CURVED DNA

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I. INTRODUCTION

For as long as 20 years after the historical paper of Watson and Crick[1] on the DNA structure, this molecule has generally been considered to be a perfectly regular double helix with strictly periodical geometry of interwound sugar-phosphate backbones identical for any sequence of bases forming the stacked core of the molecule.

This structure can be described by two gross parameters originally considered to be constant: (1) the twist angle a base-pair rotates relative to an adjacent one around the molecular axis, equal to 36° in the classical B form of DNA, and (2) the angle between the planes of two adjacent base-pairs, close to zero. The first evidence that there is an influence of base composition on the average twist between adjacent base-pairs came from DNA X-ray fiber diagrams.[2] Recent crystallographic studies[3,4] and solution measurements[5,6] clearly demonstrated that the twist angle in B DNA is nucleotide sequence dependent, varying between 30 and 40° for different combinations of adjacent base-pairs (reviewed in Reference 7).

An indication that the angle between planar base-pairs is not constant but also depends on the neighbors, came from the nucleotide sequence analysis of eukaryotic DNA, which was shown to display a weak periodicity of some dinucleotides along the sequence, with period close to the helical repeat of DNA.[8,9] It was suggested then that this periodicity might reflect unidirectional curving of the DNA molecule due to periodical appearance of non-parallel (wedge-like) combinations of base-pairs along the molecule.[8] Such sequence-dependent curving of DNA would facilitate its wrapping around histone octamers in the nucleosomes. Based on this concept a sequence-directed mapping procedure was developed which allows one to map the predicted nucleosome positions along the DNA molecules,[10,11] in good agreement with the available experimental mapping data. On the other hand, recent solution studies provide more direct evidence that certain DNA fragments indeed happen to be curved, and this curvature correlates with the periodical distribution of some dinucleotides along these molecules.[12,13] These as well as other relevant data are the subject of this review.

In what follows, the term *curved DNA* refers to molecules which are curvilinear rather than straight, without application of any external forces, as opposed to *bent DNA*, forcibly deformed. For example, the molecule could be inherently straight but deformed (bent) by thermal motion, while the inherently curved molecule will always remain curvilinear unless forcibly stretched. Kinks, DNA deformations involving unstacking of base-pairs,[14-17] and bends (forcible DNA bending induced by some proteins bound to DNA)[13,17] are outside the scope of this paper.

II. *A PRIORI* CONSIDERATIONS

In the classical regular B DNA double helix, any two adjacent base-pairs are characterized by the same constant angle between their planes, close to zero. There are ten different

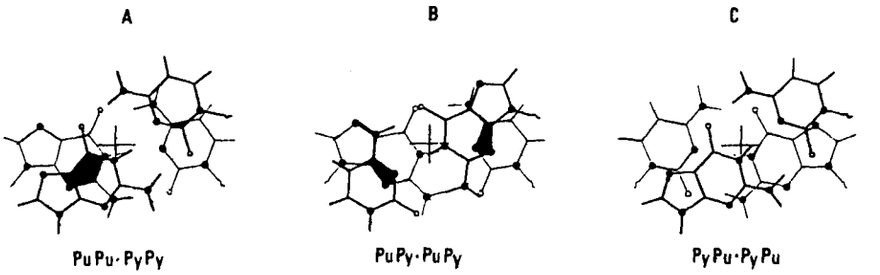


FIGURE 1. Projected views of two successive base-pairs of B DNA. Three possible cases of purine and pyrimidine base overlap are shown. Helix axes (perpendicular to the base-pairs) are indicated by crosses. Overlapping of the heterocyclic rings is shown in black. (From Arnott, S., Dover, S. D., and Wonacott, A. J., *Acta Crystallogr.*, B25, 2192, 1969. With permission.)

combinations of adjacent base-pairs in the natural DNA molecules: (AA) · (TT) [i.e., (5′ApA) · (5′TPT)], (AC) · (GT), (AG) · (CT), (AT) · (AT), (CA) · (TG), (CC) · (GG), (CG) · (CG), (GA) · (TC), (GC) · (GC), and (TA) · (TA). Strictly speaking, the overall geometry of these "minihelices" is identical only in the first approximation. Differing stereochemistries of the bases involved should result in slight differences in the geometry of the minihelices. In particular, one would expect *a priori* that the actual angles between the planes of adjacent base-pairs should be nonidentical, no matter how small the difference is. In other words, the base-pairs should be somewhat nonparallel so that each of these complementary combinations would serve as a sort of a wedge locally inclining the DNA axis in one direction or another, depending on the particular combination of base-pairs.

More specifically, the expected nonparallelness of base-pairs could be caused by the asymmetry of stacking interactions between the base-pairs. This is illustrated by Figure 1 where three typical combinations of the base-pairs are shown in projection onto the plane perpendicular to the DNA axis. For example, due to the significant overlapping of purine heterocycles in the combination (PuPu) · (PyPy) compared to the poor overlapping of pyrimidines (Figure 1, left) the van der Waals distance along the axis between the adjacent purines of one strand of DNA should be somewhat shorter than the distance between the complementary pyrimidines of the other strand. This would result in a certain small wedge angle between these base-pairs pointing towards purines.

It is noteworthy that the twist angles between various base-pairs are all different,[7] apparently because of the same reason — stereochemical nonidentity of various combinations of the base-pairs.

Let us consider a molecule with a random sequence of base-pairs. For this molecule the line connecting the imaginary centers of the slightly nonparallel base-pairs (axial line) will not be straight. Rather, it will appear somewhat broken at every joint between center-to-center segments, making a sort of randomly drifting line.

The nucleotide sequence could be biased in such a way that this axial line would be curved preferentially in one direction. This can be achieved, for example, by placing the same wedge-like combination every ten or so bases (one helical repeat of DNA) along the sequence. As a result these wedges would all point in the same direction, as illustrated schematically in Figure 2. In the general case, the total angle of inclination of the axial line of the DNA molecule in any given direction would be a sum of contributions (positive and negative) of all wedge elements of the molecule towards this direction. Since this would depend on which combinations of base-pairs are involved, the total effect of curving of the DNA axis should be sequence dependent.

