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Form Approved Through 09/30/2011

OMB No. 0925-0002

Department of Health and Human Services
Public Health Service

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ional Research Service A
owship Application

nstructions carefully.
Do not exceed character length restrictions indicated.

PI: REYES, EMIGDIO D
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Dual: CA
IRG: ZRG1 IMST-E(29) L

Council: 10/2009
Received: 04/08/2009

1. TITLE OF RESEARCH TRAINING PROPOSAL (Do not exceed 81 characters, including spaces and punctuation.)
Biochemical Characteristics of the RecN Protein in DNA Repair Mechanisms

2. LEVEL OF FELLOWSHIP	3. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT ☐ NO ☒ YES (If "Yes," state number and title)
Predoctoral	Number: PA-07-106 Title: Ruth L. Kirschstein NRSA for Individual Predoctoral

4a. NAME OF APPLICANT (Last, First, Middle)	4b. ERA COMMONS USER NAME	4c. HIGHEST DEGREE(S)
Reyes, Emigdio, D.		B.S.

4d. PRESENT MAILING ADDRESS (Street, City, State, Zip Code)	4e. PERMANENT MAILING ADDRESS (Street, City, State, Zip Code)

4f. E-MAIL ADDRESS: [redacted]

TELEPHONES AND FAX (Area code, number and extension)

4g. OFFICE	4h. HOME	4i. PERMANENT	4j. FAX NUMBER
575-646-4427			

4k.

5. TRAINING UNDER PROPOSED AWARD (See Fields of Training)		6. PRIOR AND/OR CURRENT NRSA SUPPORT (Individual or Institutional)
Discipline No.: 1130	Subcategory Name: Enzymology	☒ NO ☐ YES (If "Yes," refer to item 22, Form Page 5)

7a. DATES OF PROPOSED AWARD		7b. PROPOSED AWARD DURATION	8. DEGREE SOUGHT DURING PROPOSED AWARD	
From (MM/DD/YY):	Through (MM/DD/YY):	(in months)	Degree:	Expected Completion Date:
12/01/09	11/30/11	24	Ph.D.	12/2011

9. HUMAN SUBJECTS RESEARCH	9b. Federalwide Assurance No.	10. VERTEBRATE ANIMALS ☒ No ☐ Yes
☒ No ☐ Yes ☐ Indefinite	9c. Clinical Trial ☐ No ☐ Yes; 9d. NIH-defined Phase III Clinical Trial ☐ No ☐ Yes	10a. Animal Welfare Assurance No.
9a. Research Exempt ☐ No ☐ Yes If "Yes," Exemption No.		

11. SPONSORING INSTITUTION	13. OFFICIAL SIGNING FOR SPONSORING INSTITUTION
Name: The Regents of New Mexico State University	Name: Ancelmo Encinias
Address: Office of Grants and Contracts MSC OGC, Box 30002 Anderson Hall, E1200 Corner of Espina St & Stewart St. Las Cruces, NM 88003	Title: Assistant Director of Grants and Contracts Address: Office of Grants and Contracts Anderson Hall, E1200 Corner of Espina St & Stewart St. Las Cruces, NM 88003
12a. ENTITY IDENTIFICATION NO.: 85-6000401	12b. DUNS NO.: 17-385-1965
	Tel: 575-646-1590 Fax: 575-646-2020
	E-Mail: dgc@nmsu.edu

14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete, and accurate to the best of my knowledge, and I agree to comply with the terms and conditions of award if an award is issued as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 13. (In ink. "Per" signature not acceptable.)	DATE
Ancelmo Encinias	4/7/2009

Kirschstein–NRSA Individual Fellowship Application *(To be completed by applicant – follow PHS 416-1 instructions)*	NAME OF APPLICANT *(Last, first, middle initial)* Reyes, Emigdio, D.

SPONSOR and Co-Sponsor Information

15. NAME OF SPONSOR	16. NAME OF Co-SPONSOR *(When applicable)*
15a. NAME AND DEGREE(S) Shelley L. Lusetti, Ph.D.	16a. NAME AND DEGREE(S) Jeffrey B. Arterburn, Ph.D.
15b. ERA COMMONS USER NAME	16b. ERA COMMONS USER NAME
15c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT Chemistry & Biochemistry	16c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT Chemistry & Biochemistry
15d. MAJOR SUBDIVISION	16d. MAJOR SUBDIVISION
15e. Address: MSC 3C, Box 30001 Las Cruces, NM 88003-8001 [redacted]	16e. Address: MSC 3C, Box 30001 Las Cruces, NM 88003-8001 [redacted]

RESEARCH PROPOSAL

17. DESCRIPTION: See instructions. State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project (i.e., relevance to the **mission of the agency**). Describe concisely the research design and methods for achieving these goals. Describe the rationale and techniques you will use to pursue these goals.

In addition, in two or three sentences, describe in plain, lay language the relevance of this research to public health. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**

The appearance of resistant phenotypes in cancer cells hinders the successful treatment of cancer. Members of the Structural Maintenance of Chromosomes (SMC) protein family have been shown to contribute to resistant phenotypes in cancer cells by enhancing DNA repair. The goal of this research project is to understand the biochemical function of RecN, a bacterial SMC protein, in the DNA repair mechanism of the radio-resistant bacterium Deinococcus radiodurans (DEIRA). To accomplish this goal, two aims have been developed. Aim 1 will describe the biochemical properties of RecN by testing the hypothesis that RecN is a DNA binding, ATP hydrolyzing protein that requires these biochemical activities to facilitate the assembly of DNA fragments. Aim 2 focuses on determining the role of RecN in the biochemical processes implicated in the DNA double-strand break repair mechanism of DEIRA by testing the hypothesis that RecN facilitates the repair of DNA double-strand breaks by bridging DNA molecules together. A combination of genetic and biochemical methodology will be used to test our working hypotheses. These techniques include a spectrophotometric enzyme assay to assess ATP hydrolysis activity of RecN as well as various DNA binding techniques to examine the activity of this protein.

This project is relevant to public health because the information obtained can provide basic knowledge about a group of proteins associated with the development of resistance to cancer therapy by cancer cells. The information acquired in this study can be use to improve the efficacy of cancer therapy.

Kirschstein–NRSA Individual Fellowship Application *(To be completed by applicant – follow PHS 416-1 instructions)*	NAME OF APPLICANT *(Last, first, middle initial)* Reyes, Emigdio, D.

18. GOALS FOR KIRSCHSTEIN–NRSA FELLOWSHIP TRAINING AND CAREER

Two of my passions in life are research and education. I have great interest in the research field of cancer therapy development. As a [redacted] I am aware of the burden Cancer represents to our community. I believe that by working in this field of research I will be able to contribute to my community and to humanity. Similarly, I am interested in training young scientists, especially those who come from a Hispanic background. The Hispanic community is underrepresented in the scientific field and I want to be a key player in promoting science as a career for Hispanics. Thus, my overall career goal is to become a principal investigator and a professor at a Hispanic-serving academic institution. To attain this goal I need to complete two important training stages that will help me develop the professional skills required to become a competent investigator and professor. First, I will complete my Ph.D. level education. In this stage, I expect to develop scientific communication skills. Good communication skills are critical for obtaining an academic position as principal investigator and professor. Thus, I will write and publish scientific papers and present my research ideas, results and conclusions at national scientific meetings such as the Keystone Symposia, American Chemical Society and Gordon Research Conferences, to mention a few.

(Continues on Page 4, next)

19. ACTIVITIES PLANNED UNDER THIS AWARD: Approximate percentage of proposed award time in activities identified below. *(See instructions.)*

Year	Research	Course Work	Teaching	Clinical
First	90%	0%	10%	0%
Sec	90%	0%	10%	0%
Thir				
PREDOCTORAL FELLOWSHIPS ONLY				
Four th				
Fifth				
MD/PhD FELLOWSHIPS ONLY				
Sixt h				

Briefly explain activities other than research and relate them to the proposed research training.

Specific workshops offered at New Mexico State University can help me improve manuscript and grant writing skills. The theory learned in these workshops combined with practice can supplement my training and improvement in these specific areas. Also, teaching strategy workshops can supplement my teaching and lab student supervision experiences. Courses related to responsible counduct in research will also enhanced my training as a competent researcher.

20. TRAINING SITE(S) Is the Primary Training Site the same as the Sponsoring Institution? ☒ Yes ☐ No

If No, provide detailed information below for the Primary Training Site Location

Organizational Name:		
DUNS:		
Street 1:	Street 2:	
City:	County:	State:
Province:	Country:	Zip/Postal Code:
Project/Performance Site Congressional Districts:		

21. HUMAN EMBRYONIC STEM CELLS ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/eligibilityCriteria.asp. *Use continuation pages as needed.*

If a specific line cannot be referenced at this time, include a statement that one from the Registry will be used.

Cell Line

Goals for Kirschstein-NRSA Fellowship training and career (Continued from Page 3)

At this point of my Ph.D. training, I have completed all the coursework requirements for my degree. However, I will continue to attend seminar courses where I can practice presenting my research results and learn about other investigator research. This will help me learn to convey research results and knowledge in an articulate and efficient manner to the scientific and non-scientific communities. Furthermore, I want to develop experimental design and critical thinking skills which are important tools for identifying, testing and understanding scientific problems and thus to become a competent investigator. Therefore, I will work with my sponsors in planning and developing experiments to test hypotheses related to our research. Then, I will analyze and interpret the data obtained to develop scientific models that attempt to explain our questions. This stage of my training will have two components. The main component will be the continuation of the research project proposed here under the supervision of Dr. Shelley Lusetti. This component includes my research training in biochemistry focused in the enzymology of bacterial DNA repair mechanisms. My training at this stage will also include visiting the laboratory of Dr. Patrick Sung at Yale University, but depends on the funding of Dr. Lusetti's [redacted] Since I plan to go into the field of cancer therapy, I will attend group meetings with the research group of Dr. Jeffrey Arterburn to obtain basic knowledge about treatment development strategies. These meetings will help me gain experience in the field I plan to go to once I finish my Ph.D. training. Finally, during this stage of my training I want to improve my leadership and teaching skills. Principal investigators and professors are leaders and instructors. Thus, I need to learn good leadership and teaching skills. To develop my leadership skills, I will work with my sponsor in different activities such as supervising undergraduate students, summer interns and other junior graduate students. Presently, I am the most senior graduate student in the lab of Dr. Lusetti. Thus, I have been assigned different supervision tasks that will help me improve my leadership skills. I will train and teach techniques and theory to undergraduate and graduate students under my supervision in the laboratory. Furthermore, I will work with my department as a teaching assistant during the Fall of 2009 to develop strong teaching skills.

