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The main role of the sequence-dependent DNA elasticity in determining the free energy of nucleosome formation on telomeric DNAs

Ilaria Filesi[a], Stefano Cacchione[a], Pasquale De Santis[b], Luigi Rossetti[a], Maria Savino[a,*]

[a] *Dipartimento di Genetica e Biologia Molecolare, Fondazione Istituto Pasteur -Fondazione Cenci Bolognetti, Università di Roma 'La Sapienza', Piazzale A. Moro 5, 00185 Roma, Italy*
[b] *Dipartimento di Chimica, Università di Roma 'La Sapienza', Piazzale A. Moro 5, 00185 Roma, Italy*

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Abstract

Using a competitive reconstitution assay, we measured the free energy spent in nucleosome formation of eight telomeric DNAs, differing in sequence and/or in length. The obtained values are in satisfactorily good agreement with those derived from a theoretical model that allows the calculation of the free energy of nucleosome formation on the basis of sequence-dependent DNA elasticity, using a statistical thermodynamic approach. Both theoretical and experimental evaluations show that telomeres are characterized by the highest free energies of nucleosome formation among all the DNA sequences so far studied. The free energy of nucleosome formation varies according to the different telomeric sequences and the length of the fragments. Theoretical analysis and experimental mapping by λ exonuclease show that telomeric nucleosomes occupy multiple positions spaced every telomeric repeat. Sequence-dependent DNA elasticity appears as the main determinant of the stability of telomeric nucleosomes and their multiple translational positioning.

Keywords: Nucleosome; Telomeres; Theoretical prediction; Sequence-dependent DNA elasticity; Competitive reconstitution

* Corresponding author. Tel.: +39-06-49912238; fax: +39-06-4440812.
E-mail address: m.savino@caspur.it (M. Savino)

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1. Introduction

Telomeres, the specialized structures located at the end of eukaryotic chromosomes, generally consist of tandem arrays of 5–8 bp repeated units, characterized by 3–4 consecutive G residues on the 3′-ending strand [1,2]. Although telomeric sequences assume mainly the canonical B-DNA double helical structure, under specific ionic or pH conditions, or by means of specific binding proteins, both G-strands and C-strands can form folded secondary structures, namely G-quadruplexes [3–5] and I-motif structures [6], which have a potential functional relevance.

Recently [7,8] we found that four telomeric DNAs can be considered as the nucleotide sequences which form the highest free energy nucleosomes among the DNA sequences so far studied. The low affinity of telomeric sequences for the histone octamer can be reasonably attributed to the global straightness of telomeric DNAs, because of their period difference with the B-DNA helical repeat. However, the lack of DNA curvature can not explain the free energy differences among the various telomeric sequences, nor the behavior of other repeated sequences with a period different from the B-DNA helical repeat such as the triplet repeats, recently studied by Wolffe and coworkers [9,10]. Although DNAs containing triplet repeats can be considered globally straight as the telomeres, they are able to form highly stable nucleosomes; the authors suggested that this behavior could depend on specific interactions between the histone octamer and DNA at the nucleosome dyad axis. Therefore, sequences characterized by the lack of DNA curvature, such as telomeres and triplet repeats, form respectively the less stable and one of the most stable nucleosomes. A possible explanation of this different behavior could be the ability of the histone octamer to distinguish via specific interactions between the two types of sequences. Alternatively, the role of sequence-dependent intrinsic features, different from DNA curvature, that determine nucleosome stability could be relevant: in particular, sequence-dependent flexibility (namely bending stiffness) could be involved. It is interesting to recall that Chastain et al. [11] showed that DNA fragments containing CTG or CGG triplet repeats, derived from the human myotonic dystrophy gene, migrate up to 20% faster than expected in non denaturing polyacrylamide gels, whereas telomeric DNAs present a normal electrophoretic behavior [8]. Applying a reptation model for electrophoresis, their results are consistent with a 20% increase in persistence length of the DNA, a parameter that characterizes the bending stiffness of DNA [11,12].

According with the nucleosome structure recently obtained by Luger et al. [13], which does not show relevant specific interactions between the histone octamer and the DNA bases, De Santis and coworkers recently proposed a model [14] that, using a statistical thermodynamic approach, evaluates the free energy of nucleosome formation, in terms of sequence-dependent DNA elasticity; this last feature can be evaluated in terms of sequence-dependent DNA twisting, curvature and related flexibility. In the case of G-rich DNAs such as the telomeric sequences, the sequence-dependent DNA twisting should be sensibly influenced by the nucleosome formation. In fact GG, TG and GT dinucleotides have intrinsic twist values which differ more than AA, AT and TA dinucleotides from the average twist of nucleosomal DNA [15].

As for the sequence-dependent DNA flexibility, it is known [16] that there is a correlation between melting temperature and theoretically evaluated stacking energies of base paired dinucleotides in B-DNA geometry. Taking into consideration that base stacking is relevant in determining DNA flexibility, this feature can be correlated with DNA melting temperature experimentally measured or evaluated in terms of dinucleotide contributions. In the case of telomeric DNA the calculated temperature is plausibly near to the real one, since they can be considered as the repetition of equal microdomains.

Here we report the free energy of nucleosome formation of eight telomeric DNAs differing in sequence and/or in length, measured by competitive reconstitution, with respect to a standard sequence. The relevance of DNA length in determining the free energy of nucleosome formation is generally accepted but difficult to measure,

because of the variation of DNA sequence at increasing length. This problem has been overcome by using telomeric repeated sequences; in this type of sequences, ceteris paribus, the increase of DNA length corresponds to the decrease of the free energy of nucleosome formation according to a well predictable behavior.

The free energies of telomeric nucleosome formation, calculated using our theoretical model [14], are in good agreement with the experimental ones. Moreover, we have calculated theoretically the positioning of nucleosomes on telomeric sequences. The predicted nucleosome positions are in good agreement with those experimentally measured by λ exonuclease footprinting, and show that in all telomeres nucleosomes occupy positions spaced every telomere repeat. Both methods indicate that one of the 3–4 consecutive guanines present in the telomeric repeats is preferred at the nucleosome dyad axis of all the telomeric sequences examined.

