DNA Sequence-Dependent Contributions of Core Histone Tails to Nucleosome Stability: Differential Effects of Acetylation and Proteolytic Tail Removal†

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ABSTRACT: Modulation of nucleosome stability in chromatin plays an important role in eukaryotic gene expression. The core histone N-terminal tail domains are believed to modulate the stability of wrapping nucleosomal DNA and the stability of the chromatin filament. We analyzed the contribution of the tail domains to the stability of nucleosomes containing selected DNA sequences that are intrinsically straight, curved, flexible, or inflexible. We find that the presence of the histone tail domains stabilizes nucleosomes containing DNA sequences that are intrinsically straight or curved. However, the tails do not significantly contribute to the free energy of nucleosome formation with flexible DNA. Interestingly, hyperacetylation of the core histone tail domains does not recapitulate the effect of tail removal by limited proteolysis with regard to nucleosome stability. We find that acetylation of the tails has the same minor effect on nucleosome stability for all the selected DNA sequences. A comparison of histone partitioning between long donor chromatin, acceptor DNA, and free histones in solution shows that the core histone tails mediate internucleosomal interactions within an H1-depleted chromatin fiber amounting to an average free energy of about 1 kcal/mol. Thus, such interactions would be significant with regard to the free energies of sequence-dependent nucleosome positioning. Last, we analyzed the contribution of the H2A/H2B dimers to nucleosome stability. We find that the intact nucleosome is stabilized by 900 cal/mol by the presence of the dimers regardless of sequence. The biological implications of these observations are discussed.

Transcription and replication occur within the intricate structure of chromatin in eukaryotes. This organization of the DNA is necessary for the efficient compaction of an immense amount of genetic material, within the small volume of the eukaryotic nucleus. The fundamental building block of chromatin is the nucleosome core, in which 147 bp of DNA is wrapped almost two complete turns around an octamer of core histone proteins. This octamer is composed of a tetramer of histones $(H3/H4)_2$ together with two dimers of histones (H2A/H2B) in a tripartite assembly. An additional stretch of DNA of variable length, the linker, connects adjacent nucleosomes. This linker DNA and the nucleosome core are associated with a fifth histone, known as the linker histone or H1.

The structure−function relationship of the tripartite nucleosome assembly is best understood during replication. Following the passage of the replication fork, nucleosome assembly is thought to be a two-step process in which the $(H3/H4)_2$ tetramer is first assembled with nascent DNA (*1*, *2*) possibly by the action of complexes such as CAF-1 (*3*). Following this event, the H2A/H2B dimers bind to form the intact nucleosome (*1*), albeit in an immature state (*4*). Interestingly, the subnucleosomal−tetramer complex recognizes the same nucleosome positioning determinants as the intact nucleosome (*5*, *6*), and in this view, the role of the H2A/H2B dimers is not fully understood. However, H2A/H2B deficiency has been correlated to transcription factor access (*7*−*9*) and transcriptionally active chromatin (*10*). This favors the idea that the H3/H4 tetramer−DNA complex is relatively transparent to the binding of transacting factors.

All the core histones have a histone-fold domain that comprises the columnar body of the nucleosome core and a protruding, structurally undefined, amino-terminal tail region (*11*). The tails are highly positively charged and are subjected to posttranslational modifications, such as acetylation and phosphorylation, which are thought to regulate the accessibility, degree of condensation, and transcriptional permissibility of the chromatin fiber (*12*, *13*). Thus, the tails are important end points in a number of transcription-related signal transduction pathways in the nucleus. These modifications alter the charge of the tails and are likely to affect tail conformations and thus tail interactions with DNA or protein within chromatin (*14*).

On the level of individual nucleosomes, the removal of histone tails has been shown not to affect nucleosome positioning (*15*, *6*) and salt-dependent stability (*16*). However, the tail domains have been shown to contribute to thermal stability of the nucleosome cores containing random

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sequences (*16*). Moreover, removal of the histone tails results in the inability for complete cation-dependent chromatin folding and concomitant linker DNA bending (*17, 18*). Acetylation of the tails leads to the same behavior on nucleosomal arrays (*19*). In addition, hyperacetylation does not significantly alter nucleosome positioning or thermal stability (*20*) or the binding of H1 to individual nucleosomes (*21*). However, the stability of H1 binding is reduced in acetylated chromatin (*22*). Both removal and acetylation of the tails are thought to "loosen" the histone−DNA contacts due to the removal of positive charge in the vicinity of nucleosomal DNA. This is in line with studies in which either acetylation or removal of the tail domains has been coupled to increased transcription factor access (*22*−*26*).