The second stage of training required to achieve my overall career goal is the training at the post-doctoral level. After finishing my Ph.D. training I want to move into a post-doctoral position in the cancer research and treatment development field. At this point, I expect to have learned communication, critical thinking, experimental design, leadership and teaching skills, which I consider critical for the development of my career. Therefore, at the post-doctoral level of training, I expect to keep practicing and improving these skills. Additionally, at this stage, I expect to develop two other important scientific skills, grant writing and autonomy. To demonstrate that I am a competent scientist I need to be able to obtain competing grants. Thus, I expect to develop my grant writing skills during my post-doctoral training by writing and applying to all possible funding agencies that can support my research. I also plan to complement the practice with grant writing classes and workshops that can help me improve my writing skills. Often, grant writing workshops are offered at many institutions. Learning to be independent is another important skills I want to develop while I am a post-doc. As a principal investigator, it is important to be autonomous and feel confident making decisions by myself. Thus, I plan to look for a postdoctoral position where I have the opportunity to be more independent from the principal investigator and where I can start building the projects for a principal investigator position.

The Kirschstein-NRSA fellowship award embraces the majority of the skills I want to learn during my Ph.D. level training. For instance, preparing the application for this scholarship required me to identify a problem and to design experiments to test the hypothesis I have derived by studying the observed problem. In addition, to maintain this award I will need to produce, analyze and interpret data to be included in the yearly progress reports to the NIH. This activity will help me develop my critical thinking, scientific writing and experimental design skills since the report needs to be written in a professional scientific manner and alternative experiments will have to be designed in case the ones proposed here do not work as planned. The Kirschstein-NRSA fellowship application process has also helped me start developing these skills. Writing this research proposal and the whole application process for this award has helped me get familiarized with the NIH grant application process. This experience is critical for my grant writing training since the NIH will be one of the main funding agencies I will be applying to. In addition, the process of writing a research proposal by myself has given me the opportunity be a little more independent from my principal investigator. Finally, the Kirschstein-NRSA fellowship award will provide the required funding to accomplish the research proposed here in a timely manner, which can help me transition to the second stage of my training.

Kirschstein–NRSA Individual Fellowship Application

Previous Research Experience

(To be completed by applicant – follow PHS 416-1 instructions.)

NAME OF APPLICANT (Last, first, middle initial)
Reyes, Emigdio, D.

22. PRIOR AND CURRENT KIRSCHSTEIN–NRSA SUPPORT. List type (individual and/or institutional), level (predoctoral or postdoctoral), dates, and grant or award numbers.

N/A

23. APPLICATION(S) FOR CONCURRENT SUPPORT

☒ NO ☐ YES Using format below, list all support (training, research, supplies, travel, etc.) applied for that would run concurrently with the period covered by this application. Include the type, dates, source, and amount.

Type: Dates:
Source: Amount:
Type: Dates:
Source: Amount:
Type: Dates:
Source: Amount:

24a. TITLE(S) OF THESIS/DISSERTATION(S) (Predoctoral and Senior Fellowships omit this section.)

24b. NAME OF DISSERTATION ADVISOR OR CHIEF OF SERVICE *(If reference report not included, explain why not.)*	TITLE, DEPARTMENT, AND INSTITUTION

25. DOCTORAL DISSERTATION AND OTHER RESEARCH EXPERIENCE
(See Instructions – particularly Predoctoral and Senior Fellowships should follow special instructions for this section. Use continuation pages. Do not exceed two pages.)

Fortunately, I have had the opportunity of expanding my research experience through different job positions and education levels. My research experience began as an undergraduate student at St. Edward's University. At this institution, I optimized a protocol to detect single-nucleotide point mutations in genes using Surface Enhanced Raman Scattering. This experience allowed me to learn techniques to produce silver nanoparticles and gold nanoparticles, to anchor DNA probes onto these nanoparticles and to analyze these particles through UV-Vis Spectrometry. The results obtained from this project were presented by me at the 2004 annual American Chemical Society Meeting. Upon graduating from college, I was hired as a medical research technician by the Center for Gene Therapy at Tulane University. At this institution, I worked in projects related to adult stem cell research. For instance, I worked on adipogenic, chondrogenic and osteogenic differentiation of human, rat and murine adult stem cells. I analyzed the effects of certain bone regeneration inhibitors in mouse models, and I studied the immune response of rats to serum used in cell culturing. The experience of working at this institution was a great influence in the development of my research interest for the biomedical field. After working for a year at the Center for Gene Therapy, I accepted a lead technician position at Texas Tech University with the Department of Experimental Pathology. Here, I worked on projects related to the metabolic syndrome and nerve regeneration. In these projects, I studied the different factors that trigger hypertension in obesity and I worked on methods to manipulate the Renin-Angiotensin System (RAS) in a rat model of obesity. I also designed methods to accelerate peripheral nerve regeneration using stem cell therapy. Working at this institution also gave me the opportunity to develop neurogenic differentiation assays for adult stem cells and get trained in small mammal microsurgeries to implant telemetric blood pressure sensors and nerve "Conduits" in rats. My work at this institution helped me broaden my biomedical research experience. Now, as a graduate student in the Department of Chemistry and Biochemistry at New Mexico State University, I continue to acquire research experience working in a biochemistry laboratory. In this laboratory, I have learned basic biochemical techniques that are fundamental

(Continues on Page 10, next)

Research Experience (continued from p. 9)

for the biomedical research I want to pursue. I am currently performing research related to DNA repair systems in bacterial organisms. My current research projects include purifying proteins associated with DNA repair and characterizing the activity of these proteins through biochemical and molecular biology methods.

Research Training Plan

2. Specific Aims

Successful treatment of different types of cancers is frequently obstructed by the development of resistance to anti-cancer agents. Many chemotherapeutic drugs and radiation treatments eradicate cancer cells by fragmenting their genome through the induction of DNA double strand breaks. Thus, the resistance of cells to anti-cancer agents can be determined by their efficacy to repair DNA damage. Evidence gathered from genetic studies suggests that the proteins of the Structural Maintenance of Chromosomes (SMC) family contribute to resistant phenotypes in cancer cells. SMC proteins play essential roles in chromosomal maintenance processes. However, their precise molecular role in DNA damage repair and resistance remains poorly understood. In eukaryotes, SMC proteins form large protein complexes that contain non-SMC components essential for function. Generally, these complexes are hard to tease apart and the biochemical function of their individual components is difficult to determine. RecN is an SMC-like protein that has been implicated in the repair of DNA double strand breaks (DSBs) and in the radio-resistant phenotype of bacterial organisms. *Deinococcus radiodurans* (*DEIRA*) is an organism that exhibits extraordinary resistance to γ-irradiation and other DNA damaging agents. Several pieces of evidence suggest that the radio-resistant phenotype of this organism is in great part due to its ability to efficiently and precisely repair extensive DNA damage. The *recN* gene has been identified as an important element in the radio-resistant phenotype of *DEIRA*. The goal of this research project is to understand the biochemical function of RecN in the DNA repair mechanisms of the radio-resistant bacterium *DEIRA*. This work is significant because the knowledge obtained from *DEIRA* RecN can be used to predict the biochemical role of eukaryotic SMC proteins in DNA repair and in the development of chemo- and radio-resistance by cancer cells.

Aim 1. Define the biochemical properties of the bacterial SMC protein RecN:

RecN is a DNA binding, ATP hydrolyzing protein that requires these biochemical activities to facilitate the assembly of DNA fragments. The objective of this aim is to determine the parameters of the two different biochemical activities exhibited by RecN. This objective will be accomplished using biochemical techniques that will help us explore the DNA binding and ATP hydrolyzing activities of this protein.

Aim 2. Determine the role of RecN in the biochemical processes implicated in the DSB repair mechanism of *DEIRA*:

RecN facilitates the repair of DSBs by bridging DNA molecules together. There are two objectives for this aim. First, the stage of the biphasic DSB repair at which RecN functions will be determined. Then, guided by the results from the first objective, the contribution of RecN to different repair pathways will be established. The objectives of this aim will be reached using a combination of genetic and biochemical techniques that will allow us to determine the molecular contribution of RecN the mechanisms of DSB repair.

The two aims of this proposal have been designed to help me accomplish my long-term goal. I am interested in becoming an investigator in the field of cancer therapy development. Thus, understanding the role of proteins that hinder the treatment of this disease is fundamental for me. This knowledge can lead us to identify possible targets for therapy that will enhance the treatment of this disease. The research proposed here will give me an insight in the process of treatment develop since an early step in this process is the identification of a biological target. The completion of this research project will also benefit my short-term career goal of getting a post-doctorate position in the field of cancer treatment development.

3. Background and Significance

Development of resistance to chemo- and radiotherapy hinders the successful treatment of cancer. Many types of cancer cells are initially responsive to therapy. However, the prolonged exposure may lead to the development of resistance, and thus treatment failure. Radiotherapy and certain chemotherapeutic agents kill cancer cells by inducing DNA double strand breaks (DSBs), the most severe form of genomic damage. Substantial evidence suggests that an enhanced DNA DSB repair mediates the development of resistance [1-5]. Members of the Structural Maintenance of Chromosomes (SMC) family of proteins have been implicated in the resistant phenotypes of cancer cells. Such is the case of Rad50, a eukaryotic SMC-type protein that forms part of the MRN complex in human cells. The MRN complex is a critical component of the DNA DSB repair mechanism in mammal cells. Interestingly, Tran and colleagues showed that inactivation of this complex sensitizes human head and neck squamous carcinoma cells to the anti-cancer drug cisplatin [1]. Other SMC proteins such as cohesin and the SMC5/6 complex have been identified as targets for sensitizing breast cancer cells to the DNA damaging agent valproic acid [6]. In bacteria, SMC-type proteins like RecN have also been identified as important elements in the resistance against DNA damaging agents [7-11]. Although SMC proteins have been implicated in DSB repair and DNA damage resistance, their molecular functions in these processes remains poorly understood. The RecN protein of the radioresistant bacterium *Deinococcus radiodurans* (*DEIRA*) is a great model for studying the role of SMC-type proteins in the development of resistance to chemo- and radiotherapy by cancer cells. *DEIRA* can survive genome-shattering exposure to ionizing radiation through a DSB repair mechanism that effectively reassembles DNA fragments into intact chromosomes without detectable mutation in approximately 3 hours [12-14]. Funayama and coworkers showed that RecN plays an important role in the ability of *DEIRA* to cope with shattered chromosomes [15]. Thus, we expect to describe the biochemical properties and functions of *DEIRA* RecN so that we can predict the role of SMC proteins in the development of resistance against cancer therapies by cancer cells.

