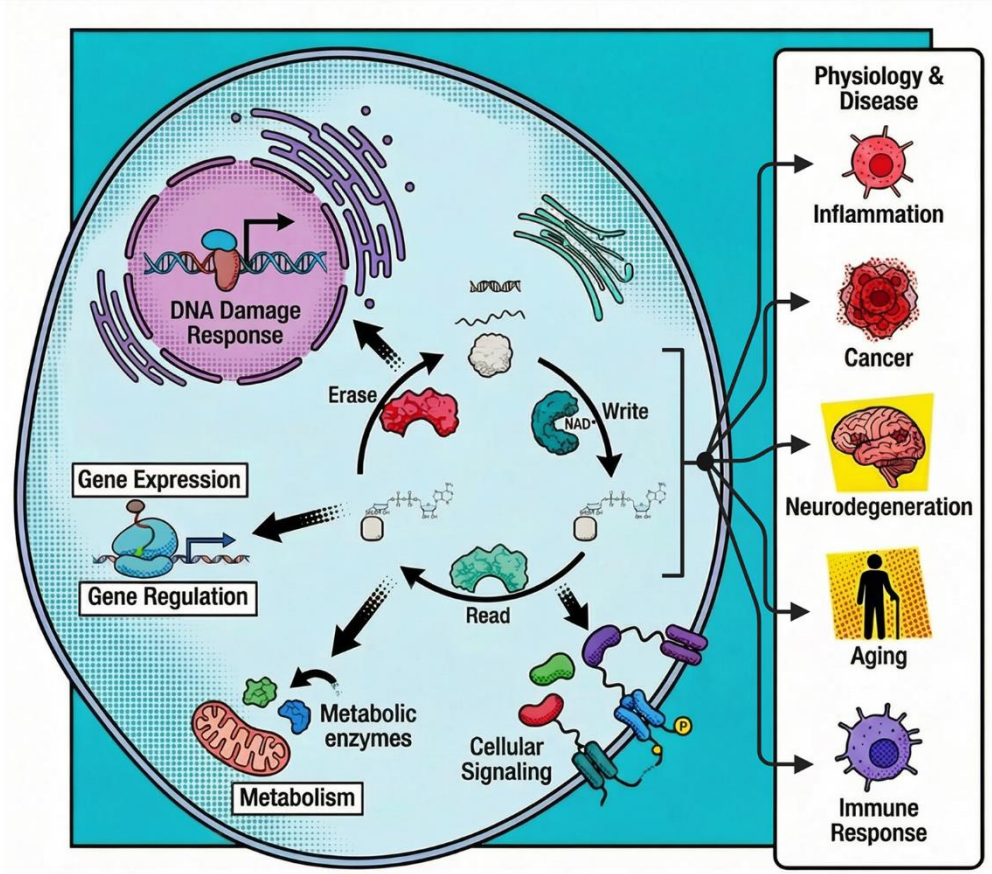
Abstracts of papers presented
at the 2026 meeting on

THE PARP FAMILY & ADP-RIBOSYLATION

March 25–March 28, 2026



CSH Cold Spring Harbor Laboratory
MEETINGS & COURSES PROGRAM

Abstracts of papers presented
at the 2026 meeting on

THE PARP FAMILY & ADP-RIBOSYLATION

March 25–March 28, 2026

Arranged by

Michael Cohen, *Oregon Health and Science University*
Lari Lehtiö, *University of Oulu, Finland*
Yonghao Yu, *Columbia University*
Shan Zha, *Columbia University* (*in absentia)*

Cold Spring Harbor Laboratory
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THE PARP FAMILY & ADP-RIBOSYLATION
Wednesday, March 25 – Saturday, March 28, 2026

Wednesday	7:30 pm – 9:00 pm	**1** Therapeutic Targeting of PARPs and Beyond in Cancer
Wednesday	9:30 pm – 10:15 pm	**Keynote Speaker**
Thursday	9:00 am – 12:00 pm	**2** Physiological Functions for ADPR Writers and Erasers
Thursday	2:00 pm – 5:00 pm	**3** PARPs in DNA Repair, Genome Stability, and Stress
Thursday	5:00 pm	*Wine and Cheese Party*
Thursday	7:30 pm – 8:00 pm	**Flash Talks**
Thursday	8:00 pm – 10:30 pm	**Poster Session**
Friday	9:00 am – 12:00 pm	**4** Structural Biology of ADPR Writers and Erasers, and ADPR Site Mapping
Friday	1:30 pm – 2:30 pm	**Panel Discussions:** Cutting-edge Technologies and Approaches in the Field *and* Drug Discovery Targeting ADP-ribose Writers and Erasers
Friday	2:30 pm – 4:30 pm	**5** Chemical Biology of ADP-ribosylation and Beyond
Friday	5:00 pm – 6:00 pm	**Keynote Speaker**
Friday	6:00 pm	*Cocktails and Banquet*
Saturday	9:00 am – 12:00 pm	**6** ADP-ribosylation in Immune and Pathogen Interactions

Mealtimes at Blackford Hall are as follows:
Breakfast 7:30 am-9:00 am
Lunch 11:30 am-1:30 pm
Dinner 5:30 pm-7:00 pm

Bar is open from 5:00 pm until late

All times shown are US Eastern: Time Zone Converter

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PROGRAM

WEDNESDAY, March 25—7:30 PM

SESSION 1 THERAPEUTIC TARGETING OF PARPs AND BEYOND IN CANCER

Chairpersons: **W. Lee Kraus,** University of Texas Southwestern Medical Center, Dallas
Karla Feijs, RWTH Aachen University Hospital, Germany

Therapeutic exploitation of tumor-selective parylation by PARG inhibition
Mike White.
Presenter affiliation: IDEAYA Biosciences, South San Francisco, California.

PARGi impairs base excision repair by sequestering PAR-binding protein-XRCC1 and its binding partners.
Isaac Dumoulin, Brian J. Lee, Chuhan Zhang, Yunyue Wang, Shan Zha.
Presenter affiliation: Columbia University, New York City, New York. 1

PARP12 in estrogen receptor-positive breast cancer—Survival pathways and beyond
Giovanna Grimaldi.
Presenter affiliation: Institute of Endotypes in Oncology, Metabolism and Immunology, Naples, Italy. 2

PARP2 drives R-loop-associated ADP-ribosylation in myelodysplastic syndromes
Hai Dang Nguyen.
Presenter affiliation: University of Minnesota, Minneapolis, Minnesota. 3

Updates to PARP trapping—A role for mono-ADPr in PARP1 release
Kira Schützenhofer, Ellen Laker, Domagoj Baretic, Rebecca Smith, Ivan Ahel.
Presenter affiliation: Sir William Dunn School of Pathology, Oxford, United Kingdom. 4

Redefining BRCA1 and PARP1 synthetic lethality
Sharon B. Cantor, Hitha G. Nair, Min Peng, Nathan MacGilvary, Joseph Edmonds, Dohoon Kim.
Presenter affiliation: University of Massachusetts Chan Medical School, Worcester, Massachusetts. 5

WEDNESDAY, March 25—9:30 PM

EMBO KEYNOTE LECTURE

Ivan Ahel
University of Oxford

THURSDAY, March 26—9:00 AM

SESSION 2 PHYSIOLOGICAL FUNCTIONS FOR ADPR WRITERS AND ERASERS

Chairpersons: **Sharon Canter,** University of Massachusetts Chan Medical School, Worcester
Gerbrand J. van der Heden-van Noort, Leiden University Medical Centre, the Netherlands

Molecular and functional aspects of PARP3 in physiological and pathological processes
Françoise Dantzer.
Presenter affiliation: CNRS, Strasbourg University, Illkirch, 67412, France. 6

Investigating the functional and regulatory roles of PARP2 isoforms in breast tissue and cancer
Holland Driscoll, Brittany Angarola, Mattia Brugiolo, Maeva Devoucoux, Hyeon Gu Kang, Olga Anczukow.
Presenter affiliation: The Jackson Laboratory, Farmington, Connecticut; University of Connecticut, Storrs, Connecticut. 7

Tankyrase switches a disease-associated RNA-binding protein from active to aggregating
James A. Gray, Alistair J. Langlands, Rachel Toth, C. James Hastie, Leeanne McGurk.
Presenter affiliation: University of Dundee, Dundee, United Kingdom. 8

Role of tankyrase scaffolding in the β-catenin destruction complex and WNT signaling
Chuo Chen, Qian Wang, Liping Li, Lin You, Lei Han, Shuai Wang, Lawrence Lum, Yonghao Yu, Jerry W. Shay.
Presenter affiliation: UT Southwestern Medical Center, Dallas, Texas. 9

Biology and mechanisms of site-specific ADP-ribosylation of C/EBPβ in obesity-related cardiac dysfunction
Yangyang Dai, Elizabeth M. Li-Fiedler, Weidan Song, W. Lee Kraus, Cristel V. Camacho, Dan Huang.
Presenter affiliation: The University of Texas Southwestern Medical Center, Dallas, Texas. 10

Tankyrase-2 regulates adipocyte differentiation through AMPK/mTOR signaling
Boglárka Rauch, Gyula Ujlaki, Szilárd Póliska, Nakayi Murahwa, Karen Uray, Peter Bai.
Presenter affiliation: University of Debrecen, Debrecen, Hungary. 11

A PARP inhibitor, rucaparib, improves cardiac dysfunction in ADP-ribose-acceptor hydrolase 3 deficiency
Jiro Kato, Sachiko Namamori, Xiangning Bu, Hiroko Ishiwata-Endo, Danielle Springer, Liu Chengyu, Zu-Xi Yu, Randy Clevenger, Karen Keeran, Martin J. Lizak, Joel Moss.
Presenter affiliation: NHLBI, National Institutes of Health, Bethesda, Maryland. 12

Cellular NAD^+-sensing and the regulation of ADP-ribosylation during adipogenesis
W. Lee Kraus.
Presenter affiliation: The University of Texas Southwestern Medical Center, Dallas, Texas. 13

THURSDAY, March 26—2:00 PM

SESSION 3 PARPs IIN DNA REPAIR, GENOME STABILITY AND STRESS

Chairperson: **John Pascal,** Université de Montréal, Canada
Katharina Höfer, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

A poly(ADP-ribose) code encodes biomolecular condensate formation, composition, and material state
Anthony K. Leung.
Presenter affiliation: Johns Hopkins University, Baltimore, Maryland. 14

ADP-ribosylation of FUS regulates the dynamics of DNA Damage repair condensate formation and maintenance
Fatmanur Tiryaki, Orsolya Leidecker, Dorothee Dormann.
Presenter affiliation: Johannes Gutenberg University, Mainz, Germany. 15

PARP1-induced nuclear RNA condensates link DNA damage to translation arrest
Pedro Ortega, Elodie Bournique, Ambrocio Sanchez, Jessie Altieri, Klemen Hertel, Rémi Buisson.
Presenter affiliation: University of California Irvine, Irvine, California. 16

PARylation-dependent regulation of replication fork dynamics by FET proteins
Celeste Giansanti, Jack C. Schultz, Jessica Jackson, Alessandro Vindigni, David Cortez.
Presenter affiliation: Vanderbilt University, Nashville, Tennessee. 17

PARG inhibition alters PARP1 dynamics at complex DNA lesions in living cells
Petar-Bogomil Kanev, Stoyno Stoynov, Radoslav Aleksandrov.
Presenter affiliation: Institute of Molecular Biology, BAS, Sofia, Bulgaria. 18

HPF1 protects cells from toxic forms of ADP-ribosylation
Lena Duma, Andrii Gorelik, Evgeniia Prokhorova, Ray W. Bowman, Joao A. Paulo, Kira Schützenhofer, Steven Gygi, Roderick O'Sullivan, Ivan Ahel.
Presenter affiliation: University of Oxford, Oxford, United Kingdom. 19

Balancing PARP activity during DNA replication through dynamic dePARylation
Litong Nie, Junjie Chen.
Presenter affiliation: The University of Texas M.D. Anderson Cancer Center, Houston, Texas. 20

PARG activity is required for cell death by parthanatos
Rafael D. Moura, Beatriz Kopel, Priscilla D. Mattos, Penélope F. Valente, Aline C. Santos, Rafael T. Nascimento, Fernanda M. Prado, Marisa H. Medeiros, João C. Setubal, Nadja C. Pinto, Nícolas C. Hoch.
Presenter affiliation: University of São Paulo, São Paulo, Brazil. 21

THURSDAY, March 26—5:00 PM

Wine and Cheese Party

THURSDAY, March 26

7:30 PM: FLASH TALKS

8:00 PM POSTER SESSION

See p. xiii for List of Flashtalks / Posters

FRIDAY, March 27—9:00 AM

SESSION 4 STRUCTURAL BIOLOGY OF ADPR WRITERS AND ERASERS AND ADPR SITE MAPPING

Chairpersons: **Anthony Leung,** Johns Hopkins University, Baltimore, Maryland
Renata Kasprzyk, ETH Zurich, Switzerland

Structural biochemistry of PARP family proteins
John M. Pascal.
Presenter affiliation: Université de Montréal, Montréal, Canada. 22

Dimerization of human PARP15 is required for NAD$^+$ binding and automodification
Anna Tuovinen, Johan Pääkkönen, Mirko M. Maksimainen, Lea Hirschen, Heli I. Hentilä, Marie Tauscher, Bernhard Lüscher, Carlos Vela-Rodríguez, Patricia Korn, Lari Lehtiö.
Presenter affiliation: University of Oulu, Oulu, Finland. 23

PARP1 exhibits an enzymatically inactive chromatin binding mode
Alexandria Fiorenza, Mahika Anand, Johannes Rudolph, Karolin Luger.
Presenter affiliation: University of Colorado Boulder, Boulder, Colorado. 24

A large-scale method to measure the stoichiometries of protein poly-ADP-ribosylation
Peng Li, Yajie Zhang, Chiho Kim, Yonghao Yu.
Presenter affiliation: Columbia University Vagelos College of Physicians and Surgeons, New York, New York. 25

Exceeding expectations with the Orbitrap Excedion Pro for large-scale ADP-ribosylation site analysis
Sara C. Buch-Larsen, Ivo A. Hendriks, Peter Krueger, Alexander Harder, Markus Kellmann, Jesper V. Olsen.
Presenter affiliation: NNF Center for Protein Research, University of Copenhagen, Denmark. 26

Deciphering the role of ADP-ribosylation in cardiolaminopathy
Deena M. Leslie Pedrioli, Nicolas Vignier, Antoine Muchir, Michael O. Hottiger.
Presenter affiliation: University of Zurich, Zurich, Switzerland. 27

How viral macrodomains erase ADP ribosylation and how to stop them
James S. Fraser.
Presenter affiliation: University of California San Francisco, San Francisco, California. 28

FRIDAY, March 27—1:30 PM

PANEL DISCUSSIONS

- Cutting-edge Technologies and Approaches in the Field
- Drug Discovery Targeting ADP-ribose Writers and Erasers

SESSION 5 CHEMICAL BIOLOGY OF ADP-RIBOSYLATION AND BEYOND

Chairpersons: **Giovanna Grimaldi**, Consiglio Nazionale delle Ricerche, Italy
Adam Hurlstone, University of Manchester, United Kingdom

Development of chemical tools to study ADPr-ubiquitylation
Gerbrand J. van der Heden van Noort.
Presenter affiliation: Leiden University Medical Centre, Leiden, the Netherlands. 29

MARUbylation as a novel hybrid post-translational modification
Rachel E. Lacoursiere, Kapil Upadhyaya, Michael S. Cohen, Jonathan N. Pruneda.
Presenter affiliation: Oregon Health & Science University, Portland, Oregon. 30

Cell-permeable nicotinamide adenine dinucleotides for exploration of cellular protein ADP-ribosylation
Renata Kasprzyk, Lea-Sophie Frings, Karina Kwapiszewska, Jaroslaw Michalski, Emma Lintfert, Robin N. Kohler, Florian Stengel, Andreas Marx.
Presenter affiliation: ETH Zurich, Zurich, Switzerland. 31

Mapping protein–poly(ADP-ribose) binding sites using a cleavable photocrosslinking proteomics probe
Maranda McDonald, Morgan Dasovich, Leilei Shi, Lu Sun, Isaiah Mixon, Sabrina Hunt, Dmitri Filippov, Michael-Christopher Keogh, Mark T. Bedford, Shao-En Ong, Marc Greenberg, Anthony Leung.
Presenter affiliation: Johns Hopkins University, Baltimore, Maryland. 32

Mono(ADP-ribosyl)ation—A marker for protein degradation?
Roko Žaja, Nonso J. Ikenga, Karla L. Feijs-Žaja.
Presenter affiliation: Institute of Biochemistry and Molecular Biology, Aachen, Germany. 33

FRIDAY, March 27—5:00 PM

KEYNOTE SPEAKER

Trey Ideker
University of California-San Diego

FRIDAY, March 27—6:00 PM

COCKTAILS and BANQUET

SATURDAY, March 28—9:00 AM

SESSION 6 ADP-RIBOSYLATION IN IMMUNE AND PATHOGEN INTERACTIONS

Chairpersons: **James Fraser,** University of California, San Francisco
Rebecca Smith, University of Oxford, United Kingdom

PARP14—An actionable node in the immunosuppressive tumour microenvironment
Chun Wai Wong, Rotem Salmi-Leshem, Kieran Sefton, Christos Evangelou, Mario Niepel, Adam Hurlstone.
Presenter affiliation: The University of Manchester, Manchester, United Kingdom. 34

Dual enzymatic activity of PARP14 and its role in interferon-induced ADP-ribosylation
Nina Dukic, Pulak Kar, Osamu Suyari, Jonas Damgaard Elsborg, Kang Zhu, Michael L. Nielsen, Dragana Ahel, Rebecca Smith, Ivan Ahel.
Presenter affiliation: University of Oxford, Oxford, United Kingdom. 35

Synthesis of a bacterial signalling molecule—ADP-ribosylated histidine
Bob van Puffelen, Hugo Minnee, Gijsbert A. van der Marel, Yang Lu, Ivan Ahel, Jeroen D. C. Codée, Dmitri V. Filippov.
Presenter affiliation: Leiden University, Leiden, the Netherlands. 36

A diversified synthetic toolkit for non-canonical ADP-ribosylation—Nucleic acid-ADPr, rifampicin-ADPr and glycocyclic-ADPr
Li Tang, Lingxiao Liu, Shuyan Jiang, Zongxing Yu, Qiang Liu.
Presenter affiliation: Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China; Zhongshan Institute for Drug Discovery, Zhongshan, China. 37

Mechanistic insight into ADP-ribosylation establishment and virulence by fungal PARPs
Adam G. Bainbridge, Tiberius-Marius Gianga, Rohanah Huassain, Nicolas Helmstetter, Rhys A. Farrer, Giuliano Siligardi, Johannes Gregor Matthias Rack.
Presenter affiliation: University of Exeter, Exeter, United Kingdom. 38

The art of RNAylation—Linking nucleic acids to proteins with natural precision to regulate cellular processes
Katharina Höfer.
Presenter affiliation: Marburg University , Marburg, Germany; Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany; Max Planck Institute for Terrestrial Microbiology, Marburg, Germany. 39

FLASH TALKS / POSTERS

Interdomain regulation of PARP14 enzymatic activity through allosteric interactions
Sofia Bali, James S. Fraser.
Presenter affiliation: University of California, San Francisco, San Francisco, California. 40

Identification of purine-based inhibitors for the SARS-CoV-2 and the MERS-CoV macrodomains
Banhi Biswas, Hien Vu, Junlin Zhuo, Rachy Abraham, Jürgen Bosch, Anuradha Roy, Alessandro Panattoni, Michal Maryska, Petr Slavik, Kryštof Šigut, Anthony R. Fehr, Barbara S. Slusher, Takashi Tsukamoto, Anthony K. Leung.
Presenter affiliation: Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland. 41

Histone mono-ADP-ribosylation as a regulatory axis in chromatin remodeling during stem cell differentiation
Remo Bode, Chrysi Kapsali, Ina Huppertz, Ivan Matic.
Presenter affiliation: Max Planck Institute for Biology of Ageing, Cologne, Germany; Cologne Graduate School of Ageing, Cologne, Germany. 42

PARP-1 mediates DDX helicases to prevent R-loop-induced DNA damage and genome instability
Jung-Kuei Chen, Wen-Ling Lin, Hung-wen Liu.
Presenter affiliation: University of Texas at Austin, Austin, Texas; National Taiwan University, Taipei, Taiwan. 43

Identification of allosteric modulators of PARP-3 activity
Khouloud Chtiba, Jean-Christophe Amé, Sergio Ortiz, Bruno Kieffer, Clothilde Le Guen, Françoise Dantzer.
Presenter affiliation: UMR7242, CNRS, Université de Strasbourg, Illkirch, France. 44

