Proc. Natl. Acad. Sci. USA
Vol. 73, No. 9, pp. 3068–3072, September 1976
Biochemistry

Organization of DNA in chromatin

(chromatin structure/intercalation/DNA-kinking)

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Communicated by F. H. C. Crick, June 15, 1976

ABSTRACT **Conformational changes in DNA that accompany drug intercalation have led us to ask if DNA first bends or "kinks" to accept an intercalative drug or dye. Kinking is made possible by altering the normal C2′ *endo* deoxyribose sugar ring puckering in B DNA to a mixed sugar puckering pattern of the type C3′ *endo* (3′–5′) C2′ *endo* and partially unstacking base-pairs. A kinking scheme such as this would require minimal stereochemical rearrangement and would also involve small energies. This has prompted us to ask more generally if a conformational change such as this could be used by proteins in their interactions with nucleic acids. In this paper we describe an interesting superhelical DNA structure formed by kinking DNA every 10 base-pairs. The structure may be used in the organization of DNA in chromatin.**

The organization of DNA in chromatin is a subject that has attracted growing interest in recent years (for a review, see Elgin and Weintraub, ref. 1). It is generally agreed that chromatin consists of a linear arrangement of bead-like structures (called ν bodies) that contain DNA and histones (2). The exact diameter of the bead is uncertain, but is probably in the order of 100 Å. Each bead is thought to contain two sets of four different histones [i.e., 2(H-2a, H-2b, H-3, H-4)] complexed with about 170 base-pairs of DNA and an additional histone (i.e., H-1) complexed with about 40 base-pairs (3, 4). This DNA is folded to about one-seventh its length, a value deduced from electron microscopy measurements of minichromosomes of simian virus 40 (SV40) and adenovirus 2 (5, 6).

The exact manner in which DNA is folded within the ν body is not known. Noll has shown that DNase I digestion of chromatin liberates DNA fragments 10, 20, 30, 40, . . . up to 200 bases long (7). This suggests that the DNA lies on the outer surface of the ν body and that some structural feature of DNA related to its helical periodicity is recognized and cleaved by the enzyme. Crick and Klug (8) have advanced a specific hypothesis to explain the arrangement of DNA in chromatin. They postulate that DNA is wound around the outer surface of the histone core not by continuously deforming DNA, but by kinking DNA every 20 base-pairs. In their scheme, kinking is accomplished by unstacking base-pairs and altering the sugar-phosphate backbone from its normal *gauche–gauche* conformation to a *gauche–trans* conformation. This allows helical sections above and below the kink to come apart and form an angle of 98° between their helical axes. In their model, kinking imparts a small negative twist to DNA, reducing the twist angle from 36° to about 15–20° at the kink. This gives rise to a left-handed (kinked) toroidal helix when DNA is complexed with histone, a structure that subsequently could be detected as a right-handed interwound superhelix in histone-free circular DNA molecules (9).

The Crick-Klug stereochemical kinking scheme predicts eight kinks per 170 base-pairs and this gives rise to a left-handed kinked toroidal helix with a diameter of about 90 Å that contains somewhat more than two turns per ν body. Although their scheme is satisfactory from the stereochemical point of view [even though their sugar-phosphate backbone conformation is *not* one of the preferred conformations currently listed (10, 11)], we would like to suggest an alternative stereochemical scheme for kinking DNA that results in much the same consequences as regards histone–DNA interaction. The scheme uses our current ideas about drug intercalation into DNA.

Stereochemistry of drug intercalation

We have recently determined the three-dimensional structures of *two* ethidium: dinucleoside monophosphate crystalline complexes [ethidium: 5-iodouridylyl(3′–5′)adenosine (12–14) and ethidium: 5-iodocytidylyl(3′–5′)guanosine (15, 16)] and *one* 9-aminoacridine: dinucleoside monophosphate crystalline complex [9-aminoacridine: 5-iodocytidylyl(3′–5′)guanosine (17, *)] by x-ray crystallography. ***All three structures demonstrate drug intercalation into miniature Watson–Crick double helices.*** Features common to these structures are a *gauche–gauche* sugar-phosphate backbone conformation with altered glycosidic torsional angles (these will be described in detail below) and the following pattern of ribose sugar ring puckering at the intercalation site: C3′ *endo* (3′–5′) C2′ *endo*. These conformational changes permit base-pairs to separate 6.8 Å and give rise to the observed twist angle between base-pairs above and below the intercalative drug or dye (estimated in these studies to be between 8 and 10°) as well as to a common relative base-pair orientation as defined by the positions of the glycosidic bonds. We have used this stereochemical information to understand the general nature of intercalative drug binding to DNA. This is shown in Fig. 1B and D.

