PI: **Jiang, Mengxi**	Title: Intersection of polyomavirus infection and host cellular responses	
Received: 06/02/2015	FOA: PA13-302	Council: 01/2016
Competition ID: FORMS-C	FOA Title: RESEARCH PROJECT GRANT (PARENT R01)	
1 R01 AI123162-01	Dual:	Accession Number: 3825771
IPF: 1288803	Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM	
Former Number:	Department: Microbiology	
IRG/SRG: VIRB	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted] Year 2: [redacted] Year 3: [redacted] Year 4: [redacted] Year 5:	Animals: N Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: Y Early Stage Investigator: Y
Senior/Key Personnel:	*Organization:*	*Role Category:*
Mengxi Jiang	UNIVERSITY OF ALABAMA AT BIRMINGHAM	PD/PI
[redacted]	University of Alabama at Birmingham	Co-Investigator

Additions for Review

Updated Pages update

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

c. Previous Grants.gov Tracking Number

2. DATE SUBMITTED | **Application Identifier**

5. APPLICANT INFORMATION

Organizational DUNS*: [redacted]

Legal Name*: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Department: Office of Sponsored Programs
Division:
Street1*: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Street2: 1720 2nd Ave. South, AB 1170
City*: BIRMINGHAM
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111

Person to be contacted on matters involving this application
Prefix: First Name*: Celesta Middle Name: Last Name*: Smith Suffix:
Position/Title:
Street1*: 1720 2nd Ave. South, AB 1170
Street2:
City*: BIRMINGHAM
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111
Phone Number*: [redacted] Fax Number: [redacted] Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Intersection of polyomavirus infection and host cellular responses

12. PROPOSED PROJECT
Start Date* Ending Date*
04/01/2016 03/31/2021

13. CONGRESSIONAL DISTRICTS OF APPLICANT
AL-007

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Mengxi Middle Name: Last Name*: Jiang Suffix:
Position/Title: Assistant Professor
Organization Name*: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Department: Microbiology
Division: School of Medicine
Street1*: BBRB 834, 845 19th Street South
Street2:
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352942170
Phone Number*: [redacted] Fax Number: Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested* [redacted]
b. Total Non-Federal Funds* [redacted]
c. Total Federal & Non-Federal Funds* [redacted]
d. Estimated Program Income* [redacted]

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:
b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Lynn Middle Name: W. Last Name*: Stedman Suffix:
Position/Title*: Director
Organization Name*: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Department: Office of Sponsored Programs
Division:
Street1*: 1720 2nd Avenue South
Street2: AB 1170
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111
Phone Number*: [redacted] Fax Number: [redacted] Email*: [redacted]

Signature of Authorized Representative*
Celesta Smith

Date Signed*
06/02/2015

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_Letter_Final.pdf

424 R&R and PHS-398 Specific Table Of Contents

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Duns Number: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: AL-007

File Name

Additional Location(s)

Funding Opportunity Number: PA-13-302. Received Date: 2015-06-02T17:13:17.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_Abstract_Final.pdf
8. Project Narrative*	Project_Narrative_Final.pdf
9. Bibliography & References Cited	References_Final.pdf
10.Facilities & Other Resources	Facilities_and_Other_Resources_Final.pdf
11.Equipment	Equipment_Final.pdf

Funding Opportunity Number: PA-13-302. Received Date: 2015-06-02T17:13:17.000-04:00

PROJECT SUMMARY/ABSTRACT

Polyomaviruses cause a variety of severe human diseases particularly in immunocompromised individuals. No specific anti-viral treatments or prophylactic approaches exist to target this family of viruses. There are several critical gaps in our current knowledge of the molecular mechanism of viral replication and tumorigenesis. Our long-term goals are to identify how these viruses subvert normal host cellular processes to facilitate viral replication, and how these interactions may result in oncogenesis.

Our previous studies revealed an intricate balanced relationship between viral replication and virus-induced host genomic instability. These results lead to our central hypothesis that an activated cellular DNA damage response (DDR) is important for facilitating viral replication and maintaining host genome stability during polyomavirus infection. Towards this hypothesis, we have identified host mismatch repair system and replicating viral DNA as novel factors contributing to DDR activation. We have also discovered that the ability of polyomavirus to cause host genomic DNA damage is linked to its ability to replicate viral DNA. Guided by strong preliminary data, we propose to pursue three Specific Aims to characterize DDR activation mechanism and how the DDR ties together viral replication and host genomic stability: (1) To define the role of host mismatch repair proteins in polyomavirus replication and polyomavirus-induced DDR activation. (2) To determine the viral DNA triggers that activate the DDR upon polyomavirus infection. (3) To elucidate the molecular mechanism by which polyomavirus induces host genome instability.

Collectively, our proposed research will broadly impact the field by characterizing the essential roles that the DDR plays in promoting viral replication and maintaining host genome stability. These studies will have the potential to uncover novel molecular mechanisms underlying polyomavirus replication as well as viral oncogenesis. These findings may be extrapolated to other DNA viruses and to our understanding of normal cellular processes.

PROJECT NARRATIVE

Polyomaviruses are a family of viruses associated with severe human diseases and a subset of them can also cause cancers. Our proposed studies aim to understand the interactions between polyomaviruses and the host DNA damage response, a cellular pathway important for both viral replication and host genome maintenance. This research will have the potential to reveal novel therapeutic host targets to treat polyomavirus-related diseases.

FACILITIES AND OTHER RESOURCES

Jiang Laboratory:

Laboratory:
Approximately 1000 square feet of laboratory space are dedicated for use by Dr. Jiang and affiliated personnel on the 8th floor of the Bevill Biomedical Sciences Research Building. The laboratory is operated at biosafety level II (BSL-2) containment level and is equipped for cell culture, molecular biology, biochemical, and cell biology techniques. There are two 4-ft biosafety cabinets and two CO_2 cell culture incubators that are suited for working with infectious viruses. There are two common equipment rooms on the 4th and 7th floor and include all major equipment needed.

Biohazards:
All of the BSL-2 work proposed in the studies will be performed in the Jiang laboratory. There are designated and approved areas in the laboratory for handling infectious agents including BK polyomavirus and lentivirus. Approval from the UAB Occupational Health and Safety Office will be obtained for all the studies involving infectious agents. An annual laboratory audit will be performed by UAB to ensure compliance with the safety requirements. All members of the lab have been trained to safely handle infectious agents and are re-trained on a yearly basis.

Office:
Dr. Jiang's office is located directly next to the lab space. It is a 105 square foot office with a window opening into the laboratory allowing ease of communication with lab members throughout the day.

Computer:
A Dell PC Desktop connected to both LaserJet and color printers is readily accessible in the Jiang lab. In addition, Dr. Jiang has a PC laptop and a Macbook Pro laptop. All computers are outfitted with a wide suite of software including Microsoft Office, Adobe Creative Suite, DNA sequence and proteomics analysis software, statistics, and graphic design software. The computers are linked to the Microbiology network, providing access to additional software, a color printer/scanner, the Internet, and a storage server for data backup. Telecommunication is available allowing Dr. Jiang to communicate with other investigators in the field of polyomavirus research on a regular basis to seek advice and collaborations if needed.

Administrative Support:
Dr. Jiang's research is supported by the administrative offices of the Department of Microbiology at UAB. Full time personnel are available for grant management and general administrative services.

[redacted] Laboratory:

Laboratory:
[redacted] has 1,800 square feet of newly renovated lab space on the first floor of Volker Hall. The lab is fully equipped with standard laboratory items and sufficient bench space for 10 individuals. Additional tissue culture rooms, common use space with major equipment, and adjacent conference rooms are readily accessible.

Office:
[redacted] has a 420 square foot office [redacted], with full secretarial and business office support staff. Dr. [redacted] has a Mac Quad-Core computer, a color laser printer, and scanner/fax machine in her office in the Department office suite, which is immediately adjacent to the lab.

Other Resources:

University core facilities:
Numerous shared core facilities are maintained at the UAB. These include: UAB high resolution imaging core capable of 3D and 2D Confocal Laser Microscopy, Electron Microscopy (EM) including transmission EM and Cryo-EM; flow cytometry facilities equipped with BSL-3 live cell sorting; UAB mass spectrometry/proteomics consortium performing both high resolution and quantitative analyses of small molecules and selected peptides; the Heflin Center for Genomic Sciences provide Next Generation Sequence analysis and standard Sanger sequencing analysis; state of the art animal facilities; biostatistics and bioinformatics cores; X-ray crystallography and NMR facilities; tissue procurement and biobanking cores; hybridoma cores for monoclonal antibody production; biofermentation cores for recombinant protein production. All core facilities are within close proximity from the PI's laboratory space.

Department support:
The Department of Microbiology is extremely supportive of Dr. Jiang's career development as a junior independent scientist. At least 80% of Dr. Jiang's effort will be protected for research activities for the next five years. The department chair also helped Dr. Jiang to form a mentoring committee consisting of senior faculty from both within and outside of the department to guide Dr. Jiang on research, publications, grantsmanship, and laboratory management. There is annual faculty retreat and department retreat, providing Dr. Jiang and her trainees opportunities to present research and seek collaborations. Please see attached Department Chair Letter of Support.

Collaborative Research Environment at UAB:
UAB is a highly collaborative and collegial research university. There are 25 University-wide Interdisciplinary Research Centers, including an NCI designated Comprehensive Cancer Center (CCC), an NIH-designated Center for AIDS Research (CFAR), Center for Clinical and Translational Science (CCTS), and the Center for Emerging Drug Discovery (which serves as the operational base for UAB participation in the Alabama Drug Discovery Alliance). As such, UAB provides a rich intellectual environment in which basic, translational and clinical investigators collaborate.

There are about 40 faculty including 15 virologists in the Department of Microbiology with most of them located in the same building as Dr. Jiang. Additionally, there are virologists in the Departments of Biochemistry, Pediatrics, Internal Medicine, and the CFAR. Scientific discussion and communication occur on a daily basis among faculty. This open environment has greatly facilitated research collaborations with our colleagues including Drs. Luo, Chow, Broker, Xu. There are weekly seminar series in the department, CFAR, and Cancer Center by invited outside speakers. Dr. Jiang holds a monthly joint meeting with Dr. Guangxiang Luo's (a senior virologist in the Department of Microbiology) group in addition to the weekly lab meeting to stimulate idea exchanges. There is also a virology discussion group at UAB consisting of faculty members, postdoctoral fellows, and students from virology labs on campus. This group meets weekly with rotating group presentations. All of these activities greatly enhance the quality and excellence of our research and education and therefore will facilitate the successful completion of our proposed studies.

EQUIPMENT

Major equipment available in the Jiang laboratory: Two tissue culture incubators, a liquid nitrogen storage tank, -80°C and -20°C freezers, a 4°C refrigerator, an inverted microscope, and a refrigerated centrifuge with a swinging bucket rotor and a Fiberlite rotor are located within Dr. Jiang's lab space for cell culture experiments. Two biosafety hoods and one chemical fume hood are installed. We also have all the necessary equipment for molecular biology, including a refrigerated microfuge, two regular microfuges, a thermocycler, electrophoresis power supplies and apparatus, analytical balances, water baths and incubators, and many small laboratory items. A walk-in cold room is located right across from the Jiang lab space for biochemical experiments and storage of reagents.

Major equipment available in the [redacted] laboratory: Instrumentation of 2-D gels and DNA fiber analyses are available in the [redacted] lab. The lab is also equipped with biosafety cabinets, incubators, water baths, phase microscopes and coulter counter. The facility also contains humidified CO2 incubators for 384 well-formatted plates, a MultiDrop Combi for liquid /media dispensing, CLARIOstar plate reader (BMG labtech) with 5 primary detection modes (absorbance, fluorescence intensity, fluorescence polarization, time-resolved fluorescence, luminescence); and an ABI QuantStudio 7 Flex sequence detection system for RT-PCR. Additional common use space includes baculovirus and bacterial culture facilities, immunofluorescent microscopes, dark room, and other large pieces of equipment, including RT-PCR machines, fluorescent plate readers, -20˚C and -80˚C freezers, and liquid N2 storage units for cell line storage.

Shared Equipment in the Department of Microbiology: A number of shared resources are available in the Department of Microbiology, which are all readily accessible within the same building as Dr. Jiang's lab. These include: ultra-, medium, and low speed centrifuges, fluorescence microscope, real-time PCR, nanodrop spectrophotometer, gel documentation and Odyssey imaging system, phosphorimager, scintillation counters, shaker incubators, dark room with film processor, autoclaves, and dishwashers. Other centralized resources and equipment are described in the *Facilities and Other Resources* section.

Overall, all the necessary equipment to successfully carry out the proposed studies are in place either in the Jiang and [redacted] labs, or as part of a well-organized shared program and UAB core facilities.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Mengxi Middle Name Last Name*: Jiang Suffix:

Position/Title*: Assistant Professor
Organization Name*: UNIVERSITY OF ALABAMA AT BIRMINGHAM
Department: Microbiology
Division: School of Medicine
Street1*: BBRB 834, 845 19th Street South
Street2:
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 352942170

Phone Number*: [redacted] Fax Number: E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: Ph.D. Degree Year: 2006

	File Name
Attach Biographical Sketch*:	Jiang_Biosketch_Final.pdf
Attach Current & Pending Support:	

Tracking Number: GRANT11922341 Funding Opportunity Number: PA-13-302 . Received Date: 2015-06-02T17:13:17.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: [redacted] Middle Name Last Name*: [redacted] Suffix:

Position/Title*: [redacted]
Organization Name*: University of Alabama at Birmingham
Department: Pharmacology and Toxicology
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*: [redacted]
Province:
Country*:
Zip / Postal Code*:

Phone Number*: [redacted] Fax Number: E-Mail*: [redacted]

Credential, e.g., agency login:

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: Degree Year:

File Name

Attach Biographical Sketch*: [redacted]

Attach Current & Pending Support:

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Jiang, Mengxi

eRA COMMONS USER NAME (agency login): [redacted]

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Fudan University	BS	06/2001	Life Sciences
University of Michigan, Ann Arbor, MI	PHD	08/2006	Molecular, Cellular & Developmental Biology
University of Michigan, Ann Arbor, MI	Postdoctoral Fellow	01/2012	Microbiology & Immunology

A. Personal Statement

The goal of the proposed research is to elucidate how the host DNA damage response (DDR) regulates viral replication and host genomic stability during polyomavirus infection. I have the technical expertise and leadership experience to perform the proposed studies. My graduate work focused on ribosome biogenesis in *E. coli*, during which I pioneered the usage of powerful quantitative proteomic methodologies to analyze defective ribosomal particles and to identify novel ribosome-associated proteins. As a postdoctoral fellow in Dr. Michael Imperiale's lab, my research focused on the molecular characterization of various aspects of host-BK polyomavirus interactions, including viral entry, viral replication, and host nuclear architecture rearrangements. I discovered that polyomavirus replication and host genome stability rely on an activated host DDR pathway during infection, which laid the groundwork for this proposal. I have extensive experience in polyomavirus molecular virology and cell biology, and have been leading and designing research projects throughout my research career. Since I started my independent research career at UAB, my laboratory has discovered that mismatch repair proteins are required for polyomavirus replication and that viral DNA replication drives the activation of the DDR. The current application builds logically on my prior work and these novel findings. Additionally, I have teamed up with [redacted] as a co-investigator to provide expertise in DNA damage and repair for this project. The characterization of the important functions of the DDR for both viral replication and host genome maintenance has the potential to broadly impact the DNA virus and tumor virology field. Collectively, I have demonstrated both research skills and productivity to be the principal investigator to address these important and fundamental questions regarding the intersection of polyomavirus infections and host cellular responses.

