H1-mediated Repression of Transcription Factor Binding to a Stably Positioned Nucleosome*

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Previously, we reported that histone H1 binding to nucleosome cores results in repression of binding of the basic helix-loop-helix upstream stimulatory factor (USF) (Juan, L.-J., Utley, R. T., Adams, C. C., Vettese-Daley, M., and Workman, J. L. (1994) EMBO J. 13, 6031–6040). We have tested whether this inhibition resulted from H1-mediated changes in nucleosome positioning (Ura, K., Hayes, J. J., and Wolffe, A. P. (1995) EMBO J. 14, 3752–3765) forcing the USF recognition sequence into less accessible locations within the nucleosome. Nucleosome boundaries were determined by assays combining micrococcal nuclease and restriction endonuclease digestion. A unique pair of boundaries were observed, indicating a single nucleosome translational position. This nucleosome position did not change on H1 or USF binding. Thus, H1 repression of USF binding was independent of nucleosome mobility, indicating an alternative mechanism of H1 repression. H1 repressed USF binding at a site 35 base pairs into the nucleosome core more effectively than at a site near the “linker” DNA, suggesting that inhibition by H1 was not simply due to steric occlusion. Instead, these data are consistent with a model by which H1 binding reduces transient dynamic exposure of the DNA from the histone octamer surface (Polach, K. L., and Widom, J. (1995) J. Mol. Biol. 254, 130–149).

Biochemical studies have implicated both nucleosome core assembly and/or the subsequent binding of the linker histone H1 in transcriptional repression (4–9). Because the affinities of sequence-specific DNA-binding proteins for nucleosomal DNA are often dramatically reduced compared with free DNA (reviewed in Ref. 10), nucleosomes repress the transcriptional process, at least in part, by inhibiting access of activator proteins to their cognate binding sites in chromatin. Factor occupancy of binding sites in nucleosomal arrays is most likely achieved through multiple mechanisms involving nucleosome disruption, nucleosome displacement in trans, or histone octamer sliding in cis (reviewed in Ref. 11). The idea of mobile nucleosomes (i.e. nucleosome sliding) is attractive in that it has the potential to create transient accessibility of factors to their binding sites. Its potential importance is further implicated in studies that illustrate the differential affinity of factors for sites at different translational and rotational positions within nucleosomes (12–15). Using a model system consisting of chromatin assembled on tandemly repeated sea urchin 5 S RNA nucleosome positioning sequences (16), Bradbury and colleagues (17, 18) have previously reported that nucleosomes adopt a dominant position surrounded by minor positions 10 base pairs apart (i.e. in the same rotational frame). These data indicate that the cluster of octamer positions is in dynamic equilibrium in low ionic strength conditions. In the presence of the linker histone H1, this mobility is inhibited (19). The same group found that this short range sliding behavior also applies to bulk mononucleosomes and nucleosomes reconstituted onto sequences of the Alu family of ubiquitous repeats. Thus, they proposed that nucleosome mobility is a general behavior (20). Moreover, H1-mediated reduction in nucleosome mobility has been implicated in repression of transcription of a dinucleosome reconstituted onto a dimerized Xenopus somatic 5 S RNA gene (2).

In our previous studies, we reported for the first time direct inhibition of factor binding by the association of H1 with nucleosome cores. The binding of H1 to form a chromatosome significantly repressed the subsequent binding of USF but only slightly inhibited GAL4-AH binding (1). In this report, we extend these studies to explore the underlying mechanisms by which H1 repressed USF binding. The results illustrate H1-mediated repression of USF binding to a stably positioned nucleosome. Thus, H1 repression in this instance occurred in the absence of nucleosome mobility. In addition, H1 repressed USF binding to a site on the opposite side of the histone octamer from the entry and exit points of the linker DNA. These data are consistent with a mechanism by which H1 stabilizes histone octamer-DNA interactions, thus reducing transient exposure of factor binding sites.