3A. DNA double-strand break repair

DSBs are a dangerous but common type of DNA lesion. They can be generated by programmed cellular events as well as by random spontaneous events that cause DNA damage. A DSB is particularly dangerous because if left unrepaired, it can trigger cell death. In addition, if the DSB is repaired improperly, it can lead to genomic instability. For these reasons organisms have evolved pathways to efficiently repair this type of lesion. In bacteria, the major mechanism used to repair DSBs is homologous recombination. The recombinational repair mechanism is divided into three fundamental stages that are conserved among organisms from the three branches of life: presynapsis, synapsis and postsynapsis. In the model organism *Escherichia coli* (*E. coli*), recombinational repair proceeds as follows. First, the DNA ends that result from a DSB are processed by the RecBCD protein complex to produce 3' single-stranded DNA tails on both ends. Once these 3' tails have been generated, the RecBCD complex loads RecA protein onto each single-stranded DNA tail in a process called nucleation. After nucleation, additional RecA protein monomers bind to the single-stranded DNA tails in a 5' to 3' direction forming a filament. This process is known as the RecA filament extension. Once established, the RecA filament catalyzes the search for homology in another chromosome so that the missing information can be recuperated. This series of events is identified as the presynapsis stage. The synapsis stage begins when the RecA filament finds a homologous DNA region. At this point, the RecA filament displaces the identical strand in the homologous chromosome and pairs with the complementary strand generating a D loop structure. The same RecA filament converts the D loop into a Holliday Junction structure by facilitating the pairing of the displaced identical strand of the homologous chromosome with its complementary strand in the damaged chromosome. Finally, the postsynapsis stage initiates when RuvA recognizes the Holliday Junction, binds to it and recruits RuvB to form the RuvAB complex. The RuvAB complex promotes branch migration, the process by which DNA is exchanged between the invaded homologous chromosomes and the damaged chromosome, to help the damaged chromosome recuperate the genetic information lost due to the damage. The process of recombinational repair concludes when the RuvC protein binds to the RuvAB complex and cleaves the Holliday Junction generating two intact DNA molecules [16].

3B. *Deinococcus radiodurans* and DSB repair

First isolated in 1957 from irradiated meat, *DEIRA* has demonstrated an unusual resistance to ionizing radiation and other DNA damaging agents [17]. *DEIRA* can withstand γ-irradiation doses as high as 0.5 Mrad without loss of viability [18]. Several mechanisms have been proposed to explain the contributions to radioresistance exhibited by *DEIRA*. For instance, it was proposed that *DEIRA* cell structure protects the genome from the insults produced by radiation exposure, thus preventing DNA damage. However, the radioresistance of *DEIRA* cannot be attributed only to DNA damage prevention since a γ-irradiation dose of 0.5 Mrad shatters the genome of this organism [19]. To put the tolerance of *DEIRA* to DSBs into context, an *E. coli* cell can survive only a few DSBs [20]. It was shown that DEIRA reassembles DNA fragments into intact chromosomes without detectable mutation in about 3 hours [12-14]. Unlike *E. coli*, *DEIRA* presumably uses a biphasic DSB repair mechanism. Daly and Minton provided evidence for this biphasic mechanism in 1996 through an elegant experiment. Briefly, cultures of wild-type and *recA* null *DEIRA* strains were exposed to 1.75 Mrad of γ-irradiation (the dose at which only 37% of the irradiated *DEIRA* cells survive). Samples from each culture were collected before irradiation and at different time points after irradiation. The total genomic DNA of each sample was purified and analyzed by pulse-field gel electrophoresis (PFGE). Before irradiation, the extracted genomic DNA leaves a characteristic smear on the gel that represents multiple copies of four different chromosomes this organism contains. After irradiation, this smear had turned into a well-defined high mobility band suggesting that the total DNA had been shatter to small fragments. As *DEIRA* repairs its DNA, the high mobility band starts to broaden until a smear similar to that observed in the pre-irradiation samples appears. The rate of DNA repair in the presence and absence of RecA in *DEIRA* was assessed by analyzing the time it took each strain to generate the wild-type DNA smear. The experiment showed that both wild-type and *recA* null strains repaired approximately one-third of the DSBs during the first 1.5 hours post-irradiation suggesting that the first stage of DSB repair occurs in a RecA-independent manner. However, *recA* null strains were not able convert their fragmented DNA back to wild-type size fragments. This observation suggested that RecA was required to complete the chromosomal repair several hours after the irradiation, but not the initial repair [21]. These results led to the conclusion that the DSB repair mechanism of *DEIRA* proceeds first in a RecA-independent fashion followed by RecA-dependent recombinational phase. Different mechanisms have been proposed to account for the first phase of the biphasic DSB repair exhibited by *DEIRA*. Some of these mechanisms include non-homologous end joining (NHEJ) and different variations of single strand annealing (SSA). In 2006, Zahradka and colleagues found that the reassembly of DNA fragments created by irradiation of *DEIRA* cultures overlapped with an increase in DNA synthesis observed in irradiated but not in unirradiated cultures. In addition, this group found that the assembled fragments were made up of DNA regions containing newly synthesized DNA as well as old or parental DNA in a manner that did not resemble common semi-conservative DNA replication. However, this pattern of DNA repair was only found in cells containing a functional DNA Polymerase A (PolA). Interestingly, no repair was observed in *polA* null mutants [22]. PolA is one of the three DNA polymerases found in *DEIRA*. Although the DNA polymerases of *DEIRA* have not been well characterized yet, this result suggests that PolA may participate in DNA repair mechanisms [23]. Based on the results obtained in this study, Zahradka and colleagues proposed a variation of SSA as the mechanism responsible for the repair during the first phase. This process is thought to help reconstruct long double-stranded DNA intermediates that can be reassembled by homologous recombination [22]. Several features of this process remain to be characterized [7]. The work presented by Zahradka and colleagues established that the DNA potymerase PolA and the recombinase RecA play distinct but key roles in the biphasic repair of DNA DSBs. Many other genes coding proteins of unknown biochemical function have been identified as important components in the DSB repair mechanism of *DEIRA*, including the *recN* gene [15, 19].

3C. The *recN* gene

Genetically, it is well established that the *recN* gene is required for the recombinational repair of DSBs. Two separate research groups first identified the *recN* gene in *E. coli* over twenty years ago. Lloyd and coworkers isolated an *E. coli* mutant that was sensitive to UV irradiation and mitomycin C (MMC) [24]. Parallel to this, Sargentini and Smith characterized an *E. coli* mutant that was sensitive to ionizing and UV irradiation and methyl methanesulfonate (MMS) treatment [25]. UV light, γ-irradiation, MMC and MMS cause DNA damage. Thus, these results suggested an involvement of the *recN* gene in DNA repair and recombination.

Further genetic characterization of the *E. coli recN* gene demonstrated that *recN* null mutants failed to repair DSBs [8]. It was also shown that the *recN* gene is required for suppression of chromosomal rearrangements and deletions [26] during the repair of a single DSB. These findings led to the hypothesis that a functional product of *recN* is necessary for the accurate repair of DSBs.

Expression of the *recN* gene in *E. coli* is regulated by the LexA repressor [8, 27], and the RecN protein is one of the most highly induced proteins during the SOS response to DNA damage [28, 29]. However, the half-life of RecN is short (~10 minutes) since it is rapidly proteolyzed by the ClpXP protease system [30]. Furthermore, the *recN* gene is widespread in bacterial species, but its genetic characterization is limited to very few organisms. In *DEIRA*, the *recN* gene is not induced following irradiation [31], unlike the *E. coli recN* gene. However, this gene is indispensable for *DEIRA* survival upon irradiation [15]. The role of the *recN* gene in the DNA damage repair pathways has also been evaluated in a few more bacterial organisms such as *Neisseria gonorrhoeae*, *Bacillus subtilis* and *Helicobacter pylori* [9-11]. In these organisms the *recN* gene is also required for the proper repair of DNA DSBs.

3D. RecN: a predicted SMC-type protein

The RecN protein is related by sequence to the SMC family of proteins (see Figure 1) [32]. SMC proteins are essential for the regulation of the structural and functional organization of chromosomes in all three branches of life. SMC proteins are generally large in size, averaging ~1000-1500 amino acids. Structurally, SMCs are characterized by globular N- and C-termini connected by a coiled-coil domain. Each globular domain contains part of a functional site for binding and hydrolyzing ATP, the Walker A and Walker B motifs, that becomes activated when these domains are brought together [33]. In contrast to SMC proteins, RecN proteins average ~550 amino acids. However, high sequence similarity is observed with the N- and C-terminal catalytic domains of the eukaryotic SMCs. Additionally, the central part of the RecN sequence has regions with a probable coiled-coil structure (like SMCs) as predicted by the programs Lupus and COILS.

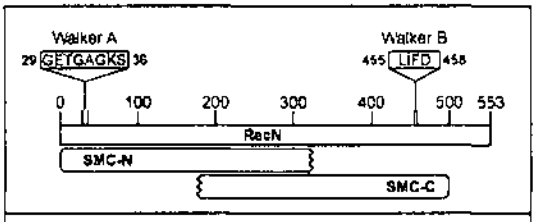

Figure 1: Linear amino acid sequences of the *E. coli* RecN protein (553 residues) showing the location of the Walker A and B motifs. Also shown are the regions of RecN that are homologous to SMC N- and C-terminal domains.

There are six well-characterized SMC proteins in eukaryotes that dimerize in specific combinations to form three large protein complexes: cohesin, condensin and the SMC5/6 complex. Each complex consists of a particular pair of SMC proteins and at least two other non-SMC proteins [34]. SMC1 and SMC3 along with two other non-SMC proteins form a functional cohesin. The cohesin complexes are essential for establishing sister chromatid cohesion following DNA replication. The condensin complex contains SMC2 and SMC4 along with three non-SMC proteins. This complex participates in the condensation of chromatin during mitosis. SMC5 and SMC 6 interact with four non-SMC proteins to form a less characterized complex, the SMC5/6 complex. Little is known about the function of the SMC5/6 complex, but it had been associated with DNA damage repair and checkpoint responses [34]. Interestingly, *smc5* and *smc6* null mutants exhibit high levels of gross chromosomal rearrangements. This phenotype has also been observed in *recN* mutant strains (see section 3C) [35]. The SMC5/6 and cohesin complexes have been implicated in the recombinational repair of DSBs. Both complexes are recruited to DSBs and play essential roles in the efficient repair of DSBs by favoring sister-chromatid recombination [36]. Furthermore, Rad50 is another SMC-type protein implicated in DSB repair. Rad50 is an essential component of the human MRN complex (Mre11, Rad50 and Nbs1). This complex is required for the repair of DNA DSBs through homologous recombination or non-homologous end joining pathways [37].

Although little is known about the biochemical function of RecN, recent studies in *B. subtilis* have provided insight into the possible function of this protein. In this organism, RecN localized (as observed via fluorescent protein fusions) to the nucleoid of live cells in response to DSBs [9,38]. Additionally, in vitro characterizations of *B. subtilis* RecN has shown that this protein binds and protects 3' single-stranded DNA extensions in the presence of ATP [39].

