pure heavy-atom soaps have been studied in some depth (*22–26*). However, because the present LB sample is a solid supported trilayer and is heterogeneous, consisting of a CdA monolayer and a ZnA bilayer, comparison with the aforementioned systems, although instructive, is not rigorous. At low temperatures, the dry powdered CdA exists in a lamellar (smectic) phase with rigid hydrocarbon chains densely packed in a crystalline arrangement, their long axis oriented normal to the bilayer surface. At 120°C, the chains undergo a conformation order/disorder transition accompanied by a transformation from lamellar to hexagonal long-range order. This high-temperature phase is of the inverted hexagonal type and it persists upon heating up to 220°C (*23*). To our knowledge no such information is available in the literature concerning the thermotropic phase properties of ZnA.

It is interesting to note that in the present mixed trilayer system a dramatic change in both the Zn-Cd separation and in the coherent fraction of Zn occurred close to the major thermotropic transition temperature observed in bulk CdA (*23*). However, because the standing wave method does not provide a direct determination of the molecular structure of the low electron density (carbonaceous) material in the assembly, one can only speculate on the underlying structural changes in the hydrocarbon core and polar headgroups of the trilayer that accompany this Zn shift. For example, this shift could be accounted for by one or a combination of the following events: hydrocarbon chain "melting," tilting, and/or interdigitation. In the present system, chain melting alone would contribute a shift of only 7 Å based on model calculations. Tilting alone, on the other hand, would require a 62.9° deviation of the long axis of the soap molecule from the normal to the planar surface to effect the measured 30.4 Å total reduction in the Zn-Cd separation. Such extreme tilting requires that a large number of methylene groups are taken out of van der Waals contact, which would prove to be an energetically expensive proposition. In contrast, interdigitation alone could account for a 28 Å shift since the hydrocarbon chain length of arachidate in its fully extended form is 28 Å and complete interdigitation of the chains is a distinct possibility. As noted previously, the observed shift may also come about from a combination of the above. The gradual "pretransitional" reduction in Zn-Cd separation could similarly be explained. It seems unlikely that the hexagonal phase is involved in the transition owing to a paucity of material in the LB film. It is possible that the fall in coherent fraction in the vicinity of 100°C derives from a coexistence in the same monolayer of soap molecules of the low- and high-temperature configuration.

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Single Strands, Triple Strands, and Kinks in H-DNA

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A naturally occurring $(dT\text{-}dC)_{18}$:$(dA\text{-}dG)_{18}$ repeat in the H conformation of DNA was shown to contain single-stranded nucleotides in the center of the TC_{18} repeat and on one half of the AG_{18} repeat. These results support the model that H-DNA is a structure containing both triple-stranded and single-stranded regions. The stability of this structure was affected by both *p*H and the degree of negative supercoiling: at *p*H 7.6 to 7.7, a high level of supercoiling was needed to keep about half of the molecules in the H conformation; at *p*H 6 and *p*H 5, normal levels of supercoiling supported H-DNA; and at *p*H 4, no supercoiling was required. At mildly alkaline *p*H, the TC/AG_{18} repeat assumed a novel conformation called J-DNA that differed from both the B and H forms. A three-dimensional model for the structure of H-DNA is proposed that accounts both for the single-strandedness of the nucleotides and for the influence of supercoiling on H-DNA formation. This model predicts and evidence is presented that H-DNA introduces a sharp kink in the DNA. Moreover, the angle of this kink appears not to be fixed, so that H-DNA is also a hinged-DNA.

THE REPEATING COPOLYMER $(dT\text{-}dC)_n$:$(dG\text{-}dA)_n$ in DNA (TC/AG_n) has an unusual structure, as revealed by its sensitivity to single-stranded specific nucleases (*1–7*) and its ability to relax negative supercoils in DNA (*5–8*). The most widely accepted model of this structure contains triple-stranded and single-stranded regions called H-DNA (*9, 10*). The need for symmetry in nucleotide sequence (*11*) and the location of bases that are sensitive to chemical or enzymatic probes (*1–7, 12*) support this model. Because all of the reagents used in those studies can react with bases that are in exposed but not single-stranded structures (*13*), those results are also consistent with other structures that have been proposed, such as self-paired cruciforms or altered double-stranded structures (*3–7, 14, 15*).

