(2)

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: (U)	1b. RESTRICTIVE MARKINGS: NA
2a. SECURITY CLASSIFICATION AUTHORITY: NA	3. DISTRIBUTION / AVAILABILITY OF REPORT: Distribution Unlimited
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE: NA	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): Massachusetts Institute of Technology	5. MONITORING ORGANIZATION REPORT NUMBER(S): NA
6a. NAME OF PERFORMING ORGANIZATION: Massachusetts Institute of Technology; 6b. OFFICE SYMBOL (If applicable):	7a. NAME OF MONITORING ORGANIZATION: Office of Naval Research
6c. ADDRESS (City, State, and ZIP Code): 77 Massachusetts Avenue, Cambridge, Massachusetts 02139	7b. ADDRESS (City, State, and ZIP Code): 800 North Quincy Street, Arlington, Virginia 22217-5000
8a. NAME OF FUNDING / SPONSORING ORGANIZATION: Office of Naval Research; 8b. OFFICE SYMBOL (If applicable): ONR	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: N00014-84-K-0300
8c. ADDRESS (City, State, and ZIP Code): 800 North Quincy Street, Arlington, Virginia 22217-5000	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 61153N; PROJECT NO. RR04106; TASK NO. 441; WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification): (U) Transcriptional Regulation in the Cell Cycle

12. PERSONAL AUTHOR(S): Alexander Rich

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
ANNUAL	FROM 6/87 TO 9/88	10/14/88	9

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	DNA CONFORMATIONAL CHANGES, PROPELLER (P) DNA, Z-DNA, HOMOLOGOUS RECOMBINATION
06	03		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The long-range objective of this project is to uncover conformational changes in DNA and understand their biological roles with some emphasis on transcriptional regulation. The conformational changes are uncovered largely by x-ray diffraction investigations. The biological roles associated with these changes are generally addressed by isolating proteins that bind to DNA with altered conformation in order to uncover their biological activities.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	(U)
22a. NAME OF RESPONSIBLE INDIVIDUAL: M. Marron	22b. TELEPHONE (Include Area Code): (202) 696-4760; 22c. OFFICE SYMBOL: ONR

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

S/N 0102-LF-014-6603

Report on Contract No. N00014-84-K-0300

Principal Investigator: Alexander Rich

Contractor: Massachusetts Institute of Technology

Contract Title: Transcriptional Regulation in the Cell Cycle

Start Date: 1 June 1987

Objective

We explore the role of conformational changes in DNA both in regulating transcription as well as influencing homologous genetic recombination. One of the important events associated with transcriptional activation is the formation of DNA loops in regulatory regions. Looping brings together regulatory proteins that are found at different positions along the regulatory segment of DNA. The looping involves bending of the DNA at specific segments. It has been known that segments of oligo(dA)·oligo(dT) are associated with DNA bending. Recently, we have solved the structure of a DNA dodecamer that incorporates a segment of $d(A_3T_3)\cdot d(T_3A_3)$. Knowledge of the details of this conformation suggest they may be important in providing bending points in DNA looping.

Z-DNA affinity columns have been used for isolating Z-DNA binding proteins. Recently we have found such proteins that are active in carrying out the strand transferase activity that is involved in homologous recombination.

Progress Report

For some time it has been known that DNA is capable of adopting a bent conformation. The sequence oligo(dA)·oligo(dT) is frequently associated with DNA bending. This is pointed out most strikingly in the DNA minicircles that are found in trypanosome in which the segments of the five base pairs of $dA_5\cdot dT_5$ are interspersed into the minicircle every ten base pairs. It is the presence of these segments that makes possible a circularization of DNA with a small number of nucleotides. DNA looping is found in the upstream regulatory regions of DNA. This looping is associated with activation of DNA for transcription. Looping phenomena bring together proteins attached to DNA that are widely separated when the DNA is linear. The looping of DNA is associated with activation of transcription.

In addition, looping is frequently stabilized by oligo(dA)·oligo(dT) stretches that are found in upstream regulatory regions.

