InFORMATION ABOUT PRINCIPAL INVESTIGATORS/PROJECT DIRECTORS(PI/PD) and co-PRINCIPAL INVESTIGATORS/co-PROJECT DIRECTORS

Submit only ONE copy of this form **for each PI/PD and co-PI/PD** identified on the proposal. The form(s) should be attached to the original proposal as specified in GPG Section II.B. Submission of this information is voluntary and is not a precondition of award. This information will not be disclosed to external peer reviewers. ***DO NOT INCLUDE THIS FORM WITH ANY OF THE OTHER COPIES OF YOUR PROPOSAL AS THIS MAY COMPROMISE THE CONFIDENTIALITY OF THE INFORMATION.***

PI/PD Name: Justin Courcelle

Gender: ☒ Male ☐ Female

Ethnicity: (Choose one response) ☐ Hispanic or Latino ☒ Not Hispanic or Latino

Race:
(Select one or more)
- ☐ American Indian or Alaska Native
- ☐ Asian
- ☐ Black or African American
- ☐ Native Hawaiian or Other Pacific Islander
- ☒ White

Disability Status:
(Select one or more)
- ☐ Hearing Impairment
- ☐ Visual Impairment
- ☐ Mobility/Orthopedic Impairment
- ☐ Other
- ☒ None

Citizenship: (Choose one) ☒ U.S. Citizen ☐ Permanent Resident ☐ Other non-U.S. Citizen

Check here if you do not wish to provide any or all of the above information (excluding PI/PD name): ☒

Pecase Eligibility: Y

REQUIRED: Check here if you are currently serving (or have previously served) as a PI, co-PI or PD on any federally funded project ☒

Ethnicity Definition:
Hispanic or Latino. A person of Mexican, Puerto Rican, Cuban, South or Central American, or other Spanish culture or origin, regardless of race.
Race Definitions:
American Indian or Alaska Native. A person having origins in any of the original peoples of North and South America (including Central America), and who maintains tribal affiliation or community attachment.
Asian. A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam.
Black or African American. A person having origins in any of the black racial groups of Africa.
Native Hawaiian or Other Pacific Islander. A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.
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WHY THIS INFORMATION IS BEING REQUESTED:

The Federal Government has a continuing commitment to monitor the operation of its review and award processes to identify and address any inequities based on gender, race, ethnicity, or disability of its proposed PIs/PDs. To gather information needed for this important task, the proposer should submit a single copy of this form for each identified PI/PD with each proposal. Submission of the requested information is voluntary and will not affect the organization's eligibility for an award. However, information not submitted will seriously undermine the statistical validity, and therefore the usefulness, of information recieved from others. Any individual not wishing to submit some or all the information should check the box provided for this purpose. (The exceptions are the PI/PD name and the information about prior Federal support, the last question above.)

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List of Suggested Reviewers or Reviewers Not To Include (optional)

SUGGESTED REVIEWERS:
Not Listed

REVIEWERS NOT TO INCLUDE:
Not Listed

COVER SHEET FOR PROPOSAL TO THE NATIONAL SCIENCE FOUNDATION

PROGRAM ANNOUNCEMENT/SOLICITATION NO./CLOSING DATE/if not in response to a program announcement/solicitation enter NSF 04-2		FOR NSF USE ONLY
NSF 02-111	07/20/04	NSF PROPOSAL NUMBER
FOR CONSIDERATION BY NSF ORGANIZATION UNIT(S) (Indicate the most specific unit known, i.e. program, division, etc.)		0448315
MCB - Microbial Genetics		

DATE RECEIVED	NUMBER OF COPIES	DIVISION ASSIGNED	FUND CODE	DUNS# (Data Universal Numbering System)	FILE LOCATION
				075461814	

EMPLOYER IDENTIFICATION NUMBER (EIN) OR TAXPAYER IDENTIFICATION NUMBER (TIN)	SHOW PREVIOUS AWARD NO. IF THIS IS ☒ A RENEWAL ☐ AN ACCOMPLISHMENT-BASED RENEWAL	IS THIS PROPOSAL BEING SUBMITTED TO ANOTHER FEDERAL AGENCY? YES ☐ NO ☒ IF YES, LIST ACRONYM(S)
646000819	0130486	

NAME OF ORGANIZATION TO WHICH AWARD SHOULD BE MADE	ADDRESS OF AWARDEE ORGANIZATION, INCLUDING 9 DIGIT ZIP CODE
Mississippi State University	Mississippi State University Mississippi State, MS. 39762
AWARDEE ORGANIZATION CODE (IF KNOWN) 0024232000	
NAME OF PERFORMING ORGANIZATION, IF DIFFERENT FROM ABOVE	ADDRESS OF PERFORMING ORGANIZATION, IF DIFFERENT, INCLUDING 9 DIGIT ZIP CODE
PERFORMING ORGANIZATION CODE (IF KNOWN)	

IS AWARDEE ORGANIZATION (Check All That Apply) (See GPG II.C For Definitions) ☐ SMALL BUSINESS ☐ FOR-PROFIT ORGANIZATION ☐ MINORITY BUSINESS ☐ WOMAN-OWNED BUSINESS ☐ IF THIS IS A PRELIMINARY PROPOSAL THEN CHECK HERE

TITLE OF PROPOSED PROJECT **CAREER: Recovery of Replication Following DNA Damage in E.coli**

REQUESTED AMOUNT	PROPOSED DURATION (1-60 MONTHS)	REQUESTED STARTING DATE	SHOW RELATED PRELIMINARY PROPOSAL NO. IF APPLICABLE
$ 745,845	60 months	04/01/05	

CHECK APPROPRIATE BOX(ES) IF THIS PROPOSAL INCLUDES ANY OF THE ITEMS LISTED BELOW

- ☐ BEGINNING INVESTIGATOR (GPG I.A)
- ☐ DISCLOSURE OF LOBBYING ACTIVITIES (GPG II.C)
- ☐ PROPRIETARY & PRIVILEGED INFORMATION (GPG I.B, II.C.1.d)
- ☐ HISTORIC PLACES (GPG II.C.2.j)
- ☐ SMALL GRANT FOR EXPLOR. RESEARCH (SGER) (GPG II.D.1)
- ☐ VERTEBRATE ANIMALS (GPG II.D.5) IACUC App. Date ________
- ☐ HUMAN SUBJECTS (GPG II.D.6) Exemption Subsection ________ or IRB App. Date ________
- ☐ INTERNATIONAL COOPERATIVE ACTIVITIES: COUNTRY/COUNTRIES INVOLVED (GPG II.C.2.g.(iv).(c)) ________
- ☐ HIGH RESOLUTION GRAPHICS/OTHER GRAPHICS WHERE EXACT COLOR REPRESENTATION IS REQUIRED FOR PROPER INTERPRETATION (GPG I.E.1)

PI/PD DEPARTMENT	PI/PD POSTAL ADDRESS
Biological Sciences	Box GY Mississippi State, MS 39762 United States
PI/PD FAX NUMBER 662-325-7939	

NAMES (TYPED)	High Degree	Yr of Degree	Telephone Number	Electronic Mail Address
PI/PD NAME Justin Courcelle	PhD	1999	662-325-2131	jcourcelle@biology.msstate.edu
CO-PI/PD				
CO-PI/PD				
CO-PI/PD				
CO-PI/PD				

Electronic Signature

CERTIFICATION PAGE

Certification for Authorized Organizational Representative or Individual Applicant:

By signing and submitting this proposal, the individual applicant or the authorized official of the applicant institution is: (1) certifying that statements made herein are true and complete to the best of his/her knowledge; and (2) agreeing to accept the obligation to comply with NSF award terms and conditions if an award is made as a result of this application. Further, the applicant is hereby providing certifications regarding debarment and suspension, drug-free workplace, and lobbying activities (see below), as set forth in Grant Proposal Guide (GPG), NSF 04-2. Willful provision of false information in this application and its supporting documents or in reports required under an ensuing award is a criminal offense (U. S. Code, Title 18, Section 1001).

In addition, if the applicant institution employs more than fifty persons, the authorized official of the applicant institution is certifying that the institution has implemented a written and enforced conflict of interest policy that is consistent with the provisions of Grant Policy Manual Section 510; that to the best of his/her knowledge, all financial disclosures required by that conflict of interest policy have been made; and that all identified conflicts of interest will have been satisfactorily managed, reduced or eliminated prior to the institution's expenditure of any funds under the award, in accordance with the institution's conflict of interest policy. Conflicts which cannot be satisfactorily managed, reduced or eliminated must be disclosed to NSF.

Drug Free Work Place Certification

By electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative or Individual Applicant is providing the Drug Free Work Place Certification contained in Appendix C of the Grant Proposal Guide.

Debarment and Suspension Certification

(If answer "yes", please provide explanation.)

Is the organization or its principals presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency? Yes ☐ No ☒

By electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative or Individual Applicant is providing the Debarment and Suspension Certification contained in Appendix D of the Grant Proposal Guide.

Certification Regarding Lobbying

This certification is required for an award of a Federal contract, grant, or cooperative agreement exceeding $100,000 and for an award of a Federal loan or a commitment providing for the United States to insure or guarantee a loan exceeding $150,000.

Certification for Contracts, Grants, Loans and Cooperative Agreements

The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, ''Disclosure of Lobbying Activities,'' in accordance with its instructions.

(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by section 1352, Title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

AUTHORIZED ORGANIZATIONAL REPRESENTATIVE	SIGNATURE	DATE
NAME Jonathan W Pote	Electronic Signature	Jul 20 2004 4:22PM

TELEPHONE NUMBER	ELECTRONIC MAIL ADDRESS	FAX NUMBER
662-325-3571	jpote@research.msstate.edu	662-325-8028

*SUBMISSION OF SOCIAL SECURITY NUMBERS IS VOLUNTARY AND WILL NOT AFFECT THE ORGANIZATION'S ELIGIBILITY FOR AN AWARD. HOWEVER, THEY ARE AN INTEGRAL PART OF THE INFORMATION SYSTEM AND ASSIST IN PROCESSING THE PROPOSAL. SSN SOLICITED UNDER NSF ACT OF 1950, AS AMENDED.

CAREER ELIGIBILITY CERTIFICATIONS

A. CAREER ELIGIBILITY CERTIFICATION

To be eligible for a CAREER award, you must meet the CAREER eligibility requirements as defined in the CAREER Program Solicitation (also refer to the CAREER FAQ for further explanations). To certify your eligibility, complete each section of the CAREER checklist below. The CAREER Eligibility Certification checklist will be included as part of the proposal and will be sent to reviewers.

I certify that by the relevant July deadline for submission of CAREER proposals, I will have met all of the following criteria.

- ☒ I will hold a doctoral degree in a field of science or engineering supported by NSF
- ☒ I will be untenured
- ☒ I will not have received an NSF PECASE or CAREER award
- ☒ I will not have competed more than two times in previous NSF CAREER Program Competitions

I certify that by October 1st following the relevant July deadline for submission of CAREER proposals I will

☒ be employed in a tenure-track position

OR

☐ be employed in a tenure-track equivalent position

☒ hold the title of assistant professor

OR

☐ hold a title that is equivalent to assistant professor

☒ be employed at an institution in the U.S., its territories, or possessions, or the Commonwealth of Puerto Rico that awards degrees in a field of science or engineering supported by NSF

OR

☐ be employed at an institution in the U.S., its territories, or possessions, or the Commonwealth of Puerto Rico that is a non-profit, non-degree granting institution such as a museum, observatory, or research lab

Directorate for Biological Sciences
Division of Molecular and Cellular Bioscience
Microbial Genetics

Proposal Classification Form
PI: Courcelle, Justin / Proposal Number: 0448315

CATEGORY I: INVESTIGATOR STATUS (Select ONE)

- ☐ Beginning Investigator - No previous Federal support as PI or Co-PI, excluding fellowships, dissertations, planning grants, etc.
- ☐ Prior Federal support only
- ☑ Current Federal support only
- ☐ Current & prior Federal support

CATEGORY II: FIELDS OF SCIENCE OTHER THAN BIOLOGY INVOLVED IN THIS RESEARCH (Select 1 to 3)

☐ Astronomy	☐ Engineering	☐ Psychology
☐ Chemistry	☐ Mathematics	☐ Social Sciences
☐ Computer Science	☐ Physics	☑ None of the Above
☐ Earth Science		

CATEGORY III: SUBSTANTIVE AREA (Select 1 to 4)

☐ BIOMATERIALS	☐ Fungal	☐ Arabidopsis-Related Plant Research
☐ COMPUTATIONAL BIOLOGY	☐ Plant	☐ REPRODUCTIVE ANIMAL BIOLOGY
☐ DATABASES	☐ Animal	☐ SPINAL CORD/ NERVE REGENERATION
☐ ENDOCRINE DISRUPTORS/ ENVIRONMENTAL ENDOCRINOLOGY	☐ HUMAN NUTRITION	☐ Modeling (general)
☐ EPIGENETICS	☐ MARINE MAMMALS	☑ Modeling of Biological or Molecular Systems
☐ EXTREMOPHILES	☐ NANOSCIENCE	☐ Computational Modeling
☐ GENOMICS (Genome sequence, organization, function)	☐ PHOTOSYNTHESIS	☐ STRUCTURAL BIOLOGY
☐ Viral	☐ PLANT BIOLOGY	☐ NONE OF THE ABOVE
☑ Microbial		

CATEGORY IV: INFRASTRUCTURE (Select 1 to 3)

COLLECTIONS/STOCK CULTURES	FACILITIES	☐ INDUSTRY PARTICIPATION
☐ Living Organism Stock Cultures	☐ Field Stations	☐ Technique Development
☐ DATABASES	☐ LTER Site	☑ NONE OF THE ABOVE

CATEGORY V: HABITAT (Select 1 to 2)

