### NEW LIMITATION CHANGE

**To**

Approved for public release, distribution unlimited

**From**

Distribution authorized to U.S. Gov't. agencies only; Proprietary Info.; Aug 2000. Other requests shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott St., Fort Detrick, MD 21702-5012.

**Authority**

USAMRMC ltr, dtd 28 July 2003
Award Number: DAMD17-99-1-9150

TITLE: Understanding Single-Stranded Telomere End Binding by an Essential Protein

PRINCIPAL INVESTIGATOR: Emily Anderson, Ph.D.
Dr. Deborah Wuttke

CONTRACTING ORGANIZATION: University of Colorado
Boulder, Colorado 80309

REPORT DATE: August 2000

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, Aug 00). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
NOTICE

USING GOVERNMENT DRAWINGS, SPECIFICATIONS, OR OTHER DATA INCLUDED IN THIS DOCUMENT FOR ANY PURPOSE OTHER THAN GOVERNMENT PROCUREMENT DOES NOT IN ANY WAY OBLIGATE THE U.S. GOVERNMENT. THE FACT THAT THE GOVERNMENT FORMULATED OR SUPPLIED THE DRAWINGS, SPECIFICATIONS, OR OTHER DATA DOES NOT LICENSE THE HOLDER OR ANY OTHER PERSON OR CORPORATION; OR CONVEY ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE, OR SELL ANY PATENTED INVENTION THAT MAY RELATE TO THEM.

LIMITED RIGHTS LEGEND

Award Number: DAMD17-99-1-9150
Organization: University of Colorado
Location of Limited Rights Data (Pages):

Those portions of the technical data contained in this report marked as limited rights data shall not, without the written permission of the above contractor, be (a) released or disclosed outside the government, (b) used by the Government for manufacture or, in the case of computer software documentation, for preparing the same or similar computer software, or (c) used by a party other than the Government, except that the Government may release or disclose technical data to persons outside the Government, or permit the use of technical data by such persons, if (i) such release, disclosure, or use is necessary for emergency repair or overhaul or (ii) is a release or disclosure of technical data (other than detailed manufacturing or process data) to, or use of such data by, a foreign government that is in the interest of the Government and is required for evaluational or informational purposes, provided in either case that such release, disclosure or use is made subject to a prohibition that the person to whom the data is released or disclosed may not further use, release or disclose such data, and the contractor or subcontractor or subcontractor asserting the restriction is notified of such release, disclosure or use. This legend, together with the indications of the portions of this data which are subject to such limitations, shall be included on any reproduction hereof which includes any part of the portions subject to such limitations.

THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.

Kathleen More 12/20/00
Telomeres are the nucleoprotein structures that protect the ends of eukaryotic chromosomes. Telomere length regulation is controlled by the enzyme telomerase and a suite of telomere binding proteins. Anomalous telomeric replication and regulation are implicated in most forms of cancer, while telomeric shortening contributes to cellular aging. Cdc13p is an essential protein from S. cerevisiae that binds to the single-stranded ends of telomeres with high specificity and affinity. Genetically, Cdc13p has been shown to protect the end of the chromosome from degradation and to load telomerase. Biochemically, Cdc13p binds yeast single-stranded telomeric DNA (sstelo DNA) in vitro with high affinity ($K_d=0.3$ nM).

We are investigating the structural basis for high affinity binding and sequence specificity of the single-stranded DNA binding domain. We have expressed and purified the ssDNA binding domain in high yield. Its binding affinity and specificity have been examined with libraries of sstelo DNA randomized at each position. In vitro photocrosslinking experiments have been performed using 5-iodouracil substituted for thymine bases. Proteolytic digestion of the crosslinked products along with micro peptide sequencing have allowed us to identify sites critical for DNA binding. These experiments complement high resolution NMR studies of the protein/DNA complex in progress in our laboratory.