PARP1 mediates NAD+-dependent nucleosome disassembly to enable chromatin accessibility during the early phase of the DNA damage response
Ashish Verma, Changlei Zhu, Bernadette Truong, Silvija Bilokapic, Mario Halic, Hai Dao.
Presenter affiliation: St. Jude Children Research Hospital, Memphis, Tennessee. 45

Novel insights in the domain function and activation of PARP12
Simone De Franceschi, Touko Paloniemi, Johan Pääkkönen, Anna Tuovinen, Carlos Vela-Rodriguez, Lari Lehtiö, Albert Galera-Prat.
Presenter affiliation: University of Oulu, Oulu, Finland. 46

A survey of PARPs' function on antiviral immunity reveals a novel regulatory mechanism to regulate energy metabolism
Dorsa Farhadnia, Julia Mergner, Pauline Krebs, Dirk Wohlleber, Carina Baer de Oliveira Mann, Andreas Pichlmair.
Presenter affiliation: Technical University Munich, Munich, Germany. 47

PARylation of RNA by PARP1 in breast cancer cells
Isaac Zin, Jeetendra K. Nag, Valentin Gogonea, Paul L. Fox.
Presenter affiliation: Cleveland Clinic Research, Cleveland, Ohio; Cleveland State University, Cleveland, Ohio. 48

Enhanced mass spectrometry strategies for linkage-specific analysis of labile ADP-ribosylation
Ivo A. Hendriks, Sara C. Buch-Larsen, Kyuto Tashiro, Jonas D. Elsborg, Bernhard Lüscher, Glen Liszczak, Ivan Ahel, Michael L. Nielsen, Jesper V. Olsen.
Presenter affiliation: NNF Center for Protein Research, ICMM, Copenhagen, Denmark. 49

ADP-ribosylation of RNA in stem cell metabolism
Chrysi Kapsali, Nikolaos Kouvelas, Virginia Kroef, Lea Hund, Ina Huppertz.
Presenter affiliation: Max Planck Institute for Biology of Ageing, Cologne, Germany; Cologne Excellence Cluster for Aging and Aging-Associated Diseases, Cologne, Germany; University of Cologne, Cologne, Germany. 50

PARP14 mediated cysteine ADP-ribosylation of SQSTM1/p62 remodels innate immune responses
David Kubon, Sudharshana Sundaresan, Deena M. Leslie Pedrioli, Michael O. Hottiger.
Presenter affiliation: University of Zurich, Zurich, Switzerland. 51

Characterizing the PAR-binding properties of the multi-PDZ scaffold protein PATJ
Mingyu Lee, Leilei Shi, Isaiah J. Mixon, Morgan Dasovich, Maranda McDonald, Anthony Leung, Mark T. Bedford.
Presenter affiliation: The University of Texas MD Anderson Cancer Center, Houston, Texas. 52

NEURL4-dependent ADP-ribosylation as a post-translational switch regulating mitochondrial function
Keyu (Chloe) Li, Ting-Yu (Claire) Fan, Maria Dafne Cardamone, Valentina Perissi.
Presenter affiliation: Boston University Chobanian & Avedisian School of Medicine, Boston, Massachusetts. 53

Catalytically inactive PARP1 drives PARP inhibitor–induced anemia
Xiaohui Lin, Zhengping Shao, Wenxia Jiang, Denitsa Yaneva, Demis Menolfi, Seema K. Bhandari, Brian J. Lee, Alan E. Tomkinson, Julian Stingele, Shan Zha.
Presenter affiliation: Columbia University, New York, New York. 54

Evaluation of full-length PARP1 structure bound to damaged DNA in the presence of PARP inhibitors
Marcelo V. Muniz Corrêa, Aleksandr Sverzhinsky, John M. Pascal.
Presenter affiliation: Université de Montréal, Montréal, Canada. 55

PARP10 is as a novel target of the next-generation PARPi saruparib
Thales C. Nepomuceno, Lindsey Nguy, Rachael H. Martin, Ou Deng, Lancia Darville, John Koomen, Uwe Rix, Mian M. Shahzad, Alvaro Monteiro.
Presenter affiliation: Moffitt Cancer Center, Tampa, Florida. 56

Optimized detection of acidic ADP-ribosylation by multi-modal mass spectrometry reveals negative autoregulation of PARP14
Steph Nivaggioli, Hannah H. Nguyen, Kiana K. Harker, Christian Mohr, Jake Wood, Mikayla Dupen, Zeeshan Javed, Ashok Reddy, Ian Carter-O'Connell. 57
Presenter affiliation: Santa Clara University, Santa Clara, California.

Generation of *PARP14*, *p62* and *RNF114* knockout cell lines by CRISPR/Cas9 technology
Fernando Pacheco-Cintra, Lilian Cristina Russo, Nícolas Carlos Hoch.
Presenter affiliation: University of São Paulo, São Paulo, Brazil. 58

Elucidating the structural basis for PARP1-HPF1 selective inhibition—A combined computational and biochemical approach
Luis E. Paez-Beltran, Johannes Rudolph, Kayla Sprenger, Karolin Luger.
Presenter affiliation: University of Colorado Boulder, Boulder, Colorado. 59

Allosteric activation of PARP2 self-PARylation by SUMO constrains DNA break-dependent catalytic function
Shivani A. Parate, Dr.Rajakumara Eerappa.
Presenter affiliation: Indian Institute of Technology, Hyderabad, India. 60

SIRT6 mono-ADP-ribosylates target proteins in a DNA and polyhistidine dependent manner
Nicholas J. Pederson, Katharine L. Diehl.
Presenter affiliation: University of Utah, Salt Lake City, Utah. 61

Mechanistic insight of dysregulated DNA damage repair and PARylation in Huntington disease
Yi Peng, Ray Truant.
Presenter affiliation: McMaster University, Hamilton, Canada. 62

Poly(ADP)-ribose polymer induces length dependent phase separation of ALS/FTD-related C9orf72 arginine-rich dipeptide repeats at sub-stoichiometric concentrations
Meenakshi Pillai, Hongrui Liu, Takeshi Yoshikawa, Maranda McDonald, Ming Xu, Hanyu Zhong, Anthony Leung.
Presenter affiliation: Johns Hopkins University, Baltimore, Maryland. 63

How do polyamines affect DNA damage repair condensates?
Megan R. Rabon, Abby J. Sundquist, Juan-Manuel Schvartzman.
Presenter affiliation: Columbia University, New York, New York. 64

RECQL4 interacts with poly(ADP-ribose) in the absence of exogenous DNA damage and its loss sensitizes cells to the next-generation PARPi saruparib
Brian B. Rodemoyer, Thales C. Nepomuceno, Alvaro N. Monteiro.
Presenter affiliation: H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida. 65

Exploring PARP12 as a modulator of ER-positive breast cancer and endocrine signaling
Ludovica Serpieri, Nadia Badolati, Domenico Palumbo, Viola Melone, Roberta Tarallo, Giovanna Grimaldi.
Presenter affiliation: National Research Council (CNR), Naples, Italy. 66

PARylation regulates DNA replication through the formation of biomolecular condensates
Celeste Giansanti, Jack Schulz, Jessica Jackson, Alessandro Vindigni, David Cortez.
Presenter affiliation: Vanderbilt University, Nashville, Tennessee. 67

A novel HSP20-PARP1 axis regulates DNA damage repair and cancer drug resistance
Yi Zhao, Sheon Samji, Elaine Brown, Will Fuller, George S. Baillie, Yuan Yan Sin.
Presenter affiliation: University of Glasgow, Glasgow, United Kingdom. 68

Molecular determinants of PARP1 catalytic activity and substrate selection for the regulation of proadipogenic gene expression
Weidan Song, Tulip Nandu, Lee W. Kraus.
Presenter affiliation: The University of Texas Southwestern Medical Center, Dallas, Texas. 69

Novel biochemical tools to study histone ADP ribosylation signaling
Lu Sun, Sabrina R. Hunt, Andrew Nguyen, Ugochi C. Onuoha, Catherine Valadez, Lily Kim, Oluwatobi A. Adeleke, Tessa M. Firestone, Jonathan M. Burg, Matthew J. Meiners, Marcus A. Cheek, Martis W. Cowles, Zu-Wen Sun, Glen Liszczak.
Presenter affiliation: EpiCypher Inc, Research Triangle Park, North Carolina. 70

PARP2 modulates sebocyte lipid metabolism and turnover
Bailasan Hassan, Szilárd Póliska, Endre Kókai, Boglárka Rauch, Christos C. Zouboulis, Gábor Balogh, Mária Péter, Magdolna Szántó.
Presenter affiliation: Faculty of Medicine, University of Debrecen, Debrecen, Hungary. 71

Akt-dependent activation of PARP1 requires nuclear EPRS1
Isaac Zin, Arnab China, Jeetendra Nag, Valentin Gogonea, Paul Fox.
Presenter affiliation: Cleveland Clinic Research, Cleveland, Ohio; Cleveland State University, Cleveland, Ohio. 72

PARP1 at the crossroad of genotoxic and inflammatory response
Yukang Wu, Xinyun Hu, Sijia Wang, Xingzhi Xu, Jingjing Cao, Zhao-Qi Wang.
Presenter affiliation: Shandong University, State Key Laboratory of Microbial Technology, Qingdao, China. 73

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PARGi IMPAIRS BASE EXCISION REPAIR BY SEQUESTERING PAR-BINDING PROTEIN-XRCC1 AND ITS BINDING PARTNERS.

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DNA strand breaks activate PARP1 and PARP2 to synthesize poly(ADP-ribose) (PAR), promoting chromatin relaxation and recruitment of DNA repair factors. Although PARP inhibitors (PARPi) have transformed the treatment of homologous recombination (HR)–deficient cancers, resistance is inevitable, motivating the development of alternative strategies such as poly(ADP-ribose) glycohydrolase (PARG) inhibitors (PARGi), whose mechanism of action remains poorly defined. Using genome-wide CRISPR screening, we show that PARG inhibition does not synergize with HR deficiency but instead displays potent synthetic lethality with PAR-binding single-strand break repair factors, including the XRCC1–LIG3 complex, DNA polymerase β, and the chromatin remodeler ALC1. Mechanistically, PARG inhibition delays the resolution of PAR-dependent repair assemblies after lesion repair, causing dose- and time-dependent sequestration of PAR-binding repair factors—particularly XRCC1 and its partners—at resolved damage sites. This aberrant retention depletes the free nuclear pool of the XRCC1–LIG3–Polβ complex, prevents its redeployment to newly arising lesions, and results in profound hypersensitivity to the alkylating agent methyl methanesulfonate (MMS). This vulnerability requires PARP1 catalytic activity and the XRCC1 BRCT1 PAR-binding domain, but is independent of PARP2. Together, these findings define an HR-independent mechanism of action for PARG inhibitors driven by sustained PAR signaling and impaired recycling of PAR-binding DNA repair factors, with important implications for biomarker-guided therapeutic strategies.

PARP12 IN ESTROGEN RECEPTOR-POSITIVE BREAST CANCER: SURVIVAL PATHWAYS AND BEYOND

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Endocrine resistance remains a major challenge in the treatment of estrogen receptor-positive (ER+) breast cancer. Although multiple targeted therapies have been developed, resistance and subsequent disease relapse frequently occur, often sustained by persistent activation of survival pathways such as PI3K/AKT/mTOR. Despite extensive research, the molecular mechanisms that maintain constitutive AKT signaling in these tumors remain incompletely understood.

Mono-ADP-ribosylation (MARylation), catalyzed by PARP enzymes, is increasingly gaining attention due to its involvement in diverse cellular processes and human diseases. In breast cancer, our work identifies PARP12 as a key regulator of AKT signaling and cell survival in ER+ tumors. We show that PARP12 mediates the MARylation of AKT, a modification essential for its full activation. Loss of PARP12 impairs AKT phosphorylation, disrupts downstream signaling, and induces apoptosis in a subset of ER+ breast cancer cells. Mechanistically, PARP12 depletion derepresses FOXO transcription factors, promoting the expression of pro-apoptotic genes. Furthermore, PARP12 loss is associated with increased DNA damage markers, revealing a functional link between PARP12 activity and the DNA damage response.

Importantly, higher PARP12 expression correlates with acquired resistance to endocrine therapies, highlighting its central role in the development and maintenance of endocrine resistance. Beyond its impact on tumor cell survival, emerging evidence from our laboratory suggests that PARP12 also modulates immune-related signaling pathways, indicating broader implications for tumor-immune interactions.

In this talk, I will discuss our current understanding of PARP12 in sustaining survival signaling and modulating immune-related pathways in ER+ breast cancer, and explore its potential as a pharmacological target. Additional mechanistic insights and therapeutic implications will also be presented.

PARP2 DRIVES R-LOOP-ASSOCIATED ADP-RIBOSYLATION IN MYELODYSPLASTIC SYNDROMES

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RNA splicing factor (SF) mutations in *U2AF1*, *SRSF2*, and *SF3B1* occur in over half of myelodysplastic syndromes (MDS) and induce widespread RNA splicing perturbations. We previously showed that SF mutations or pharmacologic splicing modulation drive accumulation of R-loops, three-stranded nucleic acid structures composed of RNA:DNA hybrids and displaced single-stranded DNA and render cells selectively sensitive to PARP inhibition in an R-loop dependent manner (PMID: 37967363). Although FDA-approved PARP inhibitors target both PARP1 and PARP2, the specific contribution of PARP2 to R-loop-associated ADP-ribosylation (ADPr) remains unclear.

Here, we show that PARP1 deletion completely abrogates ADPr signaling, whereas PARP2 deletion selectively attenuates the majority of ADPr in SF-mutant cells and following treatment with pharmacologic splicing modulators, despite minimal involvement in reactive oxygen species-induced ADPr. Importantly, PARP2 deletion selectively sensitized cells to pharmacologic splicing modulators. Together, these findings reveal a previously unappreciated and distinct role for PARP2 in the response to RNA splicing perturbation. We propose a model in which PARP1 acts as a sensor of R-loops and initiates ADPr, thereby promoting subsequent and distinct PARP2 activation to amplify ADPr signaling, facilitate R-loop resolution, and protect against R-loop-associated genomic instability in response to RNA splicing perturbation.

UPDATES TO PARP TRAPPING: A ROLE FOR MONO-ADPR IN PARP1 RELEASE.

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PARP1 is readily recruit to sites of DNA damage where it modifies proteins around the break site, including itself and histones. This ADP-ribosylation at breaks plays a number of roles in repair including initiating chromatin architecture changes, promoting the recruitment of downstream repair factors, and promoting the release of PARP1 from breaks. PARP inhibitors have for many years been used in the clinic to treat primarily homologous recombination deficient tumours and relies on their ability to inhibit the catalytic activity of PARP1, leading to their retention at sites of DNA damage, known as PARP "trapping". Thus, understanding the mechanisms PARP1 utilise to prevent trapping is essential for the design of improved inhibitors. Several models of how PARP1 trapping is prevented have been proposed including both auto-modification of PARP1 and trans-modification of histones. In this study, we delve deeper into the role of mono- vs poly-ADP-ribosylation in regulating PARP1 trapping and on the way uncover some additional effects of mono- or poly-ADPr on the efficacy of different PARP inhibitors trapping potential.

REDEFINING BRCA1 AND PARP1 SYNTHETIC LETHALITY

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Synthetic lethality between loss of BRCA1 and loss of PARP1 defines a therapeutic vulnerability exploited by PARP inhibitors, yet the replication-associated basis of this interaction remains incompletely understood. Here, we describe evidence that lagging-strand processing and replication fork–proximal events contribute to cellular responses to PARP inhibition. Our findings suggest that FEN1 positioning during DNA replication is dynamically regulated and influenced by BRCA1-, PARP1-, and 53BP1-associated processes. Disruption of this balance alters replication-associated PARP1 activity and modulates sensitivity to PARP or FEN1 inhibition. Together, these observations support a model in which coordination of replication maturation, rather than downstream DNA break repair alone, is an important determinant of PARP inhibitor sensitivity, highlighting replication-associated regulation as an emerging factor in therapeutic response.

MOLECULAR AND FUNCTIONAL ASPECTS OF PARP3 IN PHYSIOLOGICAL AND PATHOLOGICAL PROCESSES.

Françoise Dantzer

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Poly(ADP-ribose) polymerase 3 (PARP3) has been primarily characterized for its role in the DNA damage response and mitotic progression. Recent studies have expanded its functional scope to include critical roles in (i) physiological processes such as neurogenesis by promoting astrocytic differentiation and myogenesis by fostering myogenic differentiation and muscle function (ii) and in pathophysiological events precisely in tumor aggressiveness under conditions of reduced PARP1 expression. Notably, share features emerge in both contexts, particularly involving physical properties of the cells. This convergence is especially striking considering the predominantly nuclear localization of the protein. Our current research is aimed at addressing these questions and investigating how PARP3 influences cell plasticity and mechanical adaptability in aggressive tumor contexts. Current findings that begin to shed light on this issue will be presented.

INVESTIGATING THE FUNCTIONAL AND REGULATORY ROLES OF PARP2 ISOFORMS IN BREAST TISSUE AND CANCER

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Alternative RNA splicing is a tightly regulated mechanism that generates proteomic diversity in eukaryotes by facilitating the production of multiple protein isoforms with distinct functions from a single gene. This process selectively joins together different combinations of exons and introns from a single pre-mRNA transcript. Dysregulation of this process can lead to the production of aberrant protein isoforms that contribute to disease, including cancer. In breast tissue, altered splicing patterns have been shown to promote tumor initiation and progression. A defining feature of tumorigenesis is increased genomic instability, often arising from compromised DNA repair pathways. We hypothesize that dysregulated RNA splicing contributes to defective DNA repair via the production of DNA-repair protein isoforms that impair or alter repair machinery function, thereby facilitating mutation accumulation and tumorigenic signaling. In this work, we investigate a novel alternatively-spliced DNA-repair factor, PARP2, to determine how its isoforms influence breast cancer–associated cellular behaviors.

The PARP2 protein has two distinct protein isoforms resulting from alternative splicing involving the usage or skipping of an alternative 5' splice site in exon 2 of its pre-mRNA. Utilization of this splice site results in a longer PARP2 isoform containing an additional 13 amino acids within the N-terminal DNA-binding domain (DBD). We have so far identified a genomic single-nucleotide polymorphism (SNP), rs2297616G>A which acts as a "master regulator" driving the splicing of the two PARP2 isoforms at both the RNA and protein levels detected in breast cancer cell lines and primary Human Mammary Epithelial cells (HMECs). We have additionally observed that the longer PARP2 isoform is more prevalent in triple-negative breast cancer (TNBC), a highly aggressive breast cancer subtype, compared to non-TNBC patient samples in the Cancer Genome Atlas database.

We propose that the long and short PARP2 protein isoforms perform distinct biological functions through differential DBD-mediated interactions with DNA, PAR and/or protein partners which may subsequently impact response to cancer therapeutics and drugs, specifically PARPi. This work aims to define the functional consequences of PARP2 alternative splicing and to elucidate the roles of its isoforms within the broader context of RNA splicing regulation, DNA repair, and breast cancer.

TANKYRASE SWITCHES A DISEASE-ASSOCIATED RNA-BINDING PROTEIN FROM ACTIVE TO AGGREGATING

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Tankyrase, best known for its catalytic roles in signalling and protein turnover, exhibits poorly understood non-canonical functions. TAR DNA-binding protein 43 (TDP-43) is an essential RNA-binding protein whose aggregation is a hallmark of multiple neurodegenerative diseases. In disease, loss of RNA binding and pathological aggregation are tightly coupled, but the mechanism linking these events has remained unclear.

Here, we show that Tankyrase directly binds the RNA-recognition motif of TDP-43, forming an RNA-independent complex that does not require Tankyrase catalytic activity. This interaction induces a conformational rearrangement in full-length TDP-43 that abolishes RNA binding, including displacement of pre-bound RNA, and simultaneously promotes aggregation driven by the C-terminal prion-like domain. Tankyrase therefore acts as a molecular switch that structurally couples RNA-binding loss to pathological self-assembly.

These findings reveal a previously unrecognised, non-catalytic role for Tankyrase in regulating protein conformation and aggregation. More broadly, they expand the functional repertoire of PARP family substrates and suggest that substrate binding alone can drive profound changes in protein behaviour relevant to disease.

ROLE OF TANKYRASE SCAFFOLDING IN THE B-CATENIN DESTRUCTION COMPLEX AND WNT SIGNALING

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Aberrant WNT/β-catenin signaling drives tumorigenesis and metastasis in cancer. Although enzymatic inhibitors of tankyrase (TNKS) effectively block AXIN degradation and stabilize the β-catenin destruction complex (DC), they have demonstrated limited efficacy in various cancer models. Here we demonstrate that, unexpectedly, the induction of AXIN puncta represents a major barrier to achieving therapeutic efficacy. Mechanistically, catalytic inhibition of TNKS prevents TNKS turnover and drives its accumulation in the DC, wherein the scaffolding function of TNKS induces AXIN puncta formation, rigidifies the DC, and impedes β-catenin turnover. Chemically induced degradation of TNKS overcomes this limitation by stabilizing AXIN without puncta formation, providing a deeper suppression of the WNT/β-catenin pathway activity and the proliferation of colorectal cancer cells harboring dysfunctional APC mutations. Collectively, these findings provide an explanation for the unsatisfactory outcomes of drugging the WNT/β-catenin signaling pathway by TNKS inhibitors and highlight TNKS degradation as a promising approach to treat WNT/β-catenin-driven cancers.