Although almost all DNA in the nuclei is in the form of chromatin, some sequences are more prone to nucleosome formation than others. Intrinsic properties of the DNA molecule such as flexibility (*27*) and anisotropic bendability (*28*) have been shown to affect nucleosome positioning. Selection studies have shown that sequences with these properties preferentially associate with nucleosomes over the average (*29, 30*). However, regardless of DNA sequence-dependent structure in solution and the propensity for wrapping with nucleosomes, all sequences are constrained to adopt very similar conformations within the nucleosome (*6, 31*−*33*). Thus, relative free energy differences in nucleosome formation are expected to reflect the degree of DNA conformational change required for nucleosomal assembly (*31*). However, the role of histone tails, acetylation, and H2A/H2B dimers in defining these relative free energy differences has not been explored.

Here, we investigate to what degree the tails and the tripartite nature of the histone octamer modulate nucleosome stability for a variety of DNA molecules that exhibit different intrinsic properties. We find that the contribution of the tail domains to nucleosome stability is sequence-dependent, while the contribution of the H2A/H2B dimer is not. In addition, supplementary tail-dependent internucleosomal interactions were found to increase nucleosome stability in H1-depleted chromatin. Last, we find that the effect of hyperacetylation of the tails on nucleosome stability is quite different than the effect of tail removal by limited trypsin proteolysis.

EXPERIMENTAL PROCEDURES

PCR Generation of Labeled DNA Molecules. The 190 bp DNA fragments for nucleosome reconstitutions were amplified by PCR under standard conditions from the following plasmids containing the indicated inserts: pHCn41 (TATAAACGCC repeat); pHWn27 (mouse major satellite DNA); pHWn7 (CAG repeat); pHWn10 (CA repeat); and pasHC47 (TGGA repeat). The plasmids are derivatives of pCR-Script (Stratagene) with inserts cloned into the *Eco*RI/*Bam*H1 sites. The primers FWD (CGGAATTCAGATCTTCCTGGGAAAACCCTGG) and REV (CGGGATCCCGAGCTGTTTCCTGTGT) were radioactively end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase.

Preparation of Purified Chromatin and Histones. Chromatin was prepared from chicken erythrocytes and HeLa cells according to standard published procedures (*6, 29*). In brief, cells were lysed, nuclei isolated, and chromatin digested by mild micrococcal nuclease digestion. Soluble chromatin was prepared, and histone H1 was removed by ion exchange. Purified histones were prepared by hydroxyapatite chromatography (*34*). Trypsinized chromatin was prepared by agarose-immobilized trypsin digestion in batch essentially as described (*16*). Hyperacetylated chromatin was prepared from HeLa cells by the addition of 300 nM Trichostatin A in the media during the last 48 h of cell growth, and then chromatin was prepared essentially as for chicken erythrocytes. Purified octamers were isolated by acid precipitation of nucleic acids from soluble chromatin depleted of linker histones. The degree of acetylation was assessed by immunostaining assay with an acetylated histone H4 antiserum (*35*).

In Vitro Reconstitution of Nucleosomes. Nucleosomes were reconstituted by the stepwise salt dilution method (*36, 37*). Typically, 4 μg of purified histone octamers was mixed with 0.4 μg of labeled DNA fragment and 4 μg of competitor DNA in a volume of 10 μL containing high salt buffer (1 M NaCl, 0.1% NP40, 10 mM sodium phosphate [pH 7.0]). Competitor DNA was omitted when using long chromatin as the histone donor. Samples were incubated for 30 min at 37 °C and then diluted by three additions of 30 μL of low salt buffer, 20 min apart, resulting in intermediate salt concentrations of 250 mM, 140 mM, and the final concentration of 100 mM NaCl. Trial experiments in which low salt buffer was added in smaller aliquots (2 μL) yielded the same distribution of reconstituted products and the same ΔG's and $\Delta(\Delta G)$'s. In the experiments using acetylated histones and chromatin, the amounts were reduced 10-fold in all samples. Reconstituted products were separated on native 5% 19:1 polyacrylamide with 5% glycerol (loaded without dyes) at room temperature and run at 150 V for 45 min (*29*) or resolved on 0.7% native agarose gels to identify different subnucleosomal complexes (*32*). After electrophoresis, the gels were dried and analyzed by PhosphorImager (Molecular Dynamics).