4. Preliminary Studies

The progress that has been made on this project represents the work I have done in Shelley Lusetti's laboratory (New Mexico State University) during the first two years of my graduate research.

4A. Expression and purification of soluble RecN protein

The two specific aims of this project require the expression and purification of the RecN protein. The *recN* gene from *DEIRA* was cloned and expressed in *E. coli*. The *recN* construct from *DEIRA* (*DEIRA* RecN) in native form (no tag) was found to be soluble and a procedure was developed to purify it to near homogeneity (Figure 2). Mass spectrometry and N-terminal sequencing confirmed the identity of the purified protein. We have recently refined the purification protocol to a single bulk fractionation step followed by a single chromatographic fractionation. The preparation is free of nuclease contamination.

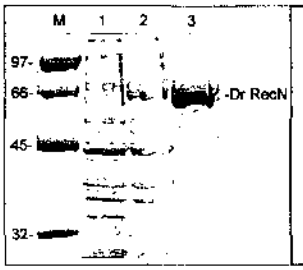

Figure 2: SDS-PAGE of *D. radiodurans* RecN expressed in *E. coli*.

M- MW markers (kDa)
1- uninduced cells
2- induced cells
3- purified RecN protein

4B. Biochemical Properties of the RecN Protein

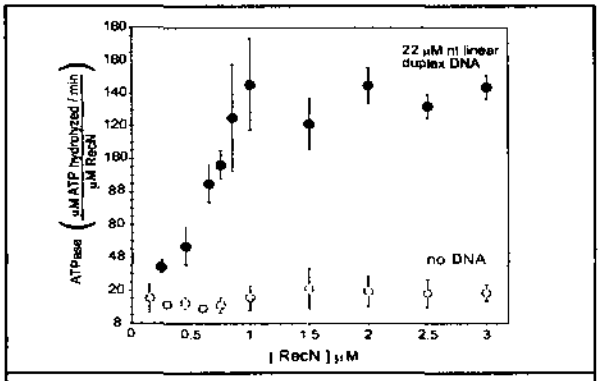

Figure 3: Turnover rate of the ATP hydrolysis reaction catalyzed by RecN in the presence (black circles) and absence (gray circles) of 22μM bp linear dsDNA.

4B.1 ATPase Activity of RecN. The RecN protein sequence strongly suggests that it has ATPase activity. To confirm this, we have used a enzyme-coupled spectrophotometric assay [40], and shown that purified DEIRA RecN protein hydrolyzes ATP. The ATPase kinetic data have been collected and analyzed. Briefly, the regeneration of ATP from phosphoenolpyruvate and ADP (produced by hydrolysis) was coupled to the oxidation of NADH (through the addition of pyruvate kinase and lactate dehydrogenase) and followed by the decrease in absorbance of NADH at 380 nm. The assays were carried out on a Varian Cary 100 dual beam spectrophotometer equipped with a temperature controller. The NADH extinction coefficient at 380 nm of 1.21 mM^{-1} cm^{-1} was used to calculate the rate of ATP hydrolysis. Figure 3 shows the plot of the turnover rate as a function of RecN concentration in the presence and absence of DNA. *DEIRA* RecN hydrolyzes ATP with an apparent turnover rate of 15 min^{-1} in the absence of DNA in a RecN concentration-independent fashion. However, in the presence of linearized double-stranded DNA (22 μM bp), the turnover rate increases approximately 7-fold. The DNA-dependent ATP hydrolysis turnover rate appears to be strongly dependent on the RecN concentration suggesting that RecN-RecN interactions are required for efficient ATP hydrolysis. The rate increased linearly to an apparent maximal rate (V_{max}) of 140 μM/min as a function of protein concentration until a ratio of approximately 1 RecN to 20 μM bp of DNA was achieved. The positive effects of linear double-stranded DNA in the ATPase activity of RecN were confirmed by titrating different concentrations of linear double-stranded DNA into the ATPase reaction with a constant RecN concentration. Figure 4 shows a plot of the rate of ATP hydrolysis as a function of the concentration of linear double-stranded DNA. The rate of ATP hydrolysis catalyzed by RecN is dependent on DNA concentration suggesting that linear double-stranded DNA is a cofactor in this reaction. These results suggest that DNA may stabilize a RecN complex; this issue will be explored further in our future work (see section 5A.2a). Furthermore, the hyperbolic curve observed in the ATPase rate as function of linear double-stranded DNA concentration plot (Figure 4) suggests that RecN may bind linear double-stranded DNA with an apparent K_d of approximately 5 μM bp. However, this will be confirmed via direct DNA binding assays in our future work (see section 5A.2b) . In other experiments, we have observed no stimulation in RecN ATPase activity by linear or circular single-stranded DNA (data not shown). This does not rule out RecN binding to these DNA substrates. It may be that higher order RecN complexes do not form on these substrates. Experiments will be conducted to determine the affinity of RecN for the different DNA substrates (see section 5A.2b).

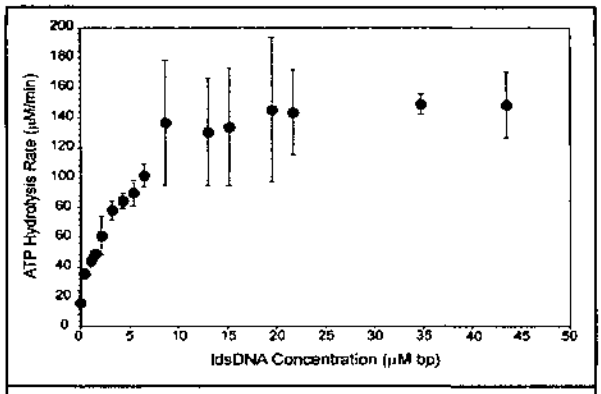

Figure 4: The rate of ATP hydrolysis catalyzed by RecN as a function of linear dsDNA (black circles)

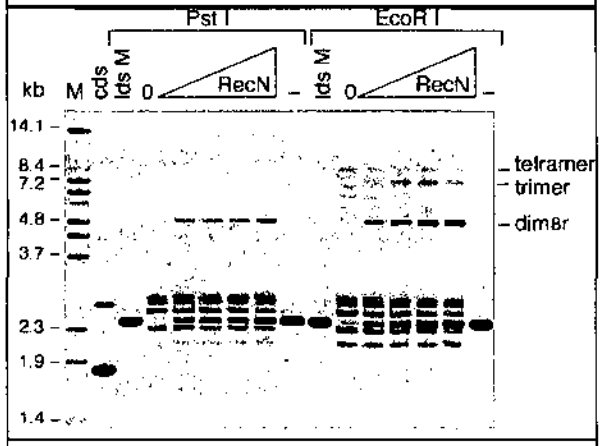

Figure 5: Stimulation of DNA ligation by the RecN protein. Ligase alone ("0" lanes) generates mostly circular products with variable topoisomers. The addition of RecN increases the generation of ligated linear dimers.

4B.2 RecN-dependent Intermolecular Ligation of linear DNA. SMC proteins have several distinctive activities, tethering DNA molecules and facilitating the formation of knots and supercoils in particular patterns depending on whether the protein works as a condensin or a cohesin [33, 34]. One activity that is characteristic of cohesin-like proteins and not displayed by condensin-like proteins is the facilitation of the ligation of linear DNA fragments [41]. We tested this by incubating RecN with plasmid DNA digested to produce short complementary single-strand overhangs and adding T4 DNA ligase. The reaction is carried out under dilute conditions such that the major product of ligation in the absence of RecN is intramolecular, forming circles in different topological states (Figure 5, lanes "0"). RecN greatly stimulates the intermolecular ligation of linear duplex DNAs (2431 bp) to form dimers, trimers, etc. This experiment revealed that RecN activity is observed with DNA ends having either a 3' single-strand overhang (generated by the PstI endonuclease) or a 5' one (generated by the EcoRI endonuclease). These results suggest that RecN can tether DNA ends from different DNA molecules, a cohesin-like activity.

A cohesin activity would help explain the need for RecN in the DSBs repair mechanism of *DEIRA*. This type of activity suggests that *DEIRA* RecN may play a role in holding DNA fragments together at the site of a double strand break to keep the DNA ends in close proximity and facilitate their repair by non-homologous end joining, single strand annealing, homologous recombination or a combinations of all of these in the biphasic repair of DSBs observed in *DEIRA*. These ideas are explored in section 5 below.

To summarize, we have achieved significant progress exploring the molecular function of the *DEIRA* RecN protein using biochemical tools. Through this preliminary research, we have developed helpful leads towards the completion of both aims of this proposal.

5. Research Design and Methods

Genetic studies have established that the product of the *recN* gene is required for the repair of DSBs and for cellular tolerance to radiation and other DNA damaging agents. In vivo fluorescence microscopy has confirmed that RecN is recruited to DSB sites on the DNA. However, these approaches have not yielded sufficient information to describe the molecular function of RecN. Thus, we have designed an approach that will help us understand the activity of RecN at the biochemical level.

5A. Define the biochemical properties of the bacterial SMC protein RecN (Aim 1)

5A.1 Introduction

We have expressed and purified *DEIRA* RecN and implemented a biochemical approach to obtain more detailed information about the enzymatic activity of this protein. Our preliminary results show that *DEIRA* RecN can hydrolyze ATP with DNA as cofactor. Moreover, indirect data suggests that RecN can bind DNA and that it facilitates the ligation of DNA fragments under certain conditions (see section 4B. 1 and 2). Thus, *the objective of this aim is to confirm the ATPase and DNA binding activities of DEIRA RecN and to characterize the biochemical parameters (binding and kinetic) of each of these activities.* This objective will be attained by testing the *hypothesis that RecN is a DNA binding, ATP hydrolyzing protein that requires these biochemical activities to facilitate the assembly of DNA fragments.* Biochemical techniques will be used to test our working

hypothesis. These techniques include a spectrophotometric assay to assess the ATP hydrolysis activity of RecN as well as electrophoretic mobility shift assays (EMSAs), fluorescence polarimetry and electron microscopy to examine the DNA binding activity of this protein. The rationale for this aim is that the results obtained from this research will provide basic knowledge about the biochemical activities of *DEIRA* RecN. The biochemical characterization of the RecN activities is essential to understand the molecular role of this protein in DSB repair mechanisms. In addition, the biochemical characterization of this bacterial SMC-type protein will confer basic knowledge about the biochemical function of the SMC proteins. After attaining the objective of this aim, we expect to understand the mechanisms and requirements for the ATPase and DNA binding activities of RecN. A deep understanding of these activities is key for deciphering the biochemical involvement of RecN in DNA repair pathways.