2. Materials and methods

2.1. DNA fragments

Synthesis, multimerization, and cloning of telomeric sequences were performed as previously described [7]; sequences from human, $(GGGTTA)_{28}$ and $(GGGTTA)_{38}$, *Arabidopsis thaliana* $(GGGTTTA)_{24}$ and $(GGGTTTA)_{30}$, *Tetrahymena thermophila* $(GGGGTT)_{26}$, and *Saccharomyces cerevisiae* $(GGTGTGTG)_{20}$ were cloned into the *Sma*I site of pUC18, whereas telomeric sequences from *Bombyx mori* $(TTAGG)_{30}$, and *Chlamydomonas rheinhardtii* $(TTTTAGGG)_{22}$ were cloned into the *Sma*I site of pBluescript II KS(−). The sequence named TAND-1 was a gift from A. Travers. All the sequences have been checked by dideoxy sequencing. For competitive reconstitution experiments DNAs were cut from the plasmid with *Eco*RI and *Bam*HI, gel-purified, and 3′-labelled by filling in with [α-^{32}P]dATP and Klenow enzyme. For λ exonuclease experiments DNA fragments were radiolabeled only on one strand as previously described [8].

2.2. Competitive reconstitution

The procedure used for competitive reconstitution was that of Shrader and Crothers [17,18] with minor modification. Two micrograms of H1- and H5-depleted polynucleosomes, obtained from chicken erythrocytes [19], was mixed with 30 ng of radiolabeled telomeric DNA and various amounts of competitor DNA (sonicated calf thymus DNA) in 1 M NaCl, 10 mM Tris–HCl (pH 8), 1 mM EDTA (pH 8), 0.1% Nonidet P40, in a final volume of 10 μl. After incubation at room temperature for 30 min, the salt concentration was lowered to 0.1 M NaCl with three additions of 30 μl 10 mM Tris–HCl (pH 8), 1 mM EDTA (pH 8), 0.1% Nonidet P40 (30 min apart, room temperature). Samples were resolved on 5% polyacrylamide gel in 0.5 × TBE buffer (90 mM Tris–Borate, 2 mM EDTA pH 8.3), 0.01% Nonidet P40. The relative quantities of reconstituted and free DNA were assayed by scanning dried gels with Instant Imager (Packard). The free energy for a given telomeric sequence (Tel) was calculated from the equation $\Delta\Delta G(\text{Tel}) = RT \ln[\alpha(\text{TAND-1})] - RT \ln[\alpha(\text{Tel})]$, where α(TAND-1) is the ratio of labeled reconstituted nucleosome to labeled free DNA for the reference sequence TAND-1, and α(Tel) is the analogous ratio for telomeric sequences [8,17]. At least five independent experiments were carried out for each fragment.

2.3. λ Exonuclease footprinting assays

Reconstituted nucleosomes, labeled on the 3′ end, were gradient purified as previously described [8]. The samples were made 2.5 mM $MgCl_2$, and digested with λ Exonuclease (final concentration 25 U/ml) for 20–40 min at 37°C; the reaction was stopped with an equal volume of 20 mM EDTA, 0.2% SDS. Samples were phenol-extracted and analyzed on 6% denaturing polyacrylamide gels, containing 30% formamide to avoid the possible formation of unusual secondary structures. Gels were fixed in 20% methanol, 5% acetic acid, dried, and autoradiographed. Autoradiographs were scanned with a BioRad GS-670 imaging densitometer.

3. Results

3.1. Comparison between experimental and theoretical free energies of nucleosome formation

We have recently shown that four telomeric sequences from *T. thermophyla*, *S. cerevisiae*, human, and *A. thaliana* have a positive free energy of nucleosome formation, when compared to average nucleosomal DNA [7]. If we compare their free energy values to those obtained for a number of different sequences studied in many different laboratories, telomeric sequences result characterized by the highest free energy of nucleosome formation. This feature seems interesting for what concerns telomere behavior in vivo, because it suggests that the telomeric nucleosome could be an intermediate in the organization of telosome [20] since its high energy could dynamically control the association between telomeric DNA and specific telomeric protein factors or alternatively the histone octamer. Taking into consideration that all telomeric DNAs are globally straight, they represent a useful database system to study the influence of different sequence motifs on the free energy of nucleosome formation.

One of the main problems in evaluating the nucleosome formation free energies of different sequences is the choice of the reference DNA. In most studies average nucleosomal DNA has been adopted as the standard DNA. The use of this type of DNA has the advantage of a clear biological meaning; however, it must be considered that its heterogeneity could give rise to a significant error. We have used as standard DNA a unique sequence from the set of 177 nucleosomal sequences cloned by Satchwell et al. [21]. Most sequences in the set should have an affinity for the histone octamer practically equal to that of average nucleosomal DNA [22]; however, we tested by competitive reconstitution some of these nucleosomal sequences [21] to be sure to choose a standard DNA with practically the same free energy of nucleosome formation of average sequence nucleosomal DNA. The sequence of this nucleosomal DNA, that we named TAND-1 (Travers Average Nucleosomal DNA) is reported in Fig. 1. Using TAND-1 as standard, we have measured again the free energy values of nucleosome formation of the four telomeric sequences previously studied [7], together with telomeric sequences from *Bombyx mori*, which has a repeating unit of 5 bp, TTAGG, and from *Chlamydomonas rheinhardtii,* with a repeating unit of 8 bp, TTTTAGGG. The obtained values are in good agreement with those previously reported, with a difference of $\Delta\Delta G$ values of approximately -0.2 kcal/mol of nucleosome (see Table 1). On this basis we decided to quantitatively test the influence of DNA length on the free energies of nucleosome formation.

It is known that the increase of DNA length should diminish the free energy of nucleosome formation since its value obviously depends on the number of positions that the histone octamer can occupy along the DNA. However, in the case of non-repeated sequences the free energy values are strongly influenced by the sequence variation. Telomeres offer the advantage that at increasing length the sequence does not vary and allows quantitative evaluation of the influence of DNA length on the free energy of nucleosome formation.

A typical experiment of competitive reconstitution is shown in Fig. 2; two telomeric DNAs (human and *A. thaliana*) having two different lengths compete for a limited amount of histone octamer. In the experimental conditions adopted, telomeric DNAs do not form unusual structures such as DNA quadruplexes [4]. In addition to the classical experiment of competitive reconstitution [7], we put sequences of different length in the same reaction tube, in order to have a direct competition (Fig. 2, lanes S + L). We obtained the same results in the two different experimental conditions. The free energy values of nucleosome formation with respect to standard DNA are reported in Table 1; increasing the length of telomeric DNAs the free energy of nucleosome formation appreciably decreases.