To construct the ethidium–DNA binding model, we have added B DNA to both sides of the intercalated dinucleoside monophosphate. This is done easily and without steric difficulty. ***An important realization that immediately emerges is the concept that drug intercalation requires a helical screw axis dislocation in DNA***; our model therefore differs in a fundamental way from other models of intercalation recently proposed (18, 19). We estimate that helical axes for B DNA above and below ethidium intercalation are displaced by about +1.0 Å. Base-pairs in the immediate region of intercalation are twisted by 10° (this value has been estimated by projecting the interglycosidic carbon vectors on a common plane and then measuring the angle between them). This gives rise to an angular unwinding of −26° at the immediate site of drug intercalation. We have also observed that intercalated base-pairs are tilted relative to one another by about 8° in both ethidium crystal structures. This results in a small residual "kink" of 8° at the intercalation site, and has been included in our ethidium–DNA binding model (Fig. 1D).

Abbreviation: SV40, simian virus 40.

*T. D. Sakore, S. C. Jain, C. C. Tsai, and H. M. Sobell, manuscript in preparation.

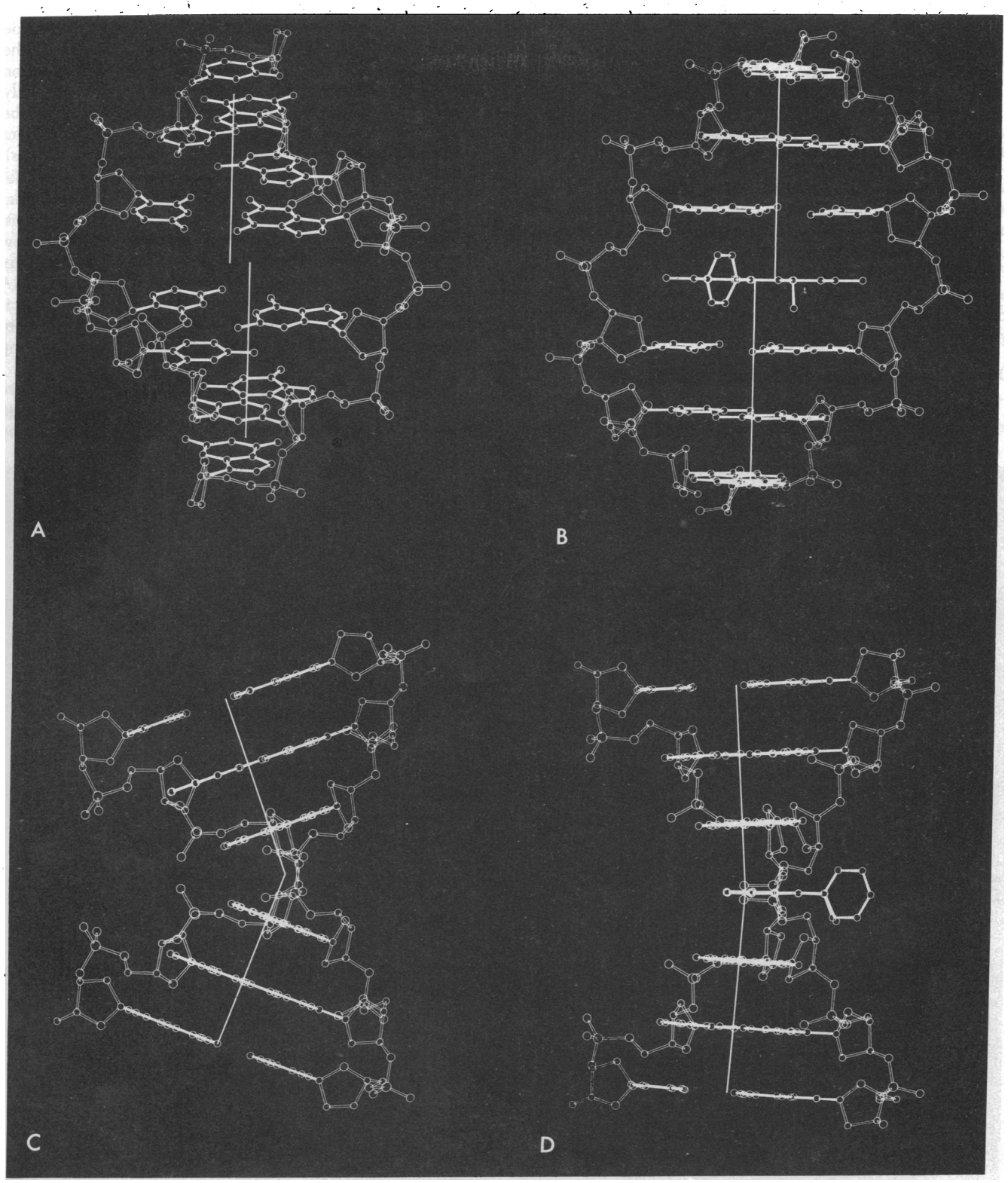

FIG. 1. Computer graphics illustration of the detailed stereochemistry for ethidium–DNA binding and the kink. (A) The kink, shown down the dyad axis. (B) Ethidium–DNA intercalation, shown down the dyad axis. (C) The kink, side view. (D) Ethidium–DNA intercalation, side view. Long solid lines indicate helix axes for B DNA sections above and below the kink and ethidium intercalation structures. Notice that these helix axes are *not* colinear. These figures were drawn by a Tektronix 4014 display console coupled with a Data General Nova 840 computer system.

The magnitude of angular unwinding predicted by our ethidium–DNA model is in good agreement with Wang's recent estimate of ethidium–DNA angular unwinding based on alkaline titration studies of superhelical DNA in cesium chloride density gradients (20). Furthermore, the C3′ *endo* (3′–5′) C2′ *endo* mixed sugar puckering (we postulate this to be an invariant structural feature common to *all* intercalative drug binding) necessarily predicts that intercalation be limited to