In order to study this problem we have solved the structure of a fragment of a DNA dodecamer that contains a d(A_3T_3)·d(T_3A_3) segment in it. Segments of DNA with only AT base pairs have a narrow minor groove. This groove can serve as a binding site for certain planar molecules. We have solved the structure of a DNA dodecamer d(CGCA_3T_3GCG). both by itself and bound to the antibiotic distamycin. The distamycin molecule was found bound in the minor groove of DNA. However, the A_3T_3 segments of the molecule had an unusual conformation both while the distamycin was present and in the DNA itself. In this conformation, the AT base pairs were found to have a strong propeller twist. This propeller twist was stabilized by bifurcated or three-centered hydrogen bonds between adenine amino NH_2 and two thymine O4 atoms on the opposite strand (see Fig. 1). The propeller twist of the base pairs put the adenine amino group into a position where it could make two hydrogen bonds rather than one.

DNA bending is associated with oligo(dA)·oligo(dT) segments. This segment of DNA in the propeller conformation is likely to have a bend at the interface between the propeller conformation and the B-DNA outside of it. The reasons for this are associated with the important stacking interactions between the bases. In the three-dimensional structure of yeast $tRNA^{Phe}$, the anticodon stem is at an angle compared to the adjacent dihydro U stem. Between these two stems is an AG base pair that is frozen in the propeller conformation. The anticodon stem stacks on one blade of the propeller, while the dihydro U stem stacks on the other blade of the propeller, thus giving rise to an angle in the orientation of these two adjacent helical segments of the molecule.

A similar situation may prevail in DNA with an oligo(dA)·oligo(dT) segment. The B-DNA segment with planar base pairs cannot stack upon both the adenine and the thymine bases in the propeller conformation. However, it may stack predominately on one blade of the propeller, which would have the effect of giving rise to an angle or bend in the helical axis of the B-DNA segment relative to the propeller DNA segment. A similar distortion could take place at the other end of the otherwise straight propeller segment. Thus, the propeller conformation may serve as an important component in stabilizing DNA looping.

Role of Z-DNA in Homologous Recombination

Homologous recombination is a process whereby two DNA molecules come together at a position where their nucleotide sequences are identical. The recombination process results in a "crossing over" of the duplex molecules. The mechanism of homologous recombination is not clearly understood. One mechanism that has been described involves an initial scission of a polynucleotide chain followed by unwinding of a DNA strand and invasion of a duplex DNA by this single strand. Such a process could ultimately lead to the formation of a holiday junction and recombination.

However, there is likely to be an alternative recombination pathway in which DNA strands sense the homology or nucleotide sequence identity by forming a recombination intermediate involving both right-handed and left-handed Z-DNA in this recombination intermediate. A process such as that, outlined in Figure 2, would involve the participation of proteins that have the ability to recognize Z-DNA. Several recombination enzymes have already been reported that have the ability to bind to Z-DNA.

We have looked for the presence of recombination proteins that bind to Z-DNA in human acute lymphoblastic leukemia cells. To do this we have constructed a Z-DNA affinity column as outlined in Figure 3. In this column the Z-DNA segments are held through biotin-Avidin linkers only at the ends of the Z-DNA segments. A column such as this was used to isolate proteins from the nuclei of human cells. The proteins were assayed for their ability to carry out strand transferase, that is assayed by measuring the combination of a single-stranded circular DNA molecule with a linearized duplex (Fig. 4). The strand transferase activity was detected in the proteins eluted from the Z-DNA affinity column. This strand transferase was characterized both in the electron microscope and in control experiments that identified its dependence on DNA sequence homology. Other experiments demonstrated the directionality of the reaction. A dependence on ATP was also found associated with this enzymatic activity. Even though the enzyme is not yet purified, the fraction containing the enzyme has considerable binding to Z-DNA as compared to B-DNA.