It is interesting to note that while in the case of the shorter DNA it is difficult to assay the presence of more than one band in the reconstituted sample, a second main band is clearly evident in

Table 1
Comparative free energies in nucleosome formation expressed relative to the DNA sequence TAND-1

N[a]	DNA	$\Delta\Delta G$ (kcal/mol)[b]
1	*Tetrahymena thermophila* telomere (185 bp) GATCCCCGTTGGG(TTGGGG)$_{26}$GTACCGAGCTCGAATT	1.80 ± 0.20
2	*Saccharomyces cerevisiae* telomere (168 bp) AATTCGAGCTCGGTACCCC(TGTGTGGG)$_{18}$GGATC	1.50 ± 0.20
3	*Human* telomere (192 bp) GATCCCCGG(TTAGGG)$_{28}$TACCGAGCTCGAATT	1.20 ± 0.05
4	*Human* telomere (254 bp) GATCCCCGG(TTAGGG)$_{38}$GGTACCGAGCTCGAATT	0.80 ± 0.20
5	*Chlamydomonas reinhardtii* telomere (198 bp) GATCCCCC(TTTTAGGG)$_{22}$GGGCTGCAGGAATT	0.80 ± 0.15
6	*Bombyx mori* telomere (171 bp) GATCCCCC(TTAGG)$_{30}$GGCTGCAGGAATT	0.70 ± 0.20
7	*Arabidopsis thaliana* telomere (195 bp) GATCCCCGG(TTTAGGG)$_{24}$GGGTACCGAGCTCGAATT	0.75 ± 0.25
8	*Arabidopsis thaliana* telomere (236 bp) AATTCGAGCTCGGTACCCGG(TTTAGGG)$_{30}$GGGATC	0.40 ± 0.20
	TAND-1 (159 bp)	0.00
	Mononucleosomal DNA (160–200 bp)	−0.20 ± 0.20
9	(CGG)$_{74}$[c]	−1.66 ± 0.03
10	TG-pentamer[d]	−2.00

[a] The numbers identify the sequences reported in Fig. 3.
[b] Values are given as mean ± S.D.
[c] Data from Godde et al. [10].
[d] Data from Shrader and Crothers [17].