14. SUBJECT TERMS
breast cancer, telomeres, telomerase, single-stranded DNA binding protein, Cdc13, nuclear magnetic resonance (NMR), structural biology, biophysical chemistry, structure-function relationships, Saccharomyces cerevisiae

15. NUMBER OF PAGES
14

16. PRICE CODE
Unlimited

17. SECURITY CLASSIFICATION OF REPORT
Unclassified

18. SECURITY CLASSIFICATION OF THIS PAGE
Unclassified

19. SECURITY CLASSIFICATION OF ABSTRACT
Unclassified

20. LIMITATION OF ABSTRACT
Unlimited
Annual Report for: Understanding Single-Stranded Telomere End Binding by an Essential Protein

Emily M. Anderson
Department of Chemistry and Biochemistry
University of Colorado at Boulder

TABLE OF CONTENTS

Front Cover...........................................................................1
SF 298.............................................................................2
Table of Contents.................................................................3
Introduction.........................................................................4
Body..................................................................................4
Key Research Accomplishments..............................................7
Reportable Outcomes............................................................7
Appendices (Meeting Abstracts)..............................................8
INTRODUCTION

Telomeres are the nucleoprotein complexes that protect the ends of linear eukaryotic chromosomes. Telomere replication and length regulation are controlled by the enzyme telomerase and a suite of telomere binding proteins. Anomalous telomeric replication is implicated in most forms of human cancer. Telomere metabolism is thus an active field in basic research for the eventual goal of developing inhibitors or modulators of telomere replication for cancer therapy. Cdc13p is an essential protein from the budding yeast *Saccharomyces cerevisiae* whose role is to protect the end of the chromosome from degradation and to load telomerase. Biochemically, Cdc13p binds to single-stranded yeast telomeric DNA with high affinity and specificity. We are investigating the structural basis for high affinity binding and sequence specificity of the DNA binding domain. One aspect of this research involves solving the high resolution solution structure of the domain complexed to DNA using heteronuclear, multidimensional NMR. Biochemical techniques are also being employed, including mapping regions of the domain in proximity to the DNA by photocrosslinking and investigating sequence specificity using libraries of DNA with varying sequences. The advantage of studying this protein using yeast as a model system is the power of combining structure, biochemistry, and genetics all in one system.

BODY

Significant progress toward accomplishment of the technical goals has been completed to date. Technical objective 1, outlined below, has been completed in full.

**Technical Objective 1:**
- Express and purify DNA binding constructs
- Conduct binding assays with site-randomized DNA
- Conduct CD experiments of protein folding and DNA binding

An optimized DNA-binding domain construct has been delineated using proteolysis and MALDI mass spectrometry. This construct has been subcloned, expressed and purified in high yield, suitable for high resolution structural characterization. The construct binds DNA with affinity comparable to that reported for the full-length protein as measured by both gel-shift binding assays and nitrocellulose filter-binding assays. Binding assays were conducted with site-randomized single-stranded DNA oligomers to determine bases in the DNA critical for binding affinity and specificity. These experiments are to be followed up by experiments involving chemical modification of the DNA with dimethylsulfate. Circular Dichroism experiments were performed to assess the secondary structural content of the domain, whether there are gross structural changes upon DNA binding, and to assess the thermostability of the domain in isolation. It was found that the domain in isolation forms a compact, stable, globular structure with both α helical and β sheet structure content. No major secondary structural changes occurred upon DNA binding.
Technical objective 2 is well on its way to completion.

**Technical objective 2:**

Conduct photocrosslinking/identify contacts 3 Months  
Design mutants/test *in vitro* and *in vivo* 6 Months

Photocrosslinking experiments with the chromophore iodouracil substituted for thymine have been performed. Upon proteolytic digestion and micro-Edman sequencing, peptides in the domain which crosslink to various substituted DNAs have been identified, along with the sites of crosslinking in the peptide. Although MALDI mass spectrometry was not successful in identifying the crosslinks, we are in the process of confirming their identity with electrospray ionization mass spectrometry, as this technique is less sensitive to peptide identity, sample preparation, etc. Mutations are currently being introduced at the sites of crosslinking to assess the effects of these mutations on DNA binding and crosslinking efficiency. The *in vivo* effect of these mutations will be assessed in the laboratory of our collaborator, Dr. Victoria Lundblad at the Baylor College of Medicine. A manuscript is currently in preparation to be published outlining the results of the photocrosslinking experiments.

