(2)

AD-A209 686

REPORT DOCUMENTATION PAGE

CTE

JUL 0 5 1989

D

1b. RESTRICTIVE MARKINGS NA	This file copy
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.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
61153N	RR04106	4413045	

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 6/88 TO 6/89	06/30/89	

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, Z-DNA AND Z-DNA BINDING PROTEINS, 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.

89 7 03 127

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	(U)

22a NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
M. Marron	(202) 696-4760	ONR

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

S/N 0102-LF-014-6603

SECURITY CLASSIFICATION OF THIS PAGE

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 1988

Objective

The major focus of work in this laboratory concerns the relationship between molecular structure and biological function. The research includes molecular biological studies in which alternative conformations of the nucleic acids may play an important role. In addition, there are a variety of x-ray diffraction studies of nucleic acids and related compounds in order to uncover the role that molecular conformation plays in interactions.

In previous years, attention was directed towards understanding the physical and chemical events that were important in the interconversion of right-handed B-DNA to left-handed Z-DNA. However, a significant component of the work of the laboratory now focuses on the biological activities of the left-handed Z form of DNA. Toward that end, several investigations involve the isolation and purification of various Z-DNA binding proteins. We seek to uncover their biological function. New methodologies have been created for isolating these proteins and separating them from the more numerous B-DNA binding proteins. There is also some evidence that the Z-DNA conformation plays a role in homologous genetic recombination. Accordingly, a number of studies have been carried out on this system.

X-ray diffraction studies have been carried out on fragments of DNA with a view towards understanding the role of nucleotide sequence in DNA conformation. This includes studies of both right-handed and left-handed helical conformations as well as their interactions with other substances.

(AW)

Progress Report

Advances in the identification of Z-DNA binding proteins have been limited by the lack of appropriate methodology to prove unequivocally that a protein is Z-DNA specific. Our approach to this problem has been to develop short oligonucleotide probes that are in the Z-DNA conformation at near physiological salt conditions. These probes are constructed by using chemically synthesized alternating purine-pyrimidine tracts to prime the enzymatic incorporation of chemically modified nucleotides, such as 5-bromo-deoxycytosine, into duplex DNA. Such probes, as judged by circular dichroism measurements and binding to a Z-DNA specific monoclonal antibody, will exist in the left-handed conformation in 50 mM sodium chloride in the presence of either magnesium chloride or cobalt hexamine chloride.

We have used these probes to test for Z-DNA binding proteins in two ways. First protein immobilized to nitrocellulose is assayed for ability to bind to the Z-DNA oligomer. Second, protein-binding to the probe is assessed by the ability of the protein to decrease the electrophoretic ability of the probe in a non-denaturing polyacryamide gel. In both assays specificity of binding is assessed by competition with large amounts of unlabeled B-DNA.

A number of purified DNA-binding proteins have been tested in these assays. These include T4 replication proteins, strand transferases from yeast, bacteria, *Drosophila* and human tissues, steroid-receptor protein, and Sph-1. None of these proteins bind to the probe. However it has been possible to identify in yeast, *Drosophila* and human nuclear extracts the presence of Z-DNA binding proteins. The activity from *Drosophila* co-purifies with histone H1. Proteins from other sources are currently being purified. It is planned to test for sequence-specific Z-DNA binding activity by using a footprint assay where such sequences are stabilized in the Z-DNA conformation by negative supercoiling.

Histone H1 appears to bind to DNA probes which are predominantly in the Z conformation in preference to DNA which is predominantly in the B conformation or single stranded. The binding of *Drosophila* H1 was initially identified by selecting nuclear proteins using brominated poly(dG-dC)-sephadex column chromatography. It has subsequently been verified using purified H1 samples. Studies are currently underway to determine whether this binding is truly Z-DNA specific and whether any specific

subfraction of H1 is involved. These studies are facilitated by a monoclonal antibody we have raised against *Drosophila* H1.

The purification of a protein from yeast which binds to the left-handed Z-conformation of DNA is described. Three assay systems were used to monitor this purification. The first was a rapid Southwestern dot blot assay. Protein fractions that had been dot blotted on nitrocellulose filters were probed with a ^{32}P end-labelled synthetic oligomer forty-four base pairs long that was stabilized in the left-handed Z-form by chemical modification. Specificity was enhanced by using a 200 fold molar excess of B-DNA and single-stranded DNA as competitor. The second was a gel retardation assay in which d(G-m^{5}C) polymer (approximately 600 bp) in the Z-form, labelled with ^{32}P and a 400 fold excess of B-DNA were incubated with the protein fractions and the mixture was subjected to non-denaturing gel electrophoresis in order to resolve the protein/DNA complex. The third was a Southwestern-PAGE/SDS assay. In this assay proteins were separated by electrophoresis on a polyacrylamide/SDS gel, electroblotted to Immobolin membrane, renatured and mixed with solution containing ^{32}P labelled Z-DNA in the presence of a 300 fold molar excess of B-DNA. The membrane was then washed several times to remove unbound DNA. These assays were used to monitor the purification of Z-DNA-binding activities from either a yeast whole cell extract or a yeast nuclear extract.