MATERIALS AND METHODS

Preparation of DNA Probes—The 183-bp probe DNAs, referred to as GU or EG probes, were either directly generated by BanHI digestion of plasmids pGALUSFBEND or pUSFGALBEND, followed by Klenow incorporation of [α-32P]dATP, or prepared by polymerase chain reaction amplification from plasmid pGALUSFBEND and body-labeled by the incorporation of [α-32P]dATP. The primers used for amplification were GUB-5′ (5′-GATCCCTCTAGAGGGAGGACA-3′) and GUB-3′ (5′-GATCCCTCTAGGGCACG-3′). These plasmids were constructed by inserting the 46-base pair oligonucleotide (5′-etagaAGAGGCACGTCCT- Cggt-taccttcgaacCACGTGgccgt-3′), containing consensus GAL4 and USF binding sites (capitalized) separated by 14 bp, into the XhoI site of the pBEND-derived vector pTK401 (21) in both orientations. The probes were purified from 8% polyacrylamide (acrylamide/bisacrylamide, 29:1)× Tris borate/EDTA (TBE) gels.

Protein Purification and Nucleosome Core and Chromatosome Recon-

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1 The abbreviations used are: USF, upstream stimulatory factor; bp, base pair(s); TBE, Tris borate/EDTA; G-H1, globular domain of histone H1; MNase, micrococcal nuclease.
in the absence or presence of H1 in 0.1 M NaCl were divided for USF binding reactions such that each binding reaction contained 0.3 mg of nucleosomal DNA. At this point, the transcription factors were added at the amounts indicated in the figure legends. Binding reactions contained 100 nM NaCl, 0.25 mg/ml bovine serum albumin, 25 mM KCl, 5 mM HEPES (pH 7.4), 0.5 mM EDTA, 10% glycerol, 2.5 mM 2-mercaptoethanol, 2.5 mM ZnCl₂, 0.5 mM phenylmethylsulfonyl fluoride, 0.05% Nonidet P-40, and 4 mM dithiothreitol. The binding reactions were carried out at 30 °C for 30 min with a final volume of 27–40 µl, loaded onto 4% acrylamide (acrylamide:bisacrylamide, 29:1)/0.5 M EDTA gels, and run at 150 V (constant voltage) for 3 h at room temperature. Gels were dried and subjected to autoradiography. Addition of each gel was quantitated using a Betascope blot analyzer (Betagen Corp.).

Nucleosome Boundary Mapping Experiments—To determine the nucleosome boundaries, we adapted the method of Meersseman et al. (18). This method uses a combination of micrococcal nuclease and restriction enzyme digestion. The exact protocol is outlined in Fig. 2B and is described in detail as follows. Nucleosomes and chromatosomes were reconstituted onto a polymerase chain reaction-generated, 32P body-tRNA. After 4–5 h of incubation at 37°C, DNA was precipitated, pelleted, and washed once with 80% ethanol. Pellets were air dried for 5 min and resuspended in 4 µl of 10 × tracking dye (0.42% bromophenol blue, 0.42% xylene cyanol FF, and 50% glycerol), followed by loading the mixtures onto a 6% acrylamide (acrylamide:bisacrylamide, 29:1)/0.5× TBE gel and running in 0.5 × TBE at 150 V (constant voltage) for 5–6 h. Gels were exposed to x-ray films as described above.

The rest of the samples were digested with micrococcal nuclease (0.05 unit, Sigma) after 3 µl of CaCl₂ was included in each reaction mixture (digestion of mock-reconstituted DNA requires 10-fold less nuclease, whereas digestion of nucleosome in the presence of two molecules of H1 requires 3-fold more nucleosome). Digestion was performed at room temperature. Aliquots (14 µl, approximately 30,000 cpm) were removed after 0, 1, 5, 15, and 30 min, stopped, and deproteinized with 25 mM EDTA (pH 8.0), and 1 mM EDTA (pH 8.0). The binding reaction was carried out at 30 °C for 30 min. Samples were then digested with micrococcal nuclease and subsequent restriction enzymes as described above.

RESULTS

Nucleosome mobility on both sea urchin and Xenopus 5 S rRNA genes has been nicely characterized (2, 17, 18, 20). It is possible that short range sliding has an impact on factor binding to nucleosomes, either by making the binding sites more accessible or by burying the sites more deeply into a nucleosome. It has been shown that H1 is capable of reducing nucleosome mobility (19) and locking the nucleosomes into a unique position concurrent with inhibition of transcription from a Xenopus 5 S rRNA gene (2).