5A.2 Experimental design

5A.2a Characterization of the ATPase activity of DEIRA RecN. We will continue to explore the ATPase activity of *DEIRA* RecN using the enzyme-coupled spectrophotometric assay described in section 4B.1 [40]. We will determine all kinetic parameters relevant for the ATP hydrolysis reaction catalyzed by RecN such as V_{max}, k_m and k_{cat} in the presence and absence of DNA. This will be accomplished by measuring the rate of the reaction at a constant enzyme concentration but at different substrate (ATP) concentrations. A plot of the rate of hydrolysis as a function of substrate concentration will allow us to analyze the kinetic behavior of this reaction catalyzed by RecN. If a hyperbolic curve is obtained from this plot, the reaction is said to follow Michaelis-Menten kinetics. A double reciprocal plot (Lineweaver-Burk) can be used to calculate the V_{max}, k_m and k_{cat} of a reaction with this type of kinetics. Alternatively, if the plot of the rate of hydrolysis as a function of substrate concentration yields a sigmoidal curve, the reaction is said to follow cooperative kinetics. A logarithmic plot known as the Hill plot will reveal the kinetic parameters of the ATP hydrolysis reaction catalyzed by RecN. Our preliminary data shows that when DNA is present, the apparent turnover rate (k_{cat}) of the ATP hydrolysis reaction is dependent on the concentration of RecN (see section 4B.1). This result suggests that DNA stabilizes RecN-RecN interactions that are required for efficient ATP hydrolysis. The nature of RecN-RecN interactions induced by DNA will be further analyzed using a mutant protein deficient in ATP hydrolysis. For this analysis, a *DEIRA* RecN protein containing a point mutation at the Walker A domain (K67R) will be used. The mutation of this specific lysine in the Walker A domain to an arginine is a classic mutation leading to a mutant protein that can bind but not hydrolyze ATP [42]. This *DEIRA* RecNK67R protein will be purified using the protocol established for wild-type RecN with procedure modifications where necessary. The pure RecNK67R protein will be titrated into our enzyme-coupled spectrophotometric assay at a constant concentration of wild-type RecN in the presence and absence of DNA. Changes in the kinetic parameters of the reaction will reflect if a multimeric conformation of RecN is required for efficient ATPase activity. RecN-RecN interactions will be further analyzed using chromatography techniques. Gel filtration columns separate molecules according size differences. This type of column can help us determine the multimeric states at which RecN exists in solution.

We will further study the effect of DNA in the ATPase activity of RecN. We have observed a positive effect of linear double-stranded DNA in the rate of hydrolysis (see figures 2 and 3). However, we have not determined if this effect if due to molecule characteristics such as structure, length and the presence or absence of ends. To assess the effect that DNA substrates of different characteristics might have in the ATPase activity of RecN we will evaluate ATPase activity of this protein in presence of linear and circular DNA molecules of different sizes at equal DNA concentrations in μM molecules. This experiment will tell us if there is a minimal size of DNA required for an optimal ATPase activity of RecN. However, if ATPase stimulation is observed as the size of circular DNA increases, we will need to further examine if the stimulation is in fact due to size or if it is due to secondary structure that may form regions of duplex DNA. Additionally, we will evaluate if DNA ends stimulate the ATPase activity of RecN. This will be assess by measuring and comparing the reaction rates in the presence of different size fragments of the same DNA molecule at an equal concentration in μM base pairs (bp). At the same concentration of base pairs, smaller fragments will provide more DNA ends to the reaction than larger fragments of the same DNA substrate. Thus, an increase in the ATPase rate of reactions containing small fragments over large fragments will suggest that DNA ends stimulate the ATPase activity of RecN. A caveat to this approach is that if a minimal DNA size is required for optimal stimulation, DNA fragments of sizes smaller than the minimal size will yield slower rates and the effect of DNA ends will not

be accurately assessed. Therefore, the size requirement for the DNA substrate will be determined first. Furthermore, we will use DNA substrates of different structures to determine if there is structure specificity in the way RecN gets stimulated by DNA. Our preliminary data suggests that the ATPase activity of RecN is greatly stimulated by duplex DNA substrates, most notably linear double-stranded DNA (see section 4B.1). Thus, we will determine whether the interaction requires a 3' or 5' single-stranded overhang or a 3'-hydroxyl or a 5' phosphate. We will further evaluate the effect of by circular duplex DNA in its relaxed and supercoiled (positively or negatively) state. The results from these studies will help us determine the requirement for the RecN ATPase reaction. In addition, these studies will provide insight into the type of reaction conditions or situations at which RecN functions in the cell.

5A.2b Biochemical description of the DNA binding activity of RecN. The positive effect of linear double-stranded DNA in the ATPase activity of RecN observed in our preliminary studies suggested a direct interaction between RecN and DNA (see figure 3). The existence of this interaction was reiterated when the rate of ATPase of RecN was plotted as a function of DNA concentration. This plot gave a hyperbolic curve that estimates apparent K_d of approximately 5 μM bp (see figure 4). To confirm that RecN can bind DNA, we will carry out direct DNA binding studies and determine the affinity of RecN for different DNA substrates. The characterization of the RecN-DNA binding interaction will be evaluated using three complementary techniques. We will start by studying the DNA binding activity of RecN using an electrophoretic mobility shift assay (EMSA). In this assay, RecN will be incubated with different types of DNA substrates and subjected to electrophoresis in non-denaturing polyacrylamide or agarose gels. The type of gel matrix used will depend on the size of the complex formed between RecN and DNA. Retardation in the gel mobility of a specific DNA substrate will be observed if RecN binds this substrate. Large DNA substrates will be visualized by ethidium bromide staining, while small DNA substrates will be fluorescently-labeled. Often, DNA-protein interactions are very dynamic and thus DNA binding is hard to detect using the EMSA technique. If our results suggest that the DNA-RecN interaction is highly dynamic, we will use glutaraldehyde to crosslink RecN and DNA prior to gel electrophoresis in the EMSA assays. The DNA binding parameters of RecN will be further confirmed by a fluorescence polarimetric technique. This technique measures the rate of molecular rotation (tumbling) of a fluorescent molecule. Briefly, the tumbling rate of fluorescently-labeled DNA oligonucleotides decreases when bound by proteins. Thus, under equilibrium conditions, we will test the effect of different amounts of RecN in the tumbling rate of labeled oligos (single and double stranded). The binding data obtained from this experiment will be used to determine the DNA binding parameters of RecN. Fluorescence polarimetry studies will be done by the applicant at the University of Arizona in the laboratory of Dr. Nancy Horton. Our characterization of the DNA binding activity of RecN will be supplemented with electron microscopy (EM) studies to explore the nature of RecN-DNA interactions. These studies will help us determine the specific regions at which RecN binds the DNA molecule and whether this protein binds DNA as a monomer or as a multimeric complex. Put simple, this technique will allow us to describe the architecture of RecN in the presence of DNA substrates. EM analysis will be performed in collaboration with the laboratory of Patrick Sung at Yale University. The techniques proposed mention in this section will also help us determine the ATP requirements for the DNA binding activity of RecN. To characterize the ATP requirements we will perform these experiments in the presence and absence of ATP and different ATP analogues. The results obtained from this section will provide information about the DNA substrate preferences, binding mechanism and the ATP requirement of RecN.

5B. Determine the role of RecN in the biochemical processes implicated in the DSB repair mechanism of *DEIRA*: (Aim 2)

5B.1 Introduction

DEIRA has been shown to repair DSBs by a biphasic mechanism. In the first phase of repair, short DNA fragments are reassembled into larger chromosomal fragments. The large chromosomal fragments are then pasted together to restore intact chromosomes during the second phase [21, 22]. RecN has been identified as an important protein for the repair of DSBs in *DEIRA*. However, the stage or stages of repair at which RecN participates has not been determined. *The first objective of this aim is to determine the stage of the biphasic DSB repair at which RecN functions.* Repair pathways such as non-homologous end joining (NHEJ), single-strand annealing (SSA) and homologous recombination (HR) seem to be responsible for the repair that occurs

at the different stages of the biphasic mechanism. NHEJ and SSA have been implicated in the first stage of repair since this stage occurs in a RecA-independent manner. The second phase is RecA-dependent suggesting that HR goes on at this stage [7]. Therefore, *the second objective of this aim is to establish the contribution of RecN to each of the repair pathways associated with the stage of repair at which RecN is required.* The objectives of this aim will be achieved by testing the *hypothesis that RecN bridges two DNA molecules to facilitate the repair of DSBs.* A combination of genetic and biochemical techniques will be used to test this hypothesis. The rationale for this aim is that the experiments proposed here will provide basic knowledge about biochemical role of RecN in the effective DNA DSB repair mechanism of DEIRA. Understanding the basic function of RecN can help us determine the general contribution of SMC-type proteins to DNA damage resistance.

5B.2 Experimental design

5B.2a Determining the stage at which RecN participates in the biphasic DNA repair mechanism of *DEIRA*. We will evaluate the requirement for RecN in the two stages of DSB repair in *DEIRA* by comparing the kinetics of DNA fragment joining between a wild-type and a *recN* null *DEIRA* strain. Fragment joining kinetics from each strain will be determined by an adaptation of a pulsed-field gel electrophoresis (PFGE) experiment described by Zahradka *et. al.* In short, cultures of wild-type and *recN* null *DERIA* strains will be grown to exponential phase. Once at this phase, the cultures will be exposed to 0.7 Mrad of γ-irradiation to produced DNA fragments between 20-30 kb of length. The irradiated cultures will be provided by John Battista at Louisiana State University. Samples from each culture will be collected before and at different time points after irradiation. These samples will be embedded into agarose plugs where the cells will be tysed and treated with the restriction enzyme *NotI*. The treated plugs will be subjected to a pulsed electric field to allow DNA fragment separation [22]. *NotI* digestion provides a characteristic DNA banding pattern for each sample. In wild-type DEIRA, we expect the pre-irradiation sample to show a well-defined banding pattern. This pattern will serve as control for completed DNA repair. The 0 hour post-irradiation sample is expected to display a high mobility DNA smear representing small DNA fragments. Samples taken between 0.5 and 1.5 hours, are expected to show the gradual disappearance of the DNA smear and the appearance of high mobility bands representing short, partially repaired chromosome fragments. Samples from these time points reflect the first phase of the repair. The second phase of the repair is reflected in samples collected at 2+ hours post-irradiation. Thus, samples from these time points are expected to show the gradual appearance of slow migrating bands (larger chromosome fragments) until the pre-irradiation pattern is recuperated at ~3 hours. Zahradka *et. al.* established a set of possible outcomes of this experiment for proteins required for the repair of DSBs in *DEIRA* [22]. *DEIRA recN* null strains have been shown to be sensitive to ionizing radiation [15]. Thus, we expect one of three possible outcomes from the *recN* null strain. First, we can obtain a banding pattern in which all the post-irradiation samples (except 0 hours) display discrete fast migrating bands only (short chromosome fragments). This can indicate that RecN functions in the second stage of repair, since only the first phase of repair is observed in the absence of RecN. Secondly, we may observe that the early banding pattern characteristic of the first phase starts appearing at late post-irradiation samples. In other words, there is a change in the rate of repair since the banding pattern appears later than in wild-type *DEIRA*. This result may suggest that RecN is required for the first phase of the repair. In a third outcome we may find that all the post-irradiation samples display a DNA smear similar to that of the 0 hours sample. This would suggest that RecN is required for both phases since no repair is observed. The results obtained from this experiment will further guide our analysis of the function of RecN in DSB repair.

