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Phased Adenine Tracts in Double-Stranded RNA Do Not Induce Sequence-Directed Bending†

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ABSTRACT: Tracts of four to six adenines phased with the DNA helix produce a sequence-directed bending of the helix axis. Here, using gel electrophoresis and electron microscopy (EM), we have asked whether a similar motif will induce bending in a duplex RNA helix. Single-stranded RNAs were transcribed either from short synthetic DNA templates or from *Crithidia fasciculata* kinetoplast bent DNA, and the complementary single-stranded RNAs were annealed to produce duplex RNA molecules containing blocks of four to six adenines. Electrophoresis on polyacrylamide gels revealed no retardation of the RNAs containing phased blocks of adenines relative to duplex RNAs lacking such blocks. Examination by EM showed most of the molecules to be straight or only slightly bent. Thus, in contrast to DNA duplexes, phased adenine tracts do not induce sequence-directed bending in double-stranded RNA. Analysis of the distribution of molecule shapes for the highly bent *C. fasciculata* DNA showed that the adenine blocks do not act cooperatively to induce DNA bending and that the molecules must equilibrate between a spectrum of bent shapes.

The folding of natural RNA creates molecules of great structural complexity. As seen in ribozymes and ribosomal RNA, folded RNA contains segments of perfectly and imperfectly paired duplexes joined by regions containing nonpaired bulges of varying size. The duplex regions themselves may contain unusual sequence arrangements, which in duplex DNA can create bends, left-handed helices, cruciforms, and slipped structures (Wells, 1988). A major challenge in understanding RNA folding is to understand the structure of each of these elements alone. In some cases, lessons can be taken from what is known for duplex DNA; as described below this appears to be true for bulges. In this paper we have investigated the possibility of "RNA bending" by phased blocks of oligo(rA).

In duplex DNA, bulges of one to five bases have been shown to create stiff kinks that can cause significant electrophoretic retardations of the bulge-containing DNA (Bhattacharyya & Lilley, 1989; Hsieh & Griffith, 1989; Rice & Crothers, 1989). On the basis of this finding, the laboratories of Lilley and Draper (Bhattacharyya et al., 1990; Tang & Draper, 1990) showed that bulges introduce kinks into the RNA helix, the magnitude depending on the number and types of bases in the

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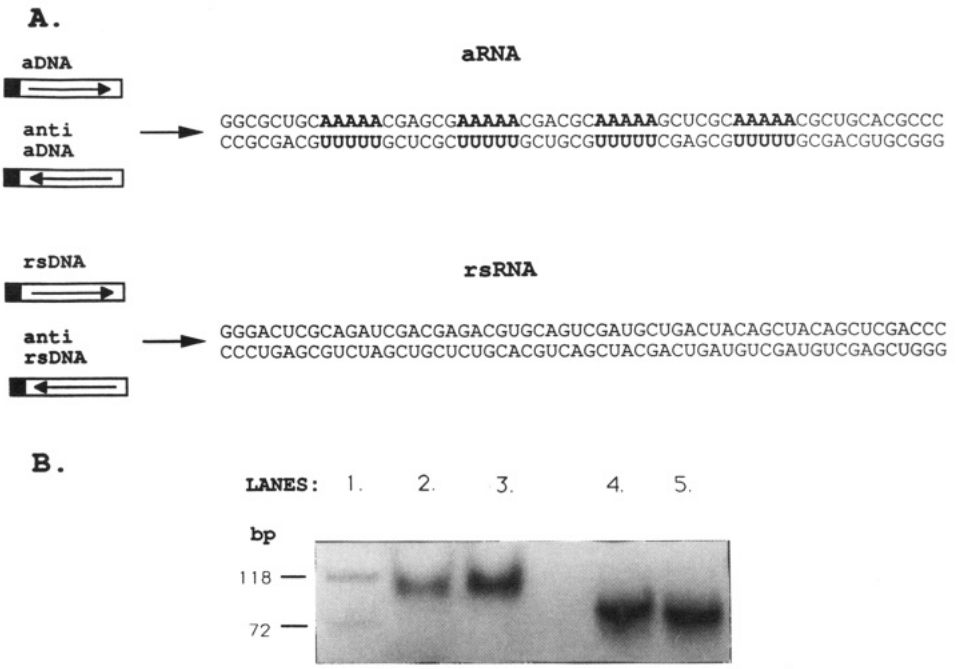


FIGURE 1: Electrophoretic mobility of 58-bp double-stranded RNA containing phased adenine blocks. (A) Represented to the left of the arrow as aDNA, anti-aDNA, rsDNA, and anti-rsDNA are the DNA templates used to transcribe the RNAs. The templates contain a T7 RNA polymerase promoter (shaded area within the box) and sequence information to encode each complementary strand for aRNA and rsRNA. The sequence for aRNA and rsRNA is given to the right. (B) Electrophoresis of aDNA, rsDNA, aRNA, and rsRNA on a 12.5% polyacrylamide gel is shown: lane 1, ^{32}P-labeled ϕX174 DNA marker; lane 2, aRNA; lane 3, rsRNA; lane 4, aDNA; lane 5, rsDNA. The numbers on the left correspond to the size of the ϕX174 markers.

bulges and their position in the fragment. Within the cell, bulges in RNA may create specific recognition sites for proteins such as the coat protein of phage R17 (Wu & Uhlenbeck, 1987) and the tat protein of human immunodeficiency virus (Weeks et al., 1990).

