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Current Opinion in Structural Biology

Poly(dA:dT) tracts: major determinants of nucleosome organization

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Homopolymeric stretches of deoxyadenosine nucleotides (A's) on one strand of double-stranded DNA, referred to as poly(dA:dT) tracts or A-tracts, are overabundant in eukaryotic genomes. They have unusual structural, dynamic, and mechanical properties, and may resist sharp bending. Such unusual material properties, together with their overabundance in eukaryotes, raised the possibility that poly(dA:dT) tracts might function in eukaryotes to influence the organization of nucleosomes at many genomic regions. Recent genome-wide studies strongly confirm these ideas and suggest that these tracts play major roles in chromatin organization and genome function. Here we review what is known about poly(dA:dT) tracts and how they work.

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Current Opinion in Structural Biology 2009, **19**:65–71

This review comes from a themed issue on
Protein-nucleic acid interactions
Edited by Elena Conti and Daniela Rhodes

Available online 7th February 2009

0959-440X/$ – see front matter

DOI 10.1016/j.sbi.2009.01.004

Functional importance of poly(dA:dT) tracts *in vivo*

Poly(dA:dT) tracts — homopolymeric stretches of deoxyadenosine nucleotides (A's), often having lengths of 10–20 bp or even greater — are highly enriched in eukaryotic genomes but, intriguingly, not in prokaryotic genomes [1], suggesting that they may have a functional role unique to eukaryotic genomes. Indeed, studies of many individual genes showed that poly(dA:dT) tracts are important for transcriptional regulation [2••,3••,4–6], recombination [7], and blocking the spread of histone post-translational modifications that are linked to transcriptional repression [8]. An early suggestion, inspired in part by *in vitro* studies described below, was that poly(dA:dT) tracts might function *in vivo* to facilitate gene activation by excluding nucleosomes [2••]. A recent genome-wide analysis showed further that poly(dA:dT) tracts are associated with and may cause nucleosome depletion at promoters, origins of DNA replication, and 3′-ends of genes; that genes whose promoters contain poly(dA:dT) tracts tend to exhibit less transcriptional noise; and that origins of replication that have poly(dA:dT) tracts in their vicinity tend to have a greater likelihood of utilization per round of DNA replication [9••].

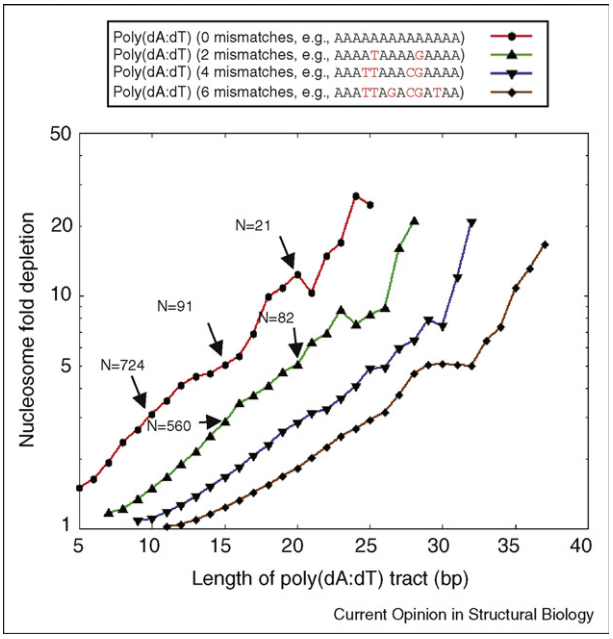
The X-ray crystallographic structure of the nucleosome shows nucleosomal DNA to be highly distorted and sterically occluded, thereby hindering interaction of the nucleosomal DNA with other DNA binding proteins [10]. Thus, nucleosomal organization of DNA may have a generally repressive effect on DNA activity [11]. If nucleosomes were excluded from poly(dA:dT) tracts *in vivo* (and from their vicinity; see below), this nucleosome exclusion would facilitate access of other proteins to the DNA, helping to explain these functional roles of poly(dA:dT) tracts.

