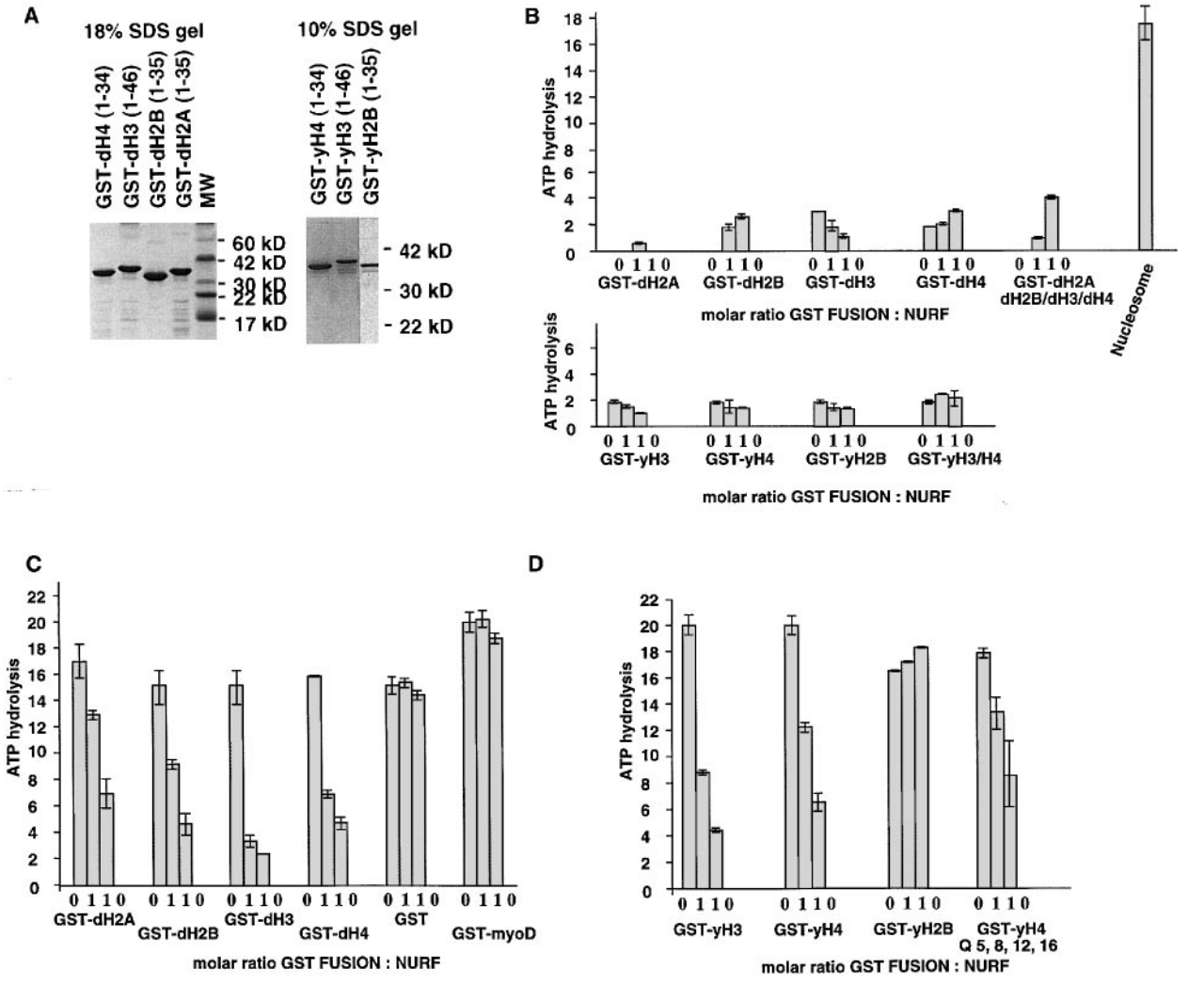

Fig. 3. Inhibition of NURF activity by GST–histone tails. (**A**) SDS–PAGE (18% gel) analysis of GST–histone tail fusions. Left panel: purified GST–*Drosophila* histone tail fusions. Right panel: GST–yeast histone tail fusions. (**B**) No substantial activation of nucleosome-stimulated ATPase activity by GST–histone tails. Increasing amounts of purified GST–*Drosophila* histone tail fusions (top panel) and GST–yeast histone tail fusions (bottom panel) were introduced with NURF in the ATPase assay (molar ratios of GST–histone tail to NURF of 1:1 and 10:1). The bar graphs display the percentage ATP hydrolysis after subtraction of the background hydrolysis contaminating the GST fusion preparation and the endogenous P_i present in the [γ-^{32}P]ATP sample (average results of three experiments). (**C**) Inhibition of nucleosome-stimulated ATPase activity by GST–*Drosophila* histone tails. Increasing amounts of purified GST–*Drosophila* histone tail fusions were introduced with nucleosomes and NURF into the ATPase assay (molar ratios of GST–histone tail to NURF of 1:1 and 10:1). The ratio of NURF to nucleosomes was 1:1.8 in all reactions. Controls using GST–MyoD and GST alone were performed in duplicate. (**D**) Inhibition of nucleosome-stimulated ATPase activity by GST–yeast histone tails (yH2B, yH3 and yH4) and by the mutant GST–yH4 Q5,8,12,16. Conditions are as in (C).

fully acetylated state of the wild-type histone H4 tail, the observed failure to abolish or greatly reduce the competitive inhibition suggests that the acetylation state of these four lysines is unlikely to be relevant for stimulating the ATPase activity of NURF.