Calculation of $\Delta(\Delta G)$. For reactions in which nucleosomes are in facile thermodynamic equilibrium with free histones source and acceptor DNA, the reaction constant is directly proportional to the intensity ratios between the nucleosome and the naked DNA bands relative to an internal reference after gel electrophoresis (*37*). This yields the following formula:

$$\Delta(\Delta G^{\ominus})_{\text{sample/reference}} = -RT \ln \frac{I_{\text{nuc,sample}}/I_{\text{DNA,sample}}}{I_{\text{nuc,reference}}/I_{\text{DNA,reference}}}$$

where T = 310 K (37 °C) and R = the universal constant 1.987 cal/K mol.

RESULTS

H2A/H2B Dimers Stabilize Nucleosomal Wrapping. We wanted to assess the effect of histone domains and modifications on the relative stability of nucleosomes containing several different DNA sequences. The DNA sequences were derived from nucleosome selection studies in vitro (*29, 38*). All the sequences used have a central internal 123 bp sequence exhibiting different inherent intrinsic properties, flanked by identical cropping sequences resulting in a total length of 190 bp (Table 1). Thus, the inherent sequence

Table 1: DNA Fragments Used in This Study and Their Intrinsic Properties

fragment	intrinsic properties	ref
TATAAACGCC repeat	flexible, bent	*29, 43*
CAG repeat	flexible	*44*
CA repeat	flexible	*29*
mouse major satellite	bent	*29*
TGGA repeat	unflexible, unbent	*38, 45*[a]

[a]The TGGA repeat has sequence similarity to telomeric repeat DNA and the same out-of-helical-phase repeat.

properties exhibited are only due to the central-most portion of the fragments but are presented as a $\Delta(\Delta G)$ for the entire fragment and include all nucleosome positions. However, the central 120 bp within the nucleosome has been shown to be the major determinant for nucleosome positioning (*29, 31, 33*).

We used the salt dilution method of in vitro reconstitution to compare the selected DNA sequences. This method of reconstitution has been shown to yield native nucleosomes (*39*). In theory, the extent of reconstitution is dictated thermodynamically by the equilibrium constant for histone−DNA association (*37, 40*), and the ratio of DNA incorporated into nucleosomes relative to free DNA allows the free energy for nucleosome formation to be calculated relative to a standard (*37, 41*). In these experiments, the reconstitution of nucleosomes was started at an initial ionic strength of 1 M NaCl, and then the salt was stepwise diluted down to 0.1 M NaCl. We choose to use the stepwise dilution method since this is less laborious than the dialysis method, and both methods do represent a true equilibrium situation. This is seen in the data from Thåström et al. (*40*) when data from both methods are plotted against each other resulting in a straight line but deviating from $k = 1$, suggesting (small) systematic influences showing up in either data set (method). In addition, we do not use the extreme sequences that were found to deviate the most in the latter study. Also, our differences we measure here are significantly smaller, and the reference state is compared to the same particular DNA in each pair.

In our experiments, competitor nucleosomal-length DNA (more than 10-fold excess) as compared to radiolabeled probe was present in each reconstitution reaction with identical amounts of purified histone octamers (as assessed by SDS−PAGE and absorption spectroscopy). The products were separated by native gel electrophoresis and analyzed by densitometry. To ensure that free exchange of histones occurs under these conditions, we also performed reconstitutions beginning at 1.3 and 2.0 M NaCl, which were then diluted down to 0.1 M NaCl, and experiments comparing slow stepwise dilution versus rapid dilution. In these experiments, we did not detect any difference in reconstitution ratios (data not shown). Moreover, the relative free energies of nucleosome formation did not depend on the level of histone donor or the amount of competitor DNA in the exchange (data not shown) (see also ref *42*).

