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Role of the Histone Amino Termini in Facilitated Binding of a Transcription Factor, GAL4-AH, to Nucleosome Cores

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Facilitated, "cooperative" binding of GAL4-AH to nucleosomal DNA occurred in response to inhibition from the core histone amino termini. The binding of GAL4-AH (which contains the DNA-binding and dimerization domains of GAL4) to nucleosome cores containing multiple binding sites initiated at the end of a nucleosome core and proceeded in a cooperative manner until all sites were occupied. However, following tryptic removal of the core histone amino termini, GAL4-AH binding appeared to be noncooperative, similar to binding naked DNA. Binding of GAL4-AH to nucleosomes bearing a single GAL4 site at different positions indicated that inhibition of GAL4 binding was largely mediated by the histone amino termini and primarily occurred at sites well within the core and not near the end. When the histone amino termini were intact, binding of GAL4-AH to sites near the center of a nucleosome core was greatly enhanced by the presence of additional GAL4 dimers bound to more-accessible positions. These data illustrate that the binding of a factor to more-accessible sites, near the end of a nucleosome, allows facilitated binding of additional factors to the center of the nucleosome, thereby overcoming repression from the core histone amino termini. This mechanism may contribute to the binding of multiple factors to complex promoter and enhancer elements in cellular chromatin.

Genetic and biochemical studies have illustrated a crucial role of chromatin structural proteins in the regulation of eukaryotic gene transcription (reviewed in references 19, 22, 29, 51, 58, and 61). For example, nucleosome cores repress basal transcription both in vivo and in vitro, which increases the dependence of promoter function on the action of upstream regulatory factors (16, 23, 24, 34, 60, 63, 64). Similarly, subsequent binding of the linker histone H1 further represses promoter function and increases the dependence of transcription on regulatory factors in vitro (15, 30). These studies implicate a crucial role of upstream activators in histone displacement as well as transcription complex formation at the core promoter (i.e., the TATA box and transcription start site). This function of upstream activators is dependent on their activation domains (5, 15, 34, 43, 64) and is affected in vivo by the stability of nucleosomes located over core promoter sequences (50) and mutations in the N termini of histone H4 (17).

Before upstream activators can act on core promoters, they must first gain access to their respective upstream binding elements (reviewed in reference 1). Studies thus far implicate at least three criteria which govern the ability of factors to access their binding sites on nucleosomes. The first is an inherent difference in the ability of factors to bind nucleosomal DNA, perhaps dictated by their particular DNA-binding motifs. Those found to bind at least in some instances include TFIIIA, the glucocorticoid receptor, and GAL4 derivatives, while those unable to bind in similar circumstances include nuclear factor 1 and the human heat shock factor (2, 31, 42, 44, 45, 52, 62). Second, nucleosome positioning has been implicated in determining factor access. The rotational phasing of the DNA helix on positioned nucleosomes containing glucocorticoid response elements often expose the major groove (to the solvent) at those sequences which may accommodate glucocorticoid receptor binding (2, 41, 42, 44). In addition, translational positioning of nucleosomes has been shown to effect function of *cis*-acting elements in studies of autonomous replicating sequence (ARS) function in *Saccharomyces cerevisiae* and in in vitro studies with phage polymerase (48, 59). In general, accessibility decreases as the binding elements are moved from the end towards the center of the nucleosome core. Finally, the composition of nucleosome cores can influence factor access. The binding of TFIIIA to nucleosome cores is enhanced when the core histone amino-terminal tails are acetylated at lysine residues or removed by proteases (30). These results suggest that the positively charged histone tails play a role in limiting factor access to nucleosomal DNA, although they are not essential for the formation of the nucleosome core (reviewed in reference 54).

These studies raise important questions with regard to the contribution of the amino-terminal tails to nucleosome position effects and the mechanisms by which factors might overcome these constraints to gain access to nucleosomal DNA. One possible mechanism is suggested by the observation that multiple factor binding sites (enhansons) are required for enhancer function in vivo (18, 40). Thus, multiple binding sites may improve the opportunity for factors to overcome nucleosome position effects which inhibit factor binding. For example, it was previously shown that GAL4 derivative binding to multiple sites on a nucleosome in vitro occurred at lower GAL4 concentrations than binding at a single site. This apparent cooperativity in GAL4 binding to nucleosomal DNA was termed facilitated binding since it involved three components (DNA, histones, and GAL4) (52). In this study we show that the amino-terminal tails of the core histones contributed to the inhibition of GAL4 binding to nucleosomal DNA. However, facilitated binding of GAL4 overcame the inhibition of the amino termini.

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Facilitated binding occurred by an apparent end-to-center mechanism, where by GAL4 initially bound sites near the edge of the nucleosome. These observations suggest that factor binding to more-accessible sites near the end of nucleosomes can increase the accessibility at more-inhibited positions near the center. Thus, facilitated binding, and the corresponding nucleosome perturbation, provides a mechanism whereby multiple factors may participate in achieving complete occupancy of complex promoters and enhancers during chromatin remodeling.

MATERIALS AND METHODS

Preparation of DNA probes with GAL4 sites. The five-GAL4-site probe (180 bp) with centered sites was created by PCR amplification from the plasmid pG5H (64) and was labeled by the incorporation of [^{32}P]dCTP. The primers used for amplification were DCG5H, 5′-ATCCGGACCGCTTGC CCC-3′, and UCG5H, 5′-ATGACCATGATTACGCCA-3′. The probes used to study the binding to one GAL4 site were generated by annealing an oligonucleotide containing a GAL4 site and *Xba*I overhanging ends. The oligonucleotide sequence used was 5′-CTAGACGGAGGACWGTCCTCCG-3′, where W = A + T. The annealed oligonucleotide was kinased and ligated into the vector pTK401, which contains a repeated polylinker (27), cut at the *Xba*I site to generate the plasmid pBEND401G1. This vector also contains an Ap1 site ligated between the *Xba*I and *Sal*I sites but does not contain bent DNA sequences (27). The ligants were transformed into SURE-competent cells (Stratagene). Digestion of plasmid pBEND401G1 with *Xho*I, *Bam*HI, or *Asp* 718 results in a 160-bp fragment with the center of the GAL4 site 74 bp (*Xho*I), 21 bp (*Bam*HI), or 40 bp (*Asp* 718) from the end. The probes were end labeled by Klenow and purified on an 8% acrylamide (acrylamide-bisacrylamide, 29:1)–1× Tris-borate-EDTA (TBE) gel.

The five-GAL4-site probe used in the footprinting experiment shown in Fig. 1 was prepared as follows. Plasmid $G_5$40HSP70CAT (52) was cut with *Nhe*I, 3′ end labeled with Klenow, and then cut with *Hin*dIII. This resulted in a 168-bp fragment with five GAL4 sites located between 21 and 114 bp from the *Hin*dIII end (the center of the first site was located at position 30). The end-labeled probe was purified on an 8% acrylamide (acrylamide-bisacrylamide, 29:1)–1× TBE gel.

For microccocal nuclease digestion experiments (see Fig. 4), probe DNAs which contained an internal label were prepared. The vector pBend401G1 was digested with *Sal*I to linearize the plasmid, and the ends were labeled with Klenow polymerase. The blunt ends were ligated in a 50-μl reaction mixture with 4 μl of ligase (Boehringer Mannheim) overnight at 16°C and the next morning were incubated at room temperature for 2 h with an additional 1 μl of ligase. The reaction mixtures were digested with *Xho*I, *Bam*HI, or *Asp* 718 to yield fragments for reconstitution. Fragments were purified on an 8% acrylamide–1× TBE gel.