Sequence-directed bending in DNA, now a well-established phenomenon (Hagerman, 1990), was first postulated by Selsing et al. (1979), who suggested that a kink or bend is produced when an A-form helix abuts a B-form helix. The first naturally occurring bent DNA was found in kinetoplast minicircles of trypanosomes and their relatives (Marini et al., 1982; Natambi et al., 1984). DNA fragments from these minicircles were found to migrate more slowly in highly cross-linked gels than expected for their size, and EM[1] (Griffith et al., 1986) directly revealed these DNAs to describe a curved shape. Since then, sequence-directed bends have been found in many other DNAs in particular at sites controlling initiation and termination of DNA replication and transcription (Bossi & Smith, 1984; Zahn & Blattner, 1985a,b; Koepsel & Khan, 1986; Ryder et al., 1986; Snyder et al., 1986; Plaskon & Wartell, 1987; Stenzel et al., 1987; Hsieh & Griffith, 1988).

Most of these bent DNAs contain runs of adenines in phase with the helix. A 211 bp[1] segment isolated from the kinetoplast minicircles of *Crithidia fasciculata* contains 18 runs of four to six adenines, 16 of the runs being in the same strand and many being in phase with each other and with the DNA helical repeat (Kitchin et al., 1986). Several models have been proposed to explain the bending of DNA by phased blocks of adenines. In the wedge model (Trifonov, 1980; Trifonov & Sussman, 1980), it is assumed that different neighboring base pairs have different dihedral angles; as if wedges were inserted between stacked base pairs. The AA·TT dinucleotide was estimated to have the largest wedge of 8.7° (Ulanovsky et al., 1986), and in this model the positioning of AA·TT wedges in phase along the DNA helix produces unidirectional bending. In the junction model (Wu & Crothers, 1984; Koo et al., 1986; Koo & Crothers, 1988), it is assumed that the oligo(dA) tracts exist in a helical form different enough from normal B-form DNA so that a kink is created at the junctions of the oligo(dA) tracts and the DNA between them. Recent X-ray diffraction studies appear to be more in support of the latter model (Coll et al., 1987; Nelson et al., 1987; Yoon et al., 1988).

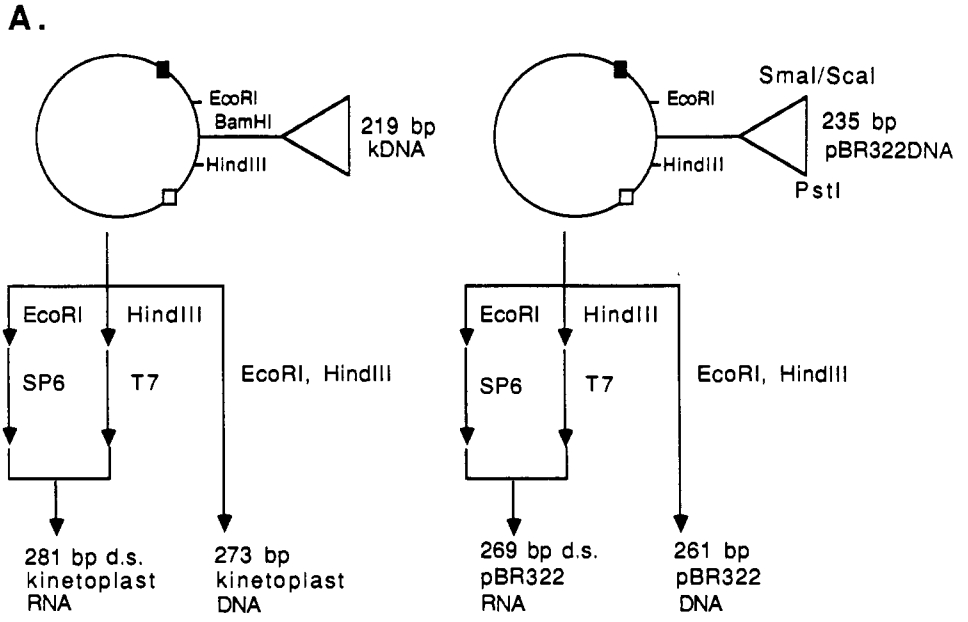
Natural double-stranded RNA normally exists in the A-form helix. Thus, it was not clear whether phased blocks of oligo(rA) in duplex RNA would induce a bending of the RNA helix axis. In this study, single-stranded RNAs were transcribed either from synthetic short DNA templates containing adenine tracts in phase with the helix or from *C. fasciculata* kinetoplast bent DNA and the complementary strands were annealed to produce duplex RNAs with phased blocks of adenines. These molecules were examined by polyacrylamide gel electrophoresis and EM. Our results show that double-stranded RNAs containing phased adenine tracts migrate at a rate similar to that of double-stranded RNAs without adenine tracts on polyacrylamide gels, and upon examination by EM most of the molecules appear straight or only slightly bent. Arguments are presented that the adenine tracts in duplex DNA act independently of each other to induce DNA bending.

