PCGI Newsletter

Issue 1 - December 2021

Innovation with MACs funded by NIH

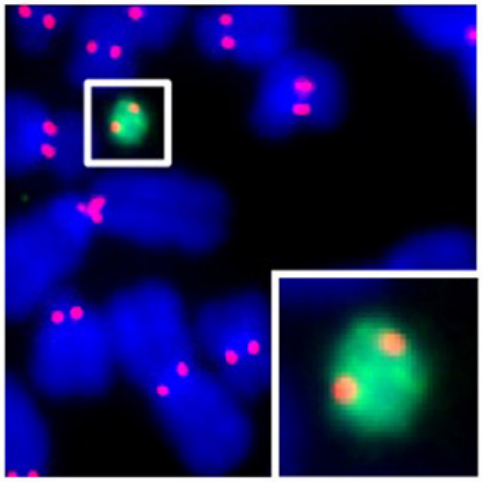
Richard Feynman, the late theoretical physicist, once said, “What I cannot create, I do not understand”

Two PCGI Leaders, Drs. Ben Black and Michael Lampson, were inspired in part by this concept to build synthetic mammalian artificial chromosomes (MACs) to advance our fundamental understanding of what comprises a mammalian chromosome. This work was recently funded by an NIH Director’s award for Transformative Research. The ultimate aim: generate an entire set of synthetic human chromosomes. The clinical applications, even short of this ambitious aim, are significant and far-reaching.

These studies build upon ten years of successful collaboration between the Black and Lampson labs on diverse studies of chromosome segregation and inheritance. For the MACs, work in the Black lab led by his PhD student, Glennis Logsdon (Logsdon GA et. al. *Cell* 2019) piqued Lampson’s interest and sparked a conversation between the two investigators. Black and Lampson recently sat down with us to explain their motivation behind the proposal.

“I saw Glennis present her work several times,” Lampson said, “and I was on her thesis committee, I think together Ben and I thought, ‘Well…wouldn’t it be exciting to try to introduce the MACs into an animal model?’”

Lampson began studying chromosome segregation during mammalian reproduction with a focus on processes occurring in oocytes. “It raises more long-term questions that we hope we can answer about how the DNA sequences in the centromeres affect segregation and bias segregation,” he said. “And MACs would give us a way to control those things that we don’t have otherwise.”



HAC is shown (green) and has two sister centromeres (red), similar to that of the natural host chromosomes (blue).

And so, they looked to the building blocks. “If you want to understand how mammalian chromosomes really work, then we want to try to build in all the functionalities we think are important,” Lampson continued. “So, in the long term, I think that’s what we’re trying to do. Not so much about what genes are on the chromosome, but everything else about the chromosome, hopefully, that controls its inheritance. The centromere which controls how it interacts with the spindle, and other parts of the

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Welcome to the first Penn Center for Genome Integrity (PCGI) newsletter. Each quarter, we will highlight some of the innovations we are fostering within the Center.

Our overarching mission is to integrate cutting-edge research in basic and clinical sciences from several key disciplines in order to advance our fundamental understanding of genome integrity and its contributions to human biology. Since our founding in January 2020, despite the COVID-19 pandemic, we have continued to grow, successfully contributing to faculty recruitment, shared resources, and the creation of a vibrant scientific program centered on genome integrity and human disease. We hope to bring you monthly features, particularly to highlight our robust trainee program.

Penn Medicine

PCGI Penn Center for Genome Integrity

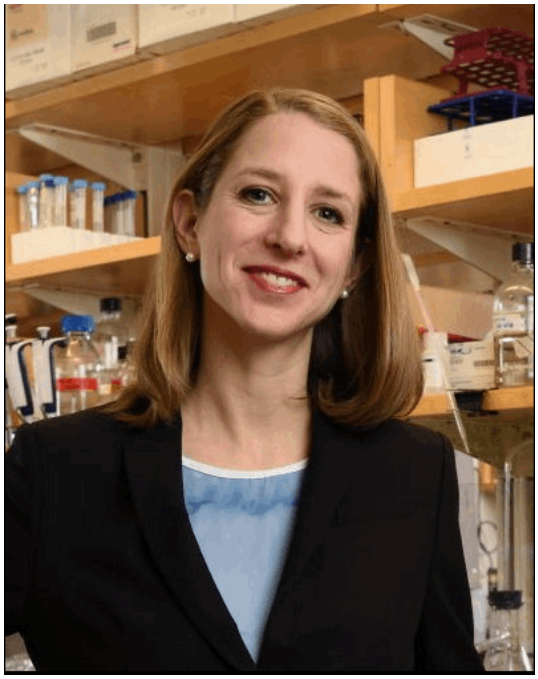
www.med.upenn.edu/pcgi
421 Curie Blvd
5th floor, BRB II/III
Philadelphia, PA 19104
P: 215-573-0908