Models for the triple-stranded nature of H-DNA were based on earlier studies of mixtures of $(dT\text{-}dC)_n$ and $(dG\text{-}dA)_n$ oligonucleotides (TC_n and AG_n repeats). A one-to-one mixture of these oligonucleotides formed a triple-stranded helix spontaneously in mild acid, with one AG_n repeat pairing both to a TC_n repeat and to a protonated TC_n^+ repeat through Watson-Crick and Hoogsteen base pairs, respectively (*16*); the polypyrimidine strands are antiparallel (*17*). Hence, Lee *et al.* (*9*) and Lyamichev *et al.* (*10*) proposed that this conformation could

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be the basis of the unusual structure of TC/AG$_n$ copolymers. In their models, half of the copolymer bends on itself, allowing one half of the TC$_n$ to form Hoogsteen base pairs with the purines in the other half of the repeat; a triple-stranded structure containing C-G·C$^+$ and T-A·T base triplets would result (*10*). This model predicts that both the bent region of the TC$_n$ repeat and the entire denatured half of the AG$_n$ repeat would be single-stranded, thereby accounting for the nonrandom distribution of sites in the structure sensitive to nuclease S1 (*2*).

We present direct evidence that the reactive nucleotides are indeed single-stranded and that the structure results from a disproportionation of a DNA duplex into a triplex plus single-stranded polypurine sequences. Our three-dimensional model for this structure explains why H-DNA relaxes negative supercoils and the model predicts that the structure introduces a severe kink in the DNA. We show that this kink exists and that flexibility at the base of the kink makes it into a hinge.

We mapped accessible nucleotides in a TC/AG$_{18}$ repeat using methoxylamine (MA) (*18, 19*) and osmium tetroxide (OT) (*20*), which are specific for exposed C and T nucleotides, respectively; accessible purines were probed with diethyl pyrocarbonate (DEPC) (*21*). Reactive nucleotides within the sequence were located by the ability of the adducts to terminate transcription chemically in vitro. After reaction, the modified DNA was repurified and used as a template for RNA synthesis in vitro. This rapid mapping procedure (*22*) makes use of a template with convergent T7 or SP6 promoters (in separate reactions) so that either DNA strand can be used as a template. The template we used, derived from a region 1.8 kb downstream of a human *U1* snRNA gene, contains 18 repeats of the dinucleotide (dT-dC)$_n$:(dA-dG)$_n$, which we call the TC/AG$_{18}$ region (Fig. 1). For convenience, we refer below to sequences at the left or right side of the structure, as drawn.

When TC/AG$_{18}$-containing plasmids (at normal bacterial supercoiling density) were treated with MA or OT at *p*H 4 to 6, the center of the TC repeat (five to seven nucleotides) and a T-rich region adjacent to the right side of the repeat revealed reactive regions (Fig. 2A, lanes 1 to 4).

Efficient H-DNA formation required mild acid consistent with the need to protonate C residues in the third strand of H-DNA. Reactivity was greatly reduced at *p*H 7 and above (Fig. 2A, lanes 5 and 6), but was retained when the DNA was highly supercoiled (Fig. 2A, lane 10). Overexposure of lanes 5 and 6 revealed a low but significant level of reactivity at *p*H 7 and 8

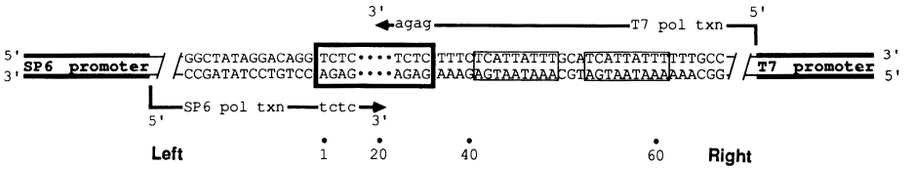

Fig. 1. Structure of the TC/AG$_{18}$ repeat and flanking sequences used for modification and transcription. The copolymeric repeat, denoted by a heavy box, contains 18 copies of (dT-dC):(dA-dG); the T:A base pair immediately to the right of this repeat may also participate in the structure because it represents half of an additional repeat. An A-T–rich direct repeat is shown by the light boxes. This segment, derived from a region located 1.8 kb downstream of the human *U1* gene *HU1-1*, was cloned as a 180-bp Sac I–Bam HI restriction fragment (*2*) into the pGEM-2 vector (Promega Corp.). The solid dots denote every 20th nucleotide starting at the 5′ end of the TC$_{18}$ repeat. Base modifications were carried out on this supercoiled DNA, and the adducts were mapped by in vitro transcription with T7 or SP6 RNA polymerases (pol txn, polymerase transcription). The lowercase letters above or below the DNA sequence represent the product RNAs. Transcription that used the TC$_{18}$ strand as template (from the T7 promoter) resulted in the shortest products coming from the 3′ side of the TC$_{18}$ strand, or the right side of the structure as drawn here; likewise, transcripts that probed the AG$_{18}$ strand (from the SP6 promoter) had their shortest products at the left side of the structure. Modification of T by OT resulted in transcripts that terminated at the corresponding A, whereas modification of C by MA resulted in transcripts ending with the nucleotide encoded by the prior nucleotide in the template (*22*); transcription also stopped one base short of DEPC-modified purines.

