Fall 2019

DNA Pitt Crew

The latest news and updates from the UPMC Hillman Cancer Center Genome Stability Program

University of Pittsburgh

UPMC | HILLMAN CANCER CENTER

Note from the Genome Stability Program Co-leaders, Patricia Opresko, PhD, and Bennett Van Houten, PhD

We are delighted to present the Fall 2019 edition of the DNA Pitt Crew newsletter, which provides recent information about UPMC Hillman Cancer Center Genome Stability Program (GSP) members, new recruits, and some of the exciting scientific accomplishments from this program. The last six months have been extremely productive, and we are exceptionally proud to share highlights of recent high-impact papers published in *Science, Nature, Molecular Cell, PNAS,* and *Nature Communications*, among others. Trainees and new recruits had the opportunity to share their exciting projects at our annual Genome Stability Symposium, the UPMC Hillman Cancer Center annual retreat, and several international meetings. We have added a new feature, the Pitt Stop, in which we highlight visits from leading scientists. These visits are chronicled by postdoctoral fellows and graduate students who played an important part in hosting our guests. This spring we were extremely fortunate to welcome eight outstanding scientists working in DNA repair and genome stability to deliver seminars and meet with faculty and trainees. We were especially fortunate to have Dr. Phil Hanawalt present a unique historical lecture on his pioneering contributions to the field of DNA repair. Not only has his laboratory helped to discover nucleotide excision repair in bacteria and transcription-coupled repair, his tireless and generous support has fostered an entire generation of outstanding scientists. All of us greatly appreciated our colleagues carving out time to stop in Pittsburgh and illuminate us with their science and helpful advice. Finally, we look forward to sharing the successes, vision, and future directions of the GSP at the upcoming NCI Cancer Center Grant renewal in January of 2020.

Faculty Spotlight — Jian Yu, PhD

Dr. Jian Yu is currently a tenured Professor of Pathology and Professor of Radiation Oncology at the University of Pittsburgh School of Medicine, and a member of Genome Stability Program at UPMC Hillman Cancer Center. She is a faculty member of the Cellular and Molecular Pathology Graduate Training Program and Pitt-CMU Medical Scientist Training Program. Dr. Yu obtained a bachelor's degree in Chemistry from Sichuan University, China, a doctorate in Human Genetics and Molecular Biology and postdoctoral training from the Johns Hopkins University under the guidance of Bert Vogelstein and Kenneth W. Kinzler. She joined the faculty at the University of Pittsburgh in 2002.

Research in Dr. Yu's lab mechanistically and therapeutically explores cancer driver-dependent regulation of cell death and stress response. Her work focuses on the role of p53, APC/Myc and RAS/RAF in controlling cell death, metabolism, and immunologic consequences in the gastrointestinal (GI) tract. Her work employs a multidisciplinary and collaborative approach using cell lines, organoids, mouse models, and human tissues. Dr. Yu currently is a principle investigator (PI) on an R01 funded through the National Cancer Institute (NCI) entitled "Translation addiction and targeting in colon cancer" and a U19 sub-award funded through the National Institute of Allergy and Infectious Diseases (NIAID) entitled "Targeting intestinal stem cell dysfunctions in Radiation Mitigation." She is co-investigator on several NCI R01s and multiple PI (MPI) on a pending NCI R01. Dr. Yu's contribution to science includes 102 research articles cited over 12,000 times with an H-index of 46, including publications in *Cell, Nature, Science, Molecular Cell, Cell Stem Cell, Science Translational Medicine, JCI* and *PNAS*. Dr. Yu is an executive editor for *Molecular Carcinogenesis,* associate editor for *Genes and Diseases,* a standing member and co-chair of the NIH-MCT1 (former BMCT) study section, and reviewer for a variety of national and international funding agencies and professional journals. Dr. Yu also holds international and U.S. patents. Her hobbies include swimming, tennis, yoga, reading, cooking, travel, and Chinese cultural and educational community work. The long-term goal of Dr. Yu's research is to reduce cancer burden and suffering through more effective and cell death mechanism-based therapy, prevention, and normal stem cell protection.

Inside this issue:

Contact Us:

For more information about the GSP, please contact:
Patty Opresko, PhD - plo4@pitt.edu
Ben Van Houten, PhD - vanhoutenb@upmc.edu

Dr. Tatiana Moiseeva

Trainee Spotlight — Tatiana Moiseeva, PhD

By Mary Byrnes

Congratulations to Tatiana Moiseeva, PhD, former postdoctoral associate in Dr. Christopher Bakkenist's lab, on her new position as a senior research scientist in the Department of Chemistry and Biotechnology at Tallinn University of Technology in Estonia. Dr. Moiseeva earned her bachelor's degree in Physics and her master's degree in Biophysics at Saint Petersburg State Polytechnical University, followed by her doctorate in Molecular Biology at the Institute of Cytology RAS, Department of Regulation and Gene Expression, in 2011. Dr. Moiseeva had been a postdoctoral associate in Dr. Bakkenist's lab since 2013. Her main research interests are roles of ATM and ATR kinases in regulation of replication proteins and replication origin firing. Dr. Moiseeva is first author on two journal publications from 2019, and one each from 2018, 2017, and 2016, in addition to several others as co-author. She has been an invited speaker to nine national and international conferences over the past two years and received numerous honors and awards including the 2018 Best Oral Presentation at the 1st Southern Genome Maintenance Conference in Mobile, Ala., the 2018 Emerging Scientist Award from the Environmental Mutagenesis and Genomics Society (EMGS), and the 2018 Best Oral Presentation at the 20th Annual Midwest DNA Repair Symposium in Cleveland, Ohio.

"Dr. Moiseeva is a natural and generous leader and helps everyone in the lab academically, technically, and is a positive force in the lab leading with tireless resilience and fortitude," said Dr. Bakkenist. The Genome Stability Program will miss Dr. Moiseeva. We wish her all the best in her new position and future endeavors.

Pitt Stop: Special Events & Visiting Speakers

Rodney Rothstein, PhD

Professor, Department of Genetics & Development and Systems Biology
Columbia University

March 5, 2019

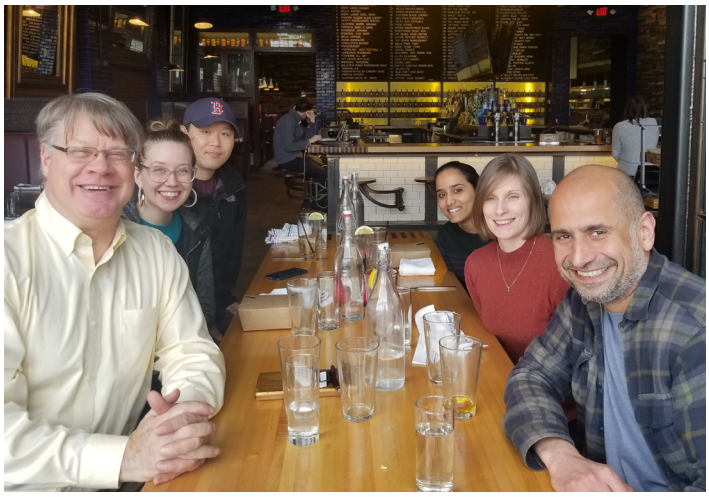
By Sarah Hengel, PhD, and Kara Bernstein, PhD

Dr. Rothstein visted UPMC Hillman Cancer Center and presented a research seminar entitled *"Using yeast to study gene overexpression, an underappreciated perturbation in cancer cells"* on March 5, 2019.