5B.2b Evaluating the contribution of RecN to repair pathways associated in the biphasic DSB repair of *DEIRA*. Many DNA repair pathways have been associated with the biphasic repair mechanism of *DEIRA*. Non-homologous end joining (NHEJ) and single strand annealing (SSA) have been implicated in the first stage of the repair. If our results from the first objective of Aim 2 indicate that RecN functions in the first or in both stages of repair, we will evaluate the molecular contribution of RecN to NHEJ and SSA pathways. Our preliminary results suggested that RecN stimulates the intermolecular ligation of DNA fragments (see section 4B.2) In eukaryotic cells, the Ku protein binds the ends of two different DNA molecules and stimulates their ligation [43,44]. Thus, we will determine if RecN has Ku-like activity and promotes NHEJ in *DEIRA*. Two different DNA ligase proteins were identified in *DEIRA*. One of these proteins depends on NAD^+ and Mn(II) ion as cofactors to catalyze the DNA ligation reaction. The second ligase is a predicted ATP-dependent DNA

ligase. However, no ligase activity has been observed for this protein *in vitro* [45]. We will test if RecN can stimulate the ligation activity of the two *DEIRA* ligases. Michael Cox at the University of Wisconsin, Madison has provided us with the purified forms of both *DEIRA* DNA ligases. These enzymes will be used to reproduce the experiments described in section 4B.2 where the ability of RecN to stimulate intermolecular ligation is assessed. These experiments will be optimized for the use of the *DEIRA* ligases, if required. To further analyze the Ku-like activity of RecN, we will perform Exonuclease III protection assays. Ku-like proteins are predicted to protect DNA ends from degradation DSB repair [43]. Thus, we will test whether RecN protects DNA ends from Exonuclease III. The DNA binding parameters of RecN determined in Aim 1 will guide the experiment set up. In short, a RecN-DNA complex will be formed by incubating RecN with a DNA substrate labeled with a fluorophore on the 5' end (Exonuclease III degrades DNA in 3' to 5' direction). The complex will be then treated with Exonuclease III and disassembled by protease treatment. The treated DNA will be isolated, concentrated and analyzed by sequencing gel electrophoresis and fluorimaging. A reaction without RecN will serve as control. We expect to see less degradation in the reactions containing RecN than in the control reaction.

In 1996, Daly and Minton proposed that the first phase of DSB repair in *DEIRA* occurred by SSA [21]. Thus, we will also test the contribution of this protein to the SSA pathway. A set of experiments developed for Rad52, a DNA strand annealing eukaryotic protein [46, 47], will be used as follows. First, the strand annealing activity of RecN protein will be determine by EMSA. Briefly, fluorescently-labeled single-strand DNA oligos and a non-labeled complementary DNA oligos will be incubated with varying amounts of RecN. Then, the annealing reaction will be stopped and the DNA products will be analyzed by polyacrylamide gel electrophoresis [46]. Single-stranded DNA substrates have fast mobility when compared to double-stranded DNA substrates. Thus, appearance of low mobility DNA bands in the presence of RecN will indicate that this protein stimulates DNA strand annealing. EMSA studies will be supplemented with a fluorimetric assay. In this assay, we will monitor the change in fluorescence of a solution containing DAPI (4',6-diamidino-2-phenylindole), complementary single-strand oligos and RecN using a spectrofluorimeter. Binding of DAPI to double-stranded DNA causes an increase in DAPI fluorescence [47]. Therefore, an increase in DAPI fluorescence in the presence of RecN will suggests that this protein facilitates DNA strand annealing.

The second phase of DSB repair in *DEIRA* occurs in a RecA-dependent manner. This suggests that the homologous recombination (HR) is responsible for the repair at this stage. Therefore, if there is any indication that RecN is required for the second stage of DSB repair, we will examine the contribution of RecN to the HR pathway. RecA promoted homologous recombination can be mimicked *in vitro* through a RecA-dependent DNA strand exchange assay. We will use this assay to test the contribution of RecN to this pathway. Contrary to *E. coli* RecA (see section 3A), *DEIRA* RecA catalyzes DNA strand exchange by binding to linear duplex DNA and aligning the homology with a circular single-stranded DNA molecule [48]. This reaction yields nicked circular double-stranded and linear single-stranded DNA products. These DNA products can be analyzed by gel electrophoresis where they can be easily distinguished from the substrates by their difference in agarose gel mobility. To test the effect of RecN in this reaction catalyzed by *DEIRA* RecA, we will set up reaction conditions at which RecN would bridge DNA molecules together. Then, we will add RecA to carry out the DNA strand exchange reaction. Under these conditions, we expect to see an increase in the rate of RecA-mediated DNA strand exchange. This will indicate that RecN stimulates homologous recombination.

In conclusion, SMC-like proteins seem to play important roles in the resistance against DNA damage in cancer cells. The RecN protein from the radioresistant organism *DEIRA* is a good model for studying the function of SMC-like proteins in DNA damage resistance. This proposal attempts to describe the biochemical function of RecN in DSB repair by testing two hypotheses. First, we will test that RecN is a DNA binding, ATP hydrolyzing protein that requires these biochemical activities to facilitate the assembly of DNA fragments (Aim 1). A biochemical approach will be used to determine the biochemical parameters of the two activities of RecN. Second, we will test that RecN facilitates the repair of DSBs by bridging two DNA molecules (Aim 2). A combinations of genetic and biochemical techniques will be used to test this second hypothesis with the purpose of describing the molecular contribution of RecN the mechanisms of DSB repair. The work proposed here is significant because the basic knowledge obtained from the biochemical characterizations of *DEIRA* RecN can be used to predict the biochemical role of eukaryotic SMC proteins in the resistance against cancer therapy.

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19. Respective Contributions:

The research training plan included in this application has been conceived and written by the applicant, Emigdio D. Reyes with minimal input from the sponsor, Shelley Lusetti, and co-sponsor, Jeffrey Arterburn. The first aim of this proposal was derived from a specific aim from a grant written by Shelley Lusetti. However, the aim proposed in this research training plan has been completely rewritten by the applicant. Aim 2 of this research training plan has been developed and written by the applicant only. The applicant assembled and wrote the Background and Significance section alone. The raw data used in the Preliminary Data Section was obtained from experiments performed by the applicant. However, the data analysis and conclusions presented in this section were developed in collaboration with the sponsor. Furthermore, the applicant wrote the Research Design and Methods section. The experiments proposed for the first aim were also derived from a grant proposal written by Shelly Lusetti, but modified to fit the objectives of this training plan. The methodology proposed for accomplishing the second aim was completely developed by the applicant. Finally, the remaining sections of this research training plan including Respective Contributions, Selection of Sponsor and Institution and Responsible Conduct of Research were all developed and written by the applicant. The sponsor performed minimal editing work in all sections of this plan. The research project proposed in this training plan does not include human subjects or animal research. Thus, sections of the training plan related to these areas of research were not prepared.

20. Selection of Sponsor and Institution:

The institution and sponsors selected to be part of this research training plan are key factors in the achievement of the goals proposed herein. New Mexico State University is a land-grant institution dedicated to teaching, research and service at the undergraduate and graduate levels. This institution strongly promotes research in several areas, including the biomedical field. This characteristic has led the institution to be ranked as the 28th in the nation (among all institutions without medical schools) by the National Science Foundation in research and development funding. In addition, NMSU is a Hispanic serving institution that promotes diversity in all education areas. Thus, NMSU seems to share similar objectives with the Ruth L. Kirschstein program announcement. The characteristics above mention have attracted me to NMSU. I believe this institution offers a great atmosphere and support to students like me in search of a scientific career.

As mentioned in section 18, my overall career goal is to become a principal investigator in the field of cancer therapy development. To become an expert in this field and a competent investigator, I believe that it is important to understand the basic enzymology of cancer and then start applying this knowledge in the medical development of efficient therapies. For this reason, I have selected two sponsors with vast expertise in the research areas I want to develop. My mentor, Dr. Shelley Lusetti, is an assistant professor in the Department of Chemistry and Biochemistry of NMSU. She is a new investigator that has extensive graduate and post-doctoral training in the biochemical mechanisms of DNA repair proteins from the laboratory of Michael M. Cox at the University of Wisconsin, Madison. Dr. Lusetti has brought fresh curricular ideas to the Department of Chemistry and Biochemistry. She is an expert enzymologist with research interest in DNA repair mechanisms. My interest in cancer enzymology encouraged me to rotate through her laboratory during my first semester at NMSU. Through this rotation I realized that her research is related to cancer at a basic level, an area of research I was looking for in my Ph.D. training. Furthermore, through this rotation I was able to experience first hand her training style. Dr. Lusetti likes to provide the basic knowledge and tools that students need to learn and develop a protocol. However, she motivates her students to figure out how to do it on their own, while still offering guidance when obstacles are encountered. In addition, I discovered some of her professional and personal qualities. She creates a training atmosphere of trust, support, motivation and open communication that just seemed the right fit for me. All these factors made me want to work and get trained by Dr. Lusetti.

Along with Dr. Lusetti, I have selected Dr. Jeffrey Arterburn to co-sponsor me in the research training. Dr. Arterburn is a professor in the Department of Chemistry and Biochemistry at NMSU. He is a well-experienced investigator with research focused on the development of therapeutic organic compounds for the treatment of diverse disorders including breast cancer. Being involved in a more applied cancer research field, he is a good candidate to train me in the field I want move into for my post-doctoral training. In addition, Dr. Arterburn has been able to obtain funding from several funding agencies including the NIH and the [redacted] which suggests that he is competent in the grant writing process. Thus, he can also help me improve my grant writing skills. I have experience his training style through classes I have taken from him as well as from thesis committee meetings. Dr. Arterburn is an active member of my graduate committee. He is familiar with my research project and he is also involved in my graduate training through the committee. I have selected Dr. Arterburn as my co-sponsor because his scientific expertise compliment the level of scientific training I am looking for in my Ph.D. education.