FIG. 2. Nomenclature to describe various kinked DNA helices. Vertical arrows indicate kinks placed 10 base-pairs apart, a structure denoted κ kinked B DNA. See *text* for discussion.

every *other* base-pair at maximal drug–nucleic acid binding ratios (i.e., a neighbor exclusion model) (21). We have examined the stereochemistry of this model carefully. The effect of having a screw displacement every other base-pair combined with an 8° kink is to give rise to a maximally unwound DNA structure possessing a slow right-handed superhelical writhe. Full information documenting these and other stereochemical points will appear elsewhere (22).

Does DNA-kinking precede drug intercalation?

The conformational changes in DNA that accompany drug intercalation have led us to ask if DNA first bends or "kinks" to accept an intercalative drug or dye. Kinking of DNA is made possible through flexibility in sugar puckering (this leads to the mixed sugar puckering pattern observed in the intercalation site) as well as by the small energies associated with partially unstacking base-pairs (depending on the magnitude of the energies involved, kinking may occur spontaneously from bending modes of DNA possible at physiological temperatures). In this scheme, base-pairs initially do not come apart parallel to each other but instead are tilted to form a V-type notch that opens from the narrow groove (see Fig. 1A and C). This permits ethidium and actinomycin binding to proceed from the narrow groove of the double helix. Subsequent conformational changes (perhaps thermally induced by torsional and longitudinal DNA vibrational modes) allow base-pairs to assume a parallel orientation so that the planar drug or dye can gain entrance between base-pairs. This step could be catalyzed by the entering drug or dye.

We have explored the stereochemistry of the kink and have related it to the intercalation stereochemistry. The kinked structure can be obtained from the intercalated structure by