1. Jiang M, Abend JR, Tsai B, Imperiale MJ. Early events during BK virus entry and disassembly. J Virol. 2009 Feb;83(3):1350-8. PubMed PMID: 19036822; PubMed Central PMCID: PMC2620883.
2. Jiang M, Entezami P, Gamez M, Stamminger T, Imperiale MJ. Functional reorganization of promyelocytic leukemia nuclear bodies during BK virus infection. MBio. 2011;2(1):e00281-10. PubMed PMID: 21304169; PubMed Central PMCID: PMC3039439.
3. Jiang M, Zhao L, Gamez M, Imperiale MJ. Roles of ATM and ATR-mediated DNA damage responses during lytic BK polyomavirus infection. PLoS Pathog. 2012;8(8):e1002898. PubMed PMID: 22952448; PubMed Central PMCID: PMC3431332.
4. Verhalen B, Justice JL, Imperiale MJ, Jiang M. Viral DNA replication-dependent DNA damage response activation during BK polyomavirus infection. J Virol. 2015 May;89(9):5032-9. PubMed PMID: 25694603; PubMed Central PMCID: PMC4403456.

B. Positions and Honors

Positions and Employment

2001 - 2005 Graduate Student Instructor, University of Michigan, Department of Molecular, Cellular & Developmental Biology, Ann Arbor, MI

2001 - 2006 Graduate Research Assistant, University of Michigan, Department of Molecular, Cellular & Developmental Biology, Ann Arbor, MI

2006 - 2012 Research Fellow, University of Michigan, Department of Microbiology & Immunology, Ann Arbor, MI

2012 - 2013 Research Investigator, University of Michigan, Department of Microbiology & Immunology, Ann Arbor, MI

2014 - Assistant Professor, University of Alabama at Birmingham, Department of Microbiology, Birmingham, AL

Other Experience and Professional Memberships

2008 - Full Member, American Society for Virology

2010 - 2012 Member, Sigma Xi, The Scientific Research Society

2011 - *Ad Hoc* Reviewer, *PloS One, Journal of Virology, International Journal of Tropical Disease & Health, Antiviral Research, American Journal of Transplantation, Journal of Cardiovascular Disease Research, Applied and Environmental Microbiology*

2014 - Member, American Heart Association

2014 - Member, American Society for Microbiology

2014 - Member, UAB Comprehensive Cancer Center

2014 - Member, UAB Center for AIDS Research

2014 - Editorial Board Member, Clinical Journal of Microbiology & Pathology

Honors

1997 Monsanto Fellowship, Fudan University

2001 Outstanding student fellowships, First Prize, Fudan University

2005 Arnold Ravin-Muriel Rogers Fellowship, National Science Foundation

2006 Rackham Graduate Student Travel Fellowship, University of Michigan

2008 Postdoctoral Travel Award, American Society for Virology

2008 Postdoctoral Fellowship, American Heart Association

2009 Postdoctoral Travel Award, American Society for Virology

2011 Postdoctoral Travel Award, University of Michigan, Department of Microbiology & Immunology

2013 Early Career Investigator Travel Fellowship, PML Consortium

2014 Annual Transplant Symposium Travel Grant, UAB Comprehensive Transplant Institute/Emory Transplant Center

2014 Faculty Development Grant, UAB

2014 CFAR Research Day People's Choice Poster Award, UAB

2015 Travel Grant, International Center for Genetic Engineering and Biotechnology

C. Contribution to Science

1. I have demonstrated the importance of the host DNA damage response (DDR) for productive polyomavirus life cycle and host genome stability during polyomavirus infection: Host DDR had been implicated in polyomavirus life cycle but the detailed roles of the two key players in the DDR pathway, namely ATR and ATM, had not been clearly understood. My work has demonstrated that both ATR and ATM are required for optimal viral replication. Additionally, in the absence of either kinase, the host genome becomes susceptible to polyomavirus-induced damage. I have further demonstrated that the activation of these two kinases is mainly dependent on an active viral DNA replication during polyomavirus infection. These findings have contributed to our basic knowledge of how polyomaviruses replicate and the molecular basis for polyomavirus-induced genome instability. I anticipate that in the long run these discoveries may

facilitate the development of novel inhibitors to combat polyomavirus infection or to treat polyomavirus-induced tumors.

a. Jiang M, Zhao L, Gamez M, Imperiale MJ. Roles of ATM and ATR-mediated DNA damage responses during lytic BK polyomavirus infection. PLoS Pathog. 2012;8(8):e1002898. PubMed PMID: 22952448; PubMed Central PMCID: PMC3431332.
b. Justice JL, Verhalen B, Jiang M. Polyomavirus interaction with the DNA damage response. Virol Sin. 2015 Apr;30(2):122-9. PubMed PMID: 25910481.
c. Verhalen B, Justice JL, Imperiale MJ, Jiang M. Viral DNA replication-dependent DNA damage response activation during BK polyomavirus infection. J Virol. 2015 May;89(9):5032-9. PubMed PMID: 25694603; PubMed Central PMCID: PMC4403456.

2. I have identified essential intracellular trafficking pathways and inhibitors for polyomavirus entry: The early trafficking pathways of polyomavirus in relevant cell types had not been well defined. I have led research projects to show that BK polyomavirus traffics through endosomes, and uses an intact microtubule network to reach the endoplasmic reticulum (ER) where viral disassembly occurs. I have also shown that the virus hijacks the host ER-associated degradation pathway in order to penetrate the limiting membrane. Furthermore, my work has demonstrated that an inhibitor targeting the host Abl kinase is able to block polyomavirus entry through down-regulation of cellular receptors for polyomavirus. These studies have revealed previously unknown trafficking pathways for polyomavirus in primary cells and will form the basis for the development of novel entry inhibitors for polyomavirus infections.

a. Jiang M, Abend JR, Tsai B, Imperiale MJ. Early events during BK virus entry and disassembly. J Virol. 2009 Feb;83(3):1350-8. PubMed PMID: 19036822; PubMed Central PMCID: PMC2620883.
b. Swimm AI, Bornmann W, Jiang M, Imperiale MJ, Lukacher AE, Kalman D. Abl family tyrosine kinases regulate sialylated ganglioside receptors for polyomavirus. J Virol. 2010 May;84(9):4243-51. PubMed PMID: 20181697; PubMed Central PMCID: PMC2863717.
c. Bennett SM, Jiang M, Imperiale MJ. Role of cell-type-specific endoplasmic reticulum-associated degradation in polyomavirus trafficking. J Virol. 2013 Aug;87(16):8843-52. PubMed PMID: 23740996; PubMed Central PMCID: PMC3754070.

3. I have applied quantitative proteomic methods to identify novel ribosome assembly factors in *E. coli*: Ribosome biogenesis is a complex biological process that requires orchestrated assembly of both ribosomal RNAs and proteins. In addition to the core structural proteins, there are also protein components that perform chaperone functions to facility the assembly. I was among one of the first researchers to apply quantitative proteomic approaches to identify such assembly factors in *E. coli*. My research provided novel insight into how ribosome biogenesis is regulated in bacteria and also demonstrated the power of using novel quantitative proteomic approaches to address biological questions. We are now adapting some of the technologies to answer polyomavirus-related questions such as host proteomic changes during infection.

a. Jiang M, Datta K, Walker A, Strahler J, Bagamasbad P, Andrews PC, Maddock JR. The Escherichia coli GTPase CgtAE is involved in late steps of large ribosome assembly. J Bacteriol. 2006 Oct;188(19):6757-70. PubMed PMID: 16980477; PubMed Central PMCID: PMC1595513.
b. Jiang M, Sullivan SM, Walker AK, Strahler JR, Andrews PC, Maddock JR. Identification of novel Escherichia coli ribosome-associated proteins using isobaric tags and multidimensional protein identification techniques. J Bacteriol. 2007 May;189(9):3434-44. PubMed PMID: 17337586; PubMed Central PMCID: PMC1855874.
c. Jiang M, Sullivan SM, Wout PK, Maddock JR. G-protein control of the ribosome-associated stress response protein SpoT. J Bacteriol. 2007 Sep;189(17):6140-7. PubMed PMID: 17616600; PubMed Central PMCID: PMC1951942.

Complete List of Published Work in My Bibliography:
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/47959635/?sort=date&direction=ascending

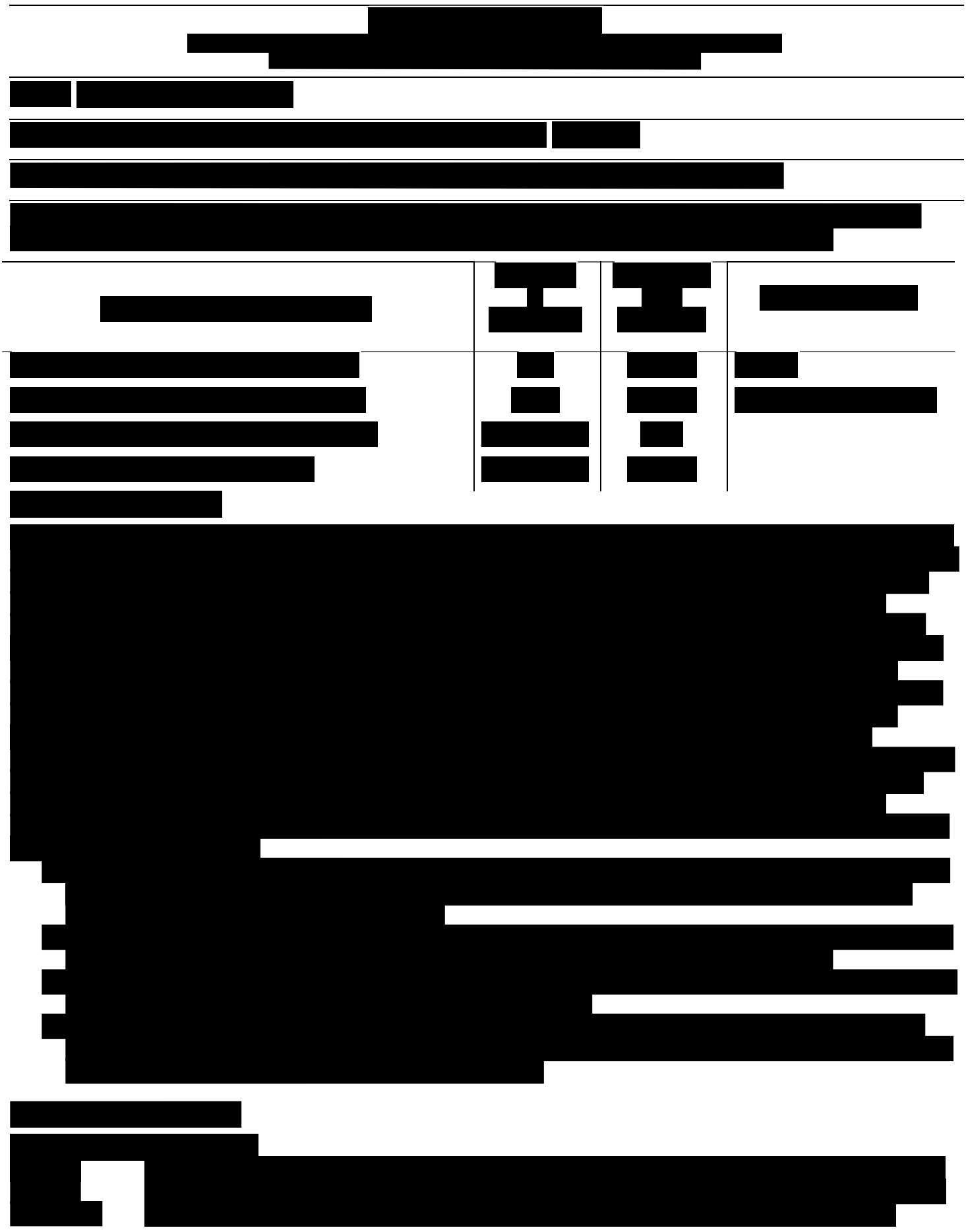
D. Research Support

Ongoing Research Support

[redacted]

Completed Research Support

[redacted]



[redacted]

[redacted]

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
First Name*: Mengxi
Middle Name:
Last Name*: Jiang
Suffix:

2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

● Yes ○ No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ❍ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ❍ Yes ❍ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ❍ Yes ❍ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Modular Budget

OMB Number: 0925-0001

Budget Period: 1

Start Date: 04/01/2016 End Date: 03/31/2017

A. Direct Costs

Funds Requested ($)

Direct Cost less Consortium F&A*	[redacted]
Consortium F&A	
Total Direct Costs*	[redacted]

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	Modified Total Direct Costs	[redacted]	[redacted]	[redacted]
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Steven Zuraf, 301-492-4855

Indirect Cost Rate Agreement Date: 09/25/2014

Total Indirect Costs: [redacted]

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): [redacted]

Funding Opportunity Number: PA-13-302. Received Date: 2015-06-02T17:13:17.000-04:00

PHS 398 Modular Budget

Budget Period: 2			
Start Date: 04/01/2017 End Date: 03/31/2018			
A. Direct Costs			Funds Requested ($)
		Direct Cost less Consortium F&A*	[redacted]
		Consortium F&A	
		Total Direct Costs*	[redacted]
B. Indirect Costs			
	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	Modified Total Direct Costs	[redacted]	[redacted]	[redacted]
2.				
3.				
4.				
Cognizant Agency (Agency Name, POC Name and Phone Number)	DHHS, Steven Zuraf, 301-492-4855			
Indirect Cost Rate Agreement Date	09/25/2014		Total Indirect Costs	[redacted]
C. Total Direct and Indirect Costs (A + B)			Funds Requested ($)	[redacted]

Funding Opportunity Number: PA-13-302. Received Date: 2015-06-02T17:13:17.000-04:00

PHS 398 Modular Budget

Budget Period: 3
Start Date: 04/01/2018 End Date: 03/31/2019

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	[redacted]
Consortium F&A	
Total Direct Costs*	[redacted]

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	Modified Total Direct Costs	[redacted]	[redacted]	[redacted]
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Steven Zuraf, 301-492-4855

Indirect Cost Rate Agreement Date: 09/25/2014

Total Indirect Costs: [redacted]

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): [redacted]

Funding Opportunity Number: PA-13-302. Received Date: 2015-06-02T17:13:17.000-04:00

PHS 398 Modular Budget

Budget Period: 4

Start Date: 04/01/2019 End Date: 03/31/2020

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	[redacted]
Consortium F&A	
Total Direct Costs*	[redacted]

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	Modified Total Direct Costs	[redacted]	[redacted]	[redacted]
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Steven Zuraf, 301-492-4855

Indirect Cost Rate Agreement Date: 09/25/2014

Total Indirect Costs: [redacted]

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): [redacted]

Funding Opportunity Number: PA-13-302. Received Date: 2015-06-02T17:13:17.000-04:00

PHS 398 Modular Budget

Budget Period: 5	
Start Date: 04/01/2020 End Date: 03/31/2021	

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	[redacted]
Consortium F&A	
Total Direct Costs*	[redacted]

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	Modified Total Direct Costs	[redacted]	[redacted]	[redacted]
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Steven Zuraf, 301-492-4855

Indirect Cost Rate Agreement Date: 09/25/2014

Total Indirect Costs: [redacted]

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): [redacted]

Funding Opportunity Number: PA-13-302. Received Date: 2015-06-02T17:13:17.000-04:00

PHS 398 Modular Budget

Cumulative Budget Information

1. Total Costs, Entire Project Period

Section A, Total Direct Cost less Consortium F&A for Entire Project Period ($)	[redacted]
Section A, Total Consortium F&A for Entire Project Period ($)	0.00
Section A, Total Direct Costs for Entire Project Period ($)	[redacted]
Section B, Total Indirect Costs for Entire Project Period ($)	[redacted]
Section C, Total Direct and Indirect Costs (A+B) for Entire Project Period ($)	[redacted]

2. Budget Justifications

Personnel Justification	Budget_Justification_Final.pdf
Consortium Justification	
Additional Narrative Justification	

Funding Opportunity Number: PA-13-302. Received Date: 2015-06-02T17:13:17.000-04:00

BUDGET JUSTIFICATION

Senior/Key Personnel:

Mengxi Jiang, Ph.D., (Principal Investigator; 4.8 calendar months) is an Assistant Professor in the Department of Microbiology at UAB. As Principal Investigator, Dr. Jiang will lead and direct the studies. She will help plan and perform experiments, assist in interpreting results, be responsible for all manuscripts, and ensure that the scientific goals and milestones of the project are achieved. She will work directly with all the members involved in the project to ensure open communication and facilitate progress in the project. She will meet with members of her group individually each week in addition to organizing weekly meetings with the entire laboratory staff in order to evaluate and analytically critique the data to establish future experiments and troubleshoot potential problems.