Dr. Rothstein is a National Academy of Sciences member and Professor in the Department of Genetics and Development and Systems Biology at Columbia University. He described his use of yeast to study gene over-expression, an under-appreciated perturbation in cancer cells. Given the gene conservation between yeast and humans, Dr. Rothstein is exploiting his expertise in yeast genetics to identify new synthetically lethal pairs to better understand protein over-expression in cancer. Dr. Rothstein introduced the idea that gene over-expression does not have to be a driver of cancer but can be an important part of the story. Gene over-expression can be targeted with therapeutics and mechanistically used to dissect how gene overexpression enables cancers. Looking outside of the 5% false discovery rate cutoff often used in synthetic screens, he found novel cisplatin-sensitive partners to the gene HRQ1. The second portion of his talk discussed the Rad5 (HLTF) protein that is over-expressed in lung carcinomas. The Rad5 protein promotes sister chromatid exchanges and plays an unknown role in replication fork restart. Using yeast genetics, Dr. Rothstein and his colleagues plan to uncover what HLTF does and how it modulates the pathways it functions in for future targeted cancer therapies. *This visit was funded by Dr. Kara Bernstein's lab and UPMC Hillman Cancer Center.*

Dr. Sarah Hengel (Bernstein lab), Dr. Rodney Rothstein, and Dr. Kara Bernstein.

Pitt Stop: Special Events & Visiting Speakers



Left, front to back: Dr. Ben Van Houten, Emily Beckwitt, Dr. Sunbok Jang; Right, front to back: Dr. Neil Kad, Dr. Alex Moores, Namrata Kumar

Neil Kad, PhD

Reader in Molecular Biophysics
University of Kent, UK

Alex Moores, PhD

BBSRC Postdoctoral Fellow
University of Kent, UK

March 7, 2019

By Ben Van Houten, PhD

Dr. Neil Kad and Dr. Alex Moores visited UPMC Hillman Cancer Center on March 7 and together delivered a talk entitled *"Walking the tightrope between DNA and actin: Single molecule insights into mechanism."* Neil started the lecture with a beautiful overview of the power of how single molecule techniques allow a dynamic view of biology. From his website, he has this beautiful quotation: "Imagine a room full of people saying the same sentence but not all at the same time, all you hear is garbled noise. But using a microphone you could amplify what a single person is saying and you'll hear the sentence. This is what we do to investigate complex processes." He first discussed his stunning work on understanding how myosin interacts with myosin to facilitate muscle contraction. Specifically, he showed a novel imaging platform that shows how myosin works in a cooperative manner to provide activation. He then switched gears and discussed his revolutionary imaging platform for protein-DNA interactions using a DNA tightrope assay coupled to fluorescently labeled purified DNA repair proteins. Finally, Alex discussed how the lab is transitioning from purified systems to watching the same bacterial DNA repair proteins in a living E. coli cell at the single molecule level. *This visit was partially funded by the UPMC Hillman Cancer Center Genome Stability Program fund.*

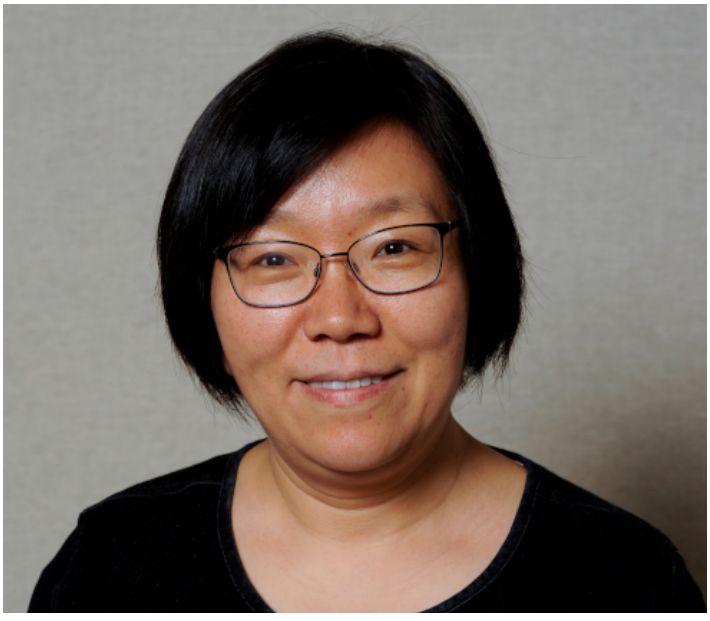
Hong Wang, PhD

Associate Professor of Physics
North Carolina State University

March 11, 2019

By Ben Van Houten, PhD

Dr. Hong Wang visited March 11 and gave an outstanding presentation on *"Unraveling the secrets at telomers, one molecule at a time."* Hong is a former postdoctoral fellow in the Van Houten laboratory, who helped establish Neil Kad's DNA tightrope platform here at the University of Pittsburgh. She also developed our capacity to image protein-DNA interactions with atomic force microscopy. Hong discussed her work on using a new AFM approach, Dual Resonance Frequency Enhanced Electrostatic Microscopy (DREEM), to study how an ensemble of proteins replicates mitochondrial DNA. Her group observed that mtSSB molecules bound to single strand regions of DNA, with one strand winding around the mtSSB tetramer. The second half of her talk she discussed how the fourth subunit of cohesion complexes, SA2 (STAG2) behaves on DNA at the single molecule level. Her group found that SA2's diffusion properties change on single versus double stranded DNA, and that a loss of SA2 leads to defects in homologous recombination mediated double-strand break repair. *This visit was partially funded by the UPMC Hillman Cancer Center Genome Stability Program fund.*



Dr. Hong Wang

Pitt Stop: Special Events & Visiting Speakers

Jeremy Stark, PhD

Professor of Cancer Genetics and Epigenetics
The Beckman Research Institute

April 1-2, 2019

By Carola Neumann, MD

We were fortunate to have Dr. Jeremy Stark visit UPMC Hillman from April 1-2, 2019. Jeremy is a leading expert on post-resection-related repair of DNA double strand breaks (DSB), namely single-strand annealing (SSA) and alternative-end joining (Alt-EJ). During his postdoctoral work in Maria Jasin's laboratory, he developed in cell GFP-reporter assays that allow specific testing of all DNA DSB repair pathways, which are now used by laboratories worldwide. Jeremy gave a wonderful lecture covering the etiology of chromosomal rearrangements and emphasized the role of ATM in classical non-homologous end joining (C-NHEJ). This topic then led into his new work, based on his recent publication in *Nature Communications,* titled "C-NHEJ without indels is robust and requires synergistic function of distinct XLF domains." He discussed the development of a new powerful assay that detects end joining (EJ) between distal ends of two Cas9-induced chromosomal breaks, that are joined without causing insertion/deletion mutations (indels). He described that such EJ requires several core C-NHEJ factors, including XLF. *This visit was funded by Dr. Chris Bakkenist's UPMC Hillman Cancer Center endowment fund.*