Technical objective 3 involves primarily the high resolution NMR solution structure of the domain. Several of the tasks have been completed, and the rest are in progress. Two significant changes to the objective have been made. First, although the domain construct of the protein we have chosen exhibits adequate DNA binding and structural compactness by CD spectropolarimetry as mentioned earlier, we have not yet succeeded at keeping the domain alone in concentrated solution conditions long enough for any of the triple-resonance NMR experiments. However, the protein/DNA complex exhibits excellent solution behavior, and our structural efforts are focused in this direction. Second, the structural studies are now being completed in collaboration with another graduate student in the lab, Rachel Mitton-Fry. Technical objective 3 was stated originally as follows:

**Technical Objective 3:**

Optimize solution conditions of sample for NMR spectroscopy 1 Month  
Protein alone –  
Collect heteronuclear NMR data for resonance assignment 6 Months  
Assign resonances in the protein domain 6 Months  
Collect heteronuclear NMR data for distance restraints 1 Month  
Determine family of structures that satisfy restraints 6-12 Months  
Protein/DNA complex –  
Titrate DNA into protein and conduct NMR experiments 6-18 Months
Solution conditions for the sample have been optimized through conducting a buffer screen of the protein/DNA complex. Samples of well over 1 mM concentration can be routinely prepared and NMR experiments can be conducted at 30 °C for several weeks to over a month. As mentioned earlier, work has focused on the protein in complex with DNA. The entire suite of triple resonance heteronuclear NMR experiments has been collected on a $^{15}$N/$^{13}$C labeled protein sample with DNA, including HNCA, HNCO, HN(CO)CA, HNCACB, CBCA(CO)NH, etc. Rachel Mitton-Fry has completed the backbone amide assignments in the domain and has completed approximately half of the side-chain resonances. Carbon assignments are in progress as well. NOE experiments to determine distance restraints have been conducted and are in the process of being assigned and interpreted. This fall I will perform experiments to measure 3-bond scalar coupling which will add more restraints to allow for structure calculation. I will also examine the conformation of the unlabeled DNA in the complex using NMR by conducting isotope filtering experiments. Also this fall, a rotation student in the lab will continue efforts at optimizing solution conditions of the protein domain alone and conduct hydrogen exchange experiments by NMR to determine packed hydrophobic residues and surface expose residues.
KEY RESEARCH ACCOMPLISHMENTS

- An optimal construct of the Cdc13p single-stranded DNA binding domain has been expressed and purified in high yield
- DNA binding experiments have been conducted using site-randomized single-stranded telomeric DNA oligomers
- CD spectropolarimetry experiments of the domain have been performed to assess the folded state and DNA binding
- Photocrosslinking experiments have mapped key residues in the protein domain located at the protein/DNA interface
- Solution conditions have been optimized to perform high resolution NMR experiments of the protein/DNA complex
- Heteronuclear NMR experiments have been performed on the complex to assign residues in the protein domain
- Main-chain assignments of the domain have been completed, and approximately half of the side-chain resonances

REPORTABLE OUTCOMES

Manuscripts: The results of the photocrosslinking study (Technical Objective 2) are currently being prepared as a manuscript for publication.

Abstracts: The work in progress has been presented as a poster at several meetings: the 2000 Colorado Protein Stability Conference (Breckenridge, CO), the 14th Symposium of the Protein Society (Student Poster Award - San Diego, CA) and the 42nd Annual Rocky Mountain Conference on Analytical Chemistry (Broomfield, CO).