Nucleosome cores and GAL4-AH purification. GAL4(1–147)-AH, a fusion protein of the amino-terminal 147 amino acids of GAL4 (containing the DNA-binding and dimerization domains) and an artifical 15-amino-acid putative amphipathic helix, was purified from bacterial strains by the method of Lin et al. (33). H1-depleted oligonucleosome cores were prepared from HeLa nuclear pellets (65) by the following procedure. Two milliliters of packed HeLa cell nuclear pellets were suspended in 8 ml of 0.6 M salt buffer with sucrose (0.6 M NaCl, 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.5], 1 mM EDTA [pH 8.0], 0.5 mM 2-mercaptoethanol, 0.5 mM phenylmethylsulfonyl fluoride [PMSF], 0.34 M sucrose) and homogenized with a B pestle until completely resuspended (20 strokes). The homogenate was then centrifuged for 20 min at 9,600 × *g* at 4°C. The supernatant from the centrifugation was dialyzed into 0.1 M salt buffer (0.1 M NaCl, 10 mM HEPES [pH 7.5], 1 mM EDTA [pH 8.0], 0.5 mM 2-mercaptoethanol, 0.5 mM PMSF) at 4°C. Following the addition of $CaCl_2$ to a 3 mM final concentration, the soluble chromatin was digested with 0.01 U of micrococcal nuclease (Sigma) per ml for 5 min at 37°C. The digestion was quenched by the addition of ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) to a final concentration of 50 mM, followed by the addition of NaCl to 0.6 M (dropwise), and the solution was clarified by centrifugation at 150,000 × *g* for 30 min at 4°C. Oligo- and single-nucleosome cores were separated from H1 and other contaminating proteins by filtration through a 280-ml Sepharose CL-6B column (2.5 by 57cm; Pharmacia) developed in 0.6 M buffer without sucrose. The column was run at 10 ml/h, and 2-ml fractions were collected. The fractions of interest were identified by agarose gel analysis of DNA lengths and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis analysis of proteins. Those fractions which contained the H1-depleted oligo- or mononucleosomes were pooled and dialyzed against 10 mM HEPES (pH 7.5)–1 mM EDTA–0.5 mM 2-mercaptoethanol–0.5 mM PMSF at 4°C. The material was concentrated about fivefold by dialysis against solid sucrose for 3 h and then dialyzed against 10 mM HEPES (pH 7.5)–1 mM EDTA–0.5 mM 2-mercaptoethanol–0.5 mM PMSF at 4°C.

Removal of the amino termini. To address the function of the histone amino-terminal tails on GAL4 binding, we utilized a standard proteolysis protocol which selectively removes the amino termini of the core histones (3, 4, 8). Nucleosome cores were treated with trypsin, to remove the N termini, and then an excess of trypsin inhibitor was added. The amino-terminal tails of the core histones (0.56 mg of nucleosome cores per ml) were removed by trypsinization at room temperature for 5 min with a trypsin (Sigma, 10,200 U/mg) concentration of 30 μg/ml. Reactions were stopped by adding a 100-fold excess (wt/wt) of soybean trypsin inhibitor (Sigma). For the experiments shown in Fig. 2 and 3, nucleosome cores were purified after trypsin digestion and addition of trypsin inhibitor by gel filtration on a Sepharose CL-6B (Pharmacia) column (40 by 1 cm) equilibrated with 0.6 M salt buffer (0.6 M NaCl, 10 mM HEPES [pH 7.5], 1 mM EDTA, 0.5 mM 2-mercaptoethanol, 0.5 mM PMSF, 0.34 M sucrose) with 50 μg of trypsin inhibitor per ml. Fractions (1 ml) were collected into tubes containing an additional 50 μg of inhibitor. Peak fractions were pooled and dialyzed overnight against 10 mM HEPES (pH 7.5)–1 mM EDTA–0.5 mM PMSF–0.5 mM 2-mercaptoethanol. Dry sucrose was poured over the dialysis bag, and the sample was concentrated at 4°C until the volume was decreased about fivefold. The concentrated cores were dialyzed against 10 mM HEPES (pH 7.5)–1 mM EDTA–0.5 mM PMSF–0.5 mM 2-mercaptoethanol to remove sucrose.

Controls for trypsin inactivation. The trypsinized nucleosome cores were subsequently used as histone donors in transfer reactions of the histone octamer onto labeled DNA probes (46). Trypsin inhibitor was included in all octamer transfer and binding reaction mixtures to avoid subsequent trypsin activity (3). The inactivation of the trypsin was tested directly by control reaction mixtures which included the same amount of trypsin but to which it was added after the

trypsin inhibitor. These controls illustrated that the core histones were not degraded by trypsin, during the reconstitution or binding reactions, once the inhibitor was present. The experiments whose results are shown in Fig. 2 and 3 utilized the gel filtration-purified nucleosome cores. While the trypsin activity in the gel filtration repurified nucleosome cores was substantially reduced, some trypsin activity remained. Thus, trypsin inhibitor was included in the nucleosome transfer reaction mixtures, dilution buffers, and binding reaction mixtures (see below). In addition, controls of GAL4-AH binding on naked DNA in the presence of nucleosome cores from trypsin-treated and untreated nucleosome cores demonstrated that the trypsin inhibitor prevented any degradation of the subsequently added factor (Fig. 2 and 3).

The experiments shown in Fig. 5, 6, and 7 utilized nucleosome cores which were not gel filtration purified prior to nucleosome transfer onto probe DNA. The following procedures with these cores rendered results identical to those shown in Fig. 2 and 3 when these five-site experiments were repeated. For Fig. 5, 6, and 7 trypsin was added to the nontrypsinized cores after the addition of trypsin inhibitor. Thus, each reaction mixture contained the same amount of trypsin and trypsin inhibitor but differed only in the order of addition. DNA control reactions were also performed to assess the integrity of GAL4-AH in the binding reaction mixtures, and mixtures contained all of the components present in the nucleosome binding reaction mixtures (i.e., donor nucleosomes, trypsin inhibitor, and trypsin). In these controls, however, the probe DNA bearing the GAL4 sites was added without histone octamer transfer (see below) and thus did not acquire histones (see Fig. 5, 6, and 7; see below). Through the use of excess trypsin inhibitor in all steps and through judicious controls, these procedures avoided previously forewarned concerns of continued proteolysis of core histones (3) or proteolysis of subsequently added transcription factors (31).

Nucleosome reconstitution. For the transfer and control reactions with the five-GAL4-site probe and the one-GAL4-site probes, 6 μg of HeLa oligonucleosome cores (10.7 μl) was mixed with 1 to 3 μl of probe DNA (approximately 27 fmol) in a final volume of 20 μl. A total of 5 μl of 5 M NaCl was added to reach a salt concentration of 1 M NaCl, and the transfer reaction mixtures were incubated at 37°C for 20 min. For the DNA control reaction mixtures, the entire dilution volume (below) was added to the nucleosome cores and was followed by 5 M NaCl and lastly the labeled probe DNA prior to GAL4-AH binding. The transfer reaction mixtures were serially diluted to 0.8, 0.6, 0.4, and 0.2 M NaCl with 50 mM HEPES (pH 8.0)–1 mM EDTA (pH 8.0)–200 μg of trypsin inhibitor per ml with incubations at 30°C for 15 to 30 min at each dilution step. The reaction mixture was brought to 0.1 M salt with GAL4 final dilution buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA [pH 8.0], 1 mM 2-mercaptoethanol, 0.1% Nonidet P-40, 20% glycerol, 100 μg of bovine serum albumin [BSA] per ml) and incubated at 30°C for 15 min. Each reaction mixture (transfer and control) was divided into six equal portions for GAL4-AH binding. These binding reactions contained 1 μg of donor nucleosome cores and approximately 0.1 nM probe DNA or probe reconstituted into nucleosome cores.

For the reconstitution with end-labeled footprinting probe (Fig. 1), 10 μl of 3′-end-labeled probe (250 ng), 75 μg of unlabeled calf thymus DNA, and 75 μg of core histones were mixed in a total volume of 50 μl with final concentrations of 2 M NaCl, 10 mM HEPES (pH 8.0), 1 mM EDTA, 1 mg of BSA per ml, and 1 mM 2-mercaptoethanol. The nucleosome cores were assembled by slow dialysis to decreasing salt and were gradient purified as described previously (64). The probe used was the five-GAL4-site end-labeled probe (see above).

For the micrococcal nuclease digestion experiments shown in Fig. 4, nucleosomal core transfers and mock reactions were performed as described above. After the final dilution and incubation, $CaCl_2$ was added to each reaction mixture to a final concentration of 3 mM. The mixture was preincubated at 37°C for 5 min, and a 0.01-U/μl final concentration of micrococcal nuclease was added to each reaction mixture (DNA control and reconstituted nucleosomes). Aliquots (35 μl) were removed after 1, 3, 9, 15, and 30 min and stopped with 0.2% SDS–20 mM EDTA. Samples were phenol-chloroform (1:1) extracted, precipitated, pelleted, and washed once with 80% ethanol. Pellets were air dried for 5 min and resuspended in 6 μl of 80% formamide–1 mg of xylene cyanol per ml–1 mg of bromophenol blue per ml–10 mM EDTA, heated to 100°C for 5 min, and resolved on a 10% 8 M urea sequencing gel.

GAL4-AH binding reactions. Serial dilutions of the GAL4-AH protein stock (5 mg/ml) were made in G4D buffer (1 mg of BSA per ml, 100 mM KCl, 10 mM HEPES [pH 7.4], 10 mM 2-mercaptoethanol, 10 μm $ZnCl_2$, 0.2 mM PMSF). GAL4-AH dilutions (3 μl) were added to 42 μl of the transferred nucleosome cores or the DNA control reactions and were incubated for 30 min at 30°C.