Materials and Methods

Preparation of Synthetic DNA Templates. Oligodeoxyribonucleotides were synthesized on an Applied Biosystems 380B DNA synthesizer and purified by polyacrylamide gel electrophoresis. The complementary oligodeoxyribonucleotides, overlapping by 19 nucleotides, were hybridized, and the molecule was made fully duplex by use of the large fragment of DNA polymerase I to produce 75-bp DNA templates containing T7 RNA promoters.

Plasmid Preparation. The plasmid pPK201/CAT (Kitchin et al., 1986) was digested with *Bam*HI, and the 219-bp bent DNA was eluted from a 2% agarose gel. After ethanol pre-

[1] Abbreviations: EM, electron microscopy; bp, base pair(s); EDTA, ethylenediaminetetraacetic acid.



B. Double-stranded kinetoplast RNA

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-------GG AUCCCCUAAA AUUCCAACCG AAAAUCGCGA GGUUACUUUU UUGGAGCCCG
------- CC UAGGGGAUUU UAAGGUUGGC UUUUAGCGCU CCAAUGAAAA AACCUCGGGC

AAAACCACCC AAAAUCAAGG AAAAAUGGCC AAAAAAUGCC AAAAAAUAGC GAAAAUACCC
UUUUGGUGGG UUUUAGUUCC UUUUUACCGG UUUUUUACGG UUUUUUAUCG CUUUUAUGGG

CGAAAAUUGG CAAAAAUUAA CAAAAAAUAG CGAAUUUCCC UGAAUUUUAG GCGAAAAAAC
GCUUUUAACC GUUUUUAAUU GUUUUUUAUC GCUUAAAGGG ACUUAAAAUC CGCUUUUUUG

CCCCGAAAAU GGCCAAAAAC GCACUGAAAA UCAAAAUCUG AACGUCUGGA UCC-------
GGGGCUUUUA CCGGUUUUUG CGUGACUUUU AGUUUUAGAC UUGCAGACCU AGG-------
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FIGURE 2: Strategy for generating double-stranded RNA from DNA templates. (A) Two plasmids (Materials and Methods) containing T7 RNA polymerase promoters (■) and SP6 RNA polymerase promoters (□) were linearized by *Eco*RI digestion to give templates for transcription by SP6 RNA polymerase (SP6) or by *Hin*dIII digestion to give templates for transcription by T7 RNA polymerase (T7). Upon annealing of the two single-stranded complementary RNAs, the 281-bp double-stranded kinetoplast RNA containing phased adenine blocks or the 269-bp double-stranded pBR322 RNA was produced. The corresponding DNA fragments were obtained by double digestion with *Eco*RI and *Hin*dIII. (B) The sequence of double-stranded RNA transcribed from kinetoplast bent DNA is shown. The dotted lines represent the vector sequence.

cipitation, the fragment was ligated into *Bam*HI-cleaved pGEM3Zf(+) (Promega Biotec), and this recombinant DNA was used to transform *Escherichia coli* DH5α. Colonies were screened by the minilysate procedure combined with restriction enzyme digestion for the presence of an insert. The appropriate size of this insert was verified on a 2% agarose gel, and the highly anomalous electrophoretic behavior of this insert was confirmed on a 6% polyacrylamide gel. A 235-bp *Pst*I$_{3609}$–*Sca*I$_{3846}$ fragment of pBR322 DNA was separated by gel electrophoresis and, following purification, was subcloned into pGEM3Zf(+) digested with *Pst*I and *Sma*I. The colonies containing the recombinant plasmid were identified by restriction digest analysis.