Dr. Jeremy Stark and Dr. Roddy O'Sullivan

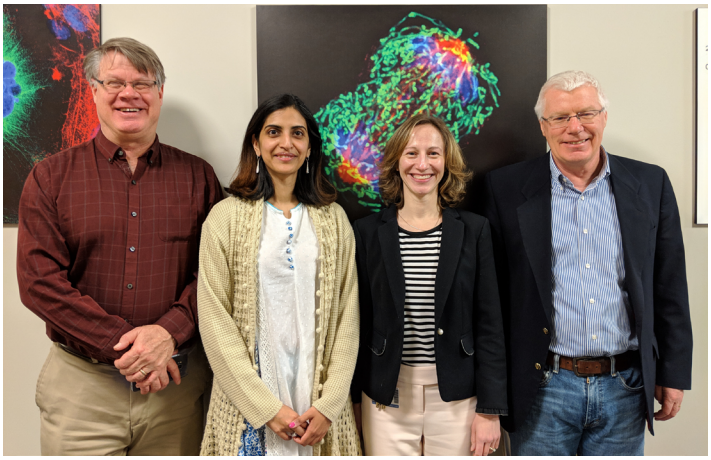
Alan Tomkinson, PhD

Professor, Division of Molecular Medicine
University of New Mexico, Department of Internal Medicine

April 4-5, 2019

By Ben Van Houten, PhD

Dr. Alan Tomkinson visited the University of Pittsburgh on April 4 and 5 and delivered a distinguished professor lecture in Pharmacology and Chemical Biology entitled *"Human DNA ligases; from mechanism to potential therapeutic targets."* Alan gave a remarkable presentation detailing his biochemical and biophysical studies of three different human DNA ligases in mammalian cells. He finished his lecture showing how he has developed a specific inhibitor of DNA ligase III which has profound effects on the physiology of mitochondria, but not the nucleus. Most importantly he showed that this Ligase inhibitor worked effectively to kill tumor cells, but normal cells were significantly less impacted by this drug. This presentation served as a keynote lecture for a mini-symposium co-chaired by Drs. Sarah Hengel (Bernstein lab) and Namrata Kumar (Van Houten lab). *This visit was partially funded by the UPMC Hillman Cancer Center Genome Stability Program fund.*

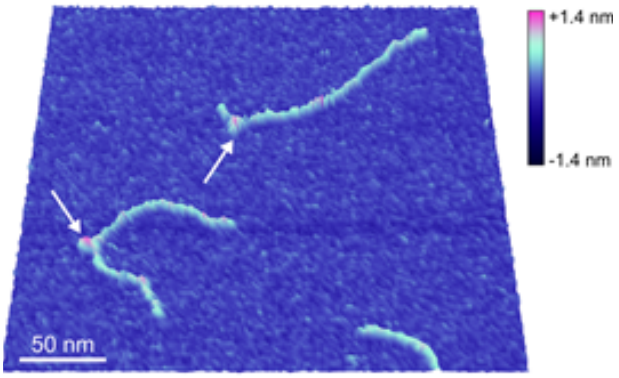


Dr. Ben Van Houten, Dr. Aditi Gurkar, Dr. Patty Opresko and Dr. Alan Tomkinson

Pitt Stop: Special Events & Visiting Speakers

The mini-symposium included presentations by:

- **Jacob-Stewart Ornstein, PhD,** Assistant Professor, Department of Computational and Systems Biology, presented *"p53 Signaling and Cell Identity."*
- **Norie Suganti (Chiba), PhD,** (Bakkenist lab) Department of Radiation Oncology, presented *"Why CD8+ T Cells Proliferate Every 6 Hours?"*
- **Meghan Sullivan, PhD,** (Bernstein lab) Department of Microbiology and Molecular Genetics, presented *"Uncovering How RAD51C Mutations Contribute to Cancer Predisposition."*
- **Emily Beckwitt, PhD candidate,** (Van Houten lab) Department of Medicine-Pharmacology and Chemical Biology, presented *"Single Molecule Analysis Reveals that XPA Bings and Bends DNA as a Monomer, Exhibiting Episodic Motion on DNA."*
- **Yael Nechemia-Arbely, PhD,** Assistant Professor, Department of Medicine-Pharmacology and Chemical Biology, presented *"Guarding the Genome: Maintaining Epigenetically Defined Human Centromeres Through Error Correction."*
- **Ryan Barnes, PhD,** (Opresko lab) Department of Environmental and Occupational Health, Graduate School of Public Health, presented *"Targeted Production of Telomeric 8-oxoguanine Induces Senescence in Normal Human Fibroblasts."*
- ***Song-My Hoang, PhD candidate,*** (O'Sullivan lab) Department of Medicine-Pharmacology and Chemical Biology, presented *"Control of Telomeric Homology Directed Repair by Poly ADP-Ribose Metabolism."*



Atomic force microscope image of the DNA repair protein, XPA specifically bound to a AAF-dG adduct in a 538 bp DNA fragment. White arrow indicates XPA binding. Image taken by Emily Beckwitt.

Bruce Demple, PhD

Professor, Department Pharmacological Sciences
Stony Brook University

May 9-10, 2019

By Mariarosaria De Rosa, PhD

According to the tradition of welcoming an expert in genome stability to visit UPMC Hillman Cancer Center, this spring Drs. Ben Van Houten and Patty Opresko invited Dr. Bruce Demple, a world-renowned expert on base excision repair, to deliver a lecture entitled *"Base Excision DNA Repair of Oxidative Damage: Pathways and Detours."* During a breakfast meeting with GSP Trainees, Dr. Demple listened to our graduate students and postdocs describe their research, and offered constructive criticism, supportive feedback, and insightful questions and ideas for experiments. So, not only coffee and food for breakfast, but also "food for thought."

He delivered an outstanding seminar, with particular attention to his contributions to the field over the years, including the first discovery of a DNA glycosylase acting on oxidative DNA damage, and pioneering work on how oxidative abasic sites and DNA-protein crosslinks originate and are repaired (PNAS USA, Dec 1991; 88(24): 11450–11454 - PNAS USA, Jul 2015;112(28):8602-7). *This visit was partially funded by the UPMC Hillman Cancer Center Genome Stability Program fund.*

Namrata Kumar, Maria Beecher, Dr. Tatiana Moiseeva, Dr. Bruce Demple, Dr. Mariarosaria De Rosa, Emily Beckwitt, Dr. Norie Sugitani

Pitt Stop: Special Events & Visiting Speakers

Phil Hanawalt, PhD

Professor Emeritus
Stanford University

April 25-26, 2019

By Ben Van Houten, PhD

First row: Maria Beecher, Dr. Tatiana Moiseeva, Namrata Kumar, Dr. Mariarosaria De Rosa, Dr. Norie Sugitani, Samuel Johnson
Second row: Dr. Phil Hanawalt, Dr. Sarah Hengel, Emily Beckwitt

