

SYNTHESIS OF A NOVEL POLYNUCLEOTIDE: POTENTIAL R-L MODEL

R. K. MISHRA and S. K. BRAHMACHARI

Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India.

ABSTRACT

Synthesis of a novel polynucleotide with potential alternating B and Z segments is described. This is the first report of a polynucleotide where the double helix undergoes change in handedness after every half a turn.

INTRODUCTION

EVER since the original proposal¹ of left-handed double helical structure of DNA and the subsequent discovery² of Z DNA in single crystals, extensive work has been carried out on left-handed Z DNA^{3,4}. Using stereochemical guidelines and taking advantage of the inherent conformational flexibility of DNA, Sasisekharan and co-workers⁵ could generate double helical DNA structures by joining five residues in right-handed and five residues in left-handed helical conformation. A composite RU and LZ helix with a stable link was proposed^{6,7} as a special case of the generalised R-L model. Crick *et al*⁸ ruled out by their studies on circular DNA, the possibility of the R-L model representing a generalized structure for DNA but the feasibility of such a structure in local segments was not tested experimentally.

Subsequent experimental studies have shown that B→Z transition can take place in oligo- and polynucleotides under a variety of conditions^{3,4}. B→Z transition in the solid state under mild conditions, first reported from this laboratory, indicated the possibility of the coexistence of these two structures⁹.

In cases where a potential Z DNA sequence is inserted in a covalently closed circular plasmid, B and Z conformations have been shown to coexist with a junction of ≥ 5 nucleotides¹⁰⁻¹². The basic difference between Watson-Crick and R-L models is that the linking number is one order of magnitude higher in the former. Recently, it has been shown¹³ that because of topological constraints several sequences adopt altered conformation in pBR322 Form V molecule with zero linking number. In one stretch of sequence starting from the *Eco* RI site of pBR322 Form V, shown below, altered (L) and normal (R) structures appear, alternately, four times in less than 40 nucleotides. Residues whose structures were probed are underlined. However, the fine details of the altered structure (L) are not known.

GAAITCTCATGTTTGACAGCTTATCATCGATAAGCTTTAA
 ————R—————L—————R—————L

So far, no attempt has been made to study a polymer half of which is in right and half in left-handed conformation. For the first time we report a strategy to synthesize a polynucleotide with potential R-L conformation. This polymer, obtained by the block polymerization of d(CGCGCGATCGAT), has alternating six-base-pair Z- and B-helicogenic regions. Preliminary studies on the dodecamer, in concatamer and polymer form, indicate the presence of R-L conformation and open up new possibilities for the investigation of the role of unusual structures in the biological functions of DNA.

MATERIALS AND METHODS

2' Deoxynucleosides, dimethoxytritylchloride, tetrazole, etc. used in the synthesis of the dodecamer were from Sigma Chemical Co., USA. Solvents were purified before use in the synthesis. T4 DNA ligase and polynucleotide kinase were from New England Biolabs, USA. Other reagents were of analytical grade.

Synthesis of d(CGCGCGATCGAT)

5'-Protected nucleosides were prepared according to Jones' procedure¹⁴. The corresponding amidites were prepared using methoxy (N,N-diisopropyl)-aminochlorophosphine and checked by ³¹P NMR¹⁵. Coupling reactions were done manually in an open column system using standard procedure^{15,16}. The dodecamer was deprotected and purified on a denaturing polyacrylamide gel¹⁷.

CD measurements

A Jasco J 500A CD spectropolarimeter was used to measure the CD of the oligonucleotide. Samples were preheated and allowed to cool slowly before