Mobility shift and footprinting assays. For the mobility shift gels, samples were loaded onto a 4% acrylamide (acrylamide-bisacrylamide, 29:1)–0.5× TBE gel immediately after the binding reactions and run in 0.5× TBE at 150 V (constant voltage) for 2.5 h. Gels were dried and placed against Kodak film with an intensifying screen overnight. In addition, each gel was counted on a Betascope blot analyzer (Betagen Corp.), and the counts in each band were determined. For the single-GAL4-site nucleosomes, the percent nucleosomes bound by factor was determined by dividing the counts in the GAL4-AH–nucleosome bands by the counts in these bands plus the counts in the unbound nucleosome bands. The percent five-dimer-bound nucleosomes from Fig. 2 was determined by subtracting the DNA contribution from the five-dimer bands and dividing by the counts of unbound nucleosomes added to the binding reaction mixture (from the control lanes without GAL4-AH). DNase I footprinting with the end-labeled five-GAL4-site probe (Fig. 1) was performed after binding reactions. Binding reaction mixtures included 8,000 cpm of probe DNA or nucleosome cores and the GAL4-AH concentrations indicated in the figure in a total volume of 5 μl. DNase I (0.002 U for DNA and 0.01 U for nucleosome cores) was added in 5 μl of 5 mM $MgCl_2$–15 mM $CaCl_2$–20 mM HEPES (pH 8.0) and was incubated at 37°C for 1 min. Reactions were stopped by the addition of 50 mM EDTA and 1% SDS, and samples were extracted with phenol-chloroform. The aqueous phase was precipitated with 0.1 volumes of 3 M sodium acetate and 2 volumes of absolute ethanol. Samples were pelleted, washed once or twice with 70% ethanol, dried, and resuspended in 5 μl of 80% formamide–1 mg of xylene cyanol per ml–1 mg of bromophenol blue per ml–10 mM EDTA. Samples were heated to 100°C for 5 min and resolved on an 8 M urea sequencing gel.

RESULTS

Facilitated binding involves initial binding to sites at the end of nucleosome cores. Previous studies have illustrated that

the binding of GAL4 derivatives to multiple sites within a nucleosome cores requires a lower concentration of factor relative to binding nucleosome cores bearing a single site. This apparent cooperativity in GAL4 binding was not observed on naked DNA and was termed facilitated since it involved three components; GAL4, DNA, and histones (52). To further investigate the mechanism of facilitated binding, we more closely examined the footprinting pattern of GAL4-AH binding to nucleosome cores bearing five sites (Fig. 1). Titration of GAL4-AH binding to a naked DNA fragment bearing five repeated GAL4 sites (lanes 1 to 7) and to the same fragment reconstituted into nucleosome cores (lanes 9 to 15) was analyzed by DNase I footprinting. The naked DNA displayed a 19-bp repeating pattern of DNase I sensitivity through the region of the repeated 19-bp GAL4 sites (17-mer binding site plus 2 bp [10]). Thus, the enzyme cut at preferred regions within this repeated sequence (lanes 2 and 3). By contrast, following reconstitution of the fragment into nucleosome cores, DNase I digestion demonstrated a digestion pattern of approximately 10 bp (lanes 9 and 10). The 10-bp pattern superseded the 19-bp pattern of the DNA alone and is indicative of the protection of one side of the DNA helix by the surface of the histone octamer (37).

At increasing GAL4-AH concentrations, protection from DNase I digestion was observed on both the naked DNA and the reconstituted nucleosome core probes. However, the order of sites bound by the factor was strikingly different in these two samples. In the case of the reconstituted nucleosome, the first site protected was at the end of the fragment (lane 11). Following complete occupancy of the end site, the remainder of the sites on the nucleosome cores were bound by GAL4-AH, resulting in a complete footprint at a three-fold-higher GAL4-AH concentration (lane 13). In the case of naked DNA, while all of the sites were substantially protected, the site which was first fully protected from digestion by GAL4-AH binding was the other end site, which was closer to the center of the DNA fragment (lane 4). A complete five-site footprint was observed at a 1.5-fold-higher GAL4-AH concentration (lane 5). These data indicate that the binding of GAL4-AH to nucleosome cores bearing five sites differed from binding to naked DNA and occurred initially at the site at the end of the nucleosome core and then by binding to the more internal sites.

Facilitated binding of GAL4-AH to nucleosome cores occurs in response to the core histone amino termini. Facilitated binding of GAL4-AH was apparent in the loading of GAL4-AH dimers onto nucleosome cores versus free DNA (Fig. 2). In the case of naked DNA, inclusion of increasing amounts of GAL4-AH in binding reaction mixtures with DNA probe bearing five GAL4 sites resulted in the generation of complexes containing increasing amounts of GAL4-AH dimers (lanes 2 to 5). Through the titration, complexes representing all of the intermediate numbers of bound dimers (from 1 to 5) were observed, indicating that the factor loads in a linear concentration-dependent manner. By contrast, a significantly different pattern of factor loading is observed when the five-site probe was reconstituted into nucleosome cores. In this case, following the binding of the first dimer, few intermediate complexes were observed (lanes 7 to 10). Instead, the complexes observed jumped from one to five bound dimers with increasing amounts of GAL4-AH. This observation illustrates that the binding of GAL4-AH beyond the first dimer proceeded in a cooperative manner to fill the remaining sites.

Removal of the core histone amino termini dramatically affected the loading of GAL4-AH onto nucleosome cores.

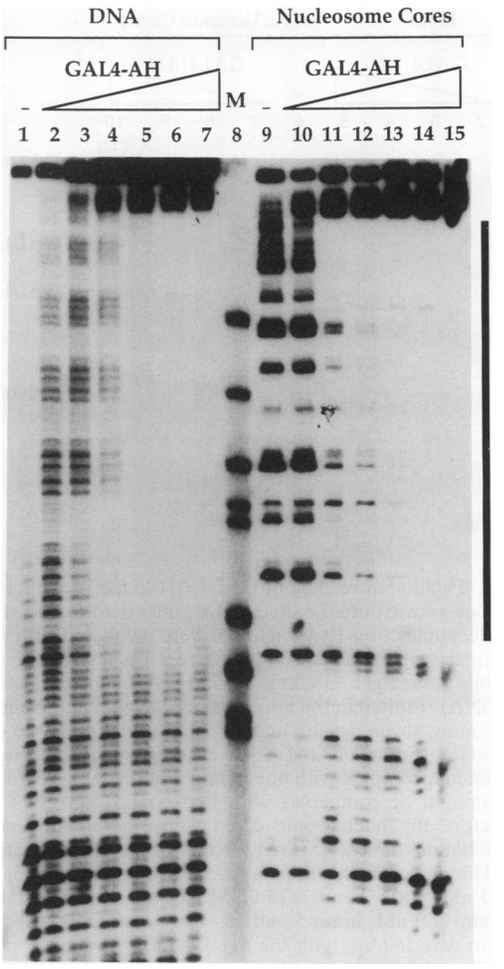

FIG. 1. GAL4-AH binding to a five-site nucleosome core initiates at the end. Naked DNA (lanes 1 to 7) and reconstituted nucleosome cores (lanes 9 to 15) bearing five GAL4 sites were incubated with increasing concentrations of GAL4-AH and then analyzed for binding by DNase I footprinting. The bar on the right marks the region of the five repeated GAL4 sites. The GAL4-AH concentrations tested for the naked DNA are 0, lane 1; 2.3 nM, lane 2; 4.6 nM, lane 3; 9.2 nM, lane 4; 13.8 nM, lane 5; 46 nM, lane 6; and 92 nM lane 7. GAL4-AH concentrations for the nucleosome lanes are 0, lane 1; 23 nM, lane 10; 46 nM, lane 11; 92 nM, lane 12; 138 nM, lane 13; 460 nM, lane 14; and 920 nM, lane 15. Lane 1 does not show a footprint but was overdigested with DNase I. Lane 8 illustrates molecular weight markers, the sizes of which were (from top to bottom) 124, 104, 89, 80 (doublet band), 64, 57, and 51 (doublet band) bases. The five-GAL4-site probe used was 168 bp, with five GAL4 sites located between 21 and 114 bp from one end, with the first GAL4 site centered at position 30. If the reconstituted nucleosome were positioned at the far end of the fragment, the center of the end GAL4 site would be 8 bp into the nucleosome (i.e., the site would be between nucleotide 1 and 17). This position would be shifted 22 bp into the nucleosome for nucleosome cores which are positioned at the other end.