BIOLOGY AND MECHANISMS OF SITE-SPECIFIC ADP-RIBOSYLATION OF C/EBPβ IN OBESITY-RELATED CARDIAC DYSFUNCTION

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Obesity is a major risk factor for cardiac dysfunction, leading to cardiac remodeling and impaired diastolic and/or systolic dysfunction. Cardiac remodeling is accompanied by reprogramming the expression of a wide array of genes that are tightly regulated by post-translational modifications (PTMs). ADP-ribosylation (ADPRylation) is an NAD^+ dependent PTM, catalyzed by the poly(ADP-ribose) polymerase (PARP) family of enzymes, including the nuclear enzyme PARP1. In mice, cardiac tissue, across all the tissues that we tested, has the highest basal levels of PARP1-mediated poly(ADP-ribosyl)ation (PARylation). Although this suggests an important function for PARP1 and PAR in the heart, our understanding of their role is limited. By using an NAD^+ analog-sensitive PARP1 (asPARP1) approach, we have characterized the PARP1-mediated ADPRylated proteome in AC16 cardiomyocytes, an immortalized human ventricular cardiomyocyte cell line. The PARP1 substrates are enriched in the gene ontology terms of transcription regulation, chromatin remodeling, and RNA splicing and processing. Among these substrates, we identified the transcription factor C/EBPβ, which has been characterized previously as a PARP1 substrate. ADPRylation of C/EBPβ mediates the effects of PARP1 on the regulation of proadipogenic gene expression during adipogenesis. To investigate the functional role of site-specific ADPRylation of C/EBPβ in vivo, we generated a unique C/EBPβ ADPRylation site (E135/E139) mutant knockin (CebpbADPRMut) mouse model. Compared to control mice, the CebpbADPRMut mice on a high fat diet exhibited a phenotype of enhanced obesity coupled with impaired cardiac function, represented by increased expression of *Nppb* gene (encoding B-type natriuretic peptide, a common indicator of heart failure) in cardiac tissue. RNA-sequencing analysis for cardiac tissue samples demonstrated a key role of site-specific ADPRylation of C/EBPβ in the regulation of cardiac gene expression. We verified site-specific ADPRylation of C/EBPβ in cardiac tissue and AC16 cardiomyocytes, and further demonstrated that mutation of the E135/E139 ADPRylation sites enhances C/EBPβ binding to its DNA element by EMSA. These results have allowed us to characterize PARP1-mediated, site-specific ADPRylation of C/EBPβ, determine the cellular and molecular mechanisms by which site-specific ADPRylation of C/EBPβ regulates gene expression, and link these molecular mechanisms with biological outcomes in obesity and obesity-related cardiac dysfunction, providing an insight into site-specific ADPRylation in vivo.

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TANKYRASE-2 REGULATES ADIPOCYTE DIFFERENTIATION THROUGH AMPK/mTOR SIGNALING

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Tankyrases (TNKSs) were originally described as enzymes safeguarding genome integrity, however, TNKSs are also associated with other biological processes, as metabolism and cellular differentiation. Deletion or pharmacological inhibition of TNKSs were shown to influence the beige/brown/white adipocyte trichotomy. We aimed to understand the role of TNKS isoenzymes in adipocyte differentiation and to decipher the complex process. The deletion of TNKS1 or TNKS2 decreased the rate of adipocyte differentiation and induced multi-pronged cellular adaptation. The deletion of TNKS2 induced changes characterized by cellular proteostasis-centered responses, AMPK activation, the suppression of mTORC1 activity and the induction of mitochondrial oxidative phosphorylation and glycolytic flux. The fraction of oligomycin-resistant respiration decreased, suggesting more coupled OXPHOS. The lower differentiation rate of TNKS2 knockout cells was salvaged by pharmacological activation of autophagy by NV-5138 and the pharmacological inhibition of LKB1 (by HY-10371), an upstream supressor of the mTOR system. TNKS2 can ADP-ribosylate LKB1, rendering it susceptible for ubiquitinylation that appears to be a key step towards committing adipocytes to differentiation. Taken together, TNKS1 and TNKS2 appears to have differential effects on adipocyte differentiation, however, both enzymes are necessary for adipocyte differentiation.

A PARP INHIBITOR, RUCAPARIB, IMPROVES CARDIAC DYSFUNCTION IN ADP-RIBOSE-ACCEPTOR HYDROLASE 3 DEFICIENCY

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ADP-ribose-acceptor hydrolase 3 (ARH3) degrades protein-linked poly(ADP-ribose) (PAR)[1] and hydrolyzes ADP-ribose serine.[2,3] *ARH3* deficiency is implicated in stress-induced childhood-onset neurodegeneration with ataxia and seizures (CONDSIAS).[4] ARH3 hydrolysis of PAR chains synthesized by poly(ADP-ribose)polymerase (PARP)-1 during oxidative stress, prevents excessive PAR accumulation that can lead to PAR-dependent cell death or parthanatos.[1] Approximately one-third of patients with homozygous mutant *ARH3* die from cardiac arrest, often described as neurogenic.[4,5] However, whether ARH3 plays a direct role in maintaining myocardial function remains unclear. To address this question, we investigated cardiac function in *Arh3*-knockout (KO) mice.

Arh3-KO male mice (C57BL6J) displayed cardiac hypertrophy and decreased contractility by MRI and dobutamine-stress echocardiography. Hearts showed decreased post-ischemic rate-pressure products, increased ischemia-reperfusion (IR) infarct size, and elevated PAR levels. In vivo IR injury demonstrated enhanced infarcts in both sexes. Treatment with rucaparib, an FDA-approved PARP inhibitor, improved contractility during stress and reduced IR infarct size. CRISPR-Cas9-generated *Arh3*-KO myoblasts and myotubes showed decreased viability after H_2O_2 exposure, with PAR-dependent cell death reduced by PARP inhibitors or transformation with *Arh3* gene. These findings demonstrate that ARH3 regulates PAR homeostasis in myocardium to preserve function and protect against oxidative stress. Further, PARP inhibitors reduce myocardial dysfunction in *Arh3* deficiency.

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CELLULAR NAD^+-SENSING AND THE REGULATION OF ADP-RIBOSYLATION DURING ADIPOGENESIS

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NAD^+ is a small molecule co-factor in metabolic redox reactions, as well as a signaling molecule. We have found that NAD^+ synthesis in mammalian cells is compartmentalized, with functionally distinct nuclear and cytosolic pools that support compartment-specific ADP-ribosylation by members of the PARP family. For example, NAD^+ synthesized in the nucleus by nicotinamide mononucleotide adenylyl transferase-1 (NMNAT-1) plays a key role in regulating gene expression programs mediated by nuclear PARPs, such as PARP1 and PARP7. Likewise, NAD^+ synthesized in the cytoplasm by NMNAT-2 plays a key role in controlling cytosolic MARylation by MARTs, including PARP16, which ultimately regulates protein translation. This compartment-specific "NAD^+ sensing" is evident in cancer cells, adipocytes, and embryonic stem cells, where alterations in the spatial and temporal use of specific NAD^+ biosynthetic pathways lead to changes in the ADPRylated proteome, as well as downstream cellular processes such as transcription, mRNA splicing, chromatin regulation, translation, and stress granule formation. In our most recent studies, we are exploring the mechanisms and biological outcomes of NAD^+ sensing by PARP1 and PARP7 in the nucleus, and PARP16 in the cytosol, during adipogenesis. This includes how site-specific ADPRylation of nuclear and cytoplasmic proteins control various processes in physiology and disease. We have found, for example, that PARP16-mediated MARylation of key ribosomal proteins during adipogenic differentiation is required for the loading of a subset of mRNAs on ribosomes and the association of those ribosomes with the endoplasmic reticulum (ER). Inhibition or depletion of NMNAT-2 or PARP16, or mutation of the sites of MARylation on the ribosomal proteins, alters mRNA loading, association with the ER, and adipogenesis. Our work has placed a particular emphasis on identifying, confirming, characterizing, and functionally analyzing specific sites of ADP-ribosylation in biologically relevant PARP substrate proteins in physiological processes in vivo. More broadly, our studies connect subcellular NAD^+ synthesis, PARP catalytic activity, and site-specific ADPRylation to downstream biological outcomes.

The ADPRylation-related research in the Kraus lab is supported by grants from the NIH/NIDDK, NIH/NCI, U.S. DOD Ovarian Cancer Research Program, the Cancer Prevention and Research Institute of Texas, and the Burroughs Wellcome Fund, as well funds from the Cecil H. and Ida Green Center for Reproductive Biology Sciences Endowment, to W.L.K.

A POLY(ADP-RIBOSE) CODE ENCODES BIOMOLECULAR CONDENSATE FORMATION, COMPOSITION, AND MATERIAL STATE

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Growing evidence suggests a broader function for poly(ADP-ribose) (PAR) in organizing intracellular space. Here, we show that its polymeric properties encode distinct biological outcomes. Using photoaffinity proteomics, we generated a census of PAR-binding proteins and found strong enrichment for components of biomolecular condensates, including multiple proteins genetically linked to neurodegeneration. Focusing on stress granules as a model system, we identify PAR itself, a subset of PARP enzymes, and PAR-degrading activities as critical granule components. Functional perturbations reveal that distinct PARPs play nonredundant roles in condensate dynamics: PARP10 acts as a local, rate-limiting source of PAR required for granule initiation following translational arrest, whereas PARP13 functions as a PAR sensor that promotes condensate maturation and turnover, establishing a feedback circuit that regulates granule size and material properties. At the molecular level, biochemical reconstitution and single-molecule analyses demonstrate that sub-stoichiometric PAR is sufficient to nucleate condensation of the stress granule RNA-binding protein FUS, while increasing PAR chain length drives a transition from dynamic, liquid-like assemblies to more solid-like aggregates. These results show that PAR chain length is a key physical parameter controlling condensate behavior, establishing a tunable “PAR code” that links enzymatic signaling to phase transitions. Together, these findings establish PAR chain length as a key physical parameter controlling condensate behavior and define a tunable “PAR code” that links enzymatic signaling to phase transitions.

ADP-RIBOSYLATION OF FUS REGULATES THE DYNAMICS OF DNA DAMAGE REPAIR CONDENSATE FORMATION AND MAINTENANCE

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FUS is a DNA/RNA-binding protein linked to the neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), forming insoluble protein aggregates in these disorders. In healthy cells, FUS primarily localizes to the nucleus, where it regulates DNA/RNA-related processes such as DNA damage repair (DDR), transcription, and alternative splicing. It can undergo liquid-liquid phase separation (LLPS) and localize to nuclear and cytosolic condensates, including DNA damage repair condensates and stress granules. Proteomics and in vitro studies suggest that FUS is ADP-ribosylated by PARP1, and that both proteins are important for DDR. However, it remains unclear whether and when FUS is ADP-ribosylated during DDR, and how this modification influences FUS phase separation and function in the DDR. Here we show that FUS undergoes ADP-ribosylation in vitro, promoting the dissolution of FUS condensates. Using an in vitro reconstitution system that models DDR condensates, we found that PARP1 and FUS form immiscible condensates with dsDNA. Upon NAD^+ addition, PARP1 becomes active, leading to fusion of PARP1/DNA and FUS condensates. Progressive ADP-ribosylation causes both condensates to dissolve. By using PARG and a PARP1 mutant (E988A), which abrogates PARylation activity but retains MARylation activity, we demonstrated that FUS is most likely short-chain PARylated and MARylated. Moreover, MARylation is not enough to suppress FUS and PARP1/DNA condensate formation. Finally, we showed that unmodified FUS can replace PARylated FUS and stabilize PARylated PARP1/DNA condensates. Our findings reveal how ADP-ribosylation regulates FUS condensate dynamics in the DDR, revealing a new regulatory mechanism of DNA damage repair foci formation and maintenance, which may play a crucial role in the DDR.

PARP1-INDUCED NUCLEAR RNA CONDENSATES LINK DNA DAMAGE TO TRANSLATION ARREST

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The DNA damage response triggers widespread transcriptional changes and modulates protein degradation, but its role in regulating translation remains largely unexplored. Here, we uncover a new role for PARP1 in suppressing cellular mRNA translation when hyperactivated in response to specific DNA lesions. Mechanistically, we find that APE1-dependent cleavage of abasic sites leads to PARP1 hyperactivation-mediated translation arrest. We demonstrate that PARP1 causes the formation of large nuclear condensates associated with RNAs, nuclear speckle factors, and other RNA-binding proteins (RBPs) involved in RNA splicing and export. PARP1-mediated nuclear condensates (PNCs) disrupt RNA homeostasis by mislocalizing multiple RBPs and sequestering RNAs within the nucleus, thereby decreasing the cytoplasmic RNA pool available for translation. Finally, we show that PARP1 inhibits the expression of many DNA damage-induced stress response factors by blocking the export of their mRNA transcripts, revealing an unrecognized function for PARP1 in uncoupling RNA transcript levels from protein expression during the DNA damage response.

PARylatioN-DEPENDENT REGULATION OF REPLICATION FORK DYNAMICS BY FET PROTEINS

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Targeting replication-associated DNA repair mechanisms, including those regulated by PARP1/2 and PARG control of ADP-ribosylation is a powerful cancer therapeutic approach. However, the mechanisms by which PARG inhibition impacts DNA replication remain unclear. We combined isolation of proteins on nascent DNA (iPOND) with quantitative proteomics and functional assays to investigate replication fork dynamics upon acute PARG inhibition. We found that FET family proteins (FUS, EWS, and TAF15) are recruited to replication forks in a PAR-dependent manner, forming condensates that slow fork progression and promote fork reversal. FET proteins control fork dynamics in response to some, but not all, replication stresses. FUS inactivation leads to unrestrained fork progression via RECQ1 and PRIMPOL, increased single-stranded DNA gaps, genome instability, and synthetic lethality with BRCA1 deficiency. These findings reveal that FET protein condensates modulate replication stress responses, influencing genome stability and the cellular response to cancer therapeutics targeting PARylation pathways.

PARG INHIBITION ALTERS PARP1 DYNAMICS AT COMPLEX DNA LESIONS IN LIVING CELLS

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Chromatin poly(ADP-ribosylation) (PARylation) is a rapid modification, which signals the presence of DNA breaks. Upon binding to DNA breaks PARP1 is activated and produces the majority of nuclear PAR, which is attached to chromatin proteins in the vicinity of the breaks as well on PARP1 itself (autoPARylation). PAR serves as a molecular platform for the rapid recruitment of various PAR-dependent DNA repair proteins, including scaffold proteins and chromatin remodelers. Within minutes, damage-induced PAR is degraded by several dePARylating enzymes, including poly(ADP-ribose) glycohydrolase (PARG) and ADP-ribosylhydrolase 3 (ARH3). Importantly, chromatin dePARylation primes damaged chromatin for downstream DNA repair events. Using a combination of rapid live-cell imaging, UV laser micro-irradiation (IR), fluorescence recovery after photobleaching (FRAP), and mathematical modeling, we recently provided a unified mechanism of PARP inhibitor (PARPi)-induced PARP1 retention at damaged chromatin sites. We showed that the catalytic inhibition of PARP1 causes repeating unproductive binding-dissociation cycles of inactive PARP1 molecules to DNA lesions, while reverse allosteric effects, which vary greatly among different PARPi, prolong the DNA lesion-bound state of PARP1. Both effects stack to define the overall retention of PARP1 at IR-induced damaged chromatin sites. Notably, the extent of PARP1 chromatin retention strongly correlated with the delay in downstream repair events and PARPi cytotoxicity, establishing PARP1 retention as major contributor to PARPi efficacy.

Herein, following our live-cell imaging approach, we decided to interrogate if and how PARG inhibitors (PARGi) influence the dynamics of the PARylation-dePARylation cycle inside living cells. To this end we explored three PARGi compounds, namely, PDD00017273, COH34, and JA2131. We uncovered that PARGi prolong the residence of PARP1 at IR-induced complex DNA lesions in a concentration-dependent manner, an effect reminiscent of that elicited by PARPi. However, in contrast to PARPi-induced PARP1 retention, we uncovered that PARGi-induced PARP1 retention at damage sites is fully dependent on its catalytic activity – inhibiting PARP1 with a PARPi fully abrogated the PARGi-induced PARP1 retention. Furthermore, the kinetics of PARylation-deficient PARP1 mutants were not affected by the PARGi treatment. Notably, PARG inhibition also induced markedly larger and brighter PARP1 DNA repair foci, as compared to untreated cells. Various genotoxins in combination with PARGi, including methyl methanesulfonate (MMS) and camptothecin (CPT), managed to induce PARP1 foci inside living cells, showing that the observed phenotype is not limited to IR-induced DNA damage foci. In summary, we uncover an unexpected effect of PARGi, which may contribute to their efficacy in the preclinical and clinical settings.

HPF1 PROTECTS CELLS FROM TOXIC FORMS OF ADP-RIBOSYLATION

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ADP-ribosylation (ADPr) is a reversible post-translational modification that can be attached to different amino acids by PARPs. This family of enzymes is involved in various cellular processes including DNA damage response (DDR). While the biochemistry of serine-ADPr, a modification catalyzed by poly(ADP-ribose) polymerase 1 and 2 (PARP1/2) in complex with the cofactor histone PARylation factor 1 (HPF1), is well-characterized, the physiological roles and mechanisms of HPF1-dependent or -independent ADPr remain poorly understood. Here, we reveal a surprising discovery that cells deficient in HPF1 are strongly dependent on the function of serine-ADP-ribose hydrolase, (ADP-ribosyl)hydrolase 3 (ARH3), when the poly-ADP-ribose hydrolase (PARG) is pharmacologically inhibited. A combined deficiency of HPF1, ARH3, and PARG leads to strong toxicity that is caused by uncontrolled, aberrant PARP1/2 activity. We show that in the absence of HPF1, PARP1 and 2 synthesize toxic ADP-ribosylation which, when not degraded by PARG or ARH3, results in telomere instability, cell cycle arrest, and apoptosis. Altogether, our findings delineate distinct functional outcomes of HPF1-dependent and -independent ADPr and highlight an underappreciated role of HPF1 in protecting the cell from toxic forms of ADP-ribosylation with potential implications for understanding cancer and neurodegenerative diseases.

BALANCING PARP ACTIVITY DURING DNA REPLICATION THROUGH DYNAMIC DEPARYLATION

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Poly(ADP-ribose) polymerase 1 (PARP1)-mediated PARylation is a central signaling mechanism in genome maintenance, yet how endogenous PARP activity is restrained during unperturbed DNA replication remains poorly understood. Here, we define a replication-coupled PARP1-PARG-CHD1L axis that suppresses toxic PARylation arising from Okazaki fragment maturation.

Using complementary CRISPR screening, genetic, and cell-cycle–resolved analyses, we show that poly(ADP-ribose) glycohydrolase (PARG) is essential for cell viability by restraining PARP1 activation specifically during S phase. In the absence of effective dePARylation, unligated Okazaki fragment intermediates trigger sustained PARP1 activation, leading to excessive poly(ADP-ribose) accumulation, prolonged PARP1 chromatin engagement, replication-associated DNA damage, and PARP1/2-dependent cell death. This endogenous S-phase PARylation is distinct from canonical DNA damage-induced signaling and reflects a fundamental housekeeping role for PARP1 during lagging-strand synthesis.

We further identify the PAR-binding chromatin remodeler CHD1L (ALC1) as a critical regulator of this process. CHD1L loss exacerbates PARG deficiency by promoting toxic S-phase PARylation originating from unprocessed Okazaki fragments. Mechanistically, CHD1L facilitates efficient turnover of replication-associated single-strand break repair factors and limits aberrant retention of PAR-dependent signaling complexes on chromatin. Both the ATPase activity and PAR-binding macrodomain of CHD1L are required to suppress this replication-linked PARylation.

Together, these findings reveal an essential PARP-PARG-CHD1L circuit that safeguards genome integrity during normal DNA replication. Disruption of this balance exposes a replication-specific vulnerability to excessive PARylation, with direct implications for PARP- and PARG-targeted cancer therapies and biomarker-guided patient stratification.