Poly(dA:dT) tracts and their flanking DNA are relatively depleted of nucleosomes *in vivo*

The possibility that poly(dA:dT) tracts might function *in vivo* to facilitate gene activation by excluding nucleosomes [2••] focused attention on the nucleosome organization around poly(dA:dT) tracts at many genes [2••,3••,4–7,12–16]. The results of these studies at individual loci were conflicting, in part because some were not carried out quantitatively. Certain assays for nucleosome occupancy can sensitively reveal the presence of nucleosome-free DNA even if a given sequence is in fact wrapped in nucleosomes across most of the cells in the population, while other assays have a converse sensitivity. Consequently, in a real situation, in which a poly(dA:dT) tract is wrapped in nucleosomes in only some fraction of the cells in the population, such analyses could report either nucleosome absence or nucleosome occupancy, depending simply on which kind of experiment was carried out. Thus, the real *in vivo* nucleosome occupancy over poly(dA:dT) tracts remained unclear.

Recently, quantitative genome-wide analyses establish that poly(dA:dT) tracts are, on average, relatively depleted of nucleosomes *in vivo* [9••,17••,18–21]. These studies reveal that nucleosomes are depleted not just over perfect poly(dA:dT) tracts, but also over imperfect tracts containing multiple basepair substitutions or containing clusters of shorter tracts that alternate between strands [9••]. The magnitude of nucleosome depletion increases

Figure 1



Nucleosome are relatively depleted over poly(dA:dT) tracts *in vivo*. Shown is the combined nucleosome fold depletion over all poly(dA:dT) tracts of length *k*, for *k* = 5, 6, 7, . . ., and for tracts with exactly 0, 2, 4, or 6 base substitutions. Each graph is trimmed at length *k* at which there are less than 10 such tracts in the *S. cerevisiae* genome, and the fold depletion at this final point is computed over all elements whose length is at least *k*. The number of underlying elements at various points in the graph is indicated (*N*). Figure adapted from Ref. [9••].

with both the length and the perfection of the poly(-dA:dT) tracts (Figure 1). The fold depletion over a perfect or imperfect poly(dA:dT) tract can be predicted from the sequence itself, and can be surprisingly large. In the yeast genome, there are hundreds of poly(dA:dT) tracts with relative nucleosome depletions of 10-fold or greater [9••].

The nucleosome depletion over poly(dA:dT) tracts extends for considerable distances also into the flanking DNA on both sides of the poly(dA:dT) tract. The depletion is maximal over the poly(dA:dT) tract, but (on average) remains significant over much greater distances, ±100–150 bp (Figure 2), comparable to the length of the nucleosomal DNA itself. This longer range nucleosome excluding behavior arises as a consequence of configurational statistics: there are a smaller number of configurations in which a nucleosome can be located nearby to a nucleosome excluding region, compared to regions that are far from such constraints [22••].

In summary, nucleosomes are, on average, strongly depleted from poly(dA:dT) tracts *in vivo*, and this depletion extends for considerable distances into the flanking DNA. Since nucleosomes occlude their wrapped DNA from interacting with many other proteins, decreased nucleosome occupancy over such an extended DNA region will have the effect of increasing the accessibility of all of the DNA in that region — both the poly(dA:dT) tract itself and its flanking DNA — to other DNA binding proteins. Such enhanced DNA accessibility could explain many of the *in vivo* functions that have been associated with poly(dA:dT) tracts.