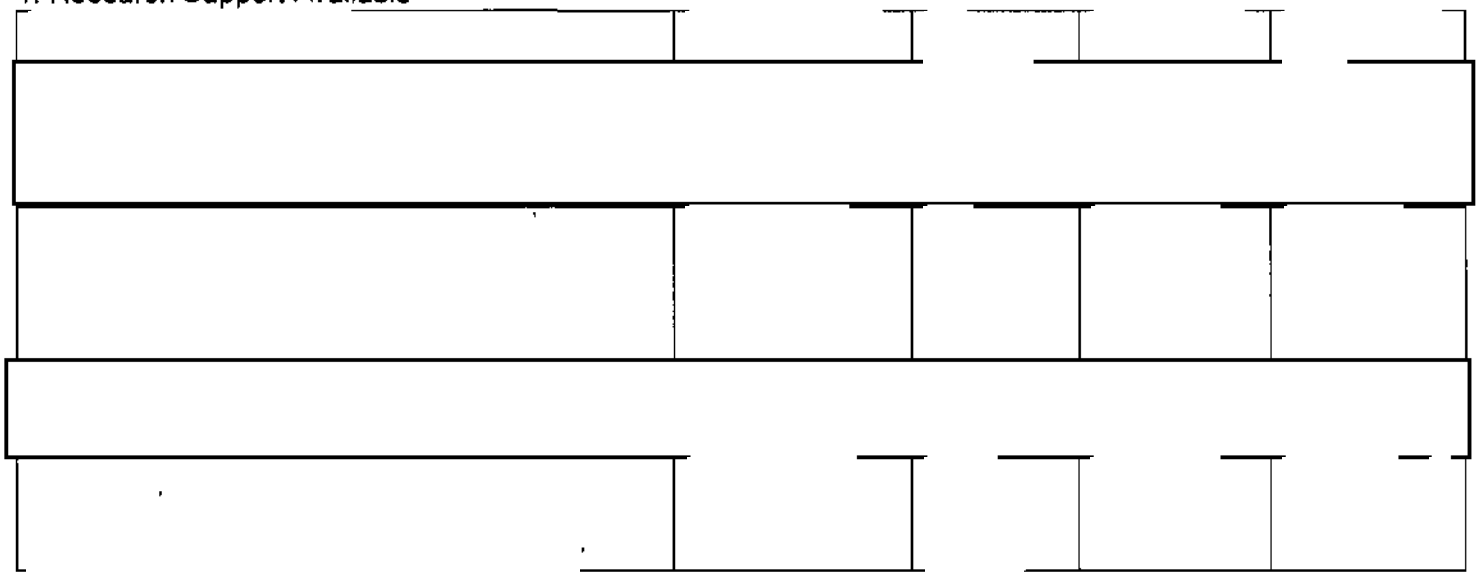
To achieve the goals of the research training plan presented in this proposal, good guidance, training and resources required. Both of the sponsors I have selected have offered me excellent guidance and training in the fields of research I want to develop. Similarly, NMSU is an institution with the resources and the environment I need to complete my training plan effectively. For these reasons, I believe that the sponsors and the institution I have selected will facilitate the achievement of my goals.

21. Responsible Conduct of Research

To comply with the NIH required training on Responsible Conduct on Research (RCR), my sponsor, Dr. Shelley Lusetti and I, Emigdio D. Reyes have developed a research ethics training plan, which I am already following. This plan includes attending to different instructive activities that cover the nine areas of research ethics most relevant to the Office of Research Integrity: data acquisition, management, sharing and ownership; conflict of interest and commitment; human subjects; animal welfare; research misconduct; publication practices and responsibilities; peer review and collaborative science. To begin with my training, I completed Philosophy 540: Ethical Issues in the Biological Sciences taught by Philosophy Professor Timothy Cleveland during the spring semester of 2007. This course covered the nine instructional areas required by the Office of Research Integrity as well as basic introduction into Philosophy and Ethics. To strengthen my training on these areas, I attended a series of research ethics workshops offered by the MBRS RISE program at NMSU during the summer of 2008. These workshops included topics such as "Introduction to Ethics and Philosophy", "Responsible Conduct in Research Requirements in NSF and NIH Training Grants" and "NMSU Research Ethics Policies". In addition, I plan to complete the online training on RCR offered by the Office of Research Integrity by the beginning of June 2009. Future activities to reinforce my education and training in RCR will include workshops and lab group activity in which the nine areas on interest will be discussed.

Section II—Sponsor and Co-Sponsor Information

1. Research Support Available



2. Sponsor's/Co-sponsor's Previous Fellows/Trainees:

2A. Shelley L. Lusetti (Sponsor)
The sponsor has been an independent researcher at New Mexico State University for less than 3 years. The following students were mentored by Shelley Lusetti during her post-doctoral position at UW-Madison in the department of Biochemistry (*co-authored publications with the sponsor):

Graduate Student Mentees:
*Julia Drees earned her Ph.D. in 2006; currently a postdoctoral fellow at the University of California, San Francisco, Department of Laboratory Medicine
*Michael Hobbs earned his Ph.D. in 2006; currently a research scientist at
Dennis Harris earned his Ph.D. in 2007; currently a postdoctoral fellow at
Department of Radiation Oncology
Undergraduate Mentees:
April Schumacher earned her Ph.D. from University of Minnesota, Masonic Cancer Center; currently a Postdoctoral fellow at the University of Connecticut Health Center
*Christopher D. Fleming, Currently in a Ph.D. program at the University of North Carolina, Chapel Hill in the laboratory of Matthew R. Redinbo
*Jeffrey J. Shaw, Currently in a Ph.D. program at Johns Hopkins University in the HHMI laboratory of Rachel Green

2B. Jeffrey B. Arterburn (Co-sponsor):
*Ian M. Fogarty earned his M.S. in 1997; "Synthesis of Functionalized Rhenium Organoimido Complexes: Possible Anticancer Radiopharmaceuticals" currently a Teacher in Enriched Chemistry and Physics at
*Marc C. Perry earned his Ph.D. in 2000; "Rhenium-Catalyzed Heteroatom Transfer Reactions"
currently an Assist. Prof. in the Dept. Chem., U. Alaska, Anchorage.
*Minghua Liu earned his M.S. in 2000 "Synthesis and Application of a Polymer-Supported Rhenium Catalyst: Alcohol Oxidation with Methyl Sulfoxide and Epoxide Deoxygenation with Triphenylphosphine" currently a Research Chemist,
*Ranjit Ramdas earned his M.S. in 2001; "The Catalytic Amination of Pyridyl Electrophiles with Protected Hydrazines: Synthesis of Pyridyl Derivatives of Estradiol" currently a Research Chemist at

*Anicele Gonzalez earned her M.S. in 2001; "Catalytic Degradation of Organic Chelating Agents in Simulated Nuclear Waste: Analysis of the Degradation Pathway Using Carbon-13 NMR" currently a Research Chemist at

*Sherry L. Nelson earned her M.S. in 2001; "Development of a Homogenous Rhenium Catalyst for Oxidation, Reduction, and Lewis Acid Activation of Organosulfur Compounds" currently a Research Chemist,

*Cesear Corona earned his Ph.D. in 2006; "Synthesis and Characterization of Carbon-Linked Biotin Derivatives" currently a Research Scientist at

*Duminda S. Liyanage earned her M.S. in 2006; "Synthesis of Fluorescent Capsaicin Derivatives for Investigation of Vanilloid Receptor Binding" currently a Research Scientist in

*Sudath S. Hapuarachchige earned his M.S. in 2006; "Synthesis of Fluorescent Rhenium(I) Tricarbonyl Hydrazone Complexes as Dual Functional Molecular Probes" he is currently a Doctoral candidate at NMSU with expected completion of the Ph.D. in Fall 2009.

*Bj K. Bryant earned his Ph.D. 2006; "Development of Tridentate Pyridin-2yl-Hydrazine Chelates for Targeting Estrogen-Binding Receptors" currently the Director of an

*Juan Jacob R. Strouse earned his Ph.D. in 2007; "Copper Mediated C-N Arylation for Synthesis of New Anti-Viral Purine Nucleoside Analogs" currently an NIH Postdoctoral Research Associate at the University of New Mexico, Health Sciences Center, Albuquerque, NM.

*Sidath C. Kumarapperuma earned his Ph.D. in 2008; "Design, Synthesis and Modeling of Novel Azole Nucleoside Analogs as Potential Therapeutics for RNA Viruses" currently a Postdoctoral Research Associate, at the University of Minnesota, Department of Medicinal Chemistry, Minneapolis, MN.

*Ritwik Burai earned his Ph.D. in 2009; "Design and Synthesis of GPR30-Selective Molecular Probes for Chemical Biology and Imaging Applications" currently searching for a Postdoctoral Research Associate position.

3. Training Plan, Environment, Research Facilities

3A: Training Plan

Emigdio has started on a path toward his long-term career goal to become a principle investigator in academia focusing his research on the cancer biology field. Emigdio has completed all formal coursework required by the Department of Chemistry and Biochemistry and the Graduate School at NMSU, allowing him to devote the majority of his time to research training. This includes training in the laboratory as well as other critical areas that will contribute to his development as a scientist consisting of workshops designed to improve written communication skills and provide ethics training, and student seminars that will give Emigdio vital practice in oral communication.

Although Emigdio has only completed 2.5 years of his graduate training, he is the senior graduate student in my laboratory. I have worked very closely with him to personally train him in experimental design, collection and documentation of data, and analysis of his results. In this respect, he has benefited from joining this laboratory early in my career since he is able to learn techniques directly from me. This direct experimental training will continue for the duration of his thesis work. I meet one on one with Emigdio each week to discuss short-term goals, trouble-shoot experiments, plan publications, discuss current literature, etc.

Additionally, several efforts have been made to ensure Emigdio has the opportunity for research training elsewhere. For example, we have made plans for Emigdio to carry out some of the DNA binding studies proposed in Aim 1 at the University of Arizona, Tucson in the laboratory of Nancy C. Horton. Dr. Horton is an Associate Professor in the Department of Biochemistry and Molecular Biophysics. She will host Emigdio for several weeks during the summer and assist him with biophysical techniques currently not available at NMSU, such as fluorescence polarimetry. Additionally, Emigdio will benefit from my own mentor, Patrick Sung. Dr. Sung is a full Professor and Vice-chair at Yale University in the Department of Molecular Biophysics and Biochemistry. The Sung lab focuses on DNA double-strand break repair mechanisms in eukaryotic systems. Dr. Sung is my formal mentor for my pending and a collaborator on the larger RecN project that includes Emigdio's thesis work. Included in our mentorship agreement is for myself and a student to travel once per year to participate in joint group meetings with Dr. Sung and his research team.