the case of the longer DNAs (see Fig. 2, the two bands C_L1 and C_L2, both in the case of *A. thaliana* and human). We tentatively assign this band, having a higher electrophoretic mobility, to nucleosome terminal positions, as previously found in a number of DNAs [23–25]. This result could be attributed to entropic effects that favor peripheral positioning of nucleosomes. This last factor has been shown to cause changes of the binding constants associated with peripheral or middle site positioning of the CAP protein [26,27]. In the case of long telomeric DNAs, the calculation of the free energy of nucleosome formation was made considering the contribution of the reconstituted nucleosome as the sum of the two bands relative to nucleosomal DNA.

```
1
GATCCCCTTTTTAATTGCTCAGCTCTAGAGGTGAAATGTC

41
TCACAGGATACTTTGGATCACATGAGCTAATAAAGTGCTT

81
CCTGCTAAACTGAGAGGAACAGCCTTACTACCTTTACATT

121
AACTCCTTGCTTTCTGTCTAAAAATGTAGGGAGGGAATT
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Fig. 1. Sequence of the nucleosomal DNA TAND-1.

The values of $\Delta\Delta G$, expressed relative to the sequence TAND-1, for the telomeric sequences examined, as well as for the TG pentamer [17] and the repeated triplet $(CGG)_{74}$ [10], measured in other laboratories, are reported in Table 1. To clarify the respective roles in determining the free energy of nucleosome formation of a global thermodynamic DNA property, namely sequence-dependent DNA elasticity, and of local specific interactions between histone octamer and DNA, we have taken advantage of the theoretical model we recently developed [14].

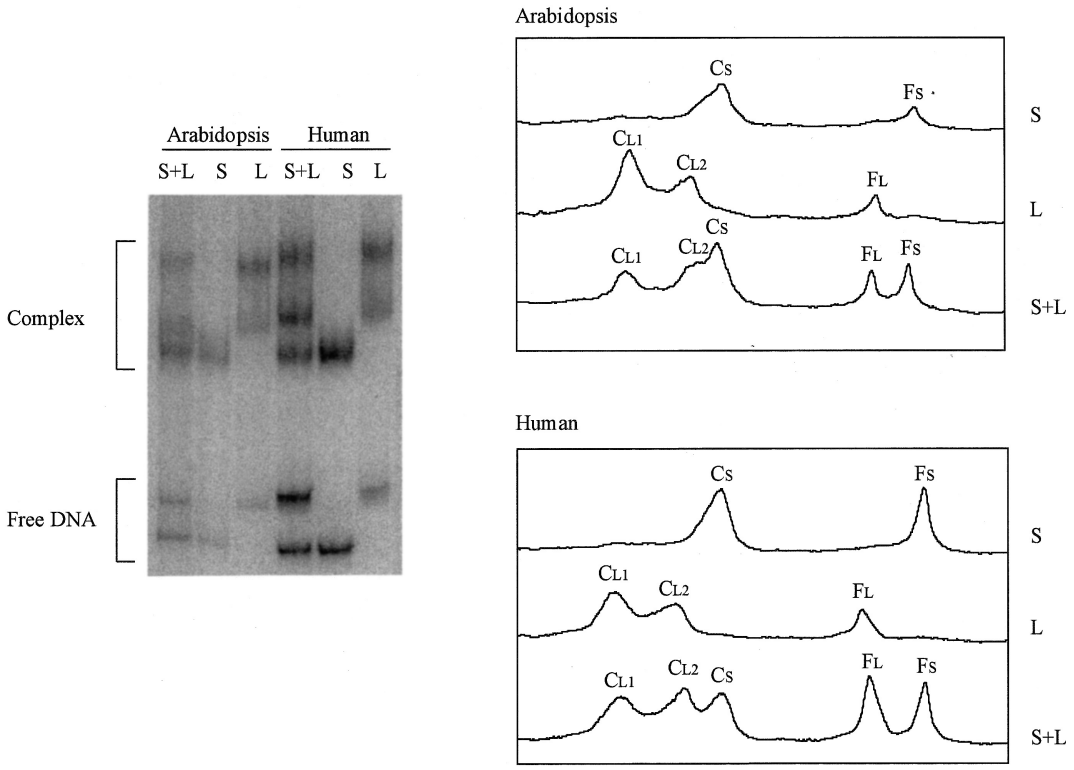

Fig. 2. Left: competitive reconstitution experiment of the *Arabidopsis* and human telomeric DNAs reported in Table 1. In the case of *Arabidopsis* S (short) and L (long) indicate respectively the 195 and 236 bp long fragments, in the case of human, S and L indicate respectively the 192 and 254 bp long fragments. Right: densitometric profiles. C and F indicate respectively the peaks relative to the complexes and to the free DNA.

The outline of the model can be summarized as follows.

The free energy to transform the DNA intrinsic superstructure into a nucleosomal structure has been predicted, by adopting a simple first order elasticity theory. The DNA intrinsic superstructure has been calculated using the same roll and tilt angles, we derived long ago [15] and that successfully worked in predicting gel electrophoretic retardation [28], cyclic permutation assay [29], and circularization equilibria [26,30]. The DNA flexibility has been derived using standard torsional and bending force constants [26,30], multiplied by the empirical normalized melting temperature for each dinucleotide step [31] to represent the differential flexibility along the sequence. This approach derives from the correlation between base stacking and melting temperature and represents at the moment the unique possibility to derive a sequence-dependent DNA flexibility.

If $\Delta G(k)$ represents the nucleosome reconstitution free energy difference from a standard nucleosome of the kth DNA tract of $L = 144$ bp along a sequence, the free energy of nucleosome pertinent to the whole DNA chain, having N bp will be:

$$\Delta G/RT = -\ln \sum_{K=L/2}^{N=L/2} \exp\left(-\Delta G(k)/RT\right) \qquad (1)$$

considering the global reaction as a sum of parallel reactions; for this reason the different equilibrium constants, pertinent to all the possible nucleosome positions, sum up.