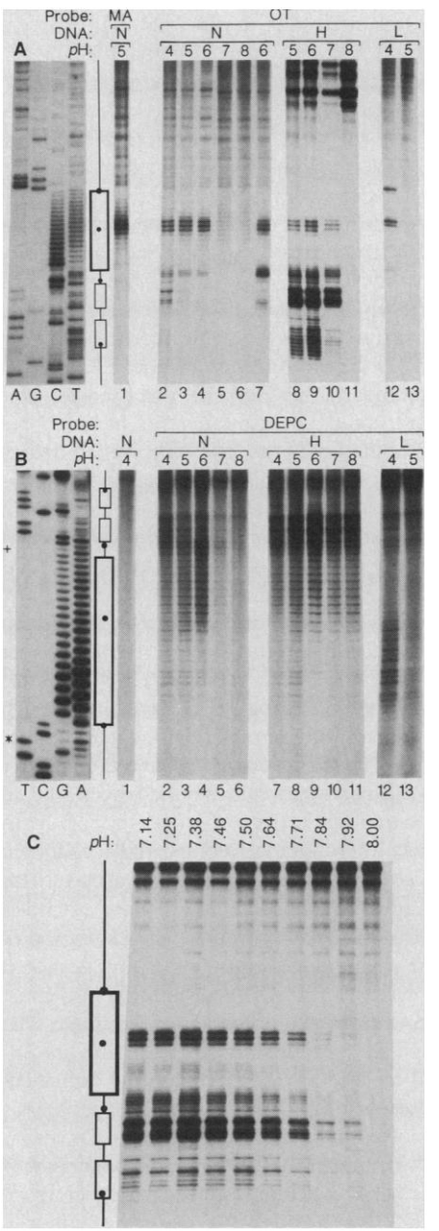

Fig. 2. Reactive regions of the TC/AG$_{18}$ copolymer, as revealed by termination of transcription at modified bases in the template. (**A**) Modification of pyrimidines in TC$_{18}$. The template DNA was modified with methoxylamine (MA) or osmium tetroxide (OT), which react with single-stranded C or T residues, respectively, at a variety of *p*Hs (*30*). DNAs in lanes 1 to 7 were at normal bacterial supercoiling density (about −0.05), whereas DNAs in lanes 8 to 11 were at >−0.1 supercoiling density [made by treating closed circular DNA with topoisomerase I in the presence of ethidium bromide (25 μg/ml)]; DNA in lanes 12 and 13 was relaxed by treatment with Sin I. After reaction, the DNAs were purified and used as templates for T7 RNA polymerase in the presence of α-[^{32}P]uridine triphosphate (*31*). In parallel, chain-terminated marker RNAs were made with 3′ deoxynucleoside triphosphate (*22, 32*) (lanes on the far left side). Transcripts were analyzed in an 8% (29:1) polyacrylamide–7*M* urea gel. Symbols N, H, and L refer to normal or high levels of supercoiling or linearized DNA, respectively; A, G, C, and T indicate the nucleotide in the template that directed incorporation of the chain-terminating nucleotide. Major landmarks in the sequence (Fig. 1) are indicated to the left of lane 1. Lane 1 was shifted up by one nucleotide in the photograph, to compensate for these products terminating one nucleotide prior to the modified base in the template. (**B**) Modification of purines in AG$_{18}$ (*33*). Markers on the left are chain-terminated RNAs made with the AG$_{18}$ strand as template and SP6 RNA polymerase. This sequencing gel was shifted by one nucleotide to compensate for termination of transcription one nucleotide before DEPC-modified nucleotides in the template. The * and + indicate misincorporated nucleotides in the sequencing ladders; from other experiments, we deduced that those should be a T and A, respectively. Landmarks are shown as in Figs. 1 and (A). (**C**) Stability of H-DNA at elevated *p*H. Highly negatively supercoiled DNA containing TC/AG$_{18}$ was reacted with OT at the *p*Hs indicated and the adducts were mapped as in (A), except that the DNAs were linearized with Sin I before transcription (*34*).

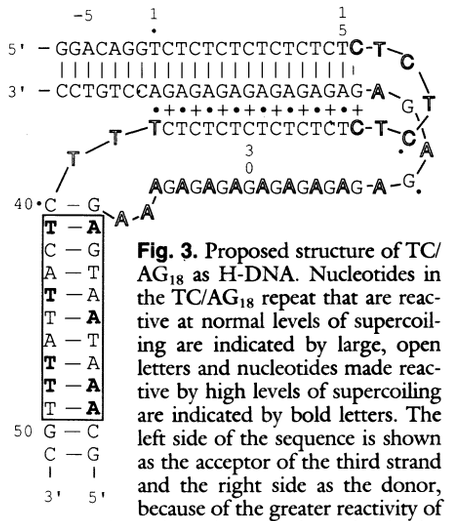

Fig. 3. Proposed structure of TC/AG_{18} as H-DNA. Nucleotides in the TC/AG_{18} repeat that are reactive at normal levels of supercoiling are indicated by large, open letters and nucleotides made reactive by high levels of supercoiling are indicated by bold letters. The left side of the sequence is shown as the acceptor of the third strand and the right side as the donor, because of the greater reactivity of purines 19 through 39 than of 1 through 16 (Fig. 2B, lanes 2 to 4) under the conditions used.

that accounted for the nuclease S1 sensitivity of TC/AG_n repeats at *p*H 7 (*2*), indicating that H-DNA may exist under physiological conditions.