Titration of GAL4-AH in binding reaction mixture containing five-site nucleosomes with the amino termini removed (Fig. 3B) demonstrated a binding pattern similar to that observed on naked DNA (Fig. 3A, lanes 7 to 10). Intermediate complexes representing increasing numbers of bound dimers (1 to 5) were apparent, in contrast to the binding to nucleosome cores, for which the amino termini were intact

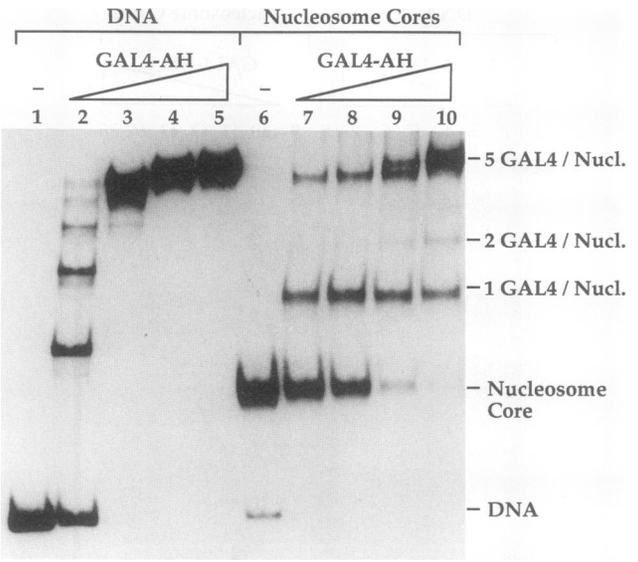

FIG. 2. Facilitated binding of GAL4-AH to five-site nucleosome cores. Mock-reconstituted naked DNA (lanes 1 to 5) and reconstituted nucleosome cores (lanes 6 to 10) were incubated with increasing concentrations of GAL4-AH before resolution of the complexes on a mobility shift gel. Binding of GAL4-AH to the naked DNA (lane 1; DNA) resulted in the appearance of five distinct bands (lane 2) representing an increasing number of bound GAL4-AH dimers. By contrast, titration of GAL4-AH binding to nucleosome cores demonstrated a complex with one bound dimer (1 GAL4/Nucl.) and a small amount of complexes with two bound dimers (2 GAL4/Nucl.) before the nucleosome cores were shifted to a complex with five bound dimers (5 GAL4/Nucl.). The concentrations of GAL4-AH included in the binding reaction mixtures were 0, lanes 1 and 6; 5.3 nM, lanes 2 and 7; 15.9 nM, lanes 3 and 8; 53 nM, lanes 4 and 9; and 159 nM, lanes 5 and 10. The probe DNA used in this experiment was 180 bp, with the five GAL4 sites exactly centered between 43 and 138 bp from the ends of the fragment. For nucleosome cores positioned against one end or the other, the first GAL4 site would be centered 18 bp into the nucleosome core.

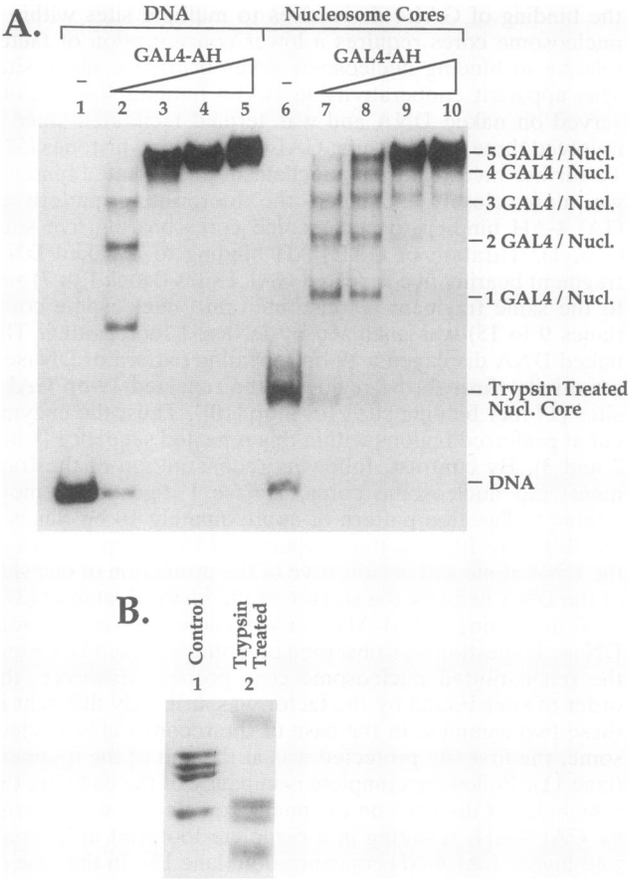

FIG. 3. Facilitated binding requires the core histone amino termini. (A). Naked DNA was mock reconstituted (lanes 1 to 5) or reconstituted (lanes 6 to 10) into nucleosome cores from which the amino-terminal tails were removed by trypsin digestion and titrated with increasing amounts of GAL4-AH as in Fig. 2. Binding of GAL4-AH to the trypsin-treated nucleosome cores resulted in the appearance of five complexes with increasing numbers of GAL4-AH dimers bound (1 GAL4/Nucl. to 5 GAL4/Nucl. [lanes 7 and 8]) in contrast to the binding to nucleosome cores in which the amino termini were intact (Fig. 2). The appearance of GAL4-AH nucleosome complexes with intermediate numbers of bound dimers resembled that on the naked DNA (lane 2), except that the complexes were supershifted relative to the DNA complexes with the same number of bound dimers (compare lanes 7 and 2). The concentrations of GAL4-AH included in the binding reaction mixtures were 0, lanes 1 and 6; 5.3 nM, lanes 2 and 7; 15.9 nM, lanes 3 and 8; 53 nM, lanes 4 and 9; and 159 nM, lanes 5 and 10. The probe DNA used was the same as in Fig. 2. (B) Protein gel of the histones from native nucleosome cores (lane 1) used in the reconstitution shown in Fig. 2 and the trypsinized nucleosome cores (lane 2) used in the reconstitution shown in panel A. The upper band in lane 2 is from the added trypsin inhibitor.

(compare lanes 7 to 10 of Fig. 2 with lanes 7 to 10 of Fig. 3A). Thus, the inhibition of factor binding, which causes the cooperative nature of GAL4-AH binding, was largely mediated by the core histone amino termini. Facilitated binding of GAL4-AH to nucleosomal DNA therefore overcame inhibition due to the core histone amino termini.

Positional inhibition of GAL4-AH binding mediated by the core histone amino termini. Previous studies have illustrated the lack of a significant rotational phasing effect on the binding of GAL4 derivatives to nucleosome cores (i.e., orientation of the GAL4 binding site with regard to the histone octamer surface [52]). The apparent insensitivity of GAL4 to rotational orientation may be due to the fact that GAL4 dimers interact with the major groove of the DNA helix on both sides (38), which might allow binding to initiate regardless of orientation. Alternatively, the inherent mobility of nucleosome cores may provide transient accessibility of the factor's DNA contacts on a rotationally phased nucleosome core as it shifts between alternative translational frames (i.e., every 10 bp [39]). In contrast to the apparent lack of a rotational phasing effect, we have found a strong translational position effect (i.e., location of a site relative to the end or center of the nucleosome) on GAL4-AH binding.