Using a statistical thermodynamic approach

[14], we evaluate $\Delta G(k)$.

$$\Delta G(k)/RT = \Delta E^{\circ}(k)/RT - 3/2L \ \ln\langle T/T^{*}\rangle + b\langle T/T^{*}\rangle A_n A_f^{\circ}/(LRT) - \ln \ J_0\left(ib\langle T/T^{*}\rangle A_n A_f^{\circ}/(LRT)\right) \quad (2)$$

where $\Delta E^{\circ}(k)$ is the minimum elasticity energy required to distort the k-th tract, having $L = 144$ bp in the nucleosomal form; b is the apparent isotropic bending force constant; T is the dinucleotide empirical melting temperature as evaluated by Gotoh and Takashira [31] and T^{*} the relative mean value; their ratio modulates the force constants along the sequence producing a sequence-dependent flexibility; A_f° represents the effective curvature, namely the Fourier term of the free DNA curvature function, which coherently contributes to the nucleosomal structure whereas fixing A_n is a sufficient condition to ensure DNA to assume a nucleosome like form. J_0 is the zero order Bessel function.

The curvature function for a defined DNA sequence was calculated using the roll, tilt and twist angles used to satisfactorily calculate other properties dependent from DNA curvature such as gel electrophoresis mobility [28], permutation assay behavior [29], and more recently the circularization propensity [26,30] and writhing transformations [30] of different DNAs. The values of the bending and twisting force constants were derived from a DNA persistence length of 450 Å and a torsional rigidity of 2.1×10^{-19} dyne cm^{-1} [30].

Fig. 3 illustrates the comparison between theoretical values of the free energies of nucleosome formation and the experimental values reported in Table 1. The agreement between theoretical and experimental values is very satisfactory ($R = 0.91$), and the influence of DNA length is quantitatively predicted. The larger differences between theoretical and experimental values for some telomeric DNAs could be attributed to local effects, which should be more relevant in the case of repeated sequences. In this connection, it is interesting to note the deviation of the *Bombyx*

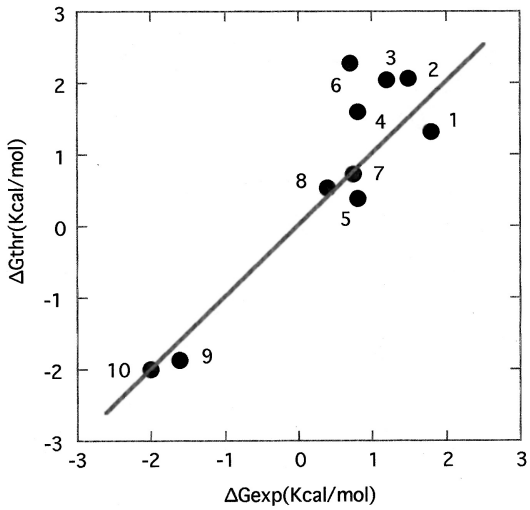

Fig. 3. Comparison between theoretical and experimental free energy of telomeric nucleosomes with respect to the sequence TAND-1. The numbers indicating different sequences are as reported in Table 1.

telomere from the general behavior. The behavior of other telomeric or synthetic sequences with a periodicity of 5 bp surely deserves further investigation.

We also report the experimental values and the theoretical calculations of $\Delta\Delta G$ of $(CGG)_{74}$ repeated triplets and the Crothers TG pentamer that many authors adopted as a reference, with respect to the nucleosomal DNA sequence we used here as standard. It is worth noting that in the case of the relevantly curved TG pentamer it must be taken into account also the contribution of curvature-dependent DNA hydration [14]. The reported results allow us to conclude that our model is capable to explain in terms of sequence-dependent DNA elasticity and length the different behavior characterizing the two sets of globally straight DNAs: telomeres and repeated triplets. Other effects due to local specific interactions between nucleosomal DNA and the histone octamer could only subtly modulate the nucleosome association process.

3.2. Telomeric nucleosome translational positioning