Through his doctoral training in my laboratory, Emigdio is learning the enzymology of DNA double-strand break repair as a tool for studying mechanisms of radiation resistance. Here we use bacterial organisms to

model these complicated processes. However, Emigdio would eventually like to expand his knowledge and experience into human cancer biology and the development of cancer therapies in a future post-doctoral position. Therefore, he has established a relationship with Dr. Jeffrey Arterburn (co-sponsor). Dr. Arterburn is an expert in organic synthesis and is developing antiviral drugs and radiopharmaceuticals. He is the PI of the NIH NCRR funded New Mexico IDeA Network for Biomedical Research, a statewide network that focuses on investigations of biomolecule structure and activity, cell signaling pathways, and pathogens. He is the co-leader of the Chemistry sub-group of the University of New Mexico's NIH Roadmap Molecular Libraries Screening Center and a member of the UNM Cancer Center. He has ongoing collaborative interdisciplinary research projects with investigators from the UNM College of Pharmacy, UNM School of Medicine, Los Alamos National Laboratory, U. Illinois, and the Southern Research Institute. In addition to the mentorship provided by Dr. Arterburn as a member of his thesis committee (see below), Emigdio will attend monthly group meetings with Dr. Arterburn's research team. This participation will expose him to many aspects of cancer chemistry, further contributing to his growth and development in the broad field of cancer research.

Emigdio will receive training in the public presentation of his work. We hold weekly group meetings, affording Emigdio the opportunity to present research findings approximately every 6 weeks. While technically informal, I require the students to prepare structured presentations. Each semester, Emigdio is also required to present current research papers in the Biochemistry division Literature club. His presentations are critiqued, in writing, by his peers and the Biochemistry faculty. Once per year, he presents a formal student research seminar for students and faculty involved in the Molecular Biology Program here at NMSU. Additionally, Emigdio will present his research in poster form annually at NMSU sponsored poster sessions. Following these various presentations, I will provide Emigdio with feedback and suggestions to improve his presentation skills. Emigdio will submit an abstract and attend at least one national meeting per year. For example, in March 2009 Emigdio and I attended the Keystone symposium on Genomic Instability and DNA repair where he presented his poster entitled "Biochemical Characterization and Mutational Analysis of a Bacterial RecN Protein." When selecting meetings for Emigdio to attend, we consider his long-term goal of moving into the human cancer biology fields. At meetings such as this latest Keystone symposia, he is exposed to the broad DNA repair field as studied in both the prokaryotic and eukaryotic fields. Another consideration when selecting meetings are the personal interactions possible. Emigdio has been able to meet and interact with my post-doctoral mentor from UW-Madison, Michael Cox and many members of his laboratory as well as many members of the DNA repair community. I will continue to strive to connect Emigdio to as many investigators, post-doctoral fellows and graduate students in the eukaryotic DNA repair field and encourage him to develop a network that we can employ when he starts to look for a post-doctoral position.

NMSU is fortunate to have an outstanding, NIH funded, graduate student training program called "RISE to the Post-doctorate." Emigdio is currently a RISE doctoral student scholar and as such, is required to attend regular professional development courses, intensive writing workshops, leadership retreats, and Responsible Conduct in Research seminars. RISE scholars are also required to maintain portfolios that are submitted and reviewed annually by a RISE mentor committee. This portfolio contains current vitae, documentation of oral presentations, writing samples, yearly and career goals, manuscripts, etc. Students are evaluated on their progress toward the doctorate. Emigdio will continue to participate in all RISE scholarly activities.

To provide ample mentoring for Emigdio, we have established a thesis committee consisting of myself, Professor Jeffrey Arterburn (co-sponsor), Professor Champa Sengupta-Gopalan, and Professor Peter Lammers. These three full professors collectively have mentored more than twenty doctoral students over the past decade. Emigdio meets with the full committee at least once per year to discuss research progress and goals. The members of this committee also attend Emigdio's seminar presentations, asking probative questions and providing constructive comment.

Finally, an important component of Emigdio's scientific training is related to Chemical and Biological safety, the handling of recombinant DNA, the responsible conduct in research (RCR), and ethics. In section 21 of his Research Training Plan, Emigdio has detailed the steps we have and will take to ensure he has been trained in the RCR policies as laid out by the NIH. Additionally, Emigdio has attended Chemical Safety, Laboratory Standard Practices, Biological Safety, and Chemical Waste management training through formal classes provided by NMSU. He is required to attend annual refresher courses in lab safety.

3B: Environment

New Mexico State University at Las Cruces is a comprehensive, state-supported land grant university

(since 1890) and is ranked among the Carnegie I research intensive institutions in the USA. NMSU is one of only six Ph.D.-granting institutions in the United States which is classified simultaneously as a Carnegie I Institution and a Minority Serving Institution. The Department of Chemistry and Biochemistry is served by 28 faculty members in the areas of Biochemistry, Organic chemistry, Inorganic chemistry, Physical chemistry, Analytical chemistry and Toxicology. This group of researchers possess considerable strength in chemical biology and biochemistry, providing Emigdio with a wealth of experience. These strengths are augmented by an interdisciplinary Molecular Biology Program which brings together faculty, students and post-doctoral fellows from 2 colleges (Arts and Sciences, Agriculture) and many departments (such as Biology and Chemistry) that utilize molecular techniques. Additional details regarding Emigdio's research environment, including the RISE program and outside contacts can be found in the Training plan above.

3C: Facilities

The Lusetti lab occupies approximately 1,000 square feet of the 3rd floor of the1997 addition of the Chemistry & Biochemistry Building. The lab includes seven lab benches, a fume hood, and safety cabinets. 100 square feet of walk-in cold room space is also available to my lab and is located on the 3rd floor. Two MacPro computers are available in the lab for students. Two laser printers are also available. The lab is also equipped with 2 Dell PCs that are dedicated for equipment operation. My office and a separate 150 square foot office space has been provided for the use of the students and lab staff. These are located on the same hall as the lab. The Lusetti lab contains that following equipment for use by Emigdio: AKTA FPLC chromatography system for protein purification housed in a refrigerated chromatography cabinet, a Varian UV/Vis spectrophotometer (Cary 100 equipped with a temperature controller and a twelve position sample chamber) for kinetic/ATPase assays, a gel documentation system (Fotodyne Investigator) equipped with a CCD camera and quantitation software for DNA and protein gel analysis, a clinical centrifuge, and a UV cross-linker for cell growth sensitivity assays. The lab also contains a full complement of common lab equipment, such as refrigerators, freezers, incubators/shakers, water baths, microfuges, power supplies, electrophoresis and blotting equipment, balances, pH and conductivity meter, micropipettors, etc. The following equipment is immediately available for Emigdio's use (we are only listing items relevant to the current proposal) and is located in the Chemistry and Biochemistry building: French pressure cell, lyophilizer, Beckman L8-70M preparative ultracentrifuge with Ti70, SW45, SW50.1, SW27 and TiV rotors, three Sorvall preparative refrigerated centrifuges with a full complement of rotors, ultrasonic cell disrupter, a dark room, autoclaves, dishwasher, Storm 860 Phosphor/Fluor-imager, and a Varian Cary Eclipse spectrofluorimeter. NMSU also maintains a Molecular Biology Core Facility where DNA sequencing services are available as well as the Pulse Field Gel Electrophoresis equipment that Emigdio will use for Aim 2.

4. Number of Fellows/Trainees to be Supervised During the Fellowship

4A. Sponsor supervises 4 pre-doctoral trainees (including the applicant, E. M. Reyes). Two of these trainees (Praveen Patidar and Megha Khandelwal) are supported by Teaching Assistantships and NM-INBRE. Lee Uranga is currently supported by the MBRS-RISE program.

4B. Co-sponsor supervises 4 pre-doctoral trainees, two are supported by NIH grants: Ritwik Burai (Ph.D. final defense 3/09), and Sudath Hapuarachchige; two are supported by a combination of Teaching Assistantships and external funds: Ali Taj Nashir, and Samuel Kadavakollu. The co-sponsor also supervises two postdoctoral Research Associates supported by external funds: Ramesh Chinnasamy (NIH), and Marjan Jeselnik (DOD); and two full time Administrative Staff supported by the NIH/NCRR NM-INBRE: Christiane Herber-Valdez, Ed.D., and Carolyn Bizzell, M.S.

5. Applicant's Qualifications and Potential for a Research Career

Emigdio is a currently a 3rd year doctoral graduate student in the Chemistry & Biochemistry Dept. at NMSU. He graduated *magna cum laude* with a B.S. degree in Biochemistry from St. Edwards University in Austin, TX in 2004. Prior to enrolling in graduate school he worked as a bio-medical research technician for 2 years. Emigdio joined my laboratory Spring 2007 and has made exceptional progress on his thesis project describing the biochemical properties of the bacterial RecN protein. RecN is a predicted chromosomal maintenance protein and is required for DNA double-strand break repair, yet very little is known regarding its

function. The reason for this is simple, the protein is difficult to work with in vitro. In a very short time, Emigdio has purified the protein and has characterized some key kinetic parameters of the enzymatic ATP hydrolysis catalyzed by the protein. He has recently determined the effects of various DNA substrates on this ATPase activity, which will help us determine the substrate specificity for RecN. Emigdio has now designed several functional DNA assays to elucidate the specific role of RecN in the double-strand break repair pathway. These complicated assays have required months of DNA substrate construction, which Emigdio is currently completing. This body of work described above is actually remarkable given that he was the first graduate student in the lab. He has no senior people to train him and has learned much of the lab techniques independently. Emigdio is also a natural teacher. He is extremely patient and organized when mentoring undergraduates and the newer graduate students in the lab. He has a strong mastery of the DNA repair literature and can disseminate knowledge easily. In fact, he was able to step in on an hour's notice and teach a lecture on DNA repair in a course I was teaching.

As mentioned above, Emigdio has completed all of the formal coursework required for his doctoral degree here at NMSU. His academic record in our department is exemplary, displaying mastery in the diverse chemical divisions of Organic, Inorganic, Analytical, Physical and Biochemistry as well as in Biology. In fact, instructors have sought me out on several occasions to communicate that Emigdio was the top student in their course.

The current proposal was written entirely by Emigdio. I have, of course, provided editing and suggestions for improvement. Emigdio has a nice writing style, which is impressive [redacted]
The first Aim of this proposal is part of my pending NIH-SCORE proposal, although re-organized and re-written by Emigdio here. I want to point out that Aim 2 was completely conceived and developed by Emigdio. Collaborative efforts described by Emigdio in this proposal are formal collaborations between myself and Patrick Sung, Nancy Horton and John Battista.

In conclusion, I believe that Emigdio has made significant progress in his research. He has approached his work with zeal and displays true interest in obtaining knowledge. He displays exceptional leadership qualities, a mastery of biochemical topics, and a good understanding of the literature related to his current field. In short, he possesses the qualities necessary to advance to a post-doctoral fellowship and a tenure-track academic research position.