TERRESTRIAL HABITATS

☐ GENERAL TERRESTRIAL	☐ SUBTERRANEAN/ SOIL/ SEDIMENTS	☐ EXTREME TERRESTRIAL ENVIRONMENT

AQUATIC HABITATS

☐ GENERAL AQUATIC	☐ MARINE	☐ EXTREME AQUATIC ENVIRONMENT
☐ FRESHWATER	☐ HYPERSALINE	

MAN-MADE ENVIRONMENTS

☑ CELL/TISSUE CULTURE (In Vitro)	☐ THEORETICAL SYSTEMS	☐ OTHER ARTIFICIAL SYSTEMS
☐ In Silico		

NOT APPLICABLE	
☐ NOT APPLICABLE	

CATEGORY VI: GEOGRAPHIC AREA OF THE RESEARCH (Select 1 to 2)

- ☐ WORLDWIDE
- ☑ NORTH AMERICA
  - ☐ United States
    - ☐ Northeast US (CT, MA, ME, NH, NJ, NY, PA, RI, VT)
    - ☐ Northcentral US (IA, IL, IN, MI, MN, ND, NE, OH, SD, WI)
    - ☐ Northwest US (ID, MT, OR, WA, WY)
    - ☐ Southeast US (DC, DE, FL, GA, MD, NC, SC, WV, VA)
    - ☐ Southcentral US (AL, AR, KS, KY, LA, MO, MS, OK, TN, TX)
    - ☐ Southwest US (AZ, CA, CO, NM, NV, UT)
    - ☐ Alaska
    - ☐ Hawaii
    - ☐ Puerto Rico
    - ☐ Canada
  - ☐ Mexico
- ☐ CENTRAL AMERICA (Mainland)
  - ☐ Caribbean Islands
  - ☐ Bermuda/Bahamas
- ☐ SOUTH AMERICA
  - ☐ Eastern South America (Guyana, Fr. Guiana, Suriname, Brazil)
  - ☐ Northern South America (Colombia, Venezuela)
  - ☐ Southern South America (Chile, Argentina, Uruguay, Paraguay)
  - ☐ Western South America (Ecuador, Peru, Bolivia)
- ☐ EUROPE
  - ☐ Eastern Europe
  - ☐ Russia
  - ☐ Scandinavia
  - ☐ Western Europe
- ☐ ASIA
  - ☐ Central Asia
  - ☐ Far East
  - ☐ Middle East
  - ☐ Siberia
  - ☐ South Asia
  - ☐ Southeast Asia
- ☐ AFRICA
  - ☐ North Africa
  - ☐ African South of the Sahara
    - ☐ East Africa
    - ☐ Madagascar
    - ☐ South Africa
    - ☐ West Africa
- ☐ AUSTRALASIA
  - ☐ Australia
  - ☐ New Zealand
  - ☐ Pacific Islands
- ☐ ANTARCTICA
- ☐ ARCTIC
- ☐ ATLANTIC OCEAN
- ☐ PACIFIC OCEAN
- ☐ INDIAN OCEAN
- ☐ OTHER REGIONS (Not defined)
- ☐ NOT APPLICABLE

CATEGORY VII: CLASSIFICATION OF ORGANISMS (Select 1 to 4)

- ☑ VIRUSES
  - ☑ Bacterial
  - ☐ Plant
  - ☐ Animal
- ☑ PROKARYOTES
  - ☐ Archaebacteria
  - ☐ Cyanobacteria
  - ☐ Eubacteria
- ☐ PROTISTA (PROTOZOA)
- ☐ FUNGI
  - ☐ Filamentous Fungi
  - ☐ Yeasts
- ☐ LICHENS
- ☐ SLIME MOLDS
- ☐ ALGAE
- ☐ PLANTS
  - ☐ N0N-VASCULAR PLANTS
  - ☐ VASCULAR PLANTS
    - ☐ GYMNOSPERMS
    - ☐ ANGIOSPERMS
- ☐ ANIMALS
  - ☐ INVERTEBRATES
    - ☐ Hexapoda (Insecta) (Insects)
  - ☐ VERTEBRATES
    - ☐ FISHES
      - ☐ Chondrichthyes (Cartilaginous Fishes) (Sharks, Rays, Ratfish)
      - ☐ Osteichthyes (Bony Fishes)
    - ☐ AMPHIBIA
    - ☐ REPTILIA
    - ☐ AVES (Birds)
    - ☐ MAMMALIA
      - ☐ Primates
        - ☐ Monkeys
        - ☐ Apes (Gibbons, Orang-utan, Gorilla, Chimpanzee)
        - ☐ Humans
      - ☐ Rodentia
        - ☐ Laboratory Rodents (Rat, Mouse, Guinea Pig, Hamster)
        - ☐ Non-Laboratory Rodents
      - ☐ Marine Mammals (Seals, Walrus, Whales, Otters, Dolphins, Porpoises)
- ☐ TRANSGENIC ORGANISMS
- ☐ NO ORGANISMS

CATEGORY VIII: MODEL ORGANISM (Select ONE)

☐ NO MODEL ORGANISM

MODEL ORGANISM (Choose from the list or input up to 9 characters)

VIRUS/BACTERIA
- ☑ Escherichia coli
- ☐ Bacillus subtilis

PROTISTA
- ☐ Chlamydomonas reinhardtii

FUNGI
- ☐ Dictyostelium
- ☐ Saccharomyces cereviseae
- ☐ Schizosaccharomyces pombe

PLANT
- ☐ Mouse-Ear Cress (Arabidopsis thaliana)
- ☐ Corn (Zea mays)

ANIMAL
- ☐ Nematode (Caenorhabditis elegans)
- ☐ Fruitfly (Drosophila melanogaster)
- ☐ African Clawed Frog (Xenopus laevis)
- ☐ Mouse, Laboratory

[Enter your own model organism - up to 9 characters]

Project Summary

Inaccurate replication in the presence of DNA damage is responsible for the majority of cellular rearrangements and mutagenesis that are observed in cancer cells. DNA damage, such as that induced by UV irradiation, severely impairs the ability of replication to copy the genomic template. Significant advances have been made to identify the gene products that are required when replication encounters DNA lesions in the template. However, the cellular mechanism(s) by which these lesions are processed during replication *in vivo* remains relatively uncharacterized. Following UV-induced DNA damage in *Escherichia coli,* replication can either arrest at the lesion site, or skip over the DNA lesion generating a gap. When replication forks are arrested, they are processed and maintained by several proteins in the *recF* pathway prior to the recovery. The processing involves a transient reversal of the replication fork that has been postulated to allow repair enzymes or translesion DNA polymerases to gain access to the blocking lesion and effect repair. The overall objective of this project is to determine how replication faithfully duplicates the genomic template in the presence of DNA damage. To achieve this objective, two specific aims are proposed. 1) Using two, previously established assays to characterize the recovery that occurs at DNA damage-blocked replication forks in vivo, we will identify the genetic requirements and conditions that determine when repair or translesion synthesis occurs at arrested replication forks to restore replication. 2.) We will identify the genes and mechanisms that operate on lesions in gapped substrates when replication fails to arrest. Through an understanding of how faithful replication resumes when it is blocked by DNA damage, we hope to identify the conditions and events that can lead to mutagenesis, genomic rearrangements, and lethality in the presence of DNA damage.

In addition, these experiments will be accomplished through a process that includes modernizing the Microbiology program at Mississippi State University by 1.) strengthening the undergraduate curriculum, 2.) creating a new molecular genetics laboratory for undergraduates, and 3.) increasing student participation in research. Mississippi State University is part of an EPSCoR state that has a significant minority population. The presence of quality research programs in this region are critical because they reach a large population of potential young scientists who may otherwise not be exposed to the career opportunities that science offers. This particular research proposal focuses on DNA replication, a topic that all students learn in high school and they generally find to be scientifically approachable. Other students are drawn into this research because of its medical relevance to both cancer and aging. These factors, taken together, make this an ideal project for undergraduate and graduate students to learn and develop as scientists, while at the same time, making significant contributions to the field through an exciting and technically manageable research experience. Support of this project will allow us to significantly expand the on the number of potential young scientists we reach and increase the quality of the program and science that students in this region are exposed to.

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For font size and page formatting specifications, see GPG section II.C.

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Project Description (Including Results from Prior NSF Support) (not to exceed 15 pages) **(Exceed only if allowed by a specific program announcement/solicitation or if approved in advance by the appropriate NSF Assistant Director or designee)**	15	
References Cited	7	
Biographical Sketches (Not to exceed 2 pages each)	4	
Budget (Plus up to 3 pages of budget justification)	8	
Current and Pending Support	2	
Facilities, Equipment and Other Resources	0	
Special Information/Supplementary Documentation	1	
Appendix (List below.) **(Include only if allowed by a specific program announcement/ solicitation or if approved in advance by the appropriate NSF Assistant Director or designee)**	3	

Appendix Items:

*Proposers may select any numbering mechanism for the proposal. The entire proposal however, must be paginated. Complete both columns only if the proposal is numbered consecutively.

RESEARCH OBJECTIVES

All cells must faithfully replicate their genomes in order to reproduce. If not repaired, damage encountered during replication can lead to a loss of genomic stability, mutations, or cell death. Despite the widely accepted view that most genomic instability and mutagenesis results from the replication of damaged DNA, the cellular mechanism(s) by which replication processes lesions during replication remains largely uncharacterized.

Recent studies indicate that two predominant substrates are produced when replication encounters DNA lesions. Lesions in the leading strand template result in an arrested replication fork, whereas lesion in the lagging strand template produce gaps in the nascent DNA but do not arrest the overall progression of replication (43, 71, 77). In previous funding from NSF, we focused upon identifying the gene products and structural intermediates that are associated with restoring replication forks arrested by UV-induced DNA damage in *E. coli*. Through these studies, we showed that:

1.) replication forks blocked by DNA damage are maintained and processed by several proteins in the RecF pathway until replication can resume,
2.) a transient regression of the blocked replication fork occurs prior to recovery that we speculate may render the offending lesion accessible to repair enzymes, and
3.) either nucleotide excision repair or translesion synthesis is required for, and capable of, allowing replication to resume following UV-induced arrest.

In this renewal, we propose to extend on these results to identify the cellular conditions that lead to mutagenesis during the recovery of arrested forks and identify the mechanisms by which the subset of lesions that do not arrest replication are processed and repaired. To achieve these goals, the following specific aims are proposed:

A. *To identify the genetic requirements and conditions that determine whether nucleotide excision repair or translesion DNA synthesis occurs at lesions that arrest DNA replication.*

Both nucleotide excision repair and translesion synthesis have been proposed to be the predominant mechanism that restores replication following arrest (19, 36, 57, 58, 75, 81, 96). However, little is known about their actual relative contributions or the conditions that determine when each process is used. Therefore, we will:

1.) Determine the relative contribution that each process has in restoring replication by quantifying the recovery of DNA synthesis, the nascent DNA processing, and the structural intermediates that occur at blocked replication forks in nucleotide excision repair and damage-inducible polymerase mutants.
2.) Identify the processing events that determine whether repair or translesion synthesis occurs at blocked replication forks by further characterizing the recovery in nucleotide excision repair or damage-inducible polymerase deficient strains with mutations in candidate processing genes.

B. *To identify the genetic requirements and mechanisms that operate on lesions that do not arrest the progression of the replication fork.*

Following UV-induced damage in vivo, nascent strand gaps are generated by non-arresting DNA lesions (33, 87). The repair mechanisms operating on these lesions remain poorly defined. Therefore we will

1.) Identify genes that process and repair lesions in nascent strand gaps using alkali sucrose gradient analysis and 2-dimensional agarose gel analysis in candidate mutants.
2.) Utilize plasmids containing site-specific lesions in either the leading or lagging strand template to identify the structures, enzymes, and mechanisms that are specifically associated with the repair and restoration of the template in each strand.

BACKGROUND AND SIGNIFICANCE

The failure to accurately replicate the genomic template in the presence of DNA damage, whether spontaneous or induced, is thought to produce most of the genetic instability and mutagenesis observed in cells of all types. DNA damage encountered during replication produces genomic rearrangements when it resumes from the wrong place, mutagenesis when the incorrect base is incorporated opposite to the lesion, or even cell death when the block to replication cannot be overcome. Several cancer prone genetic disorders clearly demonstrate the severe consequences that occur when replication inappropriately processes damaged templates. Cells from patients with classical xeroderma pigmentosum (XP) exhibit high frequencies of chromosomal rearrangements, mutagenesis, and lethality due to an inability to repair DNA lesions that block replication, rendering patients extremely sensitivite to UV and prone to skin cancers (11, 12, 24, 102). These same phenotypes are also observed in the variant form of xeroderma pigmentosum (XPV) but are instead produced specifically by the loss of a polymerase that replicates through blocking DNA lesions (15, 39, 56, 64, 98). Other genetic disorders, such as Bloom's syndrome and Werner's syndrome, are characterized by cancer predisposition and premature aging. Cells from these patients lack a RecQ-like DNA helicase that results in abnormal replication patterns and high rates of chromosomal exchanges (27, 28, 31, 35, 37, 42, 51, 54, 55, 60, 63, 94, 115). In *E.coli*, RecQ processes the nascent DNA at DNA damage-blocked replication forks prior to their resumption and is needed to suppress illegitimate recombination (18, 20, 41). These genetic disorders clearly indicate that inaccurate replication in the presence of DNA damage is likely to significantly contribute to the incidence of cancer and aging in humans.

Considering the severe consequences that result from the improper processing of damaged DNA, the molecular events that normally allow replication to accurately duplicate damaged genomic templates have been intensely studied over the years. This has resulted in the identification of a large number of candidate genes in both prokaryotes and eukaryotes which, when mutated, are known to impair the accuracy of replication in the presence of DNA damage. *A remaining challenge has been to determine the precise roles that these gene products play in the recovery process.* The mechanism of DNA replication and its associated proteins, like RecQ, are highly conserved among evolutionary diverged organisms, making *E. coli* an extremely valuable and appropriate system for dissecting the mechanism by which replication recovers from DNA damage.