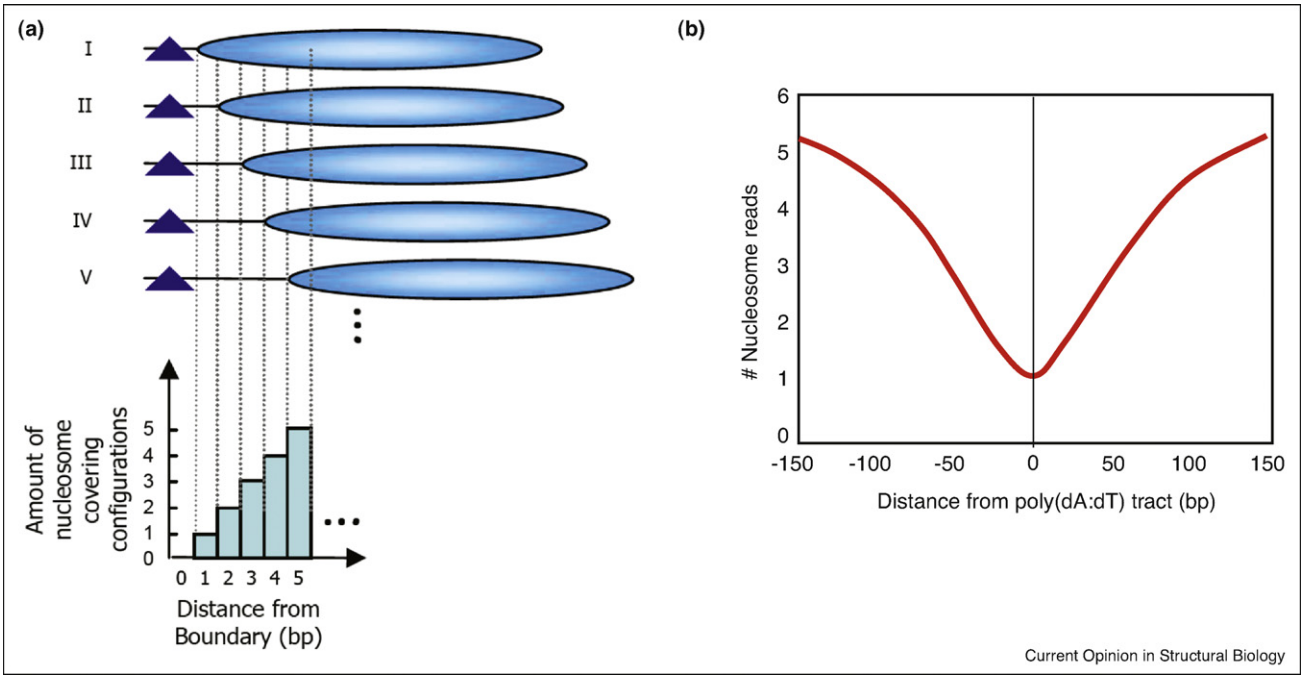
Nucleosome depletion over poly(dA:dT) tracts results chiefly from the tracts' intrinsically lower nucleosome affinity

What causes the dramatic nucleosome depletion over poly(dA:dT) tracts? The simplest hypothesis, that the nucleosome depletion is due to competition with another protein that binds specifically to poly(dA:dT) tracts, is ruled out. To date, a single protein in *Saccharomyces cerevisiae*, called Datin (Dat1p), which recognizes poly(dA:dT) tracts of length 9 bp or greater, has been identified [23]. Datin may be the only DNA binding protein in *S. cerevisiae* that binds poly(dA:dT) tracts, since yeast cell extracts in a Datin deletion do not exhibit any detectable protein binding to poly(dA:dT) tracts. Many of the studies that revealed functional roles for poly(dA:dT) tracts specifically tested the role of Datin binding by deleting the DAT1 gene [3••,5–7,15,23]. However, Datin binding was found to be important for transcriptional activation in only one case [4]. These studies prove that Datin binding is not responsible for the transcription activating function of most poly(dA:dT) tracts or for the nucleosome depletion over the poly(dA:dT) tracts.

