BIOGRAPHICAL SKETCH

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NAME: John M. Pascal

eRA COMMONS USER NAME: jmp007

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Clemson University, Clemson, SC	B.S.	05/1995	Biochemistry
University of Texas at Austin	Ph.D.	12/2000	Biochemistry
University of Texas at Austin	Postdoc	12/2001	Biochemistry & Structural Biology
Harvard Medical School, Boston, MA	Postdoc	06/2006	Biochemistry & Structural Biology

A. Personal Statement

My laboratory has a broad interest in understanding the cellular response to DNA damage, and the nucleic acid-binding proteins that correct DNA damage or mediate cell signaling and coordination of the damage response. Our research approach uses biochemical and cell biological analyses coupled with the tools of structural biology and biophysical analysis to provide important biological insights at the molecular level. A focus of our work is the poly(ADP-ribose) polymerase, or PARP, family of enzymes. PARPs create the ADP-ribose posttranslational modification to regulate virtually every aspect of human cell biology. Much of our research has focused on PARP1, the major enzyme responsible for poly(ADP-ribose) production in cells. PARP1 is a key player in the cellular response to DNA damage, has equally important roles in regulating transcription, and impacts cell fate decisions based on the level of PARP1 catalytic activity. My laboratory has been a leader in the structural biology of PARP1, most notably establishing the mode of DNA damage detection and identifying how the multiple domains of PARP1 transmit damage detection to the catalytic domain. Our work has also provided important insights into how PARP1 can be specifically targeted for cancer therapy. We are currently addressing key gaps in our understanding of PARP1 allosteric activation in response to DNA damage and de-activation in response to automodification, and key deficiencies in our understanding of PARP inhibitor mode of action. Based on my specific expertise and training, history of collaborative research, and leadership and motivation, I am fully capable of effectively executing the proposed research program.

1. Zandarashvili L, Langelier MF, Velagapudi UK, Hancock MA, Steffen JD, Billur R, Hannan ZM, Wicks AJ, Krastev DB, Pettitt SJ, Lord CJ, Talele TT, **Pascal JM***, and Black BE*. (2020) Structural basis for allosteric PARP-1 retention on DNA breaks. ***Science*** 368. PMCID: PMC7347020 (*corresponding authors)
2. Langelier MF, Zandarashvili L, Aguiar PM, Black BE*, **Pascal JM***. (2018) NAD(+) analog reveals PARP-1 substrate-blocking mechanism and allosteric communication from catalytic center to DNA-binding domains. ***Nature Communincations*** 9: 844. PMCID: PMC5829251. (*corresponding authors)
3. Dawicki-McKenna JM, Langelier MF, DeNizio JE, Riccio AA, Cao CD, Karch KR, McCauley M, Steffen JD, Black BE*, **Pascal JM*** (2015) PARP-1 activation requires local unfolding of an autoinhibitory domain. ***Molecular Cell***. 60, 755-68. PMCID: PMC4712911. (*corresponding authors)
4. Langelier MF, Planck JL, Roy S and **Pascal JM*** (2012) Structural basis for DNA-dependent poly(ADP-ribosyl)ation by human PARP-1. ***Science*** 336, 728-32. PMCID: PMC3532513 (*corresponding author)

B. Positions and Honors

Academic Positions:

2000-2001 Postdoctoral Fellow, University of Texas at Austin, Austin, TX
2002-2006 Postdoctoral Fellow, Harvard Medical School, Boston, MA
2006-2012 Assistant Professor of Biochemistry & Molecular Biology, Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA
2012-2015 Associate Professor of Biochemistry & Molecular Biology, Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA
2015-2018 Associate Professor of Biochemistry & Molecular Medicine, University of Montreal, Montreal, Quebec, Canada
2018- Professor of Biochemistry & Molecular Medicine, University of Montreal, Montreal, Quebec, Canada

Honors:

1994 Howard Hughes Undergraduate Research Fellow
1995 Honors graduate in Biochemistry, Clemson University
1998 Robert E. Eakin Centennial Award, University of Texas
1999 Dorothy Banks Charitable Trust Fellowship, University of Texas
2000 College of Natural Sciences Fellowship, University of Texas
2004 Ruth L. Kirschstein National Research Service Award, NIH
2009 American Cancer Society Research Scholar
2012 Sidney Kimmel Cancer Center Basic Science Research Award
2014 Jefferson Medical College Early Career Investigator Award
2016 Senior Research Award – Fonds de recherche du Québec, santé (FRQ-S)