Preparation of Double-Stranded RNA. Plasmids containing either the 219-bp kinetoplast DNA or the 235-bp pBR322 DNA were linearized by *Eco*RI digestion to give templates for transcription by SP6 RNA polymerase (Promega Biotec) or by *Hin*dIII digestion to give templates for transcription by T7 RNA polymerase (Promega Biotec). Seventy-five-bp synthetic DNA templates were transcribed by T7 RNA polymerase. Transcription was carried out in a buffer containing 40 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 2 mM spermidine, 10 mM NaCl, 0.05 mM rCTP, and 0.2 mM each rATP, rGTP, and UTP together with [α-^{32}P]rCTP for 2 h at 37 °C. Homogeneously ^{32}P-labeled RNAs were purified on 6 or 8% polyacrylamide–7.5 M urea gels. Equal quantities of complementary RNAs were mixed and hybridized in 0.45 M sodium citrate and 0.045 M NaCl by incubating at 65 °C for 15 min followed by slow cooling to room temperature.

Gel Electrophoresis. Radioactively labeled DNA and RNA duplexes were electrophoresed on 6 or 12.5% polyacrylamide gels (30:1 acrylamide:bisacrylamide ratio) in TBE buffer (89 mM Tris base–89 mM boric acid–2 mM EDTA[1], pH 8.0) at room temperature. Samples were visualized by autoradiography.

EM. Methods for preparing DNA and RNA samples for direct mounting have been described (Griffith & Christiansen, 1978). In brief, the samples were mixed with a buffer containing 0.15 M NaCl, 1 mM $MgCl_2$, and 2 mM spermidine, adsorbed to glow-charged copper-mesh grids covered by a thin carbon film, washed with water and a graded ethanol series, air-dried, and rotary shadowcast with tungsten. The preparation of samples by fast freezing and freeze-drying was carried out by mixing the samples with the spermidine-containing buffer and adsorbing the sample to the carbon-coated grids as above. The excess liquid was blotted away and the sample

plunged into liquid ethane chilled with liquid nitrogen. The samples were then freeze-dried at −85 °C in a Wiltek Inc. freeze etch system at 10^{-7} torr for 2 h followed by rotary shadowcasting with tungsten. Micrographs were taken on a Philips EM400 TLG, and the lengths of molecules were measured from micrographs by use of a Summagraphics digitizer coupled to an IBM PC-AT computer.

Results

Electrophoretic Behavior of 58-bp Double-Stranded RNAs Containing Phased Adenine Blocks. Examining DNAs containing phased blocks of adenines, Diekmann and Wang (1985) observed that these DNAs needed to be greater than ~100 bp to show significant electrophoretic retardations. However, due to the different helical parameters of double-stranded RNA compared to those of DNA, what is known about sequence-directed DNA bending cannot be applied to RNA. Thus, 58-bp duplex RNAs containing phased adenine tracts were synthesized to directly address this question.

DNA templates were designed from which T7 RNA polymerase can transcribe an RNA or its complementary sequence. Upon annealing, this produced 58-bp double-stranded RNAs containing either A·T blocks (aRNA) in phase with the helical repeat or double-stranded RNA with a random sequence composition (rsRNA) (Figure 1A). Transcripts were generated by use of T7 RNA polymerase and ^{32}P-labeled rCTP. Full-length transcripts were isolated by gel electrophoresis on 8% denaturing polyacrylamide gels, the complementary transcripts were hybridized, and the resulting duplexes were electrophoresed on 12.5% polyacrylamide gels (Figure 1B). The template DNAs composed of a promoter for T7 RNA polymerase and A·T blocks (aDNA) or random sequences (rsDNA) were also electrophoresed. The aDNA showed no retardation in mobility relative to the rsDNA of the same size (compare lanes 4 and 5, Figure 1B). On the same gel, aRNA and rsRNA also appeared to comigrate (compare lanes 2 and 3, Figure 1B).

Electrophoretic Properties of Larger Double-Stranded RNAs Containing Phased Adenine Blocks. The inability to detect a significant retardation in electrophoretic mobility of the double-stranded RNAs with phased blocks of adenines could be due to their short size as observed with short duplex DNAs (Diekmann & Wang, 1985; Hagerman, 1985; Koo et al., 1986). To produce a longer duplex RNA containing phased blocks of oligo(rA), we used, as a template for transcription, the highly bent 219-bp *C. fasciculata* kinetoplast DNA fragment, which contains 18 runs of four to six adenines with many of these runs in phase with each other and the helical repeat (Griffith et al., 1986; Kitchin et al., 1986). Indeed, it was noted that the average repeat of these oligo(dA) blocks is 11 bp, closer to the A helix than the B helix. The 219-bp fragment was cloned into the plasmid pGEM3Zf(+), which contains T7 and SP6 RNA polymerase promoters (Materials and Methods). A 235-bp fragment from pBR322 was used as a control template to produce double-stranded RNA lacking phased blocks of adenines and was also cloned into the same vector.