The mobility shift gels shown in Fig. 2 and 3 indicate that the initial binding of one GAL4-AH dimer to nucleosome cores occurred readily; however, the subsequent filling of the remaining sites were more difficult when the core histone amino termini were intact. The DNase I protection experiment shown in Fig. 1 indicates that the initial sites bound were at the end of the nucleosome cores. Together these results raise the possibility that initial occupancy near the end is required to alleviate inhibition at additional sites closer to the center of the nucleosome. Moreover, this possibility would implicate the core histone amino termini in nucleosome translational positioning effects on the function

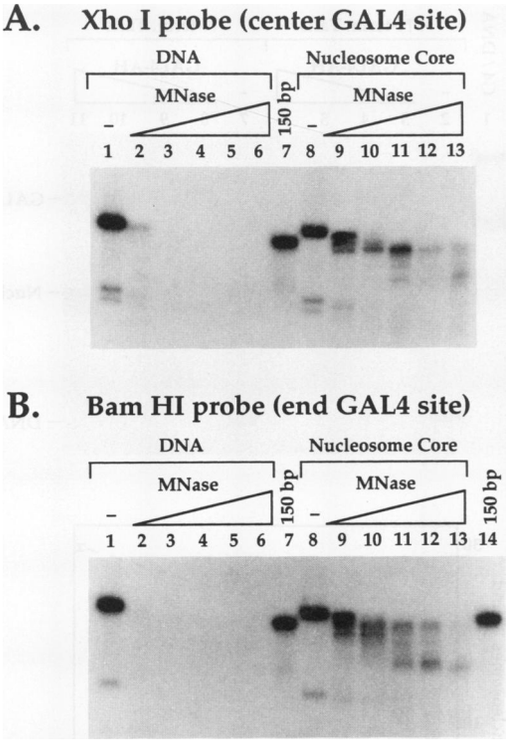

FIG. 4. Reconstituted nucleosome cores occupy nucleosome core-length DNA. (A) Naked DNA (lanes 1 to 6) and reconstituted cores (lanes 8 to 13) with one GAL4 site (center of the site 74 bp from the 5′ end) were digested with 0.01 U of micrococcal nuclease per ml for 0, 1, 3, 9, 15, and 30 min, respectively. In the mock-reconstituted DNA control lanes (lanes 1 to 6), probe is digested completely after 3 min (compare lane 1, zero time point, with lane 3) and therefore, not protected. In contrast, nucleosome core-reconstituted samples (lanes 8 to 13) were trimmed to digestion intermediates slightly smaller in size than the 150-bp marker (lane 7). The lower single stranded fragments in lanes 1 and 7 are the result of a small fraction of the double-stranded probe which ligated on only one strand after insertion of the internal label. (B) Reaction conditions and lane markers are the same as above except that the one-GAL4-site probe has the center of the site 21 bp from the 5′ end. Micrococcal nuclease digestion with the *Asp* 718-cut probe (center of the site 40 bp from the 5′ end) also produced similar results (results not shown).

of DNA elements observed in vivo and in vitro (see Introduction). To further investigate the mechanism of facilitated binding and the function of the core histone amino termini, we analyzed the binding of GAL4-AH to nucleosomes bearing a single GAL4 site.

To analyze the effect of the N termini on GAL4-AH binding at different translational positions, we constructed plasmids bearing a single GAL4 site between the repeated polylinkers of the plasmid pTK401 (27; also see 28). Digestion of these probes with different restriction endonucleases allowed the preparation of near-nucleosome-length probes (160 bp) with the GAL4 site at different positions (see Materials and Methods). These probes were then reconstituted into nucleosome cores by octamer transfer. Following reconstitution, the nucleosome cores formed protected approximately 146 bp (nucleosome core length) of each probe DNA from digestion by micrococcal nuclease. This is illustrated for two of the probe DNAs in Fig. 4. Following reconstitution, the mock-reconstituted DNA samples and the nucleosome core reconstituted samples were treated with micrococcal nuclease for increasing times, and the digestion products were extracted and analyzed on a denaturing acrylamide gel. The mock-reconstituted naked DNA was rapidly degraded by micrococcal nuclease without the appearance of intermediate digestion products (lanes 1 to 6). By contrast, the nucleosome core-reconstituted probes were first trimmed to digestion products which ran slightly faster than the 150-bp marker (i.e., nucleosome core length [lanes 8 to 13]). This indicates that the nucleosome cores reconstituted on each of the probes occupied a complete 146 bp of DNA.

Following octamer transfer from donor nucleosomes containing or lacking the amino termini, the reconstituted nucleosome cores bearing a single GAL4 site were analyzed for GAL4-AH binding relative to naked DNA controls. There are two important technical points relevant to these analyses of GAL4-AH binding to nucleosome cores bearing GAL4 sites at different positions on the probe DNAs. First, in order to observe changes in the relative affinity of GAL4-AH for its binding sequence, rather than simply measuring the molar ratio of GAL4-AH to binding site, the probe concentrations (approximately 0.1 nM) were kept below the K_d for specific binding of GAL4 to naked DNA. Thus, the nucleosome-reconstituted probe DNAs were a very small fraction of the total nucleosome cores (probe and donor nucleosomes) in the binding reaction mixtures. The low probe concentration ensured that the amount of binding observed was dependent on the GAL4-AH concentrations and reflects the affinity of GAL4-AH for its binding site within the different nucleosome reconstitutes. In principle, under these conditions the GAL4-AH concentration required for 50% binding to a specific nucleosome-reconstituted probe will equal the K_d for GAL4-AH binding to that site (discussed in reference 52 [and references therein]). Second, since the probe DNAs used in these reconstitutions were 160 bp (i.e., 14 bp longer than nucleosome core length), the position of the GAL4 site (within the nucleosome cores) within a reconstituted sample may vary by 14 bp depending on the specific location of the histone octamers on the fragments. As we have not observed precise homogeneous positioning of octamers on these fragments, we will only consider positioning of sites within this 14-bp resolution.

The binding of GAL4-AH to nucleosomes containing a GAL4 site near the end of the DNA fragment is illustrated in Fig. 5. The center of the GAL4 binding site on this 160-bp probe is 21 bp from the end. Thus, between the two extremes of translational positioning of the 146-bp nucleosome core on this fragment (i.e., against one end or the other), the center of the GAL4 site would be between 21 and 7 bp into the nucleosome core. Binding of GAL4-AH to this fragment after reconstitution into a nucleosome core is shown in Fig. 5A. Increasing concentrations of GAL4-AH led to a distinct complex of one GAL4-AH dimer bound to the nucleosome core (lanes 3 to 6). The minor bands just above the nucleosome complex and just above the GAL4-AH–nucleosome complex were due to GAL4-AH binding to the small amounts of naked DNA in the reaction mixtures. The mobility of these DNA complexes is illustrated in lane 1. Removal of the amino-terminal tails increased GAL4-AH binding at the end of the nucleosome core to a small extent (lanes 8 to 11). The effect of removal of the histone tails on the fraction of nucleosome cores bound is illustrated in Fig. 5B and results from quantitation of the gel in Fig. 5A and two additional independent experiments. At the lowest concentration of GAL4-AH (5.3 nM), there was a 2.5-fold stimula-