Modification of the purine-containing AG_{18} strand with DEPC revealed a larger area of reactive nucleotides, concentrated toward the right side of the structure (Fig. 2B). This pattern is clearest at *p*H 6 (Fig. 2B, lane 4), although the bias is evident at *p*H 4 and *p*H 5 (Fig. 2B, lanes 2 and 3). As with the polypyrimidine strand, significant reactivity at *p*H 7 or above required high levels of negative supercoiling (compare lanes 5 and 10 of Fig. 2B). The region of reactivity in the AG_{18} strand starts to the left of the midpoint of the repeat and extends into the right-hand junction region. Using a different assay, other workers have also found a strong bias toward reactivity in one half of the polypurine strand (usually the 5′ side) (*3–6, 23, 24*).

The locations of reactive nucleotides in the TC/AG_{18} repeat in mild acid agree well with a fold-back H-DNA structure for TC/AG_n repeats previously proposed (*9, 10*). In that model, the repeat contains both single-stranded and triple-stranded regions (Fig. 3). The pattern of reactivity of bases in the AG_{18} repeat in negatively supercoiled DNA at *p*H 6 is consistent with the right half of this region being single-stranded; the greater range of reactivity at *p*H 5 and *p*H 4 (Fig. 2B, lanes 2, 3, and 12) indicates that at lower *p*Hs (and in relaxed DNA; see below), some molecules have the triple-stranded structure at the other end of the TC/AG_{18} region (*2*).

Both strands of the repeat were reactive at *p*H 7 and above when the DNA was highly negatively supercoiled. At *p*Hs above 7.5, H-DNA was destabilized, as shown by the loss of reactivity to OT in Fig. 2C. This loss of pyrimidine reactivity (*p*H ≥ 7.8) reflected not a return to the B form but rather a conversion to a novel conformation in which the polypurine strand remained reactive (Fig. 2B, lane 11). This purine strand showed reactivity even above *p*H 9 (*8*). The nature of this new conformation, which we call J-DNA, is unclear: because reactivity was manifested only in one half of one strand, two nonequivalent domains must exist under these conditions.

About half of the molecules in the DNA preparation used were still in the H-DNA conformation between *p*H 7.6 and 7.7 (Fig. 2C). Thus the high degree of negative supercoiling increases the ability of the third strand to remain protonated at elevated *p*H. Because the reduction in the level of H-DNA at elevated *p*H results in formation of the novel J-DNA conformation (Fig. 2, A and B, lanes 11), this apparent *p*K of 7.6 to 7.7 reflects the equilibrium of H-DNA with J-DNA rather than with B-DNA. Protons may remain trapped in J-DNA, but that seems unlikely because of the *p*H dependence of the H ⇌ J equilibrium.

Pyrimidines in the junction between the TC/AG_{18} repeat and the normal heteropolymeric DNA were reactive under all conditions that supported the H-DNA conformation (Fig. 2). With highly supercoiled DNA, the region of reactivity extended further into flanking sequences (Fig. 2A, lanes 8 and 9, and Fig. 2C). This result probably reflected the enlargement of a denaturation bubble at the junction between the B and H forms, because the A-T–rich sequences of the flanking region are prone to breathing; this was shown by the accumulation of OT adducts when the reaction conditions were more extensive (Fig. 2A, lane 7). Recruitment of a short flanking region polypyrimidine repeat into the H-DNA structure (by forcing the accommodation of a C-G·T triplet) is unlikely in this case: if that had occurred, the region of reactivity in the middle of the TC_{18} repeat would have been shifted to the right (for example, compare lanes 4 and 9 of Fig. 2A).