News Briefs

Faculty Recruitment

The PCGI has a core mission to relate fundamental processes that control genome integrity to human biology. DNA repair and immune system responses to DNA damaging events are core components of this mission. We are thus thrilled to have successfully contributed to the recruitment of Drs. Kara Bernstein and Corneluus Taabazuing.

- The PCGI made major contributions to the recruitment of new faculty Drs. Kara Bernstein (coming from University of Pittsburgh) and Cornelius Taabazuing (joining us from Memorial Sloan Kettering Cancer Center).
- The PCGI is contributing Pilot grant funding to Dr. Cornelius Taabazuing, a new Assistant Professor in the Department of Biochemistry and Molecular Biophysics. Dr. Taabazuing uses chemical biology to investigate inflammasome function. A primary interest of his independent research program is the influence of DNA damaging events on inflammasome activation.
- The PCGI, together with the Basser Center for BRCA, joined with the Department of Biochemistry to successfully recruit Dr. Kara Bernstein to Penn. Dr. Bernstein is an Associate Professor at the University of Pittsburgh, and a renowned genome integrity researcher who has made seminal contributions to understanding homologous recombination. Her recruitment adds needed expertise in DNA repair to the PCGI and both basic and translational contributions to the Basser Center for BRCA.



We look forward to welcoming Drs. Cornelius Taabazuing (above) & Kara Bernstein (below) to the PCGI/PSOM Faculty.

Trainee Awards

The PCGI is developing a community of trainees that represent diverse aspects of genome integrity and its relationship to human biology. We hold monthly trainee seminars that consist of two 30-minute trainee talks. Trainees also frequently chair sessions during our external faculty seminar series. In addition, we are planning a PCGI retreat on June 14, 2022 to be held at the Mütter museum. This event will further showcase trainee accomplishments and form a venue for much needed trainee interactions.

Furthermore, the PCGI has contributed $1,000 in support of the Cell and Molecular Biology (CAMB) Graduate Group 24th Annual Scientific Symposium which was held virtually this year on October 21st. Distinguished CAMB alum, Dr. Scott Hensley of the University of Pennsylvania and Dr. Shawn Bediako of the NIH/ University of Maryland-Baltimore County gave Keynote lectures. Investing in events such as these is an important component to our goal in building upon PSOM graduate and postdoctoral programs that host our PCGI trainees. We celebrate the following PCGI trainee accomplishments:

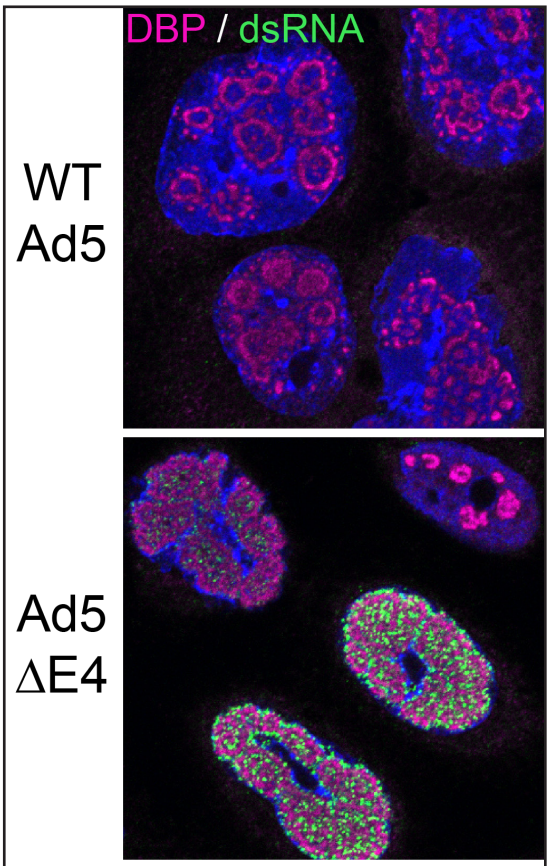
• Priyanka Verma, PhD of the Greenberg lab has started as an Assistant Professor in the Department of Medicine in the Molecular Oncology Division at Washington University School of Medicine in St. Louis.