In order to confirm these findings, we analyzed the ability of hyperacetylated nucleosomes to stimulate the ATPase activity of NURF. When hyperacetylated nucleosomes purified from sodium butyrate-treated HeLa cells or reconstituted with purified histones from untreated and sodium butyrate-treated *Drosophila* SL2 cells were tested in the NURF ATPase activity assay, no significant difference in stimulation of ATPase activity was observed (Figure 4A). The extent of histone H4 acetylation in the butyrate-treated preparations was confirmed by Triton–acid–urea gel electrophoresis (Figure 4C). Taken together, our results suggest strongly that the activity of NURF on nucleosomes, as measured by stimulation of ATP hydrolysis, is independent of the state of histone acetylation.

Minor interactions between NURF and DNA

Previously, the NURF complex was shown to have no interactions with linear DNA that could be detected by DNase I footprinting, while the footprinting pattern of NURF on nucleosomal DNA was found to be significantly altered in the presence of ATP (Tsukiyama and Wu, 1995). It was also found that the ATPase activity of NURF was hardly stimulated by the presence of free, linear DNA. Because DNA within nucleosomes is wound in a superhelix over the surface of the histone octamer, we investigated whether several DNA structures that bear a resemblance to nucleosomal DNA could show preferential stimulation of its intrinsic ATPase activity. As shown in