Two predominant substrates are generated when replication encounters DNA Damage
Irradiation of cells with 254-nm UV light induces lesions that block DNA polymerases, generating two predominant substrates when encountered during the replication of duplex DNA (6, 7, 43, 72, 91, 98, 103, 104). Lesions in the leading-strand template appear to impede the overall progression of the replication fork with the arrest of the nascent lagging-strand a short distance beyond the arrested leading-strand (30, 43, 71, 77, 99, 104). By contrast, discontinuous synthesis of the lagging strand allows replication to continue through lesions on this template, generating gaps in the nascent DNA strand opposite to the lesions Fuchs and Hanawalt, 1970, J Mol Biol, 52, 301-22; Higuchi et al., 2003, Genes Cells, 8, 437-49; Pages and Fuchs, 2003, Science, 300, 1300-3; McInerney and O'Donnell, 2004, J Biol Chem, 279, 21543-51 (**Fig1**). Events that are consistent with this can be seen on the chromosome of UV-irradiated *E. coli*. Following a moderate dose of UV-irradiation, the rate of DNA synthesis is transiently inhibited before it efficiently recovers at a time that correlates with lesion removal (14, 17, 18, 92). During this period of inhibition, some

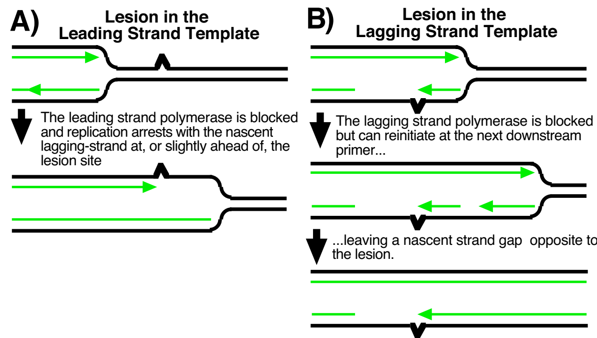

Fig 1. Substrates generated by lesions during replication.

limited DNA is still synthesized that contains gaps, consistent with replication continuing past a subset of the lesions present in the template (32, 33, 85, 87). The different substrates generated by lesions in the leading and lagging strand templates implies that unique repair mechanism may be required to repair each case. It also implies that lesions in the leading or lagging strand may carry different biological consequences for the cell with respect to lethality and mutagenesis, making it important to identify the repair pathways associated each substrate in order to understand the events that lead the normally accurate process of replication to become less than perfect.

Dealing with a Block: Maintaining the Arrested Fork Until Repair or Translesion synthesis Can Occur

Several proteins associated with the *recF* pathway, including RecA, RecF, RecO, and RecR, are required to restore replication following arrest by UV-induced damage (9, 18, 20, 22, 45). In the absence of any of these genes, UV-irradiated cells fail to recover DNA synthesis following arrest and the nascent DNA at the blocked replication fork is extensively degraded (9, 18, 20, 22, 45, 46). *In vitro*, RecA, RecF, RecO, and RecR promote pairing between single-strand DNA and homologous duplex DNA (4, 5, 50, 73, 84, 93, 111-113), an activity that was originally characterized for its role in bringing together homologous strands of DNA during recombinational processes (10). During replication, cellular assays indicate that this same enzymatic activity is also required to maintain and process the homologous strands of the replication fork when replication is prevented from proceeding normally (18-22). Other *recF* pathway proteins, RecQ, a 3'-5' DNA helicase, and RecJ, a 5'-3' single strand exonuclease, selectively degrade the nascent lagging strand at blocked replication forks prior to the resumption of DNA synthesis (18, 20). Degradation by RecJ and RecQ facilitates the timely recovery of DNA synthesis in normal cells and is thought to play a role in suppressing the frequency with which illegitimate recombination occurs, perhaps by generating a more extensive substrate for RecA to bind and stabilize at the blocked replication fork (18, 20, 41). Consistent with this interpretation, homologs of RecQ in eukaryotes have been shown to play critical roles in maintaining processive replication and suppressing the frequency of DNA strand exchanges (23, 27, 34, 37, 74, 109, 110, 115). These observations have led to our present working model in which RecA and these *recF* pathway gene products act to maintain and process the replication fork so that repair enzymes or alternative DNA polymerases can gain access to the offending lesion following arrest (**Fig 2**). In this way, processive replication would be maintained while avoiding strand exchanges that may lead to recombination or rearrangements. In addition, several other candidate genes have been speculated to process the arrested replication forks based upon their hypersensitivity to DNA damage when mutated. However, several of these genes have either not been directly examined or have been shown not to affect the recovery of replication in wild type cells.

Two mechanisms that operate to reduce the frequency of recombination and promote cell survival following DNA damage are nucleotide excision repair and translesion DNA synthesis (1, 17, 18, 52, 88)}. Both processes have been proposed to be the predominant mechanism that operates to restore replication following arrest (19, 36, 57, 58, 75, 81, 96). In *E. coli,*

the *uvrA*, *uvrB*, and *uvrC* gene products form an exinuclease that is required to initiate nucleotide excision repair of UV-induced lesions (89). Cells deficient in lesion removal are severely impaired in their ability to resume robust DNA replication and exhibit elevated levels of recombination, genomic rearrangements, and cell lethality (17, 18, 47, 88, 91). In wild-type cells, the time at which robust replication resumes correlates with the removal of the lesions by nucleotide excision repair (14, 17, 18). These observations have been interpreted to support the idea that nucleotide excision repair is the prominent mechanism that operates at replication-arresting DNA lesions (17-19, 21). However, since excision repair is required to remove all lesions throughout the genome, it remains possible that an alternative process, such as translesion DNA synthesis, predominantly operates at lesion-arrested replication forks, and that the failure to observe robust replication resume in *uvr* mutants occurs due to the re-arrest of replication at downstream lesions.

The *E. coli* genome also encodes three damage-inducible DNA polymerases that are members of a DNA polymerase superfamily found in both prokaryotes and eukaryotes (96). *In vitro*, Pol II (*polB*), Pol IV (*dinB*) and Pol V (*umuDC*) are able to incorporate bases opposite to specific lesions in template DNA with higher efficiency than the replicative polymerase, Pol III (1, 3, 8, 49, 52, 53, 76, 79, 101, 106). Both Pol II and Pol V have been reported to contribute to the recovery of DNA synthesis following UV irradiation (80, 81, 97). In addition, Pol V modestly increases the survival in UV-irradiated *E. coli* (52, 95). These observations have been interpreted to support the idea that translesion synthesis is a predominant mechanism that operates to restore DNA synthesis at lesion-arrested replication forks (36, 58, 81, 100).

Whether nucleotide excision repair or translesion DNA synthesis occurs at an arrested fork carries significant implications with respect to genome stability and mutagenesis. Whereas lesion removal by nucleotide excision repair is generally considered to be error-free, translesion DNA synthesis is responsible for majority of the mutagenesis that results from UV-induced DNA damage (1, 52, 75, 105). Therefore, in order to understand the events that lead to mutagenesis in the presence of DNA damage, it is critical to identify the contributions that nucleotide excision repair and the translesion DNA polymerases have in restoring replication forks arrested by DNA damage.

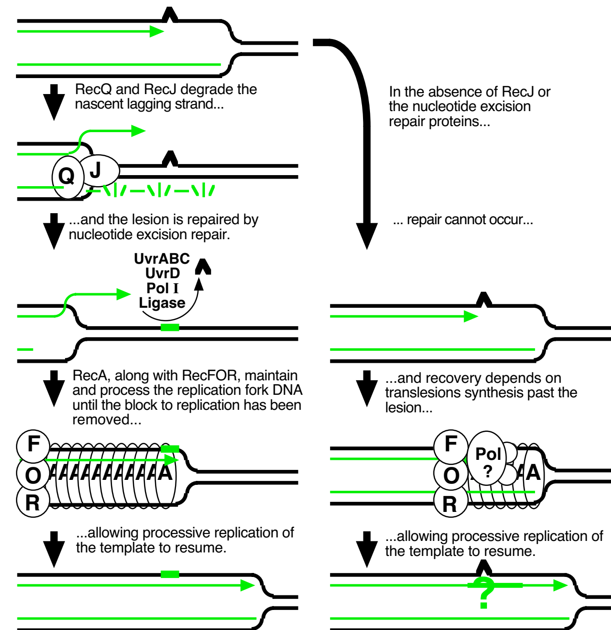

Fig 2. Our present working model for the recovery of replication forks blocked by DNA damage.

Dealing with Lesions and Gaps: The more studied, less understood situation.

Although the experiments demonstrating that replication can skip over a subset of DNA lesions (those in the lagging strand template) is very recent (43, 71, 77), the observation that the DNA synthesized immediately following UV irradiation contains gaps is among the earliest characterizations to considered the problem of replication in the presence of damage (10, 47, 48, 87). This observation led to the proposal that replication may skip over all DNA lesions, leaving gaps that could be filled in at later times through recombinational

strand exchanges (10, 47, 48, 87). A large number of studies went on to characterize the nascent strand gaps that appears after UV irradiation and demonstrated that many of the RecF pathway genes that maintain arrested replication forks are also required to process the nascent strand gaps (33, 45, 61, 85-87, 107, 108). However, because the mechanism of processing was initially presumed to operate independently from nucleotide excision repair, the vast majority of these studies were done in nucleotide excision repair mutants, in which the recovery of replication is severely impaired, survival is greatly reduced, and strand exchanges are greatly elevated. Thus, in order to understand how wild type cells faithfully process DNA lesions during replication, it will be extremely informative at this point in time to characterize this process in wild type backgrounds, at doses where the majority of cells recover and survive.

While a strictly recombinational mode of repair remains possible, an alternative mechanism may involve homologous-strand pairing to allow repair of the lesions to occur. Consistent with his possibility is the kinetics of nascent strand gap repair are much more rapid in excision proficient strains (16). A third possibility to consider is that the translesion DNA polymerases may primarily operate to fill in nascent gaps generated by nonarresting DNA lesions. This possibility would be consistent with in vitro observations that the ideal substrates utilized for translesion synthesis closely mimic those expected to occur on the nascent strand gaps in vivo (59, 78, 82, 101).

Thus, in order to understand the molecular events that can lead the normally faithful duplication of the genome to become less than perfect, *it is appropriate to utilize a model system, such as E.coli, in which the role of specific gene products and substrates can be directly examined in vivo, to critically test these different hypothesis, and determine how genomic stability is maintained in the presence of DNA damage.*

RESULTS FROM PRIOR NSF SUPPORT

a.) MCB0130486, $416,377 (4/1/02 - 3/31/05)

b.) Title: Recovery of Replication Following UV-induced DNA Damage

c.) We had previously shown that *recA*, *recF*, and *recR* are required to maintain replication forks blocked at DNA lesions and that *recJ* and *recQ* are required to process the nascent lagging strand DNA prior to recovery. These observations led us to propose that these *rec* gene products function to maintain the strands of the replication fork until repair enzymes can gain access to the lesion and effect repair (17, 19-22). The research impacted the thinking in this field because it implied that several genes which affect recombination during sexual processes, may actually have *nonrecombinational* roles (or help prevent strand exchanges from occurring) during the asexual cell cycle.

In previous funding, we proposed to identify the gene products and structural intermediates that are associated with the recovery of replication forks blocked by DNA damage.

RecO acts with RecF and RecR to maintain replication forks after UV-irradiation

Kin-Hoe Chow, a graduate student, characterized the role of *recO* during the recovery of replication following arrest. Several of RecO's genetic and biochemical phenotypes suggested that it may be epistatic with RecF and RecR. Kin-Hoe demonstrated that RecO functions together with RecF and RecR to protect and maintain replication forks that are blocked by DNA lesions (**Fig 3**). Previous studies demonstrated that RecF protects the nascent lagging strand from degradation. In the absence of all three proteins, RecF, RecO, and RecR, the extent of degradation is identical to that which occurs in the absence of any single protein, indicating that all 3 proteins act together to protect the same strand (rather than opposing strands) of blocked replication forks from degradation by RecQ-RecJ helicase-nuclease. This work was published in the *J. Biological Chemistry* (9).

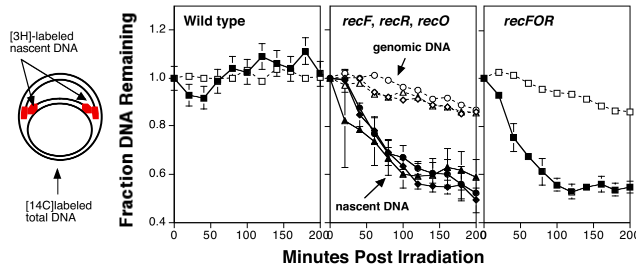

Fig 3. RecO acts together with RecF and RecR to protect replication fork structures from degradation by the RecQ-RecJ, helicase-nuclease. [3H]thymidine was added to [14C]thymine-prelabeled cells for 10 seconds immediately before the cells were filtered and irradiated in nonlabeled medium. The fraction of the radioactivity remaining in the DNA following irradiation is plotted over time. The loss of [14C]-genomic DNA (open symbols) can be compared to the loss of [3H]-DNA synthesized at the growing fork just prior to irradiation (filled symbols) for the parental strain, *recF*, *recO*, *recR*, and the triple mutant, *recFOR*.

UV-Arrested replication forks undergo a transient reversal prior to the resumption of DNA synthesis

Based upon our model, we had speculated that the repair of the blocking lesion at the replication fork may require that the polymerase and nascent DNA be transiently removed so that repair enzymes could gain access to the offending lesion and effect repair. To test this concept, we developed a technique to identify the structural characteristics of replicating plasmid molecules following DNA damage, based on 2D agarose gel analysis. Using this technique, we observed that a transient regression of the replication fork occurs on plasmid molecules following UV-induced DNA damage. The resolution of the regressed fork structure correlated with the removal of the lesions by the excision repair proteins and the recovery of replication (**Fig 4**). Consistent with our previous work, the regressed replication fork intermediate required RecA, RecF, RecO and RecR to be maintained and was processed by RecJ and RecQ. This work was published in *Science* (18).