Another possibility is that the binding of transcription factors to sites near the poly(dA:dT) tracts causes nucleosome depletion over poly(dA:dT) tracts. Indeed, such an effect is to be expected on thermodynamic grounds [22••]; the question is the relative significance of this effect. If transcription factor binding to sites flanking poly(dA:dT) tracts were a dominant cause of nucleosome depletion over the poly(dA:dT) tracts themselves, then one would expect similar nucleosome depletion over factor binding sites regardless of whether or not they are close to poly(dA:dT) tracts. However, this is not the case: nucleosomes are strongly depleted over factor binding sites that are near poly(dA:dT) tracts, but only weakly depleted over factor sites that are not near poly(dA:dT) tracts [9••]. Conversely, the extent of nucleosome depletion over poly(dA:dT) tracts is similar regardless of whether the poly(dA:dT) tracts are near to binding sites for transcription factors, or not. Thus, binding by transcription factors is not the major cause of nucleosome depletion over poly(dA:dT) tracts *in vivo*.

A remaining alternative is that poly(dA:dT) tracts are relatively nucleosome-depleted *in vivo* because the tracts themselves intrinsically disfavor nucleosome formation. This possibility is supported by both *in vivo* and *in vitro*

Figure 2



Poly(dA:dT) tracts create larger nucleosome-depleted regions. **(a)** Shown is a simple example focusing only on the immediate neighborhood of the boundary. All (five) possible nucleosome configurations are illustrated, in which a nucleosome (cyan ovals) can be placed within 5 bp of the boundary (blue triangle). The number and set of nucleosome configurations occupying each of the 5 bp immediately adjacent to the boundary are shown in the graph below. If all configurations are equally likely, then basepairs closer to the poly(dA:dT) tract will exhibit lower nucleosome occupancy simply because fewer nucleosome configurations cover those basepairs [22••]. **(b)** Schematic representation showing that nucleosome depletion caused by a poly(dA:dT) tract is maximal over the tract itself, but extends for considerable distances in either direction. Thus specific factor DNA binding sites located nearby to a poly(dA:dT) tract will have relatively enhanced accessibility compared to factor sites located far from a poly(dA:dT) tract, facilitating binding of the factor. Panel (a) adapted from Ref. [9••].

studies at specific genes. The most important of the *in vivo* experiments include: in the yeast *HIS3* promoter, a poly(dA:dT) tract, but not a Gal4 protein binding site, can induce transcription by bacteriophage T7 RNA polymerase, suggesting that the poly(dA:dT) tract acts by excluding a repressive nucleosome and not by inducing interactions with the basal transcriptional machinery [14]. At *HIS3*, *RPS28a*, and *BAR1*, replacing the poly(-dA:dT) tract by poly(dC:dG) resulted in similar transcriptional induction, with longer poly(dA:dT) tracts resulting in greater transcriptional induction, consistent with increasing nucleosome exclusion but less-so with a role for a sequence-specific DNA binding protein [3••,5]. One apparent contradictory result suggested that a poly(dA:dT) tract in the *DED1* promoter cannot function only through its nucleosome exclusion effects [24]. However, since that poly(dA:dT) tract has 7 non-A nucleotides (in a 38-bp-long tract), it may well contain a binding site for an additional site-specific DNA binding activity; and in any case, that finding does not contradict a possible additional role for an intrinsic nucleosome disfavoring activity for that imperfect poly(dA:dT) tract *in vivo*. Thus, the consensus of studies of specific genes supports a role for poly(dA:dT) tracts in causing a relative nucleosome depletion *in vivo*, with the resulting nucleosome depletion facilitating the binding of factors to specific DNA target sites.

Similarly, studies *in vitro* establish that poly(dA:dT) tracts intrinsically disfavor nucleosome organization. First, however, contrary to some claims, poly(dA:dT) tracts are *capable* of being incorporated into nucleosomes [25–29]. Any remaining question about this was definitively settled by the determination of a high-resolution X-ray crystallographic structure of a nucleosome containing a 16-bp-long perfect poly(dA:dT) tract [30]. Small structural differences caused by the poly(dA:dT) tract are detectable, but the overall wrapping of the nucleosome DNA is essentially unchanged. It follows that any effects of poly(dA:dT) tracts on nucleosome occupancy or affinity will necessarily be quantitative in nature, not absolute. Only studies that are sensitive to quantitative differences in occupancy or affinity can shed light on such questions; and early such quantitative studies showed that poly(dA:dT) tracts do indeed disfavor nucleosome formation [31,32], with a magnitude that increases with the length of the poly(dA:dT) tract [25,26]. More recent studies confirm that even a relatively short (16 bp)