Objectives for the Next Year

1. Propeller DNA: We plan to extend the investigations on the propeller conformation of DNA by seeing whether the fiber diffraction data obtained from fibers of poly(dA)·poly(dT) are fully consistent with the propeller conformation that we have found in the DNA dodecamer crystals. This will be necessary in order to demonstrate the occurrence of the propeller conformation in this sequence. It will also be needed to explain the unusual physical properties of poly(dA)·(dT) compared to the

alternating copolymer poly(dAT). The poly(dA)·poly(dT) melts almost 8 degrees higher than poly(dAT). This additional stability is probably associated with the formation of bifurcated hydrogen bonds as well as other changes associated with the propeller conformation.

Attempts will also be made to look for other DNA conformational changes that are associated with transcriptional activation. Some of the candidates that are being looked at at the present involved homopurine-homopyrimidine sequences that are known to undergo conformational changes associated with transcriptional activation.

2. Z-DNA and Homologous Recombination: Our objective here is to continue the purification of the enzymatic activity that has been detected with a view toward understanding the manner in which this strand transferase activity participates in homologous recombination. Purification of the enzyme would also make it possible to understand which other enzymatic activities may be needed to carry out the recombination step. The other enzymatic activities involved include resolvases that are known to cleave the Holiday junctions and religate the severed polynucleotide chains to form the recombined molecule. The material isolated from the Z-DNA affinity column is likely to contain, in addition to Z-DNA binding proteins, other proteins that bind to Z-DNA binding proteins. Some of these may include enzymes such as resolvases as well as ligases that are important in the recombination process.

Publications

Frederick, C.A., Saal, D., van der Marel, G.A., van Boom, J.H., Wang, A.H.-J. and Rich, A.: The Crystal Structure of d(GGm^{5}CCGGCC): The Effect ofMethylation on A-DNA Structure and Stability. *Biopolymers* **26**, S145-S160 (1987).

Burley, S.K., Wang, A.H.-J., Votano, J.R. and Rich, A. Antigelling and Antisickling Bisphenyl Oligopeptides and Peptide Analogues Have Similar Structural Features. *Biochem.* **26**, 5091-5099 (1987).

Freeman, M.J., Clark, B.D., Collins, K.L., Webb, A.C., Rich, A. and Auron, P.E.: Transcriptional Regulation of the Human Prointerleukin 1b Gene. *J. Immunol.* **138**, 3972-2979 (1987).

Rich, A.: The Left-Handed Form of DNA and Its Binding Proteins. In *Integration and Control of Metabolic Processes: Pure and Applied Aspects.* (O.L. Kon, ed.), Cambridge University Press, England, pp. 247-267 (1987).

Coll, M., Frederick, C.A., Wang, A.H.-J. and Rich, A.: A Bifurcated Hydrogen-Bonded Conformation in the d(A·T) Base Pairs of the DNA Dodecamer d(CGCAAATTTGCG) and its Complex with Distamycin. *Proc. Natl. Acad. Sci. USA* **84**, 8385-8389 (1987).

Haran, T.E., Shakked, Z., Wang, A.H.-J. and Rich, A.: The Crystal Structure of d(CCCCGGGG): A New A-Form Variant with an Extended Backbone Conformation. *J. Biomolec. Struct. & Dyn.* **5**, 199-217 (1987).

Born, W., Freeman, M., Bornstein, W., Rapoport, A., Klein, R., Khorana, H.G.,Rich, A., Potts, J.T. and Kronenberg, H.M.: Signal Sequence of Human Preproparathyroid Hormone Is Inactive in Yeast. *J. Bone and Mineral Res.*), **2**, 353-361 (1987).

Pardue, M.L., Lowenhaupt, K., Rich, A. and Nordheim A.: $(dC\text{-}dA)_n\cdot(dG\text{-}dT)_n$ Sequences have Evolutionarily Conserved Chromosomal Locations in *Drosophila* with Implications for Roles in Chromosome Structure and Function. *EMBO J.*) **6**, 1781-1789 (1987).