PARG ACTIVITY IS REQUIRED FOR CELL DEATH BY PARTHANATOS

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Cell death by a non-apoptotic pathway termed parthanatos is induced by hyperactivation of the DNA damage sensor PARP1, which uses NAD^+ as a substrate to catalyse the poly-ADP-ribosylation (PARylation) of proteins such as histones. Parthanatos has been implicated in several pathological conditions such as ischaemia-reperfusion injury and neurodegenerative processes, including Alzheimer´s and Parkinson´s diseases, as well as rare genetic disorders characterized by PARP1 hyperactivation. However, the precise sequence of molecular events by which excessive PARP1 activity causes cell death is currently unclear. Here we show that, in addition to PARP1-dependent PARylation, the execution of parthanatos also requires the hydrolysis of poly-ADP-ribose (PAR) chains by the glycohydrolase PARG. While complete inhibition of PARG activity prevents parthanatos, low levels of residual PARG activity are sufficient to support cell death by this pathway. Due to a phenotypic discrepancy between CRISPR/Cas9-generated PARG KO cells and PARG inhibitor-treated cells, we uncovered a new PARG splice variant predicted to encode an isoform of 53 kDa, termed PARG53, and provide evidence for the incorrect annotation of the previously reported isoforms PARG55 and PARG60. PARP1 hyperactivation during parthanatos leads to rapid and profound depletion of NAD^+ and ATP pools, but full PARG inhibition only prevents the depletion of ATP, indicating that the depletion of these core metabolites can be uncoupled, and that ATP depletion is more closely correlated with cell death. This work sheds light on the initial steps of parthanatos induction and provides a molecular rationale for the use of PARG inhibitors to slow pathological processes associated with parthanatos.

STRUCTURAL BIOCHEMISTRY OF PARP FAMILY PROTEINS

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PARP family members have distinct sets of regulatory domains that control catalytic output, that select for appropriate targets for ADP-ribose modification, and that interact with partner proteins, nucleic acids, and ADP-ribose. Our work focuses on the structural biology of PARP family members, addressing key questions regarding the unique modes of regulating ADP-ribose modifications and cellular functions. In the cellular response to DNA damage, PARP1 facilitates genome maintenance by rapidly detecting DNA strand breaks, recruiting repair factors to sites of DNA damage via poly(ADP-ribose), and modulating the local structure of chromatin. This talk will provide our latest research on PARP1 regulatory and signaling mechanisms in response to DNA strand break damage, the impact of PARP inhibitors on PARP1 allostery, and the mechanism of poly(ADP-ribose) synthesis.

DIMERIZATION OF HUMAN PARP15 IS REQUIRED FOR NAD^+ BINDING AND AUTOMODIFICATION

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PARP15 is a mono-ADP-ribosylating enzyme present in humans and some vertebrates. In longer PARP15 isoform 1, there is a long unstructured N-terminal tail followed by two macrodomains and conserved catalytic domain. Comparison of the activity of full-length protein and isolated catalytic domain shows that they are both active and consume NAD^+, but full-length protein is more prone to auto-ADP-ribosylation. PARP15 automodification can be hydrolysed using macrodomains or ADP-ribosyl-hydrolases, which specifically erase the modification attached to acidic residues aspartate and glutamate. By using mass spectrometry, we identified several automodified residues from the full-length protein sequence. These automodification sites are located mostly in the region of the two macrodomains.

As we discovered that full-length human PARP15 produced in insect cells was a dimer, we compared the activity and structural differences between PARP15 dimerization disrupting mutant monomer and wild-type dimer. Monomeric form of PARP15 was inactive and it binds only weakly an unhydrolyzable NAD^+ analog. This indicates that dimerization is a requirement for binding of substrate NAD^+. A crystal structure of PARP15 catalytic domain with the unhydrolyzable NAD^+ analog shows that orientation of the D-loop lining the substrate binding pocket is different compared to the apo form, which has the loop is in a collapsed conformation creating a steric hindrance for binding to NAD^+.

PARP1 EXHIBITS AN ENZYMATICALLY INACTIVE CHROMATIN BINDING MODE

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Chromatin structure and organization is critical for condensing DNA and maintaining genome integrity. Despite chromatin condensation safeguarding the genome, approximately one million DNA alterations occur per day in each cell which must be repaired in an efficient manner. Poly (ADP-ribose) Polymerase 1 (PARP1) is a highly abundant, multidomain, nuclear enzyme. Historically, PARP1 has been studied in the context of DNA damage and as a drug target for cancer therapies. However, previous and emerging studies describe an integral role for PARP1 in regulating chromatin structure and gene expression in the absence of DNA damage. In addition to binding DNA lesions, PARP1 binds undamaged DNA (plasmid), nucleosomes, and chromatin constructs, suggesting a distinct binding mode in the absence of damaged DNA. Furthermore, PARP1 promotes chromatin compaction by directly binding nucleosomes, resulting in nuclease resistant complexes. The molecular basis of how PARP1 interacts with and compacts chromatin remains largely unexplored, limiting our fundamental understanding of PARP1. We aim to define this understudied conformation by characterizing how PARP1 binds and compacts an undamaged chromatin mimic, the non-linker ended trinucleosome. Through biophysical and biochemical approaches, we present our latest efforts in determining PARP1's enzymatically inactive chromatin binding mode.

A LARGE-SCALE METHOD TO MEASURE THE STOICHIOMETRIES OF PROTEIN POLY-ADP-RIBOSYLATION

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While thousands of PARylated substrates have been identified, the specific biological functions of most PARylated proteins remain elusive. PARylation stoichiometry is a critical parameter to assess the potential functions of a PARylated protein. However, characterization of the stoichiometries of protein PARylation has been challenging. First, PARylation is a polymeric/heterogeneous modification that lacks a defined mass shift. As a result, direct detection of the PARylated species (and then comparing the ratio of the PARylated/unPARylated species to determine the PARylation stoichiometries) using MS-based methods is not feasible. Second, PARylation is of low abundance, and a robust enrichment strategy needs to be developed for its stoichiometric analyses. Finally, compared to other common PTMs, a rather unique feature of PARylation is that PARylation can occur on various amino acid acceptors. It is important to note that these PAR-amino acid linkages display drastically different stability. We previously developed a proteomic pipeline for the global and site-specific analyses of the D/E-ADP-Ribosylated proteome (Zhang et al., Nature Methods 2013 and Zhen et al., Cell Reports 2017). In the current study, we further leveraged this pipeline to develop a large-scale strategy to measure the stoichiometries of protein PARylation. Specifically, by integrating mild cell lysis, boronate enrichment and carefully designed titration experiments, we were able to determine the PARylation stoichiometries for a total of 235 proteins. Importantly, this approach enables the capture of all PARylation events, regardless of their amino acid acceptor linkages. We revealed that PARylation occupancy spans over three orders of magnitude. Most PARylation events occur at low stoichiometric values (median 0.578%). Notably, we observed that high stoichiometry PARylation (>1%) predominantly targets proteins involved in transcription regulation and chromatin remodeling. Functional analyses of this dataset revealed a remarkable breadth of regulatory events associated with the PARylated proteins, including DNA damage repair but also RNA metabolism, transcriptional regulation and chromatin remodeling. Thus, our study provides a systems-scale, quantitative view of PARylation stoichiometries under genotoxic conditions, which serves as invaluable resources for future functional studies of this important protein posttranslational modification. Finally, we will also discuss the implications of these findings in the context of future development of proteomic tools for the global analysis of protein ADP-ribosylation.

EXCEEDING EXPECTATIONS WITH THE ORBITRAP EXCEDION PRO FOR LARGE-SCALE ADP-RIBOSYLATION SITE ANALYSIS

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ADP-ribosylation (ADPr) is a dynamic and essential post-translational modification involved in diverse cellular processes, including DNA damage response, transcription, and stress signaling. Despite being discovered more than 60 years ago, comprehensive characterization of ADPr remains challenging due to its low abundance, chemical heterogeneity, and the broad range of reported amino acid acceptor sites. Importantly, understanding the biological function of ADPr requires confident localization of modification sites in their cellular context, which necessitates highly sensitive mass spectrometry approaches capable of handling limited and heterogeneous sample material - an inherent constraint in many biological and clinical settings.

Here, we evaluated the performance of the new Thermo Scientific Orbitrap Excedion Pro mass spectrometer (MS) for ADPr site identification and benchmarked it against our established, highly optimized workflows on the Orbitrap Fusion Lumos. We systematically assessed key instrumental parameters relevant for ADPr analysis, including ETD reaction times, supplemental activation energies, resolution settings, HCD triggering, and sample load. Across all tested conditions, the Orbitrap Excedion Pro MS consistently outperformed the Orbitrap Fusion Lumos tribrid MS by up to two-fold, demonstrating increased sensitivity and improved sequencing performance.

Notably, the advantages of the Orbitrap Excedion Pro MS were most pronounced under conditions relevant for biologically and clinically constrained samples, as demonstrated by dilution series, using reduced input material and shorter acquisition times. The increased speed and sensitivity enable higher sample throughput, facilitating the analysis of larger and more diverse sample collections, such as clinical cohorts or small biopsy-derived specimens. Using fractionated samples, we identified approximately 3,500 unique and confidently localized ADPr sites, reaching the same depth while requiring only half the input material and half the instrument time compared to previous studies. Together, these results establish the Orbitrap Excedion Pro MS as a powerful platform for in-depth ADPr proteomics across a wide range of sample types, particularly in settings where sample amount and instrument time are limiting.

DECIPHERING THE ROLE OF ADP-RIBOSYLATION IN CARDIOLAMINOPATHY

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Protein ADP-ribosylation is a physiologically and pathologically important post-translational modification (PTM) that regulates numerous cellular activities. ADP-ribosylation quickly alters the cellular states under acute stress conditions and disease pathologies. CardioLaminopathies are a severe form of dilated cardiomyopathy that are caused by mutations in A-type lamin gene (LMNA), which encodes a major component of of the nuclear lamina. These mutations lead to myocardial dysfunction, which ultimately results in heart failure. Unfortunately, therapies that specifically target CardioLaminopathy molecular pathophysiology are lacking. Recent studies characterizing CardioLaminopathies have provided novel perspectives on the role of the nuclear lamina and the pathological consequences of altered nuclear lamina. Interestingly, studies have revealed that nicotinamide adenine dinucleotide (NAD^+) salvage pathway was altered in the cardiac tissues carrying LMNA mutations, which drastically reduced cellular NAD^+ levels. It is interesting to speculate that these changes in NAD^+ availability could induce global ADP-ribosylome changes in striated muscle laminopathies that affect cardiac function. To address this hypothesis, we defined the ADP-ribosylomes of heart tissues isolated from an autosomal-recessive *Lmna-*$^{H222P/H222P}$ mouse model of Emery Dreifuss Muscular Dystrophy (EDMD), a striated muscle laminopathy, in which altered NAD^+ homeostasis is thought to contribute cardiac dysfunction. Compilation of the individual WT and *Lmna-*$^{H222P/H222P}$ ADP-ribosylomes using our well-established MS-based ADP-ribosylome revealed that the *Lmna-*$^{H222P/H222P}$ mutation reduced ADP-ribosylation in mouse hearts. STRING analysis of the 291 ADP-ribosylated proteins identified in the WT mouse hearts revealed a specific enrichment in proteins required for mitochondrial and muscle contraction functions. Importantly, we also discovered that the *Lmna-*$^{H222P/H222P}$ mutation specifically reduced the modification of mitochondrial, cell membrane, and secreted proteins. Importantly, this study provides novel insights into the molecular mechanisms that ADP-ribosylation governs in cardiac tissues and has allowed us identify actionable therapeutic drug targets to counteract the disease.

HOW VIRAL MACRODOMAINS ERASE ADP RIBOSYLATION AND HOW TO STOP THEM

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Interferon signaling triggers PARP-dependent ADP-ribosylation, and many viruses encode “erasers” that reverse these marks to promote replication. SARS-CoV-2 NSP3 Mac1 is a MacroD-type ADP-ribosylhydrolase that hydrolyzes protein-linked ADP-ribose and dampens innate immune responses. I will present an integrated structural and translational view of Mac1, leveraging our structural biology platform to connect catalytic mechanism to inhibitor design and in vivo efficacy.

I will describe structure-guided discovery and optimization of potent, selective Mac1 inhibitors, including orally bioavailable compounds that sustain free plasma exposures above the Mac1 IC50 and drive multi-log reductions in lung viral titers in mouse models, approaching the pharmacodynamic benchmark of a catalytic-dead Mac1 virus. I will then connect these outcomes to mechanism using high-resolution crystallography, NMR, targeted mass spectrometry, and reactive simulations that map substrate engagement and define the catalytic water network and ribose chemistry required for ADP-ribose hydrolysis.

Together, these results establish Mac1 as a tractable antiviral target, show how preserving host ADP-ribosylation can restore antiviral signaling, and provide a mechanistic blueprint for targeting viral macrodomains across families.

DEVELOPMENT OF CHEMICAL TOOLS TO STUDY ADPR-UBIQUITYLATION

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Crosstalk between different post-translational modifications, including ubiquitylation and ADP-ribosylation, has been implicated to play a role in regulating cellular processes such as DNA repair and immune responses. Notably, a direct physical connection between ADP-ribose and Ub is for instance forged by Legionella pneumophila effector enzymes in the context of bacterial infection. Such post-translational modification of a post-translational modifier also occurs in mammalian cells, where Deltex E3-ligases are able to ubiquitylate ADP-ribose. I will showcase how we explore this dual PTM using a chemical biology approach, where we synthesize a stabilized probe based on ubiquitylated ADPribose and perform a pull-down from cell lysate followed by a MS/MS analysis of the interactome. We identified several RING E3-ligases to recognize ubiquitylated ADP-ribose. Further biophysical and biochemical experiments shed light on the function of one of these E3 ligases and revealed its ability to extend the ubiquitylated ADP-ribose probe by elongating the ubiquitin chain. Through domain deletion analysis, we also identified the crucial domains involved in recognizing ubiquitylated ADP-ribose. We further show these domains to also play an essential role in recruiting the E3-ligase to sites of irradiation induced DNA damage.

MARUBYLATION AS A NOVEL HYBRID POST-TRANSLATIONAL MODIFICATION

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Cooperation among post-translational modifications provides a tiered opportunity for protein regulation. Increasingly complex layers of crosstalk are emerging between ADP-ribosylation and ubiquitylation, including directed ubiquitylation of ADP-ribosylated proteins as well as ADP-ribosylation of ubiquitin itself. As part of a growing field of noncanonical ubiquitylation, we have recently shown in cells that ubiquitylation can even take place directly on top of ADP-ribosylation marks, creating a hybrid post-translational modification that we refer to as mono-ADP-ribose ubiquitylation (MARUbylation). Prior biochemical work had implicated the Deltex family of E3 ubiquitin ligases in catalyzing this reaction, and we have confirmed in cellular studies that this unique group of enzymes responds to PARP activity to catalyze ester-linked ubiquitylation of ADP-ribose modifications. Further, we identified a small family of reader-writer E3 ligases that respond to events of MARUbylation and extend the modification with a K11-linked polyubiquitin chain. Herein, we will present our latest findings in the regulation and functional outcome of MARUbylation as a tightly regulated and highly complex new post-translational modification.

CELL-PERMEABLE NICOTINAMIDE ADENINE DINUCLEOTIDES FOR EXPLORATION OF CELLULAR PROTEIN ADP-RIBOSYLATION

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ADP-ribosylation is a key posttranslational modification in cell signaling, in which PARP enzymes transfer ADP-ribose units from NAD^+ to target macromolecules. Over the years, many NAD^+-based tools have been developed to study ADP-ribosylation, however, their limited ability to penetrate cell membranes has restricted investigations in living systems.

Here, we report the first cell-permeable NAD^+ probes that enable identification of ADP-ribosylated proteins without requiring additional carriers or subsequent chemical reactions (e.g., CuAAC). We synthesized a series of NAD^+ double conjugates bearing either affinity (desthiobiotin, DTB) or fluorescent (TAMRA) tags, combined with cholesterol or cell-penetrating polyarginine peptide modifications. The DTB-tagged probe was used for affinity enrichment of proteins ADP-ribosylated in living cells under oxidative stress. This approach revealed targets involved in host-virus interactions, DNA damage and repair, protein biosynthesis, and ribosome biogenesis, demonstrating the utility of these probes for comprehensive cellular ADP-ribosylome studies.

Fluorescent probes allowed visualization of subcellular localization changes under oxidative stress in HeLa cells. Furthermore, we demonstrated uptake of the TAMRA probe in chorionated zebrafish embryos without detectable cytotoxicity for up to 24 hours post-fertilization.

To improve sensitivity, we designed a second-generation cell-permeable NAD^+ probe incorporating a cholesteryl tag via a [4-(acetyloxy)phenyl]methyl N-carbamate bond. This modification enhanced probe stability in buffer and enabled efficient cleavage by human carboxylesterase 1b. We demonstrated the probe's ability to penetrate the cell membrane and used it to visualize ADP-ribosylated proteins and monitor fluorescence changes in HeLa cells under oxidative stress. Eventually, we used the probe to track ADP-ribosylation of G3BP1 protein upon formation of stress granules.

Together, these cell-permeable NAD^+ probes provide versatile tools for studying ADP-ribosylation dynamics in living cells and whole organisms, offering new opportunities to understand cellular responses to diverse stress stimuli.

MAPPING PROTEIN–POLY(ADP-RIBOSE) BINDING SITES USING A CLEAVABLE PHOTOCROSSLINKING PROTEOMICS PROBE

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Identifying protein–ligand binding sites at scale remains challenging. We introduce PARmap, a cleavable photoaffinity probe based on poly(ADP-ribose) (PAR) that captures PAR–protein interfaces and leaves a diagnostic mass remnant on proximal residues, enabling site-resolved identification by liquid chromatography-tandem mass spectrometry (LC–MS/MS). Applying PARmap to cytoplasmic, nuclear, and chromatin fractions of human cell extracts, we identified 1,262 PAR readers and localized 311 non-redundant PAR-binding sites on 137 proteins, supported by 6,010 peptide–spectrum matches. Benchmarking against prior studies recovered 659 previously reported PAR readers and added 603 candidates, expanding the PAR-binding proteome by one-third. PARmap sites were enriched in RNA-binding modules, intrinsically disordered segments and basic, Lys/Arg-rich regions, and overlapped with known, validated PAR binding regions. Targeted validations included APLF, histone H2B, and a 27-protein bead-based panel, confirming specificity and revealing shared PAR–RNA recognition surfaces. PARmap thus defines PAR-binding sites and motifs proteome-wide, facilitating mechanistic interrogation of PAR-regulated biology. This new tool provides residue-level hypotheses for PAR recognition by readers, complementing covalent ADPr site proteomics and enables direct tests that connect protein sequence and structure to PAR-dependent function.

MONO(ADP-RIBOSYL)ATION: A MARKER FOR PROTEIN DEGRADATION?

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ADP-ribosylation is a post-translational modification that plays a critical role in cellular stress responses. The function of poly(ADP-ribosyl)ation in for example the DNA damage response has been well studied whereas the function of mono(ADP-ribosyl)ation remains more enigmatic.

We have observed that during proteotoxic stress cellular ADP-ribosylation increases, which becomes even more pronounced when specific PARPs, including PARP7, are overexpressed, or when ubiquitination is inhibited. Using immunofluorescence approaches we found that following proteotoxic stress, ADP-ribosylated proteins accumulate in cytoplasmic foci containing ubiquitin and p62. During prolonged stress, ADP-ribosylated proteins are transported to aggresomes and processed in a ubiquitin-dependent manner. Upon inhibition of PARP7, accumulation of mono(ADP-ribosyl)ated proteins in response to proteotoxic stress is impeded. PARP7 turnover is rapid under normal conditions, however, the protein stabilises following proteotoxic stress. PARP7 thus forms an ideal proteotoxic stress sensor.

Our findings furthermore imply that contrary to the current paradigm, perhaps not all ADP-ribosylation occurs on specific sites to regulate specific protein characteristics. Instead, it may be rather promiscuous to enable efficient protein degradation to prevent irreversible damage caused by defective proteins.