We have previously shown that λ exonuclease

is able to selectively map the borders of telomeric nucleosomes [8]. λ Exonuclease degrades double stranded DNA starting from the 5′ end of the molecule. The rate of digestion of the enzyme is highly processive and independent of DNA base composition [32–34]. In a previous paper we reported that in the case of a 236-bp *Arabidopsis* telomeric DNA, the analysis with λ exonuclease is consistent with a multiple nucleosome translational positioning, characterized by the same telomeric repeat [8]. In the present research we report λ exonuclease footprinting of other telomeres differing in sequence and/or in length.

Our aim is to correlate experimental findings with theoretical predictions of nucleosome positioning, obtained using the recently developed theoretical method [14].

The theoretical model is able to calculate the variation of the free energy along the DNA sequence and thus to localize the minima characterizing the different translational positions of the nucleosome dyad axis. The free energy of nucleosome formation is theoretically obtained summing up the pertinent equilibrium constants of nucleosome formation on all the possible positions [Eq. (1)]. The integral affinity for a given DNA tract should only slightly be influenced by a change in nucleosome distribution due to 'end effects' [24]; it is worth remarking that 'end effects' do not affect multiple nucleosome dyad axis positions, whereas they surely influence the frequency of each position.

The λ exonuclease digestion analyses of the 195 bp long *A. thaliana* telomere, the 171 bp long

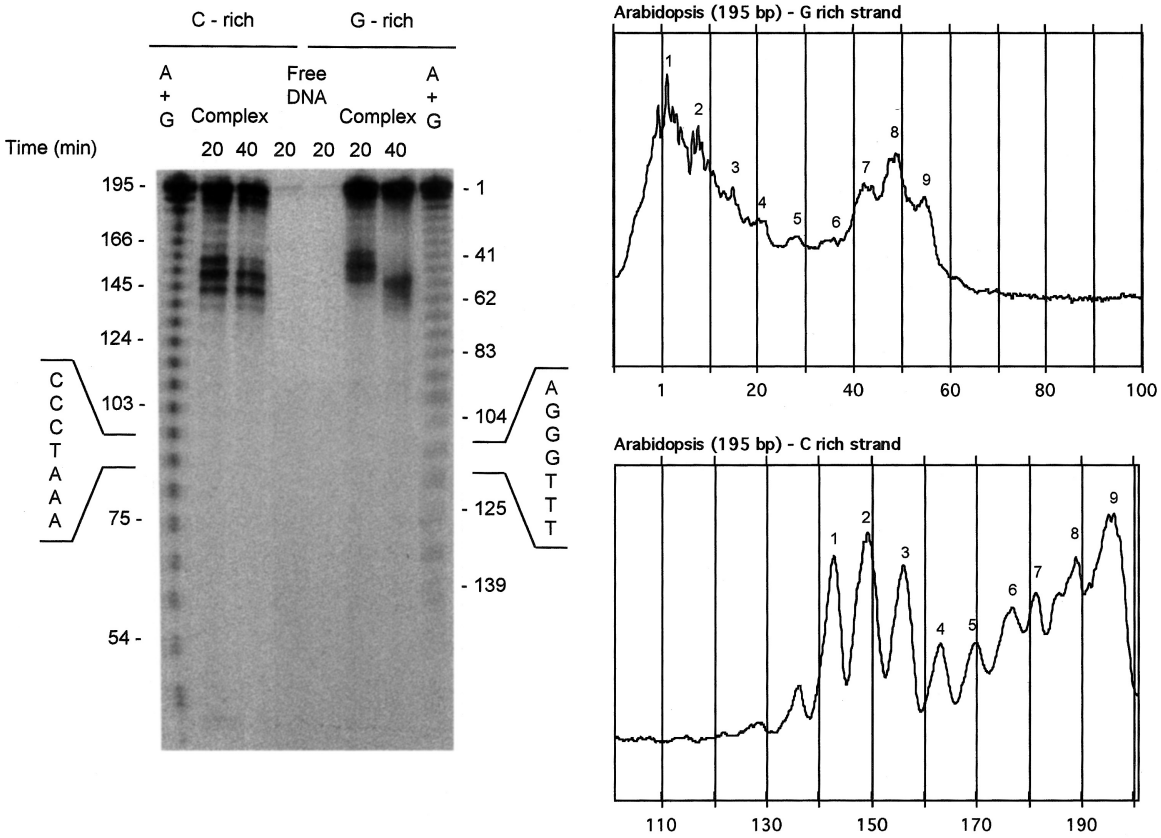

Fig. 4. Left: λ exonuclease footprinting of nucleosomes reconstituted onto a 195 bp *Arabidopsis* telomeric DNA. The sequence has been numbered starting from the 5′-end of the G-rich strand. Right: densitometric profiles. In all cases bands corresponding to the borders of the same nucleosome have been labeled with the same number.

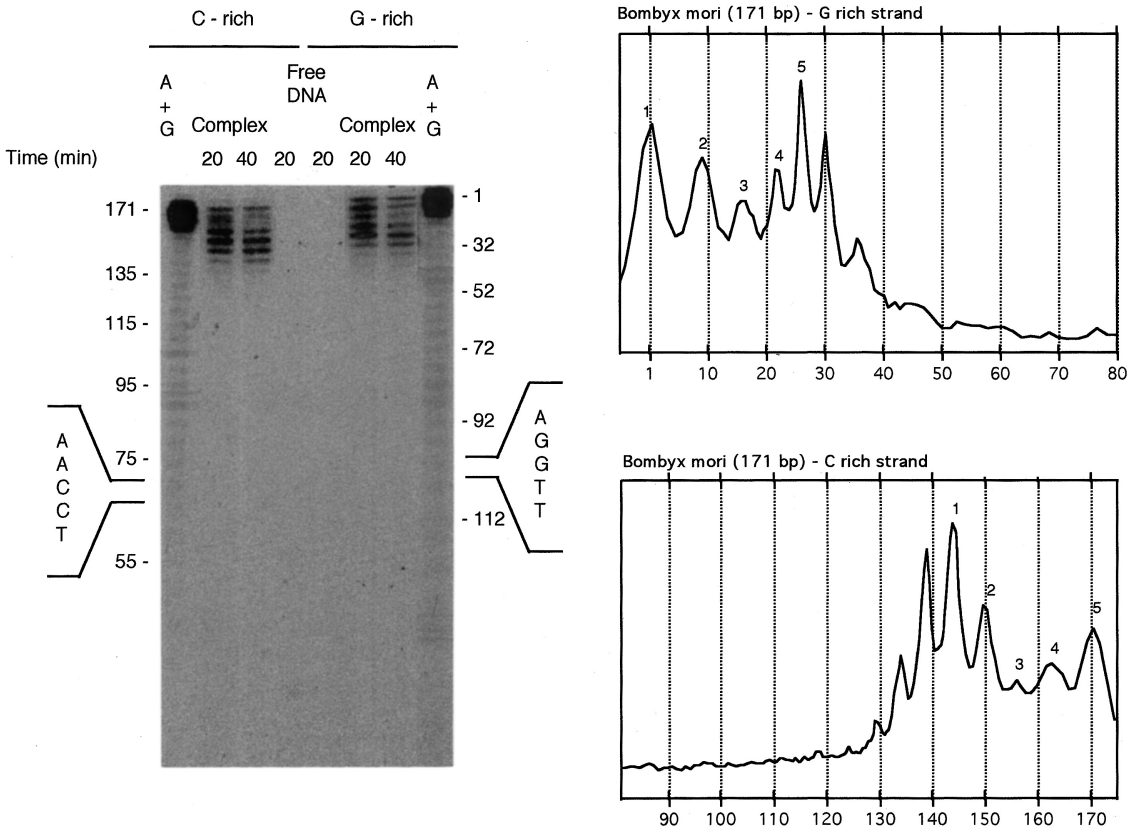

Fig. 5. λ Exonuclease footprinting of nucleosomes reconstituted onto a 171-bp *Bombyx* telomeric DNA (see the legend of Fig. 4 for the description).

Bombyx telomere, the 192 bp long and the 254 bp long human telomere are reported in Figs. 4–7, respectively. The bands correspond to the enzyme stops and identify the nucleosome borders. The borders of the nucleosomes identified on the C-rich strand correspond to those found on the G-rich strand that are 145 ± 2 bp away. These positions have been numbered on the densitometric profiles. In all cases a multiple nucleosome positioning with a periodicity equal to the telomere repeat is found; similar results were previously found in the case of *Arabidopsis* 236 bp sequence [8]. It is worth noting that the nucleosome periodicity appears slightly different at the ends of the sequence, where it is influenced by the linkers having no telomeric sequence (see Table 1). These results indicate that nucleosome multiple positioning depends mainly on the repeat of each telomeric sequence; this behavior can derive from the repetition of a relevant chemical signal or alternatively from the repetition of local curvature and flexibility motifs. The second explanation is consistent with the theoretical model, based on DNA sequence-dependent elasticity [14].