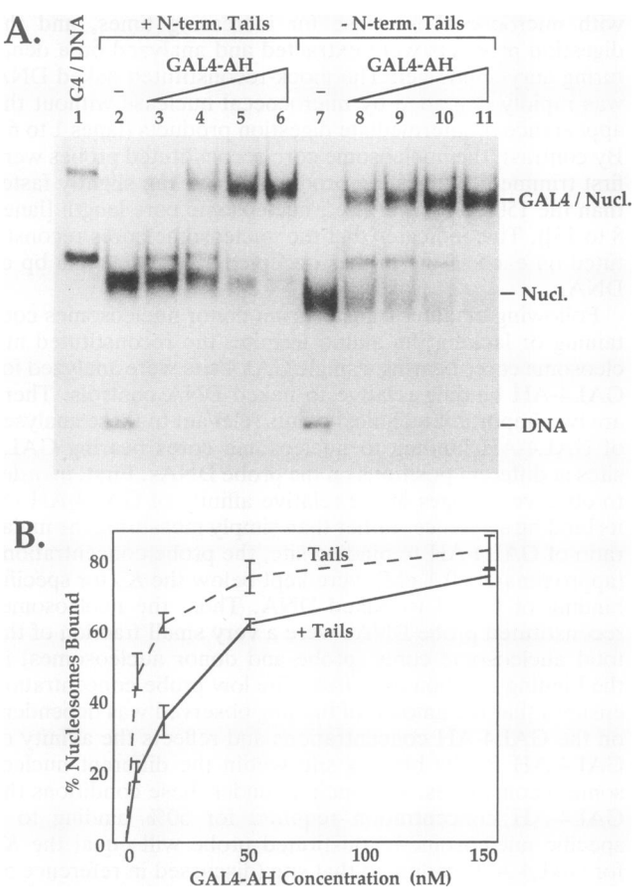

FIG. 5. Binding of GAL4-AH to nucleosome cores with a single site near the end. (A) A 160-bp probe DNA with a single GAL4 site centered at 21 bp from an end was reconstituted into nucleosome cores containing (lanes 2 to 6) or omitting (lanes 7 to 11) the amino-terminal tails. Nucleosomes were incubated with increasing concentrations of GAL4-AH, and binding was assayed by mobility shift. The mobility of the reconstituted nucleosome cores (Nucl.) and the nucleosome cores with a single GAL4-AH dimer bound (GAL4/Nucl.) are indicated. Note that the nucleosome cores without the amino termini migrate slightly ahead of the native nucleosome cores (compare lanes 2 and 7). The concentrations of GAL4-AH included in the binding reaction mixtures were 0, lanes 2 and 7; 5.3 nM, lanes 3 and 8; 15.9 nM, lanes 4 and 9; 53 nM, lanes 5 and 10; and 159 nM, lanes 6 and 11. The minor bands just above the nucleosome cores and the GAL4-nucleosome complexes resulted from GAL4-AH binding to the small amounts of unreconstituted naked DNA (DNA) present in the binding reaction mixtures. The mobilities of these complexes are illustrated in lane 1, in which the mock-reconstituted naked DNA probe was incubated with 53 nM GAL4-AH. The upper DNA band (lane 1) resulted from the nonspecific binding of a second dimer of GAL4-AH to the naked DNA at this concentration. (B) Graph of the percent nucleosomes bound by GAL4-AH at increasing GAL4-AH concentrations, with the GAL4 site located near the end. The lines are drawn through the average values derived from the experiment in panel A and two independent experiments in which GAL4-AH binding was assayed to nucleosomes containing (solid line) or omitting (dashed line) the amino-terminal tails. The percent nucleosomes bound was derived from the ratio counts in the GAL4/Nucl. complexes to total nucleosomes (i.e., GAL4/Nucl. plus Nucl.). The error bars at each point represent 2 standard deviations. The average fold stimulation resulting from removal of the amino-terminal tails [(% nucleosomes bound − tails)/(% nucleosomes bound + tails)] was 2.5 at 5.3 nM GAL4-AH, 1.9 at 15.9 nM, 1.2 at 53 nM, and 1.1 at 159 nM.

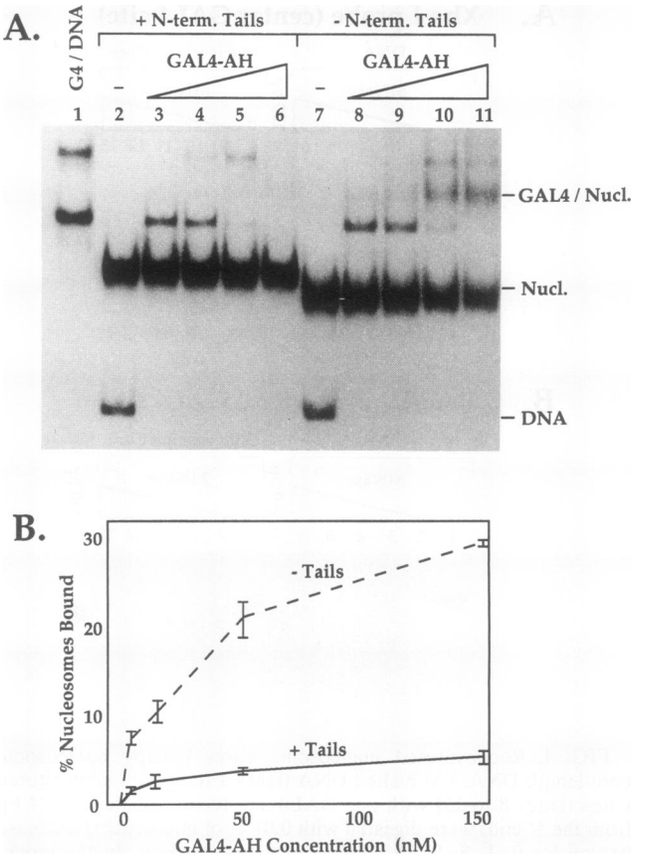

FIG. 6. Binding of GAL4-AH to nucleosome cores with a single site near the center. (A) A 160-bp probe DNA with a single GAL4 site centered at 74 bp from an end was reconstituted into nucleosome cores containing (lanes 2 to 6) or omitting (lanes 7 to 11) the amino-terminal tails and incubated with increasing concentrations of GAL4-AH. The mobility of the reconstituted nucleosome cores, GAL4-AH–nucleosome complexes, and naked DNA are indicated as for Fig. 5. The concentrations of GAL4-AH included in the binding reaction mixtures were 0, lanes 2 and 7; 5.3 nM, lanes 3 and 8; 15.9 nM, lanes 4 and 9; 53 nM, lanes 5 and 10; and 159 nM, lanes 6 and 11. Lane 1 illustrates the mobility of complexes of GAL4 with naked DNA, as in Fig. 5. (B) Graph of the percent nucleosomes bound by GAL4-AH at increasing GAL4-AH concentrations, with the GAL4 site located near the center. The lines are drawn through the average values derived from the experiment in panel A and two independent experiments. Other designations are for Fig. 5. The average fold stimulation resulting from removal of the amino-terminal tails was 4.9 at 5.3 nM GAL4-AH, 4.0 at 15.9 nM, 5.3 at 53 nM, and 5.7 at 159 nM.

tion of binding by removal of the tails. This stimulation decreased at higher GAL4-AH concentrations to 1.1-fold at 159 nM. Thus, there was only a small effect of removing the core histone amino termini on the binding of GAL4-AH to a site near the end of the nucleosome core. This result indicates that the core histone amino termini only moderately inhibit factor binding to positions near the end of the nucleosome.

Movement of the GAL4 binding site towards the center of the nucleosome significantly reduced GAL4-AH binding (Fig. 6). In this 160-bp fragment, the center of the GAL4 site was 74 bp from the end and thus between 60 and 74 bp into the nucleosome core. The binding of GAL4-AH to a nucleosome assembled on this DNA fragment after its reconstitu-

tion into a nucleosome core is significantly reduced relative to the nucleosomes bearing the GAL4 site near the end of the fragment (compare lanes 3 to 6 of Fig. 6A with lanes 3 to 6 of Fig. 5A). This difference in the affinity of GAL4-AH for binding elements located near the end versus the center of the nucleosome core illustrates that there is a difference in factor access to these two locations (see below). In contrast to the binding near the end of the nucleosome core, removal of the core histone amino termini significantly enhanced the binding of GAL4-AH at the site near the center (Fig. 6A, lanes 8 to 11). The relative levels of binding to the center site in the presence or absence of the amino-terminal tails is illustrated in Fig. 6B, which was derived from the gel shown in Fig. 6A and two independent experiments. Removal of the tails stimulated binding to the center 4.7-fold at 5.3 nM GAL4-AH, which increased to 5.5-fold at 159 nM. Thus, inhibition of factor binding to the center of the nucleosome core (relative to the end) was largely mediated by the core histone amino termini. It should be noted, however, that removal of the core histone tails did not completely remove inhibition at the center of the nucleosome core, as binding without tails was still twofold less to the center than the end site.

DNase I digestion studies have indicated that removal of the amino termini of the core histones enhances the digestion of the core DNA at 20 to 35 bp and 60 to 80 bp from the end of the core DNA (32, 57; reviewed in reference 54). This suggests that nucleosomal DNA is less accessible to the nuclease at these positions when the amino-terminal tails are present. This increased sensitivity was not apparent in hydroxyl radical footprinting, presumably because of insensitivity of this cleavage reagent to steric hindrance from the amino termini (25). In our experiments, the potential translational positions taken up by the nucleosome cores on the 160-bp fragment would place the middle of the end site (Fig. 5) between 7 and 21 bp into the nucleosome and the center site (Fig. 6) between 60 and 74 bp into the nucleosome. Thus, most of the 17-bp end site was outside of the regions protected by the tails from DNase I, while the center site most likely was located in the central, protected region. To determine whether the N-terminal tails inhibit factor binding only near the center of the nucleosome or whether significant inhibition is mediated over a broader region, we tested binding to nucleosome cores with the GAL4 site at an intermediate position.

Figure 7 illustrates the binding of GAL4-AH to nucleosome cores reconstituted on a 160-bp fragment bearing the single GAL4 site at 40 bp from one end. Potential translational frames on this nucleosome would place the center of the GAL4 site between 26 and 40 bp from the end of the nucleosome core. Binding of GAL4-AH to this nucleosome core was also significantly inhibited. While binding at this position was approximately twofold greater than the more-centered site when the tails were present, it was approximately sevenfold less than to the end site. Binding to the intermediate site was dramatically stimulated by removal of the histone tails. The stimulation observed ranged from 18-fold at 5.3 nM GAL4-AH (lanes 2 and 7) to 5-fold at 159 nM (lanes 5 and 10). This observation indicates that the core histone amino termini also inhibited binding at this intermediate position and that there was less tail-independent inhibition at this position than at the center site.