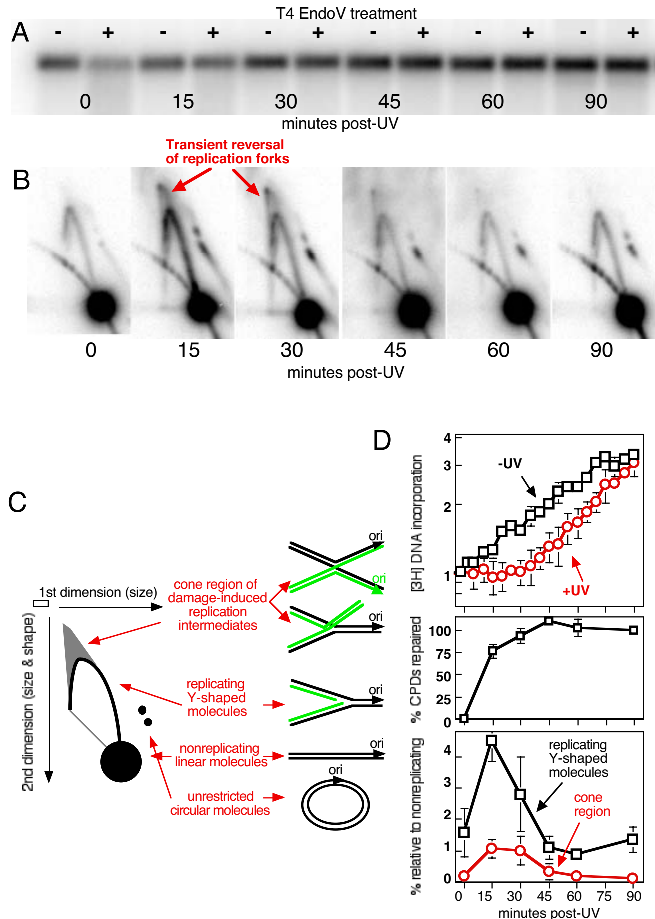

Fig 4. A transient reversal of the replication fork occurs following arrest by UV-induced DNA damage. A) UV-induced lesions are repaired from the plasmid within 30 minutes following UV irradiation. Cells containing the plasmid pBR322 were UV irradiated with 50J/m2 and genomic DNA was purified, digested with PvuII, and analyzed at the times indicated to measure the rate that the predominant UV-induced lesion, the cyclobutane pyrimidine dimer (CPD), was repaired. Lesion removal was determined by fragment sensitivity to T4 endonuclease V (TEV) which cleaves DNA containing CPDs. B) Blocked replication forks and regressed fork intermediates transiently accumulate following UV irradiation. Cells containing the plasmid pBR322 were UV irradiated with 50J/m2 and genomic DNA was purified, digested with PvuII, and analyzed by 2D agarose gels at the times indicate. C) Diagram of the migration pattern of PvuII digested pBR322 during 2D analysis. Nonreplicating plasmids run as a linear 4.4 kb fragment. Normal replicating fragments form Y-shaped structures and migrate more slowly due to their larger size and nonlinear shape, forming an arc that extends out from the linear fragment. Double Y, or X-shaped molecules migrate in the cone region. D) The replication intermediates persist until a time correlating with replication recovery and lesion removal. Replication recovery, lesion repair, and the relative amount of replicating fragments (squares) and cone region intermediates (circles) are plotted. Replication recovery was assayed by [3H]thymine incorporation for UV irradiated (circles) or mock-irradiated (squares) cultures.

Branch migration by RuvAB or RecG is NOT required to restore replication. Several groups, including ours, had speculated that enzymes may be required to catalyze the reversal of blocked replication forks in order for recovery to occur. Based on the ability of RuvAB and RecG to catalyze branch migration on synthetic replication-like structures in vitro (44, 114), it had been frequently reported in the literature that these enzymes are essential to catalyze the reversal of lesion-arrested replication forks in vivo (2, 25, 26, 38, 40, 62, 65-70, 90). However this had not yet been experimentally examined. Janet Donalson, a graduate student, constructed *ruvAB* and *recG* mutants found that arrested replication forks recovery and process the nascent DNA normally following UV-induced DNA damage. This work was published in *Genetics* (25).

The replication machinery is partially maintained at the site of blockage. Upon encountering a lesion that blocks replication, it is unknown whether the replication machinery disassembles or remains associated with with the DNA until the lesion can be repaired. To begin to address this question, Jerilyn Belle, a graduate student, compared replication forks that had been disrupted by UV to those disrupted by the inactivation of specific replication proteins. Using a thermosensitive mutant of the replication helicase, *dnaB*, Unlike the case with UV, Jerilyn observed that following disruption of the replicative helicase, large amounts of single stranded DNA began to accumulate at the replication fork which was accompanied by extensive nascent degradation that occurred irrespective of whether RecF was present or not. These differences suggest that the replication machinery is at least partially maintained following arrest at UV-induced lesions and does not simply dissociate from the DNA. Jerilyn has gone on to characterize the nucleases that degrade the disrupted replication forks identify the structural intermediates by electron microscopy. *This work is currently being prepared for publication.*

RecJ and RecQ initiate degradation on different substrates than RecBCD following UV irradiation. RecJ RecQ and RecBCD contain helicase and nuclease activities that have been proposed to operate on arrested replication forks. Kin-Hoe Chow, a graduate student, characterized the sites that were accessible to these enzymes following UV irradiation. He found that while the degradation mediated by RecJ RecQ initiated at and was limited to the nascent DNA at the arrested fork. By contrast, RecBCD could not degrade the nascent DNA. However, it would initiate at other sites in the genome, and in the absence of protection by RecA, could go on to degrade the entire genome. *This work is being submitted to Nucleic Acid Research.*

*Other results from this period are presented in the PLAN OF ATTACK section because they relate directly to proposed future experiments.

d.) Publications resulting from support thus far

∞ **Courcelle J**; Belle JJ; Courcelle CT. When replication travels on damaged templates: bumps and blocks in the road. ***Res in Microbiol*** (2004) 155 231–237**.**

∞ Donaldson JR; Courcelle CT; **Courcelle J**. RecG or RuvAB is not required for the resumption of replication following UV irradiation *Escherichia coli*. ***Genetics*** (2004) 166:1631-40**.**

∞ Chow KH; **Courcelle J**. RecO functions together with RecF and RecR to stabilize DNA damage-blocked replication forks following UV irradiation in *Escherichia coli*. ***J Biol Chem*** (2004) 279:3492-6**.**

∞ **Courcelle J**, Hanawalt, PC. RecA-dependent repair of arrested replication forks. ***Ann Rev Genet*** (2003) 37: 611-46**.**

∞ **Courcelle J,** Donaldson JR, Chow KH, Courcelle CT. UV-induced replication fork regression and processing in *Escherichia coli*. ***Science***, (2003) 299:1064-7.

(*highlight*) LeBrasseur, N. Rec'd and repaired. ***J Cell Biol*** (2003) 106:464-5

∞ Crowley DJ, **Courcelle J**. Answering the Call: Coping with DNA Damage at the Most Inopportune Time. ***J Biomed Biotech*** (2002) 2: 66-74.

e.) samples/physical collections: not applicable
f.) relation of completed work to this proposal. This renewal proposes experiments that directly extend upon results from previous funding.

RESEACH PLAN OF ATTACK

A1.) To *determine the relative contribution that nucleotide excision repair and the translesion DNA polymerases have in restoring replication by quantifying the recovery of DNA synthesis, the nascent DNA processing, and the structural intermediates that occur at blocked replication forks in nucleotide excision repair and translesion polymerase mutants.*

When replication encounters lesions in the leading strand template, replication transiently arrests, but then resumes at a time correlating with the removal of the lesions by nucleotide excision repair. Based on our working hypothesis, UV-induced lesions that block replication are normally removed by the nucleotide excision repair proteins before replication resumes. The most likely alternatives to this hypothesis are that translesion DNA polymerases synthesize past the blocking lesions. To answer this question we will utilize a combination of two assays to gain an overall picture of the events occurring at arrested forks.

Measuring the recovery of DNA synthesis.

This assay utilizes a 2 minute pulse of [3H]thymidine added to aliquots of irradiated cultures at repeated intervals throughout the recovery period to determine the *rate* that DNA synthesis is occurring (see **Fig 5A)**. We have spent considerable time and effort into refining this procedure to generate recovery curves that are sensitive to variation and highly reproducible. For these reasons, the assay contains several internal controls that include a second label, [14C]thymine, to simultaneously follow DNA accumulation, duplicate samples for each time point, a mock irradiated control, and averages of at least three independent experiments for each recovery curve. The resulting assay is able to detect and differentiate between mutants in which the recovery of DNA synthesis is delayed from those in which synthesis recovers in a timely fashion, but with a reduced rate.

Measuring the nascent DNA processing that occurs at the arrested fork. This assay utilizes a 5 second pulse of [3H]thymidine that is added to [14C]thymine prelabeled cultures just before the cells are filtered and irradiated in nonradioactive medium (see **Fig 5B)**. The [14C] prelabel allows us to compare the degradation occurring in the overall genome to that occurring specifically in the [3H] nascent DNA at the arrested replication forks. In previous studies, we have utilized his assay to show that the nascent DNA is subject to degradation by the combined action of the RecJ nuclease and RecQ helicase following arrest (20). In wild-type cells, the degradation ceases after approximately 20 minutes when replication resumes and is limited to less than 20% of the nascent DNA (**Fig5B**). (The increase in ^{3}H-labeled DNA after 60 min occurs due to the re-incorporation of the remaining intracellular pools of [^{3}H]thymidine when replication resumes). However, in mutants such as *recF*, which fail to resume DNA synthesis, the nascent DNA degradation continues and is much more extensive (13, 17, 20). This simple assay is able to identify several phenotypic abnormalities in the recovery process, including mutants which do not process the nascent DNA (20), mutants which fail to protect the nascent strands of the DNA (17), and mutants which fail to maintain the replication fork entirely (22).

Using these two assays in combination, we have now shown that nucleotide excision repair (*uvrA*), but not the translesion DNA polymerases (*umuCdinBpolB*), are required to restore DNA synthesis following UV-irradiation. **(Fig 5)**. The observation that replication resumes with nearly wild type kinetics in the absence of the three damage-inducible polymerases, argues strongly that they are not the predominant mechanism by which arrested replication forks recover. However, it does not exclude the possibility that translesion synthesis can occur at these sites in *uvrA* mutants, but that its contribution

remains below the level of detection due to the persistence of the lesions. Consistent with this possibility, we observe that the presence of these polymerases prevent the nascent DNA at the arrested replication fork from being extensively degraded in the absence of repair **(Fig 5B)**. *The observation strongly supports the idea that translesion DNA synthesis by at least one of the polymerases can act as an alternative at the blocking DNA lesion when repair does not occur*.

It will now be important to examine each polymerase, Pol II (*polB*), Pol IV (*dinB*), and Pol V (*umuDC*), individually and in combination to identify the polymerase(s) are able to act at replication forks blocked by UV-induced lesions. If the absence of any single polymerase (or two polymerases) mimics the phenotype observed in the triple polymerase mutant, then it will indicate that this polymerase is the polymerase that is active after UV-induced damage and argue against a functional redundancy in vivo. The question is significant because the three polymerases do not have equivalent fidelities. In vivo, Pol V is responsible for ALL of the mutagenesis induced by UV (1, 52, 105). (While space limits this discussion, a previous study has reported an essential role for Pol II in restoring replication after UV (80), which appears to be inconsistent with our (and other labs') present observations. We maintain very productive communications with the corresponding author of this work and are in agreement that a thorough study of all the polymerases, using multiple assays, as described above, is now useful so that further experiments can focus upon the correct factors as the mechanism by which this critical process occurs is elucidated.)