One should realize that the above description of the sequence-dependent curving of the DNA molecule is yet another idealization. The base-pairs of DNA are not exactly planar[4]

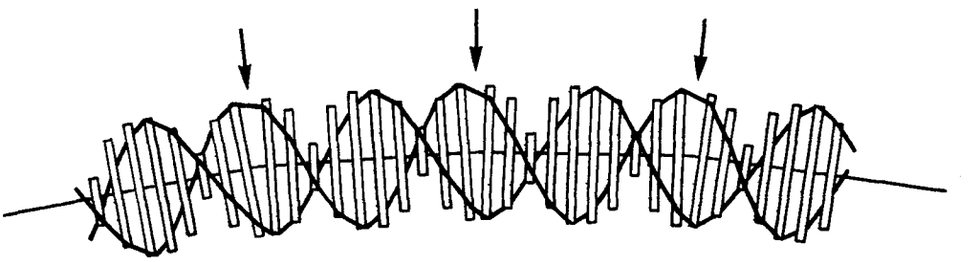


FIGURE 2. Curving of DNA molecule by periodical disposition of nonparallel (wedge-like) combinations of base-pairs (arrows). (From Trifonov, E. N. and Sussman, J. L., *Proc. Natl. Acad. Sci. U.S.A.*, 77, 3816, 1980. With permission.)

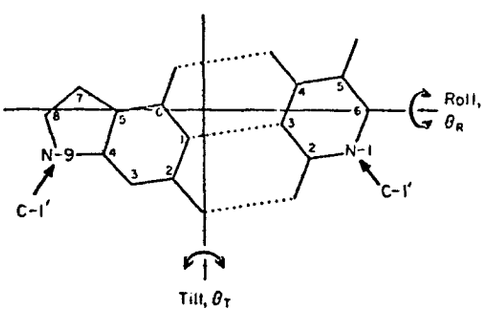


FIGURE 3. Rotational degrees of freedom of planar base-pairs. Tilt Θ_T is the rotation about pseudo-dyad axis passing through the base plane. Roll Θ_R is the rotation about an axis in the plane of the bases perpendicular to the pseudo-dyad. (From Arnott, S., Dover, S. D., and Wonacott, A. J., *Acta Crystallogr.*, B25, 2192, 1969. With permission.)

and exact definition of the wedge angles between the base-pairs should depend on how the best planes of the base-pairs are defined. The base-pairs could be shifted relative to one another in the direction perpendicular to the DNA axis (shearing distortion) and the wedge angles might depend on the next to nearest neighboring base-pairs.[19] On the other hand, the wedge angles are influenced by thermal motion. One actually deals with some mean value of this angle, not necessarily the same for different temperatures, due to the asymmetry of the potential function which characterizes the deformation.[16,20] The simple picture of the curved DNA as described above is a reasonable next approximation to the real DNA structure sufficient for the purpose of this review.

III. WEDGE ANGLES

The wedge angles between adjacent base-pairs can be presented as a sum of two components: (1) the roll angle θ_R, which expands the minor (positive θ_R) or major groove (negative θ_R), and (2) the tilt angle θ_T — in the perpendicular direction, as illustrated in Figure 3 (the notation, as in Reference 4). These angles might depend not only on the nearest base-pairs but on the next neighbors as well. Let us consider here a simple model in which only the immediate neighbors are involved. Then for a full description of the axial line of a DNA molecule, ten roll angles and ten tilt angles for ten different combinations of stacked base-pairs have to be known. Unfortunately, only very rough estimates of these angles are available from the crystallographic data[4,21] and from energy calculations.[22,23] According to

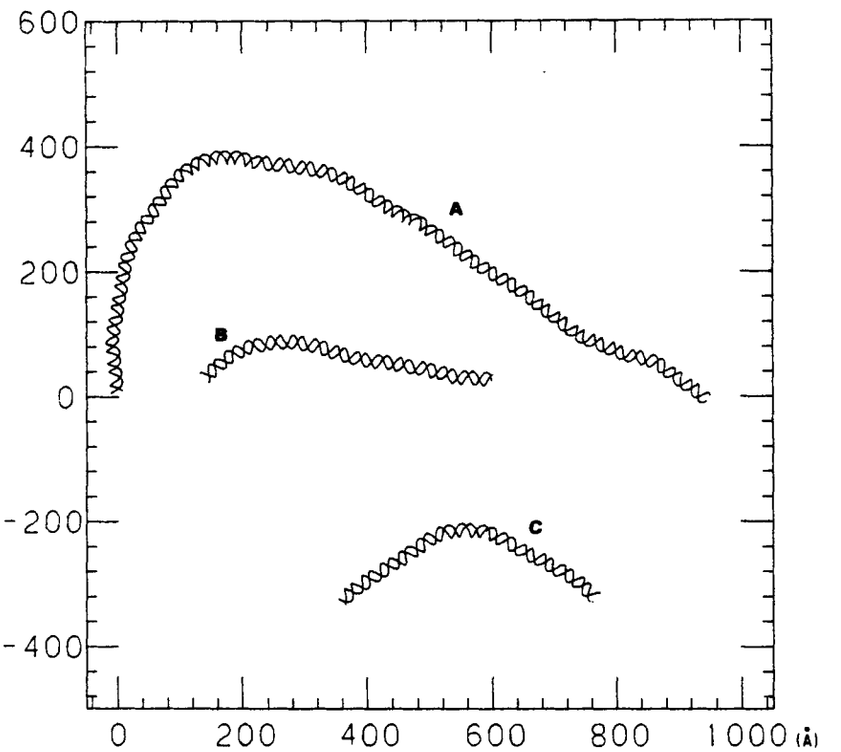


FIGURE 4. DNA helix trajectories calculated for three different DNA sequences with $\Theta_T = 5°$ at every occurrence of ApA, and other wedge angles being set equal to zero, as in Reference 25. Bending due to thermal fluctuations is ignored. (A) Kinetoplast DNA,[26] first computer drawn in a similar way in Reference 25; (B) DNA fragment of bacteriophage lambda which contains the origin of replication; (C) DNA of the nucleosome N5081 of SV40[11] which includes the origin of replication. Fragments A and B are known to exhibit strong anomaly in electrophoretic gels.[12,13,27-30] (Courtesy of L. Ulanovsky.)

these data, the absolute values of the roll angles could be as large as about 10[21] or even 18°.[22,23] The tilt angles are usually smaller; 3[21] or up to 10°.[22,23] For many reasons these estimates are rather inaccurate and often conflicting. For the combination (AA) · (TT) (e.g., according to the crystallographic data[21]), the angle $\theta_R = 0.3$ to 0.9°, while energy calculations[22,23] predict $\theta_R = -18.1°$. The tilt angle θ_T for this minihelix is -1.0 to $-2.6°$, opening towards thymines,[21] while from energy calculations[22,23] $\theta_T = -9.6°$. One independent estimate of this angle, $-11°$, was arrived at by very simple geometrical considerations linking the tilt angle and the helical twist of $(dA)_n \cdot (dT)_n$.[24] All these estimates indicate that the tilt wedge for this minihelix points towards adenines, as one would expect also from *a priori* considerations. Yet, one more estimate of the absolute value of the wedge angle for (AA) · (TT), stems from DNA trajectory model building for apparently curved fragment of kinetoplast DNA as manifested by its relaxation behavior[25] (see also below) — in the range of 5°.