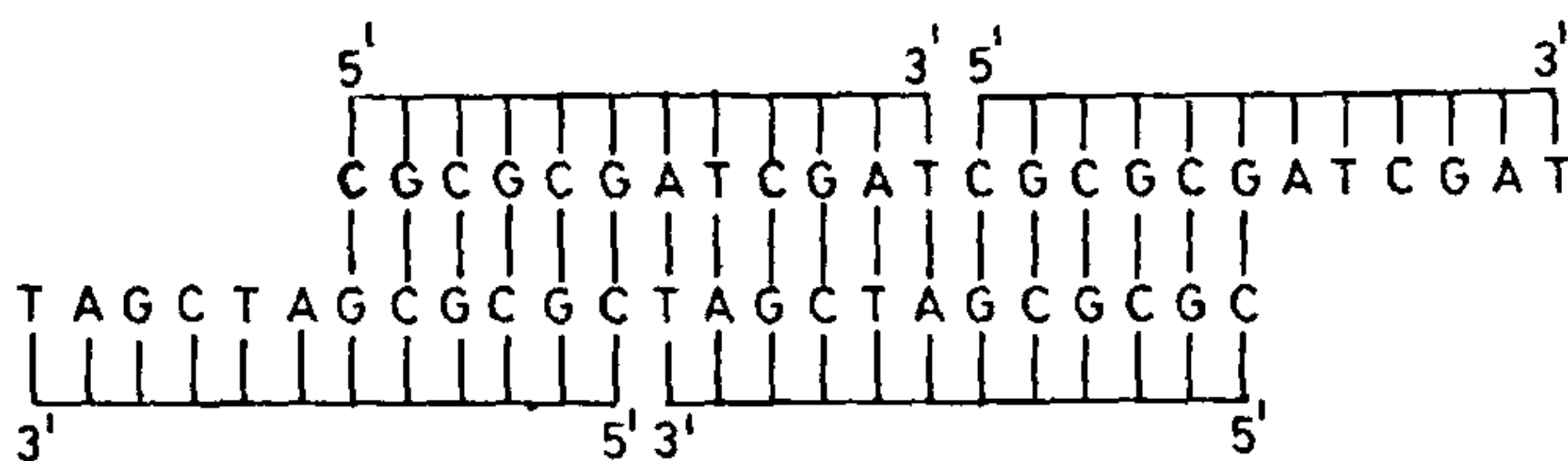


Figure 1. Concatamer duplex form of $d(\text{CGCGCGATCGAT})$ (a schematic drawing).

CD measurements. All the spectra were recorded at 20°C .

Ligation of $d(\text{CGCGCGATCGAT})$

The dodecanucleotide was phosphorylated using $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and polynucleotide kinase according to manufacturer's specification¹⁸. The labelled oligomer was heated at 90°C for 2 min, cooled slowly to room temperature and left at 4°C overnight for concatamer formation. This was followed by ligation using T4 DNA ligase¹⁸ at 16°C for 18 h. The reaction mixture was analysed on a denaturing 20% polyacrylamide gel.

RESULTS AND DISCUSSION

The dodecamer $d(\text{CGCGCGATCGAT})$ has two helical domains, the Z-helicogenic domain of CGCGCG and the B-helicogenic domain of ATCGAT. We have shown earlier¹³ that even under very high superhelical force ATCGAT remains in B conformation whereas the flanking sequences on both the sides adopt altered conformation. Con-

sidering these facts we selected the two B- and Z-favouring sequences for our studies. Earlier studies on oligomers with two helical domains¹⁹ had the limitation of chain length because of the inability of these oligomers to form concatamers. The dodecamer studied here has no choice but to adopt a concatamer duplex form (figure 1) which eventually behaves like a pseudo-polymer. When the molecule is in a concentrated solution, slow cooling after heat treatment favours duplex formation and the pseudo-polymer should dominate. CD studies show (figure 2) that in low salt conditions the oligomer exists in B form. In the presence of 5 M NaCl the emergence of negative rotation at 294 nm may be attributed to Z conformation in CGCGCG stretches. On addition of NiCl_2 in the presence of 5 M NaCl, the Z conformation is apparently stabilized. We have reported earlier that NiCl_2 in presence of high NaCl concentration brings about B \rightarrow Z transition in