To assess the contribution of the H2A/H2B dimers to nucleosome stability, we performed reconstitutions of $(H3/H4)_2$ tetramers alone or with an identical amount of tetramers and a stoichiometric amount of H2A/H2B dimers. Reconstitutions were carried out with our set of DNA fragments displaying a variety of sequence-dependent properties (Figure 1). The extent that the dimers stabilize the association of DNA in the nucleosome was then calculated from the ratio of $(H3/H4)_2$−DNA complexes as compared to naked DNA, using the nucleosome as the reference state. Interestingly, we found that the H2A/H2B dimers stabilize nucleosomal DNA wrapping about 4.5-fold ($\Delta(\Delta G) = -900$ (± 200) cal/mol) in a sequence-independent manner.

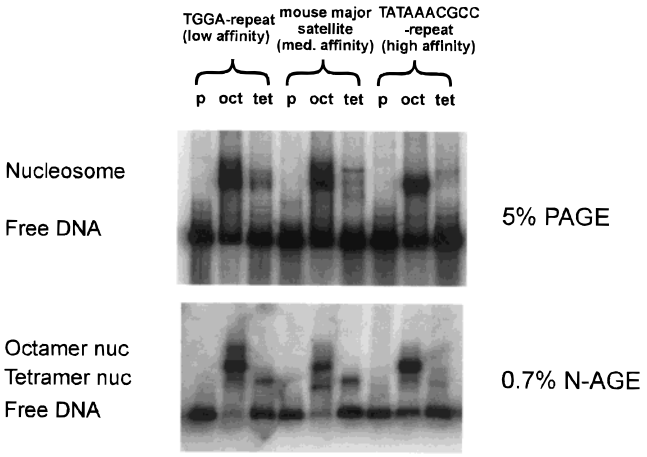

FIGURE 1: Stabilities of tetramer−DNA complexes and nucleosomes reconstituted with selected DNA fragments. Analytical reconstitutions were performed with the TGGA repeat, mouse major satellite DNA, and TATAAACGCC DNA fragment, and the products were separated on native 5% polyacrylamide and 0.7% agarose gels, as indicated. Shown are samples from reconstitutions of tetramer−DNA complexes (tet), nucleosomes (oct), and mock reconstitutions containing no histones (free DNA, p). The relative free energy of formation for tetramer−DNA complexes and nucleosomes was calculated as described in the text. Note the presence of multiple translational positions for the major satellite−tetramer complex. Mouse major satellite was used as competitor in all the individual experiments but in different amounts to yield quantitative ratios of nucleosome to free DNA.

Tail Domains Stabilize Nucleosomes Containing Intrinsically Straight and Curved DNA. We next assessed the contribution of the histone tail domains to nucleosome stability for each of the DNA fragments. Reconstitutions were performed with identical amounts of either purified normal (wt) or trypsinized histones. Results show that the negatively charged tail domains facilitate wrapping of the rigid TGGA repeat sequence 4.5-fold [$\Delta(\Delta G) = 900$ (± 250) cal/mol] (Figure 2). In addition, folding of moderately bent DNA (genomic TATAAACGCC repeat and mouse major satellite DNA) is stabilized to a lesser extent (50%) by the tail domains ($\Delta(\Delta G) = 250$ cal/mol). Interestingly, the stability of nucleosomes formed with intrinsically flexible DNA is unaffected by the presence of the tail domains.

Tail-Dependent Stability of Oligonucleosomes. To further address the effect of the tail domains on nucleosome stability, we reconstituted nucleosomes using long trypsinized and normal (wt) chromatin with comparable size distribution (data not shown) as core histone donors. While performing these experiments, we found that the absolute level of nucleosome formation on acceptor DNAs depended on the source of histone protein. Specifically, we found that the acceptor DNAs competed less efficiently for histones with long H1-depleted chromatin than with comparative amounts of purified histone octamers and short naked DNA competitor or purified nucleosome core particles. Surprisingly, we found that this effect was dependent on the presence of the core