Facilitated binding overcomes inhibition from the core histone amino termini. The data presented above suggest two mechanisms by which increased access within a nucleosome core to transcription factors might be achieved. The first is

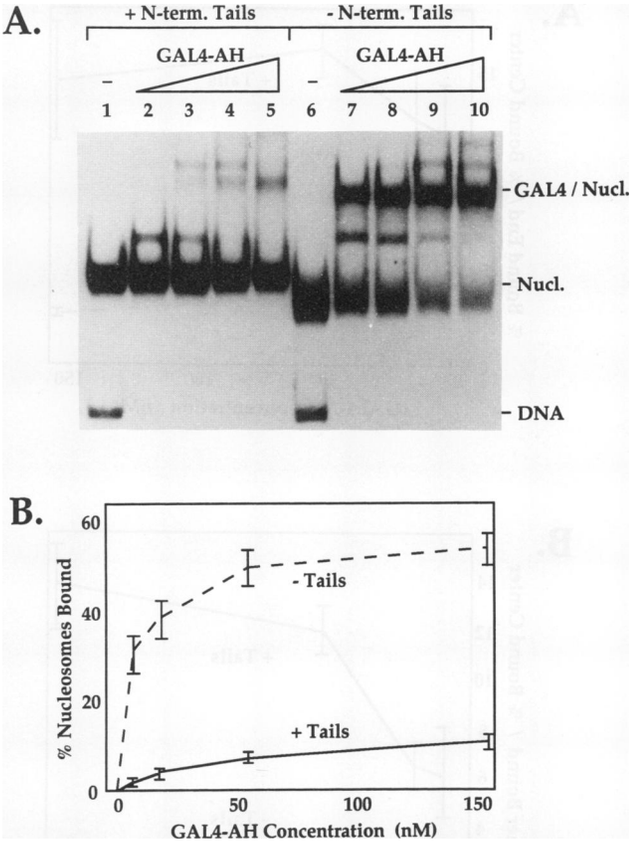

FIG. 7. Binding of GAL4-AH to nucleosome cores with a single site at an intermediate position. (A) A 160-bp probe DNA with a single GAL4 site centered at 40 bp from an end was reconstituted into nucleosome cores containing (lanes 1 to 5) or omitting (lanes 6 to 10) the amino-terminal tails and incubated with increasing concentrations of GAL4-AH. The mobility of the reconstituted nucleosome cores, GAL4-AH–nucleosome complexes, and naked DNA are indicated as for Fig. 5. The concentrations of GAL4-AH included in the binding reaction mixtures were 0, lanes 1 and 6; 5.3 nM, lanes 2 and 7; 15.9 nM, lanes 3 and 8; 53 nM, lanes 4 and 9; and 159 nM, lanes 5 and 10. (B) Graph of the percent nucleosomes bound by GAL4-AH at increasing GAL4-AH concentrations, with the GAL4 site located at the intermediate position. The values plotted are from the experiment shown in panel A, which was representative of additional experiments. Other designations are as for Fig. 5. The fold stimulation resulting from removal of the amino-terminal tails was 18 at 5.3 nM GAL4-AH, 11 at 15.9 nM, 6.8 at 53 nM, and 4.8 at 159 nM.

removal of the inhibition from the core histone amino termini (Fig. 6 and 7), and the second is facilitated binding (see below). The experiments whose results are shown in Fig. 5, 6, and 7 illustrate that it was significantly more difficult for GAL4-AH to bind a site well within the nucleosome core than a site near the end. This difference was largely due to an increased inhibition from the core histone tails. In the presence of the tails, binding at the site located near the end of the nucleosome was 12- to 18-fold greater than the site located near the center (Fig. 8A). By contrast, when the tails were removed, binding at the end was only three- to sixfold greater than in the center. Thus, the reduced affinity for the binding site located near the center versus that near the end of the nucleosome was largely a consequence of the core histone amino termini.

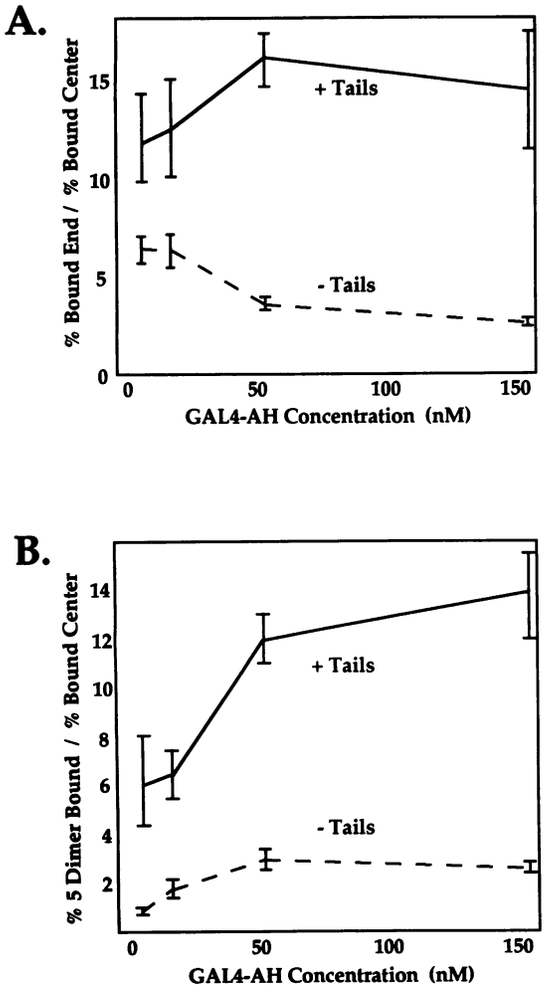

FIG. 8. Contribution of the core histone amino-terminal tails to positional and facilitated binding. (A) Position effects of nucleosomes of GAL4-AH binding are largely mediated by the core histone amino-terminal tails. The ratio of the percent nucleosomes bound at the end site (Fig. 5) over that at the most central site (Fig. 6) is plotted for nucleosomes containing (solid line) or lacking (dashed line) the amino-terminal tails. The values plotted are the average from three independent pairs of experiments, including those in Fig. 5A and 6A. The crossbars represent 2 standard deviations. The ratio of binding decreased when the tails were removed, since binding to the center site was increased to a greater extent than at the end. Thus, the effect of nucleosome position on GAL4-AH binding was reduced when the amino termini were removed. (B) Facilitated binding overcomes inhibition from the amino-terminal tails at GAL4 sites within the nucleosome core. Nucleosome cores bearing five GAL4 sites contain sites within the center of the nucleosome core, as do nucleosome cores bearing a single GAL4 site near the center. Binding of five dimers of GAL4-AH to the five-site nucleosomes therefore includes binding near the center. The ratio of five dimers of GAL4-AH bound to the five-site nucleosomes (from the experiment in Fig. 2 and two repeat experiments) versus a single dimer bound to a nucleosome with a single site near the center (from the experiment in Fig. 6A and two repeat experiments) is plotted for the GAL4-AH concentrations tested (solid line). This ratio provides an estimate of the stimulation of binding near the center attributable to the multiple binding sites (facilitated binding). The ratio of five dimers of GAL4-AH bound to the five-site nucleosomes in the absence of the tails (from Fig. 3 and two repeat experiments) versus a single dimer bound to a nucleosome lacking the amino-terminal tails with a single site near the center (from Fig. 6A and two repeat experiments) is also plotted (dashed line). The lines are drawn through the mean value of the three experiments, and the crossbars represent 2 standard deviations. In the absence of the amino-terminal tails, binding of GAL4-AH to the single center-site nucleosome approached that observed by facilitated binding, and thus the ratio was reduced. Thus, facilitated binding was required for occupancy of the center sites primarily when the amino termini were present.

The DNA fragment used for nucleosome reconstitution in Fig. 2 contained five GAL4 sites (95 bp) exactly centered on the DNA fragment. Thus, when contained in a nucleosome core, this fragment contained sites located in the center as well as near the ends. Therefore, for these nucleosome cores to become completely occupied by five-GAL4-AH dimers, GAL4-AH binding must also occur at a site which is located near the center and thus subject to the same inhibition as a single site located in the center (i.e., as in Fig. 6). However, it is clear that binding of five-GAL4-AH dimers to the five-site nucleosome (Fig. 2) was greatly enhanced relative to binding of a single dimer to the one centered-site nucleosome (Fig. 6) at all of the GAL4-AH concentrations tested. The magnitude of this effect is illustrated more clearly in the plot shown in Fig. 8B (which is derived from quantitation of the experiments shown in Fig. 2 and 6 and two repeats of each). In the presence of the core histone amino-terminal tails, occupancy of five-GAL4-AH dimers on five-site nucleosomes (including center sites) was 6- to 13-fold greater than occupancy of an individual site near the center of the core particle. These data illustrate the increased binding at centered sites (facilitated binding) which resulted from the binding of factor to more accessible sites on the same nucleosome core. In the absence of the core histone tails (Fig. 8B, − Tails), binding to the centered single-GAL4 site is only 0.8- to 3-fold reduced relative to binding the five-site nucleosome. Thus, the ability of GAL4-AH to bind the single centered site in the absence of the tails approached that achieved by facilitated binding. These observations suggest that facilitated binding of GAL4-AH dimers to more accessible sites altered the conformation of the nucleosome core in a manner which decreased the ability of the histone tails to inhibit binding within the center of the nucleosome.