The strategy for producing double-stranded RNA from both plasmids is shown in Figure 2. SP6 RNA polymerase transcribes one RNA strand with an *Eco*RI-linearized template; T7 RNA polymerase transcribes the complementary RNA strand with a *Hin*dIII-linearized template; upon annealing, the double-stranded RNAs are produced and both the kinetoplast RNA and the control RNA contain the same ends. The sequence of the double-stranded kinetoplast RNA is shown in Figure 2B. The electrophoretic mobility of the double-stranded RNA produced from the kinetoplast DNA templates was examined on a 6% polyacrylamide gel (Figure 3). The double-stranded kinetoplast RNA had a similar electrophoretic mobility compared to that of the double-stranded control RNA (lanes 4 and 5 of Figure 3), the difference being due only to the size of these double-stranded RNAs (281 bp for double-stranded kinetoplast RNA and 269 bp for double-stranded control RNA). Thus the double-stranded kinetoplast RNA does not show the anomalous electrophoretic behavior seen with kinetoplast DNA (lane 2 of Figure 3).

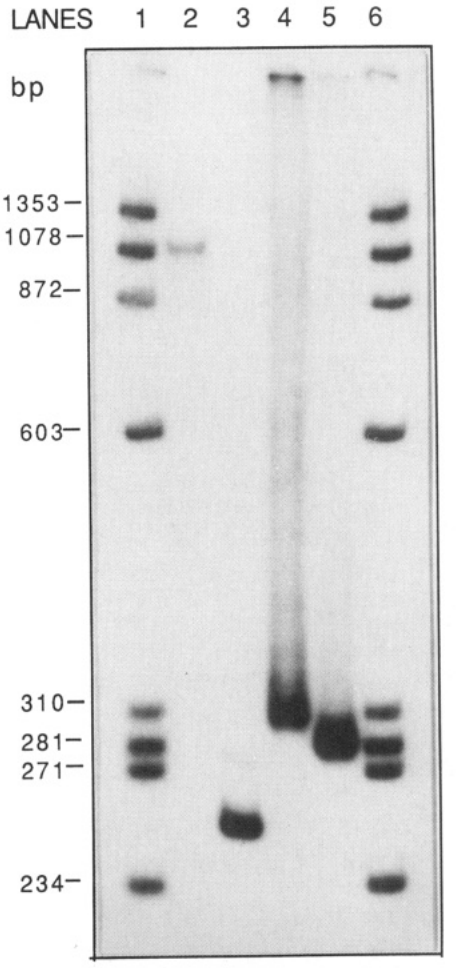

FIGURE 3: Electrophoretic mobility of double-stranded RNA transcribed from *C. fasciculata* bent DNA template on a 6% polyacrylamide gel: lanes 1 and 6, the *Hae*III-cleaved φX174 DNA fragments; lane 2, the 273-bp kinetoplast DNA fragment; lane 3, the 261-bp pBR322 DNA fragment; lane 4, the 281-bp double-stranded kinetoplast RNA; lane 5, the 269-bp double-stranded pBR322 RNA. Samples were visualized by autoradiography. The sizes of φX174 fragments are indicated on the left.

Visualization of Double-Stranded RNA Transcribed from the C. fasciculata DNA Template. As seen by EM, the 219-bp kinetoplast DNA bends smoothly over its length, with some molecules appearing as nearly perfect circles (Griffith et al., 1986). The double-stranded RNA and DNA samples synthesized above were prepared for EM by two different methods. In one, the samples were dehydrated through ethanol and air-dried, and in the other method the samples were rapidly frozen and freeze-dried prior to shadowcasting with tungsten (Materials and Methods). Upon examination of the samples (Figure 4), most of the 273-bp kinetoplast DNA fragments were highly bent or full circles, while the duplex kinetoplast and pBR322-derived RNAs and the 261-bp pBR322 DNA fragment appeared straight or only slightly

