AD_____

Award Number: DAMD17-98-1-8557

TITLE: DNA Replication Initiator Proteins & Genetic Instability: Creating a Mouse Model for Prostate Cancer

PRINCIPAL INVESTIGATOR: Peter K. Jackson, Ph.D.

CONTRACTING ORGANIZATION: Stanford University School of Medicine
Palo Alto, California 94305

REPORT DATE: September 1999

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 1999	Annual (15 Aug 98 - 14 Aug 99)

4. TITLE AND SUBTITLE
DNA Replication Initiator Proteins & Genetic Instability: Creating a Mouse Model for Prostate Cancer

5. FUNDING NUMBERS
DAMD17-98-1-8557

6. AUTHOR(S)
Peter K. Jackson, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Stanford University School of Medicine
Palo Alto, California 94305

E-Mail: pjackson@cmgm.stanford.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

14. SUBJECT TERMS
Prostate Cancer

15. NUMBER OF PAGES
6

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

___ Where copyrighted material is quoted, permission has been obtained to use such material.

___ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

___ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

X In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Peter K. Jackson, Ph.D. 3/15/00

PI - Signature Date

TABLE OF CONTENTS

Initial Goals and results:

We have suggested that limiting hyperploidy by rereplication control may be important for controlling cancer progression. The SCF ubiquitin ligases are a very broad class of enzymes that mediate ubiquitin-dependent destruction of specific proteins. Examples of SCF and related complexes have now been implicated in several tumors including renal cell carcinoma by mutations in the Von Hippel Lindau (VHL) gene.

In the fission yeast *Schizosaccharomyces pombe*, the Pop1 and Pop2 proteins are associated with SCF ubiquitin ligases and are critical for limiting re-replication in yeast. We had identified a close homolog of Pop1 in humans, called hPOP1. Mapping the chromosomal location of the gene by radiation hybrid analysis and FISH, we found the gene to be associated with a region of chromosome 9q34, which has been suggesed to contain a tumor suppressor gene for bladder cancer. We sought to explore the connection between human Pop1 and bladder cancer by looking for alterations in human Pop1 expression in bladder tumors.

Critical Progress:

1. We have obtained full length clones of human Pop1 (also the mouse homolog, called MD6). We have used these clones to verify that the the Pop1 gene is indeed associated with other components of the SCF ubiquitin ligases by expressing our gene in transfected cells and looking for coimmunoprecipitation of Skp1, Cul1, and Cdc34. These results validate that hPOP1 is indeed part of an SCF complex.

2. We have made and characterized antisera against the human Pop1 gene. The antisera appears to recognize protein species of 45 and 65 kilodaltons. Our transfection data suggests that the larger form is the correct size for our protein. The smaller form may be a closely related, but distinct protein, or a processed form of hPOP1.

Northern blot analysis also shows two messages, but at this time we don't know whether these messages encodes distinct proteins or not.

3. Pop1 is found broadly expressed, but with especially high levels of protein expression in the bladder, stomach, liver, lung, seminal vesicles, and testes. Screening through a panel of tumor cell lysates, we see very large variations in the level of hPOP1 protein. It is not clear at this time whether those variations reflect changes in cell proliferation or oncogenic activity. We hope to soon understand the consequences of hPOP1 overexpression. A number of physiological studies are underway including looking at the effect of overexpressing wild type and potential dominant negative versions of the gene in animal cells and testing for alteration in the cell cycle.

4. We have found that human Pop1 is overexpressed in half of a small number of bladder cancer samples. We are currently setting up to screen a much larger number of bladder tumors.

5. To more completely explore the importance of SCF biology in cancer, we have cloned and collected a large number (~45) novel F-box proteins. At this time, those are classified on the basis of whether they are F-box proteins containing a WD40 domain (called Fbw), a leucine-rich repeat domain (called Fbl), or are one of a catch-all class missing either of these motifs (Fbx). We are currently collecting the "complete" human set of cDNAs (not necessarily full length). At this time, there are five Fbw, 13 Fbl, and 28 Fbx proteins identified. These results were recently published in *Current Biology*.

Reference: Regan-Riemann, J., Duong, Q., Swanson, C. S., and P. K. Jackson (1999). Screening for Skp1-interacting proteins in Xenopus laevis identifies Fbl5, a novel F-box, leucine-rich repeat protein required for DNA replication. Current Biology 9, R762-763.

We intend to use these clones to design a set of PCR primers for quantitative PCR ("Taqman"), which will allow a much more reliable determination of RNA abundance for these physiologically critical genes. First, we will determine the levels of F-box proteins in normal versus the 62 transformed cell lines obtained from the Developmental Therapeutics Program (DTP). Next, we will determine the effect of critical physiological alterations (changes in media, growth factors, DNA damage, microtubule or actin poisons, cancer chemotherapeutic drugs) in normal versus transformed cells to determine whether the response of any of these F-box proteins is clearly altered in transformed cells. These correlations may be critical for understanding transformed cell biology and the response of specific tumor types to physiological changes and chemotherapy.

Future plans:

We are hoping to expand our studies on hPOP1 to find both its normal and pathological targets, to test more directly whether it is indeed a tumor suppressor by knocking-out the gene in mice, and more extensive tumor studies. The funding from the DAMD grant has been critical for our studies on hPOP1 to date. In order to further pursue the mouse knock-out, the original funding was sufficient to get to our current state with cDNA and genomic clones. We did not begin the construction of a knock-out vector until such time as we secured sufficient funding to ensure the completion of the next phase of the project.