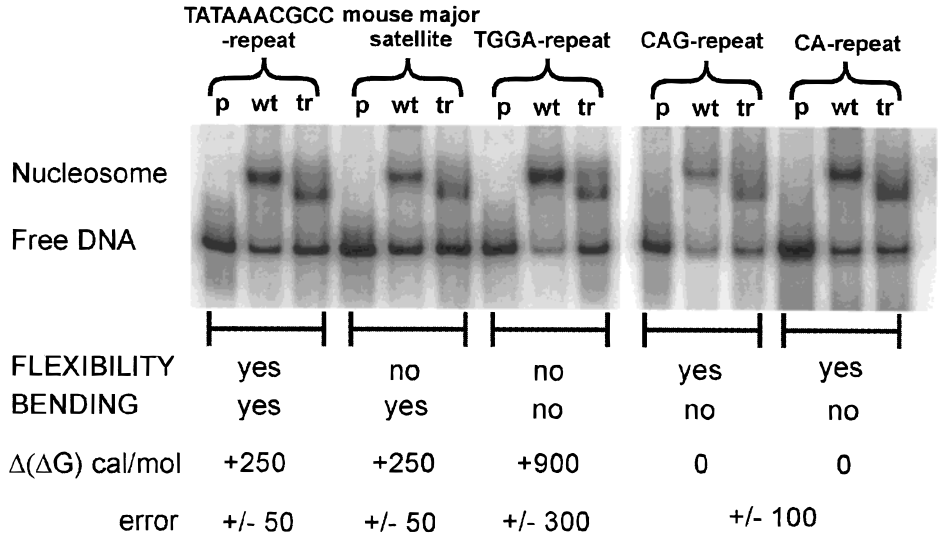

FIGURE 2: Core histone tail domains stabilize nucleosomal DNA wrapping in a sequence-dependent manner. Analytical reconstitutions were performed with native or trypsinized (tailless) core histones, and the selected DNA fragments (see Table 1) and products were analyzed on native 5% polyacrylamide gels. The relative amount of nucleosome formed with native (wt) or tailless (tr) histones was determined as described in Experimental Procedures, and the amount by which the tails stabilize nucleosomes formed on each DNA is listed below. The mouse major satellite DNA fragment was used as competitor in all experiments, and the amounts were adjusted to yield experimentally measurable ratios of nucleosomes to free DNA. The expected properties of each DNA fragment are listed below the gel. Mock reconstitutions are shown in lanes p.

histone tail domains. We assume that the stability of histones bound within oligonucleosomal arrays is the sum of two independent, but additive, contributions from intra- and internucleosomal interactions. The intranucleosomal interactions were determined in the previous experiment using purified components and short competitor DNAs. By comparing the differential nucleosome formation between trypsinized and normal (wt) long chromatin with that determined when free histones were used for nucleosome formation (Figure 2), the energetic contributions of the histone tails to "internucleosome" stabilization in the oligonucleosomal chromatin filament could be determined. As a control, we performed a titration series varying the amount of long histone donor chromatin to a constant amount of DNA probe (Figure 3). To rule out sequence-dependent variations, we used a number of different DNA fragments as in the previous experiment. The average $\Delta(\Delta G)_{\text{experiment}}$ for all sequences was found to be approximately -800 cal/mol. A similar experiment in which the amount of fragment was titrated to a constant amount of chromatin yielded the same relative free energy differences (data not shown). Note that these data should be viewed as the "unwillingness" to transfer histone octamers from the oligonucleosome filament to the acceptor DNAs to form mononucleosomes. Combining the free energies of formation from the two experiments (Table 2) yields an additional sequence-independent free energy contribution of the tail domains in nucleosome stability within the context of an oligonucleosomal array that is not present in isolated nucleosomes of about $\Delta(\Delta G) = -1$ kcal/mol. (This result is in contrast to the large range of $\Delta(\Delta G)$'s measured in Figure 2.)