poly(dA:dT) tract significantly decreases nucleosome affinity [33]; many copies of a 4–6 bp poly(dA:dT) tract greatly reduced affinity [34]; and poly(dA:dT)-tract-containing DNAs present in several different yeast promoters disfavor nucleosome incorporation [9••] by an amount comparable to that of other non-natural DNAs that were selected *in vitro* for their ability to resist nucleosome formation [35]. These findings were strongly confirmed and extended in a recent genome-wide analysis [36••]: the distribution of nucleosomes reconstituted on genomic DNA *in vitro* closely resembled the *in vivo* nucleosome distribution, with significant depletion of nucleosomes from over poly(dA:dT) tracts.

In summary, nucleosomes are on average significantly depleted from poly(dA:dT) tracts *in vivo*. This depletion in most cases is not because of competition with Datin binding specifically to the poly(dA:dT) tracts; and, while competition with other proteins binding to specific target sites located nearby the poly(dA:dT) tracts can contribute to the observed nucleosome depletion, this is not the dominant cause. Instead, the observed nucleosome depletion is due chiefly to nucleosomes intrinsically disfavoring occupancy over the poly(dA:dT) tracts; and this disfavoring is quantitative, not absolute.

Poly(dA:dT) tracts have unusual structural and dynamic properties

At the level of detailed molecular structure and mechanics, why is it that nucleosomes intrinsically disfavor wrapping poly(dA:dT) tracts relative to most other DNA sequences? The answer is not known definitively; but a growing body of studies points to a unique cooperative structure of poly(dA:dT) tracts, which in turn is associated with, and possibly owing to, a unique hydration structure of the poly(dA:dT) tracts. Deforming this unique poly(dA:dT) tract structure by forcing it into a nucleosome conformation may be much more energetically costly than are comparable deformations of generic DNA sequences.

An early hypothesis was that AA dinucleotide steps might be intrinsically stiff compared to other dinucleotide steps, and thus poly(dA:dT) tracts might have an exaggerated stiffness, maximally disfavoring the deformations required for nucleosome formation. However, analyses of newer, larger, databases of X-ray crystallographic structures of DNA and protein–DNA complexes, in which the variance among configurations in independent structures may serve as a proxy for basepair step flexibility [37], and recent molecular mechanics calculations [38,39], do not support a high intrinsic stiffness of the AA dinucleotide step. Thus, any intrinsic resistance of poly(dA:dT) tracts to nucleosome formation is not attributable to special mechanics of AA dinucleotides.

Several lines of evidence suggest instead that the structural, dynamic, and mechanical properties of poly(dA:dT) tracts may differ fundamentally from the corresponding properties of individual AA dinucleotides. Compared to generic sequence DNA, poly(dA:dT) has a shorter helical repeat [40,41], a narrow minor groove, a distinct spine of hydration within the minor grove, and maximal overlap of the bases separately within each strand [42,43]. The crystallographic studies [42,43] further suggested that poly(dA:dT) tracts also exhibit an unusual hydrogen bonding pattern (bifurcated H-bonds), in which amino groups on A bases formed hydrogen bonds both with their Watson–Crick partner and also with the O4 atom of an adjacent T base on the opposite strand. Such cross-strand H-bonds could potentially stiffen the DNA; however, subsequent higher resolution studies show the shortest (presumably, tightest) such bonds to be at the long limit for a significant H-bond [44••], so whether such bonds truly exist, and how much they might contribute to special properties of poly(dA:dT) tracts, is unclear.