PARP14: AN ACTIONABLE NODE IN THE IMMUNOSUPPRESSIVE TUMOUR MICROENVIRONMENT

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We are interested in a paradox at the heart of cancer immunotherapy: interferon-γ (IFNγ) is central to effective antitumor immunity, yet sustained IFNγ signalling can also drive immune escape and acquired resistance to PD-1 blockade. To understand how this switch occurs—and whether it can be therapeutically reversed—we use syngeneic models in immunocompetent mice, paired with chronic IFNγ conditioning of tumour cells, analyses of patient melanomas enriched for IFNG signalling, and analyses of cultures derived from lesions that progress under immune checkpoint blockade. Across these systems, we repeatedly observe induction of PARP14, suggesting it may act as a tractable node linking persistent inflammatory signalling to resistance. Consequently, we have tested whether interfering with PARP14 could restore vulnerability to PD-1 blockade. Using pharmacologic PARP14 inhibition and genetic knockdown, we found that tumours derived from IFNγ-conditioned cells regained sensitivity to α-PD-1, with a more favourable immune landscape characterised by increased effector T-cell infiltration and reduced regulatory T cells. Importantly, we also saw that tumours relapsing after an initial response to PD-1 blockade often showed renewed IFNγ pathway activity and could be re-sensitised with PARP14 inhibition, reinforcing the idea that IFNγ-driven resistance is dynamic and potentially reversible. Moreover, a PARP14 catalytic inhibitor gene signature is associated with improved response to immune checkpoint blockade therapy

Because PARP14 inhibition improved—but did not consistently deliver—complete tumour clearance, we broadened our approach to map how both immune and tumour compartments adapt to combination therapy. We used single-cell RNA sequencing to resolve cellular state changes during PARP14 inhibition plus PD-1 blockade. These analyses indicate that the combination remodels the myeloid compartment by suppressing tumour-associated macrophage programs while expanding more pro-inflammatory memory-like macrophage states. In parallel, cytotoxic T cells were less prone to terminal exhaustion; instead, they adopted a quiescent, function-preserving state that may help sustain antitumor activity over time. At the same time, we see evidence that tumour cells can engage alternative immune-evasion pathways under this pressure, consistent with adaptive resistance emerging even as immune responses are strengthened.

Together, our findings to date position PARP14 as an actionable target to counter IFNγ-associated resistance to PD-1 blockade, while revealing immune states that may be exploitable to achieve deeper and more durable responses. Ongoing work focuses on whether the quiescent T-cell program induced by combination therapy can be intentionally leveraged—through rational sequencing or additional partners—to extend functionality without re-triggering exhaustion and to drive enhanced immune surveillance toward more complete tumour control.

DUAL ENZYMATIC ACTIVITY OF PARP14 AND ITS ROLE IN INTERFERON-INDUCED ADP-RIBOSYLATION

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PARP14 is a mono-ADP-ribosyl transferase implicated in transcriptional regulation, DNA repair, carcinogenesis, and immunity, and has recently been identified as an antiviral effector against coronaviruses. Despite its biological significance, the cellular prevalence, targets, acceptor-residue specificity, and regulation of its ADP-ribosylation activity remain poorly defined. In addition to its catalytic domain, PARP14 harbours three tandem macrodomains, whose functions have not been extensively studied. Here, we demonstrate that PARP14 is a dual-function enzyme, exhibiting both ADP-ribosyl transferase and ADP-ribosyl hydrolase activities. PARP14 catalyses mono-ADP-ribosylation of numerous cellular proteins, predominantly on aspartate and glutamate residues, with modification levels tightly regulated by its hydrolytic macrodomain 1 (MD1). Imaging analyses reveal that PARP14's transferase activity drives its localisation to cytoplasmic foci, which become more numerous and smaller upon loss of MD1 hydrolase activity. Furthermore, we identify PARP14 as the primary writer of interferon-induced ADP-ribosylation, with the macrodomain-containing PARP9 and the E3 ubiquitin ligase DTX3L acting as key regulators that maintain PARP14 protein levels and fine-tune its catalytic output. Finally, we show that the SARS-CoV-2 macrodomain Mac1, which is closely related to PARP14 MD1, reverses PARP14-mediated ADP-ribosylation, providing a plausible mechanism by which Mac1 counteracts the antiviral activity of PARP14.

SYNTHESIS OF A BACTERIAL SIGNALLING MOLECULE - ADP-RIBOSYLATED HISTIDINE.

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The bacterial immune system contains several defence mechanisms to prevent pathogens from infecting the host. The Thoeris defence system is such an example that protects the bacterium against phage infection. The most studied signalling molecules in anti-phage systems that interrupt the infection process are derived from nicotinamide adenine dinucleotide (NAD+). Recently, one such molecule – ADP-ribosylated histidine (His-ADPr)- was found to play an important role in destabilising the cell membrane and thus causing cell death in phage-infected bacteria. The imidazole moiety in His-ADPr is ribosylated in a β fashion on the τ-nitrogen. This is, in contrast to His-ADPr from human ADP-ribosylated peptides, of which the exact stereo- and regiochemistry has not been conclusively established yet, but is believed to be the α. We synthesized this proposed bacterial metabolite and its close 1,4-triazole isostere (Trz-ADPr) as a potential molecular tool to study bacterial immunity. To improve on earlier used N-glycosylation approaches used for imidazole construction, we applied a novel, stereo- and regioselective method, for which little precedent exists. To introduce the 1,4-triazole, a copper-catalysed azide-alkyne cycloaddition (CuAAC) was employed, and the pyrophosphate was incorporated by applying a PIII-PV coupling procedure. We tested both compounds in an ADPr-hydrolase assay with human, bacterial and phage enzymes.

A DIVERSIFIED SYNTHETIC TOOLKIT FOR NON-CANONICAL ADP-RIBOSYLATION: NUCLEIC ACID-ADPr, RIFAMPICIN-ADPr AND GLYCOCYCLIC-ADPr

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ADP-ribosylation (ADPr) has recently expanded beyond its classical role in protein modification to include non-canonical targets such as DNA, RNA, and antibiotics. These modifications are pivotal in bacterial growth, antiphage defense, and DNA repair; however, their precise mechanisms remain elusive due to a scarcity of well-defined molecular tools. The synthesis of nucleic acid-ADPr conjugates is particularly challenging, requiring high stereoselectivity for rare glycosidic bonds and compatibility between disparate chemical frameworks. Herein, we report the chemo- and stereoselective synthesis of DNA-ADPr probes, spanning **nucleotide-phospho-ADPr** to **oligonucleotide-ADPr** containing the native TNT-ADPr-C sequence (a DarTG toxin-antitoxin target), via both solution-phase and automated strategies. These probes enabled the biochemical profiling of DNA-ADPr hydrolases (DarG, TARG1, and ARH3), revealing a strict preference for the α-anomer and enhanced activity toward oligonucleotide substrates over mononucleotdie counterparts. Furthermore, we achieved the total synthesis of **ADP-ribosylated rifampicin** and the bacterial signaling molecule **glycocyclic ADPr** (gc-ADPr), facilitating the functional study of antibiotic resistance and antiphage immunity. This comprehensive toolkit provides essential chemical scaffolds for investigating the biological landscape of non-canonical ADP-ribosylation.

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MECHANISTIC INSIGHT INTO ADP-RIBOSYLATION ESTABLISHMENT AND VIRULENCE BY FUNGAL PARPs

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Fungal disease, though historically neglected, are increasingly recognised as a growing global human health concern with current estimates of 3.8 million deaths annually from 6.5 million invasive fungal infections. *Aspergillus fumigatus* (*Af*), the causative agent of invasive Aspergillosis, is ranked in the WHO highest priority pathogen group due to its prevalence, high mortality rate, growing at-risk population, limited treatment options, and the rapid emergence of antifungal resistance. Recent studies demonstrated a role of fungal PARPs and associated protein PARylation in the regulation of fungal pathogenicity, but the underlying mechanisms remained largely elusive. We conducted a comprehensive phylogenetic and functional characterisation of *Af*-PARP1, the closest homologue to mammalian PARP2/3 as well as plant PARP2. Our data reveal *Af*-PARP1 as a DNA-dependent poly(ADP-ribosyl)transferase with unique domain architecture, DNA damage selectivity, and activation dynamics distinct from its mammalian and plant homologues. We show that the fungal specific BRCT domain plays a crucial role in both damage recognition and ADP-ribosylation signal establishment. Collectively, our findings reveal a divergence in DDR-associated ADP-ribosylation specific to fungi, highlighting the potential of this signalling pathway as target for antifungal therapy.

THE ART OF RNAYLATION: LINKING NUCLEIC ACIDS TO PROTEINS WITH NATURAL PRECISION TO REGULATE CELLULAR PROCESSES

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Nucleic acids and proteins are fundamental building blocks of life, each possessing unique biological properties. Nucleic acids, such as DNA and RNA, are characterized by their chemical programmability 1 and the ability to carry genetic information, making them valuable for genetic engineering, therapeutic approaches, diagnostics, and nanotechnology. In contrast, proteins possess structural diversity and various enzymatic functions. Combining these two biomolecules - nucleic acids and proteins - by conjugation strategies can offer a powerful approach to overcome individual limitations and unlock new functionalities. In the last few years, several chemical strategies have been developed to covalently link nucleic acids with proteins 2.
In this study, we present a novel mechanism termed RNAylation, mediated by the bacteriophage T4 ADP-ribosyltransferase (ART) ModB, which enables the covalent linkage of RNA to specific target proteins 3. Interestingly, ModB can take NAD-RNA 4 as a substrate in addition to NAD to link ADP-ribose RNA to specific arginine residues on a target protein via an N-glycosidic bond. The identified target proteins for ModB-mediated RNAylation are ribosomal proteins S1 (rS1) and L2 (rL2) of *Escherichia coli*.
Furthermore, while investigating the biological functions of RNAylation in a cellular context, we aim to use the RNAylation reaction as a technology 5 to engineer nucleic acid-protein conjugates in vitro to facilitate targeted delivery and modulation of cellular processes in eukaryotes. Specifically, we aim to: (1) define the design principles of RNAylated proteins to regulate cellular pathways; (2) develop precise delivery strategies for targeted cell-specific localization of RNAylated proteins; and (3) apply these strategies to regulate gene expression in vivo and use RNAylated proteins as therapeutic reagents.
To achieve those aims, we have increased the scope of the RNAylation to various nucleic acids, e.g., DNA, and their modifications. Using various forms of nucleic acids, we can tailor the stability of RNAylated proteins in vivo. Moreover, we have defined a protein tag that can be fused to every protein of interest to allow RNAylation of every protein of interest. Additionally, we developed a purification strategy for RNAylated proteins that can be delivered into eukaryotic cells and regulate cellular processes. Using confocal microscopy, we can already confirm the successful delivery of RNAylated proteins into eukaryotic cells.
This work will advance our understanding of RNA-protein interactions and lead to the development of next-generation RNA therapeutics, with potential implications for synthetic biology and complex disease treatment. This work not only bridges fundamental discoveries with therapeutic innovation but also positions RNAylation as a novel tool in the engineering of nucleic acid-protein conjugates.

INTERDOMAIN REGULATION OF PARP14 ENZYMATIC ACTIVITY THROUGH ALLOSTERIC INTERACTIONS

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PARP14 encodes opposing enzymatic activities within a single polypeptide: a writer (ADP-ribose Transferase, ART domain), an eraser (macrodomain 1), and multiple reader domains (macrodomains 2 and 3). This architecture enables PARP14 to suppress anti-tumor IFN-γ/STAT1 signaling while promoting IL-4/STAT6 transcription, driving immune evasion in checkpoint blockade-resistant tumors. While writer domain inhibitors show therapeutic promise, persistent immune evasion and incomplete restoration of interferon responsiveness suggest that PARP14's regulatory mechanisms extend beyond catalytic activity alone. The molecular basis for coordinating these opposing enzymatic functions remains unknown. Using coarse-grained simulations in CALVADOS, we have mapped distinct conformational states of PARP14 that depend on domain composition. Simulations reveal a dynamic structural ensemble characterized by context-dependent interdomain contacts that regulate active site accessibility. Comparison of full-length PARP14 with truncated macrodomain constructs shows reduced MD1-MD2 contact frequencies in the full-length protein, suggesting that the overall domain architecture modulates eraser-reader interfaces. Distance map analysis identifies conformational states that differentially expose or occlude the MD1 eraser active site, supporting a model in which PARP14 toggles between eraser-occluded and eraser-competent conformations through allosteric crosstalk among accessory domains.

To test functional consequences of predicted structural states, we have designed a systematic PARP14 domain variant library and established dual IL-4/STAT6 and IFN-γ/STAT1 transcriptional reporters in HEK293T cells. Preliminary analysis of PARP14 domain deletions and catalytic inactivation mutants reveals changes in cellular localization to nuclear and cytoplasmic foci, ADP-ribosylation patterns, and cytokine-dependent reporter activation. These data establish a framework for correlating structural dynamics with enzymatic output and signaling bias. This work defines PARP14 as a conformationally dynamic scaffold where interdomain communication controls the balance between ADP-ribosylation addition and removal, revealing allosteric regulatory nodes that may be targeted to reprogram immune responses in therapy-resistant tumors.

IDENTIFICATION OF PURINE-BASED INHIBITORS FOR THE SARS-COV-2 AND THE MERS-COV MACRODOMAINS

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Viral macrodomains play critical roles in viral replication and immune evasion by removing ADP-ribose moieties from ADP-ribosylated proteins. Several purine analogs, including GS-441524—the parent nucleoside of remdesivir—have been reported to inhibit the SARS-CoV-2 macrodomain (Mac1); however, their cellular target engagement has not been well defined. Using a high-throughput ADPr-Glo assay, we confirmed that GS-441524 inhibits SARS-CoV-2 Mac1 while exhibiting minimal activity against the closest human homolog, MacroD2, and the macrodomain of MERS-CoV from a distinct coronavirus subclade, indicating selectivity for the SARS-CoV-2 Mac1 over host and structurally divergent viral macrodomains. Importantly, cellular thermal shift assays (CETSA) confirmed that GS-441524 engages SARS-CoV-2 Mac1 in cells. Moreover, conversion of GS-441524 to its diphosphorylated metabolite resulted in enhanced in vitro inhibitory activity, suggesting that metabolic phosphorylation can improve target engagement. Despite these findings, GS-441524 displayed only modest activity in cellular assays. To identify more potent macrodomain inhibitors, we screened a library of 1,156 purine compounds (ChemDiv) against the SARS-CoV-2 Mac1 and MERS-CoV macrodomain using the ADPr-Glo assay at 30 µM, identifying 54 compounds exhibiting ≥50% inhibition. Subsequent counter-screening and confirmation at 10 µM and 30 µM led to the identification of two compounds with low-micromolar affinity for the MERS-CoV macrodomain. Ongoing efforts in our lab focus on optimizing the identified compounds using structure-based docking and structure–activity relationship (SAR) approaches, to develop dual macrodomain inhibitors. Collectively, our results demonstrate that purine analogs provide a tractable scaffold class for structure-guided development of selective dual coronavirus macrodomain inhibitors.

HISTONE MONO-ADP-RIBOSYLATION AS A REGULATORY AXIS IN CHROMATIN REMODELING DURING STEM CELL DIFFERENTIATION

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Cell fate transitions are governed by dynamic chromatin remodeling driven by coordinated post-translational modifications (PTMs) of histones. While acetylation and methylation are established determinants of stem cell pluripotency and differentiation, accumulating evidence indicates that poly(ADP-ribose) polymerase 1 (PARP1) is also a critical regulator of stem cell identity, transcriptional plasticity, and differentiation capacity. Despite this, the specific molecular outputs of PARP1 activity in chromatin regulation remain incompletely defined. Recent identification of PARP1/2-HPF1-mediated serine-directed mono-ADP-ribosylation (MARylation) suggests that serine ADP-ribosylation may represent a regulatory signal distinct from classical PARylation. Here, we hypothesized that serine mono-ADP-ribosylation functions as an active, differentiation-associated epigenetic signal, with histone MARylation emerging as a downstream outcome of dynamic mono- and poly-ADPr patterning during exit from pluripotency.

To test this hypothesis, we examined ADPr dynamics during differentiation of murine embryonic stem cells (mESCs) using a haploid model system differentiated by leukemia inhibitory factor (LIF) withdrawal. We observe a progressive increase in histone MARylation and global PARylation over a seven-day differentiation time course, coinciding with elevated PARP1 expression and increased γH2AX levels. These findings support a model in which ADPr signaling is dynamically regulated during differentiation and potentially linked to chromatin accessibility and genome surveillance pathways.

Biochemical enrichment of histones followed by MAR-specific immunoprecipitation identifies histone H3 as a primary MARylation target in differentiated cells. Tandem mass spectrometry further implicates serine 10 of histone H3 (H3S10) as a differentiation-specific MARylation site, raising the possibility of functional crosstalk with adjacent regulatory residues involved in transcriptional control.

Together, these data support a working model in which PARP1-HPF1-dependent histone MARylation acts as a co-determinant of chromatin state transitions during stem cell differentiation. This study establishes a framework for defining MARylation as an epigenetic regulatory layer and provides a basis for future genome-wide and functional analyses linking histone MARylation to transcriptional reprogramming during cell fate decisions.

PARP-1 MEDIATES DDX HELICASES TO PREVENT R-LOOP-INDUCED DNA DAMAGE AND GENOME INSTABILITY

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R loops occur frequently in genomes and contribute to fundamental biological processes at multiple levels. Consequently, understanding the molecular and cellular biology of R loops has become an emerging area of research. Here, it is shown that poly(ADP-ribose) polymerase-1 (PARP-1) can mediate the association of several DDX helicases, including DDX18, with R loops thereby modulating R-loop homeostasis in endogenous R-loop-prone and DNA lesion regions. DDX18 depletion results in aberrant endogenous R-loop accumulation, which leads to DNA-replication defects. In addition, DDX18 depletion renders cells more sensitive to DNA-damaging agents and reduces RPA32 and RAD51 foci formation in response to irradiation. Notably, DDX18 depletion leads to gH2AX accumulation and genome instability, and RNase H1 overexpression rescues all the DNA-repair defects caused by DDX18 depletion. Taken together, these studies uncover a function of DDX18 in R-loop-mediated events and suggest a role for PARP-1 in mediating the binding of specific DDX-family proteins with R loops in cells.

IDENTIFICATION OF ALLOSTERIC MODULATORS OF PARP-3 ACTIVITY

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Poly(ADP-ribose) polymerase 3 (PARP-3) is a member of the PARP family involved in DNA repair and cell division. Its dysregulation has been shown to restrain cancer aggressiveness while also compromising efficient muscle and neuronal stem cell differentiation, both events being key for muscle and brain recovery upon injury, thus revealing its therapeutic impact. To better understand the regulatory mechanisms of PARP-3 and to explore new therapeutic strategies, we sought to identify allosteric modulators selectively targeting this enzyme.

A chemical screening of 6,000 compounds was performed using the purified helical domain (HD) of PARP3, a structural motif known to play a central role in the allosteric regulation of DNA-dependent PARPs. This screen identified several candidate molecules capable of modulating PARP-3 enzymatic activity. Enzymatic assays revealed that one of this compound induced a strong activation of PARP-3, which was particularly pronounced in the absence of DNA, suggesting an allosteric mechanism independent of DNA binding.

Structure–activity relationship (SAR) studies were conducted to optimize this compound, and nuclear magnetic resonance (NMR) spectroscopy analyses confirmed a direct interaction between this compound and the HD. Altogether, these results highlight the HD as an exploitable allosteric platform and identify a new promising molecular tool for the selective modulation of PARP-3.

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PARP1 MEDIATES NAD+-DEPENDENT NUCLEOSOME DISASSEMBLY TO ENABLE CHROMATIN ACCESSIBILITY DURING THE EARLY PHASE OF THE DNA DAMAGE RESPONSE

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Chromatin remodeling is rapidly initiated upon DNA damage to promote chromatin accessibility at DNA lesions, thereby facilitating the recruitment and assembly of repair factors. Although this enhanced accessibility has been associated with poly(ADP-ribose) polymerase (PARP) activity during the early phase of the DNA damage response (DDR), the underlying mechanism remains unclear. PARP1 is the founding member of the PARP family and is well-established for its ability to sense DNA strand breaks and catalyze protein post-translational modification through poly(ADP-ribosyl)ation (PARylation). Using a reconstituted chromatin system, we uncovered a previously uncharacterized activity of PARP1, whereby it selectively evicts histone dimers proximal to DNA strand breaks from nucleosomes and chromatosomes to generate the corresponding hexasomes. In the presence of HPF1, PARP1 promotes both trans-PARylation of histones and nucleosome disassembly to generate PARylated subnucleosomes. By combining cell imaging and genomics, we demonstrated that PARP activity is both required and sufficient to drive chromatin accessibility and the recruitment of repair factors, with direct involvement of subnucleosomal species. Together, our findings support a model in which PARP1 drives histone eviction and trans-PARylation, leading to the formation of modified subnucleosomes that promote both DNA-dependent and PAR-dependent recruitment of repair factors to sites of damage.

NOVEL INSIGHTS IN THE DOMAIN FUNCTION AND ACTIVATION OF PARP12

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PARP12's molecular mechanisms and function of the domains have not been studied extensively. The protein features a domain architecture containing five CCCH-type zinc finger motifs, two WWE domains and an ART domain, an architecture extremely similar to the one of PARP13, its closest evolutionary relative. However, despite this similarity, the two proteins differ in a key aspect: PARP13 lacks the ADP-ribosyltransferase activity.

In PARP13, the first four zinc finger motifs are described to selectively bind CG-rich RNA sequences, but in contrast the corresponding domains of PARP12 have not shown the same specificity. However, studies suggest PARP12 can bind mRNAs and ADP-ribosylate them to inhibit their translation, this indicates the zinc finger motifs might have a key role in their recognition. These motifs have also been implicated in the recruitment of the protein to stress granules upon stress signals, and are thought to have a role in antiviral response.