[redacted]
She has extensive experience in dissecting the molecular interactions required for the cytotoxic action of experimental therapeutics, such as topotecan and inhibitors of TOR signaling, using genetics, biochemistry and mammalian cell culture. She also has expertise in the biochemical and genetic characterization of defects in DNA replication and checkpoint signaling. For this proposal, she will coordinate with Dr. Jiang on 2-D gel and DNA fiber assays. In these studies, she will direct the activities of the Research Associate, Dr. Wright, on the proposed analyses of viral DNA replication intermediates, in 2-D gels and DNA fiber assays. She will assist with data analyses and manuscript preparation.

Personnel for the project:

Joshua Justice, B.S., (Graduate Assistant; 12.0 calendar months) is a second year Microbiology Graduate theme student in the UAB Graduate Biomedical Sciences program. He joined the Jiang lab in May, 2014. He has demonstrated exceptional critical thinking and technical skills in the lab. He has presented his work at the 2014 DNA Tumor Virus meeting and will be presenting again at 2015 American Society for Virology Annual conference. He has one first-author publication from his undergraduate research. Since he joined the Jiang lab, he has already published one first-author review article and has contributed to one research paper. He will be responsible for Specific Aim 1 to examine the role of the host mismatch repair complex during polyomavirus infection. He will also be involved in parts of Specific Aim 3 to examine whether polyomavirus-induced host DNA damage is coupled with mitosis.

Brandy Verhalen, Ph.D., (Research Assistant**;** 6.0 calendar months) has received her Ph.D. degree from SUNY Upstate Medical University and completed her postdoctoral training at Vanderbilt University. She has been in the Jiang lab for over one year. Brandy has extensive molecular biology and biochemistry experience as evidenced by her 7 publications in high-impact journals over the last four years. She will be responsible for Specific Aim 2 to identify viral DNA triggers that lead to DDR activation. She will also work with Dr. Wright on Specific Aim 3 to determine whether large T antigen causes replication stress in host cells.

Christine Wright, Ph.D., (Research Associate; 2.4 calendar months) has extensive expertise in the studies of cytotoxic chemotherapeutics. For the past 8 years, she has worked in the [redacted] lab on various aspects of cellular responses to DNA damage and replicative stress, and has acquired considerable experience in the study of DNA replication intermediates. She will undertake the mechanistic studies of viral DNA replication intermediates and fork progression using 2-D gel and DNA fiber technology and will work closely with Drs. [redacted] and Jiang in discussions of date interpretation and study design.

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	Specific_Aims_Final.pdf
3. Research Strategy*	Research_Strategy_Final.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	
6. Inclusion of Women and Minorities	
7. Inclusion of Children	
Other Research Plan Sections	
8. Vertebrate Animals	
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	
12. Letters of Support	Letters_of_Support_Final.pdf
13. Resource Sharing Plan(s)	
Appendix (if applicable)	
14. Appendix	

Tracking Number: GRANT11922341

Funding Opportunity Number: PA-13-302. Received Date: 2015-06-02T17:13:17.000-04:00

SPECIFIC AIMS

Polyomaviruses are ubiquitous in human populations and cause serious life-threatening diseases including cancer, particularly in immunocompromised individuals. Currently there are no specific treatments or prophylactic approaches to target this family of viruses and their related diseases. Our *long-term goals* are to elucidate the fundamental mechanisms of polyomavirus replication, to understand how these viruses hijack and subvert normal host cellular processes to facilitate viral replication, and to dissect how these interactions may result in polyomavirus-induced oncogenesis.

One of the emerging concepts in the polyomavirus field is that cellular DNA damage response (DDR), which is a network of cellular pathways required for maintaining genome integrity, is essential for viral replication. The molecular details of DDR manipulation by polyomaviruses and the exact functions of the DDR during viral replication are not well characterized. On the host side, polyomavirus infection—in particular the virally-encoded T antigens—have been shown to cause host cell genomic instability, which could ultimately lead to oncogenesis. Currently, the underlying molecular source of such genomic instability remains unclear.

Using BK polyomavirus (BKPyV) and a primary renal proximal tubule epithelial cell culture infection model, we have recently demonstrated that (a) BKPyV activates and hijacks the DDR to promote viral replication, and that (b) BKPyV infection induces host chromosome damage, but only in the absence of viral DNA replication or DDR activation. These findings lead us to propose a novel balanced model to link virus replication, DDR activation and host genome instability together (**Fig. 1**). The *central hypothesis* of this model is that an activated DDR is important for both facilitating virus replication and maintaining host genomic stability during polyomavirus infection. We propose that viral DNA replication actively drives productive infection by inducing the host DDR, thereby stabilizing viral replication forks and preventing replication-induced DNA damage on the viral genome. We also propose that the DDR is essential to prevent host genome instability during polyomavirus infection through G2/M cell cycle arrest. To test this central hypothesis, we propose the following three *specific aims (**Fig. 1**)*:

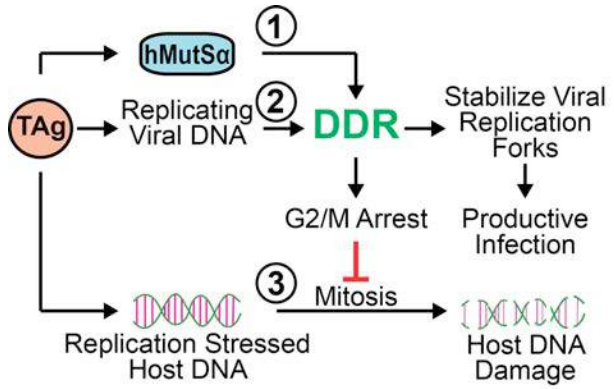


Figure 1. Model of BKPyV replication, DDR activation, and host genome instability. See text for details. The numbering refers to the three specific aims that will be pursued in this proposal.

Aim 1. To define the role of host mismatch repair proteins in polyomavirus replication and polyomavirus-induced DDR activation. Our recent quantitative nuclear proteomic analysis identified that the host mismatch repair (MMR) proteins, in particular the hMutSα complex, are required for BKPyV replication and virus-induced DDR activation. We *hypothesize* that the MMR proteins serve as novel signaling molecules to facilitate DDR activation during polyomavirus infection. The goal of this aim is to determine how the MMR proteins drive productive viral infection through DDR activation by examining viral replication products, interactions between the large T antigen (TAg) and DDR proteins, and recruitment of DDR proteins to viral DNA.

Aim 2. To determine the viral DNA triggers that activate the DDR upon polyomavirus infection. The current view in the polyomavirus field is that viral oncogenes activate the DDR. In contrast, our results suggest that a full DDR activation is dependent on viral DNA synthesis. We *hypothesize* that replicating viral DNA serves as a major trigger for DDR activation during infection. In this aim we will identify viral DNA structures that are recognized by host cells as damage signals to induce DDR activation.

Aim 3. To elucidate the molecular mechanism by which polyomavirus induces host genome instability. We *hypothesize* that via G2/M cell cycle arrest, the DDR is able to prevent host genome instability caused by TAg-induced host DNA replication stress. The goal of this aim is to investigate how TAg affects host cell DNA replication using a novel replication stress assay and to determine whether the cellular DNA damage caused by TAg is coupled with mitotic entry.

Together, our proposed studies will have a broad impact on the field by dissecting the crucial roles that the DDR play in promoting viral replication and maintaining host genome stability. In the long term, these studies may reveal novel therapeutic host targets to treat polyomavirus-related diseases.

RESEARCH STRATEGY

A. SIGNIFICANCE

Polyomaviruses are a family of small DNA tumor viruses with a ~5kb circular double-stranded DNA genome. The first two human polyomaviruses, BK polyomavirus (BKPyV) and JC polyomavirus (JCPyV), were discovered in 1971 (1, 2). Both viruses cause severe disease under certain immunosuppressed conditions (3). In the setting of kidney transplantation, BKPyV reactivates in up to 10% of transplant recipients, which can lead to severe polyomavirus-associated nephropathy and subsequent graft loss (3). Since a total of 13,299 kidney transplants were performed in US alone in 2014 (National Kidney Foundation), BKPyV-related disease represents a significant problem. While not proven, there have also been reports linking BKPyV infection to the development of urothelial and renal tubular malignancies in these patients (4-11). JCPyV is responsible for causing progressive multifocal leukoencephalopathy, a major life-threatening complication in patients with several underlying immunosuppressive conditions (12). In the past decade—due to significant improvements in DNA amplification and sequencing—a number of new polyomaviruses were discovered from various human tissues and samples (13). Among them, Merkel cell polyomavirus (MCPyV) has now been confirmed to be the causative agent for most human Merkel cell carcinomas, an aggressive human skin cancer (14-16). So far, no specific anti-viral treatments or vaccines have been developed for this group of medically important viruses.

There are several critical gaps in our knowledge of the basic biology of these viruses. First, how exactly are these viruses replicated in host cells? Over the years, Simian Virus 40 (SV40) DNA replication has been pursued as a model system to understand eukaryotic chromosome replication, and the bidirectional replication mechanism is considered a common feature between viral and eukaryotic cell DNA replication (17). In spite of the many similarities, polyomavirus DNA replication is not a sheer mimic of host cell DNA replication but exhibits several unique characteristics. One emerging concept in the polyomavirus field is that the virus manipulates the host DNA damage response (DDR) pathways to promote a productive viral replication. The DDR is composed of cellular signaling cascades that help maintain genome integrity and repair various lesions occurring on the DNA. The molecular functions of the DDR during viral replication, however, are not fully understood. Second, do these viruses cause genomic instability, and if yes, how? Recently, genomic instability induced by polyomavirus has been postulated as a potential mechanism for virally encoded large T antigen (TAg)-induced oncogenesis (18-21). The source of such TAg-induced genome instability, however, remains elusive in the field. Our research has now identified a link between productive viral infection and host genome instability through the regulation of the DDR. The major goals of this proposal are to define the importance of the DDR in polyomavirus replication and host genome stability, as well as to elucidate DDR activation mechanisms by both host and viral factors. We think that the proposed research is significant because it will greatly advance our understanding of polyomavirus biology and may in the long term reveal potential targets to prevent or treat diseases that are caused by these viruses.

Previously, we established a primary human renal proximal tubule epithelial (RPTE) cell culture system for BKPyV as a model system to study polyomavirus lytic life cycle (22-25). This is the cell type where BKPyV lytic infection is observed in humans (26). Using this system, we have begun to dissect the functional importance of DDR during viral lytic replication and we have now identified novel host and viral triggers responsible for inducing the DDR. In addition, we have uncovered host genomic instability caused by viral infection. Interestingly, this instability only appears when the ability of the virus to replicate its viral DNA or DDR activation is compromised (27). Therefore, the proposed work is significant at many levels:

A1) Our proposal investigates novel mechanisms for polyomavirus DNA replication: Using powerful quantitative proteomic approaches we have identified host mismatch repair (MMR) proteins, especially the hMutSα complex, as novel host factors required for DDR activation during viral infection. Our proposed study will determine whether TAg interaction with the MMR proteins results in the recruitment of DDR proteins to replicating viral DNA, thus activating the DDR. This will be highly significant as it will be the first characterization of the involvement of host MMR system during productive polyomavirus infection through DDR activation, which will contribute to our knowledge about the fundamental replication mechanism of polyomaviruses.

A2) Our proposal examines uncharacterized viral DNA triggers for DDR activation: The current view in the polyomavirus field is that the viral TAg activates the DDR as shown in several transformed cell lines (18, 19, 28). In contrast to results obtained from transformed cell lines, our preliminary results provide compelling evidence that TAg alone only activates a minimal DDR in primary cells and that a full DDR activation is dependent on viral DNA replication (27). These findings point to the uncharacterized role of polyomavirus DNA replication and replication-associated DNA damage in triggering DDR. Our proposed studies will determine

what DNA structures present on viral DNA during replication lead to DDR activation. This is important for two reasons: (i) DDR activation appears to be essential to produce infectious viral progeny. Therefore, dissecting the activation mechanism will allow us to further understand the requirements for a productive infection. (ii) As will be discussed below, DDR inactivation is closely linked with the appearance of host DNA damage caused by polyomavirus. A lack of DDR activation may lead to accumulation of detrimental host DNA damage. Understanding how DDR is activated in polyomavirus-infected cells will have implications in preventing host DNA damage caused by polyomaviruses.

A3) Our proposal aims to identify the molecular sources of TAg-induced host cell DNA damage: It has been postulated that TAg is able to induce replication stress and mitotic defects (29). The effect of TAg on host DNA replication has not been directly measured and whether the TAg-induced host DNA damage is associated with mitosis has not been determined. This is important because it allows us to understand the molecular basis of such DNA damage. This knowledge may also be applied in the future to selectively induce host DNA damage in polyomavirus-infected cells to help eliminate infected cells.

B. INNOVATION

B1) Our central hypothesis is conceptually innovative: we propose that polyomavirus modulates the host DDR to facilitate productive viral replication and to prevent host DNA damage. Towards this hypothesis, we have identified host and viral factors that are essential for DDR activation. Our study has also revealed a novel connection between viral DNA replication, DDR activation, and virus-induced host genome instability. These are all original concepts in the field of polyomavirus virology and tumor biology.

B2) The model system in which we will test our hypothesis is unique and highly relevant. Our proposed research will be carried out in our well-characterized primary RPTE cell culture model. This is the best cell system to test our hypotheses for several reasons: First, RPTE mimics the environment that is encountered by BKPyV *in vivo* (23). How viruses establish infection and the effects of viral infections on host cells can vary greatly depending on the cell type, therefore we believe that results generated using this system are pathologically relevant. Second, it is also extremely important to study the DDR and its contribution to viral replication in normal cells with intact cell cycle checkpoint regulation, as DDR characteristics are very different in transformed cell lines (30). Finally, our system is fully permissive to BKPyV replication and is therefore more relevant than the artificial systems that have been developed to study newly identified polyomaviruses including MCPyV (31).

