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Intrinsic Promoter Nucleosome Stability/Dynamics Variations Support a Novel Targeting Mechanism†

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ABSTRACT: Genomic processes like transcription initiation typically involve the alteration of nucleosome structure, to expose DNA elements for regulatory factor binding. Nucleosome altering/modifying complexes have been identified, but precisely how these complexes find their specific targets remains unclear. We have shown that nucleosomes can exhibit significant DNA sequence-dependent stability and dynamics variations and have suggested that these inherent variations could facilitate nucleosome recognition and thus aid in specific targeting. Here, we confirm an important prediction of the model, namely, that stability and DNA dynamics features can correlate with the transcriptional involvement of specific promoter nucleosomes. A transcriptionally inert Mouse Mammary Tumor Virus promoter-region nucleosome (MMTV-D) has greater inherent stability than and reduced dynamics compared to a nearby nucleosome (MMTV-B) that is the initial target of transcription activation-associated processes on this promoter. MMTV-D stability could help direct activation-associated events to the less stable and more dynamic target, MMTV-B.

Nucleosomes are the basic units of eukaryotic chromosome structure and are ubiquitous on genomic DNA. Their presence on regulatory DNA sequences impedes processes like transcription by restricting the access of factors to essential control elements. Processes that can modify or alter nucleosome structure to relieve this inhibition, such as histone modification and exchange or ATP-dependent remodeling, have been identified, but precisely how these inherently nonselective processes find specific targets within a large nucleosome background remains unclear.

Förster resonance energy transfer (FRET) is a powerful approach for the study of conformational features in biological complexes (*1*). FRET can occur when an excited donor fluorophore lies close (typically 1−5 nm) to an appropriate acceptor fluorophore. A sixth-power dependence on fluorophore separation makes FRET an extremely sensitive monitor of biomolecular conformation. Our laboratories (*2*−*5*) and others (reviewed in refs *2*, *3*, and *5*) have been using FRET approaches in studies of nucleosomes.

Previously, we demonstrated that both intact and H2A/H2B-depleted nucleosomes can exhibit significant, DNA sequence-dependent variation in inherent stability and dynamics features. Such variation could (1) enable specific nucleosomes to undergo functionally relevant structural transitions more easily (lower stability), (2) allow enhanced factor accessibility in specific nucleosomes (greater dynamics), and/or (3) serve as a de facto targeting mechanism, by permitting a nonselective chromatin alteration process to produce enhanced effects in a specific nucleosome(s) within a modified region (*3*, *5*). Any of these traits could facilitate specific recognition and thus aid in the crucial nucleosome targeting process.

This model would predict that individual promoter nucleosomes could show stability and/or dynamics variations that correlate with the functional role of the DNA sequences they cover; promoter nucleosomes on regulatory elements could be less stable and/or more dynamic than nucleosomes on nonfunctional sequences. Here we provide support for that model. A mouse mammary tumor virus (MMTV) promoter nucleosome that covers essential promoter elements in the inactive state and undergoes a crucial structural transition during gene activation is inherently less stable and more dynamic than the nearest (promoter region) nucleosome with no transcriptional involvement.

Positioned nucleosomes cover the inactive MMTV promoter (Figure 1). Hormone-dependent gene activation triggers a chromatin structural transition (vertical arrows, Figure 1) involving the glucocorticoid receptor (GR) and nucleosome B with the gene-proximal portion of C (*6*). This initial event leads to changes in the nucleosome A region and, ultimately, to transcription. Nucleosomes D−F undergo no structural change during gene activation and cover no regulatory elements. Nucleosome organization is known to play a crucial role in MMTV promoter function and in productive GR activity on this promoter (*6*, *7*).

Many of our studies are carried out at subnanomolar concentrations, conditions suggested to cause release of H2A/H2B from 5S and MMTV-B (*2*−*5*), from 601 (*9*), and from MMTV-D (Figure S1 of the Supporting Information) nucleosomes. H2A/H2B-depleted nucleosomes are of general interest because transcription complexes often cause release of H2A/H2B from nucleosomes (*10*−*12*) and may be of particular importance with regard to the MMTV promoter because the activation-associated structural transition of nucleosome B in vivo does not involve complete nucleosome removal and could reflect H2A/H2B loss (*13*). ATP-dependent remodeling preferentially releases H2A/H2B from MMTV nucleosome B versus neighboring nucleosomes (*14*).