Presentations: This work has been presented as a talk in two formats: the RNA Club Meeting of the University of Colorado in March, 2000 and the Biotechnology Program Summer Student Seminar in August, 2000.
2000 COLORADO PROTEIN STABILITY CONFERENCE

Presented by

The University of Colorado Center for Pharmaceutical Biotechnology

We Gratefully Acknowledge Support from Our Generous Sponsors

Amgen
Baxter
Bristol Meyers
Genencor
Genetics Institute
Immunex
McAfee Consulting
Pfizer
Zymogenetics

AstraZeneca
Biogen
Chiron
Genentech
Glaxo Wellcome
Inhale Therapeutics
Merck Research Labs.
Red Storm Software
Biochemical Investigation of a Sequence-Specific, Single-Stranded DNA Binding Protein at the Telomere

E.M. Anderson¹, R.M. Mitton-Fry¹, T.R. Hughes², V. Lundblad², D.S. Wuttke¹

¹Dept. of Chemistry and Biochemistry, U. of Colorado, Boulder, CO 80309
²Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, 77030

Telomeres are the nucleoprotein structures that protect the ends of eukaryotic chromosomes. Telomere length regulation is controlled by the replicative enzyme, telomerase, and a suite of telomere binding proteins. Anomalous telomeric replication and regulation are implicated in many forms of cancer, while telomeric shortening contributes to cellular aging. Cdc13p is an essential protein from S. cerevisiae that binds to the single-stranded ends of telomeres with high specificity and affinity. Genetically, Cdc13p has been shown to protect the end of the chromosome from degradation and to load telomerase. Biochemically, Cdc13p binds yeast single-stranded telomeric DNA (sTELO DNA) in vitro with high affinity (Kₐ=0.3 nM).

We are investigating the structural basis for high affinity binding and sequence specificity of the single-stranded DNA binding domain. We have expressed and purified the isolated ssDNA binding domain in high yield. Its binding affinity and specificity have been examined with libraries of sTELO DNA randomized at each position. In vitro photocrosslinking experiments have been performed using 5-idothymine substituted for thymine bases. Proteolytic digestion of the crosslinked products along with micro peptide sequencing and MALDI mass spectrometry have allowed us to identify sites in the protein critical for sTELO DNA binding. These experiments complement high resolution NMR studies of the protein/DNA complex in progress in our laboratory.
42nd ROCKY MOUNTAIN CONFERENCE ON ANALYTICAL CHEMISTRY

FINAL PROGRAM AND ABSTRACTS

SPONSORED BY:
Rocky Mountain Section — Society for Applied Spectroscopy
Colorado Section — American Chemical Society

July 30 – August 3, 2000
www.rockychem.com

Omni Interlocken Resort • 500 Interlocken Boulevard • Broomfield, Colorado
Telomeres are the nucleoprotein structures that protect the ends of eukaryotic chromosomes. Telomere length regulation is controlled by the telomerase enzyme. In this study, the aqueous fraction of bio-oil, generated from fast pyrolysis, was catalytically steam reformed at 825 and 875°C, high space velocity (up to 126,000 h⁻¹) and low residence time (26 ms). Using a fixed-bed micro-reactor interfaced with a molecular-beam mass-spectrometer (MBMS), the authors observed a relatively short test in which changes to the full slate of products could be monitored. A variety of research and commercial nickel-based catalysts were tested. The cobalt-promoted nickel and chromium-promoted nickel supported on MgO-LaO-α-alumina catalysts showed the best results of the research catalysts. At the reaction conditions used, progressive catalyst deactivation was observed leading to a decrease in the yields of hydrogen and carbon dioxide and an increase in carbon monoxide. The loss of activity also resulted in the formation of higher amounts of methane, benzene and other aromatic compounds. Commercial steam-reforming catalysts proved to be more efficient for hydrogen production from bio-oil than most of the research catalysts, mainly due to the higher water-gas shift activity. Supported by the U.S. DOE Hydrogen Program and the Secretaria de Estado de Universidades, Investigacion y Desarrollo (Spain).