Moreover, the unusual structural properties of poly(dA:dT) grow in cooperatively with length of the poly(dA:dT) tract, and are accompanied by unusual dynamic properties. Hydroxyl radical footprinting studies reveal a progressive decrease in reactivity with increasing distance inside poly(dA:dT) tracts, for tracts of length 4 bp or greater (also for A_2T_2), implying the existence of a distinct, length-dependent, structural state for the poly(dA:dT) tract [45]. The detailed cleavage pattern further suggested that the minor groove width decreased progressively with distance inside the poly(dA:dT) tract, a conclusion that is strongly upheld in atomic resolution crystallographic structures of A_2T_2-containing and A_3T_3-containing DNAs [44••,46,47]. Correspondingly, NMR measurements of imino proton exchange rates reveal extraordinarily long basepair lifetimes (high lifetimes) for T residues located 4 or more nucleotides inside a poly(dA:dT) tract of length 4 bp or greater (again, also for A_2T_2 and A_3T_3 tracts), with the basepair lifetimes increasing with depth inside the tract [48–50]. These results imply that not only do the poly(dA:dT) tracts possess unusual length-dependent structures, but also these structures have corresponding unusual dynamics, which could well translate into unusual mechanical properties — including, potentially, into a relatively great resistance to the bending and twisting deformations that are characteristic of DNA in the nucleosome [10]. Other evidence for cooperative formation of a distinctive DNA structure with increasing length of a poly(dA:dT) tract includes an abrupt change in gel mobility for tracts of length 4 bp or greater [51]; a remarkable cooperative premelting transition in DNAs having several poly(dA:dT) tracts of length 5 bp [52]; and structural discontinuities including local DNA bending within the poly(dA:dT) tract and at the two ends where the tract connects to arbitrary DNA sequence [53,54].

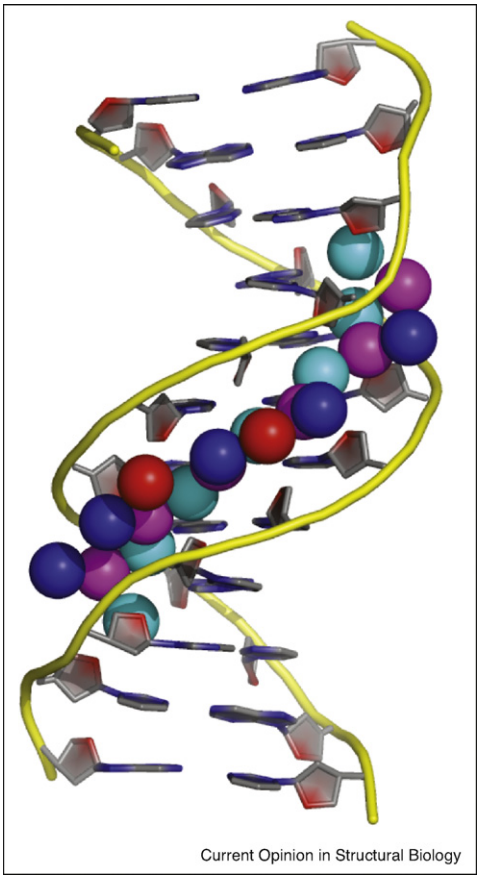
In summary, there is overwhelming evidence from diverse experiments that poly(dA:dT) tracts of lengths

of 4 bp or greater adopt a novel cooperative state whose structures, dynamics, and thermodynamic properties differ fundamentally from those of generic sequence DNA. But why does this state disfavor nucleosome incorporation?

The simplest possibility, mentioned above in connection with the results of NMR studies, is that the unique length-dependent structure of poly(dA:dT) tracts might be uniquely resistant to the deformation(s) required for nucleosome formation. Imposing such deformations on a poly(dA:dT) tract by incorporating it into a nucleosome would then incur a particularly large cost in free energy, producing a nucleosome with an intrinsically reduced stability [30], or, equivalently, causing the nucleosome to preferentially occupy locations on the DNA that exclude the poly(dA:dT) tract, as observed in the many *in vitro* nucleosome reconstitution studies mentioned above.