Thus, the wedge angles between adjacent base-pairs could be of the order of a few degrees and are, obviously, a subject of further and more accurate estimations.

Figure 4 illustrates how significantly DNA molecules could be curved due to even such small "wedges." Only one wedge angle, for (AA) · (TT), is introduced in these computer drawings. Therefore, the simplified trajectories shown are just illustrations of the sequence-dependent curving of DNA.

IV. PERIODICITY OF CHROMATIN DNA NUCLEOTIDE SEQUENCES

Periodical disposition along the DNA molecule of the wedge-like elements, identically or similarly oriented, is the simplest way to design the curved molecule, as illustrated by Figure 2. Has this design anything to do with natural DNA sequences? One obvious structure where such a curved molecule might be useful is the nucleosome. DNA in these particles is wrapped around the histone octamers apparently without disruption of base-stacking interactions.[31-33] Recent computational[10] and experimental[34] studies indicate that the DNA path in the nucleosome is somewhat oval, with several regions of high curvature where the deformation, presumably, is spread over several base-pairs.[34] Such deformation should necessarily involve some energy, and selection of regions in the DNA molecule which are already curved even when unperturbed would be energetically advantageous. Thus, one could expect that natural sequences involved in the chromatin structure might display some periodicity, with the period equal to the helical repeat of DNA in the nucleosome.

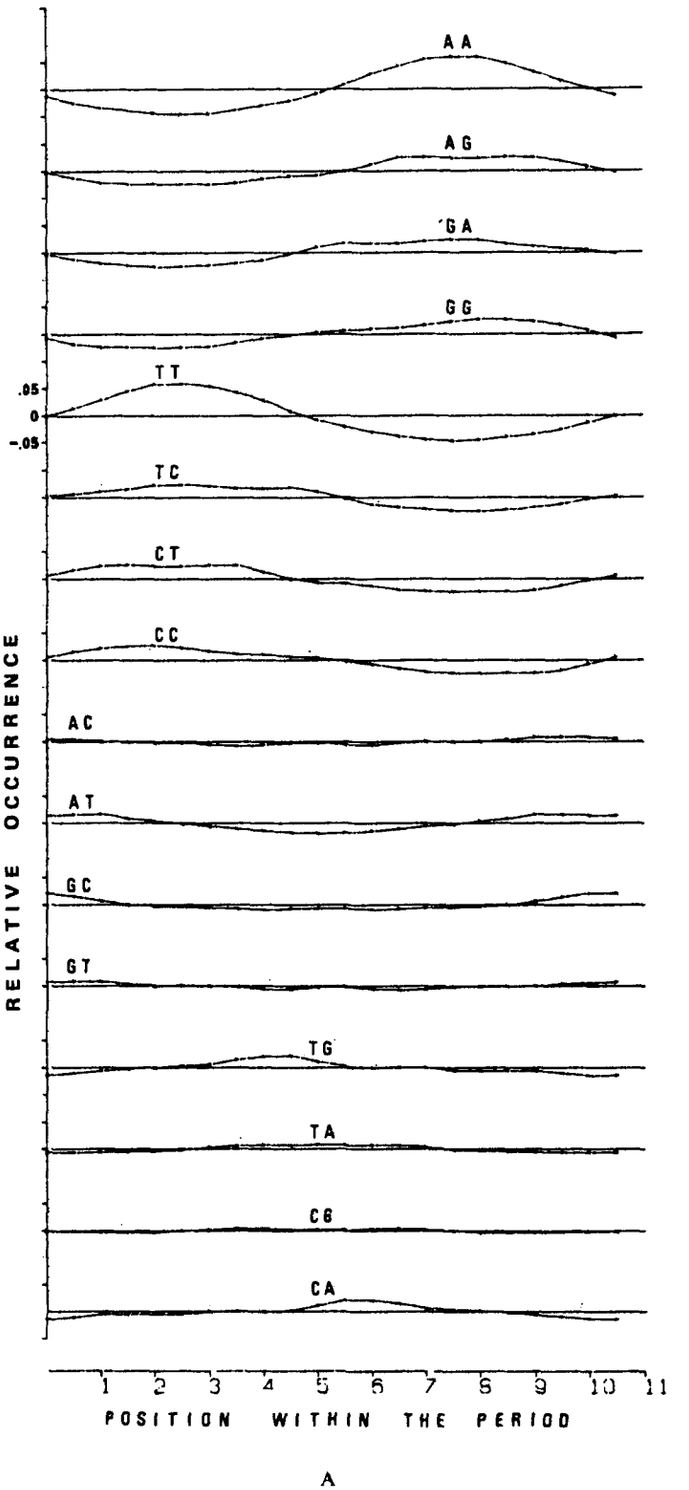
Indeed, such periodicity was observed by autocorrelation analysis of chromatin DNA sequences.[8] The distances along the sequences between dinucleotides of the same kind were scored, resulting in clear maxima in the distribution separated by 10 to 11 bases. This effect was found to be particularly strong for dinucleotides AA and TT. In other words, the combination of base-pairs (AA) · (TT), presumably, makes the biggest wedge as compared to other minihelices.

To determine how much various dinucleotides contribute to this effect and what the phase relationships between different periodical components are, another computational procedure was applied — iterative synchronous detection.[9,35] This procedure consists of a selection of pieces of sequences which manifest the 10.5 base periodicity, matching (correlating) them, and counting how frequently the 16 different dinucleotides are found in different positions within the 10.5 base repeat. This results in 16 positional distributions of the dinucleotides along the period which have to be corrected by further iterations. That is, the distributions are used then as a reference for correlation in the next round of selecting the periodical pieces of the sequences, matching them and deriving the next approximation set of distributions. After repeating this procedure many times, one arrives at the final set of functions which are not changed anymore by additional iterations. (Expressed in a matrix form, this set of functions is called the matrix of bendability.[9,10])

The 16 distributions of the dinucleotides within average 10.5 base long repeat characteristic for chromatin DNA sequences are shown in Figure 5A. The major contribution to the periodical pattern is made by PuPu (and PyPy, shifted half period) dinucleotides — what one would expect from simple *a priori* considerations (see Figure 1 and discussion). AA and TT dinucleotides appear to be the strongest. All PuPu dinucleotides are distributed similarly, preferring the same positions within the period. Most populated positions for PyPy dinucleotides, on the other hand, are all shifted by half period relative to the PuPu dinucleotides. PyPu and PuPy dinucleotides basically occupy intermediate positions (see Figure 5B).