Dr. Phil Hanawalt presented a personal view of his enormous contributions to the DNA repair field in his lecture, *"Persons and Perspectives in the history of DNA Repair"* on April 26. This delightful seminar wove together threads of his science with his outstanding organization skills in developing and chairing, often with Errol Friedberg, key DNA repair meetings at beautiful locations, like ski resorts. A wonderful account of this beautiful tapestry of his life is given in *DNA Repair* (11(5):452.e1-11, 2012). One unifying principle in his presentation was how his laboratory was always in search of new tools to answer fundamental questions about DNA damage and repair. His seminar started with his early years as a graduate student in Yale working with Dick Setlow, where he used radioactive nucleotide precursors to study the effects of ultraviolet radiation on the growth of E. coli that started his fascination with "thymineless death." After recounting his informative postdoc at Caltech, learning much from the Delbruck and Sinsheimer groups, he started his own laboratory at Stanford University in 1961. He related how 1964 was a good year for DNA repair with his and three other laboratories co-discovering nucleotide excision repair. Phil then described how after an exciting decade of discovery in the 70's, his laboratory sought to understand whether damage and repair was homogenous throughout the genome, and his early experiments with Mimi Zolan showing that alpha-DNA found in centromeres were strikingly deficient in the removal of chemical adducts. This led to the development of a new approach to look at damage and repair in specific genes developed by Will Bohr and specific DNA strands by Isabel Mellon that established actively transcribed strands are repaired faster than non-transcribed strands due to stalling by RNA polymerase at UV-induced photoproducts. He closed his seminar with his most recent work on the effects of R-loop formation during transcription and how this can cause genome instability. Phil has been a great mentor to an entire generation of scientists studying genome stability and his perpetual joy for learning was evident when he remarked that he greatly enjoyed his breakfast meeting with our trainees whose boundless enthusiasm for their science was the highlight of his trip. *This visit was partially funded by the UPMC Hillman Cancer Center Genome Stability Program fund.*

Sheila David, PhD

Professor, Department Chemistry
University of California Davis

May 14, 2019

By Sarah Hengel, PhD, and Patty Opresko, PhD

Dr. Mariarosaria De Rosa, Namrata Kumar, Dr. Sunbok Jang, Nicole Kaminski, Dr. Sarah Hengel, Dr. Sheila David, Dr. Tatiana Moiseeva, Song-My Hoang, Dr. Ryan Barnes, Dr. Norie Sugitani

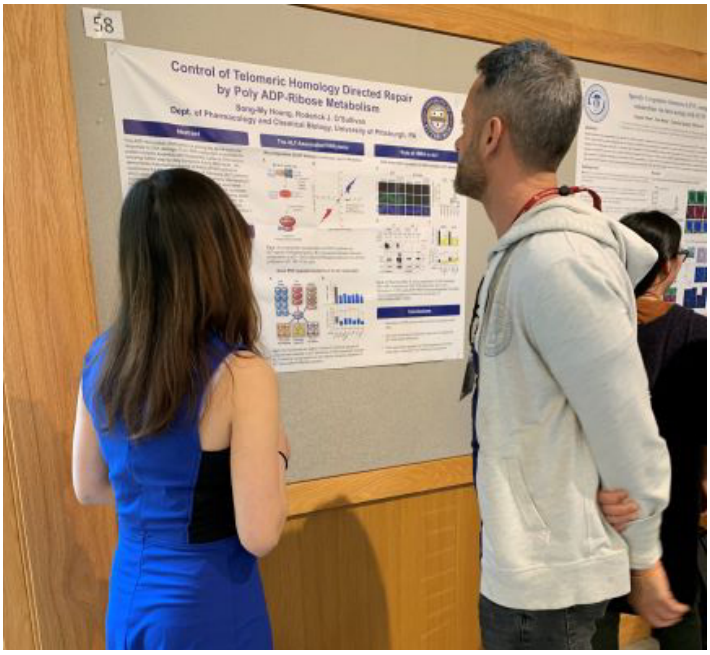
Dr. Sheila David visited UPMC Hillman Cancer Center and gave a Basic Science Seminar entitled *"When you are strange: Unusual features of the MUTYH glycosylases and implications in cancer"* on May 14, 2019. She talked about structure and chemical features of MUTYH glycosylase that allow it to recognize dA paired with 8-oxo-deoxy-guanine, and described studies investigating how variants and mutations of MUTYH in the human population alter the enzymatic activity. Mutations in MUTYH are known to cause inherited forms of colon cancer. She finished her seminar with a discussion of her lab's work exploring non-canonical roles for MUTYH, including very interesting data that suggests MUTYH may inhibit repair of alkylation base damage. Following her seminar, she graciously met with graduate student and postdoctoral trainees during lunch where she shared wisdom about her work and life in academia. Her enthusiasm and excitement for science left a marked effect on all in attendance.

Conference Highlights and Awards

DNA Pitt Crew Travels to Cold Spring Harbor Laboratory

By Song-My Hoang

Two labs from the genome stability group (O'Sullivan and Opresko labs) were represented at the eleventh Cold Spring Harbor Laboratory (CSHL) meeting on Telomeres & Telomerase. This year's organizers were Steve Artandi from Stanford University, Julia Promisel Cooper from the National Cancer Institute, and Jan Karlseder from The Salk Institute. This five-day conference brought together a diverse range of scientists who study the molecular, cellular, genetic, and clinical aspects of telomere biology and was packed with oral and poster presentations – starting from 9 a.m. and running until 10 p.m. From the Opresko lab, Ryan Barnes, PhD, gave a talk at the telomere protection session titled *"Targeted oxidative telomere base damage induces growth arrest and senescence in normal human cells."* Samantha Sanford presented the poster *"Investigating the impact of oxidized and therapeutic dNTPs on telomerase activity."* Adam Barsouk's poster asked the research question, *"Is nucleotide excision repair essential for telomere preservation following UV exposure?"* Nicole Kaminski and I were in attendance from the O'Sullivan lab, and I showcased a poster titled *"Transitional control of telomeric homology directed repair by Poly ADP-Ribose metabolism."* This CSHL conference was the perfect hub to gather feedback from telomere experts and meet new peers. This year, the organizers hosted a new event, the Meet the Speakers Lunch, where students and postdocs had lunch with prominent investigators and were able to learn from these mentors wealth of knowledge and experience. This informal lunch sparked science and career-related conversations. Overall, the CSHL meeting on Telomeres & Telomerase provided valuable insight that the Genome Stability group brought back home. It was an incredible opportunity to exchange scientific ideas and discuss the latest advances in the field. We can't wait for the next meeting in 2021!

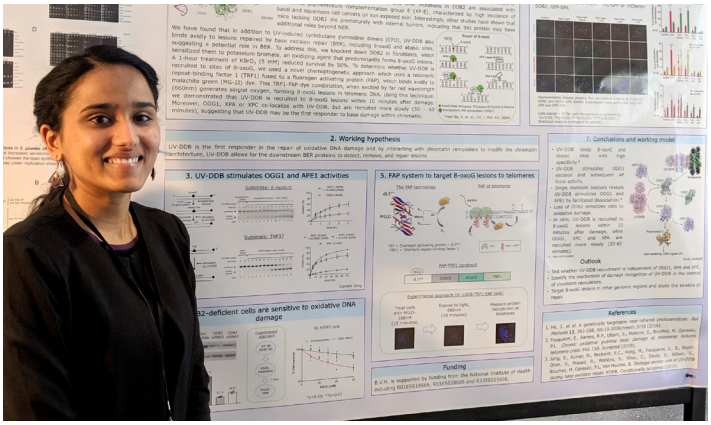


Accolades at the 31st Annual UPMC Hillman Cancer Center Scientific Retreat

Congratulations Samantha Sanford, graduate student researcher in the Opresko lab, on winning first place for the best poster in basic cancer research at the 31st Annual UPMC Hillman Cancer Center Scientific Retreat, held on June 19-20, 2019, at Soldiers & Sailors Memorial Hall in Oakland, for "Investigating the impact of oxidized and therapeutic dNTPs on telomerase activity."