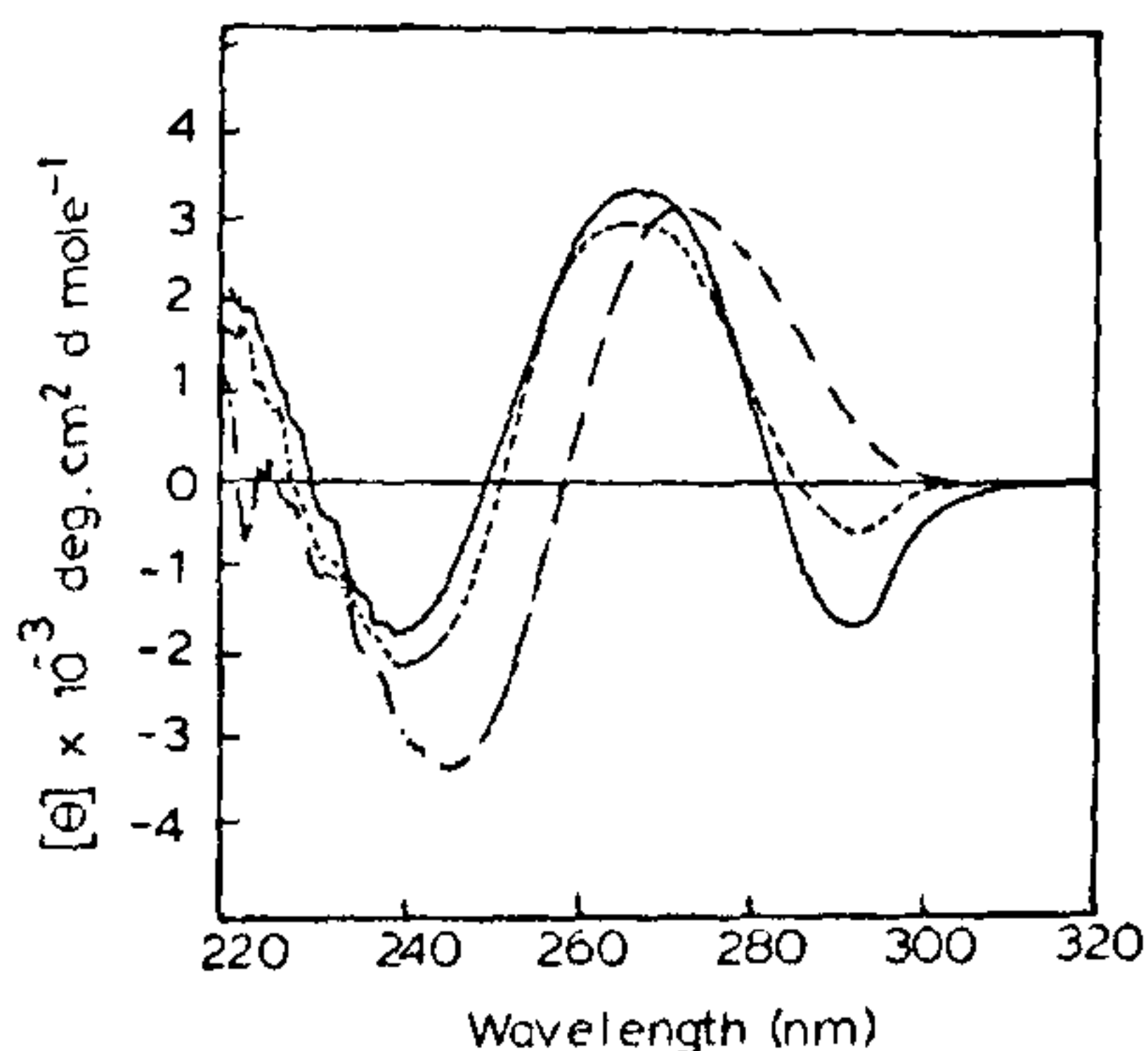


Figure 2. CD spectra of $d(\text{CGCGCGATCGAT})$ in presence of 50 mM NaCl (—·—·—·—), 5 M NaCl (----) and 40 mM NiCl_2 in 5 M NaCl (—).

Figure 3. Analysis by electrophoresis on 20% polyacrylamide gel in 8 M urea, dodecanucleotide (lane A) and ligation product of the dodecamer (lane B).