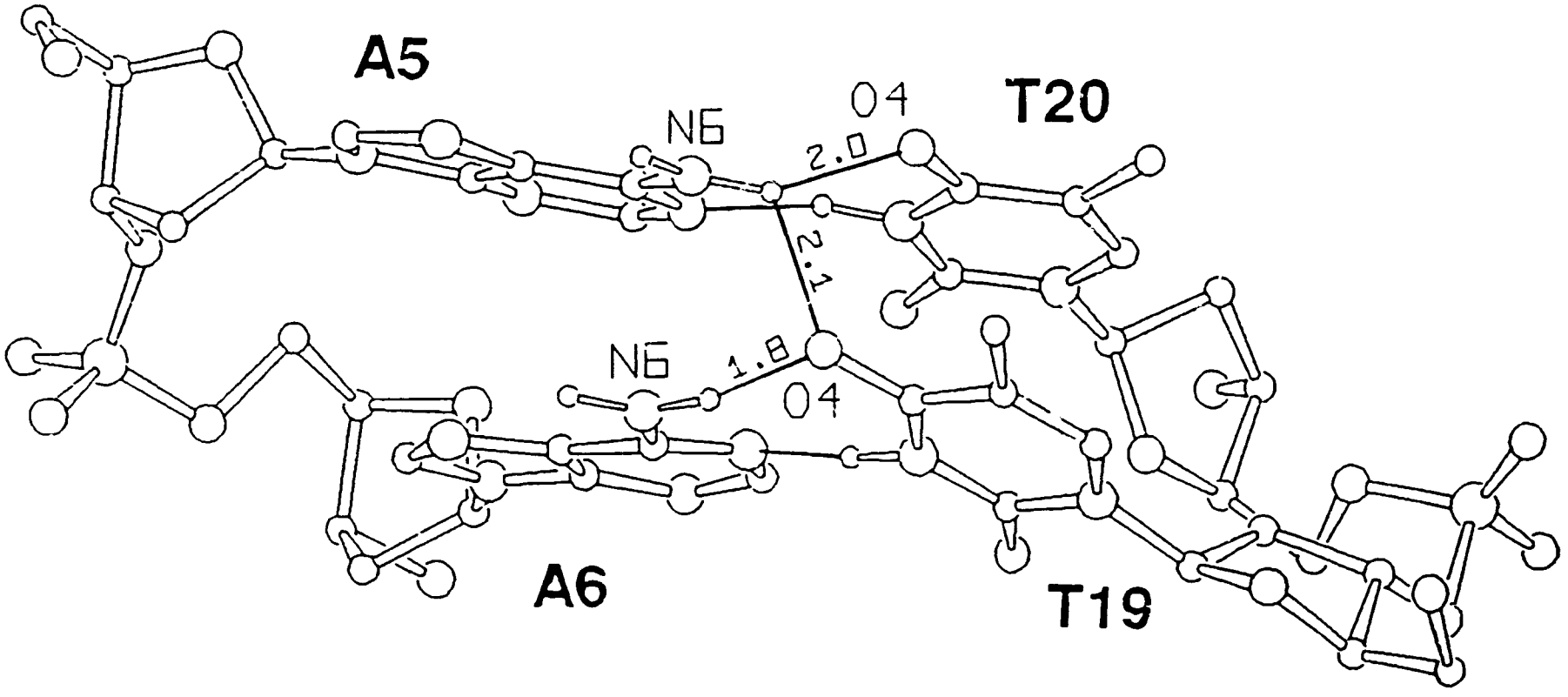
Fishel, R.A., Detmer, K. and Rich, A.: Identification of Homologous Pairing and Strand-Exchange Activity from a Human Tumor Cell Line Based on Z-DNA Affinity Chromatography. *Proc. Natl. Acad. Sci. USA* **85**, 36-40 (1988).

Ho, P.S., Quigley, G.J., Tilton, R. and Rich, A.: The Hydration of Methylated and Nonmethylated B- and Z-DNA. *J. Phys. Chem.* **92**, 935-939 (1988).