DISCUSSION

Facilitated binding of a transcription factor to nucleosomes. While 146 bp of DNA is wrapped around the histone octamer, previous studies have shown that the function of DNA elements within the nucleosome is dependent on their location. For example, the in vivo function of an ARS element is progressively reduced as the element is moved closer to the center of the nucleosome (48). Similarly, initiation of transcription by a prokaryotic polymerase in vitro was increasingly inhibited as the promoter was moved towards the center of a nucleosome core (59). In this report, we demonstrate a similar effect on the binding of a eukaryotic regulatory transcription factor, GAL4.

The binding of GAL4-AH to individual binding sites within the nucleosome core was significantly easier for a site near the end of the nucleosome than for a site more centrally located or a site at an intermediate location (Fig. 5, 6, and 7). This observation indicates that on nucleosomes which contain multiple GAL4 sites, binding would initially occur to the sites most distal from the center of the nucleosome core. Indeed binding of the factor to nucleosomes containing five sites was first observed at the end site (Fig. 1). Thus, the

affinity of GAL4-AH was greatest near the end of the nucleosome and the most reduced in the center.

Binding to end sites had a substantial impact on the subsequent affinity of GAL4-AH for central sites. Following the initial binding of the first dimer of GAL4-AH to the nucleosome cores containing five sites (occupying the central 95 bp), the remaining sites filled in a "cooperative" manner (Fig. 2). This observation indicates that binding of the second dimer of GAL4-AH altered the conformation of the nucleosome core, inducing a cascade of binding to fill all five sites. The result of this facilitated binding is that central GAL4 sites in the five-site nucleosomes had a significantly higher affinity for GAL4-AH than a single centrally located site (Fig. 8). Binding of the first dimer to the end of the nucleosome core may have altered histone DNA contacts and enhanced the affinity for the adjacent internal site (a more difficult site). Binding to the adjacent position may have further altered the conformation of the nucleosome beyond a threshold enhancing affinity of the next (central) site and the two distal sites. Close examination of footprint patterns, like those shown in Fig. 1, suggests that the second dimer loaded may have occurred adjacent to the first end dimer, consistent with this possibility. Alternatively, binding of the initial dimer to one of the end sites may have enhanced binding of the most distal site at the other end of the nucleosome. In this case, binding of two dimers at end sites may have resulted in the filling of the internal sites.

Repression from the core histone amino termini is alleviated by facilitated binding. In contrast to the apparent cooperativity in GAL4-AH binding to intact five-site nucleosome cores (Fig. 2), removal of the amino-terminal tails resulted in GAL4-AH binding to the five sites in a "noncooperative" manner similar to that observed on naked DNA (Fig. 3). This observation indicates that facilitated binding was required to overcome the inhibition to GAL4-AH binding mediated by the amino-terminal tails. This conclusion is further supported by analysis of single-site nucleosomes which illustrates that removal of the tails enhances binding primarily to sites well within the nucleosome core (Fig. 5, 6, and 7). Since the affinity of central sites is enhanced by removal of the tails, binding to these sites could occur to the five-site nucleosomes without facilitated binding and thus appear less cooperative. The observation that the tails contribute significantly to the inhibition of GAL4-AH binding might explain the inability to detect rotational phasing effects (i.e., the orientation of the binding site with regard to the histone octamer surface) on GAL4 binding to nucleosomes (52). Inhibition from the tails, which may extend over the DNA (reviewed in reference 54), might be orientation independent (i.e., affecting both sides of the DNA helix), in contrast to that expected from the histone octamer surface alone.

A recent study has implicated the core histone amino termini in restricting TFIIIA binding to nucleosome cores (31). In this study, it was found that TFIIIA binding to a site located at the end of the nucleosome core or a site 28 bp closer to the center was enhanced by either removal of the core histone tails or by their acetylation. While the former observation might appear contradictory to our end site results, in the case of TFIIIA the factor DNA contacts would extend 40 bp into the nucleosome and thus would be expected to enter the first region of DNA protected by the tails (i.e., from 20 to 35 bp into the nucleosome [32, 57]). Thus, binding of TFIIIA to the end site might more closely resemble the intermediate GAL4 position tested in Fig. 7 which was significantly inhibited. The fact that TFIIIA binding was similarly enhanced either by proteolytic removal of the tails or by the acetylation of the tails (31) is consistent with the observation that acetylation of the H4 tail peptide reduces its affinity for DNA dramatically. Acetylation of the H4 amino-terminal peptide decreased its binding constant to DNA as much as 6 orders of magnitude (26). Thus, acetylation of the core histone tails appears to alleviate the inhibition of factor binding by reducing their affinity for DNA.

The data presented in this report illustrate that facilitated binding also overcame inhibition from the core histone amino termini. The fact that facilitated binding increased the affinity of sites which in isolation are significantly repressed by the amino termini suggests that binding of adjacent factors reduces inhibition from the tails. While the mechanism for this effect is unknown, it might involve steric hindrances to tail interactions with the most repressed sites from factors bound to more accessible sites. Alternatively, binding of the factor may alter the conformation of the nucleosome such that the amino-terminal tails are unable to stably interact with the core DNA. Such a conformational change might be related to the conformation which increases the accessibility of the histone H3 cysteine to Hg columns and correlates strongly with transcription activity (14, 55, and references therein). A conformational change in the nucleosome upon factor binding is also consistent with the observation that the binding of GAL4 derivatives increases the accessibility of the core histones to displacement onto competitor DNA (64) or the histone binding protein, nucleoplasmin (13).

Implications for gene repression and activation in vivo. A long-standing question regarding the developmental control of gene expression is whether nucleosomes or higher-order chromatin structures might play a passive role in the repression of "inappropriate" gene transcription (6, 7, 9, 56). With regard to the nucleosome, there are very clear examples indicating that nucleosome positioning can repress the function of *cis*-acting elements (reviewed in reference 49). Thus, nucleosomes are clearly not transparent to DNA-binding factors. However, with some notable exceptions (47, 53), potential nucleosome position effects have not often emerged as a primary determinant of promoter function (29). This raises the possibility that in many instances nucleosome position effects, whether sequence specific or coincidental, are often overcome by *trans*-acting factors. In vivo, multiple factor binding sites (enhansons) are required in close proximity to form a functional enhancer (reviewed in reference 18). This requirement of multiple sites for transcription factor function when located at a distance from the promoter may represent a need for synergistic effects of activation domains (11, 12) and/or facilitated binding amongst factors at isolated locations in chromatin (discussed in reference 52). We suggest that multiplicity of binding sites increases the probability that some sites will be in more accessible positions within the nucleosome core (i.e., near the end). Binding of factors to these sites, in turn, would increase the accessibility of sites in more difficult positions near the center of the nucleosome. Thus, facilitated binding could bring about complete occupancy of multiple binding sites located in enhancer and promoter elements.

Repeated binding sites for constitutively active factors may generate permanent nucleosome-free regions through facilitated binding. For example, many constitutively transcribed housekeeping genes often contain multiple binding sites for the transcription factor Sp1. The repeated Sp1 sites may ensure that these promoters remain accessible to Sp1 and additional factors. The repeated Sp1 binding sites in the

simian virus 40 promoter play a predominant role in the generation of a nucleosome-free region at the simian virus 40 promoter (reviewed in references 21 and 61). Similarly, the permanent nucleosome-free regions over the *Drosophila* heat shock promoters are thought to result in part from the multiple binding of the constitutive GAGA factor to repeated CT sequences (20, 35, 36).

Facilitated binding of factors to nucleosomal DNA provides one mechanism by which repression of factor binding may be overcome at enhancers and promoters. Such mechanisms may subvert permanent passive repression of at least some eukaryotic genes. These mechanisms may also lead to the rescue of previously inactive tissue-specific genes and thereby contribute to the examples of plasticity seen in development pathways (reviewed in references 6 and 7).

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