From the λ exonuclease footprintings reported in Figs. 4–7, and from that of the 236 bp *A. thaliana* telomere (see Fig. 6b in [8]) we have derived the positions of the nucleosome dyad axis, reported in Fig. 8, indicated by the arrows. The positions have been calculated for both strands at a distance of 72 bp from the borders identified by λ exonuclease, with an approximation of ± 1 bp. There is substantial agreement between the positions found on the two strands. It is noteworthy that in all the examined telomeres the base of the

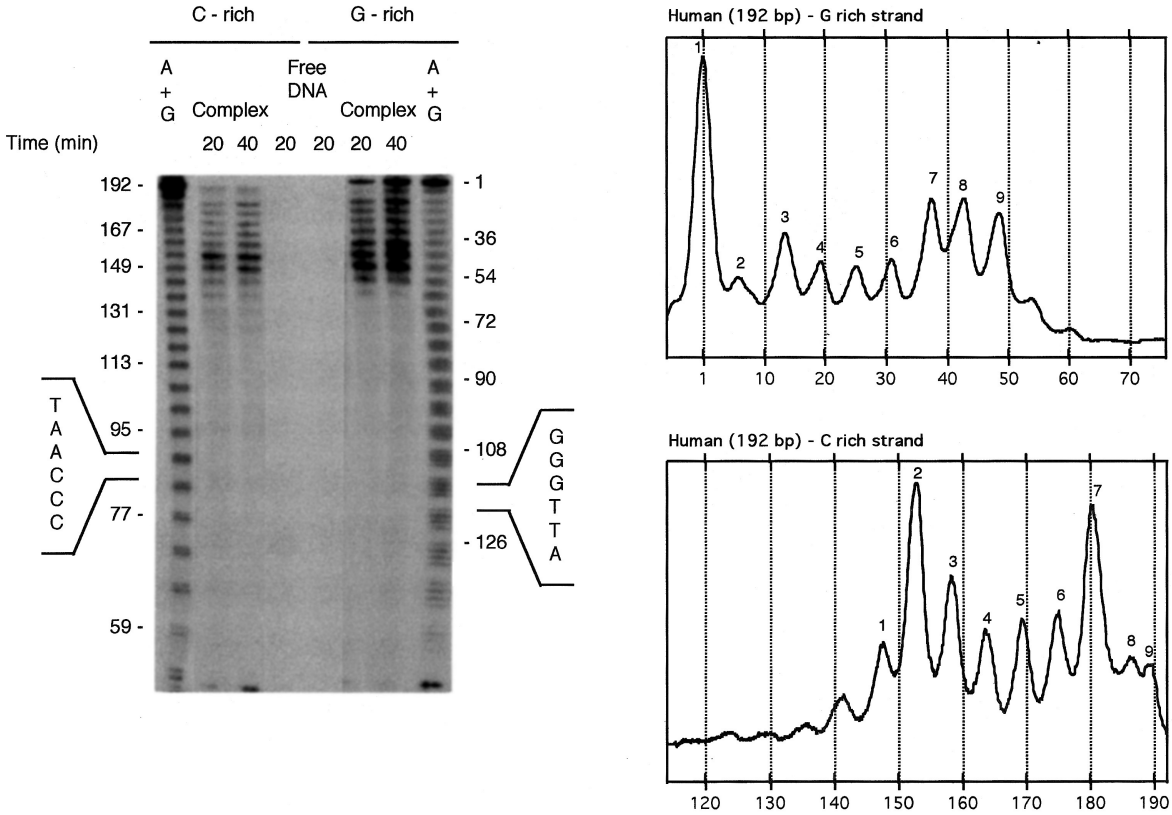

Fig. 6. λ Exonuclease footprinting of nucleosomes reconstituted onto a 192 bp human telomeric DNA (see the legend of Fig. 4 for the description).

G-rich strand situated at the dyad axis is a guanine. This result is in strikingly good agreement with the results obtained by the theoretical analysis, reported in Fig. 9. The theoretical calculations predict, in fact, nucleosome dyad axis multiple positions with a periodicity equal to the telomere repeat; moreover, the base corresponding to the dyad axis is, in all cases, a guanine, as found experimentally. Furthermore, for sake of comparison between theoretical and experimental findings a direct comparison between the nucleosome dyad axis positions deduced from λ exonuclease stops and the theoretical nucleosome dyad axis positions is reported in Fig. 10, with correlation coefficients higher than 0.99.

These results strongly suggest that a statistical thermodynamic approach based on sequence-dependent DNA elasticity is adequate to predict both nucleosome-free energy and nucleosome positioning.

4. Discussion

Nucleosome can be considered as the complex between a highly conserved histone octamer and DNAs, non-constrained by the sequence, present in eukaryotic genomes. As evidenced by Lowary and Widom [22] it is likely that the bulk of the eukaryotic genome has not evolved to favor nucleosome packaging and positioning at the level of the individual nucleosome.

Recently, from a pool of natural or synthetic DNA molecules, DNA sequences both with high and low affinities for the histone octamer have been selected [35–37]. The range of variation of

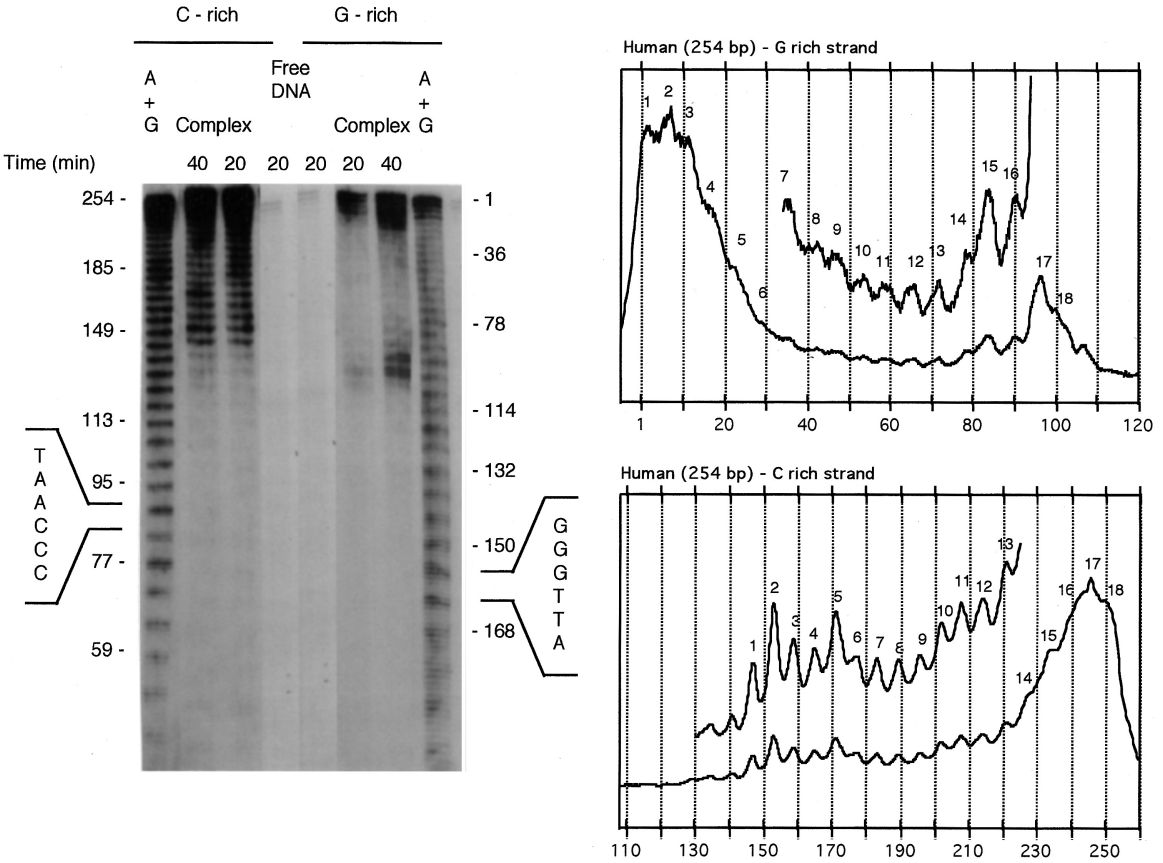

Fig. 7. λ Exonuclease footprinting of nucleosomes reconstituted onto a 254 bp human telomeric DNA (see the legend of Fig. 4 for the description).

the free energies spent in the nucleosome association process is approximately 4 kcal/mol of nucleosome; this rules out extensive specific interactions between the histone octamer and the DNA bases. Furthermore, the Richmond nucleosome structure [13] shows that the interactions between histone octamer and DNA involve mainly the phosphates. These results suggest that the process of association between DNA and the histone octamer should be driven essentially by DNA global properties. Specific interactions must be considered a second order factor, probably relevant only in specific nucleosome structure locations, such as the nucleosome dyad axis and the four kinked sites [13].

The theoretical model adopted, based on a statistical thermodynamic approach, evaluates the role of the sequence-dependent DNA elasticity in organizing nucleosome. This model is based on the concept that DNA stiffness (meant as the contrary of flexibility) can be considered a favorable parameter in the nucleosome association process, mainly for entropic reasons. Namely, a rigid sequence, in comparison with a more flexible one, is characterized by a minor decrease of the entropy in going from free DNA to nucleosome. This is an important factor in the case of sequences with the same curvature, because the actual curvature of the axis of the double helix of B DNA is relevant in determining the free energy of nucleosome formation.