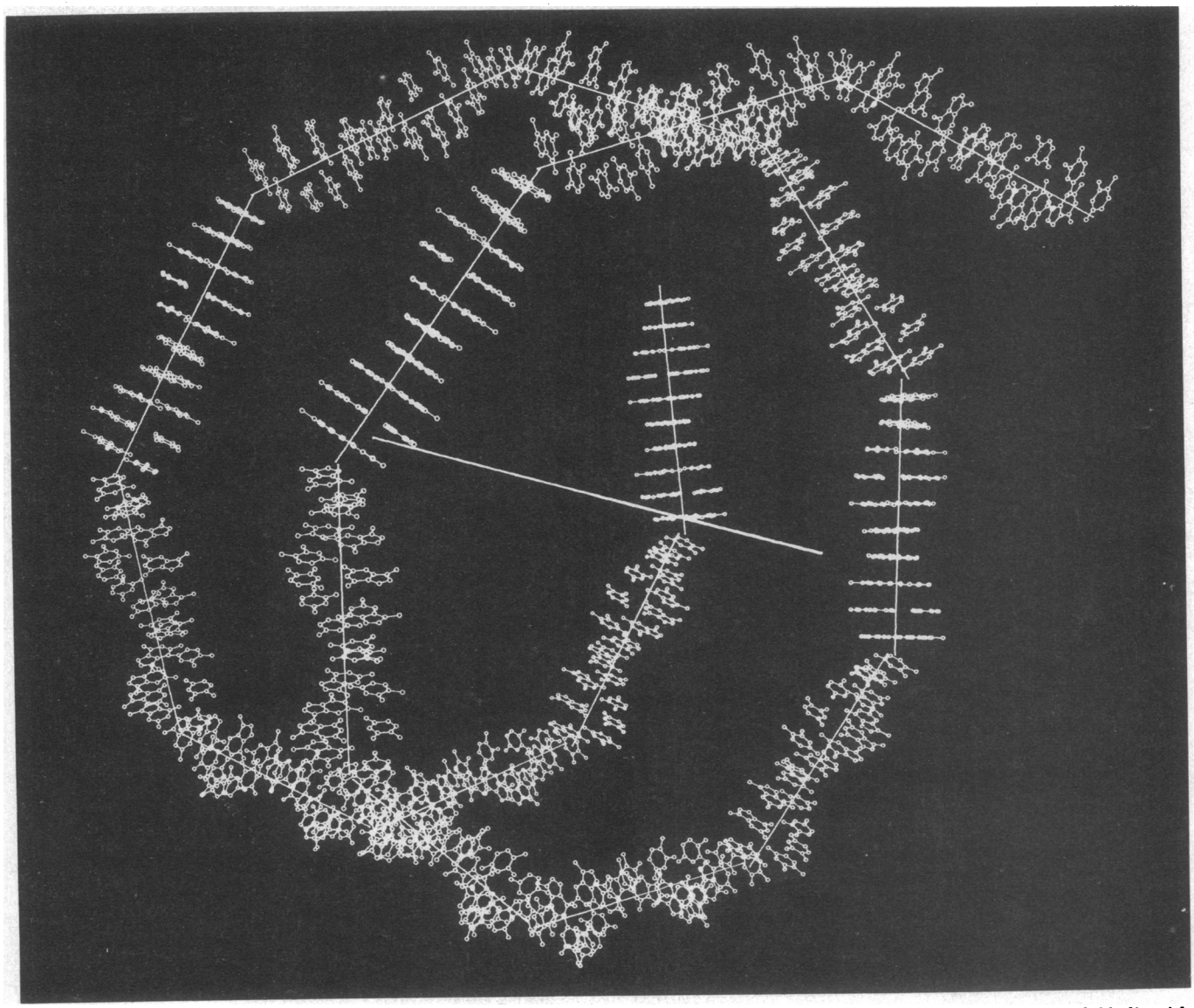

FIG. 3. Perspective illustration of κ kinked B DNA drawn by computer graphics. The structure is a left-handed kinked toroidal helix with a diameter of about 100 Å and contains somewhat less than two superhelical turns per 170 base-pairs. The long central line indicates the superhelical axis—the length shown is 90 Å. This basic structure may be used in the (partial) organization of DNA in chromatin.