**MS, GC/MS, LC/MS Oral Session—Shane Needham, Alturas Analytics, Inc., 1282 Alturas Drive, Moscow, ID 83843, Tel: 208-883-3400, Fax 208-882-9246, E-mail: sneedham@alturasanalytics.com**

**MS, GC/MS, LC/MS Poster Sessions**

**180. SURFACE ENHANCED RAMAN IMMUNOASSAY (SERIA): MEASUREMENT OF PHARMACEUTICALS AND DISEASES. Jason Quijchetteau, Roberta Sulk, Keith Carron, Robert Corcoran, University of Wyoming, Department of Chemistry, Laramie WY 82071-3838**

Surface Enhanced Raman cattering (SERS) spectroscopy offers a unique approach to immunoassays through its highly localized enhancement of materials at the surface of the metal substrate. The localized enhancement allows us to perform sandwich or competitive assays in the presence of normally interfering species. In particular, we are able to perform assays in the presence of excess reporters since only those bound directly to the antibody/antigen complex will be observed in the SERS spectrum. The aspect of our approach is very important as it eliminates the washing steps that introduce error and spread biohazardous waste in conventional immunoassays. We will discuss the instrumentation involved in the SERIA measurements and the methodologies. Appropriate dyes for tagging antibodies will be presented. Partial Least Squares (PLS) techniques were used for data quantization. Particular systems that will be discussed are human growth hormone, thyroid stimulating hormone, pesticides, illicit drugs, and prion diseases.

**MS, GC/MS, LC/MS Oral Session—Keith Carron, University of Wyoming, Department of Chemistry, Laramie, WY 82071-3838. Tel: 307 766-2811, Fax: 307 766-2807, E-mail: carron@uwyo.edu**

**181. BIOCHEMICAL INVESTIGATION OF A SEQUENCE-SPECIFIC, SINGLE-STRANDED DNA BINDING PROTEIN AT THE TELOMERE. E.M. Anderson¹, R.M. Mitton-Fry², T.R. Hughes³, V. Lundblad⁴, D.S. Wutke¹, Dept. of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309 and ¹Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, 77030**

Telomeres are the nucleoprotein structures that protect the ends of eukaryotic chromosomes. Telomere length regulation is controlled by the replicative enzyme, telomerase, and a suite of telomere binding proteins. Anomalous telomeric replication and regulation are implicated in many forms of cancer, while telomeric shortening contributes to cellular aging. Cdc13p is an essential protein from S. cerevisiae that binds to the single-stranded ends of telomeres with high specificity and affinity. Genetically, Cdc13p has been shown to protect the end of the chromosome from degradation and to load telomerase. Biochemically, Cdc13p binds yeast single-stranded telomeric DNA (sstelo DNA) in vitro with high affinity (Kd=0.3 nM). We are investigating the structural basis for high affinity binding and sequence specificity of the single-stranded DNA binding domain. We have expressed and purified the isolated ssDNA binding domain in high yield. Its binding affinity and specificity have been examined with libraries of sstelo DNA randomized at each position. In vitro photocrosslinking experiments have been performed using 5-iodouracil substituted for various thymines. Trypsin digestion of the crosslinked products along with micro peptide sequencing and MALDI mass spectrometry have allowed us to identify sites in the protein critical for sstelo DNA binding. These experiments complement high resolution NMR studies of the protein/DNA complex in progress in our laboratory.

**MS, GC/MS, LC/MS Poster Session—Emily Anderson, Dept. of Chemistry and Biochemistry, University of Colorado at Boulder, Campus Box 215, Boulder, CO, 80309-0215. Tel: (303) 492-2369, Fax: (303) 492-5894, E-mail: Emily.Anderson@colorado.edu**

**182. HIGH THROUGHPUT ANALYSIS OF COMBINATORIAL LIBRARIES BY FIA AND LC/MS ANALYSIS. Meg Hermann, Kathy Halm, Kevin Ash, Adam Cook, Mark Munson, Alan Florjancic, Gary Hingorani; Conrad Hummel; Greg Miknis; John Josey, Array BioPharma Inc., 1885 33rd Street, Boulder, CO 80301**