Sequence-Independent Facilitation of Nucleosomal DNA Wrapping by Acetylation of the Core Histone Tails. To determine to what extent acetylation of the histone tails affects wrapping of our various DNA sequences about the histone octamer, competitive reconstitutions were performed with hyperacetylated and normal histones (Figure 4). Sur-

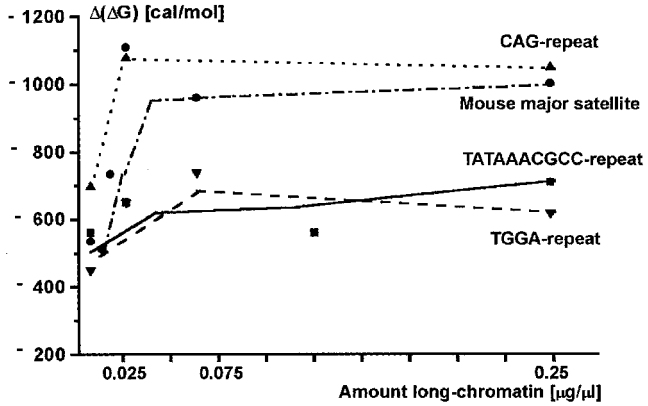

FIGURE 3: Differential histone donation from long chromatin and nucleosome cores depends on the histone tail domains. Analytical reconstitutions were performed with trypsinized and normal long chromatin as histone donor, and the extent of nucleosome formation was compared to reconstitutions performed with identical amounts of free histones and mononucleosome-sized DNAs. The difference between each is expressed as differential free energy $\Delta(\Delta G)$ and is plotted against the amount of donor material. The following DNAs were used as acceptors: CAG repeat (flexible), ▲ and dotted line, $\Delta(\Delta G) = -1050 \pm 200$ cal/mol; mouse major satellite (bent), ● and dotted-dashed line, $\Delta(\Delta G) = -1000 \pm 200$ cal/mol; TATAAACGCC repeat (flexible + bent), ■ and full line, $\Delta(\Delta G) = -650 \pm 150$; TGGA repeat (unflex + rigid), ▼ and dashed line, $\Delta(\Delta G) = -650 \pm 100$ cal/mol.

prisingly, we found that acetylation of the full octamer had the same relatively small effect on nucleosome stability for all DNA sequences studied. Moreover, in our assays, acetylation slightly enhanced nucleosome reconstitution ratios for all sequences, yielding a $\Delta(\Delta G) \sim -1$ kcal/mol relative to normal (wt) or, in terms of relative free energy, a 5-fold increase. Nucleosomes reconstituted with hyperacetylated $(H3/H4)_2$ tetramer and normal H2A/H2B dimers showed about a 2-fold stabilization ($\Delta(\Delta G) = -400$ cal/mol) relative to normal reference octamer. Thus, acetylation does not discriminate between different sequence-dependent proper-

Table 2: Resulting Folding Interactions Exerted by the Tail Domains Deduced from Oligo- and Intranucleosomal Reconstitution Experiments and Their Hypothetical Reactions

fragment	oligonucleosome[a]	intranucleosome[b]	folding interactions[c]
TATAAACGCC repeat	−650 ± 150	+250 ± 50	−900 ± 200
CAG repeat	−1050 ± 200	0 ± 100	−1050 ± 300
mouse major satellite	−1000 ± 200	+250 ± 50	−1250 ± 250
TGGA repeat	−650 ± 100	+900 ± 300	−1550 ± 400

[a] Oligonucleosomal interactions (Figure 3): chromatin + DNA ⇌ mononucleosomes + long DNA. [b] Intranucleosomal interactions (Figure 2): histone octamer + DNA ⇌ mononucleosomes. [c] Resulting folding interactions [A−B]: chromatin ⇌ histone octamer + long DNA.

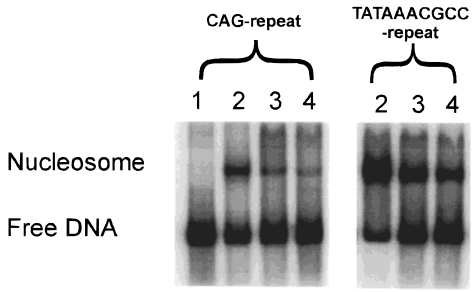

FIGURE 4: Reconstitution of nucleosomes with hyperacetylated and normal (wt) histones. Shown is an EMSA of reconstituted nucleosomes. Sample lanes are as follows: 1, free probe; 2, hyperacetylated octamer; 3, hyperacetylated tetramer and normal (wt) dimers; 4, normal (wt) octamer, respectively. In the experiment shown, flexible and bent DNA (TATAAACGCC repeat DNA) and flexible DNA (CAG repeat) were used. The differential free energy of binding between hyperacetylated and normal (wt) histones for the nucleosome and tetramer−DNA complexes is $\Delta(\Delta G) = -950$ and −400 cal/mol, respectively.

ties, in clear contrast to the results with trypsinized histones. As a control for these results, we used hyperacetylated and normal (wt) chromatin from HeLa cells. We found that when hyperacetylated chromatin was used as a donor, nucleosome stability was increased by about $\Delta(\Delta G) = -700$ cal/mol as compared to unacetylated chromatin (data not shown).