Accolades at the Midwest DNA Repair Conference

Washington University School of Medicine in St. Louis and St. Louis University hosted the 21st Annual Midwest DNA Repair Symposium on May 4-5, 2019. This yearly symposium attracts a diverse group of researchers working on the field of DNA damage and repair.



Namrata Kumar, a graduate student in Dr. Ben Van Houten's lab, received the best poster award for her poster titled "UV-DDB: an early responder in the repair of oxidative DNA damage." Hayley Rein (Bernstein lab) was also invited to give a short talk at the symposium.

Hot Papers

Top 14 hot papers to date in 2019:

1. Jang S, Kumar N, Beckwitt EC, Kong M, Fouquerel E, Rapić-Otrin V, Prasad R, Watkins SC, Khuu C, Majumdar C, David SS, Wilson SH, Bruchez MP, Opresko PL, Van Houten B. Damage sensor role of UV-DDB during base excision repair. Nat Struct Mol Biol. 2019 Aug;26(8):695-703. doi: 10.1038/s41594-019-0261-7. Epub 2019 Jul 22. PubMed PMID: 31332353; PubMed Central PMCID: PMC6684372.

2. Fouquerel E, Barnes RP, Uttam S, Watkins SC, Bruchez MP, Opresko PL. Targeted and Persistent 8-Oxoguanine Base Damage at Telomeres Promotes Telomere Loss andCrisis. Mol Cell. 2019 Jul 11;75(1):117-130.e6. doi: 10.1016/j.molcel.2019.04.024. Epub 2019 May 14. PubMed PMID: 31101499; PubMed Central PMCID: PMC6625854.

3. Barroso-González J, García-Expósito L, Hoang SM, Lynskey ML, Roncaioli JL,Ghosh A, Wallace CT, Modesti M, Bernstein KA, Sarkar SN, Watkins SC, O'Sullivan RJ. RAD51AP1 Is an Essential Mediator of Alternative Lengthening of Telomeres. Mol Cell. 2019 Aug 7. pii: S1097-2765(19)30500-3. doi: 10.1016/j.molcel.2019.06.043. [Epub ahead of print] PubMed PMID: 31400850.

4. Rosenbaum JC, Bonilla B, Hengel SR, Mertz TM, Herken BW, Kazemier HG, Pressimone CA, Ratterman TC, MacNary E, De Magis A, Kwon Y, Godin SK, Van Houten B, Normolle DP, Sung P, Das SR, Paeschke K, Roberts SA, VanDemark AP, Bernstein KA. The Rad51 paralogs facilitate a novel DNA strand specific damage tolerance pathway. Nat Commun. 2019 Aug 5;10(1):3515. doi: 10.1038/s41467-019-11374-8. PubMed PMID: 31383866; PubMed Central PMCID: PMC6683157.

5. Moiseeva TN, Yin Y, Calderon MJ, Qian C, Schamus-Haynes S, Sugitani N, Osmanbeyoglu HU, Rothenberg E, Watkins SC, Bakkenist CJ. An ATR and CHK1 kinase signaling mechanism that limits origin firing during unperturbed DNA replication. Proc Natl Acad Sci U S A. 2019 Jul 2;116(27):13374-13383. doi: 10.1073/pnas.1903418116. Epub 2019 Jun 17. PubMed PMID: 31209037; PubMed Central PMCID: PMC6613105.

6. W. Qian, N. Kumar, V. Roginskaya, E. Fouquerel, P. Opresko, S. Shiva, S. Watkins, D. Kolodiezyni, M. P. Bruchez, and B. Van Houten. Chemoptogenetic damage to mitochondria causes rapid telomere dysfunction. PNAS. 2019. Aug 26. pii: 201910574. doi: 10.1073/pnas.1910574116. [Epub ahead of print]

7. Cui Y, Parashar S, Zahoor M, Needham PG, Mari M, Zhu M, Chen S, Ho HC, Reggiori F, Farhan H, Brodsky JL, Ferro-Novick S. A COPII subunit acts with an autophagy receptor to target endoplasmic reticulum for degradation. Science. 2019 Jul 5;365(6448):53-60. doi: 10.1126/science.aau9263. PubMed PMID: 31273116.

8. Reyes J, Chen JY, Stewart-Ornstein J, Karhohs KW, Mock CS, Lahav G. Fluctuations in p53 Signaling Allow Escape from Cell-Cycle Arrest. Mol Cell. 2019 Mar 21;73(6):1306. doi: 10.1016/j.molcel.2019.02.035. PubMed PMID: 30901565; PubMed Central PMCID: PMC6544155.

9. Nechemia-Arbely Y, Miga KH, Shoshani O, Aslanian A, McMahon MA, Lee AY, Fachinetti D, Yates JR 3rd, Ren B, Cleveland DW. DNA replication acts as an error correction mechanism to maintain centromere identity by restricting CENP-A to centromeres. Nat Cell Biol. 2019 Jun;21(6):743-754. doi:10.1038/s41556-019-0331-4. Epub 2019 Jun 3. PubMed PMID: 31160708.

10. Tan X, Tong J, Wang YJ, Fletcher R, Schoen RE, Yu J, Shen L, Zhang L. BET Inhibitors Potentiate Chemotherapy and Killing of SPOP-Mutant Colon Cancer Cells via Induction of DR5. Cancer Res. 2019 Mar 15;79(6):1191-1203. doi:10.1158/0008-5472.CAN-18-3223. Epub 2019 Jan 23. PubMed PMID: 30674532; PubMed Central PMCID: PMC6420862.

11. Chen D, Ni HM, Wang L, Ma X, Yu J, Ding WX, Zhang L. p53 Up-regulated Modulator of Apoptosis Induction Mediates Acetaminophen-Induced Necrosis and Liver Injury in Mice. Hepatology. 2019 May;69(5):2164-2179. doi: 10.1002/hep.30422. Epub 2019 Mar 11. PubMed PMID: 30552702; PubMed Central PMCID: PMC6461480.

12. Veglia F, Tyurin VA, Blasi M, De Leo A, Kossenkov AV, Donthireddy L, To TKJ, Schug Z, Basu S, Wang F, Ricciotti E, DiRusso C, Murphy ME, Vonderheide RH, Lieberman PM, Mulligan C, Nam B, Hockstein N, Masters G, Guarino M, Lin C, Nefedova Y, Black P, Kagan VE, Gabrilovich DI. Fatty acid transport protein 2 reprograms neutrophils in cancer. Nature. 2019 May;569(7754):73-78. doi: 10.1038/s41586-019-1118-2. Epub 2019 Apr 17. PubMed PMID: 30996346; PubMed Central PMCID: PMC6557120.

13. Cucinotta CE, Hildreth AE, McShane BM, Shirra MK, Arndt KM. The nucleosome acidic patch directly interacts with subunits of the Paf1 and FACT complexes and controls chromatin architecture in vivo. Nucleic Acids Res. 2019 Jun 21. pii:gkz549. doi: 10.1093/nar/gkz549. [Epub ahead of print] PubMed PMID: 31226204.

14. Singh M, Tian XJ, Donnenberg VS, Watson AM, Zhang J, Stabile LP, Watkins SC,Xing J, Sant S. Targeting the Temporal Dynamics of Hypoxia-Induced Tumor-Secreted Factors Halts Tumor Migration. Cancer Res. 2019 Jun 1;79(11):2962-2977. doi: 10.1158/0008-5472.CAN-18-3151. Epub 2019 Apr 5. PubMed PMID: 30952634; PubMed Central PMCID: PMC6548579.

Cool Science

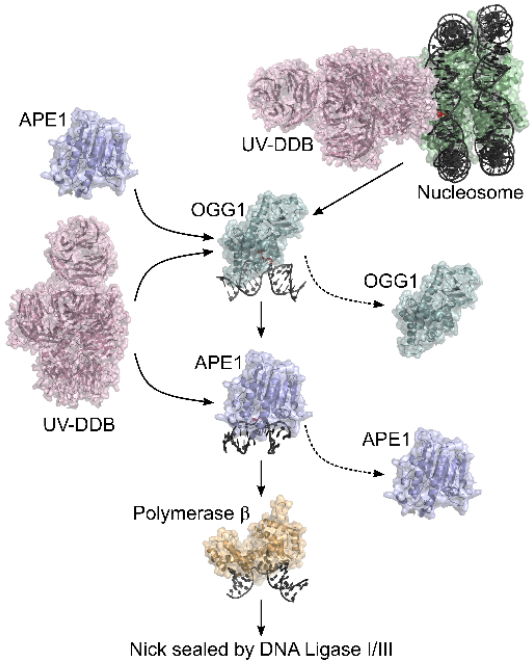
By Patty Opresko, PhD, and Ben Van Houten, PhD

Damage Sensor Role of UV-DDB During Base Excision Repair

UV-DDB plays an important role in the recognition of UV-induced photoproducts in the context of chromatin during nucleotide excision repair. Other studies have shown that mice lacking UV-DDB prematurely die due to internal cancers suggesting a broader role of UV-DDB in genome maintenance. This intra-programmatic collaboration between Ben Van Houten, PhD, working with Patty Opresko, PhD, Marcel Bruchez, PhD, and Simon Watkins, PhD, used biochemical, single molecule and cell biology approaches to show that UV-DDB stimulates several enzymes involved in the processing and removal of 8-oxoG damage during base excision repair (Jang et al., Nature Structural & Molecular Biology 2019 Aug;26(8):695-703). UV-DDB was found to enhance the activities of OGG1, MUTYH, and APE1, three-, five-, and eight-fold, respectively. Single molecule analysis revealed that this stimulation occurs through a direct protein-protein interaction causing enzyme turnover. Knockdown of UV-DDB caused enhanced sensitivity to the oxidizing agent, potassium bromate. Chemoptogenetic targeting of 8-oxoG to telomeres showed that UV-DDB is the first responder to this damage arriving prior to OGG1.

Impact: Biochemical, single molecule, and cell biology tools revealed a paradigm shift in how oxidative damage is recognized in chromatin of mammalian cells.

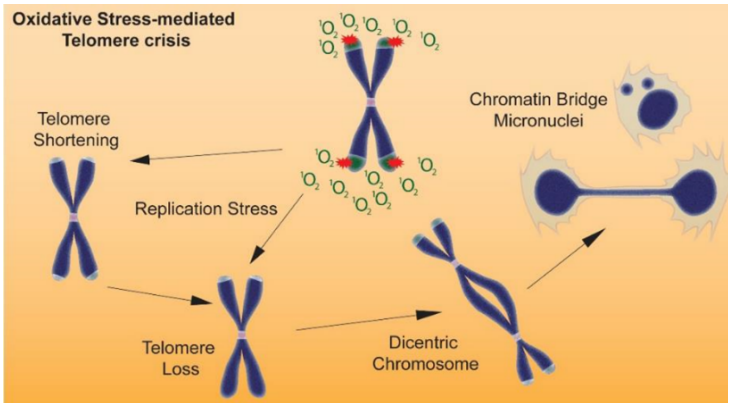
Funding: R01ES019566 and R01ES028686 to BVH R33 ES025606 to PO and BVH; R01EB017268 to MPB, R01CA067985 to SSD; ZIAES050159 and ZIAES050158 to SHW; ECB was supported by T32 GM088119; CK was supported by T32 ES007059 P30A047904 to UPMC Hillman Cancer Center.



UV-DDB is the first responder and stimulates BER enzyme.

Targeted and Persistent 8-Oxoguanine Base Damage at Telomeres Promotes Telomere Loss and Crisis

Oxidative stress and chronic inflammation accelerate telomere shortening, but the mechanism was unclear because oxidants have pleiotropic cellular effects. Telomeres are hypersensitive to 8-oxoguanine (8-oxoG) formation, suggesting this lesion may impact telomere maintenance. In an intra-programmatic collaboration, Patty Opresko, PhD, working with Marcel Bruchez, PhD, and Simon Watkins, PhD, developed a chemoptogenetic tool that selectively produces 8-oxoG damage exclusively within the telomere duplex DNA (Fouquerel, et al., *Molecular Cell* 2019 Jul 11;75(1):117-130). Working with Opresko lab members Elise Fouquerel, PhD, and Ryan Barnes PhD, this team discovered that chronic telomeric 8-oxoG induction shortens telomeres and impairs cancer cell growth. Accumulation of telomeric 8-oxoG in cells rendered deficient in 8-oxoG repair by disrupting OGG1 glycosylase, disrupts telomere replication, resulting in telomere loss and chromosome fusions, driving overall genomic instability. This article was selected for F1000Primer and was featured in a SciShow video that has received nearly 200K hits: https://www.youtube.com/watch?v=9gxogiUvVkk&feature=youtu.be.



Chemoptogenetic tool produces 8-oxoG damage exclusively at telomeres causing telomere loss, and chromatin bridges.

Impact: Oxidative DNA damage at telomeres directly drives telomere shortening & dysfunction in human cancer cells. Telomeric 8-oxogaunine impairs cell growth and causes genomic instability. High oxidative stress in cancer cells can be leveraged to promote telomere loss.

Funding: K99ES027028 to EF; R21ES025606 to PLO, SCW and MPB; R01CA207342, R01ES028242 and R01ES022944 to PLO; R01EB017268 to MPB; P30CA047904 to UPMC Hillman Cancer Center.

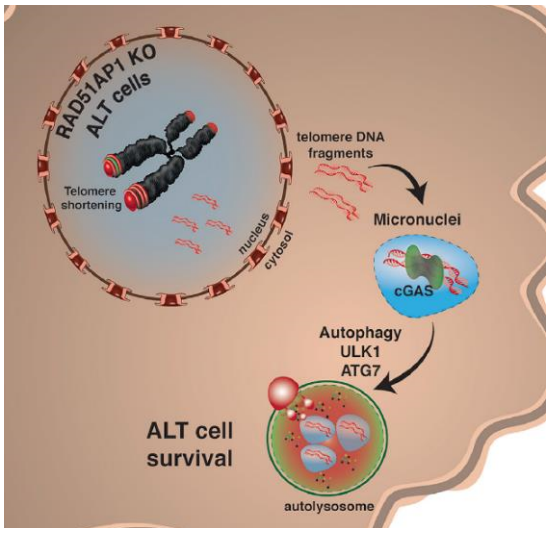
More Cool Science

RAD51AP1 Is an Essential Mediator of Alternative Lengthening of Telomeres

Alternative lengthening of telomeres (ALT) is a homology-directed repair mechanism that enables telomere maintenance and continued proliferation of aggressive cancers that lack telomerase activity. Roderick O'Sullivan, PhD, working with other GSP members Kara Bernstein, PhD, and Simon Watkins, PhD, and Saumendra Sarkar, PhD, from the Cancer Immunology and Immunotherapy Program, discovered that disrupting the homologous recombination (HR) factor RAD51AP1 caused telomere shortening and fragmentation in ALT positive cancer cells (Barroso-González et al., Molecular Cell 2019 October 3,76: 1–16). The telomere fragments are sensed by cGAS leading to ULK1-ATG7-dependent activation of autophagy. This team further showed that RAD51AP1 is stabilized in ALT positive cancers through SUMOylation.