B3) Our approach is technically innovative by bridging together methodologies in virology and DNA damage fields. We have teamed up with experts in the DNA damage and repair field to adapt a series of approaches including two-dimensional agarose gels and DNA fiber assays to examine viral DNA replication intermediates and the effects of viral infection on host DNA replication. All of these innovative methods will allow us to address important yet previously unexplored questions related to polyomavirus-induced DNA damage and viral replication strategy.

C. APPROACH

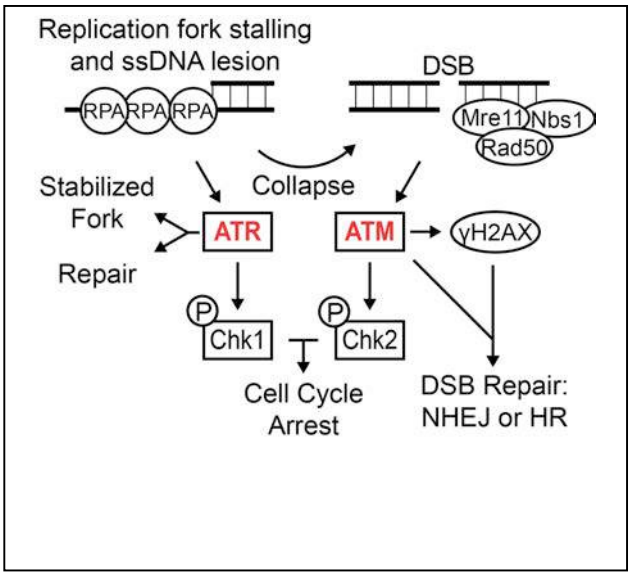
Based on our preliminary results, we propose the following model for the interplay between BKPyV DNA replication, DDR activation, and host genome instability (**Fig. 1**): 1) During productive BKPyV infection, TAg expression upregulates the mismatch repair hMutSα complex, which mediates DDR activation. 2) TAg-mediated viral DNA replication is the main driving force for DDR activation and the activated DDR helps stabilize viral replication forks and repair replication-associated viral DNA damage. 3) In the absence of viral DNA replication and its associated DDR activation, TAg-induced host cell DNA replication stress coupled with mitosis can lead to genome instability; however, this genome instability is prevented during normal infection through G2/M arrest resulting from virus replication-induced DDR activation. These three independent yet related specific aims are directed at understanding: (Aim 1) the roles of host mismatch repair proteins in polyomavirus replication, DDR activation, and host genome instability; (Aim 2) the viral DNA triggers that lead to DDR activation during polyomavirus infection; (Aim 3) the mechanisms of TAg-induced DNA damage and how the damage is connected to DDR activation.

C1) INTRODUCTION

Polyomavirus DNA replication. Following polyomavirus entry into a host cell, the viral DNA genome is delivered into the nucleus where replication occurs. The viral TAg is a multifunctional protein that orchestrates the viral replication cycle (32). TAg binds directly to the viral origin of DNA replication through its origin-binding domain (OBD) and forms a double-hexamer. TAg also possesses helicase activity that allows it to unwind viral DNA to enable viral replication. Because of the small viral genome size and hence limited coding capacity, viral

replication relies heavily on host replication machinery. In particular, TAg recruits replication protein A (RPA), DNA polymerase α-primase, and topoisomerase I to initiate replication of viral DNA. Even though the basic replication mechanism is considered similar between viral and eukaryotic DNA replication, viral DNA replication possesses its own distinct features. For example, polymerase ε is important for extending the leading strand during eukaryotic DNA replication, but it appears to be dispensable for polyomavirus DNA synthesis (33). Since TAg itself is a helicase itself, polyomavirus replication is not dependent on the cellular Cdc45/Mcm2-7/GINS helicase complex (34). Moreover, recent findings suggest that there are additional unknown host components, especially the DNA damage sensing and repair proteins, playing important yet undefined roles during viral replication. For example, polyomavirus infections have been shown to recruit several proteins involved in the DDR, such as Mre11 and Rad51, into TAg-positive viral replication foci in the nucleus (22, 35). How these viruses activate the DDR and the precise molecular functions that DDR proteins serve during viral replication still remain poorly understood.

TAg and cellular transformation. It is well established that TAg is capable of inhibiting normal functions of the tumor suppressors retinoblastoma protein (pRb) and p53. TAg binding to pRb relieves pRb inhibition of E2F, which is a transcription factor for many genes that are important for S phase progression and DNA synthesis (36, 37). TAg stabilizes and functionally inactivates p53, thereby avoiding p53-dependent apoptosis (38, 39). Although these are important avenues of TAg driving tumorigenesis, inactivating pRb and p53 is not sufficient for cellular transformation by TAg (40) and additional oncogenic activities of TAg are required. Recently, a number of polyomavirus TAgs have been reported to induce host DNA damage as determined by either comet assays or sensitivities to specific-DNA damaging treatments (18-21). Since chromosome instability is a hallmark for many cancer cells, DNA damage induction could be another mechanism contributing to TAg-induced oncogenesis.



DNA damage response (DDR). DDR is considered the guardian of the genome. There are two major phosphatidylinositol 3-kinase-related kinases: ataxia-telangiectasia mutated (ATM), and ATM and Rad-3-related (ATR). These kinases govern the activation of the DDR pathways (**Fig. 2**). ATR is activated by single-stranded DNA (ssDNA) lesions and is important for resolving replication stress from conditions such as stalled replication forks (41). ATM responds mainly to double-stranded breaks (DSBs) resulting from conditions including ionizing radiation (IR) (42) or collapsed replication forks (43). Both ATR and ATM can phosphorylate and activate numerous downstream targets that are involved in DNA repair and cell cycle arrest. These include the checkpoint kinases Chk1 (mostly by ATR) and Chk2 (mostly by ATM), ATM itself (from ATM activation-induced auto-phosphorylation), and a histone variant H2AX (termed γH2AX when phosphorylated). γH2AX is thought to mark the sites of DSBs (44) and is the first step in recruiting and localizing DNA repair proteins (45). Activated checkpoint kinases, especially Chk1, play essential roles in coordinating cellular responses to replication stress. These include suppressing inappropriate replication origin firing, stabilizing stalled replication forks, and triggering G2/M arrest (46, 47). For the past decade, DDR has become an intensive area of research in virology as a number of viruses, especially many DNA viruses, have been shown to modulate components of the DDR pathway thereby promoting viral replication or oncogenesis (48, 49).

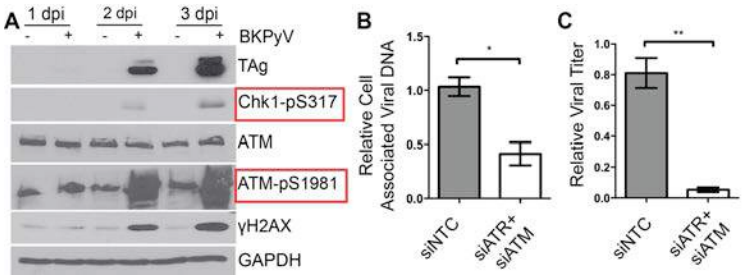


Figure 3. BKPyV activates the DDR and requires the DDR for productive infection. (A) RPTE cells were infected with BKPyV at an MOI of 5 infectious units (IU)/cell. Whole cell lysates were harvested at 1-3 days post infection (dpi) and immunoblotted for ATR and ATM activation markers. (B+C) RPTE cells were transfected with indicated siRNAs followed by BKPyV infection at an MOI of 0.5 IU/cell. Cell-associated viral DNA (B) and virus titer (C) were determined at 2 dpi by a real-time PCR assay and a fluorescence IU assay, respectively. Data were normalized to no siRNA control. *, p< 0.05; **, p<0.01.

C2) PRELIMINARY STUDIES

DDR is important for productive viral infection and host genome maintenance during polyomavirus infection. Our preliminary results show that: 1) BKPyV infection is able to activate both ATR- and ATM-dependent DDR signaling pathways in RPTE cells as determined by an increase in Chk1-pS317 (ATR activation marker) and ATM-pS1981 (ATM activation marker) in BKPyV-infected cells (**Fig. 3A, previous page**). 2) Using siRNA knockdowns, we have shown that double knockdown of ATR and ATM partially inhibited viral DNA replication as determined by a real-time PCR assay (**Fig. 3B**) and more dramatically inhibited infectious viral progeny production (**Fig. 3C**). 3) Since the real-time PCR assay can only measure the quantity of the viral DNA that is recognized by the primer pair, it does not reveal whether these viral DNAs are intact genomes that can be packaged into virions. To determine whether there was a change in the quality of the viral DNA, which may explain a greater defect with the infectious viral titer, we used Southern blotting with a specific viral probe to examine the nature of the viral DNA. We observed that inhibition of the DDR by siRNA knockdowns resulted in an accumulation of higher molecular weight dimeric and concatemeric viral DNA products (**Fig. 4A**). This is consistent with published work on SV40 (50), suggesting that the DDR functions to maintain viral replication fork integrity and prevent the accumulation of abnormal replication products. In the absence of the DDR, stalled viral replication forks cannot be stabilized and therefore are inclined to collapse. Alternatively, an unrepaired DSB at one replication fork can leave the other fork to replicate unidirectionally. Both of these events can lead to an accumulation of aberrant viral DNA intermediates (**Fig. 4B**).

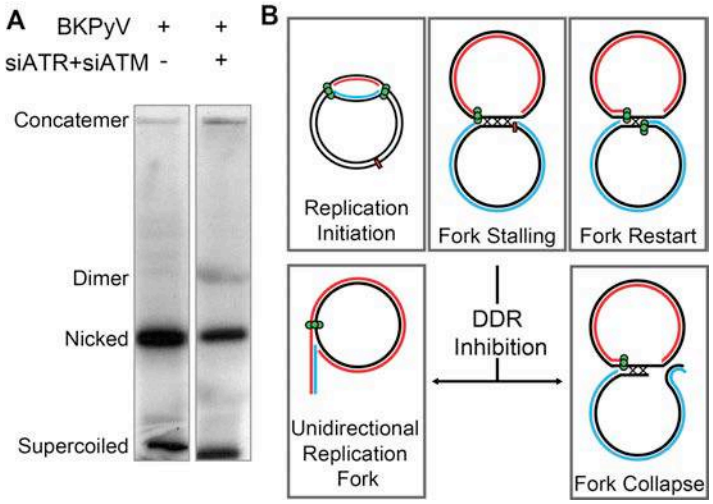


Figure 4. DDR maintains replication fork integrity during polyomavirus infection. (A) Control cells or cells that were knocked down for ATR and ATM were infected with BKPyV at an MOI of 0.5 IU/cell. Total DNA were isolated at 2 dpi and subjected to Southern blotting using a viral-specific probe. (B) Model for DDR contribution to viral replication integrity. TAg (green circle) unwinds viral DNA to initiate viral replication. When a replication fork stalls, DDR inhibition can lead to either replication fork collapsing or unidirectional replication. Both events can cause an accumulation of aberrant viral replication intermediate products.

Since DDR is important to maintain genomic stability under many genotoxic conditions, we also examined whether DDR inhibition affected host genome stability in the context of polyomavirus infection. Knockdown of ATR and ATM in BKPyV-infected cells resulted in abnormal nuclear morphology as seen by an increase in TAg-positive micronuclei formation (**Fig. 5A**). Micronuclei are biomarkers of genotoxic stress and chromosomal instability, and they are usually formed from mis-segregated chromosomes or chromosome fragments (51). To further confirm that the observed micronuclei represent true DNA damage, we performed metaphase spread experiments to visualize direct damage on the host DNA. We consistently observed that a large proportion of the metaphases became severely damaged and displayed a “shattered metaphase” appearance in BKPyV-infected cells when the DDR was inhibited (**Fig. 5B+C**). This damage was restricted to BKPyV-infected cells and was not detected in mock-infected cells that were knocked down for ATR and ATM. Based on these findings, we conclude that the DDR is important for both productive polyomavirus infection and the maintenance of host genome stability during infection. Most of these data have been published (22).

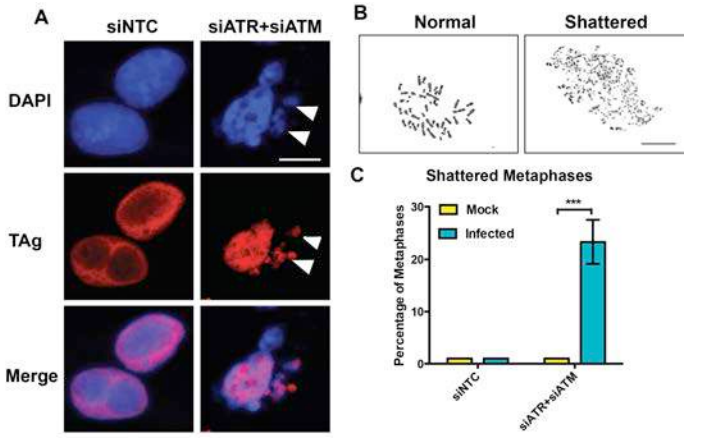


Figure 5. DDR inhibition in BKPyV-infected cells causes host DNA damage. (A) RPTE cells were transfected with the indicated siRNAs and infected with BKPyV at an MOI of 0.5 IU/cell. Cells were fixed at 3 dpi and stained for DAPI (blue) and TAg (red). Arrowheads point to micronuclei. Bar, 10 µm. (B) Representative pictures of normal and shattered chromosomes. (C) Cells were transfected and infected as in (A). Shattered metaphases were scored at 3 dpi. ***, $p<0.001$.

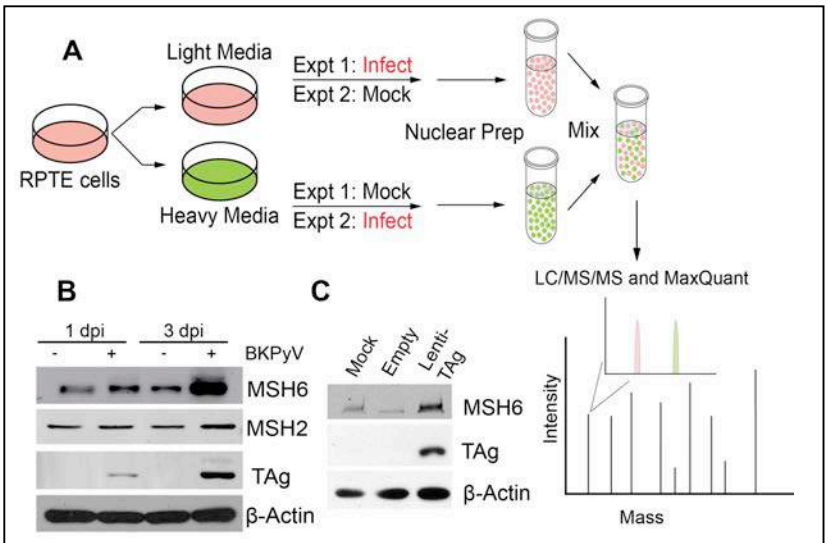


Figure 6. Quantitative proteomics identifies that host mismatch repair proteins are upregulated by BKPyV. (A) SILAC nuclear proteomic analysis set up. RPTE cells were labeled with light or heavy media. In two independent repeats, differentially labeled cells were infected with BKPyV to ensure that any change observed was not due to the differential labeling. Nuclear proteins were isolated, mixed, and subjected to 2D LC-MS/MS. (B+C) RPTE cells were infected with BKPyV at an MOI of 0.5 IU/cell (B) or transduced with a lentivirus expressing TAg or an empty control lentivirus (C). Total proteins were immunoblotted for indicated proteins.