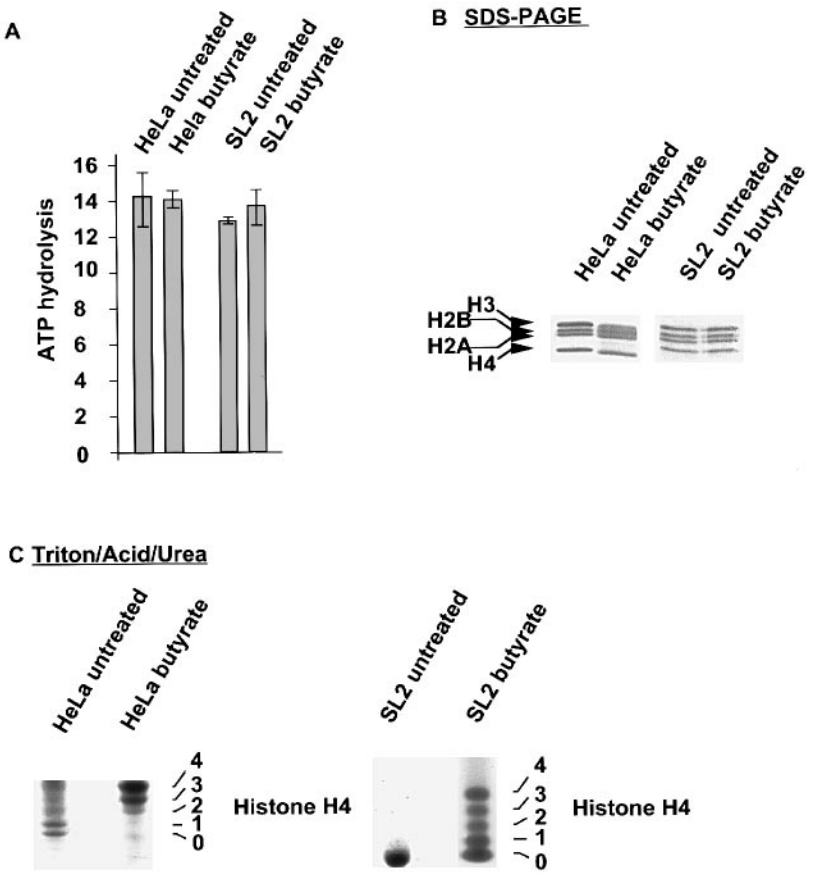

Fig. 4. Effect of hyperacetylated nucleosomes on NURF ATPase activity. (**A**) Nucleosome-stimulated ATPase activity of NURF is not significantly affected by the presence of hyperacetylated histones. Similar amounts of normal and hyperacetylated mononucleosomes were utilized for the NURF ATPase assay. (**B**) SDS–PAGE (18% gel) analysis of core histones. Coomassie blue staining of purified normal (untreated) and hyperacetylated (sodium butyrate-treated) HeLa and SL2 cell nucleosomes. (**C**) 15% Triton–acid–urea gel. Silver staining of the same set of histones (untreated or sodium butyrate-treated) shows the changes in the level of acetylation of histone H4. The mono-, di-, tri- and tetra-acetylated forms are denoted by the numbers on the side. Histone acetylation was also confirmed by immunoreaction with antibodies against acetylated histone H3 and H4 (not shown).

Figure 5A, neither supercoiled, relaxed, bent nor four-way junction DNAs at similar (10 nM) concentrations displayed a stimulation of the ATPase activity of NURF that was substantially greater than the low-level stimulation exhibited by linear DNA. Similar results were obtained using equivalent amounts by mass of each DNA template (data not shown). On average, the ATPase activity of NURF was stimulated only 1.5-fold by free DNA, while a 7- to 10-fold stimulation was observed for the SWI/SNF complex (Figure 5A; Côté *et al.*, 1994). We conclude, therefore, that structured DNA alone does not serve as a major determinant for a productive interaction with NURF.

We further assessed whether free DNA could serve as a competitive inhibitor of the nucleosome-stimulated ATPase activity of NURF. As shown in Figure 5B, a 4-fold molar excess of free linear or four-way junction DNA to NURF displayed no significant inhibition of the ATPase activity. Significant inhibition was observed with substantially higher amounts of DNA (22- and 44-fold molar excess of DNA to NURF), and this inhibition appeared slightly greater for four-way junction DNA than for linear DNA (by 1.3-fold). However, when compared with the inhibition of ATPase activity observed by the introduction of equimolar amounts of the GST–histone tails, the inefficient inhibition conferred by linear or structured DNA suggests that the interaction between NURF and DNA in nucleosomes is substantially weaker. These findings reveal an additional difference in behavior between NURF and the SWI/SNF complex, which recently was found to have a special affinity for structured (four-way junction) DNA (Quinn *et al.*, 1996).

We were unable to analyze the inhibitory effects of supercoiled or bent DNA on the nucleosome-stimulated ATPase activity of NURF, as the incubation of nucleosomes with these DNAs resulted in a loss of nucleosome structure as measured by electrophoretic mobility shift analysis (data not shown); such loss is most likely due to preferential exchange of histones from the nucleosome to the structured DNAs. Control experiments using linear or four-way junction DNAs showed no loss of nucleosome structure in the presence or absence of NURF even at the highest levels of competitor DNA (Figure 5C), indicating that the inefficient inhibition of the nucleosome-stimulated ATPase activity by these DNAs was not a trivial consequence of histone exchange. Overall, our results point toward a possible role for DNA in the recognition of nucleosomes by NURF, but this role is likely to be minor when compared with the role of the histone tails.