In addition, these assays will allow us to determine which the nucleotide excision repair genes are required for repair to occur at the replication fork. In humans, it is well established that the repair of lesions that arrest transcription do not require, XP-C, a helicase subunit of the incision complex, presumably

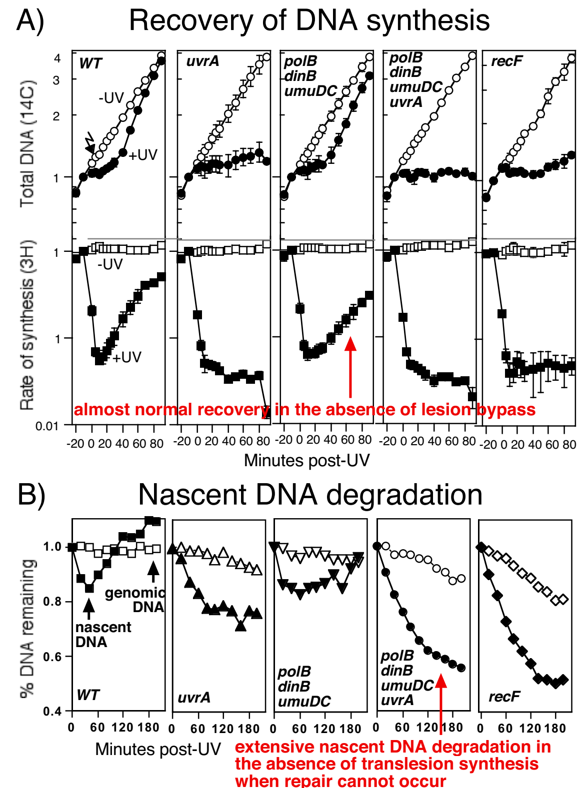

Fig 5. Either nucleotide excision repair or translesion DNA polymerases can act at the arrested replication fork. A) *Replication recovers in umuCdinBpolB mutants, but not uvrA mutants, with nearly normal kinetics*. [^{3}H]thymidine is added to [^{14}C]thymine-prelabeled cultures for 2 min at the indicated times following either UV irradiation with 27 J/m^2 (filled symbols) or mock-irradiation (open symbols). The amount of total ^{14}C-DNA (circles) and rate of ^{3}H-DNA synthesized/2min (squares), as measured by TCA precipitation, is plotted. Graphs represent an average of at least three independent experiments. Error bars represent one standard deviation. B) *The nascent DNA remains protected from extensive degradation unless both nucleotide excision repair and translesion synthesis are absent*. A 5 s pulse of [3H]thymidine is added to [14C]thymine prelabeled cells immediately before the cells are filtered and irradiated with 25 J/m2 in nonlabeled medium. The fraction of the radioactivity remaining in the DNA, as measured by TCA precipitation, is plotted against time. The loss (or degradation) of 14C genomic DNA (open symbols) can be compared to the loss of the 3H DNA synthesized at the growing fork just prior to irradiation (closed symbols). Graphs represent an average of at least three independent experiments. Error bars represent one standard deviation.

because the lesion is exposed or unwound bythe transcription machinery. Similarly, in *E. coli*, not all subunits of the incision complex may be required for incising lesions at replication forks. Alternatively, the repair complex at replication forks may utilize

additional subunits. *E.coli*, encodes a second, functional *uvrC* homolog, termed Cho, which will be characterized for its role in recovery. If inactivation of any repair component results in extensive degradation of the nascent DNA in strains that also lack the translesion DNA polymerases, it would strongly suggest that this gene product is specifically required for the repair of lesions at the arrested replication fork as shown in **Fig 5B**.

While straightforward, the potential for mutagenesis when the wrong polymerase is utilized or repair cannot occur make it important to identify the relative contribution and genetic requirements that determine when either translesion synthesis or repair occur during the recovery process.

A2.) *To identify the processing events that determine whether repair or translesion synthesis occurs at blocked replication forks by further characterizing the recovery in nucleotide excision repair or damage-inducible polymerase deficient strains with mutations in candidate processing genes.*

Several models have speculated that blocked replication forks will require enzymes to displace the nascent DNA and the replication machinery from the blocking lesion before recovery can occur. The observed regressed intermediate would be consistent with this mechanism occurring (18). If true, what enzymes are needed to displace the nascent DNA and/or replication machinery? And are the processing events required for repair the asme as those required for translesion synthesis? Several candidate helicases and nucleases, which confer recombination deficiencies or hypersensitivity to DNA damage when mutated, have been speculated to perform such roles. However, few have been directly examined for their role in vivo.

We hypothesize that recovery, when it occurs by repair, will require unique enzymes and processing events than when translesion DNA synthesis occurs. Consistent with this hypothesis, we find that the nascent DNA

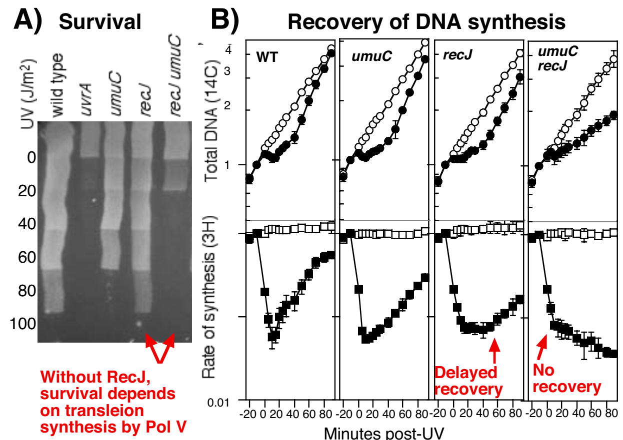

Fig 6. RecJ processing is required for the timely recovery of replication and avoids mutagenic bypass by Pol V. a) In the absence of the nascent strand processing by RecJ, survival after UV-induced damage depends upon lesion bypass by Pol V (*umuC*). B) The recovery of DNA synthesis was assayed as in Fig 5. RecJ processing is required for replication to recover in a timely fashion and depends upon lesion bypass by Pol V (*umuC*) in its absence.

processing (degradation) by RecJ is required for the normal recovery of replication to occur. In the absence of RecJ, the recovery of replication is delayed and cell survival and the recovery of replication become entirely dependent upon translesion synthesis by Pol V (**Fig 6**). *The data strongly suggest that the nascent lagging strand processing by RecJ is required for repair to occur at the arrested replication fork, but that it is not required for translesion synthesis by Pol V to occur* (**Fig 2**). It is possible that the RecJ degradation serves to move the junction of the replication fork backwards, restoring the lesion containing region to duplex DNA so that incision by repair enzymes can occur.

It will now be important to examine other candidate genes to identify those factors are required to allow either repair translesion DNA synthesis to occur. To this end, we will examine the recovery of candidate mutants in either repair deficient or polymerase deficient backgrounds to identify where they operate. As seen in *recJ*, cells deficient in translesion synthesis rely upon repair for recovery to occur. Thus inactivation of processing enzymes that are required for repairing lesions

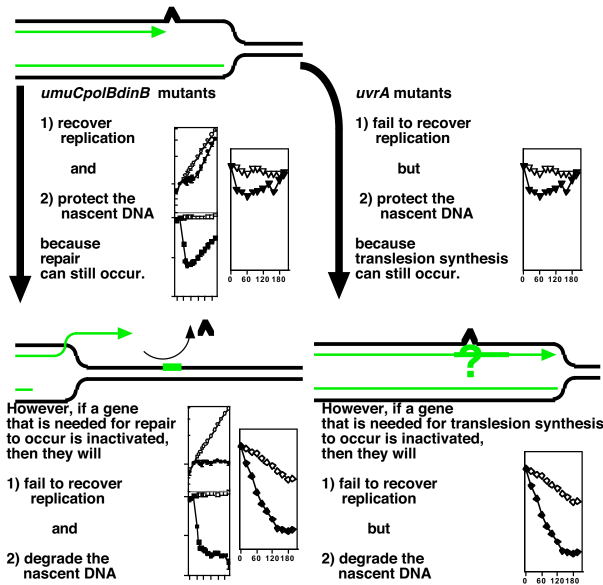

Fig 7. Detection of genes required for repair or translesion synthesis at arrested replication forks.

at the replication fork will render cells UV sensitive, result in a failure to recover and extensive nascent DNA degradation. Similarly, in repair deficient cells, translesion synthesis is able to protect the nascent DNA from degradation. However, mutations that prevent translesion synthesis from occurring would result in extensive nascent DNA degradation (**Fig 7**). Important candidates to examine in this role include: RecQ, a helicase that operates on the nascent DNA at arrested forks; the branch migration enzymes RuvAB and RecG, which are not required for recovery in a wild type cells but may participate in one or the other of these redundant pathways; RecBCD, a helicase and nuclease important for recombination; XonA, a nuclease that associates with RecA; and SbcDC, a nuclease and functional homolog of the eukaryotic Mre11-Rad50.

B1.) *To identify the gene products that are specifically required to process lesions that do not arrest DNA replication and identify where they act in the repair process.*

To answer this question, we will utilize a combination of two assays that allow us to identify and monitor the substrates that are specifically associated with lesions that do not arrest replication.

Measuring the repair of nascent strand gaps

This assay utilizes alkali sucrose gradient sedimentation to monitor the repair of the nascent strand gaps that are produced by lesions that do not block replication. [3H]thymidine is added to [14C]prelabeled culitres to label the DNA synthesized during the first two minutes after irradiation. Then at the indicated times, samples are run in an alkali sucrose gradient to determine the size of the nascent DNA fragments relative to the genomic DNA. As the gaps are repaired (joined) over time, the size of the nascent DNA fragments increase and are comparable to the overall genomic DNA. As mentioned in the BACKGROUND section, a large number of studies have examined this process in repair deficient backgrounds. However, in order to understand how these lesions are normally repaired, it will be extremely informative to characterize this process with respect to wild type cells, so that the contribution that repair, translesion synthesis, and other processing enzymes have in this process can be considered. In support of the view, we examined the gap repair that occurred in mutants lacking either the translesion DNA polymerases or RuvAB. Both mutants exhibit

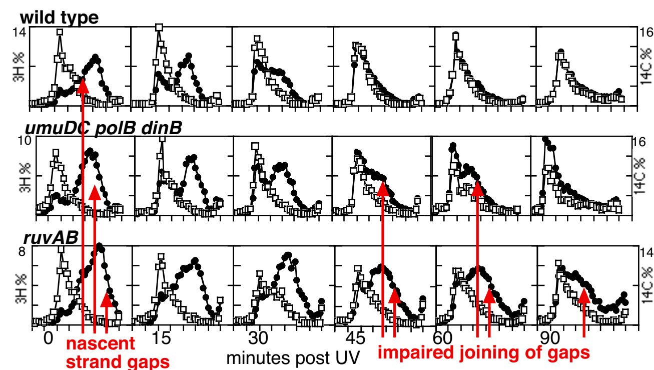

Fig 8. Translesion polyerases and RuvAB participate in the repair of nascent strand gaps generated by UV-induced damage. [3H]thymidine is added to [14C]thymine prelabeled cultures for the first 2 minutes following UV irradiation to label the post irradiation DNA synthesis. Then at the indicated times, samples are run in an alkali sucrose gradient to determine the relative size of the genomic and nascent DNA fragments. Wildtype cells "repair" the gapped nascent strands within 45 minutes after irradiation. By contrast, mutants lacking RuvAB or Pol II Pol IV Pol V exhibit a delayed joining of nascent strand gaps as compared to wild type cells.

a delayed joining of the nascent strand gaps when compared to wild type cells (**Fig 8**), despite the fact that these mutants recover replication following arrest. These results indicate that this assay will be useful in identifying mutants that are specifically impaired in the repair of nascent strand gaps.

Identifying structural intermediates induced by DNA damage. A second assay that proves useful in identifying intermediates associated with nonarresting lesions involves examining the replication on damaged DNA plasmids, such as pBR322, using 2-dimensional (2D) agarose gel analysis (18, 29). Briefly, cells containing the plasmid pBR322 are UV-irradiated and the genomic DNA is purified, digested with Pvu II, which cuts the plasmid just downstream of the unidirectional origin of replication, and analyzed by 2D agarose gels. In this technique, nonreplicating plasmids migrate as linear 4.4-kb fragments whereas replicating fragments form Y-shaped structures and migrate more slowly due to their larger size and nonlinear shape. These replicating fragments form an arc that extends out from the linear fragment (Fig4BC). Replication intermediates that arise as the result of the DNA damage and contain either gaps or branched molecules can then be resolved and identified based upon their unique size and/or shape. The utility of this procedure to identify various structures that accumulate and can be observed in different mutants is shown in **Fig 9**. Of particular interest, are intermediates observed in *ruvAB* mutants. We observed the accumulation of fully replicated branched intermediates that contain unresolved holliday junctions and gaps. The intermediate is distinct from the regressed replication fork intermediate that occurs following arrest and accumulates in *recFrecQ* mutants.

The structure of these replication intermediates will be confirmed by electron microscopy. With the assistance of Jack Griffith at UNC Chapel Hill, we have established the ability to purify

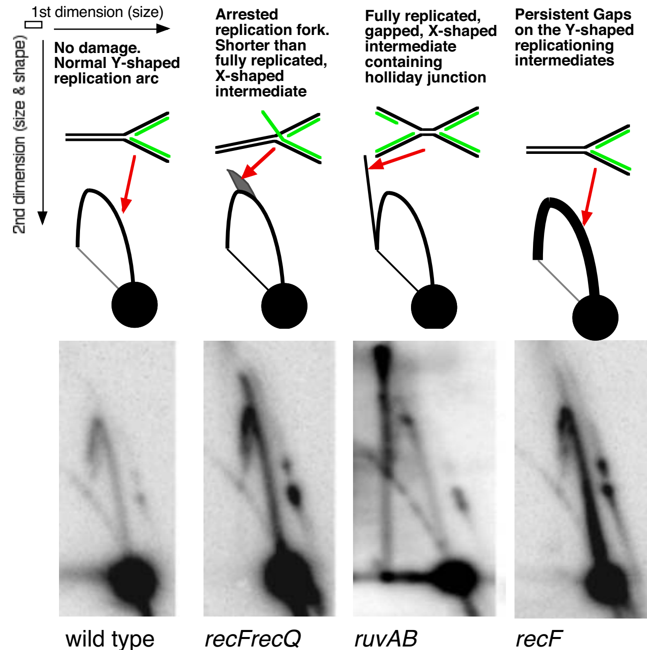

Fig 9. Identification of Replication intermediates that are associated specifically with the repair of leading or lagging strand lesions. The an arrested replication fork intermediate accumulates in *recFrecQ* mutants (*). By contrast, fully replicated molecules that contain holliday junctions and fail to resolve accumulate in *ruvAB*. Persistent gaps on replicating molecules in *recF* mutants are observed as an increase in the intensity of the Y-arc.

and examine DNA by transmission electron microscopy at Mississippi State University's microscope facility (**Fig 10**). Our preliminary experiments indicate that increasing our loading sample by a factor of ten is amply sufficient to visualize the X-shaped intermediates that occur in wild type cells, indicating that this will be a very useful in identifying structures from the 2D analysis.

These results, in combination, demonstrate that these two assays will allow us to identify the gene products that participate in processing lesions that fail to arrest the replication machinery and characterize where they act in the repair process.