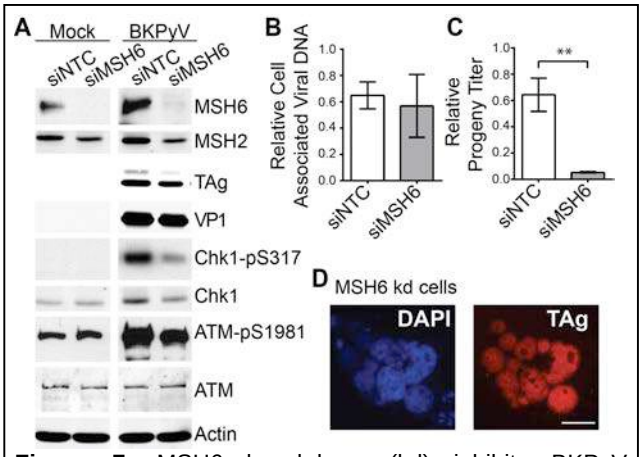


Figure 7. MSH6 knockdown (kd) inhibits BKPyV productive infection and DDR activation. RPTE cells were transfected with siRNAs and infected with BKPyV as in Fig. 3. (A) Western blots of viral proteins and DDR markers. (B+C) Viral DNA and infectious viral titer were determined as in Fig. 3. All data were normalized to no siRNA control. **, p<0.01. (D) MSH6 kd caused micronuclei formation in TAg-positive cells. Bar, 20 µm.

Identification of host mismatch repair (MMR) proteins as important players for polyoma–virus replication and DDR activation. To determine further which nuclear components are required for BKPyV replication, we used a quantitative proteomic approach, stable isotope labeling by amino acids in cell culture (SILAC) coupled with two-dimensional liquid chromatography and tandem mass spectrometry (2D LC-MS/MS) to specifically examine total nuclear proteome changes in BKPyV-infected cells compared with mock-infected cells (**Fig. 6A**). We grew RPTE cells in defined medium supplemented with either light amino acids (^{12}C, ^{14}N) or heavy amino acids (^{13}C, ^{15}N) under conditions where ~98% labeling efficiency was achieved. We infected one population with BKPyV (either light or heavy-labeled cells in two separate experiments), isolated the nuclear fractions from both mock and infected cells, mixed them and subjected the nuclear proteins to 2D LC-MS/MS (**Fig. 6A**). Identical peptides of different stable-isotope composition were differentiated and peak intensity ratios were used for quantitation. From the proteomic screen, we identified that the host mismatch repair (MMR) proteins are among the most significantly upregulated group of proteins during BKPyV infection. The MMR system normally corrects DNA mismatches during DNA replication and it is highly conserved from prokaryotes to humans. MSH6 and MSH2 form the hMutSα heterodimer, which preferentially recognizes base-base mismatches and short insertion/deletion (in/del) loops. MSH2 also interacts with MSH3 to form the hMutSβ complex that recognizes larger in/del loops (52, 53). Our proteomic data show that MSH6 is upregulated by 4.3-fold and MSH2 is upregulated by 2.7-fold by BKPyV infection. We confirmed the upregulation of MSH6 and MSH2 in BKPyV-infected cells by Western blotting (**Fig. 6B**), and we further showed that TAg expression alone was sufficient to increase MSH6 (**Fig. 6C**). What is more interesting is that in addition to its function in mismatch repair, hMutSα, particularly MSH6, has recently been shown to be important for DNA damage signaling and DNA repair pathways (54). This raises the possibility that the host MMR system may also mediate BKPyV-induced DDR.

Since MSH6 is one of the highly upregulated proteins upon BKPyV infection, we decided to focus on the role of hMutSα during polyomavirus infection. We used siRNA targeting MSH6 to specifically inactivate the hMutSα complex and assessed the effects on BKPyV gene expression, viral DNA level, infectious viral titer, and DDR activation (**Fig. 7**). Our data revealed that inactivation of the hMutSα complex did not significantly change either early viral protein TAg or late viral protein VP1 levels (**Fig. 7A**). Viral DNA levels were not affected either (**Fig. 7B**); however, the amount of infectious viral progeny was greatly reduced with MSH6 knockdown (**Fig. 7C**). We also examined whether the hMutSα complex regulates ATR or ATM-mediated DDR activation. There is a noticeable decrease in Chk1-pS317 and a slight decrease in ATM-pS1981 upon MSH6 knockdown, indicative of a defect in DDR activation (**Fig. 7A**). Additionally, we detected micronuclei and

aberrant TAg staining patters in BKPyV-infected, MSH6 knocked down cells (**Fig. 7D**). Both the infectious viral titer and the nuclear morphology phenotypes are similar to those seen in infected cells lacking DDR signaling (**Fig. 3 and 5**), suggesting that MSH6 or the hMutSα complex contributes to ATR-mediated, and to a lesser extent, ATM-mediated DDR responses during polyomavirus infection.

DDR activation during polyomavirus infection is depedent on viral DNA replication. Apart from host factors, we also went on to determine whether there are any viral components that trigger DDR activation. The current view in the field is that TAg is a major inducer for DDR activation, as has been suggested with polyomavirus infections in several immortalized cell lines (18, 19, 28). Our results, however, indicate the involvement of viral DNA in DDR activation (**Fig. 8**). When we delivered TAg into RPTE cells using lentivirus transduction, we found that TAg expression alone cannot fully activate the ATR response as demonstrated by a minimum induction of Chk1-pS317 compared with infection conditions that have similar levels of TAg (**Fig. 8A,** compare lane 3 with lane 5). We cannot determine the contribution of TAg itself to ATM activation since the empty lentivirus also induces an ATM response (**Fig. 8A**). To further determine whether viral DNA replication is required for DDR activation, we constructed a TAg mutant virus (D501A) based on a homologous SV40 helicase mutant (55). This mutation abrogates TAg helicase activity, while leaving other functions of TAg such as stabilizing p53 intact (55). We were able to grow this mutant virus in the HEK-293TT cell line, which expresses a high-level of SV40 TAg that can complement the BKPyV TAg mutation (56, 57). When we used this mutant virus to infect RPTE cells, we found that it was unable to replicate viral DNA as expected (**Fig. 8B**). Interestingly, this mutant virus cannot activate either ATR or ATM-mediated DDR pathways (**Fig. 8C**). These data suggest that although the presence of viral oncogenes such as TAg may contribute to DDR activation, viral DNA replication is the major trigger for full DDR activation during polyomavirus infection. These data were recently published in (27).

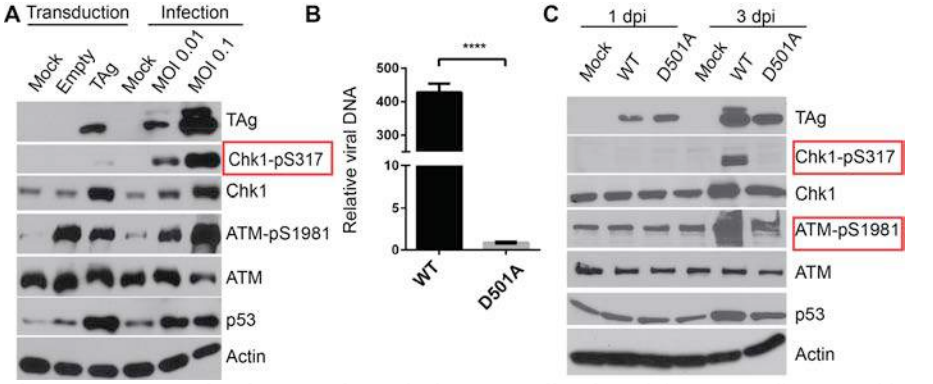


Figure 8. DDR activation requires viral DNA replication. (A) RPTE cells were either transduced with empty or TAg-expressing lentivirus, or infected with BKPyV at the designated MOIs for 3 days. Whole cell lysates were immunoblotted for DDR markers. (B+C) RPTE cells were infected with wild-type or TAg D501A mutant BKPyV at an MOI of 0.5 IU/cell. (B) Viral DNA level was measured by qPCR at 3 dpi and normalized to input DNA at 1 dpi. ****, p<0.0001. (C) Whole cell lysates were harvested at 1 dpi and 3dpi, and immunoblotted for DDR markers.

An inverse relationship between DDR activation and host DNA damage during polyomavirus infection. We observed an intriguing inverse correlation between viral DNA replication and the presence of host DNA damage (**Table 1**). During normal infection when DDR is activated, we do not detect host DNA damage (22). When the RPTE cells were infected with the TAg D501A virus, viral DNA could not be replicated and there was no DDR activation (**Fig. 8C**); however, host DNA damage accumulated with this mutant virus as shown by a comet assay and the formation of micronuclei (27). These phenotypes are similar to those seen in cells only expressing TAg itself. In those cells ATR-mediated DDR was only minimally activated (**Fig. 8A**) and there was an accumulation of micronuclei (**Table 1** and data not shown), indicative of host DNA damage. All of these results suggest that host DNA damage is only evident when DDR is not fully activated during polyomavirus infection.

	Viral DNA replication	DDR activation	Host DNA damage
Normal BKPyV infection	**+**	**+++**	**-**
Replication-deficient (TAg D501A) mutant virus infection	**-**	**-**	**+**
TAg expression alone	**-**	**+**	**+**

Table 1. Summary comparison of WT virus infection, TAg D501A mutant virus infection, and TAg expression by lentivirus.

Overall, our preliminary results demonstrate the importance of the DDR for both viral replication and host genome stability during polyomavirus infection. We have identified novel host (hMutSα) and viral (replicating viral DNA) components that contribute to DDR activation. These studies form the basis for our proposed research to examine further the relationship between viral DNA replication, DDR activation, and host genome stability.

C3) RESEARCH PLAN

Aim 1. To define the role of host mismatch repair (MMR) proteins in polyomavirus replication and polyomavirus-induced DDR activation. The goal of this Aim is to test the *hypothesis* that the MMR proteins promote polyomavirus replication and host genome maintenance through DDR activation. To address this hypothesis, we will: (i) determine whether MMR proteins are important to maintain viral replication integrity and host genome stability during infection; (ii) determine the mechanism of MMR-mediated DDR activation during viral replication.

Aim 1.1 Determine whether MMR proteins are required for viral replication integrity and host genome stability during BKPyV infection. *Rationale:* One emerging concept in the DNA damage field is that MMR proteins, especially the hMutSα complex, are critical for activating certain DDR signaling pathways and for the repair of DSBs (54). Both MSH6 and MSH2 have been found to interact directly with ATR and have been implicated in the recruitment of ATR to sites of DNA damage to initiate ATR-mediated DDR and checkpoint signaling (58-61). These results are consistent with our findings that a deficiency in MSH6 during BKPyV infection decreases the Chk1-pS317 level (**Fig. 7A**). We *hypothesize* that hMutSα is a key regulator of DDR, especially for ATR activation during polyomavirus replication. Since aberrant viral replication products and host genome damage are two prominent phenotypes that we observed when BKPyV-infected cells were inhibited for DDR, we will first examine whether hMutSα is important for regulating these two processes in infected cells.

Experimental Design. We will perform siRNA knockdown experiments for either MSH6 or MSH2 followed by mock or BKPyV infection. We will then perform the following three experiments (i) isolate total DNAs and subject them to traditional one-dimensional electrophoresis and Southern blotting using a viral specific probe to determine whether there is an accumulation of aberrant viral DNA; (ii) determine the degree of host DNA damage by metaphase spread experiments as in **Fig. 5C**; (iii) perform cell cycle analysis using propidium iodide staining followed by flow cytometry.

Expected Outcomes. (i) If our hypothesis is correct, we expect to see an accumulation of larger viral DNA products in the MMR knockdown cells, which will be similar to those seen in infected but DDR-deficient cells (**Fig. 4A**). If we detect these products, we will examine in further detail the molecular nature of these viral DNA molecules. We will then use two-dimensional agarose gel electrophoresis coupled with Southern blotting to resolve replication intermediates (62). In this method, restriction enzyme-digested DNAs are first separated by mass in the first dimension, followed by separation by topology in the second dimension. This analysis will allow for the resolution of various intermediates including unidirectional replication products and collapsed replication forks (**Fig. 4B**). Infected RPTE cells that are knocked down for ATR and ATM will serve as positive controls. It is also highly likely that ATR and ATM may contribute differently to viral replication integrity as suggested by Fanning's work (50). Therefore we will perform these analyses with single knockdown and different double knockdown combinations of hMutSα, ATR, and ATM to establish the individual and combinatorial roles of these proteins during virus replication. Dr. [redacted]i of the Department of Pharmacology and Toxicology at UAB is a leading expert in the DNA damage field and has extensive experience with this technique examining eukaryotic DNA replication intermediates, and therefore she will serve as a co-investigator and assist us to adapt these assays for viral DNA (see attached letter). (ii) We also expect to see an increase in DNA breaks and gaps as well as "shattered metaphases" of host DNA in MMR-deficient infected cells, which will suggest a DDR-deficient phenotype. (iii) One of the functional outcomes of DDR activation is cell cycle arrest to prevent DNA damage being passed onto daughter cells before the cells repair the damage (63). Consistent with this, we have detected that super G2 polyploid (>G2) cells accumulate during infection and that such accumulation is abolished when DDR is inhibited (**Fig. 9**). If the hMutSα complex is involved in DDR signaling, we expect that the G2/M arrest will be abrogated in the absence of hMutSα in infected cells.

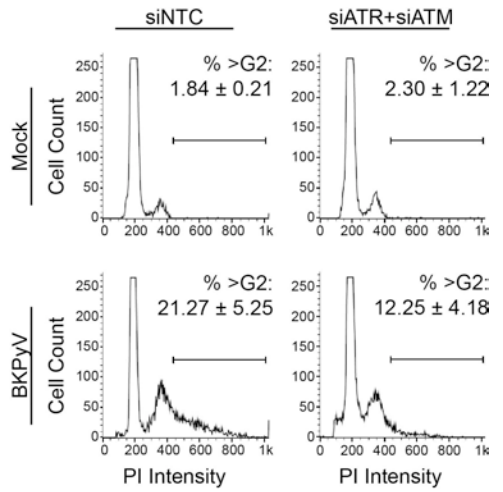


Figure 9. DDR is required for BKPyV-induced G2/M arrest. RPTE cells were transfected with indicated siRNAs and infected with BKPyV at an MOI of 5 IU/cell. At 3 dpi, cells were stained with propidium iodide (PI) followed by flow cytometry.

If we detect the above described phenotypes which are indicative of the involvement of hMutSα in activating the DDR during

infection, we will then perform the following experiments: First, we will use lentivirus to introduce an siRNA-resistant form of MSH6 or MSH2 into RPTE cells and determine whether the expression of these constructs will rescue the phenotype both in virus production and host genome stability. Second, we will determine whether the MMR-mediated DDR activation is linked to the mismatch repair function of the hMutSα complex. During mismatch repair, after hMutSα recognizes and binds to DNA mismatches, hMutLα (composed of MLH1 and PMS2) is recruited to DNA. Assembly of the hMutSα and hMutLα complexes activates the endonuclease activity of PMS2, which generates single-strand breaks near the mismatch and allow for the exonuclease EXO1 to degrade the strand containing the mismatch (64). Defects in hMutLα inhibit mismatch repair (65-67). We will perform siRNA knockdown targeting MLH1 and PMS2 and determine whether viral infection and host genome stability will be affected using the assays described above. If we do not detect the same defects in DDR activation, viral titer, and genome instability, it will suggest that the hMutSα-mediated DDR activation is independent of its mismatch repair function.

Potential Problems and Alternatives. We do not anticipate major technical problems since the knockdowns of MMR proteins are previously well described in cell culture (68), and our preliminary data show that MSH6 can be effectively knocked down in RPTE cells without affecting cell viability (**Fig. 7A** and data not shown). Southern blotting, metaphase spread, and cell cycle analyses are all well developed in our laboratory (22). One potential problem is that since MSH2 knockdown affects both hMutSα and hMutSβ, it is possible that we will get different results when we knockdown MSH6 vs. MSH2. MSH3 competes with MSH6 to bind to MSH2 and forms the hMutSβ complex, which recognizes larger in/del loops (53). Our proteomic analysis revealed a preferential increase in MSH6 compared with MSH3 (data not shown). Therefore, polyomavirus infection perhaps tips the balance between the hMutSα and hMutsβ complexes. The role of the hMutSβ in DDR activation is less understood. If we observe differences between MSH6 and MSH2 knockdowns, we will also include MSH3 knockdown in our studies.

Although we did not discuss in detail, we are cognizant that we may find that our original hypothesis is incorrect: instead of resolving viral replication intermediates, there may be other aspects of the virus life cycle affected by MMR. It could be at the particle assembly step, or alternatively, the assembled particles may contain damaged DNA in MMR knockdown cells thereby leading to a decrease in infectivity. To test the former possibility, we will determine whether viral particles still form in MSH6 or MSH2 knockdown cells by CsCl gradient purification of viral particles followed by immunoblotting for the capsid proteins VP1/2/3 and transmission electron microscopy. If the particles appear normal, we will measure the amount of viral DNA present in these particles using a real-time PCR assay and determine the viral DNA/infectious unit ratios. A high ratio will indicate that the DNA packaged in these particles is defective. These steps can also be affected during DDR inhibition and have not been previously explored; therefore we will also extend these studies under DDR inhibition conditions.

Aim 1.2 Determine how MMR proteins contribute to DDR activation during polyomavirus replication. *Rationale:* There are two possible mechanisms for hMutSα-dependent DDR activation during infection. The first is an indirect activation model that involves mismatch repair-induced accumulation of ssDNA (69, 70). The second is a direct activation model where hMutSα directly recruits ATR and ATM to DNA (59). We believe that the second model is more probable because MSH2 and, to a greater extent, MSH6 protein levels both increase markedly with BKPyV infection (**Fig. 6B**). Furthermore, the upregulation of MSH6 can be achieved by TAg expression alone (**Fig. 6C**). Although MSH2 and MSH6 transcription is increased upon BKPyV infection as determined in a microarray analysis (71), the increase at the protein level is much greater than at the transcript level (data not shown), suggesting some degree of post-transcriptional regulation. One possibility is that TAg directly binds to MSH6 and MSH2 and stabilizes these proteins. In the context of infection, we *hypothesize* that the interaction between TAg and MMR proteins serves to recruit MMR proteins to sites of viral DNA replication, which in turn recruits ATR and ATM and facilitates DDR activation.

Experimental Design. To test whether hMutSα recruits DDR proteins to sites of viral replication, we will perform the following experiments: (i) Co-immunoprecipitation (co-IP) to examine the protein-protein interactions between these proteins. We will use a monoclonal antibody, pAb416, that is known to be able to IP TAg (72) and determine whether we can pull down hMutSα, ATR, or ATM in BKPyV-infected cells. Isotype-matched IgG and mock-infected cells will be used for negative controls. The immunoprecipitated proteins will be eluted from the TAg antibody-conjugated protein G beads followed by Western blotting against MSH6, MSH2, and ATR. Reciprocal co-IP will also be performed, and antibodies targeting ATR, ATM, MSH6, and MSH2 are all commercially available (61, 73). (ii) Chromatin-IP (ChIP) to determine whether DDR proteins are associated with viral DNA and whether these associations occur in an hMutSα-dependent manner. We will

immunoprecipitate ATR or ATM using commercially available antibodies in BKPyV-infected cells, and the immunoprecipitated viral DNA will be quantified using real-time PCR. We will perform these experiments both in normal cells and in cells that are knocked down for MSH6 or MSH2.

Expected Outcomes. (i) If our hypothesis is correct, we will find that TAg is able to co-IP with both hMutSα and DDR proteins. Since we observe a greater increase in MSH6 levels compared with MSH2 upon BKPyV infection, we expect that we will more likely detect an interaction between TAg and MSH6. Likewise, since MSH6 knockdown cells have a greater defect in ATR activation compared with ATM activation, we expect that TAg or MMR protein co-IP experiments are more likely to pull down ATR. If we can confirm the interactions between these proteins through reciprocal co-IP experiments, we will perform the TAg co-IP experiment in MSH6 or MSH2 knockdown cells and determine whether a lack of hMutSα will result in a decreased ability of TAg to interact with ATR and ATM. We will also conduct these experiments in cells that are transduced with a lentivirus expressing TAg (**Fig. 8A**) or in cells that are infected with a replication-deficient mutant virus (**Fig. 8B**) to determine whether the interaction is dependent on viral DNA replication. (ii) For the ChIP analyses, we expect to detect an association of ATR and ATM with viral DNA. If our hypothesis that hMutSα recruits DDR proteins to viral DNA is correct, we will find a decreased association of viral DNA with ATM, and more likely with ATR, in MSH6 or MSH2 knockdown cells.

Potential Problems and Alternatives. These experiments are straightforward and we have all of the necessary reagents and expertise. A potential technical limitation is that the interactions between these proteins may be weak and transient. To ensure that nuclear protein-protein interactions are preserved, we will isolate nuclei from infected cells first followed by harvesting lysates under relatively mild conditions (low salt and detergent). We will also include crosslinkers such as NHS-ester derivatives (Life Technologies) to preserve interactions if necessary.

It is possible that our direct-recruitment model is incorrect; and the results could instead support the indirect model in which it is the mismatch repair-induced accumulation of ssDNA that activates the ATR (69, 70). In this case, we may not detect an interaction between hMutSα and DDR proteins by co-IP even though we may still detect a decreased association of viral DNA with DDR proteins by ChIP assays. If we get this result, we will test the indirect model by siRNA knockdowns of EXO1, which is the exonuclease required to generate ssDNA (74), or MLH1, the EXO1-recruiting protein during mismatch repair (75). If the indirect model is correct, we expect to see that DDR activation will be diminished with EXO1 or MLH1 knockdown in infected cells and that there will be a decreased association of viral DNA with DDR proteins. It is also possible that hMutSα is involved in other aspects of DDR function other than recruiting the proteins to viral DNA. In this case, we may detect protein-protein interactions between TAg, hMutSα, and DDR through co-IPs, but we will not detect changes in viral DNA association with DDR by ChIP. If we get this result, we will investigate the involvement of DNA repair pathways such as non-homologous end joining (NHEJ) or homologous recombination (HR) during BKPyV replication. We will examine whether these pathways are affected by hMutSα during infection, as hMutSα has previously been shown to regulate both pathways (68, 76, 77). Finally, if we do not detect any interaction between hMutSα and TAg using the co-IP approach, we will determine whether these proteins at least interact with the replicating viral DNA independent of each other, using methods described in Aim 2.

Aim 2. To determine the viral DNA triggers that activate the DDR upon polyomavirus infection. In this Aim we design a series of experiments to test the *hypothesis* that replicating viral DNA is in fact the major trigger for DDR activation during polyomavirus infection. These studies will challenge the current paradigm that TAg is the main driver of DDR activation during polyomavirus infection. To our knowledge, this will be the first study to directly examine potential viral DNA damage during polyomavirus replication in cells with intact cell cycle checkpoint control.

Aim 2.1 Is viral DNA replication sufficient to activate the DDR? *Rationale:* Our results with TAg-expressing lentivirus and replication-deficient virus establish that viral DNA replication is necessary for DDR activation (**Fig. 8**). In this sub-Aim we will test the *hypothesis* that replicating viral DNA is sufficient to activate the DDR.

Experimental Design. We will construct a plasmid containing the BKPyV non-coding control region (78), which includes the BKPyV origin of replication. This plasmid or a control plasmid lacking BKPyV origin of replication sequences will be transfected into RPTE cells that are transduced with a lentivirus expressing TAg (27). To identify cells that are transfected with the BKPyV origin plasmid, we will use fluorescent *in situ* hybridization (FISH) with a probe that recognizes the plasmid sequence. We will compare DDR activation between cells that have TAg alone and cells that contain both TAg and viral origin of DNA replication. Because of the relatively low transfection efficiency of RPTE cells, we will not be able to perform Western blotting on whole cell lysates

to probe for DDR activation. Instead, we will use immunofluorescence staining for specific DDR activation markers (such as Chk1-pS317) to quantify on a single-cell level if there is elevated DDR activation in cells that contain both TAg and the origin sequence.

Expected Outcomes. If our hypothesis is correct, we predict that there will be a much greater DDR activation when both TAg and viral origin are present compared with TAg expression alone. If we get this result, we will also introduce mutations in either TAg or the origin of replication to ensure that the activation is dependent on viral DNA replication. We will first clone the D501A mutant TAg into a lentivirus expression vector since we have shown that this mutation abrogates viral DNA replication. We will also create another mutant TAg that lacks a functional origin-binding domain (28). Finally, we will construct a plasmid that contains mutations in the origin of viral replication that render the viral DNA incapable of being replicated by TAg (79). We expect that RPTE cells transduced with either mutant form of TAg with the wild-type origin, or wild-type TAg with the mutant origin will display decreased DDR activation compared with the wild-type TAg and origin combinations.

Potential Problems and Alternatives. We have extensive experience in FISH techniques (80), but if the sensitivity of FISH becomes a problem, we will engineer a fluorescent protein onto the origin-containing plasmid to allow identification of transfected cells. If we cannot detect DDR activation by the combination of wild-type TAg and viral origin of DNA replication, additional viral components may be involved. We will then create expression constructs to introduce other viral proteins including the virally-encoded small T antigen or the truncated T antigen (81), deliver them into RPTE cells, and assess their effects on DDR activation with or without viral origin.

Aim 2.2 Are there ssDNA lesions and double-strand breaks (DSBs) on the viral DNA? *Rationale:* During normal cellular replication, the uncoupling of MCM helicase and DNA polymerase during DNA synthesis results in ssDNA accumulation, which activates ATR (82). ATM is mainly activated by DSBs, which can arise from collapsed replication forks during replication stress (83). In addition, DSB-mediated fork restart is one mechanism to repair stalled replication forks (84). Our preliminary data show that viral DNA replication drives the activation for both ATR and ATM, leading to the *hypothesis* that both ssDNA lesions and DSBs occur on replicating viral DNA, which serve as DDR activation triggers.

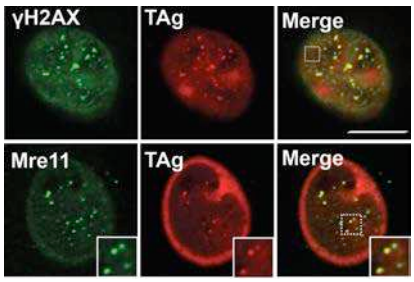


Figure 10. γH2AX and Mre11 are recruited to TAg foci. RPTE cells were infected with BKPyV infection at an MOI 0.5 IU/cells. Cells were immunostained for γH2AX, TAg, and Mre11 at 3 dpi. Bar, 10 μm.

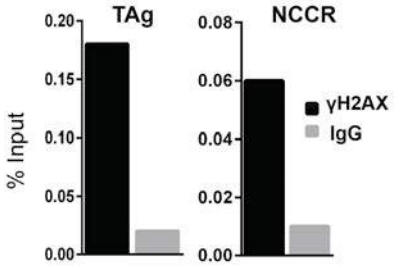


Figure 11. γH2AX associates with BKPyV viral DNA. RPTE cells were infected with BKPyV at an MOI of 0.5 IU/cell and cell lysates were subjected to ChIP analyses using antibodies against γH2AX or IgG at 3 dpi. The immunoprecipitated DNA were analyzed using real-time PCR with primers amplifying TAg or NCCR region on the viral genome and data were shown as percent of input controls.

Experimental Design. (i) To determine if there is extensive viral ssDNA accumulation, we will use a non-denaturing FISH technique using a nick-translated viral probe to detect viral ssDNA on a single-cell level (83). The average FISH signal intensities will allow us to quantify the amount of ssDNA present on viral DNA (83). (ii) To determine whether there are DSBs on viral DNA, We will perform ChIP experiments in infected cells with a commercially available antibody against γH2AX, a protein known to mark sites of DSBs and recruit DNA repair proteins to these sites (85). The IP will be followed with real-time PCRs using primers against different regions on the viral chromosome. γH2AX level is markedly increased with BKPyV infection (**Fig. 1A**). We also observed that Mre11 (an early DSB sensor) and γH2AX are both recruited to TAg-containing nuclear foci during infection (**Fig. 10**). Therefore we reason that γH2AX-associated viral DNA will be a good initial marker for DSBs on viral DNA. In these experiments, DNA damaging reagents such as doxorubicin and the known damage sites on the host chromosome will be used as positive controls (86). Our preliminary data did show that γH2AX is associated with viral DNA (**Fig. 11**). (iii) We will also examine the accumulation of viral ssDNA lesions and DSBs in cells treated with hydroxyurea (HU), which is a ribonucleotide reductase inhibitor that can cause replication stress (83) and therefore may enrich these signals that are indicative of damage on viral DNA.

Expected Outcomes. If our hypothesis is correct, we predict that both viral ssDNA and the association of γH2AX with viral DNA will be detected during normal infection, and that there will be an increase in viral ssDNA lesions and γH2AX-associated viral DNA in HU treated replication stress conditions. If we get these results, we will perform the following

experiments: (i) Determine whether we can detect the opposite phenotype by supplementing infected cells with nucleotides to reduce viral replication stress during normal infection. (ii) Since the uncoupling of MCM helicase and host cell DNA polymerase α has been proposed to be the main mechanism for ATR activation (82), we will test whether this is also the case on viral DNA, i.e., the uncoupling between TAg helicase and DNA polymerase α results in the accumulation of ssDNA and activation of ATR. We will briefly treat the infected cells with aphidicolin, a DNA polymerase α inhibitor (82) and measure whether there is any increase in ssDNA lesions and DSBs on viral DNA. (iii) We will also measure DSB formation via γH2AX ChIP assays in ATR knockdown cells. If DSB formation is a result of replication fork destabilizing and collapsing, we will expect to see an increase of DSBs in the absence of ATR. If we do not observe such an increase, it suggests that the DSBs are derived from other sources, for example, oxidative damage caused by viral infection.

Potential Problems and Alternatives. If the sensitivity of single-cell FISH is not high enough, we will modify our Southern procedure to perform a non-denaturing Southern blotting instead (78). Another caveat of the proposed study is that from a single DSB, γH2AX can also be spread several kilobases away from the damage site (87). Therefore it is possible that occupancy by γH2AX does not reflect the actual location of the DSB. To circumvent this problem and as an alternative method to map DNA damage, we will use ligation-mediated PCR (88) to directly map DSBs on the viral DNA. In this method, low-molecular weight DNA with broken ends from infected cells will be ligated with a biotinylated double-stranded oligonucleotide followed by restriction enzyme digest. This allows for the purification of these damaged DNA with streptavidin beads and the subsequent regular or real-time PCR to reveal sequence information and to perform quantitative analysis.