Our previous studies have shown that USF binding is repressed by H1 binding to a 183-bp nucleosome core bearing a USF site (E-box) (1). In principle, this inhibition of USF binding could also be explained by an H1-mediated restriction of nucleosome positions. H1 binding could lock the core particle into a position where the USF site is less accessible, i.e., deeper into the nucleosome (27). Alternatively, efficient USF binding may require movement of the nucleosome to an accessible location, which might be inhibited by H1 binding. To test these possibilities, we have analyzed the position of the histone octamers on the 183-bp USF site containing probe (GU) alone and after binding of histone H1 or USF.

The 183-bp, polymerase chain reaction-generated DNA template is derived from plasmid pGALUSF (see “Materials and Methods”). This DNA is long enough to contain one nucleosome and may allow limited mobility of the nucleosome core, since nucleosome cores have been shown to occupy 146 bp of DNA (28–30). USF binding to nucleosome cores reconstituted with this DNA probe was inhibited by the association of intact histone H1 (Fig. 1, lanes 9–14). Concurrent with inhibition of USF binding, H1 association resulted in a decrease in the electrophoretic migration of the nucleosome (chromatosome shift; Ref. 31), illustrating the relationship between H1 binding...
**Fig. 2.** Protocol for mapping nucleosome core positions. A, the relative positions of the restriction enzymes used and of the USF site on the DNA probe. The 183-bp DNA probe (GUB183-USF45) was a polymerase chain reaction-amplified product labeled with [α-32P]dATP throughout. A USF binding motif is located with the center of the binding site 45 bp from the 5'-end of this fragment. The fragment contains a unique site for the restriction enzyme Sall at 70 bp from the 5'-end and a second unique site for the enzyme BstEII 28 bp from the 5'-end. B, diagram of experimental strategy for nucleosome boundary mapping. For details see “Materials and Methods.”

and reduced USF binding (Fig. 1, A and B, lanes 9–14). Repression of USF binding was also observed with only the globular domain of histone H1 (G-H1) (Fig. 1, lanes 2–7), although in this instance repression was never complete. Interestingly, the binding of G-H1 increased the migration of the nucleosome particles (Fig. 1, A and B, lanes 2–7). The differences in electrophoretic migration of chromatosomes containing G-H1 versus intact H1 are attributed to the tail domains of H1, which are absent in G-H1. The increased migration suggests that G-H1 binding restricted the “linker” DNA at the edge of the core particles, thereby reducing its apparent size despite the increase of molecular weight. G-H1 binding has previously been shown to provide nuclease protection of linker DNA adjacent to the nucleosome core (32, 33).

To investigate whether H1 repression of USF binding by intact H1 and/or G-H1 resulted from an alteration in nucleosome positioning; we mapped the location of the histone octamer in each instance. The nucleosome mapping protocol used here was derived from that of Meersseman et al. (18) and is illustrated in Fig. 2. Control nucleosome cores reconstituted by salt dilution transfer onto the 183-bp probe (Fig. 2A) were digested to core particle length DNA with micrococcal nuclease and separated on a nondenaturing acrylamide gel (Fig. 2B). 32P body-labeled core particle DNAs were purified by excision and elution of the 146-bp bands from the gel. The core particle DNAs were then digested with two restriction enzymes to determine the locations of the micrococcal nuclease (MNase) cuts and, thus, the boundaries of the nucleosome cores. Subsequent analysis on denaturing polyacrylamide gels enabled a determination of the core particle boundaries relative to the unique restriction sites in the DNA sequence. A diagram of the relative positions of the USF binding site (45 bp from the 5'-end) and restriction enzyme sites is shown in Fig. 2A. MNase-generated 146-bp core particle DNAs purified from the nondenaturing gel were first cut with Sall (70 bp from the 5'-end), followed by a second digestion with BstEII (28 bp from the 5'-end). Cutting with the first enzyme yields two fragments of different sizes for each nucleosome position, and cleavage by the second enzyme subsequently reveals which fragment specifies the 5'-end boundary.