The half-period shift between PuPu and PyPy dinucleotides has an obvious explanation. If, e.g., combination of (AA) · (TT) makes a wedge towards adenines, then putting TT in place of AA will turn the wedge in the opposite direction. Placing dinucleotide TT about five bases away from this position will turn the wedge 180° so that it will point in the original direction again.

Of course, the chromatin sequences are far from being perfectly periodical, otherwise there would be no room for other encoded information carried by the sequences. The pattern described should be considered only as a periodical component of the sequences which is presumably more pronounced in the regions involved in the nucleosomes. This secondary message can only be superimposed on the primary protein coding message due to the degeneracy of both the triplet code and the aminoacid sequence.[36]



A

FIGURE 5. (A) Averaged distributions of the dinucleotides within the period, calculated from chromatin DNA nucleotide sequences;[10] (B) the same distributions combined in four groups (PuPu, PyPy, PuPy, and PyPu). Only oscillating components are shown.

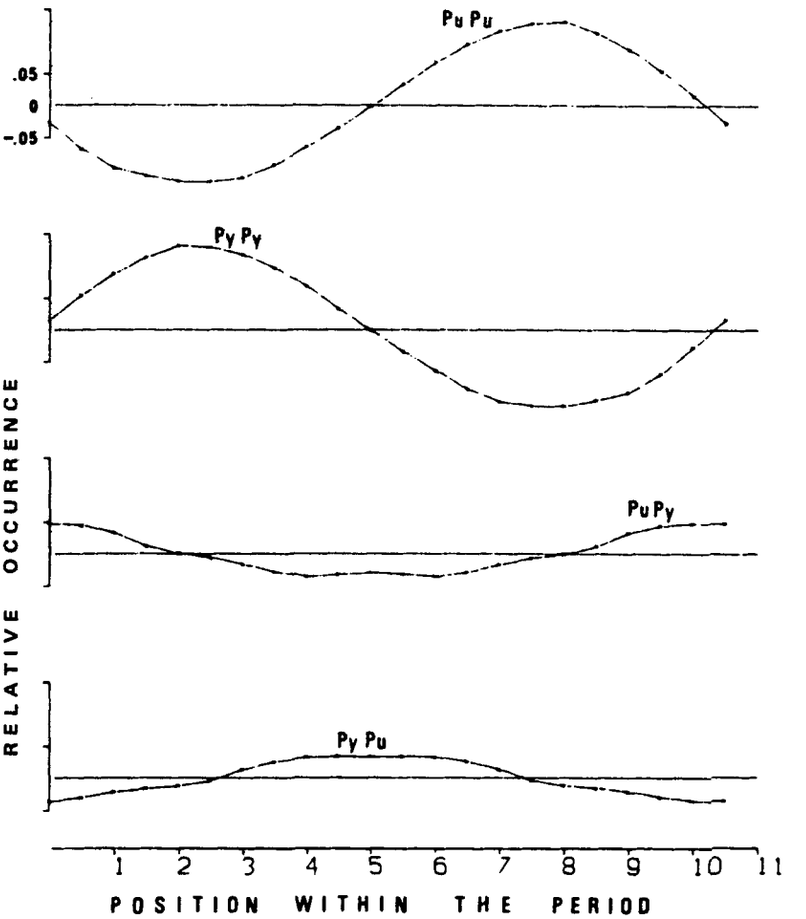


FIGURE 5B

The matrix of bendability discussed above has been calculated from the chromatin sequences without discriminating between nucleosomal and linker regions. A direct analysis of the sequences involved in the nucleosomes would provide more appropriate data concerning the expected periodicities. The results of such direct analysis[37,38] of nine nucleosomal DNA sequences (see Reference 10) demonstrate that, indeed, the sequences experimentally proven to belong to the nucleosomes manifest a clear periodical pattern, very similar to the distributions shown in Figure 5. AA and TT dinucleotide periodicities again appear to be the strongest.[37,38]

A similar periodicity in the distribution of mononucleotides along nucleosomal DNA was documented experimentally[39] by sequencing DNA extracted from a bulk preparation of nucleosomes, believed to be distributed randomly along the DNA. This periodicity was interpreted as an artifact of sequence specificity of the micrococcal nuclease.[39] In our opinion, however, this is simply a reflection of nonrandomness of the positioning of the nucleosomes along the nucleotide sequences. Even after the nucleosomes are allowed to slide and occupy new positions, these positions, apparently, are selected by the histone octamers, which we believe, bind preferentially to the curved portions of DNA, characterized by the dinucleotide and, therefore, mononucleotide periodicity.

It is noteworthy, that these curved portions of free DNA are subject to further bending in the nucleosome. Therefore, the periodical pattern characteristic for chromatin DNA se-

quences (Figure 5), presumably, not only reflects the DNA curvature but the histone-DNA interactions as well.

V. SEQUENCE-DEPENDENT AND -INDEPENDENT DEFORMATIONAL ANISOTROPY

In describing deformational properties of DNA, one has to differentiate between the anisotropy due to the helical structure of DNA[16,40] and the sequence-dependent anisotropy.[9] As was indicated first by Schellman,[40] the DNA molecule might be locally more bendable towards its grooves rather than in the perpendicular direction, irrespective of the nucleotide sequence. This point is clearly illustrated by energy calculations by Zhurkin et al.[16,20] These calculations also suggest that not only DNA bendability towards the grooves but the absolute values of the roll angles might be higher than the tilt angles toward the bases.[20,22,23,41] Thus, one would expect that not only forcible bending of DNA should be basically due to the bends towards the grooves, but the sequence-dependent curving of (unperturbed) DNA should also mostly result from local DNA axis inclinations toward the grooves. It was found[20,22,23,41] that the roll angles for (PuPy) · (PuPy) combinations of base-pairs and for (PyPu) · (PyPu) combinations have a different sign. The sequence PuPyXXXPyPuXXX was suggested, therefore, as a major repeating motif of unidirectionally curved molecules.[41,42] (See also Reference 29.)