As for the telomeres nucleosome multiple positioning, the finding that at the dyad axis one of the three to four consecutive guanines present in

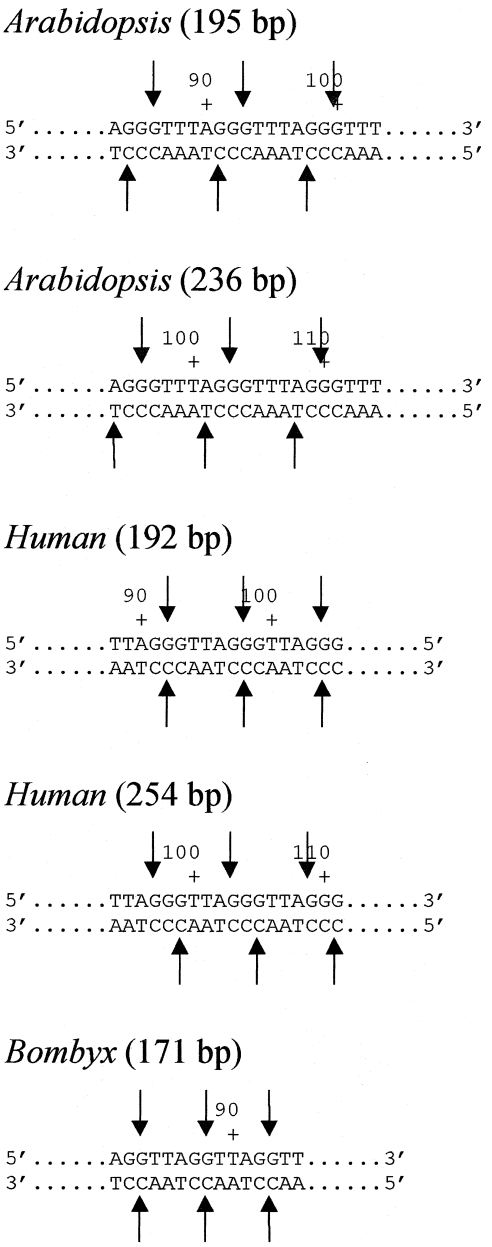

Fig. 8. Nucleosome dyad axis multiple positions derived from the λ exonuclease footprintings. The nucleotide corresponding to the dyad axis on the two DNA strands are indicated by arrows.

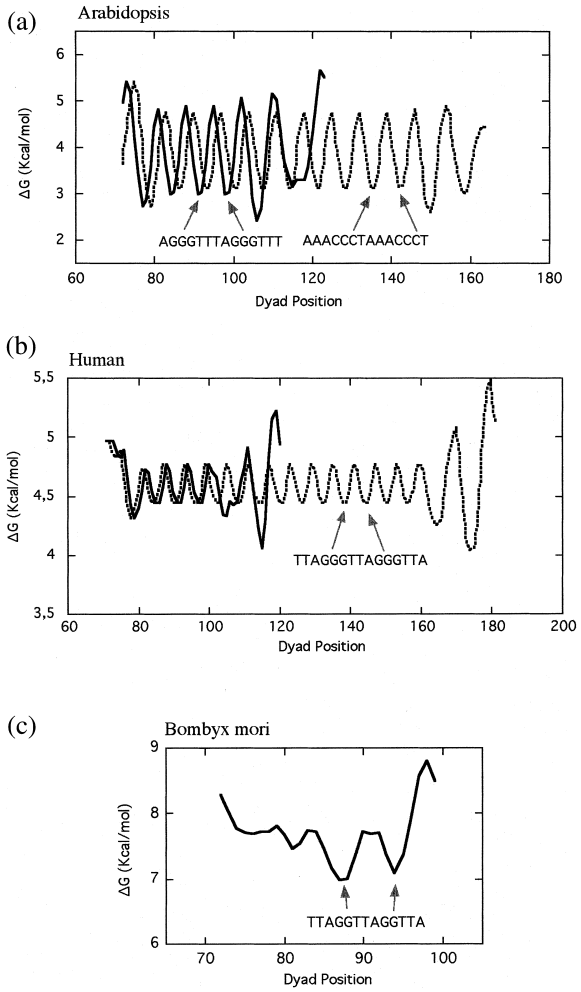

Fig. 9. Theoretical nucleosome dyad axis multiple positioning of (a) the *Arabidopsis* telomeric DNAs 236 bp (dotted line) and 195 bp long (solid line); (b) of the human telomeric DNAs 254 bp (dotted line) and 192 bp long (solid line); and (c) of the *Bombyx* telomeric DNA 171 bp long. In all cases, the arrows indicate the nucleotide corresponding to the positions of the nucleosome dyad axis, characterized by the minima of nucleosome free energy.

the telomeric repeat is preferred, is consistent with the well known rule, derived from the pioneering research of Drew and Travers [38] which locates the nucleosome AT-rich minor groove inside and the GC rich minor groove outside. In fact the Richmond nucleosome structure is characterized by the dyad axis in the major groove, exposed to the histone octamer and thus the G-rich regions present the major groove toward the histone octamer inside and the minor groove exposed to solvent and enzymes such as DNase I outside.

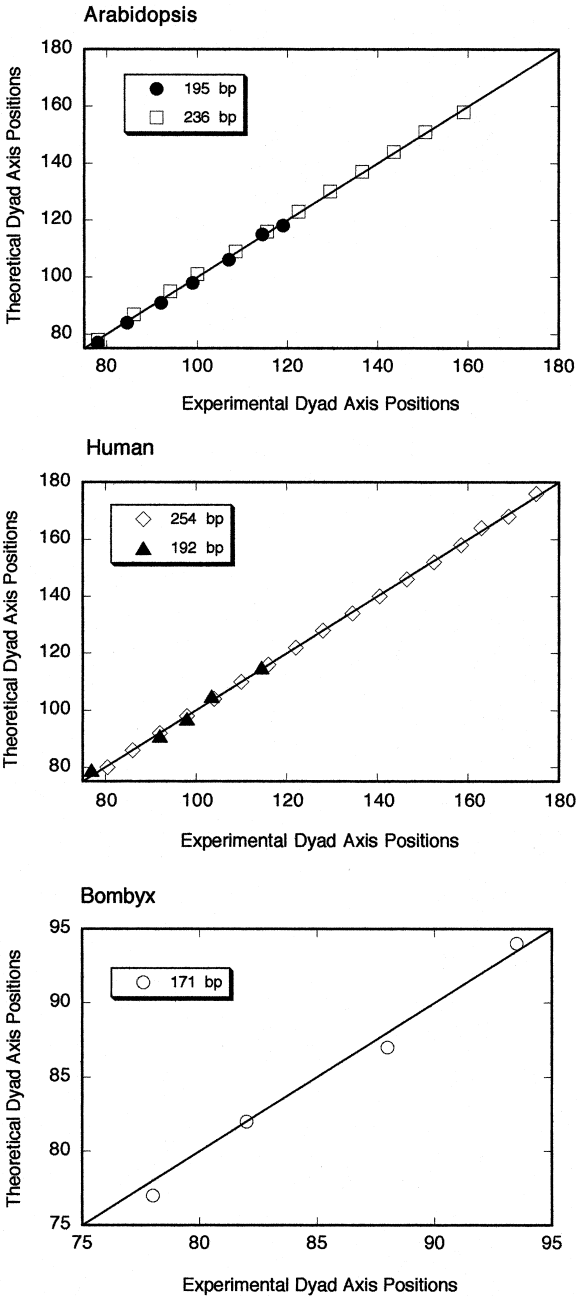

Fig. 10. Comparison between the nucleosome dyad axis positions deduced from λ exonuclease stops and the theoretical nucleosome dyad axis positions.

The high free energy of telomeric nucleosome formation with respect to average DNA sequences and its high mobility, that can be inferred from the multiple isoenergetic positions of nucleosome dyad axis, appear relevant both from the physico-chemical and the biological point of view. In fact, the satisfactorily good correlation between theoretical and experimental free energy of nucleosome formation demonstrates that DNA sequence-dependent elasticity has a main role in determining nucleosome stability, while specific local interactions represent only a second order parameter. The peculiar features of telomeric nucleosome appear relevant in telomeric chromatin organization. In higher eukaryotes, where telomeres are several kilobases long, telomeres are organized in long arrays of tightly spaced nucleosomes [39,40]; on the contrary, in lower eukaryotes nucleosomes seem absent [41] or present only in a fraction of telomeres [20]. As proposed by Cohen and Blackburn with their dynamic model of telomeric chromatin [20], this difference between higher and lower eukaryotes could be quantitative rather than qualitative. The formation of nucleosomes or telomeric specific structures should derive from a competition between histone octamer and specific proteins for telomeric sequences. Since telomere structure and length vary along with the cell cycle and cell divisions [42,43], the low affinity of telomeric sequences for the histone octamer and the high nucleosome mobility should surely favor the dynamic rearrangement of telomeric chromatin.

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