DISCUSSION

DNA Wrapping in the Nucleosome. Upon wrapping into nucleosomes, most all DNA sequences are constrained to adopt very similar conformations due to the precise histone−DNA contacts made with the histone-fold domains within the histone octamer (*31, 33*). From our data, we can conclude that the histone dimers facilitate wrapping of nucleosomal DNA about 4-fold. It is interesting in this aspect to note that the tetramer alone directs sequence-dependent positioning on the particular stretch of DNA (*5, 6*) by contacting the central 60 bp of DNA within the nucleosome (*33*). This is likely due to the altered path and helical periodicity of this DNA as it passes through the nucleosomal dyad (*28, 33, 46*). The H2A/H2B dimers are responsible for the contacts made outside of the central 60 bp around the dyad, and our data indicate that the role of the dimers is to complete nucleosomal wrapping regardless of sequence determinants. Noteworthy, the dimers are found to exchange rapidly between complexes (*47*), and this offers a window of opportunity for transcription factors to bind (*7*−*9*). Furthermore, it has been shown that association of H2A/H2B dimers generates a structure specifically recognized by linker histones (*48*).

Histone Tail Domains Mediate Wrapping of Nucleosomal DNA. The histone tails stabilize the wrapping of DNA in a nucleosome (*16*). Interestingly, our data indicate that the extent of this stabilization is sequence-dependent. In general, we find that reconstitution of flexible DNA into nucleosomes is relatively unaffected by the absence of the tail domains. However, we find the tail domains stabilize formation of nucleosomes on rigid, intrinsically bent DNA sequences by 250 cal/mol and rigid but straight DNAs by 900 cal/mol. It is possible that the tail domains interact with DNA and facilitate wrapping of DNA within the nucleosome in a DNA sequence-dependent manner since cross-linking experiments with individual histones have shown that the tail domains contact the DNA at precise locations (*49, 50*). Alternatively, the tail domains are likely to provide neutralization of DNA charge, thereby making the rigid DNAs behave like the flexible sequences with regard to nucleosomal wrapping. This neutralization may occur in an asymmetric fashion once the DNA is oriented during nucleosome assembly. Conversely, the effect of tail-dependent charge neutralization would be minimal for flexible sequences that readily conform to the nucleosomal path.

Oligonucleosomal Interactions. In oligonucleosomal arrays, the tails mediate cation-dependent nucleosome−nucleosome interactions and are essential for folding of the array into a compact fiber (*17, 18*). Our results suggest that nucleosomes within long arrays exhibit greater stability than mononucleosomes (Table 2). Specifically, equivalent masses of H1-depleted oligonucleosomes and mononucleosomes were observed to exchange histones differentially with the acceptor DNAs. This effect did not depend on the sequence of the acceptor but was dependent upon the presence of the histone tails (even though some influence of sequence was noted). We assume that at the level of salt in which free exchange takes place additional nucleosome−nucleosome interactions serve to stabilize histone binding within the oligonucleosome arrays. Since it is difficult to determine exactly at what stage of the reconstitution free exchange ceases to take place, we do not know precisely the conformation of the oligonucleosomal fiber relevant to these results. However, it is likely that these oligonucleosomal interactions are relevant to histone transactions that occur within the chromatin fiber at physiological salt. The tails mediate nucleosome−nucleosome interactions with regard to histone exchange to a free energy of −1 kcal/mol (Table 2). This value is an approximation of the interactions since different degrees of folding of the chromatin filament could affect and possibly invoke different degrees of nucleosome−nucleosome interactions. Recently, experiments using laser tweezers pulling single chromatin fibers have been used to measure a higher-order internucleosomal stabilization of about −2.0 kcal/mol in the filament (*51*). This value was obtained regardless of any modifications of the tail domains and thus, taking in account data presented here, implies that

the majority of the stabilization energy is mediated by the core histone tails. Interestingly, we note that the internucleosomal stabilization energy is in the same order as sequence-dependent nucleosome positioning and thus is expected to be significant in vivo. Furthermore, our results support the view that the tail domains perform several separate and distinguishable functions within chromatin (*52*).