This attractive and almost obvious idea on the priority of the roll angles in determining both the sequence-dependent and -independent deformational anisotropy of DNA is, however, in conflict with the sequence analysis data discussed in the previous section. It appears that PuPu (and PyPy) rather than PuPy and PyPu dinucleotides are the main carriers of the periodical signal in the chromatin DNA. Moreover, the corresponding PuPu and PyPy distributions are shifted by one half period from one another (Figure 5), which indicates that at least in the nucleosomes this wedge is tilt-like, pointing toward bases (e.g., adenines, see above) rather than roll-like (toward grooves). Otherwise, the distributions would not be shifted at all, the AA dinucleotide being replaceable by TT (same phase along the sequence) without changing the roll angle. Such replacement involves only rotation of the minihelix (AA) · (TT) around the axis entering its grooves (point made by L. Ulanovsky, personal communication).

It is important to note, however, that on one hand, the sixteen dinucleotide distributions within the typical repeat of the chromatin sequences[9,10] shown in Figure 5 are not independent functions. Periodicity in the distribution of any particular dinucleotide should cause smaller periodicity of other dinucleotides as well, either due to linking by the sequence (YZ is linked to XY) or due to simple exclusion from positions occupied by the periodically repeating dinucleotides. It is not obvious, therefore, how many and which of the 16 dinucleotides are actually responsible for the periodicity observed in the chromatin sequences. On the other hand, since the chromatin DNA periodical pattern contains both curving and histone-induced bending components (see above), the roll and tilt preference might be different for DNA in the nucleosome and free curved DNA. There is, probably, no way to solve the roll-or-tilt controversy, except by direct measurements of the wedge angles for different combinations of base-pairs in free DNA.

VI. SEQUENCE-DIRECTED MAPPING OF THE NUCLEOSOMES

The periodicity of the nucleosomal DNA sequences discussed above has been recently successfully utilized for the mapping of the nucleosomes along the chromatin nucleotide sequences.[10,11,42] In one variant of the mapping procedure all 16 dinucleotide distributions were used,[10] while the other mapping algorithm involved only alternation of PyPu and PuPy dinucleotides.[41,42]

In both cases, the mapping algorithms search for nucleosomal-size pieces of DNA sequences which manifest pronounced periodicity correlating with one or another periodical pattern of dinucleotides. Though both procedures are in good accordance with experimental nucleosome mapping data available, the 16-function algorithm[10] appears to be in better agreement with the data.

This mapping procedure was recently used for construction of a complete nucleosome map of the SV40 minichromosome.[11] The map, based exclusively on the nucleotide sequence analysis, locates 25 nucleosomes of SV40 chromatin in positions fairly consistent with the experimental data available. The portions of this map are shown in Figure 6, compared to very recent mapping results by exonuclease digestion of the chromatin linearized by restriction enzymes Bgl I, Msp I, and Bam HI,[43] and DNase I digestion of the SV40 minichromosomes.[44] The sites which are resistant to the DNase I digestion, as well as the regions where the rate of propagation of the exonuclease Bal 31[43] is slowed down, obviously belong to the nucleosomes, as mapped by the sequence-directed computer procedure[11] (Figure 6). The nucleosome N5241 marked as a less probable alternative appears to actually exist in the linearized minichromosome (see also below).

From the data discussed in the last two sections one can conclude that the weak 10 to 11 base periodicity in chromatin DNA sequences, most pronounced for the AA and TT dinucleotides, is probably the major feature recognized by the histone octamers. Ascribing certain wedge angle to every occurrence of the (AA) · (TT) in the nucleosomal DNA one can visualize what the nucleosomal DNA trajectory might look like. The curved piece of DNA shown in Figure 4C is one such example of the free nucleosomal DNA trajectory calculated for the nucleosome N5081[11] (see also Figure 6). This particular DNA fragment possesses a strong periodicity of AA(TT) dinucleotides, which is the reason why the nucleosome is mapped within this fragment which also includes the origin of replication of SV40 (see Section XI). Some physical evidence which links this periodicity with presumed unidirectional curving of the nucleosomal DNA is discussed in the following sections.

VII. ORIENTATIONAL RELAXATION TIME MEASUREMENTS

One way to detect permanent curving of the DNA molecules is to measure their rotational diffusion after application of an electric field to orient molecules. The relaxation time can be measured either by electric dichroism[12,46-48] or by electric birefringence[49,50,29] techniques. The time depends on the molecular size, and is smaller for more compact (shorter) molecules. Some of the fragments studied appear to be of smaller length than their size known from the nucleotide sequences, thus revealing their inherent curving which makes the fragments effectively shorter. One specific fragment of kinetoplast DNA of *T. tarentolae* which is characterized by strong periodicity of AA dinucleotides along its nucleotide sequence has been found to be especially compact by transient electric dichroism studies of its relaxation behavior.[12,13] These measurements of relaxation time unequivocally correlate the compactness (curving) of the DNA fragments with the 10-11 base periodicity of their nucleotide sequences.

VIII. ANOMALOUS ELECTROPHORESIS OF DNA

The electrophoretic mobility of DNA molecules in the gel is known to depend on their molecular mass and size, which is frequently used for the molecular weight determinations (see, for example, References 51 to 53). However, certain restriction fragments of DNA from various sources behave anomalously in the gel, their apparent molecular weight conflicting with the actual value expected from their nucleotide sequences.[12,53,54-61] The local curving of the DNA molecule could be one possible reason for such anomaly. On one hand, the sequence-dependent local curving of DNA would result in some compaction of the