Traditional sequential medicinal chemistry methods have been augmented by combinatorial synthesis methods yielding a higher number of compounds for high throughput screening against a disease target and consequently a greater number of compounds that need to be analyzed. Initially, the analysis of compounds was accomplished by flow injection mass spectrometry of all wells in a 96-well plate. A well was passed or failed based on the presence of the molecular ion with at least 25% base peak intensity. In trying to coordinate library information with the RP-HPLC/UV purity data it became obvious that having LC/UV/MS data on libraries was crucial. Therefore, LC/UV/MS analysis was performed on the same wells that were being analyzed in parallel by RP-HPLC/UV for purity. The sampling protocol for purity determination at Array BioPharma is three columns per plate. This work was performed using a Gilson 215 autosampler with a TSP 4000 pump and TSP 2000.
THE PROTEIN SOCIETY

Fourteenth Symposium
San Diego, CA
August 5–9, 2000

PROGRAM & ABSTRACTS

Future Protein Society Symposia
4th European Symposium, 18–22 April 2001, Paris, France
15th Annual Symposium, July 28–August 1, 2001, Philadelphia, PA
16th Annual Symposium, August 17–21, 2002, San Diego, CA
**BIOCHEMICAL MECHANISM AND ENZYME FUNCTION**

**486-T**

Dynamic studies of Single Molecule E. Coli RNA Polymerase D. Schleske, B. Kashefi, C. L.Waster, B. Landick, L. Eganman (1). Howard Hughes Medical Institute and Department of Molecular and Cell Biology and Department of Physics, University of California, Berkeley, CA 94720-3265. USA. Reptilase mechanisms are defined by RNA-dependent RNA polymerase (RdRp) which are typically large, multi-subunit enzymes. It has been shown that E. Coli RNA polymerase accumulates along the DNA discontinuously during the elongation phase of transcription, spending proportionally more time at some template positions, known as pause sites. Thus, it is of interest to determine the biochemical basis of transcriptional pausing and arrest by and substrate chemistry on the enzymatic reaction pathway. To this end, we are the crosslinked products along with micro peptide sequencing and MALDI mass spectrometry.

**487-S**

BIOCHEMICAL INVESTIGATION OF A SEQUENCE-SPECIFIC SINGLE-STRANDED DNA BINDING PROTEIN AT THE TELOMERE E.M. Anderson, R.J. Whitley, B.R. Hughes, C. Lambert, D.S. Markey, Dep of Chemistry and Biochemistry U of Colorado Boulder, CO 80309 and Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030.

Telomeres are nucleoprotein structures which protect the ends of linear eukaryotic chromosomes. Telomere length regulation is controlled by telomerase and a subset of telomere binding proteins. Anchoring telomere replication is implicated in most forms of cancer while telomere shortening contributes to cellular aging. Cdc13p is an essential protein from S. cerevisiae. Generally, Cdc13p interacts at the end of the chromosome from degradation and helps to load telomerase. Biochemically, Cdc13p binds single-stranded telomeric DNA (ssDNA) in vitro with high affinity (Kd=0.3 nM). We are investigating the structural basis for high affinity binding and sequence specificity of the single-stranded DNA binding domain. We have re-engineered and purified the isolated adenovirus binding domain in high yield. Its binding affinity and specificity have been examined with libraries of ssDNA randomized at each position. These phage-displaying experiments have been performed using 5'end-labeled substituent for thymine bases. Trypsin digestion of the crosslinked products along with micro peptide sequencing and MALDI mass spectrometry have allowed us to identify sites in the protein critical for ssDNA binding. These experiments complement high resolution NMR studies of the protein/DNA complex in progress in our laboratory.

Funded by: NIBR. American Cancer Society, CU Faculty Development Award. R21/1 Howard Hughes Medical Institute, ESA 1 US Army Breast Cancer Research Program

**488-M**

Characterization of CR52: A Group II Intron Splicing Factor. J. Davenport, R.J. Whetre, G.J.L. Wittke, T. Latel. Ohio State University, Columbus, OH 43210.

Group II introns are widespread in yeast and plant mitochondria and chloroplasts. All cellular organisms use specialized RNA polymerases called "primases" to synthesize RNA primers for the initiation of DNA replication. In this presentation, we will describe the first high-resolution crystal structure of an active protein domain, comprising the catalytic core of the E. coli DnaG protein. The core contains an active site architecture that is conserved between DNA and RNA polymerase palm folds, but is instead related to the primase fold. Based on the structure, it is likely that DnaG binds nucleic acid in a groove clustered with invariant residues and that DnaG is positioned within the replicosome to accept DNA directly from the replicative helicase. Supported by The Jane Coffin Childs Memorial Fund for Medical Research (JCK) and the G. Harold and Leila Y. Macfarlane Charitable Foundation (JMB).

**489-T**

Structure of the RNA polymerase domain of E. coli primase J.L. Koch, B.E. Rotha, A.L. Lynch, J.L. Berger, Dept. MCB, Univ. California, Berkeley, CA 94720.1 (JKB) T. Takeda, South San Francisco, CA 94080 (DOR, ASL). All cellular organisms use specialized RNA polymerases called "primases" to synthesize RNA primers for the initiation of DNA replication. In this presentation, we will describe the first high-resolution crystal structure of an active protein domain, comprising the catalytic core of the E. coli DnaG protein. The core contains an active site architecture that is conserved between DNA and RNA polymerase palm folds, but is instead related to the primase fold. Based on the structure, it is likely that DnaG binds nucleic acid in a groove clustered with invariant residues and that DnaG is positioned within the replicosome to accept DNA directly from the replicative helicase. Supported by The Jane Coffin Childs Memorial Fund for Medical Research (JCK) and the G. Harold and Leila Y. Macfarlane Charitable Foundation (JMB).

**490-S**

Zinc finger is the nuclear localization signal of transcription factor Sgl J. Kono, H. Yamada, T. Kitajima, T. Morita, A. Watanabe, K. Uchi. Faculty of Pharmaceutical Sciences, University of Edogawa, Tokyo 120-8551, Japan. The bidirectional traffic between the nucleus and cytoplasm of a growing mammalian cell is assured through nuclear pore complexes (NPCs). Globular proteins of greater than 60 kDa can not cross the NPC by simple diffusion at a significant rate and therefore, transport of large proteins into the nucleus is an active process that requires presence of a suitable nuclear localization signal (NLS). Sgl is an ubiquitous transcription factor isolated from HeLa cell. Although the protein is suggested to be actively transported into the nucleus because of its relatively large molecular mass (65 kDa:110 kDa), none of NLS has been reported. The aim of our research is to identify the NLS of Sgl and to understand its nuclear transport mechanism. We show here subcellular localization of Sgl. Full length or truncated fragments of human Sgl cDNA were ligated to the green fluorescent protein (GFP) gene from very fish, that is expressed under control of CMV promotor. The plasmid constructs were transfected into HeLa cell by lipofection. Localization of truncated recombinant GFP-Sgl fusion protein was detected using confocal laser scanning microscope. Fusion of mouse Sgl accumulated GFP in the nucleus of HeLa cell. Whereas GFP alone was localized throughout the cell. We found that the three contiguous repeats of GFP-25 kDa fusion tag were sufficient to localize GFP in the nucleus. These results suggest that the three functional domains for DNA binding and nuclear localization could be spatially close or could overlap each other.

**491-M**

Stereoinduction Study of WT DNA Polymerase Beta and Mutant D276R J. Liu, X. Zhong and M.D. Tsai, Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH 43210. DNA polymerase beta (Pol β) is a DNA repair enzyme that requires magnesium ions to catalyze the nucleotide transfer reaction. In the current study, based on the crystal structures and rational prediction, pre-existing stereo kinetic analysis, stereo directed mutations and stereo phosphorylated isotopes (dATPdS) were used to study the stereoselectivity of Pol β. Several important findings have been obtained: (1) Sp isomer was highly preferred for WT Pol β in the presence of Mg2+, while the stereoinducitiveness towards Sp was decreased dramatically when Mg2+ was replaced by Mn2+. This result suggests that metal ions are one of the important factors that control the stereoinducitiveness of Pol β. (2) A new mutagenesis of D276R was designed to introduce an extra hydrogen bonding interaction between the Arg side chain and the gamma sp oxygen of deoxyphosphate. The kinetic data showed that the stereoselectivity of D276R is "relaxed" compared with WT Pol β, suggesting that the newly introduced protein side chain interacts with deoxyphosphate and influences the stereoselectivity of the enzyme. This work was supported by the NIH.
MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