Using these assays, we will examine the daughter strand gap repair and structural intermediates in each of the single and double polymerase mutants to determine which polymerases are operating following UV irradiation. In addition, we will also examine and compare the rate of daughter strand gap

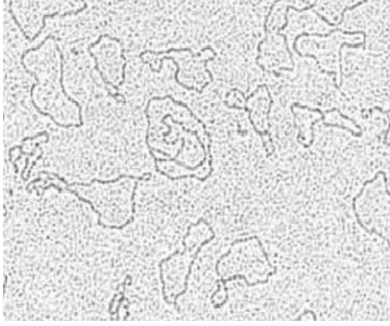

Fig 10. Gel-purified plasmid DNA prepared and examined at MSU's electron microscope facility by Janet Donaldson.

repair in *uvr* mutants to determine the relative role that it has in this process. The possibility that recombinational repair can occur independently of these processes will be addressed by examining mutants lacking both repair and translesion syntheisis. Candidate processing enzymes, as demonstrated by RuvAB, will also be characterized for their role in repairing this class of lesions.

B2.) To *utilize plasmids containing site-specific lesions in either the leading or lagging strand template to identify the structural intermediates, enzymes, and mechanisms that are specifically associated with the repair and restoration of the template in each strand.*

To complement and further extend the results obtained by the experiments described in the previous specific aims, we will also characterize the replication that occurs on plasmids containing a single CPD lesion in either the leading strand or lagging strand. While the previous methods are essential to identify the repair mechanisms operating on the chromosome of the cell, they suffer from the fact that large quantities of leading and lagging strand lesions are generated and processed simultaneously, making the intermediates and enzymes specifically associated with each type of lesion more difficult to identify.

Therefore, we have established a collaboration with Dr. Zafer Hatahet at UTHC at Tyler (see letter attached), whose lab frequently constructs several different site-specific lesions in DNA templates and plasmids. Dr. Hatahet is providing us with plasmids that contain a single CPD constructed into either the leading or lagging strand of the *lacZ* gene, inserted in opposite orientations of the puc-based vectors, pLac1 and pLac2.

We are transforming these plasmids into wild type, *uvr* mutants, and translesion DNA polymerase mutants to identify and confirm which processes are required to process leading strand and lagging strand DNA lesions. Successful replication of the lesion containing plasmids, as scored by transformation frequency, will be determined in each case. Transformation of a second, undamaged control plasmid, pMAL-p2, is included in all experiments to normalize for cell competency. A reduction in the transformation efficiency of plasmids with leading strand lesions relative to plasmids with lagging strand lesions in a given mutant will indicate that this gene is predominantly active in repairing leading strand lesions. Similarly, a reduced transformation efficiency of plasmids with lagging strand lesions, would indicate that this gene is active on lagging strand lesions.

In a second approach, these plasmids will be transformed into *E.coli* and recovered so that intermediates, specifically associated with the repair of leading or lagging strand lesions, can be identified by 2D gel analysis. A similar approach has recently been used to show that replication can bypass lagging strand lesions (77). In discussions with the authors of this work, the quantity of plasmid needed for transformation and detection should allow us to visualize replication intermediates by this technique. Intermediates observed in each case will be correlated with those observed in mutants from Aim B1. We are currently optimizing the conditions and timing of plasmid recovery to detect these intermediates.

These approaches, in combination, are expected to allow us to identify the cellular conditions that lead to mutagenesis during the recovery of arrested forks and identify the mechanisms by which the subset of lesions that do not arrest replication are processed and repaired.

EDUCATIONAL OBJECTIVES

The longterm objective is to strengthen the Microbiology program and the Biology Department at this institution such that undergraduates and graduates are able compete favorably with students nationally as they pursue careers. Three specific aims are proposed that will establish the foundation necessary for this to occur during the period of this proposal.

Aim 1. *To strengthen and update the undergraduate curriculum to reflect the practical and scientific skills that are essential for students in this field.*

Aim 2. *To establish a molecular biology course and a journal club course that provides students with current scientific techniques and teaches them how to identify and approach scientific questions.*

Aim 3. *To actively recruit students from this region to increase the number of students that participate and gain research experiences.*

Background and Significance

Mississippi State University is actively attempting to develop as a research institution to serve the state and region by strengthening its graduate programs, increasing federally funded research, and increasing the overall participation of underrepresented groups in research and education. Among its priorities are to raise our research ranking from 58th into the top 50 public research universities as reported by the NSF, to increase the total doctoral degrees awarded each year to more than 100, to increase the proportion of African-Americans in graduate programs from 6 percent to 10 percent, and to achieve a 20% overall African-American enrollment and increase the overall diversity represented at this institution. The university's five-year goals can be found at http://msuinfo.ur.msstate.edu/priorities/. Our president, dean, and faculty all realize the contribution and potential that the biology department can have in achieving these goals and they have committed to 1) renovating our facilities, hiring a new department head that has experience in building research and graduate programs (Dr. Nara Gavini), and the addition of four new faculty over the next two years.

While enthusiastic about our future directions, we currently suffer from low standards in our undergraduate curriculum, a lack of lab courses, and a low number of students who participate in research. I believe that the following three aims, as described, will have a substantial impact on improving these areas and establish a foundation to build a quality undergraduate and graduate program.

Plan of attach and methods

Aim 1. *To strengthen the curriculum.*

Our curriculum has not been significantly revised or updated in over 10 years. Based upon the subject material found in standardized tests and the ASM course recommendations (116), we, as a department, are initiating changes in the curriculum to provide the fundamental skills required to successfully compete in this field. We have already, or are in the process of addressing several primary changes, including:

a. Raising the Math requirement to include at least one semester of calculus for microbiology majors. Previously, algebra was sufficient.

b. Requiring a statistics course for biology majors.

c. Raising the minimum passing grade from a D to a C for majors in microbiology courses.

d. Restructuring the core Biology curriculum to eliminate redundancies and make room for new lab courses and capstone courses.

Aim 2. *To establish a new lab and journal club*

As a method to lower operating costs over past years, the number of lab courses offered to undergraduates has been drastically reduced. There is currently NO genetics or molecular course offered in our program that exposes students to basic techniques such as DNA purification, PCR, southern analysis, mutagenesis, or selection techniques. The majority of our seniors graduate without seeing or knowing what an agarose gel is.

To complement a microbial genetic course that I teach, I have developed a new lab course

that will be required for microbiology majors, and open to all biology majors. It has been approved by the academic board and will hopefully commence next fall given funding. The course involves lab exercises designed to expose the students to 1.) mutagenesis and selection, 2.) basic genetic techniques and strain constructions, and 3.) DNA purfication, cloning, southern analysis, and gene expression. A forth lab is designed to have students pursue a research question of their own. Lab reports are designed to teach students the scientific style of writing, experimental design, and scientific referencing. These are features not currently encountered in our department's degree program.

Interested students will be identified and encouraged to pursue independent research projects with faculty in our department. Although undergraduate research is presently offered in our course listing, a lack of lab courses and "advertising" produces few students who take advantage of this opportunity. Thus far, five undergraduates have tried small projects with our group and, of these, two have chosen to continue with graduate studies in our program and two have gone on to postgraduate nursing programs (one I just lost track of). New lab courses are expected to increase the number of students who choose to participate in research at this institution or apply to do so elsewhere.

The journal club is now listed as a course and open for students throughout the school. Since its initiation, in addition to Biology students, we have had students from Plant and Soil Sciences, Biological Engineering, and Biochemistry also participate. This type of exercise is critical for students in a small research setting, so they can discuss novel approaches, techniques, and results that may not be available or present at this institution.

Aim 3. *To increase the number of students that participate and gain research experiences.*

This aspect will be accomplished through several approaches. As mentioned above, as new lab courses are added, undergrduates will be encouraged to pursue independent projects, increasing the number of students in research.

To attract students from other regional universities, I have, and continue to actively seek out opportunities to visit regional institutions, meet with students, and discuss research programs at MSU. In the past two years, this involved visiting 13 colleges to give seminars and meet with students.

We are actively recruiting underrepresented minorities students to apply for summer research experiences with our group and department. This includes promoting our research to organization such as AGEM (Alliance for Graduate Education in Mississippi) whose purpose is to assist under-represented students in science and engineering in the transition to graduate studies. In addition, MFGN (the Mississippi Functional Genomics Network), funded by the NIH, serves to establish research links between Mississippi schools and promote undergraduate research (117). Both organizations have networks to assist students in finding compatible labs and offer funding for the initial period. Through these programs, one student has joined us for a summer research experience and is now pursuing graduate studies at MSU.

Our research has successfully attracted a diverse group of students from different social, educational, and ethnic backgrounds while establishing a productive research focus. DNA replication is a topic that all students learn in high school and they generally find to be scientifically approachable. Other students are drawn to our research because of its medical relevance to cancer and aging. These factors make this an ideal project for students to learn and develop as scientists, while at the same time, making significant contributions to the field. Support of this project will allow us to significantly expand the on the number of potential young scientists we reach and increase the quality of science that students in this region are exposed to.

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replication forks. Cell **95**:419-430.
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92. **Setlow, R. B., and W. L. Carrier**. 1963. The disappearance of thymine dimers from DNA: an error-correcting mechanism. Proc Natl Acad Sci U S A **51**:226-231.
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97. **Sutton, M. D., and G. C. Walker**. 2001. umuDC-mediated cold sensitivity is a manifestation of functions of the UmuD(2)C complex involved in a DNA damage checkpoint control. J Bacteriol **183**:1215-124..
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101. **Tang, M., X. Shen, E. G. Frank, M. O'Donnell, R. Woodgate, and M. F. Goodman**. 1999. UmuD'(2)C is an error-prone DNA polymerase, Escherichia coli pol V. Proc Natl Acad Sci U S A **96**:8919-824..
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108. **Wang, T. V., and K. C. Smith**. 1984. recF-dependent and recF recB-independent DNA gap-filling repair processes transfer dimer-containing parental strands to daughter strands in Escherichia coli K-12 uvrB. J Bacteriol **158**:727-729.
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110. **Watt, P. M., E. J. Louis, R. H. Borts, and I. D. Hickson**. 1995. Sgs1: a eukaryotic homolog of E. coli RecQ that interacts with topoisomerase II in vivo and is required for faithful chromosome segregation. Cell **81**:253-260.
111. **Webb, B. L., M. M. Cox, and R. B. Inman**. 1995. An interaction between the Escherichia coli RecF and RecR proteins dependent on ATP and double-stranded DNA. J Biol Chem **270**:31397-31404.
112. **Webb, B. L., M. M. Cox, and R. B. Inman**. 1997. Recombinational DNA repair: the RecF and RecR proteins limit the extension of RecA filaments beyond single-strand DNA gaps. Cell **91**:347-356.
113. **Webb, B. L., M. M. Cox, and R. B. Inman**. 1999. ATP hydrolysis and DNA binding by the Escherichia coli RecF protein. J Biol Chem **274**:15367-15374.
114. **Whitby, M. C., S. D. Vincent, and R. G. Lloyd**. 1994. Branch migration of Holliday junctions: identification of RecG protein as a junction specific DNA helicase. Embo J **13**:5220-5228.
115. **Yamagata, K., J. Kato, A. Shimamoto, M. Goto, Y. Furuichi, and H. Ikeda**. 1998. Bloom's and Werner's syndrome genes suppress hyperrecombination in yeast sgs1 mutant: implication for genomic instability in human diseases. Proc Natl Acad Sci U S A **95**:8733-8738.
116. ASM course recommendations. http://www.asm.org/education/index.asp?bid=10054.
117. Mississippi Functional Genomics Network. http://mfgn.usm.edu/mfgn/.

Biographical Sketch, Courcelle, Justin

a. Professional Preparation

University of Vermont; Burlington, VT	Biology	B.S., 1992
Stanford University; Stanford, CA	Cancer Biology	Ph.D., 1999
Institute Jaques Monod; Paris France	Mutagenesis	Post-Doc, 1999-2000

b. Appointments

Asst Professor, Dept. of Biol Science; Mississippi State University; 2001-present
Post Doctoral Research with Miroslav Radman; Longterm EMBO Fellow;1999-2000
Doctoral Research with Philip C. Hanawalt; Graduate Student Traineeship; 1994-99
Graduate Research with Michael R. Lieber, NSF Fellowship; 1992-94
Undergraduate Research with Susan S. Wallace; *HELiX* minigrant; 1990-92

c. Publications

i.) 5 Closely related publications

- Donaldson JR; Courcelle CT; **Courcelle J**. RecG or RuvAB is not required for the resumption of replication following UV irradiation *Escherichia coli*. ***Genetics*** (2004) 166:1631-40**.**
  http://www.genetics.org/cgi/content/full/166/4/1631
- Chow KH; **Courcelle J**. RecO functions together with RecF and RecR to stabilize DNA damage-blocked replication forks following UV irradiation *Escherichia coli*. ***Journal of Biological Chemistry*** (2004) 279:3492-6**.**
  http://www.jbc.org/cgi/content/full/279/5/3492
- **Courcelle J**, Hanawalt, PC. RecA-dependent repair of arrested replication forks. ***Annu Rev Genet*** (2003) 37: 611-46**.**
  http://arjournals.annualreviews.org/doi/abs/10.1146/annurev.genet.37.110801.142616?cookieSet=1
- **Courcelle J,** Donaldson JR, Chow KH, Courcelle CT. UV-induced replication fork regression and processing in *Escherichia coli*. ***Science***, (2003) 299:1064-7.
  http://www.sciencemag.org/cgi/content/full/299/5609/1064

  (*highlight*) LeBrasseur, N. Rec'd and repaired. ***Journal of Cell Biology*** (2003) 106:464-5
- Courcelle J., Carswell-Crumpton C., and Hanawalt P.C. *recF* and *recR* are required for the resumption of replication at DNA replication forks in *Escherichia coli*. ***Proceedings of the National Academy of Sciences of the United States of America*** (1997) 94:3714-9.
  http://www.pnas.org/cgi/content/full/94/8/3714

  (*commentary*) Kogoma T. Is RecF a DNA replication protein? ***Proceedings of the National Academy of Sciences of the United States of America*** (1997) 94:3483-4.

i.) 5 other related publications

- **Courcelle J**; Hanawalt PC. Participation of recombination proteins in rescue of arrested replication forks in UV-irradiated *Escherichia coli* need not involve recombination. ***Proceedings of the National Academy of Sciences of the United States of America*** (2001) 98: 8196-8202.
  http://www.pnas.org/cgi/content/full/98/15/8196

- **Courcelle J**; Ganesan AK; Hanawalt PC. Therefore, what are recombination proteins there for? ***BioEssays*** (2001) 23:463-470.
  http://www3.interscience.wiley.com/cgi-bin/abstract/80002935/ABSTRACT

  (*editorial*) Gene names: the approaching end of a century-long dilemma Wilkins A.S. ***BioEssays*** (2001) 23:377-378.