The WWE domains are found also in other PARPs and proteins involved in ubiquitination pathways. Typically, the domain is binding ADP-ribose-derived structures, for example the WWE2 of PARP13 has specificity for the terminal ADP-ribose unit of the poly(ADP-ribose) chain, while RNF-146 binds uniquely iso-ADP-ribose.

Furthermore, PARP12's activity also appears to be regulated by phosphorylation, as in the context of basolateral transportation of E-cadherin from the trans-Golgi network, the phosphorylation from PKD enables PARP12 to then ADP-ribosylate Golgin97.

In this study, we combine biophysical and biochemical methods of recombinant PARP12 constructs with computational modelling to investigate the specificity of the domains towards ADP-ribose, iso-ADP-ribose and RNA, as well as the activation mechanism of the protein upon phosphorylation. We aim to study the structure of the protein and the presence of any conformational rearrangement upon its activation.

A SURVEY OF PARPs' FUNCTION ON ANTIVIRAL IMMUNITY REVEALS A NOVEL REGULATORY MECHANISM TO REGULATE ENERGY METABOLISM

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ADP-ribosylation (ADPr) is increasingly recognized as a modulator in antiviral immunity. Some PARPs have been reported as interferon-stimulated genes, and viral eraser enzymes have been identified, indicating that ADPr is a virus- and immunity-relevant PTM. However, very little is known about the subset of ADP-ribosylated proteins targeted by infection-relevant PARPs, or about the functional consequences of this modification. Here, we investigate the role of ADPr in anti-viral immunity from two perspectives: (a) We characterized the anti- and pro-viral activities of PARPs using a loss-of-function screen testing for replication of a diverse set of viruses covering four RNA viruses from different classes. The anti-viral functional screen revealed surprising heterogeneity in anti-viral activities of PARP proteins, indicating an unexpected virus-specific relevance of PARP activity. Notably, we observed antiviral effects of PARP1 and PARP12 against both influenza virus A (IAV) and yellow fever virus (YFV). (b) Additionally, we use an affinity purification mass spectrometry approach to identify ADPr target proteins and their modification sites that are differentially ADP-ribosylated during YFV virus infection. Our analysis revealed 102 regulated and significantly enriched unique ADPr-sites in YFV-infected A549 cells 12 hours post-infection (hpi). Gene overrepresentation analysis of the regulated ADPr-sites revealed enrichment of metabolism-related terms associated with ADPr of downstream glycolytic enzymes GAPDH, PGK1, PGAM1, ENO1, and PKM, from which the identified PKM ADPr-sites S77 and E272 are positioned in the active center. In our functional studies, we demonstrate that PARP1 inhibition decreases PKM activity, leading to metabolic reprogramming towards aerobic glycolysis. Moreover, we confirm PARP1's antiviral activity during YFV infection by demonstrating its dependence on oxidative phosphorylation on YFV growth. Thus, we conclude that PARP1-mediated ADPr of PKM negatively regulates glycolysis and oxidative phosphorylation by shifting the glycolytic flux towards aerobic glycolysis, thereby limiting YFV growth.

PARylation OF RNA BY PARP1 IN BREAST CANCER CELLS

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PARP1 [poly(ADP-ribose) polymerase 1] modifies, i.e., PARylates, target proteins by covalent addition of NAD+-derived ADP-ribose to select amino acids to form long linear or branched poly(ADP-ribose) (PAR) chains. PARP1 is activated by DNA strand-breaks and PARylates multiple proteins involved in the DNA damage response (DDR). DNA damage induces cytoplasm-to-nucleus translocation of the bifunctional aminoacyl-tRNA synthetase EPRS1 (Glu-Pro-tRNA synthetase), enhancing PARP1 PARylation activity and DNA damage repair in breast cancer cells (I. Zin et al., PNAS, 2024). Based on the mRNA-binding activity of EPRS1, we considered the possibility that nuclear-localized EPRS1 induces PARylation of RNA as well as protein. RNA extracted from MDA-MB-468 human breast cancer cells, which exhibit a high constitutive level of nuclear EPRS1, exhibited robust PARylation as shown by dot blot analysis with anti-PAR antibody. DNA isolated from the same cells was not PARylated. These results suggest PARylation of the free 2'hydroxy ribose group in RNA that is absent in DNA, but structurally similar to the terminal 2'hydroxy group of ADP-ribose targeted by NAD+ during PAR chain elongation. Oligo(dT) purification of RNA revealed mRNA as a PARylation target. Inhibition of PARP1 expression by siRNA, or PARP1 activity by the inhibitor talazoparib, completely abolished RNA PARylation, revealing PARP1 dependence. Knockdown of PARP10, which induces PARylation at the 5'cap of mRNA in vitro, did not inhibit RNA PARylation in these cells indicating PARP-type specificity. Cell treatment with an inhibitor of the PAR eraser PARG (PAR glycohydrolase) enhanced RNA PARylation, revealing a dynamic system with PARP1 as writer and PARG as eraser. siRNA-mediated knockdown of EPRS1 completely suppressed RNA PARylation, suggesting an adaptor function of EPRS1. Knockdown of leucyl-tRNA synthetase was ineffective, indicating effector specificity. PARylation was cell type-specific – RNA PARylation was not detected in PTEN-positive MCF-7 cells, in contrast to robust RNA PARylation in PTEN-negative MDA-MB-468 cells, consistent with a critical role of AKT, a downstream effector negatively regulated by PTEN. Finally, DNA-damaging radiation exacerbated RNA PARylation, that was enhanced by PARG inhibition and suppressed by AKT inhibition. These results suggest PARylated RNA, like PARylated protein, might have a role in the DDR. About 170 distinct modifications of RNA have been identified, mostly on ribosomal RNAs and tRNAs, but only about 10 mRNA modifications have been shown. Thus, PARylation is a significant addition to the panoply of mRNA modifications.

ENHANCED MASS SPECTROMETRY STRATEGIES FOR LINKAGE-SPECIFIC ANALYSIS OF LABILE ADP-RIBOSYLATION

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ADP-ribosylation (ADPr) is a regulatory post-translational modification targeting nine amino acid residues, yet comprehensive and site-specific analysis remains challenging due to the chemical diversity and instability of distinct ADPr linkages. In particular, ester-linked glutamate- and aspartate-ADPr (Glu/Asp-ADPr) is poorly detected by conventional mass spectrometry (MS) workflows.

Here, we systematically assessed ADPr linkage stability using synthetic peptides and demonstrated that Glu/Asp-ADPr is lost under alkaline conditions, elevated temperatures, and through hydrolysis by wildtype Af1521. Based on these findings, we developed an acidic enrichment workflow incorporating an Af1521 mutant that preserves labile ADPr linkages, enabling robust site-specific MS analysis. Applying this strategy to cytokine-stimulated A549 and HeLa cells, we identified over 600 Glu/Asp-ADPr sites and more than 200 cysteine-linked ADPr sites.

Residue-specific profiling by EThcD-based LC-MS/MS analysis revealed that Glu/Asp-ADPr preferentially targets cytoplasmic protein networks enriched in immune and antiviral functions, in contrast to serine ADPr, which predominantly localizes to nuclear and chromatin-associated proteins. Quantitative analyses showed reproducible, cell-type- and stimulus-specific ADPr patterns across interferon treatments, identifying a conserved network of antiviral PARPs and associated cofactors extensively modified on Glu/Asp residues. We uncovered PARP10-dependent Glu/Asp modification of ubiquitin, highlighting crosstalk between ADP-ribosylation and ubiquitin signaling. Finally, we find evidence for MARUbylation of ubiquitin itself, i.e. Ub-MAR-Ub, hinting at the existence of mixed ubiquitin and ADPr chains in immune signaling.

Ongoing work in our lab is focused on implementation of new MS hardware, optimization of MS data acquisition strategies, further development of enrichment strategies for peptides modified by endogenous ADPr, and enhanced interpretation and understanding of ADPr MS proteomics data. Together, we have established an MS-based workflow for comprehensive and linkage-specific analysis of ADP-ribosylation, expands the detectable ADP-ribosylome, and reveals residue-specific ADPr dynamics in immune signaling.

ADP-RIBOSYLATION OF RNA IN STEM CELL METABOLISM.

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Protein ADP-ribosylation has been shown to regulate essential processes including DNA damage repair, chromatin remodeling, and immune responses. Recent studies have identified RNA as a novel target of ADP-ribosylation, catalyzed by specific ADP-ribosyltransferases using NAD+ as a substrate and reversed by ADP-ribosylhydrolases in vitro. However, the prevalence and function of RNA ADP-ribosylation and its link to cellular metabolism remain poorly understood.

Embryonic stem cells (ESCs) undergo a well-characterized metabolic transition during differentiation, shifting from glycolysis to oxidative phosphorylation. Here, we investigate whether RNA ADP-ribosylation contributes to this metabolic switch and cell fate determination in general. We show that RNA ADP-ribosylation levels increase significantly after seven days of spontaneous differentiation of mouse ESCs. RNA sequencing of ADP-ribosylated RNA reveals widespread modification of mRNAs, suggesting a broad regulatory potential.

To explore the functional consequences of this modification, we are assessing the impact of RNA ADP-ribosylation on translation using polysome profiling and identifying associated RNA-binding proteins through mass spectrometry and proximity ligation assays. These analyses will also define the ribosyltransferases and ribosylhydrolases responsible for RNA ADP-ribosylation in ESCs. Together, our findings link ADP-ribosylation to metabolic remodeling during ESC differentiation and highlight a previously unrecognized layer of post-transcriptional regulation with implications for stem cell biology and PARP-targeted therapeutic strategies.

PARP14 MEDIATED CYSTEINE ADP-RIBOSYLATION OF SQSTM1/P62 REMODELS INNATE IMMUNE RESPONSES

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Recognition of viral nucleic acids activates interferon (IFN) programs that induce hundreds of interferon-stimulated genes (ISGs), including several mono-ADP-ribosyltransferases (mono-ARTs). Among these, PARP14 is a prominent IFN-inducible mono-ART, yet the role of its catalytic activity in shaping innate antiviral signaling remains incompletely defined.
Here, we define the PARP14-dependent ADP-ribosylome in lung epithelial cells and identify the autophagy adapter SQSTM1/p62, along with multiple innate immunity regulators, as IFN-responsive MARylation substrates. We demonstrate that PARP14 catalyzes MARylation of p62 on specific cysteine residues in an IFN-dependent manner. This modification is reversed by the SARS-CoV-2 macrodomain (Mac1) and abrogated by pharmacological inhibition of PARP14. Functionally, p62 assembles into cytosolic condensates that co-localize with ADP-ribose and diverse ubiquitin species. Inhibition of PARP14 depletes ADP-ribose from these condensates without disrupting the underlying p62 scaffolds, yet markedly alters downstream signaling outputs. While canonical autophagic functions of p62 are preserved, depletion of either p62 or PARP14 in inflammatory cell models significantly attenuates pro-inflammatory signaling. These findings identify a PARP14-p62 signaling axis in which IFN-induced MARylation modulates innate immune responses.

CHARACTERIZING THE PAR-BINDING PROPERTIES OF THE MULTI-PDZ SCAFFOLD PROTEIN PATJ

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Poly(ADP-ribosyl)ation (PARylation) is a rapid post-translational modification catalyzed by PARP family enzymes, generating negatively charged poly(ADP-ribose) (PAR) chains that act as inducible signaling scaffolds to recruit effector proteins (“PAR readers”). Our recent protein array-based studies demonstrated that a selective subset of RNA recognition motifs (RRMs) can recognize PAR chains in a length-dependent manner, supporting the concept that modular protein domains can directly decode PAR signals beyond canonical DNA damage pathways.

Here, we extended this domain-centric framework to PDZ domain-containing proteins and performed a C-terminal Domain Readers Array screening to identify candidate PAR-interacting modules. This approach nominated PATJ (PALS1-associated tight junction protein), a multi-PDZ scaffold implicated in epithelial polarity and tight junction organization, as a candidate PAR-interacting protein. PAR association was supported using complementary biochemical validation approaches in both recombinant and cell-based assays.

To explore the biological relevance of PAR-related regulation of PATJ, we examined PATJ protein abundance following pharmacologic inhibition of PARP family enzymes. Tankyrase inhibition led to a marked reduction in PATJ protein levels, which was prevented by proteasome inhibition, supporting a proteasome-dependent mechanism regulating PATJ stability. We are currently expanding our investigation into how PARylation regulates PATJ function and tight junction assembly dynamics.

Together, these results establish a PDZ-domain–focused screening and validation strategy for identifying candidate PAR-interacting proteins and identify PATJ as a PAR-associated tight junction scaffold whose protein abundance is regulated in a tankyrase-dependent manner.

NEURL4-DEPENDENT ADP-RIBOSYLATION AS A POST-TRANSLATIONAL SWITCH REGULATING MITOCHONDRIAL FUNCTION

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ADP-ribosylation is a reversible, NAD^+-dependent post-translational modification that modulates protein activity, stability, and macromolecular interactions across cellular compartments. Although ADP-ribosylation of mitochondrial proteins has been widely reported, a comprehensive characterization of the mitochondrial PARylome and its functional consequences remains lacking.
NEURL4 recently emerged as a mitochondrial matrix–localized ADP-ribosyltransferase that couples NAD+ availability to organelle homeostasis. In contrast to nuclear PARPs activated by genotoxic stress, NEURL4 appears to mediate ADP-ribosylation of mitochondrial proteins under basal conditions, suggesting a role in metabolic regulation.
Our initial characterization of NEURL4 activity in HeLa cells established NEURL4 as a regulator of mitochondrial genome integrity via ADP-ribosylation of mitochondrial DNA ligase III (mtLIG3), supporting base excision repair and limiting mtDNA instability. However, NEURL4 activity likely extends beyond mtDNA repair. Here, we broadly define downstream effectors of NEURL4-dependent ADP-ribosylation involved in cellular energy handling and explore their physiological relevance. Using comparative analyses in NEURL4-wild-type and CRISPR-engineered NEURL4-deficient human cells, we enriched ADP-ribosylated peptides and identified candidate mitochondrial substrates whose modification depends on NEURL4. Ongoing validation efforts focus on proteins implicated in fatty acid import, β-oxidation, and metabolite exchange across the inner mitochondrial membrane. Functional assays indicate that re-expression of NEURL4 or restoration of NAD+ availability partially rescues mitochondrial respiratory defects in NEURL4-deficient cells, supporting a role for NEURL4-mediated, NAD+-consuming ADP-ribosylation in maintaining mitochondrial metabolic function.
In parallel, initial in vivo studies using brown adipose tissue-specific NEURL4 deletion suggest altered lipid handling and stress-responsive metabolic programs. Transcriptional profiling reveals coordinated changes in mitochondrial and energy metabolism pathways, accompanied by differences in lipid storage and fatty acid oxidation signatures between WT and NEURL4-deficient tissues.
Together, these findings support a model in which NEURL4-mediated ADP-ribosylation acts as a post-translational regulatory layer integrating NAD^+ metabolism with mitochondrial fatty acid utilization.

CATALYTICALLY INACTIVE PARP1 DRIVES PARP INHIBITOR–INDUCED ANEMIA

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Dual PARP1/2 inhibitors (PARPi) selectively and successfully target BRCA1/2- deficient cancers. But recently their maintence use are impeded by severe anemia and increased leukeima risk. This is unexpected, since loss of Parp1 that accounts for most DNA-damage induced PARylation causes no anemia. Meanwhile Parp2 loss or inactivation both impair erythropoiesis, leading to the development of PARP1-selective inhibitors. Here we showed that FDA-approved PARPi – niraparib depleted erythroblasts, lymphocyte progenitors and myeloid cells in *WT* and *Parp2*$^{-/-}$ mice, but not in *Parp1*$^{-/-}$ mice, suggesting the hematopoietic toxicity is an on-target effect of inhibiting Parp1. Using conditional and catalyticaly inactive Parp1 (*Parp1*$^{C/E988A}$), we found that somatic expression of inactive PARP1 in adults induces lethal bone marrow failure and thrombopoiesis, whereas deletion of both Parp1 and Parp2 causes only transient anemia and no lethality. Mechanistically, inactive PARP1—unlike PARP1 loss—physically blocks DNA repair at diverse lesions, including nicks, gaps, and Top1-lesions, impairs PARP2 recruitment and end-ligation, leading to replication fork collapse and markedly elevated sister chromatid exchanges, which were not observed in *Parp1*$^{-/-}$ cells. Correspondingly, PARP1-selective inhibitor saruparib causes more hematopoietic stress than veliparib, a weak-trapping dual PARP1/2 inhibitor. Thus, the presence and trapping of PARP1 underscore PARPi associated hematopoietic toxicity.

EVALUATION OF FULL-LENGTH PARP1 STRUCTURE BOUND TO DAMAGED DNA IN THE PRESENCE OF PARP INHIBITORS

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Poly(ADP-ribose) polymerase (PARP1) is a medically relevant, multifunctional protein best known for its key role in the DNA damage response. Upon binding to DNA strand breaks, PARP1 is activated, thus modifying itself and other proteins with poly(ADP-ribose) (PAR), a signalling molecule that recruits repair factors. PARP1's central role in DNA repair makes it an attractive therapeutic target in genomic instability-driven diseases, such as cancer. Indeed, four PARP inhibitors (PARPi) are currently approved to treat a subset of cancers, but their efficiency at killing cancer cells differs considerably, which is thought to arise partly because PARPi trigger unique allosteric effects on DNA-bound PARP1. Specifically, type I PARPi, underrepresented in the clinic, promote PARP1 retention on DNA, while type III support its release, and type II are neutral towards this interaction, as shown by our biophysical studies. Of note, DNA-trapped PARP1 prevents normal DNA metabolism and repair, leading to toxic lesions and consequent cell death. For many years, crystal structures of a subset of PARP1 domains afforded the most comprehensive models of PARP1 recognizing DNA strand breaks. Recently, we exploited cryo-electron microscopy (cryo-EM) and negative stain EM to gain initial views of full-length PARP1 engaging a DNA break in the presence of a type I PARPi and histone PARylation factor 1 (HPF1), an important binding partner and regulator of PARP1 activity. Unexpectedly, we found that PARP1 remains highly flexible even when bound to damaged DNA, adopting compact and extended conformations due to mobility of the ADP-ribosyl transferase (ART) domain. The complex flexibility prevailed regardless of the employed DNA probe length, DNA damage type (nick vs gap), or potentially stabilizing HPF1:DNA interactions, further indicating that the observed conformations are inherent to PARP1. This data suggests that DNA-bound PARP1 may act on distant targets through its mobile catalytic ART domain, thus opening avenues for drug design. Our ongoing efforts focus on overcoming innate challenges in our initial cryo-EM studies (i.e., sample denaturation during vitrification and orientation bias), which limited the resolution of cryo-EM maps. In addition, we plan to expand our studies to obtain a complete view of DNA-bound PARP1 in the presence of different PARPi types and better understand the phenotypic implications of observed conformations.

PARP10 IS AS A NOVEL TARGET OF THE NEXT-GENERATION PARPi SARUPARIB.

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Ovarian cancer (OC) is the most lethal cancer of the female reproductive system. The understanding of the biological roles of BRCA1/2 in maintaining genomic integrity through homologous recombination (HR) repair prompted the development of targeted treatment with PARP1 inhibitors (PARPi) in *BRCA1/2*-linked OC cases. However, almost 50% of patients do not respond to PARPi, despite their HR proficiency status. More recently, a next-generation PARPi (saruparib), highly selective for PARP1 over PARP2, demonstrated anti-proliferative effects in *BRCA*-mutant xenograft models and is currently being evaluated in late-stage clinical trials for OC. However, the potential polypharmacology of saruparib is still unexplored. Here, we chart the landscape of saruparib's targets to expand its efficacy in treating OC cases. We use chemical proteomics (CP), a powerful mass spectrometry-based tool for proteome-wide identification of cellular drug binders, to uncover novel non-canonical saruparib targets. PARPi CP screenings often recover stable PARP1-containing protein complexes, which hinder the accurate identification of direct drug targets. For that, we developed a genetically defined PARP1 knockout (KO) model to identify novel selective saruparib targets. We used the high-grade ovarian serous adenocarcinoma cell line, HEYA8, to engineer PARP1 KO cells using CRISPR/Cas9 and isolated two HEYA8 PARP1 KO clones that harbor unique frameshift mutations in the PARP1 locus, resulting in 25% increase in survival to 10 μM saruparib treatment compared to the parental cells. Couplable drug analogues of olaparib (c-ola) and saruparib (c-saru) were derivatized for independent CP screenings, which identified PARP10 as a saruparib-selective target, independent of the PARP1 background. Competition with free olaparib disrupts the interaction between c-saru and PARP1, but not PARP10, corroborating the selectivity of the c-saru/PARP10 interaction. The c-saru/PARP10 interaction was further confirmed in HR-proficient and -deficient OC cells. Interestingly, PARP1 KO in the BRCA1/2-proficient CAL51 breast cancer cell line results in increased PARP10 expression, leading to c-saru/PARP10 interaction only in the PARP1 KO background. In summary, our findings reveal that saruparib, previously recognized for its PARP1 selectivity, also targets PARP10 in OC cells. This expanded target profile might contribute to its mechanism of action for treating OC patients and produce additional benefit over other PARPi.