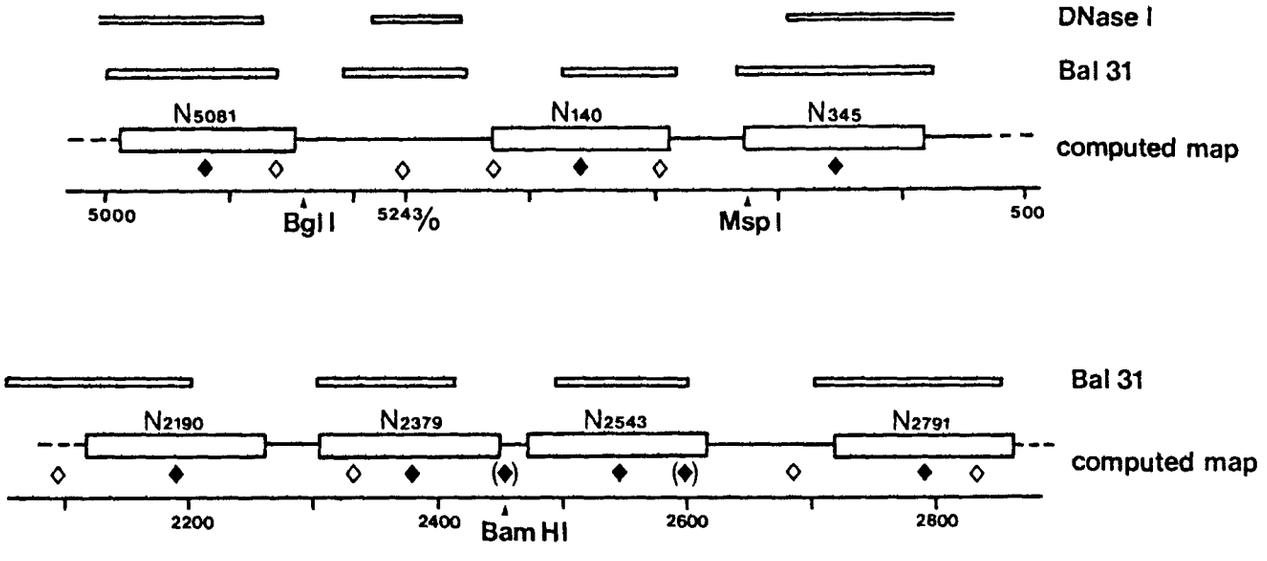


FIGURE 6. Comparison of computed nucleosome map of SV40 minichromosome[11] (numbered bars) with experimentally determined sites resistant to DNase I digestion[44] and the pause sites during exonucleolytic digestion by Bal 31 nuclease[43] (thin bars). Two regions of the SV40 genome are shown for which the experimental data are available. By filled diamonds the major positions (centers) of the "strongest" nucleosomes are marked, which presumably correspond to inactive minimal energy state of the minichromosome, while the symbols in parentheses and empty diamonds indicate some of less probable alternative positions.[11] The SV40 nucleotide sequence is numbered according to Reference 45.

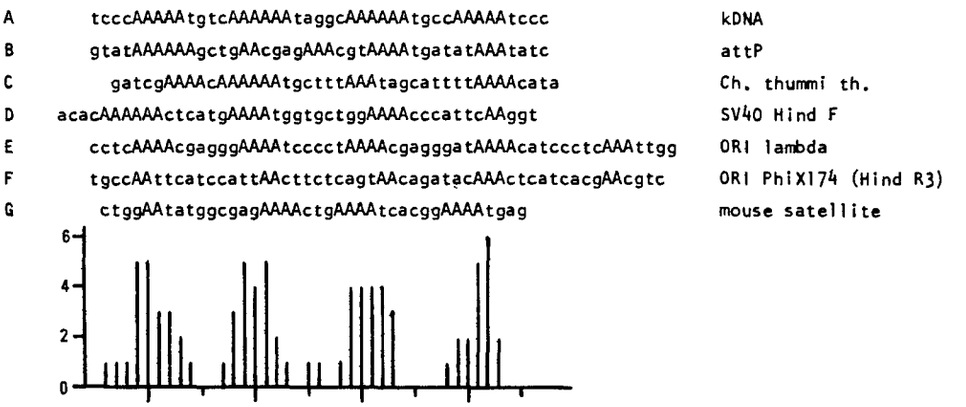


FIGURE 7. Partial nucleotide sequences of the fragments anomalously migrating in the polyacrylamide gels. (A) Kinetoplast DNA;[12,13,26-29] (B) recombination site attP of bacteriophage lambda[60,66] (C) fragment of *Chironomus thummi thummi* DNA;[61,67,68] (D) Hind F restriction fragment of SV40 DNA;[54,56] (E) restriction fragment containing origin of bacteriophage lambda replication;[30,69] (F) Hind R3 fragment of φx174 DNA, which includes origin of replication;[55,70] and (G) mouse satellite DNA.[59] Only parts of the sequences containing periodically repeating runs of adenines (capital As) are shown. Sequences B and F are complementary to respective original sequences. The diagram at the bottom presents distribution of AA dinucleotides along the sequences.

molecule, additional to its Gaussian coiling characteristic for half-rigid polymer chains[62] of which DNA is a classical example.[63] More compact coils would migrate faster in the gels with pore size comparable with the size of molecular coil, like agarose gels. The apparent molecular weight of the curved molecules in such gels might appear then somewhat smaller than the actual one. During the gel filtration the more compact coils would penetrate in smaller cavities in the gel which would result in their delayed elution from the gel (smaller apparent molecular weight). In small-pore-size gels (polyacrylamide) where the DNA molecules are believed to migrate by a head-on reptation mechanism,[64,65] the local curving would provide some additional friction for the molecule reptating through the narrow channels in the gel, thus resulting in retardation of the curved molecules (anomalously high apparent molecular weight). Exactly this behavior has been observed in the case of the above-mentioned fragment of kinetoplast DNA[12] which is also characterized by faster relaxation in electric dichroism experiments and by sequence periodicity.

Using various combinations of restriction endonucleases one can get many subfragments of DNA covering the same region. This allows one to locate the sites in the anomalously migrating molecules which are responsible for the anomaly. Such analysis has been performed in two cases: kinetoplast DNA of *T. tarentolae*[13,27,29] and att P site of bacteriophage lambda.[60] The anomaly-generating regions have been precisely located within the sequences presented in Figure 7(A,B). These two sequences both display one common feature: 3- to 6-base-long runs of adenines repeating every 10 to 11 bases. In all other cases of electrophoretic anomaly documented so far, similar periodical sequences are always found within the fragments. Some examples are shown in Figure 7(C to G). All pBR322 DNA fragments with abnormal mobility contain such a repeating motif.[53] Many subfragments of minicircular kinetoplast DNA of *Trypanosoma brucei* are anomalous,[58] and inspection of the sequences of the minicircles reveals several groups of repeating runs of adenines. One striking example is the perfect tandem repeat of five decanucleotides GTAAAGTTAG in the minicircle DNA 201 of *T. brucei*.[58]

Thus the sequence periodicity (in particular, periodical distribution of AA(TT) dinucleotides) is a characteristic feature of the DNA fragments anomalously migrating in the polyacrylamide gel. This strongly indicates that the curved shape of these fragments is the cause of their retardation in the gel. The simplified DNA trajectories calculated, for illustration

purposes only, for the nucleotide sequences of two of the anomalously migrating fragments, are shown in Figure 4(A,B). The most curved parts of these trajectories involve the nucleotide sequences shown in Figure 7(A,E).

On the basis of the data discussed in the previous sections, an obvious conclusion can be suggested that the 10.5 base periodicity of some dinucleotides along the DNA sequences, the retardation of DNA fragments during electrophoresis in polyacrylamide gel, and the faster relaxation of these fragments after their orientation in the electric field are all manifestations of the same phenomenon: sequence-dependent curving of the DNA double helix.

IX. ON THE PERSISTENCE LENGTH OF DNA

The DNA molecule is known to be a half-rigid polymer chain.[40,48,49,63,71] Long molecules of DNA in solution adopt the conformation of a random Gaussian coil due to slight multiple bendings of these molecules caused by thermal motion. The mutual orientation of any two segments of the coiled DNA molecule in solution depends very much on the separation between these segments along the molecule. At long distances the random bends accumulate, resulting in complete independence of the orientation of the segments, while at short distances the segment orientations are still correlated. The stiffness of the half-rigid wormlike molecules is characterized by the so-called persistence length, which is the distance along the molecule at which the average direction cosine drops to 1/e of its initial value (1).[62] According to most recent estimates the persistence length of natural DNA under physiological conditions is about 100 to 150 base-pairs.[49,71]

The question arises to what extent this measured value is influenced by the inherent curving of the DNA molecule. The curving distributed along the molecule in a sequence-dependent way should provide a certain initial compaction of the molecule. Together with random thermal bending this should result in a somewhat tighter coiling than on the basis of molecular rigidity alone. In other words, the size of the DNA coil in solution as well as the apparent persistence length should be smaller for an inherently curved molecule than for a similar inherently straight one.

The anomalous gel filtration of the curved kinetoplast DNA[12] illustrates this point. Elution of these molecules is delayed, apparently due to the additional compaction of the molecular coils by the local curving of the molecules. To separate the "static" and "dynamic" contributions to the apparent persistence length, some hydrodynamic experiments should be conducted involving both inherently curved and straight molecules of the same molecular weight. Poor accuracy of the wedge angles known (see Section III), unfortunately, impedes any reliable theoretical estimation of the "static" contribution to the persistence length. It might be comparable with the fluctuational contribution, since the wedge angles (ranging from 0 to at least 10°, Section III) could well have an average of the same order of magnitude as the fluctuational angle of local deformation — about 6°.[40]

X. ALTERNATIVE MODELS OF THE INHERENTLY CURVED DNA

The local structure of DNA, being very much sequence dependent,[3,4] could deviate substantially from the standard B DNA structure. Some pieces of the DNA molecule might even exist in alternative non-B forms. In this case two straight axes of the neighboring pieces of different DNA structures could be noncolinear and nonparallel, thus locally inclining the DNA axis. One possible example is the junction between the B- and A-form DNA[72] where the corresponding molecular axes are inclined one relative to another by 26°, without disruption of the base-stacking interaction at the junction. By alternating segments of B and A DNA of appropriate lengths one can design a curvilinear DNA structure with effective radius of curvature as small as 40 Å.[72]

Another model of this kind has been suggested for explanation of anomalous electropho-

retic behavior of kinetoplast DNA[13] which contains 4- to 6-base-pair long stretches of $(dA)_n \cdot (dT)_n$. It is proposed that these stretches have an altered helix structure[73] so that the junctions between the normal and altered structures deflect the DNA axis. Deflections of about 12° each, distributed along the kinetoplast DNA, could explain its anomalous electrophoretic and hydrodynamic behavior.[12] Prunell et al.[24] estimated this angle to be 18°, assuming that the stacked A·T base-pairs make a tilt wedge of 11°. Interestingly, the wedges between adjacent base-pairs cumulatively result in a helical structure,[24] very similar to the heteronomous model of Arnott et al.[73] for $(dA)_n \cdot (dT)_n$.

XI. BIOLOGICAL RELEVANCE OF CURVED DNA

The nucleosomes are shown to involve parts of DNA with the periodical distribution of PuPu (and PyPy) dinucleotides along the molecule.[10] This means, first of all, that the histone octamers preferentially bind to certain sites along the genome which could result in specific modulation of the DNA template activities. Comparison of the nucleosomal map of SV40 minichromosome[11] with the distribution of replication pause sites along the SV40 genome[74] shows that, indeed, the nucleosomes provide a barrier for propagation of DNA replication forks.[75] The major transcription initiation sites of SV40, on the other hand, are located in the linkers between the nucleosomes while the 3′-ends of SV40 mRNA are mapped within the nucleosomes.[11,75] The most spectacular demonstration of the modulating role of the nucleosomes in the transcription process is provided by experiments in which a shift of the nucleosome from its original site resulted in a manyfold change in the rate of mRNA synthesis.[76]

Various patterns of binding of the histone octamers to alternative sequence-dependent positions in DNA could correspond to different stages of gene expression. In this respect the regulatory regions as in SV40 minichromosome in which several alternative positions could be occupied by the nucleosomes[11] (see also Figure 6) are of special interest. DNase I digestion experiments[44] indicate that within the so-called "gap-region" of SV40 minichromosome[77,78] there is a 90- to 110-base-pair-long protected area centered at about 5240. In the same area, the Bal 31 exonuclease is shown to pause.[43] According to the computer-calculated nucleosomal map[11] this would correspond to nucleosome N5241 (Figure 6). On the other hand, the Bal 31 experiments demonstrate that there is one more region where the exonuclease pauses during digestion of the minichromosome — 100 ± 20 base-pairs centered at about position 170. This would correspond to the calculated position N140.[11] These two nucleosomes, however, cannot be formed simultaneously since the center-to-center distance between them is shorter than 145 base-pairs, the size of DNA involved in the nucleosome core particle. The nucleosomes N5241 and N140 might correspond to alternative chromatin structures of the "gap-region." There is, indeed, some evidence of possible existence of subpopulations of the SV40 minichromosomes with apparently difference chromatin structures.[43] Formation of the nucleosomes in alternative positions could be one possible mechanism by which these "regulatory nucleosomes" might modulate gene expression.