- Viridiana Herrera, PhD, of the Black lab, has accepted a position as an Assistant Professor at Lincoln University.

Continued on p. 3...

News briefs, continued from p. 2...

- Postdoctoral Fellow Tim Lippert from the Greenberg Lab received the Cancer Research Institute Irvington Fellowship.
- Xin Liu, PhD, a postdoc in the Shin lab, has received an NIH/NHLBI K99 Pathway to Independence award to support her research and transition to a faculty position.
- Alex Price, PhD, a Research Associate in the Weitzman lab, was awarded a K99/R01 from NIAID for his work to study how RNA processing pathways regulate the formation of double-stranded RNA during infection by human DNA viruses and the cellular responses that lead to effects on protein translation. This work demonstrates the importance of viral control of RNA splicing and it overturn the dogma on host responses that are counteracted by viral strategies. His paper on this work was published in the October 22, 2021 issue of *Nucleic Acids Research:* https://pubmed.ncbi.nlm.nih.gov/34671803/.
- Nootan Pandey, PhD, in the Black lab was awarded a postdoctoral fellowship paper from the Basser Center.
- Rachel Richards, a summer intern in the Shin lab, has been named a White House HBCU Scholar. Rachel is also a 2021 Barry Goldwater Scholar.
- Shin lab graduate student Nawar Naseer co-authored an Expert Opinion piece for the Philadelphia Inquirer in July entitled "Vaccines give these Philly researchers hope, but the COVID-19 variants make them cautious of letting their guard down: https://www.inquirer.com/health/expert-opinions/covid-variants-vaccine-20210702.html
- Shin lab graduate student Marisa Egan was appointed a Penn Center for Teaching and Learning Graduate Fellow for Teaching Excellence. She also received the 2021 Penn Prize for Excellence in Teaching by Graduate Students.
- Kurtis McCannell, a joint graduate student in the Levine and Lampson labs, has received an NSF Graduate Research Fellowship to support his dissertation research.
- Sung-Ya Lin, a graduate student in the Levine lab, has received a "Taiwanese Government Scholarship" to support her dissertation work.



Wildtype adenovirus (top) infects human cells but, counter to dogma, produces no detectable double-stranded RNA (dsRNA, green). Mutant adenovirus that can no longer process it's viral RNA efficiently (bottom) produces abundant ds-RNA composed of intermolecular unspliced viral transcripts that is contained to the nucleus.

New Funding

PCGI Investigators are regularly receiving awards for their ground-breaking research:

- Mike Lampson and Ben Black were awarded a transformative R01 Award on developing Mammalian Artificial Chromosomes (see feature article, p. 1 and continued on p. 6)
- Roger Greenberg received a score of 2nd percentile on his R01 renewal from NIGMS.
- Roger Greenberg is a co-investigator on a grant from the Wellcome Trust that was awarded to collaborator Dr. Elton Zeqiraj of the University of Leeds, in Leeds, United Kingdom.
- Matthew Weitzman received a multi-PI R01 with Ben Garcia to continue their studies of viral modulation of epitranscriptomics and RNA modifications.
- Sunny Shin successfully renewed an R01 award, and also received an R21.
- PCGI-funded PI Luca Busino was awarded two new R01 grants.
- PCGI-funded PI Junwei Shi was awarded a new R01 grant
- PCGI leadership submitted a P01 application on DNA damage activation of anti-tumor immune responses. While the first submission of this application did not receive a fundable score, there is considerable enthusiasm at the NCI and a resubmission was invited. The resubmission is planned for the May 2022 deadline.

Publications

Research in Cell from PCGI Leaders Drs. Michael Lampson and Mia Levine reveals that centromere proteins have evolved with centromeric chromatin structure to prevent chromosome segregation bias. This mitigates "cheating" by chromosomes with large centromeres and limits their advantage in widing up in the egg during cell division:

- Tomohiro Kumon, Jun Ma, R. Brian Akins, Derek Stefanik, C. Erik Nordgren, Junhyong Kim, Mia T. Levine, Michael A. Lampson. Parrallel pathways for recruiting effector proteins determine centromere drive and suppression. *Cell* 2021 Aug 20;S0092-8674(21)00940-5.