Holbrook, S.R., Wang, A.H.-J., Rich, A. and Kim, S.-H.: Local Mobility of Nucleic Acids as Determined from Crystallographic Data: III. A Daunomycin-DNA Complex. *J. Mol. Biol.* **199**, 349-357 (1988).

Johnston, B.H., Ohara, W. and Rich, A.: Stochastic Distribution of a Short Region of Z-DNA within a Long Repeated Sequence in Negatively Supercoiled Plasmids. *J. Biol. Chem.* **263**, 4512-4515 (1988).

McCarthy, J.G., Sander, M., Lowenhaupt, K. and Rich, A.: A Sensitive Homologous Recombination Strand Transfer Assay: Partial Purification of a *Drosophila* melanogaster Enzyme and Detection of Sequence Effects on the Strand Transfer Activity of recA Protein. *Proc. Natl. Acad. Sci.* **85**, 5854-5858 (1988)



Bifurcated Hydrogen Bond Lengths with Hydrogen Atoms

Fig. 1

PARANEMIC PATHWAY IN HOMOLOGOUS RECOMBINATION

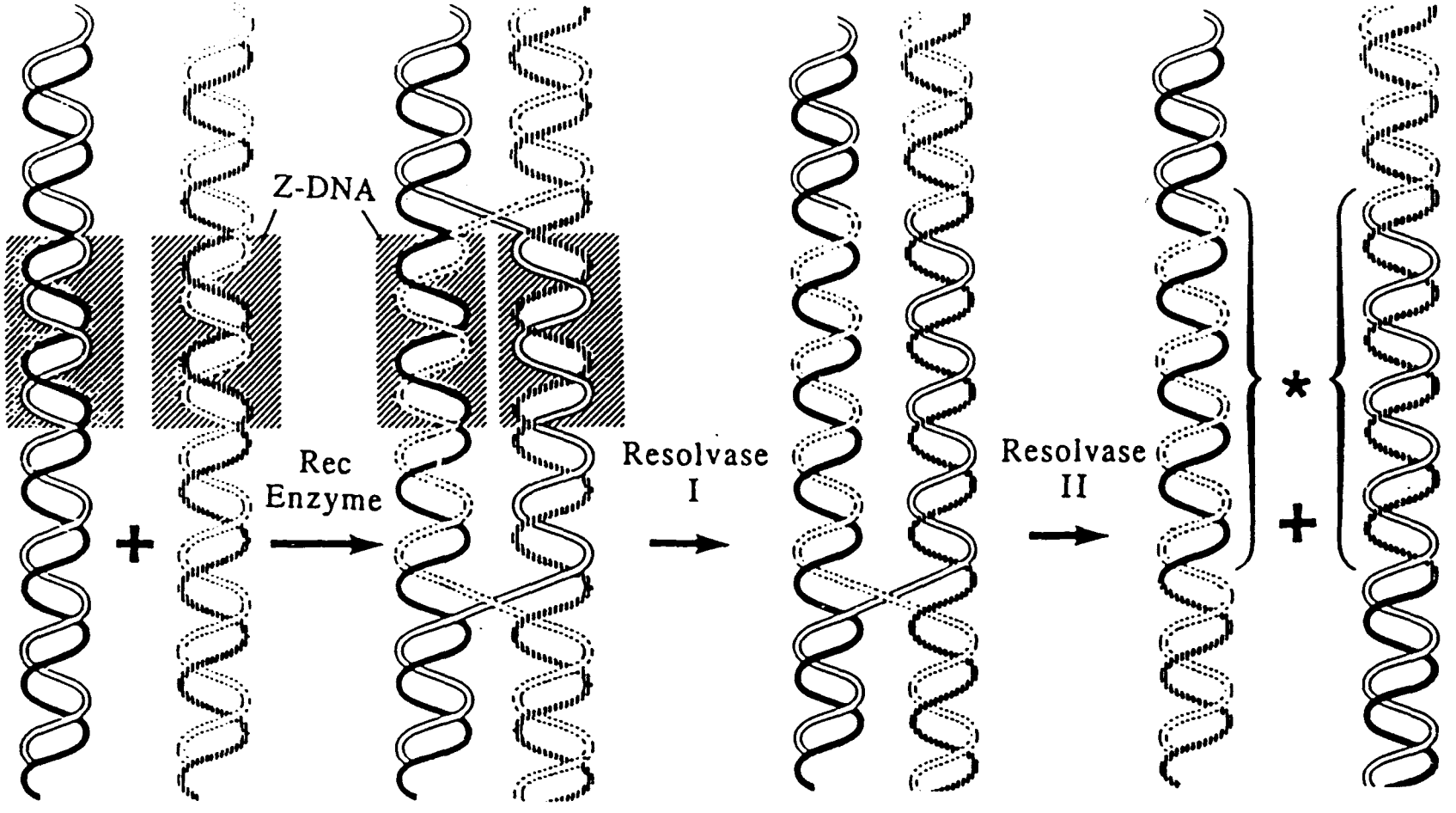


Fig. 2