MNase digestion of the reconstituted nucleosome cores was analyzed by 6% nondenaturing polyacrylamide gel electrophoresis (Fig. 3). With incubation times increasing from 0 to 15 min, a digestion product of an approximately 146-bp-length DNA was observed (Fig. 3, lanes 6–9). Importantly, this digestion intermediate was not apparent in the digestion pattern for mock-reconstituted DNA (Fig. 3, lanes 1–4). We similarly analyzed the MNase digestion products from chromatosomes reconstituted with intact H1 (Fig. 3, lanes 11–14 and 16–19) and G-H1 (Fig. 3, lanes 21–24 and 26–29). In each case a 146-bp nucleosome core digestion product was observed. These 146-bp digestion products were excised from the gel and used to establish the position of the histone octamer in each instance. As is evident from Fig. 4A, lane 3, only one major pair of bands appeared after digestion of the 146-bp MNase product derived...
from the nucleosome cores with SalI (lane 3). These products were 86- and 60-bp fragments adding up to 146 bp. Cleavage with the second enzyme, BstEII (B), did not change the intensity of the 86-bp fragment, but the 60-bp fragment was cut into two smaller fragments of 42 and 18 bp (lane 4). After incubation with one molecule of H1 (lanes 5–7), two molecules of H1 (lanes 8–10), one molecule of GH1 (lanes 11–13), and two molecules of GH1 (lanes 14–16), the pattern of digestion products was same as that of nucleosome (Nuc.) alone. Lane 1 contains the molecular weight marker. Lanes 2, 5, 8, 11, and 14 are undigested (U) 146-bp core particle DNA, B, location of the histone octamer on the DNA template GUB183-USF45. The restriction mapping indicates that the nucleosome cores were precisely positioned between 10 and 156 bp from the 5'-end of this DNA fragment regardless of H1 binding. This result is based on the digestion of two restriction enzymes, SalI and BstEII. The nucleosome is indicated by the shaded oval.

To determine whether H1 altered the position of the histone octamer, we similarly mapped the 146-bp MNase digestion products (Fig. 3) generated from chromatosomes reconstituted with either intact H1 or G-H1. To investigate the exact location of the histone octamer in the presence of H1, the 146-bp core particle DNAs were excised from gels after digestion with MNase, followed by restriction enzyme mapping. This approach allows the determination of the octamer position without bias from the controversial issue of whether H1 binds symmetrically or asymmetrically to nucleosomes (reviewed in Ref. 34). The 146-bp core particle length DNAs, generated from the reconstituted chromatosomes, were excised from the non-denaturing gel, eluted, purified, and digested with SalI and BstEII, as in the case of the nucleosome core samples. Interestingly, we detected no difference between the restriction enzyme digestion pattern of chromatosome-derived nucleosome core DNA and that of nucleosome cores without H1 (Fig. 4A, compare lanes 5–16 with 2–4). In the presence of either one or two molecules of intact H1 or G-H1 per nucleosome core, the nucleosome cores were still positioned precisely at the unique site. Thus, H1-mediated nucleosome repositioning did not occur on this probe under conditions in which H1 effectively repressed USF binding.

An alternative possible mechanism by which H1 might repress binding to this nucleosome is if USF binding induces a change in the position of the nucleosome core, which is prevented by the association of histone H1. To investigate this possibility the same approach was used to test whether USF binding moved the nucleosome core when interacting with its binding site. The nucleosome cores were incubated with high concentrations of USF in which more than 50% of the nucleosomes were bound (Fig. 5A, lane 2). These reactions were then digested with MNase, and 146-bp core particle DNAs were obtained (Fig. 5B). Subsequent analysis of these DNAs by restriction endonuclease digestion revealed that no novel nucleosome core positions arose as a consequence of USF binding (Fig. 5C, compare lanes 5–10 with 2–4).