At low *p*H (*p*H 4), supercoiling was not required for H-DNA formation (Fig. 2, A and B, lanes 12 and 13), which explains our earlier finding (*2*) that supercoiling is not essential for formation of a nuclease S1-sensitive structure at low *p*H: presumably, protonation of single-stranded A residues altered the relative stability of H-DNA structure over duplex DNA so that negative supercoiling energy was not required. Curiously, in this structure the other half of the polypurine repeat was reactive, indicating

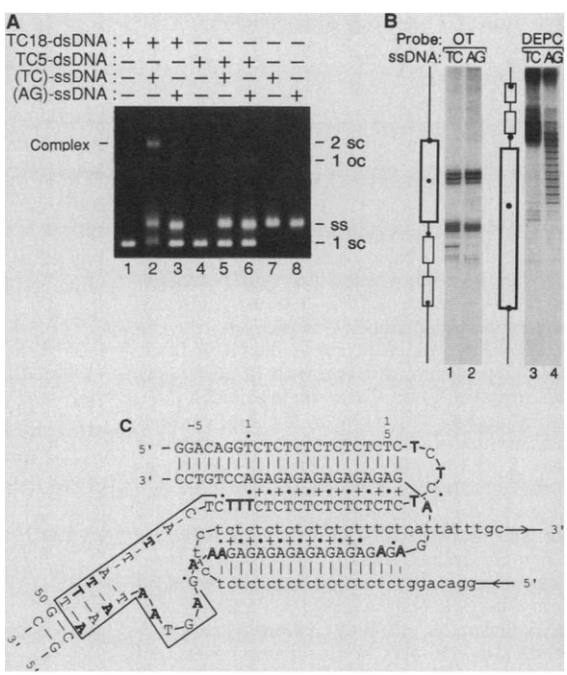

Fig. 4. Complex formation between TC/AG_{18} in H-DNA and TC_{18} in single-stranded phage M13 DNA. (**A**) Agarose gel analysis of complexes formed between negatively supercoiled duplex plasmid DNA containing TC/AG_{18} and single-stranded phage DNA containing TC_{18} (*35*). Symbols: 1 sc = monomer supercoils; 2 sc = dimer supercoils; 1 oc = monomer open circles; and ss = single-stranded M13 DNA. The complex between the DNAs containing TC/AG_{18} and TC_{18} is indicated. (**B**) Effect of complex formation on the reactivity of nucleotides in the TC/AG_{18} repeat. Complexes between duplex plasmid DNA containing TC/AG_{18} and single-stranded phage DNA containing TC_{18} (lanes 1 and 3) were treated at *p*H 5.0 with OT or DEPC, as indicated (*36*). Plasmid DNA incubated with phage DNA containing AG_{18} was isolated as an uncomplexed supercoiled circle and analyzed in an analogous fashion (lanes 2 and 4). TC_{18} and AG_{18} indicate the sequence of the insert in the phage DNA. (**C**) Model for the complex between duplex and single-stranded DNAs containing TC/AG_{18} and TC_{18}, respectively. The triple-stranded structure on the top is the same as that in Fig. 3. The one below illustrates hybridization by Watson-Crick pairing of the previously reactive polypurine region of the plasmid TC/AG_{18} to part of the TC_{18} sequence in the phage DNA (shown by lowercase letters). Because such hybrids are helical, the TC_{18} sequence in the phage DNA must also unwind its way back out of the plasmid D-loop; this can be done by traversing back down the helix, which would allow for a second (intermolecular) region of H-DNA. Bold letters indicate flanking region nucleotides in plasmid DNA that are made accessible as a result of the association of the phage and plasmid sequences.

that in nonsupercoiled DNA the H-DNA was oriented with the left side of the polypyrimidines as the denoted third strand.

The reactivity of purines within the repeat to chemical probes (Fig. 2B) was consistent with this region being in a single-stranded, stacked configuration, but these bases could also be in alternate types of base-paired structures (*3–7, 12–15*). Thus we tested the ability of the reactive polypurines to hybridize to a circular single-stranded complementary DNA. The purines were indeed single-stranded, since they formed a 1:1 complex with a single-stranded TC_{18} repeat (in phage M13mp9 DNA; Fig. 4A, lane 2). Complexes were not observed when the double-stranded DNA contained TC/AG_5 (which is insensitive to nuclease S1; 2) (Fig. 4A, lanes 4 to 6) or when the single-stranded DNA instead contained AG_{18} (Fig. 4A, lane 3). Supercoiling of the plasmid DNA was required for the interaction with the circular single-stranded M13 DNA, although supercoiling is not required for formation of triplexes between TC/AG_n repeats in duplex DNA and short TC_n oligonucleotides (*9, 16, 17, 25*). This difference indicates that the association of sequences in the plasmid and M13 DNAs was not simply by hybridization of the duplex TC/AG_n repeat by single-stranded TC_n, as occurs with oligonucleotides (*17, 25*).

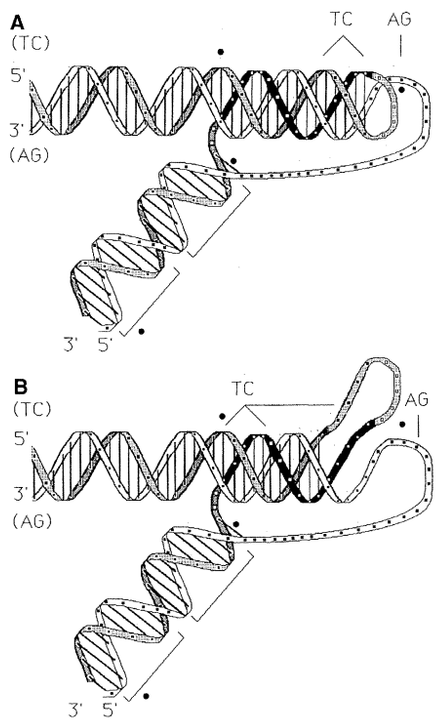

Fig. 5. Three-dimensional model for H-DNA. (**A**) The model is based on reactivity (Fig. 2) and topological (*8*) arguments. The strands containing the AG_{18} repeat and TC_{18} repeat are drawn as white and shaded ribbons, respectively; the donated polypyrimidine repeat is drawn in black. Helices are arbitrarily drawn with 11 nucleotides per turn. The form of the single-stranded polypurine region is unknown, but our evidence indicates that the bases are stacked, probably in pairs (*2, 4*). For orientation, the A-T–rich repeats in the right flanking region are indicated by thin lines, and every 20th nucleotide is indicated by a dot. (**B**) Demonstration that the triple-stranded region has no net linking. In theory, the bent-back polypyrimidine strand could be unfolded from the triple helix without disturbing the B-DNA helices in flanking regions. The figure shows the first part of this process which, if extended to the base of the triplex, would yield two unpaired strands containing the copolymeric repeat sequences. This lack of net local linking within H-DNA means that nucleotides that were originally in approximately three turns of B-DNA are topologically denatured. This denaturation results in a reduction of the negative supercoiling density in the remainder of the DNA molecule, accounting for the influence of negative supercoiling on H-DNA formation.

To ensure that the intermolecular complexes resulted from association of the phage TC_{18} repeat with only the 18- to 21-residue-long reactive purine repeat, rather than with the entire TC/AG_{18} duplex (*17, 25*), plasmid-derived sequences in the intermolecular complex were probed for reactivity. As shown in Fig. 4B (lanes 1 and 3), only the bases in the middle of both strands were reactive. (Controls in lanes 2 and 4 of Fig. 4B showed that this pattern depended on complex formation.) The central region of reactivity was incompatible with a uniform intermolecular triplex structure, showing that the single-stranded TC_{18} repeat was associated with the half of the polypurines that had previously been accessible to the reagents. The purines in the middle should be reactive, since they are needed as a bridge between the ends of the triple-stranded plasmid DNA and the complex with the TC_{18} repeat of M13 DNA. The short extension of reactivity into the flanking region presumably results from steric requirements imposed by the new intermolecular complex; however, the lack of significant new regions of reactivity indicates that the M13 DNA did not induce the formation of a new structure in the plasmid DNA.

The intertwining of the two closed DNA circles (that is, the M13 DNA and the D-loop of the single-stranded AG repeat) probably resulted in formation of an additional triple-stranded H-DNA structure (Fig. 4C). Base pairing of the TC and AG sequences would result in a double-stranded helix composed of two intertwined circles that could exist only if the circular single-stranded M13 DNA also unwound its way back out. This unwinding could be accomplished by folding the TC repeat of the phage DNA back on itself and screwing its back down the helix. This path would also facilitate formation of the intramolecular H-DNA triple-stranded structure (Fig. 4C). By sequestering the single-stranded purines, this second H-DNA helix would stabilize the intermolecular H-DNA structure, allowing it to survive exposure at *p*H 7.9 for 18 hours during electrophoresis. This hybridization of the TC_{18} repeat in M13 DNA to the previously reactive region of the polypurines in the plasmid DNA unequivocally demonstrates that the latter sequence was indeed single-stranded when the TC/AG_{18} repeat was in the H conformation.

We propose a three-dimensional model for H-DNA (Fig. 5A) in which the TC/AG_{18} region is topologically denatured (has no net local linking of strands) between the left and right ends of the repeat. The folded-back polypyrimidine strand (gray and black) could be unwound from the polypurine strand back to the bottom of the triplex, resulting in the release of two nonintersecting denatured strands (Fig. 5B). Because this reduction in local linking lowers the negative supercoiling density in the rest of the molecule, H-DNA formation is promoted by negative supercoiling. The same unlinked intertwining of strands shown in Fig. 5 is responsible for the intermolecular triple-stranded complex illustrated in Fig. 4C. The model shown in Fig. 5 uses the left half of the repeat as the acceptor of the third strand; a similar model accommodates a right-half acceptor conformation (*8*).

A prediction of our model (Fig. 5A) is that H-DNA should introduce a severe kink in DNA molecules. Such a kink would retard the electrophoretic mobility of a DNA fragment, and this retardation would increase as the kink is placed closer to the center of the fragment (*26*). Because TC/AG_{18} assumes the H-DNA conformation in relaxed linear DNA at *p*H 4 (Fig. 2, A and B, lanes 12), we could test this prediction directly. Plasmid DNAs containing TC/AG_{18} repeats were digested with restriction enzymes to produce fragments about 400 nucleotides long with the repeat at various distances from the center (Fig. 6A). As a control we used identical fragments with a shorter insert, TC/AG_5, which does not form H-DNA even in negatively supercoiled plasmids (*2, 8*).

Formation of H-DNA at *p*H 4 reduced fragment mobilities (Fig. 6B, left), and the effect on the mobility was much more pronounced when the repeat (and hence the H-DNA) was near the middle of the fragment. No retardation of mobilities was observed in the absence of H-DNA, with TC_5 or at *p*H 5 (Fig. 6B, right).

The kink introduced by H-DNA differs significantly from bends that are introduced into DNA by short repeats such as dA-dA:dT-dT (*27*), in that kink formation is reversible and the angle of the kink is vari-

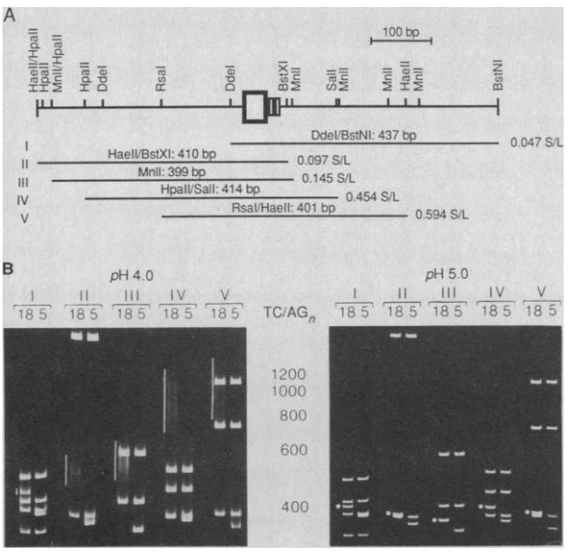

Fig. 6. Kinking in H-DNA, as demonstrated by retardation of electrophoretic mobilities. (**A**) Map of restriction fragments containing the TC/AG repeat at various locations. The fragments were between 399 and 437 bp long, including the 36-bp TC/AG_{18} repeat. Fragments with the TC/AG_5 repeat were 26 bp shorter and had a T→C transition 57 nucleotides to the right of the repeat; *S/L* indicates the ratio of the number of base pairs in the shorter and longer flanking regions in each fragment. (**B**) Electrophoresis of restriction fragments containing TC/AG repeats that formed H-DNA. Numerals I to V refer to the fragments mapped in (A) and 18 or 5 refers to the number of TC/AG repeats in the fragment. The gel on the left was run at *p*H 4.0, a condition that allows H-DNA formation in relaxed DNA fragments containing TC/AG_{18} but not TC/AG_5. The vertical lines on the left indicate the mobilities at *p*H 4.0 of the fragments containing TC/AG_{18}. As the *S/L* ratio increases, the fragments migrate more slowly and the band becomes smeared. The gel on the right was run at *p*H 5.0, under conditions that do not allow H-DNA formation in relaxed DNA fragments. The white dots on the right indicate the mobilities at *p*H 5.0 of the fragments containing TC/AG_{18}. The numbers between the gels indicate the approximate lengths (base pairs) of linear fragments containing B DNA. Prior to electrophoresis, DNAs were treated with electrophoresis buffer for 1 hour at room temperature. Electrophoresis was in a 19-cm-long 5% (60:1) polyacrylamide gel containing 33.3 m*M* sodium citrate, *p*H 4.0 or *p*H 5.0, at room temperature for 11 to 12 hours at 100 volts (43 to 53 mA); the gel had been made in 5 m*M* tris, 5 m*M* borate, 90 m*M* NaCl, 0.1 m*M* EDTA, and was equilibrated with the citrate buffer by prerunning for 3 hours.

able. Besides retarding the mobilities of fragments, H-DNA causes fragments to migrate in broad, streaky bands at *p*H 4.0 (Fig. 6B). This streakiness is primarily a result of interconversion between B and H forms during electrophoresis; at progressively higher *p*Hs (conditions that favor B-DNA), the streaks migrated progressively faster (*8*). Strikingly, the fragment migrated as a discrete band at either *p*H 4.29 or *p*H 3.86 with normal or anomolously slow mobilities that indicated being completely B- or H-DNA, respectively (*8*). The sharp transition between B- to H-DNA over a *p*H change of only 0.43 unit implies that H-DNA formation is highly cooperative.

Even at *p*H 3.86, where the fragment migrated as a band, the band was broader than others in the same gel (*8*), indicating H-DNA heterogeneity. This heterogeneity probably resulted from flexibility of the single-stranded nucleotides at the bottom of the triple helix that would allow the angle between the duplexes on either side of the H-DNA (that is, the angle of the kink) to vary. This hinge-like flexibility at the kink is an additional justification for the name H-DNA, since it is hinged-DNA.

The existence of reactive regions characteristic of H-DNA even at neutral *p*H (lanes 5 and 6 of Fig. 2, A and B) demonstrates that this structure can exist, albeit at low levels, under physiological conditions. It is unclear if H-DNA actually exists in cells, although there is some evidence that this is the case (*28*). The presence of proteins and polyvalent cations in cells and the high degree of negative supercoiling generated behind transcription or replication complexes (*29*) might thus support H-DNA formation. The kink introduced into DNA by this structure could serve both to bring DNA binding proteins into proximity with each other and to inhibit assembly of stable chromatin, thereby promoting active and continued transcription.

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30. For MA (lane 1) DNA (100 μg/ml) was treated with 3*M* methoxylamine diethylamine at 23°C for 120 min in 25 m*M* sodium acetate, *p*H 5.0 (lane 1). For OT (lanes 2 to 13), DNA (50 μg/ml) was treated at 22°C with 2.5 m*M* OT for 5 min (lanes 4 and 9) or 65 min (lanes 3, 8, and 13) or with 10 m*M* OT for 5 min (lanes 5 to 7, 10, and 11) or 60 min (lanes 2 and 12). Buffers, which contained 2% pyridine, were 100 m*M* sodium acetate, *p*H 4.0 (lanes 2 and 12); 100 m*M* sodium acetate, *p*H 5.0 (lanes 3, 8, and 13); 100 m*M* sodium cacodylate, *p*H 6.0 (lanes 4, 7, and 9); 100 m*M* sodium Hepes, *p*H 7.0 (lanes 5 and 10); and 100 m*M* sodium cacodylate, *p*H 8.0 (lanes 6 and 11).
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33. DNAs at normal (lanes 1 to 6) or high (lanes 7 to 11) supercoiling densities or relaxed DNA (lanes 12 and 13) were reacted with 10 μl of DEPC for 40 min (with vortexing every 5 min) in 400 μl of 100 m*M* buffer. DNA in lane 1 was incubated at *p*H 4 without DEPC. DNA concentrations were 19 μg/ml (except in lanes 4 and 9, 12.5 μg/ml). Buffers for each lane: sodium acetate, *p*H 4.0 (lanes 1, 2, 7, and

12); sodium acetate, *p*H 5.0 (lanes 3, 8, and 13); sodium MES [2-(*N*-morpholino)ethanesulfonic acid], *p*H 6.0 (lanes 4 and 9); Hepes, *p*H 7.0 (lanes 5 and 10); and Hepes, *p*H 8.0 (lanes 6 and 11). After incubation, DNAs were reisolated, cut with Sin I, and used as templates as described above, except SP6 RNA polymerase and α-[^{32}P]guanosine triphosphate were used and products were separated in a 15% (29:1) polyacrylamide–7*M* urea gel.

34. DNA was further supercoiled by treatment with topoisomerase I in the presence of ethidium bromide (25 μg/ml). The modification was in 1 m*M* OT, 100 m*M* sodium Hepes–2% pyridine for 7 min at room temperature.
35. pGEM DNA (0.1 μg) with a TC/AG_{18} or a TC/AG_5 insert was incubated with or without 0.2 μg of M13mp9 DNA (with the TC_{18} or AG_{18} insert shown in Fig. 1), as indicated, in 20 μl of 200 m*M* sodium acetate, *p*H 5, at 37°C for 24 hours. Electrophoresis was in 1% agarose at 12° to 14°C for 18 hours in 40 m*M* tris, 25 m*M* sodium acetate, *p*H 7.9, and 0.9 m*M* sodium EDTA.
36. Complexes were formed by using 15 μg/ml plasmid DNA and 30 μg/ml phage DNA in 100 m*M* sodium acetate, *p*H 5.0, at 37°C for 18 hours. The DNAs were either treated with 10 m*M* OT, as in lane 3 of Fig. 2A (lane 1), or with DEPC, as in lane 2 of Fig 2B (lane 3). After extraction and purification, complexes were isolated by agarose gel electrophoresis at *p*H 7.8 to 7.9 at 8°C and analyzed by transcription by using T7 (lane 1) or SP6 RNA polymerases (lane 3). RNA products were analyzed along with marker chain-terminated transcripts, as in Fig. 2.
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