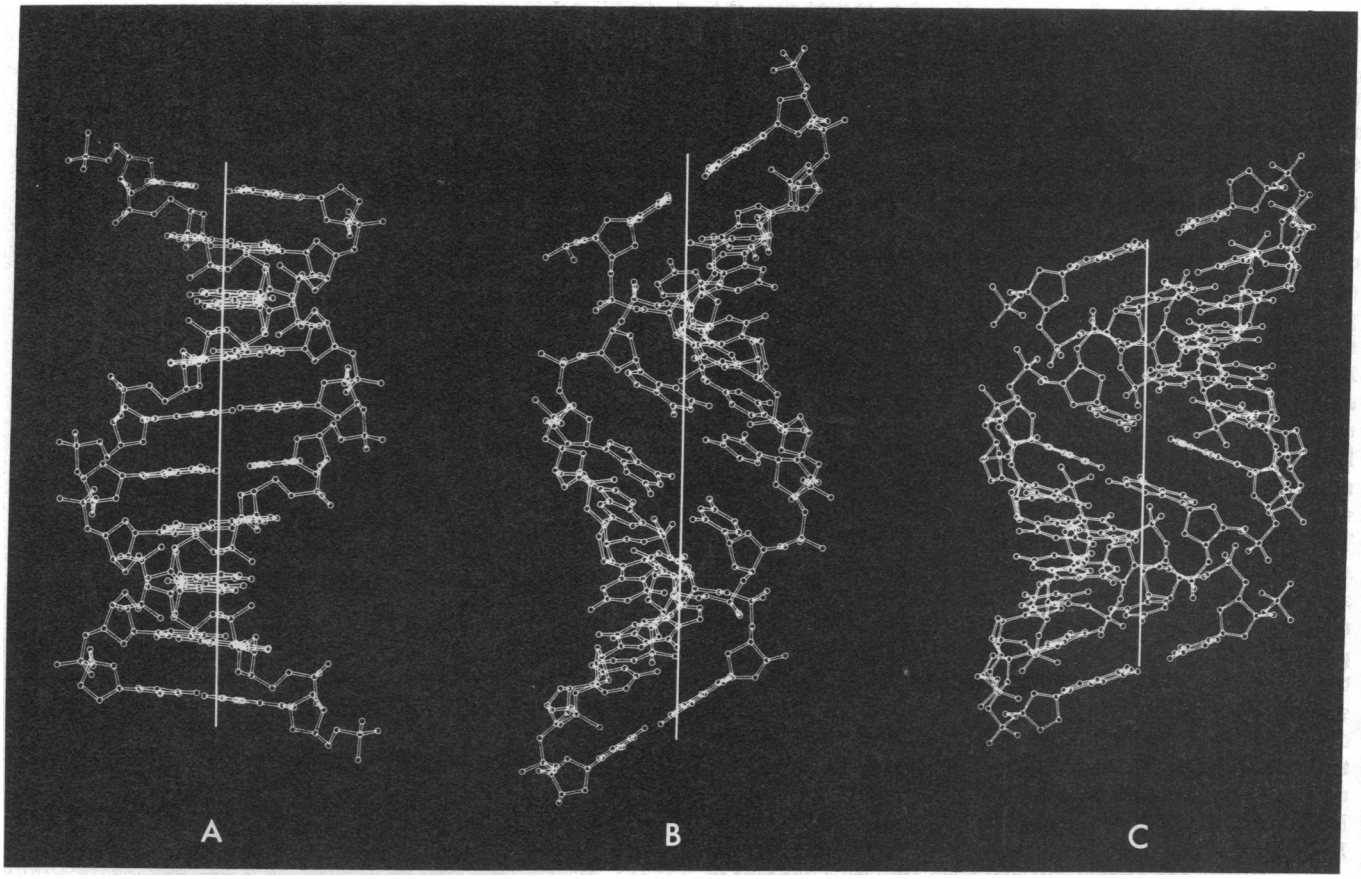

FIG. 4. Computer graphics illustration of various DNA structures. (A) B DNA. (B) β kinked B DNA. (C) A DNA. Localized domains of the β kinked B DNA structure could exist immediately prior to thermal DNA denaturation, exposing base-pairs to interactions with water molecules. *A structure such as this could be induced by the RNA polymerase enzyme when binding to (and denaturing) the promoter.* See *text* for discussion.

altering the glycosidic torsional angles as follows: χ_{CN} (for the C3′ *endo* deoxyribose sugars) from 29° to 36°, χ_{CN} (for the C2′ *endo* deoxyribose sugars) from 90° to 65°. In addition, there are numerous small (but systematic) changes in the sugar-phosphate torsional angles and some minor changes in bond angles. Although the overall features of the kink and intercalation sugar-phosphate geometries are very similar, an important difference concerns the positions of the helical axes of B DNA above and below the kink. Helix axes are displaced in the *opposite* sense in the kinked structure (i.e., −1.0 Å) (see Fig. 1A). Base-pairs at the kink are twisted by 26° (this value has been estimated as described earlier for intercalation); this gives rise to an effective unwinding of 10°. Other important parameters are (see Crick and Klug, ref. 8, for definitions): α = 40°, D = 2.30 Å, θ = 9.4°. The stereochemistry of the kink appears to be primarily determined by the sugar-phosphate geometry; however, back contacts between van der Waals surfaces of adjacent base-pairs at the kink may play an important additional role in determining the precise geometry of the kink. It is possible that different nucleotide sequences give rise to small variations in the kink parameters. We will discuss these and other stereochemical points in detail elsewhere.