These experiments indicate that the histone octamer present on this particular DNA sequence was tightly positioned. This position was not altered with H1 or USF binding. Thus, in this instance, H1-mediated repression of USF binding was independent of nucleosome mobility, indicating an alternative mechanism of H1 repression. There are at least two alternative mechanisms by which H1 might repress USF binding to this nucleosome: 1) the binding of H1 to the nucleosome core may sterically hinder the interaction of USF with its recognition sequence; and 2) the binding of H1 may stabilize interactions between the histone octamer and DNA, thus indirectly inhibiting the ability of USF to access its binding site. In this regard, it is interesting that the USF site within the positioned nucleosome is 35 bp into the nucleosome core (Fig. 4B). This places the USF site almost on the opposite side of the histone octamer from the nucleosome dyad and linker DNA, where H1 is thought to bind (reviewed in Ref. 35).

To further examine how the position of the USF site might affect its repression by H1, we compared USF binding with a nearly identical nucleosome core but with the USF site nearer the edge of the nucleosome. Fig. 6A illustrates the position of the USF site on the nucleosome core used in all of the experiments above (GU probe) and the related probe, which has only 46 bp inverted (UG probe). Fig. 6, B and C, illustrates USF binding to nucleosome cores reconstituted on the UG and GU probes, respectively, in the absence (lanes 1–5) or presence (lanes 6–10) of H1. The affinity of USF for the UG probe nucleosome core was greater than for the GU probe nucleosome core because of translational position effects, as shown previously (27). However, the binding of USF to the UG nucleosome was less inhibited by H1 than was binding to the GU nucleo-
some. H1 binding resulted in a 7-fold reduction of USF binding to the GU nucleosome but only a 1.2-fold reduction of USF binding to the UG nucleosome. Thus, H1 had a greater inhibitory effect on factor binding to a position that is more distal from the edge of the nucleosome, consistent with an indirect mode of H1 repression.

**DISCUSSION**

The fact that histone octamers are able to move in cis on a DNA fragment was reported 18 years ago (36). More recently, studies by Bradbury and co-workers (17) have illustrated an inherent localized mobility of histone octamers on DNA over short distances of 10s of base pairs. Furthermore, the linker histone H1 was reported to reduce the mobility of positioned nucleosomes (19). Ura and colleagues (2) detected a correlation between the ability of H1 to reduce nucleosome mobility and transcriptional repression of a dimerized 5S rRNA gene nucleosome, leading to the suggestion that H1 could inhibit factor access by reducing nucleosome mobility. The experiments presented here indicate a different mechanism of H1 repression.

**FIG. 5. USF binding does not move the positioned nucleosome.** A, mobility shift of USF binding to nucleosome cores. Lane 1 contains 0.4 μM USF, and lane 2 contains 1.2 μM USF. The positions of the nucleosome core (Nucl.) and the USF-bound nucleosome core (USF/Nucl.) are indicated. B, micrococcal nuclease digestion of the 183-bp nucleosome core in the presence of 0.4 μM (lanes 1–5) or 1.2 μM (lanes 7–11) USF. The digestion times are indicated above each lane. Lanes 6 and 12 contain the molecular weight DNA marker. C, restriction enzyme digestion products of the 146-bp micrococcal nuclease core particle DNA product after the nucleosome cores were incubated with 0.4 μM (lanes 5–7) or 1.2 μM (lanes 8–10) USF. The pattern of restriction endonuclease digestion products was same as that of nucleosome alone (lanes 2–4). Lanes 1 and 11 contain the molecular weight marker. Lanes 2, 5, and 8 are undigested (U) 146-bp core particle DNA.

**FIG. 6. H1-mediated repression of USF binding is most dramatic within the nucleosome core.** A, diagram of the 183-bp probe DNAs, which are identical except for the orientation of the 46-bp oligonucleotide containing the USF binding site. For the GU probe, the center of the USF binding site is 45 bp from the 5’-end. For the UG probe, the center of the USF site is 19 bp from the same end. To test H1 inhibition of factor binding, nucleosomes (Nucl.) were reconstituted with or without H1 onto either the UG probe (B) or the GU probe (C). USF binding was then assayed directly by mobility shift in 4% acrylamide gels. The amounts of USF added are listed above each lane. Protein-DNA complexes representing USF bound to naked DNA (USF/DNA) and USF bound to nucleosomes (USF/Nucl.) are indicated. In the presence of 107 nM H1, the USF-nucleosome complexes were reduced to a greater extent on the GU probe than on the UG probe (compare C, lanes 9 and 10 versus lanes 4 and 5 with B, lanes 7–9 versus lanes 2–5).

We have previously reported that H1-mediated repression of USF binding to a reconstituted nucleosome core. In this study, we have shown that the histone octamer takes up a precise location on this particular 183-bp DNA fragment from 10 to 156 base pairs relative to the 5’-end. This position of the histone octamer was unchanged after the binding of either H1 or USF. Thus, H1-mediated repression of USF binding in this instance involved neither changes in nucleosome position nor a reduction of nucleosome mobility. This example, therefore, clearly
illustrates a mechanism of H1 repression, which is independent of movement of the histone octamer.

Histone H1 is thought to bind to linker DNA at the point at which DNA enters and exits the nucleosome core particle and may also interact with DNA at the nucleosome dyad (reviewed in Ref. 35). This results in the protection of DNA at the edge of the nucleosome core particle from nucleosome cleavage. By contrast, we have observed that the inhibition of USF binding by histone H1 was more pronounced at a site 35 bp into the nucleosome core than at a site near the nucleosome edge (i.e. 9 bp into the nucleosome core). Thus, the more highly repressed USF site was located on the opposite side of the histone octamer from the entry point of the linker DNA and from the nucleosome dyad. In addition, Pruss and colleagues (37) have recently proposed a model of asymmetric H1 binding to nucleosome cores reconstituted on 5 S rRNA genes. Even if such asymmetric binding occurred on the reconstituted USF site, nucleosome cores used here, the highly repressed USF site 35 bp into the nucleosome core would be well beyond the proposed site of H1 binding, whereas the less repressed site 9 bp into the nucleosome core would be located directly at the proposed site of H1 binding. Thus, H1-mediated repression of USF binding is unlikely to have occurred by a simple process of steric occlusion of the transcription factor from its binding site. Instead, this observation suggests an indirect pathway of H1 repression.

Polach and Widom (3) have proposed a dynamic equilibrium model for transcription factor access to recognition elements within nucleosomes, which is consistent with several in vitro experiments. Their model suggests that DNA on the surface of the histone octamer is dynamic, transiently exposing stretches of DNA to transcription factors. Importantly, site exposure decreases as the site is moved further into the nucleosome core, consistent with an “unpeeling” mechanism and numerous reports of translational position effects (reviewed in Ref. 10). Thus, sites deeper into the nucleosome core are more sensitive to the extent of DNA unpeeling. Histone H1 has been shown by physical studies to stabilize the interactions of linker DNA and additional DNA within the nucleosome core with the histone octamer (38–40). Moreover, microscopic studies and topological analysis further support a role of histone H1 in preventing the “peeling” of DNA from the surface of the histone octamer (reviewed in Ref. 35). Our observation that H1 represses factor binding to a site 35 bp into the nucleosome core (a position not thought to interact with H1 directly) is consistent with a model whereby H1 indirectly represses factor binding by reducing transient unpeeling of DNA from the histone octamer surface, thus sealing the nucleosomal DNA. By such a mechanism, H1 binding could lead to repression of factor access over a broader region of DNA beyond its specific sites of direct interaction.

The function of H1 in repressing factor binding to distal sites on a nucleosome may result from the binding of H1 generating a more compact nucleosome structure. This could result from additional charge shielding of nucleosomal DNA by H1 and/or by stabilization of the wraps of nucleosomal DNA by the binding of histone H1 to both the entering and exiting DNA helices. A contribution from the basic amino- and carboxyl-terminal tails of H1 is consistent with the observation that intact H1 provided a slightly greater level of repression than the central globular domain of H1 alone (Fig. 1). However, the fact that the globular domain of H1 alone provided the majority of the observed repression (Fig. 1) indicates that repression is largely mediated by the binding of the globular “winged helix” structure (41). Indeed, the observation that H1 binding to reconstituted nucleosome cores is dependent on the presence of linker DNA (i.e. DNA beyond the 146 bp contained in the nucleosome core; Ref. 31) indicates that correct binding of the central globular domain is crucial to mediating the repressive actions of H1.

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