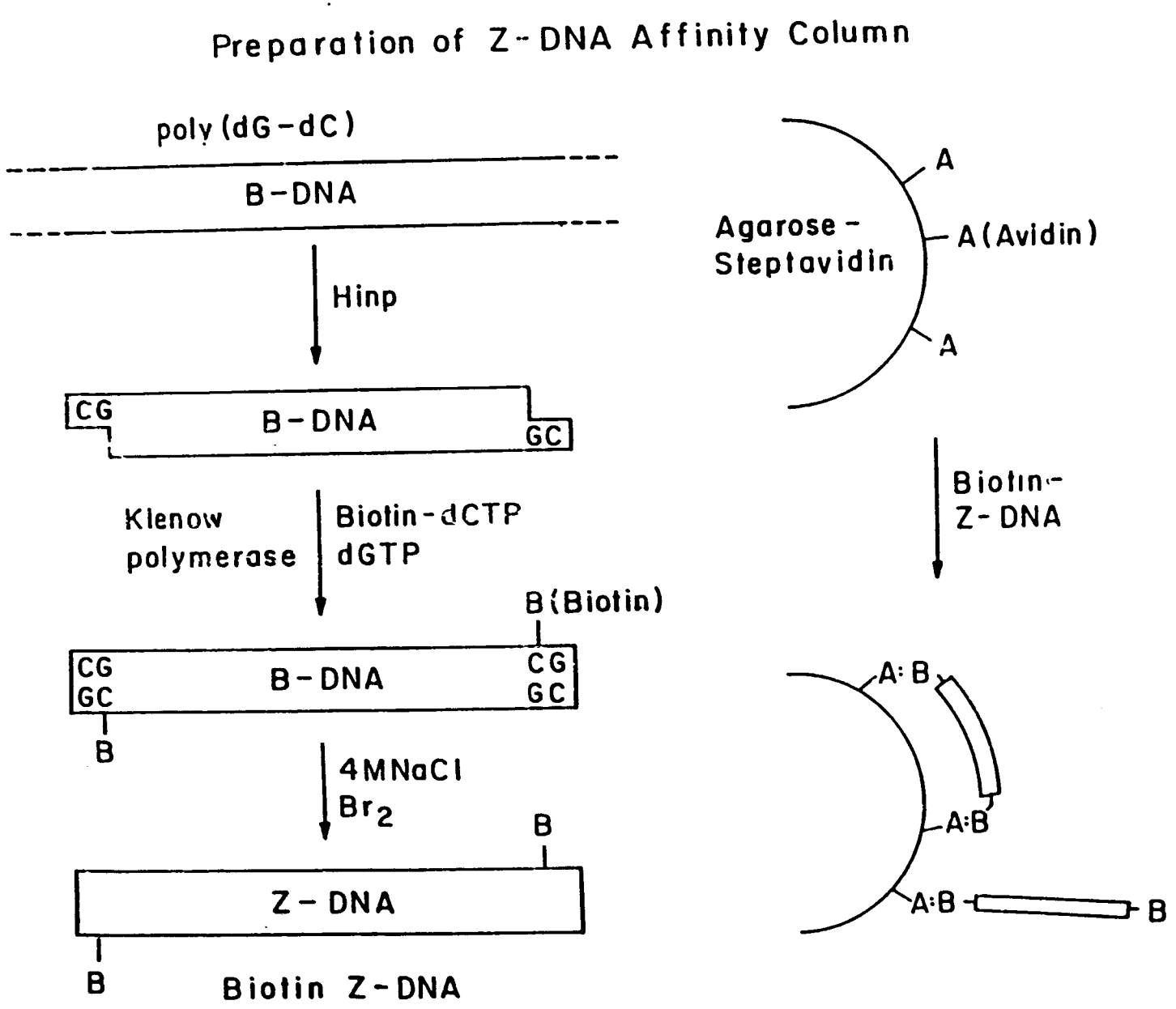
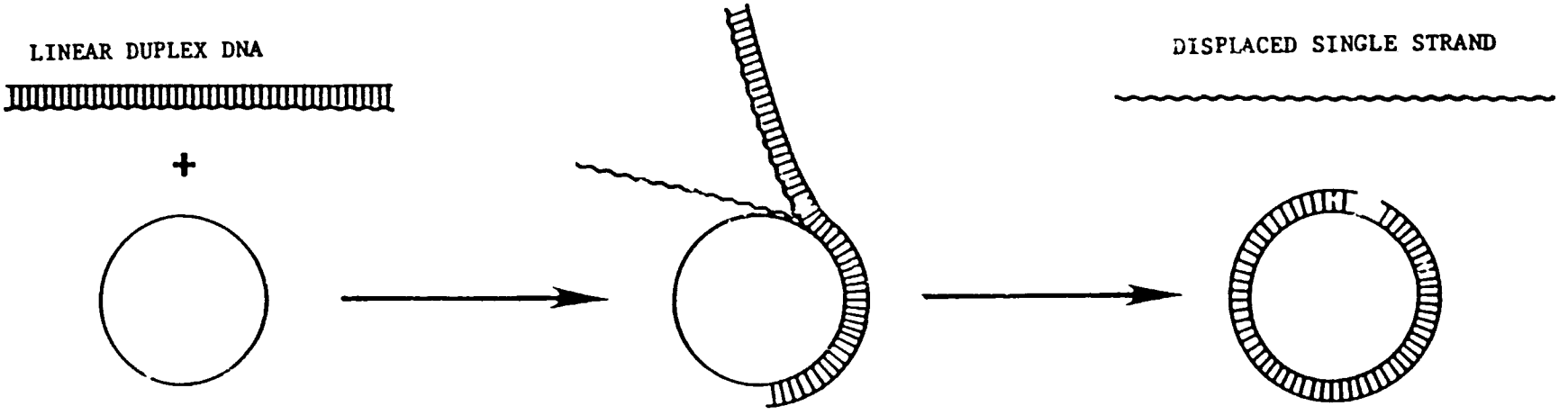

Preparation of Z-DNA Affinity Column
poly (dG-dC)
B-DNA
Hinp
CG
B-DNA
GC
Klenow polymerase
Biotin-dCTP
dGTP
B (Biotin)
CG
GC
B-DNA
CG
GC
B
4MNaCl
Br2
B
Z-DNA
B
Biotin Z-DNA
Agarose-Steptavidin
A
A (Avidin)
A
Biotin-Z-DNA
A:B
A:B
A:B
B

Fig. 3

STRAND TRANSFERASE



SINGLE STRANDED CIRCLE

α-FORM SEEN IN ELECTRON MICROSCOPE

NICKED CIRCLE (FORM II DNA)

PRODUCTS SEEN IN EM

Fig. 4