Kinking of DNA in chromatin—κ kinked B DNA

The kinking scheme we propose requires minimal stereochemical rearrangement and probably involves small energies. This has prompted us to ask more generally if a conformational change such as this could be used by proteins of their interactions with nucleic acids.

A particularly interesting superhelical DNA structure possibly used in the organization of DNA in chromatin can be obtained by kinking B DNA every *10* base-pairs, a structure we have called κ kinked B DNA (see Fig. 2 for nomenclature). This structure (shown in Fig. 3) is a left-handed (kinked) toroidal helix with a diameter (estimated from the radius of the point in the middle of each kink) of about 100 Å. Each residue of the helix contains *10* base-pairs. The helix is generated from this residue by a twist of −41.1° and a translation along the helix axis of 5.26 Å. (The position of the helix axis and the parameters of the helix have been determined by a least squares procedure, using a computer program written by Dr. John M. Rosenberg and modified for our own use.) The dimensions of this basic structure are in reasonable agreement with current estimates of the size of the ν body and neutron diffraction data of calf-thymus chromatin (23). We have therefore asked if multiple domains of such a structure could give rise to the observed topological properties of covalently closed circular DNA molecules (24). We are not, however, able to provide a detailed answer to this question for several reasons.

First, if adjacent ν bodies were held together at still a higher level of superhelical organization and, related to this, if the 40 base-pair spacer region between ν bodies had its own distinctive structure, then this would alter the overall topological winding number. Second, connecting domains of left-handed toroidal helix, such as shown in Fig. 3, can be achieved in a number of ways and this, rather dramatically, can affect its overall topological properties (25). Finally, it is possible that DNA held in a nicked relaxed covalently closed circular duplex structure is

not *exactly* B DNA but some unwound variant of B DNA and, if this were true, this would affect estimates of the linking number and a comparison with that predicted by our model. We will therefore postpone a detailed discussion of these as well as other topological questions for a later paper.

Other kinked DNA structures

In addition to κ kinked B DNA, we have calculated the three-dimensional structures of B DNA that is kinked various numbers of base-pairs apart. We will publish the detailed structures elsewhere (β, γ, δ, and ϵ kinked B DNA are *right-handed* superhelices, whereas ζ, η, θ, and ι are *left-handed* superhelices); however, we wish to call attention to one structure of particular interest—β kinked B DNA (Fig. 4B). This structure (a structure analogous to the neighbor exclusion model for ethidium intercalation) has a variety of interesting properties. Since it is kinked every other base-pair, it is maximally unwound. This reduction in twist is at least partially compensated for by the structure's assuming a slow right-handed superhelical writhe. The structure has very similar linking to B DNA. It also has similar dimensions to B DNA. Important differences, however, are the dimensions of the narrow groove [this groove is enormously broadened compared to B DNA (Fig. 4A) and compared to A DNA (Fig. 4C)] and the accessibility of base-pairs to solvent. As Crick and Klug mention in their paper, kinking should be easiest for (A+T)-rich regions in DNA. Localized domains of β kinked structure could form immediately prior to thermal DNA denaturation [kinking could begin at (A+T)-rich regions, exposing base-pairs to interactions with water molecules]. *A structure such as this might also be induced by RNA polymerase when binding to (and denaturing) the promoter.* We find the latter a particularly attractive concept in view of the (A+T)-rich regions identified in the *lac* and λ promoters (26, 27), and the magnitude of angular unwinding associated with RNA polymerase binding (28). We will discuss these as well as other points in detail in a subsequent communication.

This work has been supported in part by grants from the National Institutes of Health, the American Cancer Society, and the Energy Research and Development Administration (ERDA). This paper has been assigned Report no. UR-3490-988 at the ERDA, the University of Rochester. We wish to thank Francis H. C. Crick for critically reading the manuscript and offering several important communications regarding the nature of superhelical DNA and chromatin.

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