Discussion

What are the structural determinants of nucleosomes that are important for the activity of NURF? Based on the loss

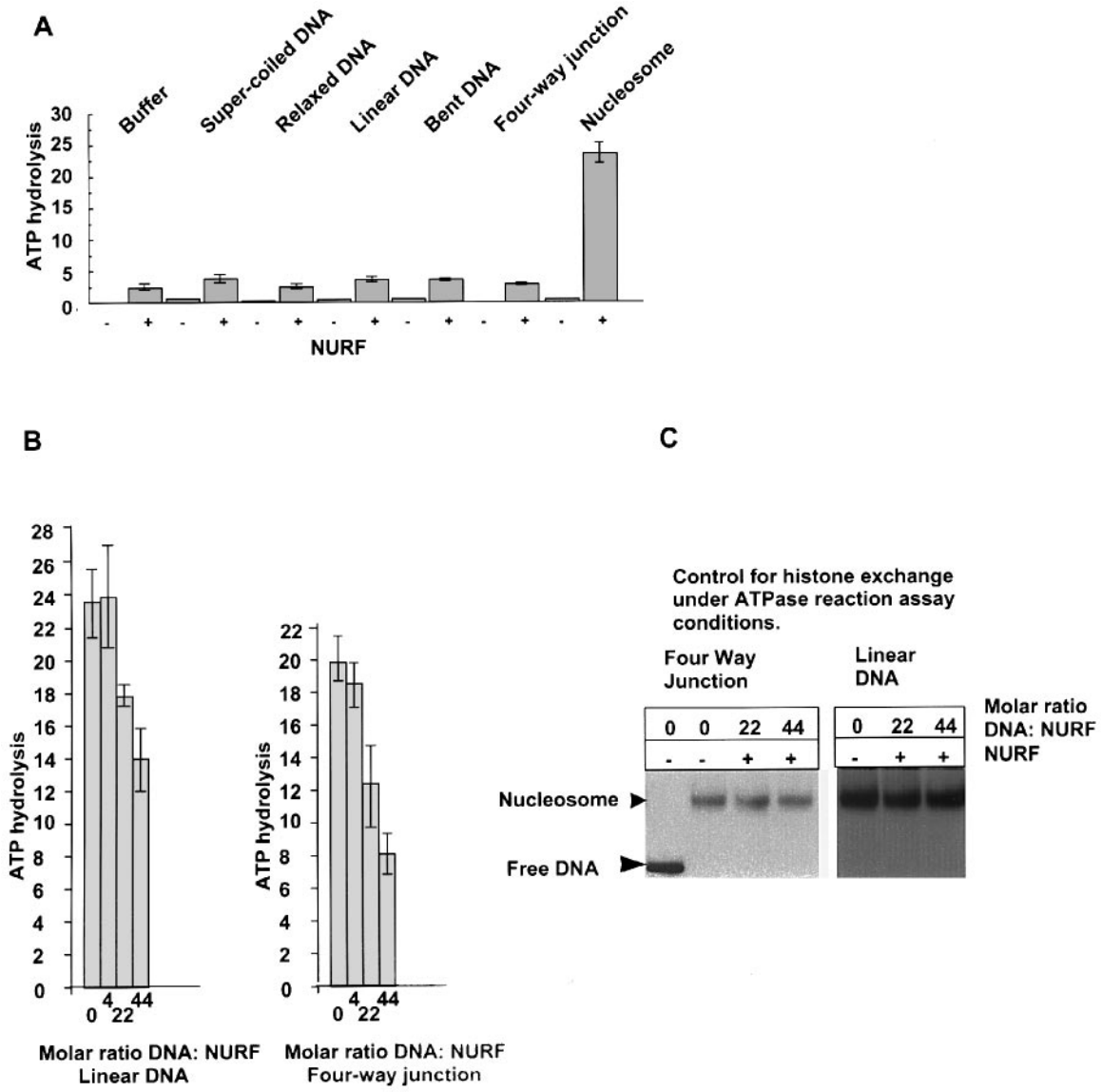

Fig. 5. Effects of DNA on ATPase activity of NURF. (**A**) The ATPase activity of NURF was tested in the presence of 10 nM concentrations of: plasmid pdHSP70 DNA, either supercoiled, relaxed by topoisomerase I or linearized by *Xho*I cleavage, bent DNA fragment from the sea urchin 5S gene positioning sequence (208 bp) (Georgel *et al.*, 1993) and synthetic four-way junction DNA, in a final reaction volume of 5 µl. Nucleosomes were utilized as a positive control. (**B**) Inhibition of nucleosome-stimulated ATPase activity by DNA. Increasing amounts of *E.coli* genomic DNA of average length of about 500 bp (right panel) or four-way junction DNA (left panel) were introduced with nucleosomes and NURF into the ATPase assay (DNA:NURF molar ratios of 4:1, 22:1 and 44:1). The bar graphs display the percentage ATP hydrolysis after subtraction of the background hydrolysis from the endogenous P_i present in the [γ-^{32}P]ATP sample (average results of three experiments). (**C**) Control for histone exchange. Electrophoretic mobility shift assay of reconstituted nucleosomes incubated with increasing amounts of free DNA under conditions of the NURF ATPase assay. No histone exchange could be detected (monitored by the appearance of free labeled DNA) at the highest DNA concentrations tested. Moreover, the integrity of the reconstituted nucleosomes in the lanes lacking competitor DNA confirms the stability of nucleosomes (5.9 ng DNA/µl) under the conditions of DNase I footprinting and the ATPase assay.

of the nucleosome-stimulated ATPase activity of NURF and the diminution of the DNase I footprint when the histone tails are removed by limited proteolysis, we suggest that the flexible tails of the *Drosophila* core histones are critical elements for interaction with NURF. This conclusion is strengthened by the inhibition of NURF ATPase activity by GST–histone fusions. The effects of cross-linking the core histones in nucleosomes are also consistent with a contribution from the histone tails, although contributions from the globular domains of the nucleosome core histones cannot be excluded by this technique. Finally, a minor role for nucleosomal DNA is indicated by the modest inhibitory effects of DNA on the nucleosome-stimulated ATPase activity of NURF. These several determinants, individually insufficient for stimulating the ATPase activity of NURF, may be required in a combinatorial manner for achieving ATP-dependent perturbation of nucleosome structure. It will be of interest to relate the recognition of these determinants to one or more subunits of the NURF complex, and to analyze how this recognition is transduced to nucleosomal reorganization coupled with the utilization of chemical energy. Although a discrete supercomplex of NURF and a nucleosome has not been detected by native gel electrophoresis (P.T.Georgel, unpublished observations), it will also be important, when sufficient amounts of NURF become

available for systematic studies, to define the interactions between NURF and nucleosomes quantitatively by biophysical methods, and to determine the histone composition of the remodeled nucleosome.

The requirement for the *Drosophila* histone tails in nucleosomal interactions with NURF and the lack of strong binding specificity for structured DNA, a property of the SWI/SNF complex (Quinn *et al.*, 1996), provides further evidence for separate modes of action for the NURF and SWI/SNF chromatin remodeling complexes, which share related ATPase subunits ISWI (Tsukiyama *et al.*, 1995) and SWI2/SNF2 (Côté *et al.*, 1994), and the ability to alter chromatin structure *in vitro* in an ATP-dependent manner (Côté *et al.*, 1994; Tsukiyama and Wu, 1995; Owen-Hughes *et al.*, 1996). Genetic studies have shown that the histone H2A/H2B tails and the histone H3/H4 tails are essential for viability in yeast (Ling *et al.*, 1996). For histones H3 and H4, the tails are also important for repression of basal transcription, for telomeric and silent mating locus repression and for activation and repression of some genes (Thompson *et al.*, 1993; Grunstein *et al.*, 1995). The H3 and H4 tails were shown recently to bind *in vitro* with the yeast silencing information regulators SIR3 and SIR4 (Hecht *et al.*, 1995), providing direct evidence that these extended regions may form specific binding sites for protein regulators of nucleosome structure and function. Tup1, a repressor of transcription of yeast **a**-cell specific genes, has also been demonstrated to interact directly with the tails of histones H3 and H4 (Edmonson *et al.*, 1996). Together with our present findings, these results suggest that the flexible tails of the histone octamer serve as common sites of interaction with several distinct nuclear protein complexes that affect nucleosome stability in a positive or negative manner.

Other biochemical studies have demonstrated that the basic histone tails partially restrict binding of transcription factors to nucleosomal DNA (Lee *et al.*, 1993; Juan *et al.*, 1994; Vettese-Dadey *et al.*, 1994). This restricted accessibility of nucleosomal DNA imposed by the histone tails can be alleviated upon neutralization of charged lysines by acetylation (Lee *et al.*, 1993; Turner and O'Neill, 1995; Vettese-Dadey *et al.*, 1996). However, as indicated by the ability of the GST–yH4 (Q5,8,12,16) mutant protein to retain competitive inhibition of the nucleosome-stimulated ATPase activity of NURF, the four acetylatable lysines of histone H4 in yeast do not seem to be of crucial importance for interaction with NURF, as measured by the ATPase assay. These lysine positions are strictly conserved in the *Drosophila* histone H4 tail and also undergo acetylation (for reviews, see Turner, 1993; Loidl, 1994; Turner and O'Neill, 1995). Hence, the remaining conserved amino acid residues of the *Drosophila* histone H4 tail are likely to be involved in the interaction with NURF, and this interaction, at least for histone H4, could be independent of the state of lysine acetylation. It should be noted that our results do not exclude an interaction between NURF and other lysine residues of the histone tails that are not subject to acetylation. Nonetheless, the observed ability of hyperacetylated nucleosomes to stimulate the ATPase activity of NURF as well as normal nucleosomes, in the case of both HeLa cell and *Drosophila* histones, is consistent with the possibility that NURF may act independently of the histone acetylation pathway of nucleosome destabilization. It will be of interest to elucidate, by site-directed mutagenesis, the precise nature of the interaction between NURF and the histone tails, to understand the mechanism by which this interaction leads to nucleosomal reorganization and to define the parallel or sequential nature of the pathways of nucleosome reorganization by chromatin remodeling and histone modifying activities.

Materials and methods

Preparation of mononucleosomes

Core histones were prepared from 0–20 h *Drosophila* embryos (Simon and Felsenfeld, 1979). Mononucleosomes were reconstituted as described previously (Tsukiyama and Wu, 1995). *Escherichia coli* DNA carrier (500 ng) plus 500 ng of bovine serum albumin and 1.16 µl of a 0.53 mg/ml solution of purified histones (in 10 mM Tris pH 7.6, 1 mM EDTA and 2 M NaCl) were added to 2.5 µl of 0.2 nmol/µl of a gel-purified 161 bp *hsp70* promoter fragment generated by PCR with a 5′ end-labeled primer. Mononucleosomes were assembled by salt dialysis using a gradient from 2 M to 50 mM NaCl in 10 mM Tris pH 7.6, 1 mM EDTA, 1 mM β-mercaptoethanol and 0.05% NP-40 (Neubauer and Horz, 1989). Reconstituted nucleosomes were stored on ice at 4°C. Only when reconstitution of the 161 bp fragment to mononucleosomes was 95%, as assayed by enzyme-linked immunosorbent assay, were the samples utilized for subsequent experiments. The final concentration of nucleosomes was 10 ng DNA/µl in 50 µl volume. Hyperacetylated mononucleosomes purified from sodium butyrate-treated HeLa cells as described by Ausio and van Holde (1986) were a generous gift from Dr M.Bustin and Dr L.Trieschmann.

Hyperacetylated and control *Drosophila* histones were prepared from 250 ml cultures of SL2 cells grown in HyQ-CCM 3 (HyClone) medium supplemented with gentamycin. Hyperacetylated histones were obtained by adding sodium butyrate to the medium to a final concentration of 10 mM and incubating for 24 h at room temperature. After harvesting (3600 *g* for 10 min), the cells were washed twice in buffer containing 137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 7\ H_2O$, 1.4 mM KH_2PO_4 and 1 mM AEBSF. All the buffers used to isolate the hyperacetylated histones were adjusted to 10 mM sodium butyrate. The cells, resuspended in 20 volumes of 10 mM Tris–HCl pH 8.0, 3 mM $MgCl_2$, 0.25 M sucrose, 1% NP-40 and 1 mM AEBSF, were lysed using a Dounce homogenizer with a B pestle. The nuclei were pelleted for 20 min at 3600 *g* and washed twice in the lysis buffer followed by two washes in the same buffer without NP-40. The nuclei were resuspended in 5 ml of 0.4 M NaCl, 10 mM Tris–HCl pH 8.0, 1 mM EDTA and 1 mM AEBSF and then stirred gently for 15 min at 4°C. After pelleting and washing twice in the same buffer, the nuclei were resuspended in 5 ml of 0.6 M NaCl, 50 mM $NaPO_4$ pH 6.8 and 1 mM AEBSF and stirred for 10 min at 4°C to lyse the nuclear membrane. Two g of dry Bio-Gel HTP hydroxylapatite (Bio-Rad) were added to the lysate and the resin was allowed to swell to a paste, poured into a column and washed with 10 volumes of the same buffer. The histones were eluted in 2.5 M NaCl, 50 mM $NaPO_4$ pH 6.8 and 1 mM AEBSF. The histone purity was analyzed by SDS–PAGE and Coomassie blue staining. Mononucleosomes were then reconstituted as described above using either untreated or hyperacetylated SL2 histones.

Digestion of histone tails with trypsin

Reconstituted mononucleosomes (50 µl) were treated with 1 µl of 0.066 ng/µl of trypsin (10 and 30 min at room temperature) (Ausio *et al.*, 1989). The reaction was stopped by addition of 2 µl of a 100 mM AEBSF solution plus 1.85 µg of TLCK (Vettese-Dadey *et al.*, 1994). Samples were stored on ice at 4°C and used directly for ATPase and DNase I footprinting assays. The extent of histone cleavage was monitored by SDS–PAGE and silver staining.

Dimethyl suberimidate (DMS) cross-linking

Mononucleosomes (50 µl volume) were dialyzed against 1 l of 10 mM HEPES pH 7.6, 1 mM EDTA, 50 mM NaCl and 0.05% NP-40 overnight at 4°C. DMS (22 mg) was dissolved in 1 ml of 10 mM HEPES pH 10.5, 1 mM EDTA and 50 mM NaCl. Five µl of the 22 mg/ml solution of DMS were added to the dialyzed nucleosomes and incubated for 15–60 min at room temperature (Thomas, 1989). The reaction was stopped by dialyzing the mixture first against 25 mM Tris pH 6.8, 1 mM EDTA,

50 mM NaCl and 0.05% NP-40 for 90 min at 4°C and then a second time against 10 mM Tris pH 7.6, 1 mM EDTA, 50 mM NaCl and 0.05% NP-40 overnight at 4°C. Aliquots were taken and analyzed by 4–15% gradient SDS–PAGE followed by silver staining to verify the extent of cross-linking as monitored by the disappearance of the individual histones and the appearance of a new protein band migrating at ~90 kDa.

ATPase assay

The ATPase assay was performed in a final volume of 5 µl using 0.5 µl of partially purified NURF (P-11 fraction, the penultimate fraction in HEMGN + 0.3 M KCl) (Tsukiyama and Wu, 1995), 0.5 µl of 10 mM $MgCl_2$, 0.25 µl of [γ-^{32}P]ATP (3000 Ci/mmol, 10 µCi/µl, Amersham or NEN DuPont), 0.5 µl of 300 µM ATP, 0.75 µl of HEMGN + 0.3 M KCl (HEMGN: 25 mM HEPES KOH pH 7.6, 0.1 mM EDTA, 12.5 mM $MgCl_2$, 10% glycerol, 0.05% NP-40) added to 2.5 µl of mononucleosomes (untreated, trypsinized or cross-linked) to a final nucleosome concentration of 5.9 ng DNA/µl. After 30 min of incubation at 26°C, the samples were analyzed by thin-layer chromatography on PEI cellulose plates (Sigma) in 0.5 M LiCl and 1 M formic acid. Quantification was performed with a Fuji BioImage Analyzer. Depending on the commercial supplier and the freshness of the [γ-^{32}P]ATP, the percentage of ATP hydrolysis varies from an average of 16 to 24%.