The small sequence dependence we note in the extent of exchange from oligonucleosomal arrays as compared to mononucleosome donors is possibly due to the different affinities of the acceptor fragments for histone proteins. In theory, the efficiency of exchange from these two donors should not be dependent upon acceptor sequence. However, it is possible that a small amount of transfer occurs from the ends of the donor chromatin fragments, which is more apparent for DNAs with stronger affinities for histones. This would have the effect of reducing the apparent difference between long chromatin and mononucleosome donors.

Since the oligonucleosomal interactions in the condensed chromatin fiber are believed to influence nucleosome positions in arrays (*53*), it is interesting to compare the effects we have measured in regard to nucleosome positioning power of particular sequences. For example, the defined strong natural somatic 5S gene has a nucleosome positioning power of about −1.7 kcal/mol relative to average nucleosomal DNA (*54*). The herein reported free energy of −1 kcal/mol from oligonucleosomal interactions would then give a lower limit for nucleosome positioning sequences on the chromatin level to ensure nucleosome phasing.

Sequence-Independent Effects of Acetylation on Nucleosome Stability. It was early recognized that acetylation is involved in transcription activation (*55*) and renders chromatin more accessible for nucleases (*56*). Recent studies have emphasized the role of acetylation in relieving DNA constraints within the nucleosome and in destabilization of the condensed chromatin fiber (*19*, *24*, *57*−*59*). However, our results suggest that acetylation does not distinguish between DNA sequence properties in its effects on nucleosome stability. Thus, with regard to nucleosome stability, acetylation of the tails does not simply recapitulate the effect of wholesale tail removal by limited trypsin proteolysis (Figure 4). This result has significance for many studies in which the removal of the core histone tail domains is used as a crude approximation of the effects of histone acetylation. These results support the view that the effects of acetylation are derived from a defined change in the specific structures and conformations adopted by the tail domains rather than a general charge-neutralization effect (*14*, *50*).

Interestingly, we find that acetylation actually resulted in a small increase of nucleosome stability for all sequences tested by about −1 kcal/mol as compared to the unacetylated state (Figure 2). The finding that acetylation has a slightly stabilizing effect on nucleosomal DNA wrapping was unexpected. The basis for this effect may be related to the observation that acetylation reduces the amount of negative DNA supercoils constrained within the nucleosome by about 20% (*60*). Since the helical periodicity of DNA within the nucleosome is not altered upon acetylation, it has been proposed that the reduction in supercoil density might be due to an opening of the entry−exit angle of nucleosomal DNA and thus a reduction in overall DNA writhe (*32*). This would reduce the energetic cost of DNA bending within the acetylated nucleosome. A more open exit−entry angle of nucleosomal DNA would also reduce the approach of the two repulsive negatively charged DNA ends and thus may increase the overall stability of the nucleosome as compared to the unacetylated complex. In this view, the reduction in the free energy cost for DNA conformational change upon nucleosome formation (i.e., less wrapping) more than offsets the loss of a few favorable electrostatic interactions (reduction of charge between acetylated lysines with DNA) causing the acetylated mononucleosome to be overall slightly more stable by approximately −1 kcal/mol over the unacetylated state. Noteworthy, this is of the same order of magnitude as the energies involved in sequence-dependent variations in nucleosome positioning and stability.

An alternative explanation for our results is that histone transfer occurs at a faster rate from the donor chromatin and, perhaps, at lower salt concentrations with acetylated histones than with unacetylated proteins. Thus, exchange may occur under conditions in which the forces defining the stability of the nucleosome are slightly different between the acetylated and the unacetylated complexes. These forces are expected to be quite large, and thus small changes may lead to the differential stability that we measure here. We note that histone hyperacetylation has been shown previously to facilitate nucleosome formation in vitro (*61*, *62*). However, regardless of the basis for this observation, it is clear that acetylation results in relatively minor changes in nucleosome stability that are not dependent upon DNA sequence.

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