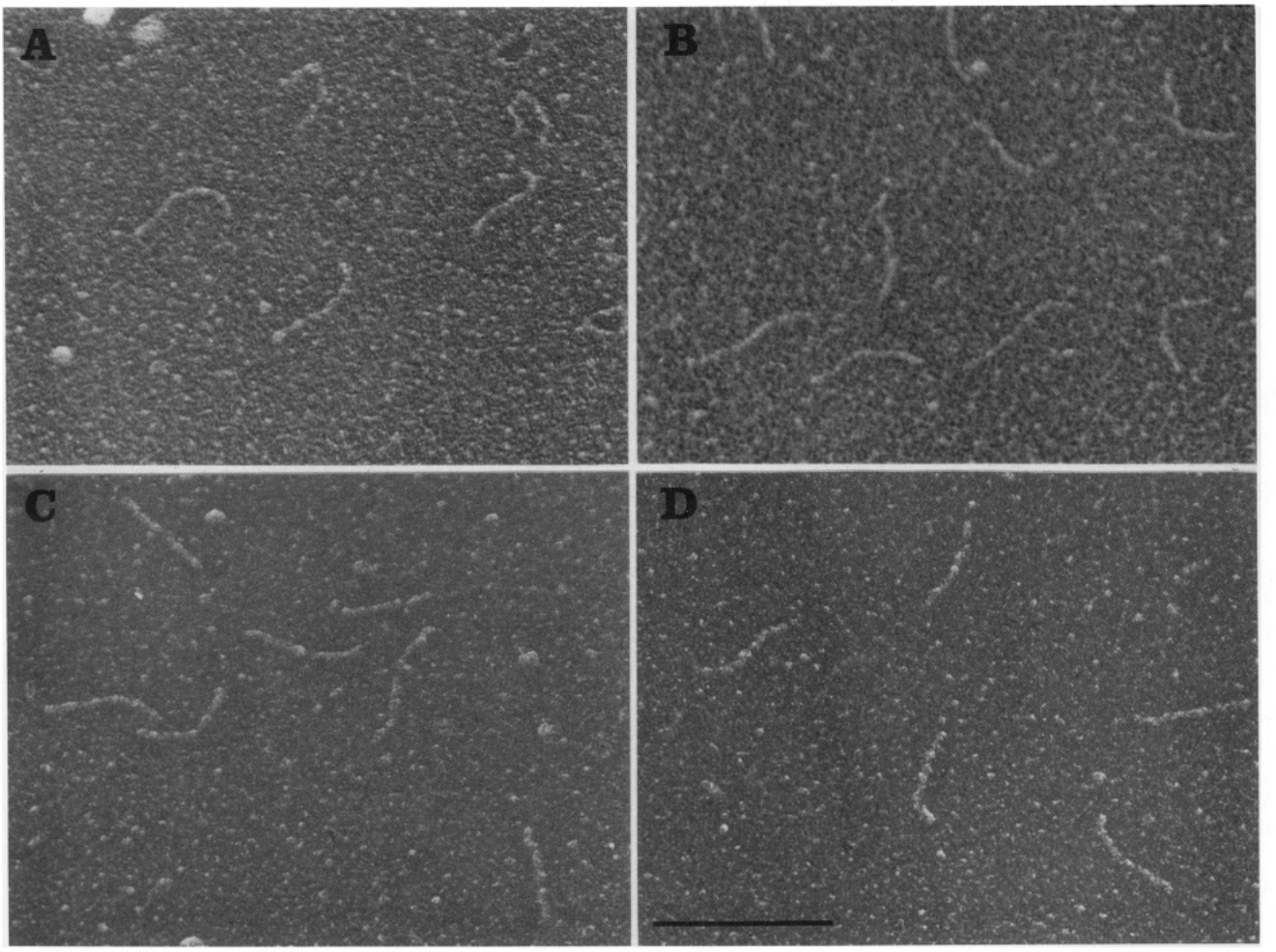

FIGURE 4: EM visualization of RNA samples. Kinetoplast bent DNA (A), pBR322 DNA (B), double-stranded kinetoplast RNA (C), and double-stranded pBR322 RNA (D) were prepared for EM by direct adsorption to thin carbon films, passage through graded ethanol washes, and rotary shadowcasting with tungsten (see Materials and Methods). Micrographs are shown in reverse contrast. The bar equals 0.1 μm.

bent. Preparation of the samples by either method produced molecules that appeared the same.

A simple means of quantitating the degree of bending of these molecules involves measuring the straight line distance between the two ends of each molecule and dividing that value by its curvilinear length. The values range from 0 to 1.0, 1.0 representing a straight line and 0 a circle. Close to 100 molecules from each sample were evaluated (Figure 5). The distribution of the values for the kinetoplast DNA is broad, and the peak of the distribution is between 0.3 and 0.4, indicating that these molecules are highly bent. Also, very few molecules were in the range of 0.8–1.0 compared to the other three samples in which 63% of kinetoplast RNA and 72% of pBR322 DNA and RNA molecules have values between 0.8 and 1.0.

Discussion

In this study, we used gel electrophoresis and EM to examine the structural features of double-stranded RNAs containing adenine tracts in phase with the RNA helix. RNA samples were transcribed either from short synthetic DNA templates or from *C. fasciculata* kinetoplast bent DNA. On polyacrylamide gels, these RNAs migrated at a rate similar to that of the duplex RNAs without adenine tracts, and upon EM examination most of the molecules were straight or only slightly bent. These results suggest that double-stranded RNA containing phased adenine tracts is not bent. This conclusion is in agreement with recent gel electrophoretic and hydrodynamic analysis of a 180-bp double-stranded RNA molecule containing four A_5 tracts in phase with the helical repeat (F.-U. Gast and P. J. Hagerman, personal communication). In addition, we observed that the double-stranded RNA migrates more slowly on polyacrylamide gels than DNA of similar size (Figure 3), a result also noted by Bhattacharyya et al. (1990) previously.