MMTV-D (−484 to −644 bp on the promoter), MMTV-B and 5S DNA fragment labeling, reconstitution into nucleosomes, and fluorescence analyses [determination of nucleosome concentrations, single-molecule counting, and fluorescence correlation

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spectroscopy (FCS)] were conducted as described in detail in refs (*2*−*4*) and at http://www.public.asu.edu/~lkelbaus (see also the Supporting Information). Numerous controls indicate that fluorophore emission properties in these constructs are not differentially affected by experimental conditions (see refs *2* and *4*). Also, we study relative differences between complexes, which are unlikely to arise from dye photophysical effects since all are made and studied in the same way.

Inherent Stability. Equilibrium constant values reflect the relative inherent stabilities of these nucleosomal complexes. For intact nucleosomes, K_{eq} values are ordered as follows: MMTV-B < 5S < MMTV-D (Table 1). K_{eq} values for H2A/H2B-depleted nucleosomes ("particles") are ordered as follows: MMTV-B < MMTV-D < 5S. Therefore, both intact and H2A/H2B-depleted MMTV-D nucleosomes are inherently more stable than the corresponding MMTV-B complexes. MMTV-D nucleosomes are also more stable than 5S, one of the most stable natural nucleosomes known. H3/H4 complexes of 5S are exceptionally stable (*15*), which probably accounts for the 5S and MMTV-D nucleosome versus particle stability reversal.

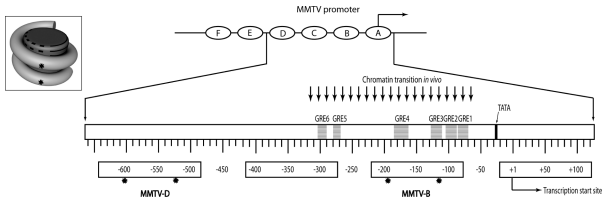

FIGURE 1: Nucleosome organization on the MMTV promoter. The positioned nucleosomes A−F (ovals) and the transcription start site (arrow) are shown at the top; the glucocorticoid receptor (GR) binding sites (patterned areas) and the chromatin region that undergoes the activation-associated transition (vertical arrows) are shown below (based on ref *6*). Stars represent FRET fluorophore locations in the specific MMTV nucleosomes analyzed here. Our studies use ~160 bp DNA fragments labeled with donor (Cy3) and acceptor (Cy5) fluorophores at sites 80 bp apart bracketing the center of the fragment. Reconstitution of this labeled DNA into nucleosomes brings the donor and acceptor into the proximity of one another, via the nucleosomal DNA wrap (inset, at left), allowing efficient energy transfer from Cy3 to Cy5 and a strong FRET signal (*8*). FRET occurs only in canonically wrapped complexes. The dashed and solid lines in the inset represent the regions of H2A/H2B histone-DNA and H3/H4 histone-DNA contact in this part of the nucleosome, respectively. The various nucleosomes are made and analyzed in exactly the same manner; only the DNA sequence varies. This system can monitor nucleosome conformational features at bulk (≥100 nM) and single-molecule (< 1 nM) concentrations, yielding static (time-average) and dynamic (time-dependent) information (*2*−*5*).

Population Distributions. Single-molecule approaches can determine the precise partitioning of molecular conformations in a sample. For nucleosomal particles, these distributions are biphasic (*2*). Molecules fall mainly into a high-FRET (0.8−1.0) efficiency class or a low-FRET (0−0.2) efficiency class; few molecules with intermediate (0.2−0.8) FRET efficiency values are found (cf. Figure 2), even during the salt-induced disruption of high-FRET efficiency particles (*2*). Thus, nucleosomal particles exist mainly in either a high-FRET or low-FRET efficiency conformational state, at least as monitored by these internal, DNA-bound fluorophores. High-FRET efficiency molecules reflect particles in which DNA is strongly associated and canonically wrapped on the histones (mainly the H3/H4 tetramer), thus keeping the fluorophores close enough, on average, for very efficient energy transfer. In low-FRET efficiency particles, the fluorophores are, on average, too far apart for significant energy transfer to occur. Histone-free DNA would also produce low-FRET efficiency molecules, but their contribution is minor in these samples (Figure 2, legend).