C. Contributions to Science

1. *PARP activation during the cellular response to DNA damage.* My laboratory has provided new structural and biochemical insights into the PARP enzymes that are activated during the DNA damage response: PARP-1, PARP-2, PARP-3. For PARP-1, we discovered the presence of a key regulatory zinc-binding domain with a unique protein fold, we were the first to define how PARP-1 detects DNA damage, and we determined the molecular mechanism for coupling DNA damage detection to the catalytic domain (key references listed in Section A). The PARP-1 crystal structure in complex with DNA damage has also provided a platform for understanding inhibitor interactions with the catalytic domain in the presence of regulatory domains, and has suggested new strategies for targeting specifically targeting PARP-1 for cancer therapy (Steffen et al., 2014 – listed in Section A). We have established that PARP-2 and PARP-3 are responsive to particular types of DNA repair intermediates, and have determined domain requirements for localization to sites of DNA damage, and this has provided new insights into PARP involvement at specific stages of DNA repair pathways.

a. Eustermann S, Wu WF, Langelier MF, Yang JC, Easton LE, Riccio AA, **Pascal, JM** Neuhaus, D. (2015) Structural basis of detection and signaling of DNA single-strand breaks by Human PARP-1. ***Molecular Cell*** 60, 742-54. PMCID: PMC4678113.
b. Riccio AA, Cingolani G, **Pascal JM*** (2016) PARP-2 domain requirements for DNA damage-dependent activation and localization to sites of DNA damage. ***Nucleic Acids Research*** 44,1691-702. PMCID: PMC4770219. (*corresponding author)
c. Langelier MF, Riccio AA, **Pascal JM*** (2014) PARP-2 and PARP-3 are selectively activated by 5' phosphorylated DNA breaks through an allosteric regulatory mechanism shared with PARP-1. ***Nucleic Acids Research*** 42, 7762-75. PMCID: PMC4081085 (*corresponding author)
d. Langelier M-F, Planck JL, Roy S and **Pascal JM*** (2011) Crystal structures of poly(ADP-ribose) polymerase-1 (PARP-1) zinc fingers bound to DNA: structural and functional insights into DNA-dependent PARP-1 activity. ***Journal of Biological Chemistry*** 286, 10690-701. PMCID: PMC3060520 (*corresponding author)

2. *PARP family structural biology.* Using x-ray crystallography as a primary tool, my laboratory has provided novel insights into the regulation of PARP family members involved in the DNA damage response, as noted in the previous two sections. We have also studied the regulation of the PARP enzyme known as Tankyrase, which regulates multiple cell signaling pathways. Our work has focused on the regulatory domains that are unique to Tankyrase and dictate its cellular functions. We have also worked with Dr. Tanaji Talele to define the binding mode of novel PARP inhibitor compounds.

a. Riccio AA, McCauley M, Langelier MF, **Pascal JM***. (2016) Tankyrase Sterile α Motif Domain Polymerization Is Required for Its Role in Wnt Signaling. *Structure*. 24:1573-81.
b. Eisemann T, McCauley M, Langelier MF, Gupta K, Roy S, Van Duyne GD, **Pascal JM***. (2016) Tankyrase-1 Ankyrin Repeats Form an Adaptable Binding Platform for Targets of ADP-Ribose Modification. *Structure*. 24:1679-1692.
c. Eisemann T, Langelier MF, **Pascal JM**. (2019) Structural and functional analysis of parameters governing tankyrase-1 interaction with telomeric repeat-binding factor 1 and GDP-mannose 4,6-dehydratase. *J Biol Chem*. 294: 14574-90.
d. Velagapudi UK, Langelier MF, Delgado-Martin C, Diolaiti ME, Bakker S, Ashworth A, Patel BA, Shao X, **Pascal JM**, Talele TT. (2019) Design and Synthesis of Poly(ADP-ribose) Polymerase Inhibitors: Impact of Adenosine Pocket-Binding Motif Appendage to the 3-Oxo-2,3-dihydrobenzofuran-7-carboxamide on Potency and Selectivity. *J Med Chem*. 62: 5330-5357.

3. *PARP-1 involvement in transcriptional regulation*. PARP-1 has key cellular functions outside of the DNA damage response, most notably as a regulator of gene transcription. Our structural, biochemical, and cell-based analysis of PARP-1 has provided new tools to probe PARP-1 function as co-regulator of androgen receptor-mediated transcription in collaboration with Karen Knudsen's laboratory. Our biochemical and structural work has also provided new insights into the domain requirements for PARP-1 interaction with chromatin. This previous work builds toward our current interest in defining PARP-1 contacts with nucleosomes in order to better define its role in regulating chromatin structure.

a. Visochek L, Grigoryan G, Kalal A, Milshtein-Parush H, Gazit N, Slutsky I, Yeheskel A, Shainberg A, Castiel A, Seger R, Langelier MF, Dantzer F, **Pascal JM**, Segal M, Cohen-Armon M (2016) A PARP1-ERK2 synergism is required for the induction of LTP. ***Science Reports*** 6:24950. PMID: 27121568
b. Schiewer MJ, Goodwin JF, Han S, Brenner JC, Augello MA, Dean JL, Liu F, Planck JL, Ravindranathan P, Chinnaiyan AM, McCue P, Gomella LG, Raj GV, Dicker AP, Brody JR, **Pascal JM**, Centenera MM, Butler LM, Tilley WD, Feng FY, Knudsen KE (2012) Dual roles of PARP-1 promote cancer growth and progression. ***Cancer Discovery*** 2, 1134-49. PMID: 22993403
c. Langelier M-F, Ruhl DL, Planck JL, Kraus WL, and **Pascal JM*** (2010) The Zn3 domain of human poly(ADP-ribose) polymerase-1 (PARP-1) functions in both DNA-dependent poly(ADP-ribose) synthesis activity and chromatin compaction. ***Journal of Biological Chemistry*** 285, 18877-87. PMCID: PMC2881810 (*corresponding author)

4. *Mechanisms of DNA strand break repair*. DNA ligases correct breaks in the backbone structure of DNA as the final step of nearly all cellular DNA transactions. I determined the crystal structure of human DNA ligase I in complex with a nicked DNA reaction intermediate, which provided the first view of any ligase enzyme bound to DNA, and provided a framework for developing specific ligase inhibitors as biological probes and potential therapeutics. DNA repair pathways involve multiple enzymes acting sequentially to modify repair intermediates, and the DNA sliding clamp PCNA is master regulator of DNA replication and repair. My research with repair proteins from *Sulfolobus Solfataricus* provided new insights into the molecular mechanism of PCNA coordinating ligation repair, and also provide the first structural view of the assembly of a heterotrimeric PCNA ring.

a. Song W, **Pascal JM**, Ellenberger T, and Tomkinson AE. (2009) The DNA binding domain of human DNA ligase I interacts with both nicked DNA and the DNA sliding clamps, PCNA and hRad9-hRad1-hHus1 (2009) ***DNA Repair*** 8, 912-9. PMCID: PMC2759717
b. **Pascal JM** (2008) DNA and RNA ligases: structural variations and shared mechanisms. ***Current Opinion in Structural Biology*** 18, 96-105.

c. **Pascal JM**, Tsodikov OV, Hura GL, Song W, Cotner EA, Classen S, Tomkinson AE, Tainer JA and Ellenberger T. (2006) A flexible interface between DNA ligase and a heterotrimeric sliding clamp supports conformational switching and efficient ligation of DNA. ***Molecular Cell*** 24, 1-13.
d. **Pascal JM**, O'Brien PJ, Tomkinson AE and Ellenberger T. (2004) Human DNA ligase I completely encircles and partially unwinds nicked DNA. ***Nature*** 432, 473-8.