Structural studies provide further evidence that is suggestive of such a picture. The atomic resolution crystallographic studies of DNAs containing the poly(dA:dT) tracts A_2T_2 and A_3T_3 [44••,46] and NMR solution studies [55] reveal highly ordered spines of hydration, at least four water-layers deep for A_3T_3 (Figure 3), restricted to the narrowed minor groove regions of the tracts themselves. In certain cases specific high occupancy and/or long lifetime-bound cations can also be detected [46,56]. The existence of such highly ordered waters (and localized cations when present) strongly suggests that they must be held in place by favorable energetic interactions. Similarly, the long bound-state lifetimes of these localized waters are analogous to those of water molecules in the interiors of globular proteins, which are integral parts of the proteins' structure [55], further suggestive of a net favorable energetic interaction. The extensive H-bonding of these waters with both DNA and each other, including between the successive water layers, would be expected to contribute to a length-dependent cooperative formation of the poly(dA:dT) tract's special structure.

Figure 3



Narrow minor groove and multilayer spine of hydration in a poly(dA:dT) tract. Shown is a representation of the atomic resolution X-ray crystallographic structure of $[d(CGCAAATTTGCG)]_2$ [44••]. The DNA backbones are shown as yellow curves, with the bases shown in a partial-charge-coded stick representation. The narrow minor groove of the A_3T_3 stretch has many high occupancy water molecules, four layers deep, shown here as spheres, color coded according to their layer from innermost to outermost as cyan, purple, blue, and red. The multiple layers, extensive hydrogen bonding, and high occupancy of these waters all suggest that they may have strongly favorable energetic interactions with themselves and the DNA. This figure was kindly provided by Prof. LD Williams (Georgia Tech.)

The DNA structural deformations required for nucleosome formation could likely disrupt such energetically favorable water–DNA or specific cation–DNA interactions, thereby causing the poly(dA:dT) tracts to have a decreased affinity (less-negative free energy change) for nucleosome formation. Consistent with this view, a model for the sequence-dependent free energy of DNA wrapping in nucleosomes suggests that the curvature-dependent DNA hydration changes coupled to sharp DNA bending plays a significant role in the energetics of nucleosome formation [57].

All of these facts — together with the experimentally proven intrinsic resistance of poly(dA:dT) tracts to nucleosome incorporation *in vitro* — suggest that poly(dA:dT) tracts intrinsically resist the structural deformations required for nucleosome formation, relative to generic DNA sequences. But is this true? Taken at face value, the available literature does not support this conclusion: some studies suggest that poly(dA:dT) tracts are not more resistant to bending and twisting, but less-so, than are other simple sequences [58], while other studies suggest stiffnesses that are within the normal range [59,60]. However, these experiments are somewhat indirect; moreover, they monitor DNA flexibility in situations in which the DNA is rather less distorted than is DNA in nucleosomes. Thus, the requirements of nucleosome organization would greatly exaggerate the effects of what

might otherwise be only small differences in the mechanics of differing DNA sequences, such that the differences are not detectable with presently available methods.

Conclusions

In summary, poly(dA:dT) tracts strongly resist incorporation into nucleosomes *in vitro*, and, if incorporated into nucleosomes, reduce the stability of those nucleosomes. This intrinsic resistance to incorporation into nucleosomes may be because of an intrinsic resistance of the poly(dA:dT) tracts to adopting the substantially distorted structures required by the nucleosome, although this idea remains unproven. Whatever the physical mechanism for their preferential avoidance of nucleosome incorporation, poly(dA:dT) tracts are dominant determinants of the *in vivo* nucleosome organization of eukaryotic genomes, and strongly influence genome function, by controlling the accessibility of other nearby specific DNA target sites to their cognate regulatory proteins. Better ways of analyzing the sequence-dependent mechanical properties of DNA are plainly needed.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.sbi.2009.01.004.

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