OPTIMIZED DETECTION OF ACIDIC ADP-RIBOSYLATION BY MULTI-MODAL MASS SPECTROMETRY REVEALS NEGATIVE AUTOREGULATION OF PARP14

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ADP-ribosylation is a highly dynamic process that is increasingly linked to a diverse set of cellular functions and pathophysiological outcomes. The modification of target substrates (e.g. proteins, DNA, RNA) involves ligation to an array of different chemical moieties. Recent work has shown that the ester bonds formed between ribose and Glu/Asp residues are particularly labile, with half-lives on the order of 2-6 hours in mild conditions - like those found in a cell or commonly used in tandem mass spectrometry (MS/MS) workflows. A common strategy to overcome this lability is the replacement of the ADP-ribose (ADPr) with hydroxamic acid (HA) via incubation with hydroxylamine. The attachment of the HA molecule results in a permanent +15 Da change in mass at Glu/Asp residues that were modified by ADPr. However, this method suffers from two notable limitations: the presence of HA for long periods of time can result in the labeling of Glu/Asp sites that are solvent exposed, but not modified by ADPr; and the competing hydrolysis reaction will still occur in the conditions routinely used for HA labeling. Herein, we optimize an MS/MS workflow that overcomes these drawbacks. As a proof-of-principle, we used a truncated form of PARP14 that has been well established in the literature and assessed its ability to label peptides and itself. We utilized an ultrathin matrix-assisted laser-desorption/ionization time-of-flight (TL-MALDI) method to test a variety of conditions that would speed up the nucleophilic attack by HA over the background rate of hydrolysis using ADPr modified peptides. Our efforts resulted in a doubling of HA labeling efficiency and complete loss of ADPr within an hour. Using our optimized conditions we performed a series of MS/MS experiments to determine the sites of PARP14 automodification using both standard HA labeling conditions (overnight, pH 7.0, 25 °C) and our optimized conditions. Importantly, we also performed the same experiments in the absence of NAD^+ to establish the background rate of non-ADPr-dependent HA modification. Our optimized conditions facilitated the identification of 9 ADPr sites on PARP14; 4 of which were only identified in the optimized conditions and 6 of which have not been previously identified. A series of mutagenesis experiments revealed a patch that plays a negative regulatory role on PARP14 activity, and another set that appears to be preferentially labeled at short time points. Taken together, our efforts have improved the true positive site identification rate using HA and have revealed a novel role for several automodification sites in PARP14 regulation.

GENERATION OF *PARP14*, *P62* AND *RNF114* KNOCKOUT CELL LINES BY CRISPR/CAS9 TECHNOLOGY

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ADP-ribosylation (ADPr) is a reversible post-translational modification (PTM) that has diverse cellular functions, such as in the interferon (IFN) response and antiviral mechanisms. Interestingly, several PARPs are induced by interferon, and we and others have recently demonstrated that the activation of the IFN response indeed induces ADPr in human cells. This PTM appears to have an important function in host-pathogen interactions, since several viruses, including coronaviruses, encode a macrodomain that hydrolyses this modification. However, the molecular functions of IFN-induced ADPr are not yet known.

More specifically, the active IFN pathway triggers the assembly of novel cytosolic structures in human cells that are enriched in ADP-ribose, termed ICABs (Interferon-induced Cytosolic ADPr Bodies). Our laboratory has interest in the cell signaling associated with ICABs and in the regulatory roles of proteins in the IFN response, with a particular emphasis on three key players. Central to the process is PARP14, the main protein responsible for mediating IFN-induced ADP-ribosylation and a member of the PARP14-PARP9-DTX3L axis. Furthermore, p62, a scaffold protein essential for selective autophagy, has been shown to co-localise with the IFN-induced ADPr, suggesting a possible interplay between IFN-induced signalling and cellular degradation pathways. Finally, given the emerging crosstalk between ubiquitination (Ub) and ADPr, RNF114 stands out as a possible player in these pathways, as this E3 Ubiquitin Ligase features a bipartite binding module, termed the Di19-UIM, which recognises hybrid ADPr-Ub modifications. Therefore, elucidating the roles of these proteins will increase the understanding of IFN-induced ADPr.

To this end, the objective of this work was to generate, via CRISPR/Cas9, knockout hTERT-RPE1 cell lines for *PARP14*, *p62*, and *RNF114* genes. The establishment of these clonal lineages was successful, with the loss of protein expression rigorously validated through western blot analysis and genomic editing confirmed by Sanger sequencing. Thereby, these novel cell lines are currently undergoing phenotypic characterisation and integrated experiments, aiming to elucidate the catalytic and/or regulatory roles of these proteins, and of ADPr itself, within the IFN response.

ELUCIDATING THE STRUCTURAL BASIS FOR PARP1-HPF1 SELECTIVE INHIBITION: A COMBINED COMPUTATIONAL AND BIOCHEMICAL APPROACH

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Poly-ADP-Ribose Polymerase 1 (PARP1) inhibitors (PARPi) are clinically effective for BRCA-deficient cancers but suffer from dose-limiting toxicities and bind all forms of this highly abundant protein. The discovery that the much less abundant Histone PARylation Factor 1 (HPF1) forms a composite active site with PARP1 provides a unique opportunity to specifically target DDR-specific PARP1 activity, potentially reducing off-target effects. However, the structural determinants driving selectivity for this composite site remain poorly understood. To elucidate the mechanism of PARP1-HPF1 selective inhibition, we employed an integrated computational and biochemical strategy. We generated a chimeric computational model of the PARP1 catalytic domain (incorporating both HD and ART subdomains) in complex with HPF1. All-atom molecular dynamics (MD) simulations in aqueous biological conditions validated the model's stability and characterized the relaxed conformational ensemble of the PARP1-HPF1 complex. Leveraging a curated library of PARPi from our lab, we identified a series of structurally related analogs with distinct inhibitory profiles. Notably, we isolated ligand pairs with minimal structural differences (e.g., single-atom substitutions) that showed drastic differences in selectivity for inhibiting the PARP1-HPF1 complex versus PARP1 alone. We are investigating the structural basis of this divergence by docking them into the relaxed hybrid model, followed by MD simulations and comparative trajectory analysis. Our approach aims to identify key protein-ligand interactions that are crucial for modulating the complex's stability and capable of inducing conformational changes at the binding and protein-protein interface sites. Our findings provide insights into the structural rationale for the design of next-generation, high-precision PARP inhibitors.

ALLOSTERIC ACTIVATION OF PARP2 SELF-PARYLATION BY SUMO CONSTRAINS DNA BREAK-DEPENDENT CATALYTIC FUNCTION

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Poly(ADP-ribose) polymerase 2 (PARP2) is a key player in sensing DNA breaks and initiating DNA damage repair by catalyzing the transfer of ADP-ribose units from NAD^+ to target proteins, a process known as Poly(ADP-ribosyl)ation (PARylation). Post-translational modifications (PTMs) such as phosphorylation, ubiquitylation, SUMOylation, and PARylation are intricately linked to the DNA damage response (DDR) and repair. However, it is often overlooked that physical interactions between these enzymes and PTMs lead to DNA damage detection, DDR, and DNA repair. SUMOylation plays a vital role in DDR and DNA repair through covalent modification and non-covalent interactions. Here, we report new insight that Small ubiquitin like modifier (SUMO) binds with human PARP2 through non-covalent interactions, predominantly mediated by the N-terminal region (NTR) of PARP2. Surprisingly, SUMO stimulated PARP2 self-PARylation activity but hampered the DNA-dependent stimulation. Further competition binding studies suggest that SUMO binding promotes DNA release from PARP2. Altogether, our work uncovers a novel mechanism of SUMO-mediated allosteric regulation of PARP2 function, providing new insights into the possible interplay between SUMOylation and PARylation in DDR and DNA repair.

SIRT6 MONO-ADP-RIBOSYLATES TARGET PROTEINS IN A DNA AND POLYHISTIDINE DEPENDENT MANNER

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Sirtuins are the NAD^+-dependent class III lysine deacylases (KDACs). Members of this family have been linked to longevity and a wide array of different diseases, motivating the pursuit of sirtuin modulator compounds. Sirtuin 6 (SIRT6) is a primarily nuclear KDAC that deacetylates histones to facilitate gene repression. In addition to this canonical posttranslational modification "eraser" function, SIRT6 can use NAD^+ instead to "write" mono-ADP-ribosylation (mARylation) on target proteins. SIRT6 mARylation has been less investigated than its KDAC activity but has been associated with DNA damage and PARP1 activity. SIRT6 mARylation has been difficult to study in a cellular context due to not understanding the precise cellular mechanisms that it occurs. In addition, there are only a few known SIRT6 targets, and there remains ambiguity due to other ADP-ribosyltransferaces. We have shown that dsDNA stimulates SIRT6 to mARylate both known and novel proteins containing polyhistidine tracks and this is dependent on the presence of the polyhistidine tack as well. Our investigations have still been elusive in understanding the role of the target proteins polyhistidine tracks. We are continuing to probe mechanistic insight into the SIRT6 and polyhistidine relationship. Recently, polyhistidine tracks have been shown to induce liquid liquid phase separation, including the known SIRT6 target (by ourselves and others) YY1. We are also working to elucidate the precise SIRT6 mARylation acceptor residue. This work provides important context for SIRT6 mARylation activity, in contrast to its KDAC activity, and generates a list of new potential SIRT6 mARylation substrates based on the polyhistidine motif.

MECHANISTIC INSIGHT OF DYSREGULATED DNA DAMAGE REPAIR AND PARYLATION IN HUNTINGTON DISEASE

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Aberrant DNA damage repair and poly(ADP-ribosyl)ation (PARylation) have been implicated in the pathogenesis of neurodegenerative disorders, including Huntington disease (HD). HD is an autosomal dominant neurodegenerative disorder caused by the expansion of the CAG repeat in the huntingtin gene. Multiple genome-wide association studies have implicated DNA damage repair mechanisms as a major modifier of disease. Others have also shown elevated levels of DNA damage and dysregulated PAR signaling in HD and implicated a role of the huntingtin protein in DNA damage repair. Here, we characterized a potential mechanism of HD pathology through hypoPARylation and the hypoactivation of XRCC1. In patient-derived fibroblast cell lines, we investigated the recruitment of PARP1 and the formation of PAR chains at DNA damage sites. PARP1 recruitment and PAR formation were elevated in homozygous HD cells compared to wild-type cells, while levels of PAR formation were significantly lower in heterozygous HD cells and in control lines with huntingtin knockdown, implicating a regulatory role of wild-type huntingtin in PARP1 recruitment and PAR formation. Compared to wild-type cells, HD cells also had lower energy levels under basal conditions and under oxidative stress and HD cells had higher susceptibility to genotoxic stress, indicating potential impairments in the DNA damage response. XRCC1 is an important scaffolding protein involved in single-stranded break repair and it is regulated by PARylation. Under basal conditions and in response to oxidative stress, XRCC1 and phospho-XRCC1 levels were also reduced in HD. The unrepaired single-stranded breaks coupled with energy deficiency could result in the higher susceptibility to genotoxic stressors seen in HD, leading to neurodegeneration. Collectively, these results characterize the impact of the dysregulated DNA damage repair response in HD and implicate the hypoactivation of XRCC1 in HD pathology.

POLY(ADP)-RIBOSE POLYMER INDUCES LENGTH DEPENDENT PHASE SEPARATION OF ALS/FTD-RELATED C9ORF72 ARGININE-RICH DIPEPTIDE REPEATS AT SUB-STOICHIOMETRIC CONCENTRATIONS

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C9orf72 hexanucleotide repeat expansion is the most common cause of familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Elevated PAR levels are observed in the C9 ALS/FTD patients, indicating a potential rise in PAR concentration or longer PAR chains in the diseased condition. The arginine-rich dipeptide repeats (R-DPRs) translated from C9orf72 hexanucleotide expansions are shown to manifest disease symptoms only above a critical number of repeats, indicating a length dependent effect governing disease onset. Recent research has shown that loss of PAR through PARPi suppresses neurodegeneration in C9ALS/FTD fly model.PAR interacts with R-DPR to induce phase separation, but the molecular basis of assembly remains unresolved. In this study, we show that R-DPR expression in cultured cells increases PAR level & co-localizes with PAR in the nucleolus. Our in-vitro LLPS assay and DLS measurements demonstrate that sub-micron sized species (<1 μm) termed nanoclusters are triggered by PAR. The formation of nanoclusters is rate-limited by the concentration of PAR. By modulating the PAR level, we can shift from nanoclusters to micron-sized phase separated assemblies. By increasing the length of the PAR and R-DPR, we observe that the phase separation occurs at a much lower concentration, suggesting that the polymer length could play a role in determining the robustness of the phase separation process. Interestingly, the length of R-DPR and PAR also determines its dynamicity in the condensates. Using FRAP, we found that GR peptide length has a pronounced impact on PAR dynamics within the condensates, whereas PAR length itself has a comparatively modest effect. Condensates formed with shorter GR peptides and shorter PAR chains are highly dynamic, exhibiting rapid molecular exchange with the surrounding environment. In contrast, increasing the length of either GR or PAR leads to the formation of a more solid-like, immobile fraction that slows or even abolishes molecular exchange, a behavior reminiscent of aggregation observed in neurodegenerative conditions. Such alterations in condensate dynamics are likely to compromise nucleolar function, which is a well-established hallmark of neurodegenerative disease. Our findings strongly suggest that both the length and the concentration of PAR play a pivotal role in reducing the kinetic barrier for association and in modulating the dynamics of the local molecular environment. Together, these effects implicate a possible role of PAR in accelerating the aggregate formation in the diseased condition.

HOW DO POLYAMINES AFFECT DNA DAMAGE REPAIR CONDENSATES?

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When a DNA break occurs, protein complexes involved in DNA damage repair are recruited to the break site forming liquid-like compartments. PARP1 (poly-ADP-ribose polymerase 1) plays a critical role in sensing DNA damage and initiating condensation. PARP1 activates the synthesis of poly(ADP-ribose) chains, recruiting DNA repair proteins to the lesion. Although PAR chains are required for condensation, PARylation has been shown to destabilize the condensates due to repulsive forces between negatively-charged groups. Thus, positively charged molecules including Mg2+, Ca2+, and polyamines have been proposed to stabilize assemblies of PARylated PARP1. The polyamines putrescine, spermidine, and spermine are polycationic metabolites present at high concentrations in eukaryotic cells. Polyamines have been implicated in many biological processes, in part due to their ability to form electrostatic interactions with nucleic acids and protein acidic domains. This interaction can alter chromatin accessibility and transcriptional efficiency in vitro, but the cell biological function of polyamines remains poorly understood. Given their electrostatic properties, small diffusible size, and high concentration in cells, we proposed that polyamines increase the stability of DNA repair condensates. Here we show that polyamines are localized to the nucleus, and that following a microirradiation-induced DNA break, recruitment of PARP1 and the DNA damage response protein XRCC1 require high levels of polyamines to efficiently repair sites of damage. Ongoing work will explore the dynamics of polyamine localization to sites of DNA damage, the effects of polyamine pathway perturbation on replication stress and DNA damage repair and the main targets of polyamine in the context of DNA dynamics.

RECQL4 INTERACTS WITH POLY(ADP-RIBOSE) IN THE ABSENCE OF EXOGENOUS DNA DAMAGE AND ITS LOSS SENSITIZES CELLS TO THE NEXT-GENERATION PARPi SARUPARIB

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RECQL4 is a member of the highly conserved family of RecQ DNA helicases, which is encoded by the *RECQL4* gene located at genomic locus 8q24.3. Loss of RECQL4 function is associated with three rare genetic disorders: Rothmund-Thomson Syndrome type II (RTS type II), Baller-Gerold Syndrome (BGS), and RAPADILLINO. All three disorders predispose patients to premature age-related diseases including an array of cancers. Like other RecQ helicases, RECQL4 has been shown to be critical for metabolic processes to facilitate genome stability including roles in HR, NHEJ, Alt-NHEJ, replication initiation, mitochondrial genome maintenance, and telomere stability. Recent evidence suggests that some of these processes, including HR and NHEJ, may also be dependent on interaction with PARP1 and ADP-ribosylation signaling. While most recent studies have focused on RECQL4 interaction with poly(ADP-ribose) (PAR) following DNA damage, we sought to identify whether RECQL4 interacts with PAR in the absence of exogenous DNA damage. Here, we find constitutive binding of RECQL4 to PAR and explore possible functional and binding mechanisms. It has also been hypothesized, based on RECQL4's critical role in early-stage DNA double-strand break repair (DSBR), that RECQL4-deficient cells may display synthetic lethality to PARP inhibitors like BRCA1/2-deficient cells. Here, utilizing both short- and long-term viability assays, we show that CRISPR *RECQL4* knockout HAP1 cells display a significant increase in sensitivity to the FDA-approved PARP inhibitors olaparib and talazoparib. Moreover, we also identify that loss of RECQL4 sensitizes cells to the next-generation highly selective PARP1-specific inhibitor saruparib, which is currently in late-stage Phase III clinical development. Together, our data suggests that RECQL4 is involved in ADP-ribosylation signaling even in the absence of exogenous DNA damage and modulates the response to PARPi treatment. Future work will focus on whether *RECQL4* synthetic lethality with PARPi's is mediated through RECQL4 functions outside of DSBR to identify additional potential targetable genetic vulnerabilities.

EXPLORING PARP12 AS A MODULATOR OF ER-POSITIVE BREAST CANCER AND ENDOCRINE SIGNALING

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PARP12, a mono-ADP-ribosyltransferase of the PARP family originally described for its roles in antiviral defense, stress response, and intracellular membrane trafficking, is emerging as a modulator of signaling pathways driving estrogen receptor–positive (ER^+) breast cancer. We previously showed that PARP12 mono-ADP-ribosylates the kinase AKT, thereby promoting AKT activation and survival of ER^+ breast cancer cells. Consistently, PARP12 loss increases DNA damage and apoptosis, highlighting its functional relevance in breast cancer biology and suggesting a role in the DNA damage response.

To further define the molecular consequences of PARP12 depletion, we performed RNA-seq analysis in MCF7 cells following PARP12 knockdown. PARP12 loss resulted in a marked down-regulation of ERα-regulated genes, identifying ERα signaling as a major downstream pathway affected by PARP12 depletion.

Accordingly, PARP12 knockdown reduced ERα protein levels, phosphorylation, and nuclear localization, together with decreased expression of estrogen-responsive genes. Notably, inhibition of AKT with Capivasertib, a selective AKT inhibitor, produced similar effects on ERα signaling, suggesting that PARP12 and AKT share pathway regulating ERα activity.

In line with these transcriptional effects, analysis of histone methylation levels revealed that PARP12 loss decreased methylation of histone H3 at lysine 27, a hallmark of repressive chromatin. This finding suggests that PARP12 may influence the interplay between ERα and chromatin regulators to control estrogen-dependent gene expression.

In parallel, we are investigating the role of PARP12 in tamoxifen-resistant MCF7 cells to assess its contribution to endocrine therapy resistance. These studies aim to determine whether PARP12 supports persistent ERα signaling and therapy escape, complementing our mechanistic analyses in parental cells.

Overall, our data indicate that PARP12 integrates hormone signaling with chromatin-based transcriptional control, thereby shaping ERα-driven proliferation and therapeutic response. Targeting PARP12 may therefore represent a strategy to improve endocrine therapy efficacy in ER^+ breast cancer.

PARYLATION REGULATES DNA REPLICATION THROUGH THE FORMATION OF BIOMOLECULAR CONDENSATES

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The dynamic regulation of poly-ADP-ribose (PAR) at replication forks is critical for DNA replication. While PARG travels with unperturbed forks to limit PAR, its exclusion during replication stress leads to PAR accumulation and stalled DNA replication forks. This direct regulatory role reveals a fundamental replication vulnerability that must be understood as PARG inhibitors enter clinical development. To define how PAR accumulation controls replication, we used iPOND-coupled proteomics to profile the replication fork proteome, complemented by functional assays measuring fork progression and structure. We identified that PAR accumulation recruits FET family proteins (FUS, EWSR1, TAF15) to stabilize biomolecular condensates at forks. These condensates are required to slow fork progression and promote fork reversal. This FET-mediated pathway is necessary for the response to specific replication stresses, including the topoisomerase poison camptothecin and H_2O_2, but not hydroxyurea, and is epistatic with fork reversal factors. Conversely, FET inactivation leads to unrestrained fork progression via RECQ1 and PRIMPOL, causing single-stranded DNA gaps, genome instability, and synthetic lethality with BRCA1 deficiency. We conclude that PARylation orchestrates replication stress responses by triggering the assembly of protective FET protein condensates at replication forks, a mechanism that impacts genome stability and the efficacy of therapies targeting the PARylation pathway.