On the cover of JCI Insight, PCGI Lab of Dr. Elizabeth McDonald, along with collaborators including Dr. Sean Carlin of Penn Radiology, demonstrated a significant advancement toward precision breast cancer care:

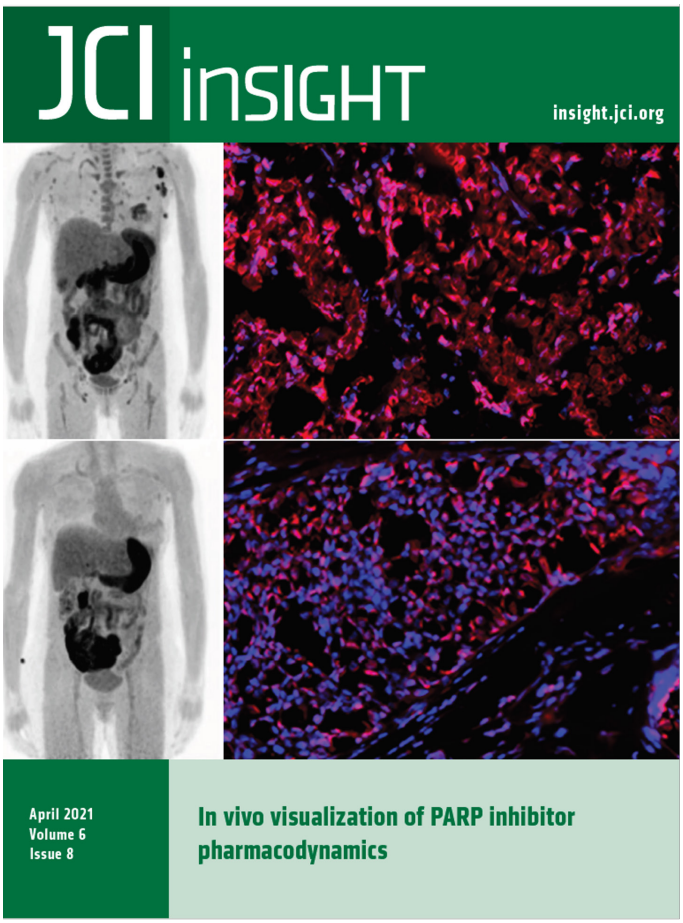
- Elizabeth S. McDonald, Austin R. Pantel, Payal D. Shah, Michael D. Farwell, Amy S. Clark, Robert K. Doot, Daniel A. Pryma, Sean D. Carlin. In vivo visualization of PARP inhibitor pharmacodynamics. *JCI Insight* 2021 Apr 22;6(8):e146592.

The Weitzman lab published a study in the journal mSystems which uses global proteomics approaches to define how the DNA tumor virus Adenovirus remodels the host proteome and affects the cellular factors that associate with the viral genome. They used a combination of whole cell proteomics together with isolation of proteins on nascent DNA (iPOND) to identify cellular DNA repair and replication proteins that are manipulated by virus infection:

- Joseph M. Dybas, Krystal K. Lum, Katarzyna Kulej, Emigdio D. Reyes, Richard Lauman, Matthew Charman, Caitlin E. Purman, Robert T. Steinbock, Nicholas Grams, Alexander M. Price, Lydia Mendoza, Benjamin A. Garcia, Matthew D. Weitzman. Adenovirus Remodeling of the Host Proteome and Host Factors Associated with Viral Genomes. *mSystems* 2021 Aug 31;e0046821. doi: 10.1128/mSystems.00468-21.

Drs. Roger Greenberg and Priyanka Verma, a postdoctoral researcher in his lab, published a review on how chromatin regulates homology-directed repair and genome integrity:

- Priyanka Verma, Roger A. Greenberg. Communication between chromatin and homologous combination. *Curr Opin Genet Dev*. 2021 Jun 4;71:1-9.