[Signature]

PHYLIS M. RINEHART
Deputy Chief of Staff for Information Management
<table>
<thead>
<tr>
<th>ADB233865</th>
<th>ADB264750</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADB265530</td>
<td>ADB282776</td>
</tr>
<tr>
<td>ADB244706</td>
<td>ADB286264</td>
</tr>
<tr>
<td>ADB285843</td>
<td>ADB260563</td>
</tr>
<tr>
<td>ADB240902</td>
<td>ADB277918</td>
</tr>
<tr>
<td>ADB264038</td>
<td>ADB286365</td>
</tr>
<tr>
<td>ADB285885</td>
<td>ADB275327</td>
</tr>
<tr>
<td>ADB274458</td>
<td>ADB286736</td>
</tr>
<tr>
<td>ADB285735</td>
<td>ADB286137</td>
</tr>
<tr>
<td>ADB286597</td>
<td>ADB286146</td>
</tr>
<tr>
<td>ADB285707</td>
<td>ADB286100</td>
</tr>
<tr>
<td>ADB274521</td>
<td>ADB286266</td>
</tr>
<tr>
<td>ADB259955</td>
<td>ADB286308</td>
</tr>
<tr>
<td>ADB274793</td>
<td>ADB285832</td>
</tr>
<tr>
<td>ADB285914</td>
<td></td>
</tr>
<tr>
<td>ADB260288</td>
<td></td>
</tr>
<tr>
<td>ADB254419</td>
<td></td>
</tr>
<tr>
<td>ADB282347</td>
<td></td>
</tr>
<tr>
<td>ADB286860</td>
<td></td>
</tr>
<tr>
<td>ADB262052</td>
<td></td>
</tr>
<tr>
<td>ADB286348</td>
<td></td>
</tr>
<tr>
<td>ADB264839</td>
<td></td>
</tr>
<tr>
<td>ADB275123</td>
<td></td>
</tr>
<tr>
<td>ADB286590</td>
<td></td>
</tr>
<tr>
<td>ADB264002</td>
<td></td>
</tr>
<tr>
<td>ADB281670</td>
<td></td>
</tr>
<tr>
<td>ADB281622</td>
<td></td>
</tr>
<tr>
<td>ADB263720</td>
<td></td>
</tr>
<tr>
<td>ADB285876</td>
<td></td>
</tr>
<tr>
<td>ADB262660</td>
<td></td>
</tr>
<tr>
<td>ADB282191</td>
<td></td>
</tr>
<tr>
<td>ADB283518</td>
<td></td>
</tr>
<tr>
<td>ADB285797</td>
<td></td>
</tr>
<tr>
<td>ADB269339</td>
<td></td>
</tr>
<tr>
<td>ADB264584</td>
<td></td>
</tr>
<tr>
<td>ADB282777</td>
<td></td>
</tr>
<tr>
<td>ADB286185</td>
<td></td>
</tr>
<tr>
<td>ADB262261</td>
<td></td>
</tr>
<tr>
<td>ADB282896</td>
<td></td>
</tr>
<tr>
<td>ADB286247</td>
<td></td>
</tr>
<tr>
<td>ADB286127</td>
<td></td>
</tr>
<tr>
<td>ADB274629</td>
<td></td>
</tr>
<tr>
<td>ADB284370</td>
<td></td>
</tr>
<tr>
<td>ADB264652</td>
<td></td>
</tr>
<tr>
<td>ADB281790</td>
<td></td>
</tr>
<tr>
<td>ADB286578</td>
<td></td>
</tr>
</tbody>
</table>