Impact: Disruption of RAD51AP1 inhibits telomere elongation by ALT in cancer cells, driving telomere loss. cGAS-ULK1-ATG7-dependent autophagy is activated upon telomere dysfunction. RAD51AP1 is specifically stabilized and regulated by SUMOylation in ALT cancer cells.

Funding: St. Baldrick's Foundation and American Cancer Society RSG-18-038-01-DMC to RJO; R01ES024872 and American Cancer Society grant 129182-RSG-16-043-01-DMC to KAB; 1S10OD19973-01 to SCW; P30 A047904 to UPMC Hillman Cancer Center.



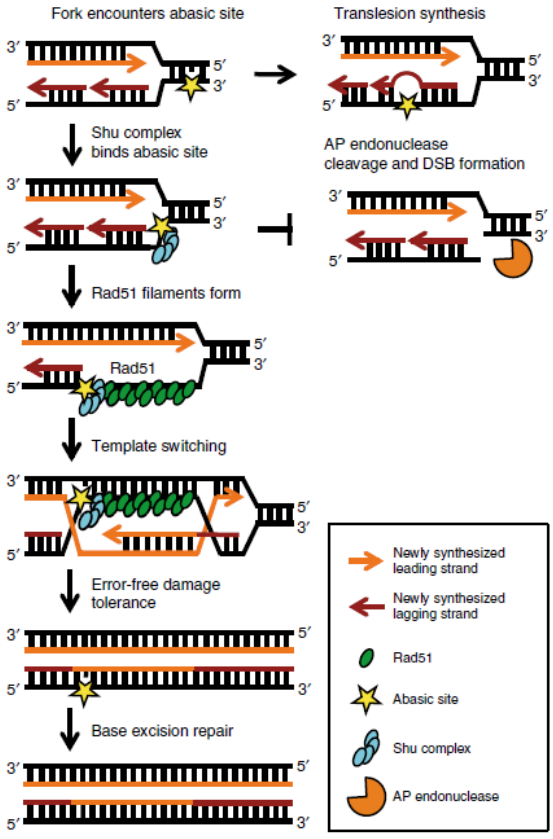
Disruption of RAD51AP1 inhibits telomere elongation by ALT in cancer cells, driving telomere loss. Release of telomeres into cytoplasm activates cGAS-ULK1-ATG7-dependent autophagy.

The Rad51 Paralogs Facilitate a Novel DNA Strand-Specific Damage Tolerance Pathway

Abasic sites block the progression of DNA replication forks, requiring either low-fidelity translesion DNA synthesis or the preferred high-fidelity homologous recombination (HR) to bypass the lesion in a damage tolerance pathway. RAD51 paralogs facilitate RAD51-mediated HR. Kara Bernstein, PhD, in collaboration with Ben Van Houten, PhD, discovered that the Shu complex, which contain RAD51 paralogs, associates with chromatin during S-phase and enables tolerance of primarily lagging-strand abasic sites (Rosenbaum et al., *Nature Communications* 2019 Aug 5;10(1):3515). In biochemical studies they show that purified Shu complex binds an abasic analog on model replication forks and prevents cleavage of the fork into a double strand break by AP endonuclease. Consistent with this, methyl methansulfonate induces greater growth reduction and mutations in cells expressing Shu complex DNA binding mutants compared to wild type.

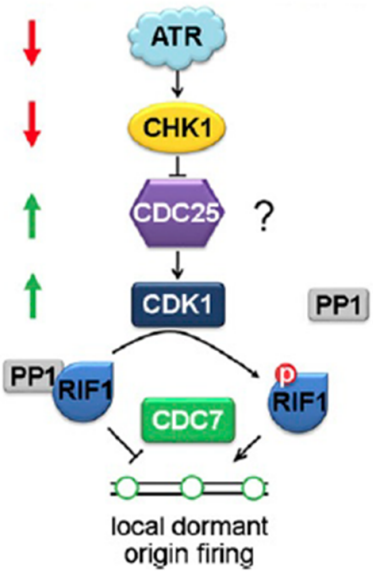
Impact: The Shu complex recognizes abasic sites at replication intermediates, where it recruits the homologous recombination machinery to mediate strand specific damage tolerance.

Funding: ES024872 to KAB and APV; ES019566 to BVH; ES007061 to PS; CA218112 to SAR and the American Cancer Society 129182-RSG-16-043-01-DMC to KAB; KP is supported by grant from the European Research Council (ERC Stg Grant: 638988-G4DSB); P30CA047904 to UPMC Hillman Cancer Center.



Working model of how the Rad51 paralogs, the Shu complex allows replicative bypass of abasic sites.

More Cool Science



ATR and CHK1 kinases stabilize RIF1 and PP1 phosphatase interaction to counteract CDC7-mediated origin licensing.

An ATR and CHK1 Kinase Signaling Mechanism that Limits Origin Firing During Unperturbed DNA Replication

Inhibitors of ATR and CHK1 kinases are being used in clinical trials and this study by Chris Bakkenist, PhD, working with Simon Watkins, PhD, sought to understand the molecular mechanism of how ATR inhibitors caused increase in replication origin firing (Moiseeva, et al., *PNAS,* 2019 July 2, 116:13374-13383). This study showed that unperturbed DNA replication is associated with a low level of ATR and CHK1 kinase signaling and that inhibition of this signaling induces dormant origin firing at sites of ongoing replication throughout the S phase. They further showed that ATR and CHK1 kinase inhibitors induce RIF1 Ser2205 phosphorylation in a CDK1-dependent manner, which disrupts an interaction between RIF1 and PP1 phosphatase. Thus, ATR and CHK1 signaling suppresses CDK1 kinase activity throughout the S phase and stabilizes an interaction between RIF1 and PP1 in replicating cells. PP1 dephosphorylates key CDC7 and CDK2 kinase substrates to inhibit the assembly and activation of the replicative helicase.

Impact: This study discovered how ATR inhibitors induce excessive replication origin firing. ATR and CHK1 kinases stabilize RIF1 and PP1 phosphatase interaction to counteract CDC7-mediated origin licensing.

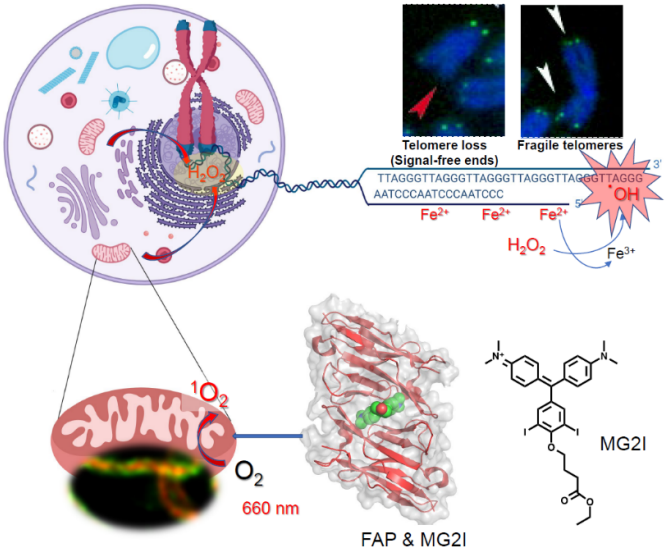
Stabilization is by suppressing CDK1-dependent RIF1 phosphorylation. This mechanism limits origin firing during unperturbed DNA replication. ATR are being used clinically to treat cancer.