In both MMTV-D and 5S distributions, the major peak is at the highest FRET efficiency (> 0.90); thus, there are many very high FRET efficiency molecules. In the MMTV-B population, the major peak is at the lowest FRET efficiency and there are relatively fewer very high FRET efficiency molecules. Thus, MMTV-D (and 5S) populations contain relatively more very stable nucleosomal particles compared to MMTV-B. The MMTV-B distribution resembles that of another transcriptionally labile nucleosome, yeast *GAL10* (*2*).

DNA Dynamics. These H2A/H2B-depleted nucleosomal complexes undergo a reversible conformational transition between the high-FRET (closed) and low-FRET (open) efficiency states (*2*). Fluorescence correlation spectroscopy (FCS) can provide the average time a complex spends in each of the two conformational states, i.e., dynamics (time-dependent) information. MMTV-B complexes reside for longer times in the open than in the closed state (Table 1). In contrast, MMTV-D complexes, like 5S complexes, spend roughly equal time in the open and closed states, and open residence times are much shorter (roughly half as long, on average) than for MMTV-B particles. 5S and MMTV-D open residence times are similar (Table 1).

In the closed (high-FRET efficiency) state, DNA is wrapped around the histones and the fluorophores are close enough for efficient energy transfer; in the open (low-FRET efficiency) state, the fluorophores are too far apart for significant energy transfer to occur. The (reversible) transition between closed and open states probably involves partial dissociation, i.e., spontaneous unwrapping, of DNA from the histone complex (*2*). For

Table 1: Stability and Dynamics Values for Intact and H2A/H2B-Depleted Nucleosomal Complexes[a]

complex	K_{nucl}[b] (relative units)	K_{part}[c] (relative units)	τ_{open}[d, e] (ms)	τ_{closed}[d, e] (ms)
MMTV-B	1.00 ± 0.10	0.69 ± 0.08	58 ± 19	40 ± 13
MMTV-D	1.6 ± 0.2	0.91 ± 0.03	33 ± 11	28 ± 8
5S rDNA	1.20 ± 0.12	1.20 ± 0.14	34 ± 7	41 ± 8

[a] All values are means ± the standard error of the mean. [b] Relative equilibrium constant values for intact nucleosomes (bulk concentration of ~150 nM) and for H2A/H2B-depleted complexes (subnanomolar concentrations of ~0.2 nM), calculated as described in ref *2*. [c] Relative equilibrium constant values for intact nucleosomes (bulk concentration of ~150 nM) and for H2A/H2B-depleted complexes (subnanomolar concentrations of ~0.2 nM), calculated as described in ref (*2*). [d, e] Open and closed residence times, calculated as described in ref *2* for H2A/H2B-depleted complexes (0.2 nM). These dynamics data are consistent with the single-molecule data (Figure 2), namely, more high-FRET efficiency, i.e., dynamically stable, molecules in MMTV-D than in MMTV-B populations. We have conducted these dynamics (FCS) experiments on intact nucleosomes (bulk concentrations), but the results have been highly variable. The cause of this is unclear; it may be technique- or template-associated. For example, dynamics may be more complex in intact nucleosomes and thus not well approximated by a two-state model, as required for this analysis.