If we do not detect either ssDNA lesions or DSBs on viral DNA, it may suggest that the DDR is activated by a different mechanism. One possibility is that TAg recruits DDR proteins to sites of viral replication and the prolonged association of these proteins with viral DNA or chromatin activates the DDR without any actual DNA lesion. This possibility has been shown previously by targeting DNA repair factors to the host chromatin (89). To test this idea, we will use a recently developed technique iPOND (isolate proteins on nascent DNA) (43, 90), to investigate whether DDR proteins such as ATR, ATM, and MMR proteins are associated with replicating viral DNA. Infected cells will be labeled with biotinylated EdU, and proteins bound to the newly replicated DNA will be purified using streptavidin following click chemistry and be confirmed by Western blotting. To ensure that we are examining proteins associated with replicating viral DNA instead of cellular DNA, we will isolate the viral minichromosomes (33) prior to the purification. We have already performed EdU labeling experiments and have confirmed that the infected cells can be efficiently labeled (data not shown).

Aim 3. To elucidate the molecular mechanism by which polyomavirus induces host genome instability. It is well accepted in the field that TAg is able to induce DNA damage (18, 19), but little is known about the exact mechanism of how the damage arises. The goal of this aim is to probe the mechanistic link between viral DNA replication and host DNA damage induced by TAg. It has previously been shown by one of the classic cell fusion experiments that fusion of an S-phase cell with a mitotic cell can result in a “shattered chromosome” phenotype (91). We *hypothesize* that TAg-induced host replication stress coupled with mitosis leads to host genome instability, which can be prevented by virus-replication induced DDR activation via G2/M arrest.

Aim 3.1 Is polyomavirus-induced host DNA damage associated with mitosis? *Rationale:* According to our model (**Fig. 1**), TAg-induced aberrant host DNA replication stress followed by mitosis causes chromosome damage. This damage is not observed during normal infection because virus replication activates the DDR and G2/M checkpoint, which inactivates Cdc25C, a phosphatase necessary for mitotic entry (92). If our model is correct, we predict that if we force cells into mitosis during normal infection, we will detect DNA damage similar to that seen in DDR-deficient infected cells.

Experimental Design. To bypass the G2/M checkpoint and induce the cells into mitosis, we will use two independent strategies (i) siRNA knockdown of Wee1, and (ii) overexpression of Cdc25C and cyclin B1 using lentivirus constructs. Wee1 is a kinase that inhibits mitotic entry through phosphorylation of Cdk1 (93). Overexpression of Cdc25C and cyclin B1 has previously been shown to induce mitosis (94). Timing is important in these experiments. We will first infect RPTE cells for two days to allow for S-phase induction by virus infection before we perform the siRNA knockdowns or lentivirus transductions. Metaphase analyses will be used following siRNA transfection or lentivirus transduction to examine the degree of chromosome damage.

Expected Outcomes. In cells that are knocked down for Wee1 or overexpressed with Cdc25C and cyclin B1, we predict that the chromosome damage will be more severe in infected cells compared with uninfected cells. Among the infected cells, we predict that by driving the cells into mitosis we will observe an increase in abnormal chromosomes compared with normal infected cells. If we observe this, we will also directly measure

the mitotic status of the cells using a well-established mitotic index assay (Life Technologies). During mitotic entry, histone H3 is phosphorylated at the Ser10 position with chromatin condensation (95) and therefore can serve as a marker for mitosis. We will examine the following conditions for mitotic index: (i) wild-type BKPyV infection, (ii) TAg D501A mutant virus infection, and (iii) lentivirus-expressing TAg. We predict that the mitotic index will increase with the latter two conditions compared with normal virus infection, as the G2/M checkpoint is not activated in these cells.

Potential Problems and Alternatives. If we do not detect an increase in chromosome damage from the above experiments, there could be two explanations. One possibility is that our experimental conditions do not completely remove other checkpoints for mitosis, for example, the spindle assembly checkpoint (SAC). To test this idea, we will also combine the previous experiments with SAC inactivation, for example, using a small inhibitor Gö6976 (96) to determine whether we can induce more damage in infected cells. Another possibility is that the chromosome damage in DDR-deficient cells is caused by DNA repair inhibition instead of a loss of checkpoint in infected cells. We will then inhibit the two major DNA repair pathways (SCR7 inhibitor to target NHEJ (97) and Rad51 siRNA knockdown to inhibit HR (98)) in infected cells and examine the chromosome damage under these conditions.

Aim 3.2 Does large T antigen induce host cell replication stress? *Rationale:* There are increasing reports on oncogenes inducing replication stress thereby leading to DNA damage (99-101). In our system, TAg expression alone can cause DNA damage. Moreover, SV40 TAg-induced DNA damage can be reversed by exogenous supplementation of nucleotides (29). Together, these observations lead to the *hypothesis* that TAg induces host DNA replication stress.

Experimental Design. To directly determine the impact of TAg on host DNA replication, we will use a novel DNA fiber assay (102) to measure how TAg affects host DNA replication initiation and elongation in lentivirus-TAg transduced cells. This is a quantitative approach that labels nascent DNA *in vivo* by sequential incorporation of two halogenated nucleotides. Individual DNA fibers are stretched onto a microscope slide, and the labeled DNA replication tracts can be visualized by antibody staining and fluorescence microscopy. Replication origin firing, replication elongation, and replication fork terminations can all be quantitatively measured based on different labeling patterns. [redacted] laboratory is experienced in this technique and she will provide expertise for these analyses (please see attached letter). We will also inhibit replication initiation in these cells using siRNA targeting the origin licensing factor Cdc6 (100) and determine whether it will prevent TAg-induced replication stress and DNA damage.

Expected Outcomes. We expect to see increased origin firing and impaired replication fork progression in the presence of TAg. We also expect that these defects will be abolished through Cdc6 knockdown. If we observe this, we will introduce several mutations in TAg and assess which function of TAg is required for the replication stress phenotype: (i) A pRb-binding mutant since TAg may induce replication stress through inactivation of pRb, thus allowing E2F to drive cells into S phase (36, 72). (ii) An origin-binding domain mutant and a helicase mutant, as it is possible that TAg binds to and unwinds non-specific or pseudo-origins of replication sequences present on host DNA, thereby triggering aberrant replication (103). This idea has been proposed for the human papillomavirus E1 helicase (104). (iii) An RPA-binding mutant, since it has been shown that TAg binds to RPA and this interaction may prevent normal function of RPA during cellular replication (20, 105).

Potential Problems and Alternatives. The DNA fiber assay only involves a short period of halogenated nucleotide labeling. If we do not detect any difference between lentivirus-TAg transduced cells and control cells, we will create an inducible-TAg system by cloning TAg under a tetracycline-controlled promoter (106) to better control the relative timing between TAg expression and DNA labeling. If we do not detect any significant replication stress defect induced by TAg, we will then test the hypothesis that TAg directly causes DNA damage either by melting host DNA or through a potential nuclease activity. As a first step to test this idea, we will perform a ChIP-sequencing experiment to determine whether there are specific host DNA sequences that are associated with TAg.

C4) OVERALL SUMMARY AND CONCLUSIONS. We believe these studies will allow us to gain a more in-depth understanding of how polyomaviruses usurp the host DDR to promote viral replication and how the DDR impacts polyomavirus-induced genome instability. We have gathered sufficient preliminary results and developed the expertise to ensure that the studies proposed here are highly feasible to accomplish. This research will have significant impact on our understanding of oncogenic polyomavirus replication and will lay the groundwork for the future development of DDR inhibitors to selectively target polyomavirus-infected cells.

Literature Cited

1. **Gardner SD, Field AM, Coleman DV, Hulme B.** 1971. New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet **1:**1253-1257.
2. **Padgett BL, Walker DL, ZuRhein GM, Eckroade RJ, Dessel BH.** 1971. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. Lancet **1:**1257-1260.
3. **Jiang M, Abend JR, Johnson SF, Imperiale MJ.** 2009. The role of polyomaviruses in human disease. Virology **384:**266-273.
4. **Nickeleit V, Singh HK, Goldsmith CS, Miller SE, Kenan DJ.** 2013. BK virus-associated urinary bladder carcinoma in transplant recipients: productive or nonproductive polyomavirus infections in tumor cells? Hum Pathol **44:**2870-2871.
5. **Bulut Y, Ozdemir E, Ozercan HI, Etem EO, Aker F, Toraman ZA, Seyrek A, Firdolas F.** 2013. Potential relationship between BK virus and renal cell carcinoma. J Med Virol **85:**1085-1089.
6. **van Aalderen MC, Yapici U, van der Pol JA, de Reijke TM, van Donselaar-van der Pant KA, Florquin S, Bemelman FJ, Ten Berge IJ.** 2013. Polyomavirus BK in the pathogenesis of bladder cancer. Neth J Med **71:**26-28.
7. **Alexiev BA, Randhawa P, Vazquez Martul E, Zeng G, Luo C, Ramos E, Drachenberg CB, Papadimitriou JC.** 2013. BK virus-associated urinary bladder carcinoma in transplant recipients: report of 2 cases, review of the literature, and proposed pathogenetic model. Hum Pathol **44:**908-917.
8. **Pino L, Rijo E, Nohales G, Frances A, Ubre A, Arango O.** 2013. Bladder transitional cell carcinoma and BK virus in a young kidney transplant recipient. Transpl Infect Dis **15:**E25-27.
9. **Emerson LL, Carney HM, Layfield LJ, Sherbotie JR.** 2008. Collecting duct carcinoma arising in association with BK nephropathy post-transplantation in a pediatric patient. A case report with immunohistochemical and in situ hybridization study. Pediatr Transplant **12:**600-605.
10. **Geetha D, Tong BC, Racusen L, Markowitz JS, Westra WH.** 2002. Bladder carcinoma in a transplant recipient: evidence to implicate the BK human polyomavirus as a causal transforming agent. Transplantation **73:**1933-1936.
11. **Narayanan M, Szymanski J, Slavcheva E, Rao A, Kelly A, Jones K, Jaffers G.** 2007. BK virus associated renal cell carcinoma: case presentation with optimized PCR and other diagnostic tests. Am J Transplant **7:**1666-1671.
12. **Ferenczy MW, Marshall LJ, Nelson CD, Atwood WJ, Nath A, Khalili K, Major EO.** 2012. Molecular biology, epidemiology, and pathogenesis of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clin Microbiol Rev **25:**471-506.
13. **DeCaprio JA, Garcea RL.** 2013. A cornucopia of human polyomaviruses. Nat Rev Microbiol **11:**264-276.
14. **Houben R, Shuda M, Weinkam R, Schrama D, Feng H, Chang Y, Moore PS, Becker JC.** 2010. Merkel cell polyomavirus-infected Merkel cell carcinoma cells require expression of viral T antigens. J Virol **84:**7064-7072.
15. **Dalianis T, Hirsch HH.** 2013. Human polyomaviruses in disease and cancer. Virology **437:**63-72.
16. **Feng H, Shuda M, Chang Y, Moore PS.** 2008. Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science **319:**1096-1100.
17. **Fanning E, Zhao K.** 2009. SV40 DNA replication: from the A gene to a nanomachine. Virology **384:**352-359.
18. **Boichuk S, Hu L, Hein J, Gjoerup OV.** 2010. Multiple DNA damage signaling and repair pathways deregulated by simian virus 40 large T antigen. J Virol **84:**8007-8020.
19. **Li J, Wang X, Diaz J, Tsang SH, Buck CB, You J.** 2013. Merkel cell polyomavirus large T antigen disrupts host genomic integrity and inhibits cellular proliferation. J Virol **87:**9173-9188.
20. **Banerjee P, DeJesus R, Gjoerup O, Schaffhausen BS.** 2013. Viral interference with DNA repair by targeting of the single-stranded DNA binding protein RPA. PLoS Pathog **9:**e1003725.
21. **Trojanek J, Croul S, Ho T, Wang JY, Darbinyan A, Nowicki M, Del Valle L, Skorski T, Khalili K, Reiss K.** 2006. T-antigen of the human polyomavirus JC attenuates faithful DNA repair by forcing nuclear interaction between IRS-1 and Rad51. J Cell Physiol **206:**35-46.
22. **Jiang M, Zhao L, Gamez M, Imperiale MJ.** 2012. Roles of ATM and ATR-mediated DNA damage responses during lytic BK polyomavirus infection. PLoS Pathog **8:**e1002898.
23. **Low J, Humes HD, Szczypka M, Imperiale M.** 2004. BKV and SV40 infection of human kidney tubular epithelial cells in vitro. Virology **323:**182-188.

24. **Jiang M, Entezami P, Gamez M, Stamminger T, Imperiale MJ.** 2011. Functional reorganization of promyelocytic leukemia nuclear bodies during BK virus infection. MBio **2:**e00281-00210.
25. **Jiang M, Abend JR, Tsai B, Imperiale MJ.** 2009. Early events during BK virus entry and disassembly. J Virol **83:**1350-1358.
26. **Randhawa PS, Finkelstein S, Scantlebury V, Shapiro R, Vivas C, Jordan M, Picken MM, Demetris AJ.** 1999. Human polyoma virus-associated interstitial nephritis in the allograft kidney. Transplantation **67:**103-109.
27. **Verhalen B, Justice JL, Imperiale MJ, Jiang M.** 2015. Viral DNA Replication-Dependent DNA Damage Response Activation during BK Polyomavirus Infection. J Virol **89:**5032-5039.
28. **Orba Y, Suzuki T, Makino Y, Kubota K, Tanaka S, Kimura T, Sawa H.** 2010. Large T antigen promotes JC virus replication in G2-arrested cells by inducing ATM- and ATR-mediated G2 checkpoint signaling. J Biol Chem **285:**1544-1554.
29. **Hu L, Filippakis H, Huang H, Yen TJ, Gjoerup OV.** 2013. Replication stress and mitotic dysfunction in cells expressing simian virus 40 large T antigen. J Virol **87:**13179-13192.
30. **Mao Z, Bozzella M, Seluanov A, Gorbunova V.** 2008. Comparison of nonhomologous end joining and homologous recombination in human cells. DNA Repair (Amst) **7:**1765-1771.
31. **Tsang SH, Wang X, Li J, Buck CB, You J.** 2014. Host DNA damage response factors localize to merkel cell polyomavirus DNA replication sites to support efficient viral DNA replication. J Virol **88:**3285-3297.
32. **Topalis D, Andrei G, Snoeck R.** 2013. The large tumor antigen: a "Swiss Army knife" protein possessing the functions required for the polyomavirus life cycle. Antiviral Res **97:**122-136.
33. **Zlotkin T, Kaufmann G, Jiang Y, Lee MY, Uitto L, Syvaoja J, Dornreiter I, Fanning E, Nethanel T.** 1996. DNA polymerase epsilon may be dispensable for SV40- but not cellular-DNA replication. EMBO J **15:**2298-2305.
34. **Moyer SE, Lewis PW, Botchan MR.** 2006. Isolation of the Cdc45/Mcm2-7/GINS (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase. Proc Natl Acad Sci U S A **103:**10236-10241.
35. **Zhao X, Madden-Fuentes RJ, Lou BX, Pipas JM, Gerhardt J, Rigell CJ, Fanning E.** 2008. Ataxia telangiectasia-mutated damage-signaling kinase- and proteasome-dependent destruction of Mre11-Rad50-Nbs1 subunits in Simian virus 40-infected primate cells. J Virol **82:**5316-5328.
36. **Chellappan S, Kraus VB, Kroger B, Munger K, Howley PM, Phelps WC, Nevins JR.** 1992. Adenovirus E1A, simian virus 40 tumor antigen, and human papillomavirus E7 protein share the capacity to disrupt the interaction between transcription factor E2F and the retinoblastoma gene product. Proc Natl Acad Sci U S A **89:**4549-4553.
37. **Harris KF, Christensen JB, Radany EH, Imperiale MJ.** 1998. Novel mechanisms of E2F induction by BK virus large-T antigen: requirement of both the pRb-binding and the J domains. Mol Cell Biol **18:**1746-1756.
38. **Bargonetti J, Reynisdottir I, Friedman PN, Prives C.** 1992. Site-specific binding of wild-type p53 to cellular DNA is inhibited by SV40 T antigen and mutant p53. Genes Dev **6:**1886-1898.
39. **Jiang D, Srinivasan A, Lozano G, Robbins PD.** 1993. SV40 T antigen abrogates p53-mediated transcriptional activity. Oncogene **8:**2805-2812.
40. **Sachsenmeier KF, Pipas JM.** 2001. Inhibition of Rb and p53 is insufficient for SV40 T-antigen transformation. Virology **283:**40-48.
41. **Cimprich KA, Cortez D.** 2008. ATR: an essential regulator of genome integrity. Nat Rev Mol Cell Biol **9:**616-627.
42. **Jackson SP.** 2002. Sensing and repairing DNA double-strand breaks. Carcinogenesis **23:**687-696.
43. **Sirbu BM, Couch FB, Feigerle JT, Bhaskara S, Hiebert SW, Cortez D.** 2011. Analysis of protein dynamics at active, stalled, and collapsed replication forks. Genes Dev **25:**1320-1327.
44. **Dickey JS, Redon CE, Nakamura AJ, Baird BJ, Sedelnikova OA, Bonner WM.** 2009. H2AX: functional roles and potential applications. Chromosoma **118:**683-692.
45. **Podhorecka M, Skladanowski A, Bozko P.** 2010. H2AX Phosphorylation: Its Role in DNA Damage Response and Cancer Therapy. J Nucleic Acids **2010**.
46. **Zachos G, Rainey MD, Gillespie DA.** 2003. Chk1-deficient tumour cells are viable but exhibit multiple checkpoint and survival defects. EMBO J **22:**713-723.
47. **Maya-Mendoza A, Petermann E, Gillespie DA, Caldecott KW, Jackson DA.** 2007. Chk1 regulates the density of active replication origins during the vertebrate S phase. EMBO J **26:**2719-2731.