The locally curved DNA could be a sequence-dependent feature recognized by some other proteins as well. The prokaryotic *E. coli* RNA polymerase appears to be such an example. The nucleotide sequences of *E. coli* promoters are found to contain a periodical component similar to what is found in the nucleosomal DNA sequences.[79] The distribution of PuPu and PyPy dinucleotides along the nucleotide sequences of 168 *E. coli* promoters is presented in Figure 8. The 10.5 ∓ 0.5 base periodicity of the dinucleotides is clearly seen, indicating that the promoter containing pieces of DNA are on the average somewhat curved. This feature, however, is not necessarily characteristic of every particular promoter, being apparently only one of several signal components contributing to distributional recognition of the promoters by the RNA polymerase.[79]

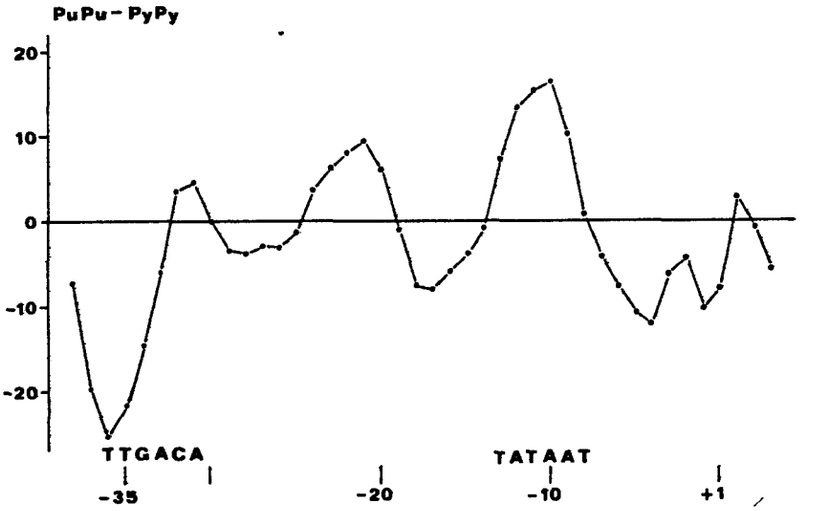


FIGURE 8. Distribution of PuPu and PyPy dinucleotides along the *E. coli* promoters. The promoter sequences analyzed are taken from Reference 80. The excess of PuPu dinucleotides over PyPy dinucleotides is shown as a function of position within the promoter. The distribution is smoothed by averaging occurrences at every 5 consecutive positions. Coordinate + 1 corresponds to 5'-end of the mRNA.

The origins of replication, both prokaryotic and eukaryotic, are another possible example of involvement of curved DNA. Two of seven DNA fragments with strongly anomalous electrophoretic behavior shown in Figure 7 contain origins of replication which are also characterized by strong periodicity of AA dinucleotides. Figure 4(B,C) presents an illustration of the DNA helix trajectories calculated for the origin-containing fragments of bacteriophage λ and SV40 DNA.

According to our preliminary analysis, the origins of plasmid pBR 322, of bacteriophages φ80, φ82, M13, F1, and fD, and of human mitochondria are strongly periodical (not shown), as well as are the origins of bacteriophages λ and φX174 presented in Figure 7, all displaying periodically repeating runs of adenines. While in case of polyoma virus, BKV, OriC *E. coli* K12, rat mitochondria, and bacteriophage G4, the periodicity is manifested by PuPu (PyPy) dinucleotides rather than AA(TT) dinucleotides (not shown). The nucleotide sequences of the origins of replication are presumably involved in specific DNA protein structures responsible for initiation of replication, replisomes.[81] Although these structures are still hypothetical, there is some experimental evidence suggesting that the replisomes indeed exist. The specific protein complex, involving DNA-polymerase I, DNA ligase, DNA primase, and DNA topoisomerase II has been recently found to bind to the origin of replication of 2*M* yeast plasmid and to the autonomously replicating sequence ars1.[82] Another example of the origin-specific DNA protein structure is the complex observed in *Drosophila* mitochondrial chromosomes.[83] The origin of replication of SV40 virus maps together with the nucleosome N5081[11] which might be involved in the early stages of the SV40 DNA replication.

Bacterial histone-like HU protein[84] and its complexes are of special interest in this respect. In presence of the eukaryotic type I DNA topoisomerase this protein binds to DNA, condensing it into nucleosome-like particles.[85] It is also shown that the HU protein is involved in the enzymatic replication of chromosomal origin of *E. coli*.[86]

Formation of nucleosome-like structures in prokaryotes is a broadly discussed possibility.[87,88] These structures might well involve pieces of curved DNA which would be energetically favorable. One interesting example is a possible involvement of the att P site in the nucleosome-like structure which seems to be required for integrative recombination in

bacteriophage lambda.[89,90] This site is characterized by strong periodicity of AA dinucleotides (see Figure 7), as well as by anomalous electrophoresis[60] — typical attributes of the curved DNA, as discussed above. The anomaly-generating periodical fragment is located at the center of the region involved in the nucleosome-like complex of the attP site with the int protein.[89]

The distributions of PuPu (PyPy) dinucleotides along the *E. coli* promoter sequences (Figure 8) and AA(TT) dinucleotides along the fragments migrating anomalously in the gel (Figure 7, bottom) are both periodical, with the apparent period of about 10.5 bases. Bearing in mind that this feature is supposed to be characteristic of unidirectionally curved fragments of DNA (see Figure 2 and discussion), one can consider the period as an estimate of the DNA helical repeat in vivo. As one would expect, this value is close to the estimates obtained for DNA in solution.[5,6] A more accurate analysis involving a bigger ensemble of natural periodical sequences will show whether the in vivo and in-solution values are indeed the same.

XII. CONCLUDING REMARKS

The bulk of evidence discussed in this review and the conclusion on the existence of the inherently curved DNA can hardly be argued against. A direct proof, however, is not yet available. The curving of DNA could probably be observed by high-resolution electron microscopy. In this case, though, some statistical analysis of the micrographs would be necessary to discriminate between molecules with inherent curvature and originally straight molecules, bent during adsorption on the supporting surface. The arc-like curved fragments of DNA would probably adsorb to the surface more frequently by the two sides of the arcs, very much like hotdogs on a table or short pieces of a rubber tube which are almost never straight. One encouraging evidence in favor of such side-wise adsorption is provided by experiments with nucleosomal DNA fragments on the mica surface.[91] DNase I digestion of these uniform length fragments with labeled 5′-ends generates single-stranded pieces of lengths that are a multiple of about 5 bases. This result can be explained if one assumes that the nucleosomal DNA is curved and that the molecules of every subpopulation of the nucleosomal DNA with the same nucleotide sequence bind to the surface by one or another of their two flat sides. Supplemented by appropriate control experiments with straight molecules this technique could provide an elegant way to experimentally test whether any given specific fragment is curved.

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