Extracts from a protease-minus mutant in early log phase were fractionated by means of a phosphocellulose ion exchange column, a Z-DNA affinity column, a Superose-12 gel filtration column and a Mono-S ion exchange column. Results from the three different assays were consistent and it was possible to identify a single peak of binding activity in each column. The major protein in our most highly purified preparations has a molecular weight of roughly 55,000 daltons which showed a positive signal when Z-DNA was used in the Southwestern assay. The protein is currently being microsequenced. Our goal is to isolate the corresponding gene and study it using biochemical and genetic approaches.

Previous work in this laboratory has demonstrated that Z-DNA binding proteins can be co-purified with SV40 minichromosomes. A component of this heterogenous population of proteins appears to bind to a specific sequence (GCATGCAT) within the SV40 enhancer element when it is in the Z-conformation. The goal of this research project is to purify this putative sequence-specific Z-DNA binding

protein (SZ) from HeLa cells and to assess its biological function. The approach being developed to assay the binding activity of SZ involves the use of ^{32}P labelled, supercoiled minicircles as affinity probes: The minicircles contain the SV40 sequence which adopts the Z-DNA conformation as a result of the superhelix density. These affinity probes could be employed for gel shift assays or "southwestern" analysis.

Objectives For the Next Year

We plan to purify Z-DNA binding proteins and will try to uncover their biological role. Histone H1 will be purified to find the subtype that binds to Z-DNA. The yeast binding proteins will be cloned, then deleted from yeast to uncover their biological function.

We also plan to continue the purification of the Z-DNA binding enzymatic activity that has been detected with a view toward understanding the manner in which the strand transferase activity participates in homologous recombination.

PUBLICATIONS

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. USA* **85**, 5854-5858 (1988).

Lafer, E.M., Sousa, R.J. and Rich, A. Z-DNA-Binding Proteins in *Escherichia coli*: Purification, Generation of Monoclonal Antibodies and Gene Isolation. *J. Mol. Biol.* **203**, 511-516 (1988).

Frederick, C.A., Quigley, G.J., van der Marel, G.A., van Boom, J.H., Wang, A.H.-J. and Rich, A. Methylation of the EcoRI Recognition Site Does Not Alter DNA Conformation: The Crystal Structure of d(CGCGAm^{6}ATTCGCG) at 2.0-Å Resolution. *J. Biol. Chem.* **263**, 17872-17879 (1988).

Fishel, R. and Rich, A. The Role of Left-Handed Z-DNA in General Genetic Recombination. In *Mechanisms and Consequences of DNA Damage Processing*, ed by E.C. Friedberg and Philip C. Hanawalt, Alan R. Liss, Inc., New York (1988), pp. 23-32.

Frederick, C., Wang, A., Rich, A., Regan, L. and Schimmel, P. Crystallization of a Small Fragment of an Aminoacyl tRNA Synthetase. *J. Mol. Biol.* **203**, 521-522 (1988).

Coll, M., Aymami, J., van der Marel, G.A., van Boom, J.H., Rich, A. and Wang, A.H.-J. Molecular Structure of the Netropsin-d(CGCGATATCGCG) Complex: DNA Conformation in an Alternating AT Segment. *Biochem.* **28**, 310-320 (1989).

Coll, M., Saal, D., Frederick, C.A., Aymami, J., Rich,A. and Wang, A.H-J. Effects of 5-fluorouracil/guanine Wobble Base Pairs in Z-DNA: Molecular and Crystal Structure of d(CGCGFG). *Nucl. Acids Res.* **17**, 911-923 (1989).

Wittig, B., Dorbic, T. and Rich, A. The Level of Z-DNA in Metabolically Active, Permeabilized Mammalian Cell Nuclei is Regulated by Torsional Strain. *J. Cell. Biol.* **108**, 755-764 (1989).

Lowenhaupt, K., Rich, A. and Pardue, M.L.. Nonrandom Distribution of Long Mono- and Dinucleotide Repeats in *Drosophila* Chromosomes: Correlations with Dosage Compensation, Heterochromatin, and Recombination. *Mol. Cell. Biol.* **9**, 1173-1182 (1989).

Aymami, J., Coll, M., Frederick, C.A., Wang, A.H.-J. and Rich, A.. The Propeller DNA Conformation of poly(dA)•poly(dT). *Nucl. Acids Res.* **17**, 3229-3245 (1989).

Manning, G.S., Ebralidse, K.K., Mirzabekov, A.D. and Rich, A. An Estimate of the Extent of Folding of Nucleosomal DNA by Laterally Asymmetric Neutralization of Phosphate Groups. *J. Biomol. Struct. & Dyn.* **6**, 0739-1102 (1989).