5. *Structural basis for activation of Ca^{2+}-sensing potassium channels*. Small- and intermediate-conductance potassium channels are activated by Ca^{2+}-bound calmodulin, and they have important roles in regulating membrane excitability. My collaboration with Ji-fang Zhang has provided key structural insights into the conformational plasticity of calmodulin, key structural features that couple calmodulin binding to channel activation, and the functional binding pocket of compounds that target these channels.

a. Zhang M, Meng X-Y, Cui M, **Pascal JM**, Logothetis D, and Zhang J-f (2014) Modulation of PIP2 sensitivity of the CaM-SK channel complex through selective phosphorylation. ***Nature Chemical Biology*** 10, 753-9.
b. Zhang M, **Pascal JM**, and Zhang J-f (2013) Unstructured to structured transition of an intrinsically disordered protein peptide in coupling Ca^{2+}-sensing and SK channel activation. ***PNAS*** 110, 4828-33. PMID: 23487779.
c. Zhang M, **Pascal JM**, Schumann M, Armen RS and Zhang J-f (2012) Identification of the functional binding pocket for compounds targeting small-conductance Ca^{2+}-activated potassium channels. ***Nature Communications*** 3, 1021. PMID: 22929778
d. Zhang M, Abrams C, Wang L, Gizzi A, He L, Lin R, Chen Y, Loll PJ, **Pascal JM**, and Zhang J-f (2012) Structural basis for calmodulin as a dynamic calcium sensor. ***Structure*** 20, 911-23. PMID:22579256

Complete List of Published Work in MyBibliography:
https://pubmed.ncbi.nlm.nih.gov/?term=pascal+jm&sort=date&size=100

D. Research Support

Current Support

NIH/NCI 9/1/21-8/31/26
Structural Cell Biology of DNA Repair Machines
The goal of this multi-investigator, multi-institutional, interdisciplinary Program Project is to structurally and mechanistically define the complex and dynamic network of DNA repair machines that maintain integrity of the genome, together with their coordination with DNA damage responses and with replication, in order to aid prediction and intervention for cancer biology. SBDR Program funding leverages individual laboratory resources and funding by supporting productive collaborative interactions among SBDR Senior Investigators and Cores to efficiently accomplish the proposed goals of the SBDR Program.
Role: Co-investigator (PI: John Tainer)

Canadian Institutes of Health Research 10/1/20-9/30/25
Structural biology and biochemistry of PARP enzyme regulation in the DNA damage response and in cancer therapy
This grant supports crystal structure and cryo-EM analysis of PARP-1, and its interaction with key regulatory factors.
Role: PI

Canadian Institutes of Health Research 4/1/17-3/31/22
Structural Biology of Tankyrase Activity and Regulation in Cell Signaling
This grant supports the structural, biochemical, and cell biological analysis of the PARP enzyme Tankyrase.
Role: PI

Past Support

Natural Sciences and Research Engineering Council of Canada (NSERC) 7/1/15-6/30/20

Structural Biochemistry of Coordinated DNA Damage Repair Pathways

This grant supports the structural and biochemical analysis of DNA repair proteins involved in Okazaki fragment repair (ligase, polymerase, endonuclease, PCNA) from the thermophilic archaeon *Sulfolobus Solfataricus*.

Role: PI

Canadian Institutes of Health Research 7/1/15-6/30/20

Structural Biology of DNA Damage-Dependent PARPs

This grant supports crystal structure analysis of DDR-PARP (PARP-1, PARP-2, and PARP-3) catalytic domains with inhibitors, small-angle x-ray scattering analysis of PARP solution structures, and screening efforts to identify small molecules that specifically inhibit PARP-1 regulatory domain functions.

Role: PI

NIH/NIGMS (#GM087282)

Structural Biochemistry of PARP-1 12/1/10-11/30/17

The goal of this study was to use x-ray crystallography and biochemical analysis to establish the molecular basis for PARP-1 interaction with DNA damage, and to determine the structural basis for PARP-1 coupling of damage detection to catalytic activation. A supplement to this grant supported collaborative work with the group of Ben Black.

University of Montreal/Merck Sharp & Dohme Corporation Fund 7/1/15-6/30/17

Developing Novel Chemical Probes Targeting Allosteric Regulation of PARP-1

The goal of this study is to identify small molecules that will specifically inhibit the function of PARP-1 regulatory domains and thereby serve as biological probes and potentially therapeutic agents.