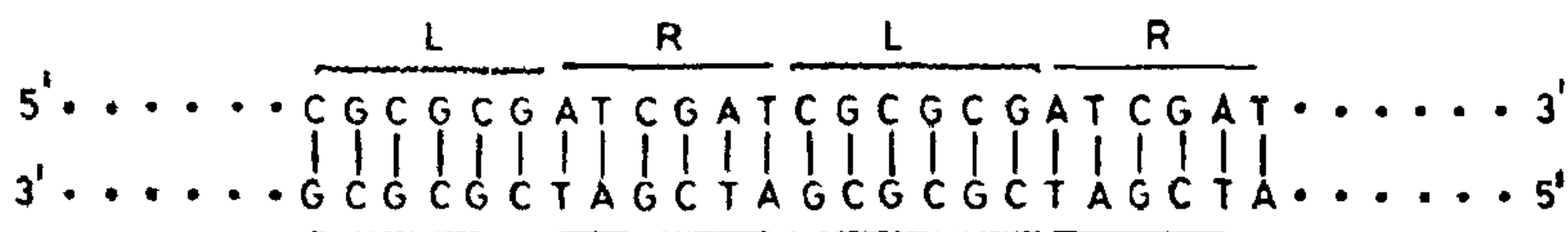


Figure 4. B and Z helical regions of the polymer in R-L form (a schematic drawing).

oligonucleotides²⁰. Here, however, the transition is not complete and thus the oligomer provides the building block for a polymer which could contain B and Z conformations simultaneously under Z-favouring conditions.

After proper reannealing of the dodecamer followed by ligation using T4 DNA ligase, high molecular weight DNA appears upon electrophoresis in an 8M urea-20% polyacrylamide gel (figure 3). The efficient ligation of oligomer units confirms concatamer formation as shown in figure 1. The resulting polymer has stretches of B- and Z-helicogenic regions as shown in figure 4. This novel polymer would adopt R-L conformation in Z-inducing conditions.

Restriction enzymes have been used as a sensitive probe of Z-conformation¹². The polymer contains *Hha* I (GCGC) and *Fnu* DII (CGCG) sites in Z-helicogenic regions and *Cla* I (ATCGAT) site in B-helicogenic regions. In addition, under conditions favouring Z conformation, there will be both B-Z and Z-B junctions which are known to be accessible for S1 and Bal 31 nucleases. This molecule therefore provides sites for several enzyme probes within the short twelve-base-pair stretches.

Preliminary studies using restriction enzymes indicate that the CGCGCG part is, indeed, in the left-handed conformation under Z-inducing conditions and ATCGAT remains in B-conformation. Detailed studies are in progress and are to be published elsewhere. For a right-handed conformation six base pairs give a +216° twist whereas six base pairs in the left-handed conformation give a -180° twist resulting in +36° overall twist per twelve base pairs. So this molecule is expected to have R-L conformation with one link per 120 base pairs under specified conditions. The ability of the ATCGAT sequence to remain in B conformation even when flanked by sequences in Z conformation enabled us to test the feasibility of an R-L duplex. It is gratifying to note that such a double helical polynucleotide is stable at room temperature. To the best of our knowledge, this is the first report of a polynucleotide where the double helix undergoes

change in handedness with a stable link after every half a turn.

The feasibility of an R-L conformation in a polymer under experimental conditions has tremendous implications for the role of conformation in the biological functions of DNA. Alternating purine-pyridine sequences are known to exist in genomic DNA^{3,4}. The presence of such sequences, interspersed between B-helicogenic sequences, would result in such unusual R-L conformation within the long stretch of B DNA. Such R-L regions would have distinct properties since they have linking number one order of magnitude lower and could be stabilized by negative supercoiling. The electrophoretic mobility of such a DNA with inherent flexibility in the junction region could be different from that of the usual B or Z DNA and this might lead to new interpretation of many results obtained on the basis of electrophoretic mobility. Further, since Z DNA has been shown to be a poor template for transcription and replication^{21,22}, it would be of interest to study the effect of R-L structure on transcription and replication processes.

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ANNOUNCEMENT

INTERNATIONAL WORKSHOP CUM SEMINAR ON PREVENTION OF GENETIC DISEASES

An international workshop cum seminar on 'Prevention of Genetic Diseases' is being organized at Bhopal from 12 to 17 December 1988. The cytogeneticists and biochemical geneticists are requested to write to the organizers immediately if they have anything to demonstrate for wider, future use.

Special sessions and lectures will be organized on 'Prevention of mental retardation, Recurrent abortions, Haemoglobinopathies and Cancer.

Those who are willing to attend are requested to write to: Dr H. K. Goswami, Department of Genetics, Bhopal University, Bhopal 462 026.