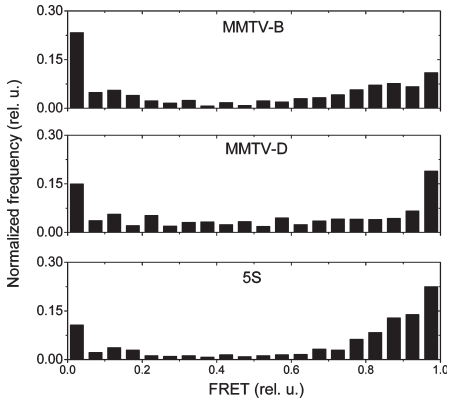

FIGURE 2: Typical single-molecule population distributions. FRET efficiency is measured one molecule at a time for a large number of individual molecules. Results are plotted as FRET (x-axis) vs the fraction of molecules with that FRET value (y-axis) for an MMTV-B sample (top panel), an MMTV-D sample (middle panel), and a 5S rDNA sample (bottom panel), each at 0.2 nM (H2A/H2B depletion conditions). Note that unreconstituted or dissociated DNA molecules, as well as nucleosomal particles in the "open state" (see the text), can yield low FRET efficiency. All three samples have similar (small) amounts of unreconstituted DNA, as shown by gel analysis (data not shown), and dilution to these concentrations does not produce significant DNA dissociation (*2*, *4*). The slightly increased numbers of molecules with intermediate FRET efficiency values in the MMTV-D distribution may reflect weaker positioning and thus greater heterogeneity in fluorophore location than in the (strongly positioning) 5S or MMTV-B DNA or an increased likelihood of undergoing a transition to the open state while in the beam, due to shorter closed state residence times (Table 1). Significant preparation-dependent sample-to-sample variations in the shape of these distributions are not observed ((*2*, *4*); not shown).

example, the average separation of the DNA-bound fluorophores differs by at least 50 Å between closed and open states. This process must involve internal regions of the complex; otherwise, it would not affect the separation of these fluorophores, which lie ~30−40 bp from the termini. DNA sequences should be significantly more exposed and spontaneously more available for factor binding in nucleosomal complexes in the open compared to the closed state. The longer open residence times for MMTV-B complexes mean that MMTV-B sequences spend more time in this exposed state than MMTV-D sequences. Intact nucleosomes probably exhibit similar relative dynamics differences, but we have been unable to test this directly (see the footnotes of Table 1).

In summary, this work shows that MMTV promoter nucleosome B, which covers essential transcriptional control elements and undergoes a crucial structural transition during in vivo transcription activation, is inherently less stable than MMTV nucleosome D, the closest promoter region nucleosome to MMTV-B that is not affected by (or involved in) the transcription initiation process. H2A/H2B-depleted nucleosomal complexes show these same inherent stability differences, and MMTV-B complexes also exhibit enhanced DNA dynamics compared to MMTV-D complexes.

These differences involve intrinsic properties and thus are likely to influence behavior in vivo. For example, studies with yeast suggest that low intrinsic nucleosome stabilities can facilitate the accessibility of promoter regions (*16*). Our approach can directly determine relative intrinsic stability levels. Also, K_{eq} and dynamics values (Table 1) reflect processes operating at all concentrations. Thus, our studies should provide valid information for physiological concentrations and the in vivo state.

Lower inherent stability could enable (intact) MMTV-B nucleosomes to undergo structural transitions, such as the one occurring in vivo during GR-mediated transcription activation, more readily than MMTV-D nucleosomes. This transition may involve H2A/H2B loss, and preferential release of H2A/H2B from MMTV nucleosome B by remodeling complexes has been observed (*14*). The reduced stability of H2A/H2B-depleted MMTV-B versus MMTV-D complexes suggests that depleted MMTV-B complexes could more easily undergo further structural alteration. The dynamics data indicate that the DNA sequences in H2A/H2B-depleted MMTV-B complexes spontaneously spend more time exposed and available for interactions with regulatory factors than the DNA in MMTV-D complexes. The enhanced stability and lower dynamics of MMTV-D suggest that in vivo, this nearby (to MMTV-B) nucleosome could provide a stability and/or dynamics boundary that helps focus factor-mediated events onto the downstream, less stable/more dynamic nucleosome B region, whether MMTV-D is intact or H2A/H2B-depleted.

These function-correlated differences thus support the suggestion (*3*, *5*) that inherent, DNA sequence-dependent, stability/dynamics variations could help facilitate the recognition of crucial nucleosomes and perhaps act as a de facto targeting mechanism to help direct factors to specific nucleosome targets during genomic processes like transcription initiation. Notably, the characteristics of the MMTV-B nucleosome (lower stability, enhanced dynamics) are shared by another labile, promoter element-containing nucleosome we have studied, the TATA-containing nucleosome from the *GAL10* promoter (*2*, *3*). Such features may be characteristic of many of these critical types of nucleosomes, thus enabling stability/dynamics variations to facilitate their recognition.

SUPPORTING INFORMATION AVAILABLE

Figure depicting MMTV-B and MMTV-D FRET concentration dependence and materials and methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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