Funding: R01 CA204173 to CJB; R01 GM108119, American Cancer Society Grant 130304-RSG-16-241-01-DMC, and V Foundation for Cancer Research Grant D2018-020 to ER; R00CA207871 to HUO; P30CA047904 to UPMC Hillman Cancer Center.

Chemoptogenetic Damage to Mitochondria Causes Rapid Telomere Dysfunction

While widely cited in the literature, actual direct cause and effect evidence that mitochondrial dysfunction causes nuclear damage is lacking. In an intra-programmatic collaboration, Wei Qian, PhD, and Ben Van Houten, PhD, working with Patty Opresko, PhD, Marcel Bruchez, PhD, and Simon Watkins, PhD, developed a chemoptogenetic tool to selectively damage mitochondria with a burst of singlet oxygen (Qian, et al., *PNAS,* 2019 released Aug 22). This singlet oxygen induced rapid mitochondrial dysfunction and generated a secondary wave of superoxide anion radical and hydrogen peroxide that caused a cascade of cellular responses, including activation of ATM kinase, cell cycle arrest and DNA replication stress. This study indicated that hydrogen peroxide released from damaged mitochondria caused oxidation of nuclear proteins, alterations in nuclear morphology, but not gross genomic damage. Analysis of telomeres indicated that this targeted mitochondrial damage led to telomere double-strand breaks, fragility and loss within 48hrs of the initial insult. For an interview with Ben Van Houten, see: https://www.upmc.com/media/news/082619-pnas-van-houten; his work has been picked up by multiple news feeds: https://www.pnas.org/content/116/37/18435/tab-article-info.

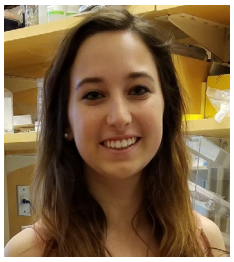
Impact: We developed a targeted chemoptogenetic tool to induce mitochondrial dysfunction through highly reactive singlet oxygen. These damaged mitochondria release hydrogen peroxide into the nucleus causing oxidation of nuclear proteins and telomere damage. This study has important ramifications for the role of mitochondrial signaling in aging and cancer among other human diseases. This is the first cause and effect study of its kind.



Mitochondrial dysfunction induced by targeted singlet oxygen causes rapid telomere damage.

Funding: R33ES025606 to PLO MPB and BVH; R01EB017268 to MPB; P30CA047904 to UPMC Hillman Cancer Center.

Faculty and Staff News



Nicole Kaminski

Namrata Kumar

Samuel Johnson

Congratulations to **Nicole Kaminski** (O'Sullivan lab), **Namrata Kumar** (Van Houten lab), and **Samuel Johnson** (Opresko lab) on passing their qualifying exams: SUMO regulation of Alternative Lengthening of Telomeres, UV-DDB: An Early Responder in the Repair of Oxidative DNA Damage, and Biophysical and Structural Investigation of Citrullinated Myelin Basic Protein, respectively.

Congratulations to **Heath Skinner, MD, PhD,** and his family on arrival of new baby girl, Catherine Anne Skinner, born April 17, 2019 weighing in at 8 lbs. 8 oz.

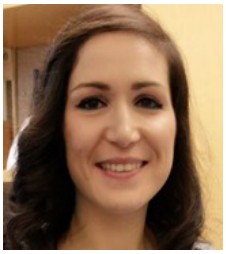
We wish a fond farewell to **Laura Garcia Exposito, PhD**, who is heading home to Spain after many years in the O'Sullivan lab.

Left to right: Ariana Detwiler, Dr. Ryan Barnes, Song-My Hoang, Nicole Kaminski, Dr. Laura Garcia Exposito, Michelle Lynskey, Dr. Mariarosaria De Rosa, Dr. Norie Sugitani, Katie Lemon, Dr. Tatiana Moiseeva, Sachi Dhakal, Lyubov Kublo

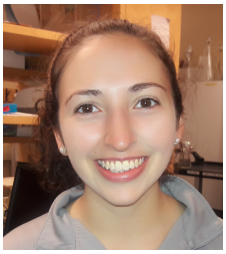
We're pleased to welcome (and in some cases, welcome back) the following new staff members:

- **Mariarosaria De Rosa, PhD,** postdoctoral associate, and **Sanjana Thosar,** graduate student, joined Dr. Patty Opresko's lab earlier this year.
- **Rebecca Raphael,** new research specialist, joined Dr. Yael Nechemia-Arbely's lab in May 2019.
- **Shayla (Shay) Goller,** new research technician, joined Dr. Jacob-Stewart-Ornstein's lab in June 2019.
- **Lyubov Kublo,** new research technician in Dr. Jacob-Stewart-Ornstein's lab, returned to UPMC Hillman Cancer Center in February 2019.
- **Maria Beecher,** graduate student, joined the Van Houten lab.
- **Fujun Dai, PhD,** visiting scholar, **Denise Resnik,** postdoc from Argentina, and **Lin Shen,** visiting student from China, joined the Lin Zhang lab.
- **Azrin Jamalruddin** and **Hayley Rein**, graduate students, joined the Bernstein lab.
- The Jian Yu lab welcomed **Jie Long,** a visiting MD/PhD student from Hunan, China, in September, and **Guangyi Zhao, PhD** (Pitt), a postdoctoral associate, joined in March 2018.
- **Ragini Bhargava, PhD,** postdoctoral fellow, and **Michelle Lynskey,** graduate student, joined the O'Sullivan lab.

SPECIAL ACKNOWLEDGEMENT AND THANKS to **Namrata Kumar** and **Maria Beecher**, graduate students, (Van Houten lab) who took time from their lab work to carefully read and edit this newsletter.



Mariarosaria De Rosa, PhD



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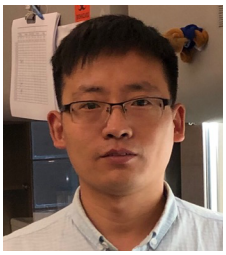
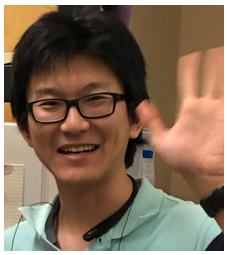
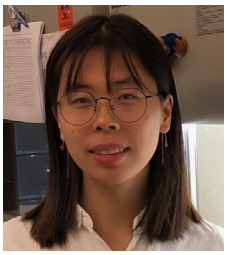


Fujun Dai, PhD



Guangui Zhao, PhD

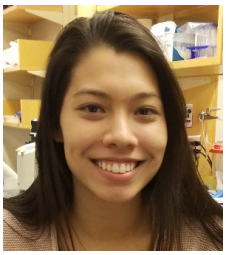
Hayley Rein



Jie Long

Lin Shen

Lyubov Kublo



Michelle Lynskey