X-ray fiber diffraction studies have shown that the helical repeat of A-form RNA is 11 bp (Arnott et al., 1968); however, two recent studies measured the periodicity of RNA helices in solution as 11.3 ± 0.1 bp/turn (Bhattacharyya et al., 1990) and 11.8 ± 0.2 bp/turn (Tang & Draper, 1990). The double-stranded kinetoplast RNA used in this study contains phased adenine blocks with an average repeat of 11 bp throughout the helix, and the central nine blocks have an average repeat of 10.4 bp. If adenine blocks induce bends in double-stranded RNA, this central region containing a phase shift from the RNA helix would produce a spiral rather than planar bend. Results with duplex DNAs containing adenine blocks phase shifted from the duplex DNA repeat (Koo et al., 1986) would argue that such a minor shift should still produce a marked retardation, if adenine blocks bend duplex RNA. Moreover, during its preparation for EM, a molecule containing a spiral bend will be flattened into the plane of the supporting film and thus EM would be expected to be less sensitive to minor phase shifts than gel electrophoresis.

Double-stranded RNA normally exists in the A-form helix. The junction model in which DNA bends are produced at the boundary between different helical forms (Wu & Crothers, 1984; Koo et al., 1986; Koo & Crothers, 1988) would predict

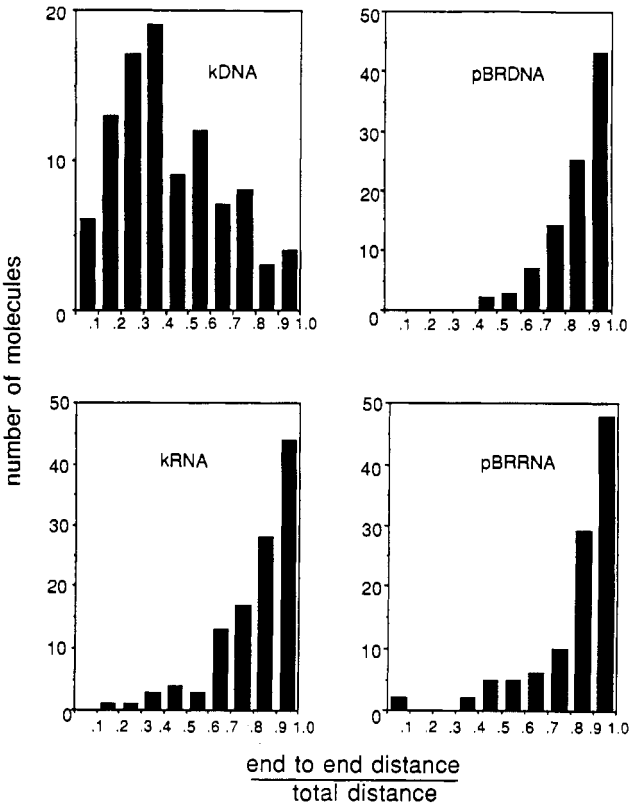

FIGURE 5: Distribution of the degree of bending of kinetoplast and pBR322 DNA and RNA samples. The degree of bending is represented by dividing the straight line distance between two ends of each molecule by its curvilinear length. Close to 100 molecules from each sample were evaluated. The value 1.0 represents a straight line and 0 represents a full circle. The data was obtained from the samples prepared by the quick freezing and freeze-drying procedure. No significant difference was observed compared to samples prepared by ethanol dehydration.

that double-stranded RNA containing phased adenine tracts would not be bent. However, if bending is produced by wedges, phased tracts of adenines in duplex RNA might be expected to bend the helix axis. Recently, on the basis of X-ray crystallographic results, three groups (Coll et al., 1987; Nelson et al., 1987; Yoon et al., 1988) concluded that oligo(dA) tracts appear internally unbent and A·T base pairs have a high positive propeller twist to give an unusual conformation in this region. These results thus favor the junction model. Moreover, Burkhoff and Tullius (1988) used hydroxyl radicals to examine the local conformation of two oligonucleotides: $(GT_4A_4C)_n$ and $(GA_4T_4C)_n$. Their results argue against the wedge model in which the local conformation of an AA dinucleotide is the same for any sequence.