A NOVEL HSP20-PARP1 AXIS REGULATES DNA DAMAGE REPAIR AND CANCER DRUG RESISTANCE

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Resistance to DNA-damaging chemotherapy and PARP inhibitors remains a major obstacle in cancer treatment, particularly in tumours with proficient DNA repair mechanisms. Identifying novel regulators of DNA repair is therefore critical for overcoming therapeutic resistance. Using proximity labelling proteomics, we identified a previously unrecognised protein complex containing HSP20 and PARP1 that regulates DNA repair in cancer cells. This interaction was validated using co-immunoprecipitation, immunocytochemistry and proximity ligation assays, revealing a strong nuclear association between the two proteins. Peptide array mapping identified the HSP20-PARP1 binding interface and guided the development of a cell-permeable disruptor peptide that selectively blocks this interaction. Functional studies were conducted in human cancer cell lines with differing homologous recombination (HR) status, namely A549 (lung, HR-proficient) and OVCAR8 (ovarian, HR-deficient). Immunocytochemistry showed that disruption of the HSP20-PARP1 complex resulted in a marked reduction in nuclear PARP1 levels. Comet assays revealed impaired DNA repair following disruptor peptide treatment, suggesting a regulatory role for the HSP20-PARP1 axis in maintaining genome integrity. qPCR and immunoblotting showed that disruption of the HSP20-PARP1 interaction suppressed BRCA1 expression in HR-proficient A549 cells, effectively reprogramming them toward an HR-deficient-like state, while exerting minimal impact on HR-deficient OVCAR8 cells. Concurrent reductions in XRCC1 and RAD51 expression further indicate impairment of both base excision repair (BER) and homologous recombination (HR) pathways, highlighting a role for the HSP20-PARP1 axis in coordinating DNA repair pathway choice. Importantly, perturbation of this regulatory axis sensitised both cell lines to PARP inhibitors (e.g. talazoparib) and platinum-based agents (e.g. cisplatin), producing synergistic anti-proliferative effects at substantially reduced drug doses. Taken together, our findings establish the HSP20-PARP1 axis as a key regulator of DNA repair pathway selection, with direct implications for therapeutic resistance. Targeting this axis represents a mechanistically distinct approach to extend DNA repair-targeted therapies beyond classical HR-deficient tumours.

MOLECULAR DETERMINANTS OF PARP1 CATALYTIC ACTIVITY AND SUBSTRATE SELECTION FOR THE REGULATION OF PROADIPOGENIC GENE EXPRESSION

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Adipogenesis, a complex and tightly regulated process that plays a vital role in multiple aspects of human health, is regulated by a carefully orchestrated gene expression program. We have previously shown that ADP-ribosylation (ADPRylation), a posttranslational modification of proteins, regulates multiple steps during the differentiation of preadipocytes to adipocytes. Enzymes in the PARP family catalyze the covalent attachment of ADP-ribose units from NAD^+ on specific amino acid acceptors in proteins. PARP1-mediated, site-specific ADP-ribosylation on substrate proteins controls multiple components of the proadipogenic gene regulatory machinery, including histone H2B and the transcription factor C/EBPβ. Our recent studies have revealed that small nucleolar RNAs (snoRNAs) can activate PARP1 catalytic activity in a DNA damage-independent manner and that snoRNA-activated PARP1 ADPRylates substrate proteins in a site-specific manner to control adipogenesis. Using an NAD^+ analog-sensitive PARP1 approach with a "clickable" NAD^+ analog and copper-catalyzed azide-alkyne cycloaddition ("click chemistry") developed in our lab, we identified PARP1 substrate proteins preferentially ADPRylated by snoRNA-activated PARP1, including the basic region/leucine zipper (bZIP) transcription factor MafG. These results suggest that snoRNA-activated PARP1 can regulate gene expression through site-specific ADPRylation of MafG. We are currently determining how site-specific ADPRylation of MafG by snoRNA-activated PARP1 regulates adipogenesis. Our results suggest that MafG acts downstream of C/EBPβ to regulate proadipogenic gene expression. Collectively, these studies will link snoRNA-mediated PARP1 activation and site-specific ADPRylation of key substrate transcription factors to the functions of PARP1 in the regulation of adipogenesis.

NOVEL BIOCHEMICAL TOOLS TO STUDY HISTONE ADP RIBOSYLATION SIGNALING

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Histone ADP-ribosylation (ADPr) is a post-translational modification (PTM) involved in diverse cellular processes. At DNA damage sites, PARP1/HPF1 complex modifies multiple histones, relaxing chromatin and facilitating access for repair factors. The mechanistic interrogation of such key signaling events heavily relies on detecting and distinguishing various forms of chromatin ADPr with residue- and state- specific antibodies and/or reader proteins. However, there is a dearth of reagents validated to physiologically relevant substrates, or robust biochemical and cellular assays that leverage these tools.

To address this, we have employed chemoenzymatic labeling and native chemical ligation to produce a collection of fully PTM defined nucleosomes (dNucs) with biologically relevant ADPr moieties (mono-, di-, or tri-) at specific serine residues on each of the four core histones. We then used a high throughput, multiplexed platform (Captify® Luminex) to interrogate the capability (specificity and efficiency) of commercially available antibodies and >50 potential ADPr reader domains (the Queries) towards our nucleosomal ADPr panel (the Targets). As with other PTM classes, the highly charged nucleosome has a dramatic impact on target engagement, highlighting the importance of using physiologically relevant substrates to derive meaningful data. The dataset reveals the distinct ADPr preference of several reader domains, and highlights an understudied nucleosomal mark with potential biological relevance for future investigations. These new insights are now being employed in cellular assays to interrogate the chromatin ADPr landscape in response to DNA damage and other disease states. Through this comprehensive resource, we demonstrate how nucleosome-based approaches can power the study of chromatin ADPr and its pathways.

PARP2 MODULATES SEBOCYTE LIPID METABOLISM AND TURNOVER

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PARP2 is increasingly recognized as a regulator of lipid metabolism. Deletion of PARP2 in mice disrupts lipid storage in white adipose tissue, and we previously showed that loss of PARP2 enhances mitochondrial biogenesis, lipid oxidation and cholesterol biosynthesis in liver and skeletal muscle. Genome-wide association data further link PARP2 variants to circulating lipid traits, supporting a broader role in systemic lipid homeostasis.
Sebaceous glands contribute to the maintenance of the epidermal barrier by secreting lipid-rich sebum, but the involvement of PARP2 in sebocyte biology is unknown. Here, PARP2 was deleted in SZ95 human sebocytes and transcriptomic and lipidomic changes were characterized. RNA-seq revealed differential expression of more than 500 genes enriched in lipid metabolic processes. Principal Component Analyses of lipidomic data showed clear separation between control and PARP2-deficient sebocytes. The most prominent alterations occurred in polar lipid classes, including cardiolipin, phosphatidylethanolamine and glycosphingolipids, suggesting mitochondrial membrane remodeling and enhanced autophagosome formation in PARP2-deleted cells. Consistently, PARP2 knock-out sebocytes displayed increased autophagic activity and elevated cell death. Overall, these data indicate that PARP2 influences sebocyte physiology. PARP2 may modulate sebocyte differentiation and sebaceous gland integrity and may have a role in lipid-driven skin pathologies, a possibility that merits further investigation.

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AKT-DEPENDENT ACTIVATION OF PARP1 REQUIRES NUCLEAR EPRS1

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Glutamyl-prolyl-tRNA synthetase (EPRS1) is a bifunctional aminoacyl-tRNA synthetase (aaRS) essential for the interpretation of the genetic code. Here, we show that EPRS1 is present in both the cytoplasm and the nucleus of breast cancer cells with constitutively low PTEN expression. EPRS1 is primarily cytosolic in PTEN-expressing cells, but chemical or genetic inhibition of PTEN, DNA damage, or other chemical/stress-mediated activation of its target, AKT, induces EPRS1 nuclear localization. Nuclear EPRS1 interacts with poly(ADP)-ribose polymerase 1 (PARP1). EPRS1 is a critical regulator of PARP1 activity, as shown by markedly reduced ADP-ribosylation in EPRS1 knockdown cells. Moreover, EPRS1 and PARP1 knockdown comparably alter the expression of multiple tumor-related genes, inhibit DNA-damage repair, and reduce tumor cell survival in breast cancer cells. In the presence of an EPRS1 knockdown, the potency of Talazoparib was increased 3-fold. In vivo, we found via a limiting dilution assay, a 5-fold reduction in tumor-initiating cells with an EPRS1 knockdown compared to the control and a 50% reduction in tumor size. Loss of EPRS1 reduces global (ADP)-ribosylation (ADPr), including PARP-1 auto-activation and H3S10 ADPr. EPRS1-mediated regulation of PARP1 activity provides a new mechanistic link between AKT activation and PARP1 activation, and cell survival and tumor growth. Targeting this non-canonical activity of nuclear EPRS, without inhibiting canonical tRNA ligase activity, provides a new clinically relevant approach, with potential to supplement existing PARP1 inhibitor treatments.

PARP1 AT THE CROSSROAD OF GENOTOXIC AND INFLAMMATORY RESPONSE

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Poly(ADP-ribose) polymerase 1 (PARP1), the founding member of the PARP superfamily, conducts the majority of poly-ADP-ribosylation (PARylation). It is well known that PARP1 is involved in DNA damage repair; yet it also plays an important role in the NF-κB-mediated inflammatory response. However, it remains controversial how PARP1 participates in the NF-κB pathway. To dissect the specific function of PARP1's activity and scaffold in both above, we generated hypo-PARylation mutant mice and cells carrying a PARP1-D993A point mutation (which targets the catalytic domain) and PARP1-K548R mutation (which compromises PARP1 UFMylation), both of which exhibit impaired PARylation activity. We found that the PARP1-D993A mutation compromised DNA damage repair, indicating that PAR homeostasis, rather than PARP1 itself, protects cells and organisms from acute DNA damage. However, in response to LPS, bone marrow-derived macrophages (BMDMs) of PARP1-D993A mice showed a normal activation of NF-κB, suggesting that PARP1's protein scaffold, but not its activity, is important for the NF-κB signaling. Interestingly, the UFMylation mutant PARP1-K548R, which also impairs DNA repair, rendered BMDMs a defective p65 (a NF-κB subunit) recruitment to chromatin, resulting in a low expression of inflammatory cytokines in response to LPS, indicating that PARP1-K548R scaffold is insufficient for the inflammatory response. Altogether, PARP1 activity is essential for DNA repair yet dispensable for inflammation; PARP1 protein scaffold requires proper post-translational modification, i.e., UFMylation, for both DNA repair and inflammation.

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CODE OF CONDUCT FOR ALL PARTICIPANTS IN CSHL MEETINGS

Cold Spring Harbor Laboratory (CSHL or the Laboratory) is dedicated to pursuing its twin missions of research and education in the biological sciences. The Laboratory is committed to fostering a working environment that encourages and supports unfettered scientific inquiry and the free and open exchange of ideas that are the hallmarks of academic freedom. To this end, the Laboratory aims to maintain a safe and respectful environment that is free from harassment and discrimination for all attendees of our meetings and courses as well as associated support staff, in accordance with federal, state and local laws.

Consistent with the Laboratory's missions, commitments and policies, the purpose of this Code is to set forth expectations for the professional conduct of all individuals participating in the Laboratory's meetings program, both in person and virtually, including organizers, session chairs, invited speakers, presenters, attendees and sponsors. This Code's prohibition against discrimination and harassment is consistent with the Laboratory's internal policies governing conduct by its own faculty, trainees, students and employees.

By registering for and attending a CSHL meeting, either in person or virtually, participants agree to:

1. Treat fellow meeting participants and CSHL staff with respect, civility and fairness, without bias based on sex, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, nationality or national origin, citizenship status, disability status, veteran status, marital or partnership status, age, genetic information, or any other criteria prohibited under applicable federal, state or local law.

2. Use all CSHL facilities, equipment, computers, supplies and resources responsibly and appropriately if attending in person, as you would at your home institution.

3. Abide by the CSHL Meeting Alcohol Policy (*see below)*.

Similarly, meeting participants agree to refrain from:

1. Harassment and discrimination, either in person or online, in violation of Laboratory policy based on actual or perceived sex, pregnancy status, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, creed, nationality or national origin, immigration or citizenship status, mental or physical disability status, veteran status, military status, marital or partnership status, marital or partnership status, familial status, caregiver status, age, genetic information, status as a victim of domestic violence, sexual violence, or stalking, sexual reproductive health decisions, or any other criteria prohibited under applicable federal, state or local law.

2. Sexual harassment or misconduct.

3. Disrespectful, uncivil and/or unprofessional interpersonal behavior, either in person or online, that interferes with the working and learning environment.

4. Misappropriation of Laboratory property or excessive personal use of resources, if attending in person.

BREACHES OR VIOLATIONS OF THE CODE OF CONDUCT

Cold Spring Harbor Laboratory aims to maintain in-person and virtual conference environments that accord with the principles and expectations outlined in this Code of Conduct. Meeting organizers are tasked with providing leadership during each meeting, and may be approached informally about any breach or violation. Breaches or violations should also be reported to program leadership in person or by email:

- Dr. David Stewart, Grace Auditorium Room 204, 516-367-8801 or x8801 from a campus phone, stewart@cshl.edu
- Dr. Charla Lambert, Hershey Laboratory Room 214, 516-367-5058 or x5058 from a campus phone, clambert@cshl.edu

Reports may be submitted by those who experience harassment or discrimination as well as by those who witness violations of the behavior laid out in this Code.

The Laboratory will act as needed to resolve the matter, up to and including immediate expulsion of the offending participant(s) from the meeting, dismissal from the Laboratory, and exclusion from future academic events offered by CSHL.

If you have questions or concerns, you can contact the meeting organizers, CSHL staff.

For meetings and courses funded by NIH awards:

Participants may contact the Health & Human Services Office for Civil Rights (OCR). See this page for information on filing a civil rights complaint with the OCR; filing a complaint with CSHL is not required before filing a complaint with OCR, and seeking assistance from CSHL in no way prohibits filing complaints with OCR. You may also notify NIH directly about sexual harassment, discrimination, and other forms of inappropriate conduct at NIH-supported events.

For meetings and courses funded by NSF awards:

Participants may file a complaint with the NSF. See this page for information on how to file a complaint with the NSF.

Law Enforcement Reporting:

- For on-campus incidents, reports to law enforcement can be made to the Security Department at 516-367-5555 or x5555 from a campus phone.
- For off-campus incidents, report to the local department where the incident occurred.

In an emergency, dial 911.

DEFINITIONS AND EXAMPLES

Uncivil/disrespectful behavior is not limited to but may take the following forms:

- Shouting, personal attacks or insults, throwing objects, and/or sustained disruption of talks or other meeting-related events

Harassment is any unwelcome verbal, visual, written, or physical conduct that occurs with the purpose or effect of creating an intimidating, hostile, degrading, humiliating, or offensive environment or unreasonably interferes with an individual's work performance. Harassment is not limited to but may take the following forms:

- Threatening, stalking, bullying, demeaning, coercive, or hostile acts that may have real or implied threats of physical, professional, or financial harm
- Signs, graphics, photographs, videos, gestures, jokes, pranks, epithets, slurs, or stereotypes that comment on a person's sex, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, nationality or national origin, citizenship status, disability status, veteran status, marital or partnership status, age, genetic information, or physical appearance

Sexual Harassment includes harassment on the basis of sex, sexual orientation, self-identified or perceived sex, gender expression, gender identity, and the status of being transgender. Sexual harassment is not limited to sexual contact, touching, or expressions of a sexually suggestive nature. Sexual harassment includes all forms of gender discrimination including gender role stereotyping and treating employees differently because of their gender. *Sexual misconduct* is not limited to but may take the following forms:

- Unwelcome and uninvited attention, physical contact, or inappropriate touching
- Groping or sexual assault
- Use of sexual imagery, objects, gestures, or jokes in public spaces or presentations
- Any other verbal or physical contact of a sexual nature when such conduct creates a hostile environment, prevents an individual from fulfilling their professional responsibilities at the meeting, or is made a condition of employment or compensation either implicitly or explicitly

MEETING ALCOHOL POLICY

Consumption of alcoholic beverages is not permitted in CSHL's public areas other than at designated social events (wine and cheese reception, picnic, banquet, etc.), in the Blackford Bar, or under the supervision of a licensed CSHL bartender.

No provision of alcohol by meeting sponsors is permitted unless arranged through CSHL.

Meeting participants consuming alcohol are expected to drink only in moderation at all times during the meeting.

Excessive promotion of a drinking culture at any meeting is not acceptable or tolerated by the Laboratory. No meeting participant should feel pressured or obliged to consume alcohol at any meeting-related event or activity.

VISITOR INFORMATION

EMERGENCY (to dial outside line, press 3+1+number)	
CSHL Security	**516-367-8870 (x8870 from house phone)**
CSHL Emergency	**516-367-5555 (x5555 from house phone)**
Local Police / Fire	**911**
Poison Control	**(3) 911**

CSHL SightMD Center for Health and Wellness ***(call for appointment)*** Dolan Hall, East Wing, Room 111 **cshlwellness@northwell.edu**	**516-422-4422** **x4422 from house phone**
Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2000**
Dentists Dr. William Berg Dr. Robert Zeman	 **631-271-2310** **631-271-8090**
Drugs - 24 hours, 7 days Rite-Aid 391 W. Main Street, Huntington	**631-549-9400**

GENERAL INFORMATION

Meetings & Courses Main Office

Hours during meetings: M-F 9am – 9pm, Sat 8:30am – 1pm
After hours – See information on front desk counter
For assistance, call Security at 516-367-8870
(x8870 from house phone)

Dining, Bar

Blackford Dining Hall (main level):
Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
Blackford Bar (lower level): 5:00 p.m. until late

House Phones

Grace Auditorium, upper / lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

Books, Gifts, Snacks, Clothing

CSHL Bookstore and Gift Shop
516-367-8837 (hours posted on door)
Grace Auditorium, lower level.

Computers, E-mail, Internet access

Grace Auditorium
Upper level: E-mail and printing in the business center area
WiFi Access: GUEST (no password)

Announcements, Message Board Mail, ATM, Travel info

Grace Auditorium, lower level

Russell Fitness Center
Dolan Hall, east wing, lower level
PIN#: **(On your registration envelope)**

Laundry Machines
Dolan Hall, lower level

Photocopiers, Journals, Periodicals, Books
CSHL Main Library
Open 24 hours (with PIN# or CSHL ID)
Staff Hours: 9:00 am – 9:00 pm
Use PIN# (On your registration envelope) to enter Library
See Library staff for photocopier code.
Library room reservations (hourly) available on request between 9:00 am – 9:00 pm

Swimming, Tennis, Jogging, Hiking
June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m.
Two tennis courts open daily.

Local Interest

Fish Hatchery	631-692-6758
Sagamore Hill	516-922-4788
Whaling Museum	631-367-3418
Heckscher Museum	631-351-3250
CSHL DNA Learning Center	x 5170

New York City
Helpful tip -
Take CSHL Shuttle OR Uber/Lyft/Taxi to Syosset Train Station
Long Island Railroad to Penn Station
Train ride about one hour.

TRANSPORTATION

Limo, Taxi

Syosset Limousine	516-364-9681
Executive Limo Service	516-826-8172
Limos Long Island	516-400-3364
Syosset Taxi	516-921-2141
Orange & White Taxi	631-271-3600
Uber / Lyft	

Trains

Long Island Rail Road	718-217-LIRR (5477)
Amtrak	800-872-7245
MetroNorth	877-690-5114
New Jersey Transit	973-275-5555

CSHL's Green Campus

Cold Spring Harbor Laboratory is pledged to operate in an environmentally responsible fashion wherever possible. In the past, we have removed underground oil tanks, remediated asbestos in historic buildings, and taken substantial measures to ensure the pristine quality of the waters of the harbor. Water used for irrigation comes from natural springs and wells on the property itself. Lawns, trees, and planting beds are managed organically whenever possible. And trees are planted to replace those felled for construction projects.

Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a "single stream waste management" system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory's efforts can be improved. This book, for example, is printed on recycled paper.

Cold Spring Harbor Laboratory Bookstore & Gift Shop

Main campus, lower level of Grace Auditorium

Store Hours

Did you miss your chance to shop at the CSHL Bookstore and Gift Shop during the conference?

No problem! Visit our Online Bookstore and Gift Shop.

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cshlvirtualstore.com

CSHL Campus Map

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