DNase I footprinting

Five µl of mononucleosomes (untreated, trypsinized or cross-linked) plus 1 µl of 10 mM ATP, 0.5 µl each of 10 mM $MgCl_2$ and HEMGN + 0.3 M KCl (to a final volume of 8.5 µl) were incubated for 30 min at 26°C in the presence or absence of NURF (P-11 fraction, 1 µl). Nucleosomes (final concentration 5.9 ng DNA/µl) were digested with 1 µl of a 0.1 U solution of DNase I in 1 mM $CaCl_2$ and 5 mM $MgCl_2$ at room temperature for 1 min. The DNA was electrophoresed on an 8% sequencing gel.

Discontinuous acetic acid–urea–Triton X-100 gels

Discontinuous acetic acid–urea–Triton X-100 gels were prepared as described (Bonner *et al.*, 1980; Turner and O'Neill, 1995). Briefly, for 30 ml of resolving gel, 14.61 ml of 30.8% acrylamide, 1.8 ml of glacial acetic acid, 150 µl of TEMED, NH_4OH to 45 mM (final) and 14.4 g of urea were mixed and the volume was adjusted to 27.4 ml. After dissolution of the urea, 150 µl of Triton X-100 were added. Then 2.0 ml of 0.004% riboflavin stock solution were added in dim light, and the final volume was adjusted to 30 ml. The solution was poured into a 0.75 mm thick gel shell and polymerized next to a light box (four 15 W fluorescent lamps). The stacking gel was composed of 2.2 ml of 30.8% acrylamide, 1.2 ml of glacial acetic acid, 100 µl of TEMED, NH_4OH to a final 45 mM and 9.6 g of urea. The volume was brought up to 18.7 ml. After dissolution of the urea, 1.3 ml of the 0.004% riboflavin stock solution was added and the volume adjusted to 20 ml. The running buffer was 0.1 M glycine and 1 M acetic acid. The loading buffer was 15% glycerol, 6% acetic acid, 100 mM NH_4OH and 5% β-mercaptoethanol. The gels were electrophoresed overnight at constant current (5–10 mA). After electrophoresis, the gels were silver stained.

Preparation of four-way junction DNA

A synthetic four-way junction was generated by annealing sequentially four oligonucleotides in pairs of two (oligos 1 and 2 and oligos 3 and 4) and then combining the two complexes (Panyutin *et al.*, 1995). The oligonucleotide sequences are: oligo 1, ACCATGCTCGAGATTACGAGCAGCTTCGATCGTCG; oligo 2, AATTCGACGATCGAAGCTGAATACGTGAGGCCTAGGATC; oligo 3, AATTCGCATGCATGCATCGATATCTCGTAATCTCGAGCATGGT; oligo 4, GATCCTAGGCCTCACGTATTATATCGATGCATGCG.

Construction, expression and purification of GST–Drosophila histone tails

GST–*Drosophila* histone fusions of the N-termini of the four core histones were constructed by inserting PCR-generated DNA fragments containing respectively: residues 1–35 (histones H2A and H2B), residues 1–46 (histone H3) and residues 1–36 (histone H4). The size of the inserts was based on similar GST fusion proteins made with yeast histones (Hecht *et al.*, 1995). The PCR fragments were inserted into the *Bam*HI and *Eco*RI sites of the plasmid pGEX1 (Smith and Johnson, 1988). The fusion proteins were expressed in *E.coli* BL 21 (DE3 Lys E). The GST fusion products were purified from *E.coli* lysates in HEMGN + 0.2 M KCl using 100 µl of glutathione beads (Pharmacia). After 2 h of incubation at 4°C, beads were washed three times with phosphate-buffered saline and then eluted in 500 µl of 50 mM Tris–HCl pH 8.0, 10 mM glutathione. The purity of the GST–histone tail fusions (80–95%) was monitored by SDS–PAGE and Coomassie blue staining.

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