48. **Hollingworth R, Grand RJ.** 2015. Modulation of DNA Damage and Repair Pathways by Human Tumour Viruses. Viruses **7:**2542-2591.
49. **Chaurushiya MS, Weitzman MD.** 2009. Viral manipulation of DNA repair and cell cycle checkpoints. DNA Repair (Amst) **8:**1166-1176.
50. **Sowd GA, Li NY, Fanning E.** 2013. ATM and ATR activities maintain replication fork integrity during SV40 chromatin replication. PLoS Pathog **9:**e1003283.
51. **Fenech M, Kirsch-Volders M, Natarajan AT, Surralles J, Crott JW, Parry J, Norppa H, Eastmond DA, Tucker JD, Thomas P.** 2011. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis **26:**125-132.
52. **Drummond JT, Li GM, Longley MJ, Modrich P.** 1995. Isolation of an hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells. Science **268:**1909-1912.
53. **Matton N, Simonetti J, Williams K.** 2000. Identification of mismatch repair protein complexes in HeLa nuclear extracts and their interaction with heteroduplex DNA. J Biol Chem **275:**17808-17813.
54. **Edelbrock MA, Kaliyaperumal S, Williams KJ.** 2013. Structural, molecular and cellular functions of MSH2 and MSH6 during DNA mismatch repair, damage signaling and other noncanonical activities. Mutat Res **743-744:**53-66.
55. **Jiao J, Simmons DT.** 2003. Nonspecific double-stranded DNA binding activity of simian virus 40 large T antigen is involved in melting and unwinding of the origin. J Virol **77:**12720-12728.
56. **Buck CB, Pastrana DV, Lowy DR, Schiller JT.** 2005. Generation of HPV pseudovirions using transfection and their use in neutralization assays. Methods Mol Med **119:**445-462.
57. **Broekema NM, Imperiale MJ.** 2012. Efficient propagation of archetype BK and JC polyomaviruses. Virology **422:**235-241.
58. **Pabla N, Ma Z, McIlhatton MA, Fishel R, Dong Z.** 2011. hMSH2 recruits ATR to DNA damage sites for activation during DNA damage-induced apoptosis. J Biol Chem **286:**10411-10418.
59. **Yoshioka K, Yoshioka Y, Hsieh P.** 2006. ATR kinase activation mediated by MutSalpha and MutLalpha in response to cytotoxic O6-methylguanine adducts. Mol Cell **22:**501-510.
60. **Wang Y, Qin J.** 2003. MSH2 and ATR form a signaling module and regulate two branches of the damage response to DNA methylation. Proc Natl Acad Sci U S A **100:**15387-15392.
61. **Liu Y, Fang Y, Shao H, Lindsey-Boltz L, Sancar A, Modrich P.** 2010. Interactions of human mismatch repair proteins MutSalpha and MutLalpha with proteins of the ATR-Chk1 pathway. J Biol Chem **285:**5974-5982.
62. **Dandjinou AT, Larrivee M, Wellinger RE, Wellinger RJ.** 2006. Two-dimensional agarose gel analysis of DNA replication intermediates. Methods Mol Biol **313:**193-208.
63. **Abraham RT.** 2001. Cell cycle checkpoint signaling through the ATM and ATR kinases. Genes Dev **15:**2177-2196.
64. **Kunkel TA, Erie DA.** 2005. DNA mismatch repair. Annu Rev Biochem **74:**681-710.
65. **Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindblom A, et al.** 1994. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature **368:**258-261.
66. **Liu B, Parsons R, Papadopoulos N, Nicolaides NC, Lynch HT, Watson P, Jass JR, Dunlop M, Wyllie A, Peltomaki P, de la Chapelle A, Hamilton SR, Vogelstein B, Kinzler KW.** 1996. Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. Nat Med **2:**169-174.
67. **Prolla TA, Christie DM, Liskay RM.** 1994. Dual requirement in yeast DNA mismatch repair for MLH1 and PMS1, two homologs of the bacterial mutL gene. Mol Cell Biol **14:**407-415.
68. **Shahi A, Lee JH, Kang Y, Lee SH, Hyun JW, Chang IY, Jun JY, You HJ.** 2011. Mismatch-repair protein MSH6 is associated with Ku70 and regulates DNA double-strand break repair. Nucleic Acids Res **39:**2130-2143.
69. **Li GM.** 1999. The role of mismatch repair in DNA damage-induced apoptosis. Oncol Res **11:**393-400.
70. **York SJ, Modrich P.** 2006. Mismatch repair-dependent iterative excision at irreparable O6-methylguanine lesions in human nuclear extracts. J Biol Chem **281:**22674-22683.
71. **Abend JR, Low JA, Imperiale MJ.** 2010. Global effects of BKV infection on gene expression in human primary kidney epithelial cells. Virology **397:**73-79.
72. **Harris KF, Christensen JB, Imperiale MJ.** 1996. BK virus large T antigen: interactions with the retinoblastoma family of tumor suppressor proteins and effects on cellular growth control. J Virol **70:**2378-2386.

73. **Sun Y, Xu Y, Roy K, Price BD.** 2007. DNA damage-induced acetylation of lysine 3016 of ATM activates ATM kinase activity. Mol Cell Biol **27:**8502-8509.
74. **Burdett V, Baitinger C, Viswanathan M, Lovett ST, Modrich P.** 2001. In vivo requirement for RecJ, ExoVII, ExoI, and ExoX in methyl-directed mismatch repair. Proc Natl Acad Sci U S A **98:**6765-6770.
75. **Tran PT, Simon JA, Liskay RM.** 2001. Interactions of Exo1p with components of MutLalpha in Saccharomyces cerevisiae. Proc Natl Acad Sci U S A **98:**9760-9765.
76. **Spell RM, Jinks-Robertson S.** 2003. Role of mismatch repair in the fidelity of RAD51- and RAD59-dependent recombination in Saccharomyces cerevisiae. Genetics **165:**1733-1744.
77. **Nicholson A, Hendrix M, Jinks-Robertson S, Crouse GF.** 2000. Regulation of mitotic homeologous recombination in yeast. Functions of mismatch repair and nucleotide excision repair genes. Genetics **154:**133-146.
78. **Broekema NM, Abend JR, Bennett SM, Butel JS, Vanchiere JA, Imperiale MJ.** 2010. A system for the analysis of BKV non-coding control regions: application to clinical isolates from an HIV/AIDS patient. Virology **407:**368-373.
79. **Kwun HJ, Guastafierro A, Shuda M, Meinke G, Bohm A, Moore PS, Chang Y.** 2009. The minimum replication origin of merkel cell polyomavirus has a unique large T-antigen loading architecture and requires small T-antigen expression for optimal replication. J Virol **83:**12118-12128.
80. **Jiang M, Entezami P, Gamez M, Stamminger T, Imperiale MJ.** 2011. Functional Reorganization of Promyelocytic Leukemia Nuclear Bodies during BK Virus Infection. mBio.
81. **Abend JR, Joseph AE, Das D, Campbell-Cecen DB, Imperiale MJ.** 2009. A truncated T antigen expressed from an alternatively spliced BK virus early mRNA. J Gen Virol **90:**1238-1245.
82. **Byun TS, Pacek M, Yee MC, Walter JC, Cimprich KA.** 2005. Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint. Genes Dev **19:**1040-1052.
83. **Couch FB, Bansbach CE, Driscoll R, Luzwick JW, Glick GG, Betous R, Carroll CM, Jung SY, Qin J, Cimprich KA, Cortez D.** 2013. ATR phosphorylates SMARCAL1 to prevent replication fork collapse. Genes Dev **27:**1610-1623.
84. **Branzei D, Foiani M.** 2010. Maintaining genome stability at the replication fork. Nat Rev Mol Cell Biol **11:**208-219.
85. **Lobrich M, Shibata A, Beucher A, Fisher A, Ensminger M, Goodarzi AA, Barton O, Jeggo PA.** 2010. gammaH2AX foci analysis for monitoring DNA double-strand break repair: strengths, limitations and optimization. Cell Cycle **9:**662-669.
86. **Seo J, Kim SC, Lee HS, Kim JK, Shon HJ, Salleh NL, Desai KV, Lee JH, Kang ES, Kim JS, Choi JK.** 2012. Genome-wide profiles of H2AX and gamma-H2AX differentiate endogenous and exogenous DNA damage hotspots in human cells. Nucleic Acids Res **40:**5965-5974.
87. **Price BD, D'Andrea AD.** 2013. Chromatin remodeling at DNA double-strand breaks. Cell **152:**1344-1354.
88. **Villalobos MJ, Betti CJ, Vaughan AT.** 2006. Detection of DNA double-strand breaks and chromosome translocations using ligation-mediated PCR and inverse PCR. Methods Mol Biol **314:**109-121.
89. **Soutoglou E, Misteli T.** 2008. Activation of the cellular DNA damage response in the absence of DNA lesions. Science **320:**1507-1510.
90. **Sirbu BM, Couch FB, Cortez D.** 2012. Monitoring the spatiotemporal dynamics of proteins at replication forks and in assembled chromatin using isolation of proteins on nascent DNA. Nat Protoc **7:**594-605.
91. **Johnson RT, Rao PN.** 1970. Mammalian cell fusion: induction of premature chromosome condensation in interphase nuclei. Nature **226:**717-722.
92. **Karlsson-Rosenthal C, Millar JB.** 2006. Cdc25: mechanisms of checkpoint inhibition and recovery. Trends Cell Biol **16:**285-292.
93. **Den Haese GJ, Walworth N, Carr AM, Gould KL.** 1995. The Wee1 protein kinase regulates T14 phosphorylation of fission yeast Cdc2. Mol Biol Cell **6:**371-385.
94. **Karlsson C, Katich S, Hagting A, Hoffmann I, Pines J.** 1999. Cdc25B and Cdc25C differ markedly in their properties as initiators of mitosis. J Cell Biol **146:**573-584.
95. **Hirota T, Lipp JJ, Toh BH, Peters JM.** 2005. Histone H3 serine 10 phosphorylation by Aurora B causes HP1 dissociation from heterochromatin. Nature **438:**1176-1180.

96. **Stolz A, Vogel C, Schneider V, Ertych N, Kienitz A, Yu H, Bastians H.** 2009. Pharmacologic abrogation of the mitotic spindle checkpoint by an indolocarbazole discovered by cellular screening efficiently kills cancer cells. Cancer Res **69:**3874-3883.
97. **Srivastava M, Nambiar M, Sharma S, Karki SS, Goldsmith G, Hegde M, Kumar S, Pandey M, Singh RK, Ray P, Natarajan R, Kelkar M, De A, Choudhary B, Raghavan SC.** 2012. An inhibitor of nonhomologous end-joining abrogates double-strand break repair and impedes cancer progression. Cell **151:**1474-1487.
98. **Koch K, Wrona A, Dikomey E, Borgmann K.** 2009. Impact of homologous recombination on individual cellular radiosensitivity. Radiother Oncol **90:**265-272.
99. **Dominguez-Sola D, Ying CY, Grandori C, Ruggiero L, Chen B, Li M, Galloway DA, Gu W, Gautier J, Dalla-Favera R.** 2007. Non-transcriptional control of DNA replication by c-Myc. Nature **448:**445-451.
100. **Jones RM, Mortusewicz O, Afzal I, Lorvellec M, Garcia P, Helleday T, Petermann E.** 2013. Increased replication initiation and conflicts with transcription underlie Cyclin E-induced replication stress. Oncogene **32:**3744-3753.
101. **Bester AC, Roniger M, Oren YS, Im MM, Sarni D, Chaoat M, Bensimon A, Zamir G, Shewach DS, Kerem B.** 2011. Nucleotide deficiency promotes genomic instability in early stages of cancer development. Cell **145:**435-446.
102. **Schwab RA, Niedzwiedz W.** 2011. Visualization of DNA replication in the vertebrate model system DT40 using the DNA fiber technique. J Vis Exp doi:10.3791/3255.
103. **Galli I, Iguchi-Ariga SM, Ariga H.** 1993. Mammalian genomic sequences can substitute for the SV40 AT stretch in sustaining replication of the SV40 origin of replication. FEBS Lett **318:**335-340.
104. **Sakakibara N, Mitra R, McBride AA.** 2011. The papillomavirus E1 helicase activates a cellular DNA damage response in viral replication foci. J Virol **85:**8981-8995.
105. **Ning B, Feldkamp MD, Cortez D, Chazin WJ, Friedman KL, Fanning E.** 2015. Simian virus Large T antigen interacts with the N-terminal domain of the 70 kD subunit of Replication Protein A in the same mode as multiple DNA damage response factors. PLoS One **10:**e0116093.
106. **Urlinger S, Baron U, Thellmann M, Hasan MT, Bujard H, Hillen W.** 2000. Exploring the sequence space for tetracycline-dependent transcriptional activators: novel mutations yield expanded range and sensitivity. Proc Natl Acad Sci U S A **97:**7963-7968.