Research from the McDonald lab on triple negative breast cancer cells featured on the cover of JCI Insight, April 2021

Dr. Shelley Berger and collaborators including PCGI Investigator Dr. Eric Joyce demonstrated that p53-driven speckle association of its target genes increases their expression, contributing to the tumor suppressor functions of p53:

- Katherine A. Alexander, Allison Cote, Son C. Nguyen, Liguo Zhang, Omid Gholamalamdari, Paula Agudelo-Garcia, Enrique Lin-Shiao, K M A Tanim, Joan Lim, Nicolas Biddle, Margaret C. Dunagin, Charly R. Good, Mariel R. Mendoza, Shawn C. Little, Andrew Belmont, Eric F. Joyce, Arjun Raj, Shelley L. Berger. p53 mediates target gene association with nuclear speckles for amplified RNA expression. *Mol Cell* 2021 Apr 15;81(8):1666-1681.
- Press release in Penn Med News

The Weitzman lab published a paper in EMBO Reports in which they report proteomics to identify interacting proteins that regulate the APOBEC3A deaminase enzyme which is linked to mutations in cancer genomes.

Continued on next page (p. 5)...

Publications, continued from p. 4...

They discovered that the CCT chaperonin complex interacts with APOBEC3A, and suggest that disruption of CCT complex in cancers may result in increased APOBEC-driven mutational activity. The first author on this paper is Dr. Abby Green who started this work at CHOP/Penn and is now an Assistant Professor at Washington University in St Louis:

- Abby M. Green, Rachel A. DeWeerd, David R. O'Leary, Ava R. Hansen, Katharina E. Hayer, Katarzyna Kulej, Ariel S. Dineen, Julia H. Szeto, Benjamin A. Garcia, Matthew D. Weitzman. Interaction with the CCT chaperonin complex limits APOBEC3A cytidine deaminase cytotoxicity. *EMBO Rep*. 2021 Aug 4;e52145.

The PCGI labs of Junwei Shi and R. Babak Faryabi, in collaboration with colleagues in the Berger and Bernt labs, published a paper on ZMYND8 regulation of the IRF8 transcription axis:

- Zhendong Cao, Krista A. Budinich, Hua Huang, Diqiu Ren, Bin Lu, Zhen Zhang, Qingzhou Chen, Yeqiao Zhou, Yu-Han Huang, Fatemah Alikarami, Molly C. Kingsley, Alexandra K. Lenard, Aoi Wakabayashi, Eugene Khandros, Will Bailis, Jun Qi, Martin P. Carroll, Gerd A. Blobel, Robert B. Faryabi, Kathrin M. Bernt, Shelley L. Berger, Junwei Shi. ZMYND8-regulated IRF8 transcription axis is an acute myeloid leukemia dependency. *Mol Cell* 2021 Jul 28 ;S1097-2765(21)00587-6.

Praveen Allu, PhD, a postdoc in the Black lab, contributed to a collaborative study led by Dr. Katya Grishchuk's lab on the connections between chomosomes and the microtubule-based spindle that guides their inheritance at cell division:

- Ekaterina V. Tarasovetc, Praveen Kumar Allu, Robert T. Wimbish, Jennifer G. DeLuca, Iain M. Cheeseman, Ben E. Black, Ekaterina L. Grishchuk. Permitted and restricted steps of human kinetochore assembly in mitotic cell extracts. *Mol Biol Cell*. 2021 Jun 15;32(13):1241-1255.

Dr. Roger Greenberg and Tianpeng Zhang, a postdoc in his lab, with collaborators at WUSTL and UT Southwestern, published a paper in the Journal of Cell Biology revealing an unexpectedly dominant role of ATR in human pluripotent stem cells (hPSCs):

- Alexandre T. Vessoni, Tianpeng Zhang, Annabel Quinet, Ho-Chang Jeong, Michael Munroe, Matthew Wood, Enzo Tedone, Alessandro Vindigni, Jerry W. Shay, Roger A. Greenberg, Luis F Z Batista. Telomere Erosion in human pluripotent stem cells leads to ATR-mediated mitotic catastrophe. *J Cell Biol*. 2021 Jun 7;220(6):e202011014.

Tim Lippert (Greenberg Lab) published a review article on the "Abscopal Effect" accepted to the Journal of Clinical Investigation:

- Timothy P. Lippert and Roger A. Greenberg. The absopal effect: a sense of DNA damage is in the air. *J Clin Invest*. 2021 May 3;131(9):e148274.