- **Courcelle J**; Khodursky A; Peter B; Brown PO; Hanawalt PC Comparative gene expression profiles following UV exposure in wild type and SOS deficient *Escherichia coli*. ***Genetics*** (2001) 158: 41-64.
  http://www.genetics.org/cgi/content/abstract/158/1/41
- **Courcelle J**; Hanawalt PC. RecQ and RecJ Process Blocked Replication Forks Prior to the Resumption of Replication in UV-Irradiated *Escherichia coli.* ***Molecular and General Genetics*** (1999) 262:543-51.
  http://link.springer.de/link/service/journals/00438/bibs/9262003/92620543.htm
- **Courcelle J**; Crowley DJ; Hanawalt PC. Recovery of DNA replication in UV-Irradiated *Escherichia coli* requires both excision repair and RecF protein function. ***Journal of Bacteriology*** (1999)181:916-22.
  http://jb.asm.org/cgi/content/abstract/181/3/916

d. Synergistic activities

- Since 2001 Instructor, Course Development, Departmental development at Mississippi State University*.* Teach Microbial Genetics. Initiated, developed, and have University approval to begin teaching a Molecular Genetics Laboratory for Microbiology and Biology majors. Organize and run departmerntal seminar series to bring in outside speakers. Initiated and run a Molecular Biol. Journal Club for graduate and advanced undergraduate students within the department.
- Since 2001 Committee work. New Faculty Recruitment Committee. University Research Enhancement Committee. Undergradaute Curriculum Committee for Biology and Microbiology. Radiation Safety Committee.
- Since 1998 Reviewer/Referee. For PNAS, Science, Genetics, Mol Gen Genet, Cell, and J Bact, FEMS Letters, Mol Micro, and ad hoc NSF proposals.
- Since 2003 NSF Review Panelist Microbial Genetics (served only once thus far).
- 2003-4. Involved in Recruitment of New Dept Head for Biol Science with a focus of increasing research and undating the curriculum within the Dept of Biology. Nara Gavini from Bowling Green Univ. with interests in Microbial Ecology, Proteomics and Cancer Biology will be starting in August

e. Collaborators and other affiliatations

i.) Collaborators

Carney J, University of Baltimore	Korolev S, St Louis Univ Sch of Med
Brown PO, Stanford University	Ganesan, AK, Stanford University
Khodursky A, University of Minnesota	Mokarski ES, Stanford University
Peter B, Stanford University	Pritchard MN, Univ Alabama, Birmingham

*no collaborators are involved on the current proposal.

ii.) Graduate and Post-Doctoral Advisors

Hanawalt PC, Stanford University — Radman M, University of Paris

iii.) Thesis advisor and Postgraduate-Scholar Sponsor

current: Janet Donaldson (PhD), Jerilyn Belle (MS), Aparna Nandiraju (PhD).
graduated: Kin-Hoe Chow (MS) currently pursuing PhD at Univ Utah.
rotational students: Larhonda Jefferson (selected Thibaudeau Dev Biol), Padmiini Jayaraman (selected Pinchuk Immun), Cynthia Doffitt (selected Vet Med).
undergraduates: Misty Hubethal, (nursing U Texas), Melissa Morton (nursing Vanderbuilt). Joshua McGuire (not yet graduated), Krista Mophett (high school, currently senior).

Biographical Sketch, Courcelle, Charmain

a. Professional Preparation

UC Berkeley, Berkeley, CA;	Mol. & Cell. Biol	B.S., 1993
Stanford University; Stanford, CA	Micro & Immuno	Ph.D., 2000
Miss. State Univ, Miss. State, MS	Genetics	Post-Doc, 2002-2004

b. Appointments

∞*Post-Doc*, *Mississippi State University* with Dr. Justin Courcelle. DNA replication, repair, and mutagenesis. NRSA from the NIH 2002-present.

∞*Science Editor and Writer*, MAFES Magazine (Mississippi Agricultural and Forestry Experiment Stations). A quarterly magazine highlighting the science and research occurring around the state of Mississippi. 2001-2002.

∞*Science Writer,* Longerliving.com webbased public science magazine. 1999-2000.

∞*Graduate Research* with Dr. Edward Mocarski, Graduate Student Traineeship 1994-1999

∞*Undergraduate Research*, *UC Berkeley* with Dr. Alex Nichols 1993.

c. Publications

i.) 5 Closely related publications

- Donaldson JR; **Courcelle CT**; Courcelle J. RecG or RuvAB is not required for the resumption of replication following UV irradiation *Escherichia coli*. ***Genetics*** (2004) 166:1631-40**.**
  http://www.genetics.org/cgi/content/full/166/4/1631
- Courcelle J; Belle JJ; **Courcelle CT**. When replication travels on damaged templates: bumps and blocks in the road. ***Research in Microbiology*** (2004) 155:231-7**.**
  http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6VN3-4C2PYFS-2&_coverDate=05%2F31%2F2004&_alid=184092362&_rdoc=1&_fmt=&_orig=search&_qd=1&_cdi=6167&_sort=d&view=c&_acct=C000053407&_version=1&_urlVersion=0&_userid=1512607&md5=6f17593667ebeea1a2761e155c906435
- Courcelle J**,** Donaldson JR, Chow KH, **Courcelle CT**. UV-induced replication fork regression and processing in *Escherichia coli*. ***Science***, (2003) 299:1064-7.
  http://www.sciencemag.org/cgi/content/full/299/5609/1064

  (*highlight*) LeBrasseur, N. Rec'd and repaired. ***Journal of Cell Biology*** (2003) 106:464-5
- **Courcelle CT**; Courcelle J; Prichard MN; Mocarski ES. Requirement for uracil-DNA glycosylase during the transition to late-phase cytomegalovirus DNA replication. ***Journal of Virology*** (2001) 75(16):7592-601.
  http://jvi.asm.org/cgi/content/full/75/16/7592?view=full&pmid=11462031
- Wolf DG; **Courcelle CT**; Prichard MN; Mocarski ES. Distinct and separate roles for herpesvirus-conserved UL97 kinase in cytomegalovirus DNA synthesis and encapsidation. ***Proceedings of the National Academy of Sciences of the United States of America*** (2001) 98(4):1895-900.
  http://www.pnas.org/cgi/content/full/98/4/1895

i.) Other significant publications

- Mocarski ES and **Courcelle CT**. 2001. Cytomegaloviruses and their replication. p.2629-2673. In D.M. Knipe and P.M. Howley (ed.), **Fields' Virology**. Lippincourt-Raven, New York.
- Mocarksi ES, Prichard MN, **Tan CS** and Brown JM. 1997 Reassessing the organization of the UL42-43 region of the human cytomegalovirus strain AD169 genome. ***Virology*** 239(1):169-75. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WXR-45K15FV-J&_coverDate=12%2F08%2F1997&_alid=184094344&_rdoc=1&_fmt=&_orig=search&_qd=1&_cdi=7165&_sort=d&view=c&_acct=C000053407&_version=1&_urlVersion=0&_userid=1512607&md5=226cb3459d573b0323b25701f062da77
- Gong EL, **Tan CS**, Shoukry MI, Rubin EM and Nichols AV. 1994 Structural and functional properties of human and mouse apolipoprotein-AI. ***Biochim. Biophys. Acta***, 1213(3):335-42

* Previously used name --- Tan, Charmain S.

d. Synergistic activities

- 2002 College Public Relations Association of Mississippi Award, Institutional Magazine
- 1999-2002. As a journalist/science produced over 50 articles published in newspapers, science magazines, and web based journals. These publications serve an important contribution to the scientific community by increasing the communication and understanding between scientists and the general public.
- 2001-present. Member of Agricultural Communicators in Education
- 2001-2003. MSU Presidential Commission on the Status of Minorities.
- 2002. Miss School for Math and Science High School mentorship program.

e. Collaborators and other affiliatations

i.) Collaborators

Carney J, University of Baltimore
Brown PO, Stanford University
Khodursky A, University of Minnesota
Peter B, Stanford University
Korolev S, St Louis Univ Sch of Med
Ganesan, AK, Stanford University
Mokarski ES, Stanford University
Pritchard MN, Univ Alabama, Birmingham

*no collaborators are involved on the current proposal.

ii.) Graduate and Post-Doctoral Advisors

Mocarski ES, Stanford University
Courcelle J, Mississippi State Univ

SUMMARY PROPOSAL BUDGET

YEAR 1

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months)	
	Proposed	Granted
AWARD NO.		

ORGANIZATION
Mississippi State University

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR
Justin Courcelle

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Justin Courcelle - PI	0.00	0.00	2.00	$ 10,511	$
2. Charmain T Courcelle - Res Assoc	9.00	0.00	0.00	32,143	
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (2) TOTAL SENIOR PERSONNEL (1 - 6)	9.00	0.00	2.00	42,654	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (1) GRADUATE STUDENTS				19,000	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				61,654	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				14,731	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				76,385	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				3,000	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				19,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				2,000	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				21,000	
H. TOTAL DIRECT COSTS (A THROUGH G)				100,385	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) Total Direct - Student Tuition (Rate: 43.0000, Base: 96725)					
TOTAL INDIRECT COSTS (F&A)				41,592	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				141,977	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 141,977	$

M. COST SHARING PROPOSED LEVEL $ 0 — AGREED LEVEL IF DIFFERENT $

PI/PD NAME
Justin Courcelle

ORG. REP. NAME*
Jonathan Pote

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

 *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

SUMMARY PROPOSAL BUDGET

YEAR 2

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months)	
	Proposed	Granted
AWARD NO.		

ORGANIZATION
Mississippi State University

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR
Justin Courcelle

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Justin Courcelle - PI	0.00	0.00	2.00	$ 11,037	$
2. Charmain T Courcelle - Res Assoc	9.00	0.00	0.00	33,750	
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (2) TOTAL SENIOR PERSONNEL (1 - 6)	9.00	0.00	2.00	44,787	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (1) GRADUATE STUDENTS				19,000	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				63,787	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				15,080	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				78,867	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				3,000	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS 1. STIPENDS $ 0 2. TRAVEL 0 3. SUBSISTENCE 0 4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				19,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				2,000	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				21,000	
H. TOTAL DIRECT COSTS (A THROUGH G)				102,867	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) Total Direct - Tuition (Rate: 43.0000, Base: 99207)					
TOTAL INDIRECT COSTS (F&A)				42,659	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				145,526	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 145,526	$

M. COST SHARING PROPOSED LEVEL $ 0 — AGREED LEVEL IF DIFFERENT $

PI/PD NAME
Justin Courcelle

ORG. REP. NAME*
Jonathan Pote

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

 *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

SUMMARY PROPOSAL BUDGET — YEAR 3

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months) Proposed	Granted
AWARD NO.		

ORGANIZATION
Mississippi State University

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR
Justin Courcelle

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Justin Courcelle - PI	0.00	0.00	2.00	$ 11,589	$
2. Charmain T Courcelle - Res Assoc	9.00	0.00	0.00	35,438	
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (2) TOTAL SENIOR PERSONNEL (1 - 6)	9.00	0.00	2.00	47,027	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (1) GRADUATE STUDENTS				19,000	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				66,027	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				15,847	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				81,874	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				3,000	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				19,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				2,000	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				21,000	
H. TOTAL DIRECT COSTS (A THROUGH G)				105,874	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) Total direct - student tuition (Rate: 43.0000, Base: 102214)					
TOTAL INDIRECT COSTS (F&A)				43,952	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				149,826	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 149,826	$

M. COST SHARING PROPOSED LEVEL $ 0 — AGREED LEVEL IF DIFFERENT $

PI/PD NAME
Justin Courcelle

ORG. REP. NAME*
Jonathan Pote

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

 *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

SUMMARY PROPOSAL BUDGET   YEAR 4

	FOR NSF USE ONLY		
ORGANIZATION **Mississippi State University**	PROPOSAL NO.	DURATION (months)	
		Proposed	Granted
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR **Justin Courcelle**	AWARD NO.		

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. **Justin Courcelle - PI**	0.00	0.00	2.00	$ 12,168	$
2. **Charmain T Courcelle - Res Assoc**	9.00	0.00	0.00	37,209	
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (2) TOTAL SENIOR PERSONNEL (1 - 6)	9.00	0.00	2.00	49,377	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (1) GRADUATE STUDENTS				19,000	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				68,377	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				13,647	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				82,024	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				3,000	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS 1. STIPENDS $ 0 2. TRAVEL 0 3. SUBSISTENCE 0 4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				19,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				2,000	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				21,000	
H. TOTAL DIRECT COSTS (A THROUGH G)				106,024	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) **Total Direct - Tuition (Rate: 43.0000, Base: 102364)**					
TOTAL INDIRECT COSTS (F&A)				44,017	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				150,041	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 150,041	$

M. COST SHARING PROPOSED LEVEL $ 0	AGREED LEVEL IF DIFFERENT $		
PI/PD NAME **Justin Courcelle**	FOR NSF USE ONLY INDIRECT COST RATE VERIFICATION		
ORG. REP. NAME* **Jonathan Pote**	Date Checked	Date Of Rate Sheet	Initials - ORG

 *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

SUMMARY PROPOSAL BUDGET   YEAR 5

ORGANIZATION **Mississippi State University**				FOR NSF USE ONLY PROPOSAL NO.	DURATION (months) Proposed / Granted
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR **Justin Courcelle**				AWARD NO.	

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. **Justin Courcelle - PI**	0.00	0.00	2.00	$ 12,776	$
2. **Charmain T Courcelle - Res Assoc**	9.00	0.00	0.00	39,070	
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (2) TOTAL SENIOR PERSONNEL (1 - 6)	9.00	0.00	2.00	51,846	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (1) GRADUATE STUDENTS				19,000	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				70,846	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				17,076	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				87,922	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				3,000	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS 1. STIPENDS $ 0 2. TRAVEL 0 3. SUBSISTENCE 0 4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				19,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				2,000	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				21,000	
H. TOTAL DIRECT COSTS (A THROUGH G)				111,922	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) **Total Direct - Tuition (Rate: 43.0000, Base: 108262)**					
TOTAL INDIRECT COSTS (F&A)				46,553	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				158,475	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 158,475	$

M. COST SHARING PROPOSED LEVEL $ 0	AGREED LEVEL IF DIFFERENT $
PI/PD NAME **Justin Courcelle**	FOR NSF USE ONLY INDIRECT COST RATE VERIFICATION
ORG. REP. NAME* **Jonathan Pote**	Date Checked / Date Of Rate Sheet / Initials - ORG

 ***ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET**

SUMMARY PROPOSAL BUDGET — Cumulative

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months)	
	Proposed	Granted
AWARD NO.		

ORGANIZATION: **Mississippi State University**

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR: **Justin Courcelle**

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Justin Courcelle - PI	0.00	0.00	10.00	$ 58,081	$
2. Charmain T Courcelle - Res Assoc	45.00	0.00	0.00	177,610	
3.					
4.					
5.					
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (2) TOTAL SENIOR PERSONNEL (1 - 6)	45.00	0.00	10.00	235,691	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (5) GRADUATE STUDENTS				95,000	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				330,691	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				76,381	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				407,072	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				15,000	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				95,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				10,000	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				105,000	
H. TOTAL DIRECT COSTS (A THROUGH G)				527,072	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)					
TOTAL INDIRECT COSTS (F&A)				218,773	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				745,845	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 745,845	$

M. COST SHARING PROPOSED LEVEL $ 0 — AGREED LEVEL IF DIFFERENT $

PI/PD NAME: **Justin Courcelle**

ORG. REP. NAME*: **Jonathan Pote**

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

C *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

Budget Justification

i.) Salaries and Wages

Justin Courcelle, Ph.D., Principal Investigator (100% effort, Summer) will be responsible for the overall administration and direction of the project. $10511 yr1; $11037 yr2; $11589 yr3; $12168 yr4; $12776 yr5 is requested to provide 2 months of summer salary.

Charmain Courcelle, Ph.D., Research Associate (100% effort) will be responsible for constructing site specific lesions and play a large role in directing and mentoring graduate students. A stipend of $32143 yr1, $33750 yr2, $35438 yr3, and $37209 yr4; $39070 yr5 is requested for the project.

1 graduate student (100% effort). A yearly stipend of $19000 is requested to cover living expenses while (s)he is enrolled in the graduate program.

ii.) Fringe Benefits

Yearly fringe benefits totaling $2680 yr1; $2814 yr2; $2955 yr3; $3103 yr4; $3258 yr5 (@25.5% of salary) are requested for Justin Courcelle.

Yearly fringe benefits totaling $8196 yr1; $8606 yr2; $9037 yr3; $9489 yr4; $9963 yr5 (@25.5% of salary) are requested for Charmain Courcelle.

Yearly fringe benefits of $3855/yr are requested to cover tuition ($3660 yr) and worker's compensation ($195/yr) for the graduate student position.

iii.) Equipment

iv.) Travel

b.) Domestic travel for two meetings each year is requested ($3,000). These meetings are necessary to remain updated on recent advances pertaining directly to this proposal. These meetings are also provide a platform to present our results to the group of scientists who are most likely able to make use of their implications and extend them into applied strategies.

vi.) Other Direct Costs

a.) **Materials and Supplies**

Consumables ($11000/year) include plastic petri dishes, pipet tips, 15ml and 50ml conical tubes, 5ml disposable test tubes, glass fiber filters, general cell filtration membranes, scintillation vials, latex gloves, autoclave bags, and 3MM blotting paper, nylon membranes, blotting paper towels, ultracentrifuge tubes, disposal containers for radioactive waste generated by culture media, ultra centrifugation, and scintillation counting procedures.

Enzymes ($2000/year) include the purchase of restriction endonucleases for genomic DNA digests, DNA repair enzymes for detection of DNA damage, and in vitro transcription/nick translation kits to generate radiolabelled RNA/DNA probes.

Chemical Reagents and Media ($3000/year) include Agarose, Buffer Reagents and Salts, Minimal and Complex Culturing Media for Bacterial Growth and Cultures.

Radionucliotides ($3000/year) include 32P-dCTP and 32P-CTP for southern blot detection.

b.) **Publication/Documentation/Dessimation**

Publication costs ($2,000/year) are requested to cover page charges and reprints following publication of results that are generated by this proposal.

viii.) Indirect Costs

Yr1 = 43%(Total direct costs – student tuition)
43%(100385– 3660) =
43% (96725) =
$41592

Yr2 = 43%(Total direct costs – equipment – student tuition – (Subcontracts)
43%(102867– 3660) =
43% (99207) =
$42659

Yr3 = 43%(Total direct costs – student tuition
43%(105874– 3660) =
43% (102214) =
$43952

Yr4 = 43%(Total direct costs – student tuition
43%(106024– 3660) =
43% (102364) =
$44017

Yr5 = 43%(Total direct costs – student tuition
43%(111922– 3660) =
43% (108262) =
$46553

Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

Investigator: **Justin Courcelle**

Other agencies (including NSF) to which this proposal has been/will be submitted.

Support: ☒ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: **Recovery of Replication Following UV-induced DNA Damage**

Source of Support: **National Science Foundation**
Total Award Amount: $ **416,377** Total Award Period Covered: **04/01/02 - 03/31/05**
Location of Project: **Mississippi State University**
Person-Months Per Year Committed to the Project. Cal: **0.00** Acad: **0.00** Sumr: **2.00**

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title:

Source of Support:
Total Award Amount: $ Total Award Period Covered:
Location of Project:
Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title:

Source of Support:
Total Award Amount: $ Total Award Period Covered:
Location of Project:
Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title:

Source of Support:
Total Award Amount: $ Total Award Period Covered:
Location of Project:
Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title:

Source of Support:
Total Award Amount: $ Total Award Period Covered:
Location of Project:
Person-Months Per Year Committed to the Project. Cal: Acad: Summ:

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.

Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

Investigator: **Charmain Courcelle**

Other agencies (including NSF) to which this proposal has been/will be submitted.

Support: ☒ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: **DNA Replication Termination in Escherichia coli**

Source of Support: **NIGMS (NIH) NRSA Fellowship**
Total Award Amount: $ **156,708** Total Award Period Covered: **07/01/03 - 06/30/06**
Location of Project: **Mississippi State University**
Person-Months Per Year Committed to the Project. Cal: **12.00** Acad: **0.00** Sumr: **0.00**

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title:

Source of Support:
Total Award Amount: $ Total Award Period Covered:
Location of Project:
Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title:

Source of Support:
Total Award Amount: $ Total Award Period Covered:
Location of Project:
Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title:

Source of Support:
Total Award Amount: $ Total Award Period Covered:
Location of Project:
Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title:

Source of Support:
Total Award Amount: $ Total Award Period Covered:
Location of Project:
Person-Months Per Year Committed to the Project. Cal: Acad: Summ:

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*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.

 USE ADDITIONAL SHEETS AS NECESSARY

Justin Courcelle

Facilities, Equipment, and Other Resources

Laboratory:

Within the Department of Biological sciences there is 1,300 sq ft. of personal laboratory space equipped with yellow lighting for DNA damage and recovery experiments. 225 sq ft. Common Equipment Rooms containing centrifuges, ultracentrifuges, and a scintillation counter. Autoclave and dishwashing room. 655 sq ft Conference room. 191 sq ft Dark room.

Clinical:
Not applicable

Animal:
Not applicable

Computer:

The lab contains, 3 Macintosh G4 computers, and one PC, Pentium 4 computer, Color inkjet printers, and scanners. Ethernet connections are present in the lab and office. The university's computer network is served by the Information Technology Services (ITS) Department.

Office:

The PI has 171 sq ft. of personal office space. In addition, departmental offices share 429 sq ft for administrative work.

Major Equipment:

Bacterial Incubators, Electrophoresis Units and Power Supplies, -80C Freezer, -20C freezer, Refrigerators, Shaking Incubators, PCR machine, Hybridization ovens, Filtering Manifolds, Heating Blocks, Refridgerated water baths, Microfuges, pH meter, Compound Microscopes, UV lamps, UV meters, UV/Vis spectrophotomer, yellow lighting (for UV irradiation experiments) Gas, and Distilled Water, entrifuges, Ultracentrifuges, Multi-Channel Scintillation Counter, Autoclaves, Industrial Washer, Dark Room, and Phosphoimager.

Other Resources:

MSU has recently established a Life Sciences Biotechnology Institute that contains several additional pieces of equipment that are available for use, including DNA sequencers, real-time PCR, fluorescent/radioisotope phosphoimager, plate readers, centrifuges, and the core facility and equipment for proteomic analysis.

MSU also has a microscope facility that contains a confocal microscope, transmission election microscope, and scanning electron microscope with a full support staff and technicians.

Department of Biochemistry

May 27, 2004

Dr. Justin C. Courcelle
Department of Biological Sciences
Mississippi State University

Dear Justin,

I would be glad to provide my resources and expertise to assist in your project studying the processing and repair of DNA damage during replication.

As you are aware, the research in my lab focuses on the enzymology of base excision repair and the mutagenic outcome of DNA lesions introduced by reactive oxygen species. As such, we have extensive reagents and expertise which should be helpful in your proposed studies. In particular, we have a large collection of oligonucleotide substrates containing most of the known stable DNA lesions. These include cyclobutane dimers, thymine glycol, cytosine glycol, uracil glycol, 8-oxoadenine, 8-oxoguanine, 5-hydroxyctosine, 5-hydroxyuracil, dihydrothymine, dihydrouracil, and tetrahydrofuran (Hatahet et al., 1993, Nucleic Acids Res, 21, 1563-8). Similarly, we have established reliable protocols to introduce these lesions site-specifically into plasmid DNA and establish their mutagenic outcome in vivo {Hatahet et al., 1998, Proc Natl Acad Sci U S A, 95, 8556-61; Hatahet et al., 1999, J Mol Biol, 286, 1045-57).

Looking forward to this collaboration and best of luck with your endeavor.

Sincerely,

Zafer Hatahet, Ph.D.
Associate Professor

11937 US Highway 271, Tyler, Texas 75708. Phone: 903/877-7446, Fax: 903/877-7940
Equal Opportunity/Affirmative Action Institution

Dr. Nara Gavini, Head
Department of Biological Sciences
PO Box GY
Mississippi State, MS 39762
phone (662) 325-3483
fax (662) 325-7939
ng95@msstate.edu

National Science Foundation
Reviewing committee members

Re: Department Endorsement of Justin Courcelle,
CAREER proposal

To whom it may concern,

I have read and I endorse this career-development plan. I attest that Dr. Courcelle's career-development plan is supported by and integrated into the educational and research goals of the department and the organization. I personally commit to the support and professional development of the PI.

Dr. Justin Courcelle is developing into an exceptional young scientist, whose integrated research and teaching efforts epitomize the objectives of our department and those targeted by the CAREER program. Although Justin is a young member of our department, he has become one of its leaders in its goal to create a nationally competitive research-based learning environment for undergraduates and graduates in our program. His own research program has already attracted several graduate and undergraduate students who have produced high quality work through his mentoring. This past May, the college awarded him the Faculty Research Award which, I am told, typically has been given to more senior faculty. His receipt of this award attests to the University's belief in the future of Dr. Courcelle's academic career and success here at Mississippi State.

As a newly recruited department head of Biological Sciences, I enthusiastically support and share the goals found in Dr. Courcelle's CAREER proposal. His proposed aims to initiate of a Molecular Lab course, participate in our planned curriculum development, and proactively recruit regional undergraduates and graduates into research is clearly something he has proven to be capable of and will be essential to the future health of our program. In a very short time, Dr. Courcelle has established himself as a leading researcher and teacher at this institution and was a large factor in my decision to join this department as its new Department Head this year. I am committed to ensuring the future success of Dr. Courcelle and, in addition to my own mentoring, will be able to provide through the department teaching stipends for one or two graduate students, course release time for Dr. Courcelle towards the development of curriculum and proposals to obtain instructional equipment, as well as continue to provide travel funds for meetings when required.

In addition, I wish to point out that Mississippi State University also shares Dr. Courcelle's commitment to developing a strong Biological Sciences department in its future. They have committed to adding five faculty positions, including my own, over the next two years and plan to renovate the Biological Sciences building within the next three years. They have established a Life Sciences and Biotechnology institute that provides core services and houses state of the art proteomic facilities, pulsed-field gel electrophoresis, DNA sequencers, real-time PCR, imagers, and plate readers which are readily available to Justin's research program.

Lastly, I can verify that the CAREER eligibility information that the PI self-certified in the proposal submission process is accurate. Dr. Courcelle is an assistant professor on a tenure track position. He is a U.S. citizen and has not applied for a CAREER award in the past.

Sincerely,

Dr. Nara Gavini, Department Head
Biological Sciences
Mississippi State University
July 13, 2004