Analysis of the distribution of end-to-end distances of the DNA and RNA samples was informative. For the kinetoplast DNA, this distribution was continuous with molecules exhibiting degrees of bending from straight to fully circular with no indication of any cooperativity between different states. This observation is in agreement with data published earlier (Griffith et al., 1986). There, as shown here, the highly bent 223-bp *C. fasciculata* DNA appeared by EM as molecules with varying degrees of bending, some describing almost perfect circles. When this DNA was electrophoresed on 6% polyacrylamide gels, it migrated as a single species with an apparent size of 890 bp. However when this same DNA was covalently closed with ligase, a sharp band of DNA was observed with an apparent size of >1300 bp. Had the linear 223-bp fragment in solution had a homogeneous shape typical of the most highly bent molecules seen by EM, then simply closing the ends would not have been expected to cause such a dramatic further reduction in mobility. Further, had the molecules not been moving rapidly between the different states of bending, then the DNA would have electrophoresed as a very broad band. Indeed, it was observed (Figure 2 in Griffith et al. (1986)) that when some of the adenine blocks were disrupted by thymine dimers, creating molecules that could not assume the most bent shape, then the DNA did migrate as a smear. Thus, both the EM and gel results argue that in solution and in the gels the linear 223-bp bent DNA exists in a rapid equilibrium between many different bent states, that the adenine blocks act independently of each other, and finally that the mobility observed describes the average state of the population. Hagerman (1990) has discussed EM and other results that argue for at least two distinct states of the adenine-tract DNA and the need for caution in interpretations of gel mobility and cyclization results on the basis of a single highly bent state of adenine-tract DNA.

Registry No. Adenine, 73-24-5.

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Solution Structure of an Oncogenic DNA Duplex Containing a G·A Mismatch

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ABSTRACT: The DNA duplex 5'-d(GCCACAAGCTC)·d(GAGCTGGTGGC), which contains a central G·A mismatch has been studied by one and two-dimensional NMR techniques. The duplex corresponds to the sequence 29-39 of the K-*ras* gene. The mismatch position is that of the first base of the Gly12 codon, a hot spot for mutations. The observed NOEs of the nonexchangeable protons show that both of the bases of the mismatched pair are intrahelical over a wide range of pH. However, the structure of the G·A mispair and the conformation of the central part of the duplex change with pH. This structural change shows a p*K* of 6.0. At low pH, the G·A bases are base paired with hydrogen bonds between the keto group of the G residue and the amino group of the A residue and, secondly, between the N7 of the G and a proton on N1 of A. This causes the G residue to adopt a syn conformation. On raising the pH, the N1-H proton of the protonated A residue is removed, and the base pair rearranges. In the neutral G·A base pair both residues adopt an anti conformation, and the mismatch is stabilized by hydrogen bonds. Our results on the exchangeable and A(H2) protons of the mismatched pair indicate a shift from a classical face-to-face two hydrogen-bonded structure to a slipped structure stabilized by bifurcated hydrogen bonds. This may be a particular characteristic of this oncogenic sequence in which the G·A error is poorly repaired.

The presence of enzymatic systems specialized in the repair of noncomplementary DNA base pairs (mismatches) is now well established. In *Escherichia coli*, three different pathways have been described. First, the postreplicative mismatch repair is dependent on the *mutS*, *mutL*, *mutH*, *mutU*, and *ssb* genes and acts shortly after DNA synthesis to eliminate the incorrect base (Radman & Wagner, 1986). Detection of the mismatch by the MutS and MutL proteins is followed by a series of events resulting in excision of the newly synthesized strand at nonmethylated GATC sites (Lu et al., 1984; Längle-Rouault et al., 1987). The repair efficiency depends on the type of mismatch, on the surrounding sequence (Kramer et al., 1984; Dohet et al., 1985), and correlates with the affinity of the MutS protein for the mismatch (Su et al., 1988). The second pathway characterized by excision of short sequences (very short patch repair) is independent of the *mutH* and *mutU* genes but requires MutS and MutL proteins (Lieb, 1987). This system specifically corrects G·T to G·C in the sequence TAGG, thus diminishing the mutagenic effect of 5-methylcytosine deamination to thymine (Jones et al., 1987). The third pathway requires the *mutY* gene and specifically corrects A·G to C·G (Au et al., 1988; Su et al., 1988).

Recently, mismatch-specific enzymatic activities have also been found in frog eggs (Brooks et al., 1989) and in human cells (Brown & Jiricny, 1988). In other studies, the mismatch repair specificity in rat liver extracts was tested on synthetic substrates mimicing replication errors that would lead to oncogenic mutations in the K-*ras* codon 12 sequence; the specificity thus observed resembles that of mismatch repair in *E. coli* (Dimitrijevic et al., private communication).

To better understand the mechanism of mismatch recognition by this repair system, we have studied by NMR the G·A-containing heteroduplex corresponding to the sequence of the K-*ras* gene in which the repair system was tested. This G·A-containing heteroduplex leads to a GGT → TGT mutation in the K-*ras* Gly12 codon found in colon carcinomas (Nishimura & Sekiya, 1987, and references therein). The recognition and repair of the G·A mismatch in this sequence may play an important role in the carcinogenesis.

Materials and Methods

The oligonucleotides corresponding to the sequence 29-39 of the K-*ras* gene mutated noncoding strand (Cys12, strand

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