PCGI Core Leadership Member, Michael Lampson, along with fellow School of Arts & Sciences colleague Jun Ma, in collaboration with Whitehead Institute researchers Iain Cheeseman and Nolan Maier, published an article revealing how a key protein, Meikin, enables the process of meiosis to unfold:

- Nolan K. Maier, Jun Ma, Michael A. Lampson, Iain M. Cheeseman. Separase cleaves the kinetochore protein Meikin at the meiosis I/II transition. *Dev Cell* 2021 Aug 9;56(15):2192-2206.e8.

The Lampson lab also published a review on cell division:

- Geng-Yuan Chen, Michael A. Lampson. Chemical tools for dissecting cell division. *Nat Chem Biol*. 2021 Jun;17(6):632-640.

PCGI Investigators Drs. Michael Lampson and David Chenoweth authored a paper showing how Aurora B kinase and intracellular forces regulate kinetochore-microtubule interactions dividing cells:

- Geng-Yuan Chen, Fioranna Renda, Huaiying Zhang, Alper Gokden, Daniel Z. Wu, David M. Chenoweth, Alexey Khodjakov, Michael A. Lampson. Tension promotes kinetochore-microtubule release in response to Aurora B activity. *CSHL bioRxiv*.

Researchers in the Chenoweth lab published a paper describing a new method for probing both the formation and function of chromatin-associated condensates on telomeres:

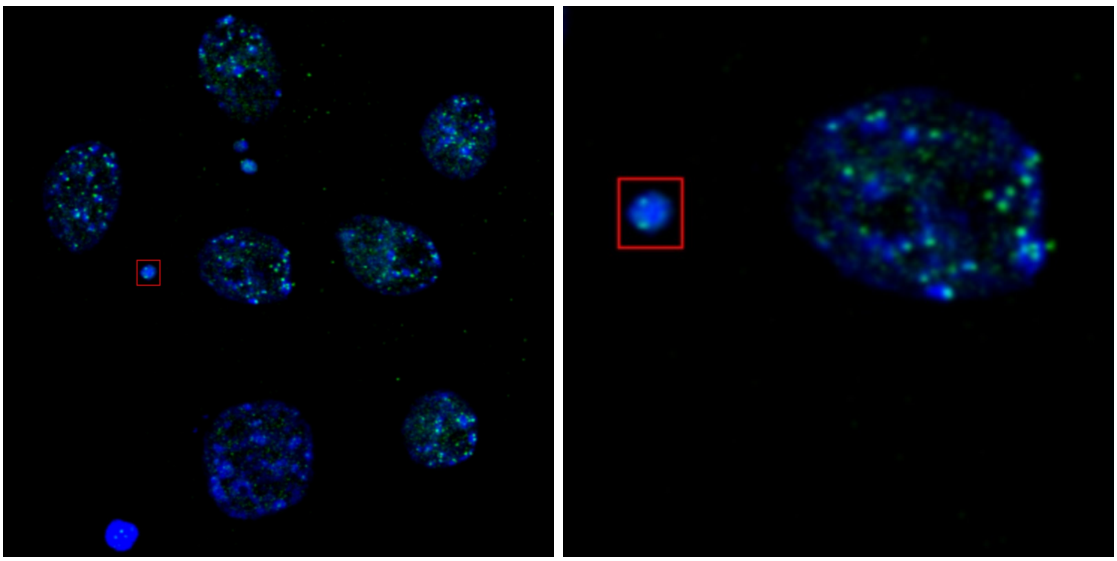
- Rongwei Zhao, David M. Chenoweth, Huaiying Zhang. Chemical Dimerization-Induced Protein Condensates on Telomeres. *J Vis Exp*. 2021 Apr 12;(170):10.3791/62173.

Did we miss your article? Don't forget to notify PCGI Administrative Director Laura Murillo when your lab publishes research: murillo@upenn.edu

Innovation with MACS, continued from p. 1...

chromosome which control how chromosomes pair and combine in meiosis, and the telomeres that are important for maintaining the chromosome without it having damage at the ends; without shortening… those are all things that we're trying to build into an artificial chromosome, with the idea being that look, if we understand all these things, we should be able to build it… and if we don't understand it, we'll find out when we try to build it."

The centromere has proven to be the biggest challenge so far of creating MACs, because mammalian centromeres are not encoded in the DNA sequence. It had remained unclear how to build synthetic chromosomes containing this crucial element.



(Left) an 8-cell embryo, and (right) just one cell from the embryo. "The red box is representative of something [Lampson Lab researcher] Jun Ma sees frequently in embryos injected with one of our MAC vectors (but not in control embryos)," Lampson says. "It looks like a micronucleus, which may be an intermediate to chromosome rearrangements that are frequently seen during MAC formation. The green shows CENP-A staining, which is an important centromere component. This result is not the final endpoint that we're looking for, but it is an indication that we may be on the right track."

"It was evident that what was minimal function for a MAC in the context of a typical cell going through mitosis was not going to suffice in the reproductive system, in a germ cell," Black said. "So those kinds of special cell divisions in meiosis present an extraordinary challenge. We thought, well, what can we do to these chromosomes to make them work in meiosis? Because we can design them and build them. If we can do that, we can test, like Mike said, how much do we really know about chromosomes? Do we know enough to be able to design the MACs to do these things that our natural chromosomes obviously know how to do?"

To solve these problems, the labs plan to hijack the existing cellular machinery for assembling centromere chromatin and incorporate additional genetic elements to ensure meiotic pairing and recombination. This effort requires innovation at multiple levels: designing MAC vectors encoding key functional elements, assembling large synthetic DNA constructs, and ultimately creating animals to test MACs *in vivo*.

And if they see unexpected outcomes, they have already given it a lot of thought and are prepared to change tactics.

"This was actually an interesting part of this process, to apply for this award, because pivoting at various inflection points was what we spent a lot of our time figuring out," Black said. "We knew what we wanted to do grand scale, and then thinking about each step of the process, what landmarks we could get to, and then what would happen if we had unexpected outcomes… this is exciting because I think we're going to learn at each step, how much do we know to design that step? And then the problem solving that we scientists like to do, that's going to help us make something that's teaching us about centromeres and chromosomes. But also, at the same time, making these molecules and the MAC approach more powerful, as we go."

The outlook is that each landmark reached in this project will provide unprecedented genome engineering capabilities that will further the field, a mission central to PCGI. Center investigators are already very excited about all the potential applications.

Black concludes the conversation: "That's, I think, inspiring for the people in the lab working on it, because you know when they're making an advance, even though it might seem like an incremental step, it's actually a big advance for the whole chromosome and genome integrity field. That's a fun place to be for scientists in the thick of it."

PCGI External Seminar Series

The PCGI hosts a monthly seminar series on a broad range of topics in genome integrity. This has been held in a virtual format with three Internationally-renowned scientists speaking in each session. These mini-symposia have been an excellent educational opportunity for faculty and trainees alike, while also allowing them to make connections to prominent scientists. Speakers are typically introduced by trainees or junior faculty, who also chair the sessions. This series will continue in a virtual format through the first half of 2022, which while protecting against the spread of COVID-19, also allows us the unique opportunity to include multiple International speakers.

On December 6, 2021, we enjoyed the following talks on the session topic of ***"Genome Editing"***:

Alexis C. Komor, PhD
Assistant Professor, Department of Chemistry and Biochemistry, University of California, San Diego

"Enhancing Genome Editing Precision Using Secondary gRNAs"

Alberto Ciccia, PhD
Associate Professor, Department of Genetics and Development and Member, Herbert Irving Comprehensive Cancer Center & Columbia Stem Cell Initiative, Columbia University Irving Medical Center

"Functional interrogation of DNA damage response variants with base editing screens"

Jacob E. Corn, PhD
Professor of Genome Biology, D-BIOL, Institute of Molecular Health Sciences, ETH, Zürich, Switzerland

"Genome editing at work in human cells"

Next month we are looking forward to a session on the topic of ***"Chromatin in Genome Integrity"*** on Monday, January 10, 2022 featuring:

Evi Soutoglou, PhD
Professor of Genome Stability (Genome Damage and Stability), University of Sussex, Brighton, UK

Gaëlle Legube, PhD
Investigator, Chromatin and DNA Repair, Toulouse Centre for Integrative Biology, France

Irene Chiolo, PhD
Assistant Professor of Biological Sciences and Gerontology, University of California, San Diego

The seminars are held Mondays from 10:00 am to 12:00 pm via Zoom:

Meeting Link:
https://pennmedicine.zoom.us/j/92784777880
Passcode: 923137

The talk titles are coming soon. If you would like to be added to our mailing list, or would like more information, please contact Laura Murillo via email at murillo@upenn.edu.

Scan this QR code for our full events calendar: