BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Tilini Wijeratne

eRA COMMONS USER NAME (credential, e.g., agency login): twijerat

POSITION TITLE: Ph.D. Candidate

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
CSU Long Beach, Long Beach CA	B.S.	05/2017	Biochemistry
UC Santa Cruz, Santa Cruz CA	Ph.D.	05/2023	Biochemistry

A. Personal Statement

My long-term research interest is in structural studies of transcription factors that bind chromatin and understanding the molecular mechanisms of how these transcription factors recognize their nucleosome substrates for gene regulation. My research thus far has equipped me with an excellent scientific background and valuable tools to probe bio-molecular structure and function, including mammalian tissue culture, recombinant protein expression, CRISPR/Cas9 genetic engineering, isothermal calorimetry (ITC), NMR spectroscopy, fluorescence polarization spectroscopy, transmission electron microscopy, and x-ray crystallography. As an undergraduate at CSU Long Beach, I spent two years working in the laboratory of Dr. Paul Weers, who is a pioneer in determining the structure and function of exchangeable lipoproteins which are critical in lipid circulation and metabolism. My project focused on characterizing the structural and functional changes of apoliphorin III in its lipid bound state and lipid-free state. Through Dr. Weers' mentorship, I not only gained the laboratory skills, scientific knowledge, and confidence to carry out independent research but I also took advantage of opportunities to apply for fellowships, present my data at the CSU Program for Education and Research in Biotechnology Conference in January 2017 (Santa Clara, CA) and ultimately publish my work as a first author in the journal *Molecular Cell Biochemistry* (April 2019). For the above reasons, my experiences as an undergraduate researcher provided me with a strong foundation in preparation for my PhD studies in biochemistry at UCSC.

My PhD advisor at UCSC, Dr. Seth Rubin, is an internationally recognized figure in the field of structural biology as pertains to cell-cycle regulation. My doctoral research is focused on better understanding how the critical cell-cycle regulating transcription factor B-Myb activates transcription in the S-phase of the cell-cycle. Unlike traditional transcription factors, B-Myb is a "pioneer" transcription factor which means that it does not recognize a sequence specific binding site in the cell-cycle promoters. Chromatin accessibility of pioneer transcription factors like B-Myb and how these TFs induce changes in chromatin prior to the time of transcription activation is poorly understood. Thus, I am specifically interested in understanding the mechanism by which B-Myb alters the inactive chromatin state at cell-cycle dependent promoters to modulate an active chromatin state by elucidating the structural basis for B-Myb's interaction with the nucleosome complex.

However, while my initial attempts to investigate this complex interaction have employed traditional x-ray crystallography methods, Cryo-EM provides an enhanced modality to probe the near-atomic structural details of large macromolecules like nucleosomes and circumvents the need to purify large amounts of purified complexes for crystallization. Furthermore, because nucleosomes have multiple substrate recognition sites for chromatin binding proteins to interact with DNA and/or histones, Cryo-EM allows for better visualization of these modes of interactions in their near-native state, without having to excessively truncate the flexible

regions of B-Myb or the flexible tails of histones which may also be important for the complex assembly. Therefore, obtaining expertise in Cryo-EM through the training opportunity provided by the NCCAT will equip me with the ideal skillset to elucidate the structural mechanism of B-Myb and contribute to an important area of study within the field of epigenetic regulation. Moreover, having completed this invaluable training, I will be well equipped to apply this knowledge once the UCSC Cryo-EM facility opens in March 2019 and to also help other members of the UCSC scientific community in expanding their use of Cryo-EM techniques.

B. Positions and Honors

ACTIVITY/OCCUPATION	DATE (mm/yy)	FIELD	INSTITUTION/ COMPANY	SUPERVISOR/ EMPLOYER
Undergraduate Researcher	08/2015-05/2017	Biochemistry	CSU Long Beach	Paul Weers
Graduate Student Researcher	08/2017-present	Biochemistry	UC Santa Cruz	Seth Rubin

C. Contributions to Science

Wijeratne T.U. & Weers P.M.M. *Mol Cell Biochem* (2019) Lipid-bound apoLp-III is less effective in binding to lipopolysaccharides and phosphatidylglycerol vesicles compared to the lipid-free protein. 458: 61. PMID: 31016454

Wijeratne, T. (2017, January). CSU Program for Education and Research in Biotechnology Conference in January 2017 (Santa Clara, CA)

Vorster P, Goetsch P, **Wijeratne, T. U**., Guiley K. Z., Andrejka L, Tripathi S, Larson B. J., Rubin S. M., Strome S, Lipsick J. S. A *bioRxiv.* (2019) Long Lost Key Opens an Ancient Lock: *Drosophila* Myb Causes a Synthetic Multivulval Phenotype in Nematodes**.**

D. Additional Information: Research Support and/or Scholastic Performance

CSULB Student Summer Research Award 2016

BIOGRAPHICAL SKETCH

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NAME: Seth M. Rubin

eRA COMMONS USER NAME: SRUBIN

POSITION TITLE: Professor of Chemistry and Biochemistry

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	Dates	FIELD OF STUDY
Columbia University, NY	B.A.	1998	Chemistry
University of California at Berkeley, CA	Ph.D.	2003	Chemistry
Memorial Sloan-Kettering Cancer Center, NY	Postdoc	2003-06	Biochemistry

A. Personal Statement

My long-term career research goal is to understand molecular mechanisms of cell division and their relationship to disease by elucidating the structure and biochemical function of cell-cycle regulatory proteins and their interactions. Current projects in the laboratory focus on the structure and function of cell-cycle transcription factors, including Rb-E2F and FoxM1, Myb, and the Cyclin dependent kinases (Cdks) that regulate them. We are using the results of our structural and biochemical studies to innovate novel classes of breast cancer therapeutics. To achieve these goals, we have employed a wide array of approaches ranging from structural biology, biochemistry, and cell biology.

My laboratory is eager to use cryo-electron microscopy toward structural analysis of macromolecular assemblies. The project most related to our proposed use of the NCCAT facility focuses on Myb, which activates transcription of genes needed for mitosis. With access to electron microscopy instrumentation, we will determine structures that reveal how Myb binds nucleosomes to activate mitotic gene expression. My laboratory is ideally positioned to execute this project. We made breakthroughs reconstituting Myb-nucleosome complexes for biochemical and structural studies, and we published important insights on the structure of Myb bound to other co-regulators.

A complimentary goal of mine is to train students such as Tilini Wijeratne as research scientists at the undergraduate, graduate, and postdoctoral level. I have worked with over twenty-five undergraduate researchers, ten graduate students, and four postdoctoral fellows. I believe in a training program that is structured yet allows the trainee to develop independence as a research scientist. My approach uses individual development plans (IDPs) to tailor training to the career goals and strengths and weaknesses identified by each trainee with my input. Tilini is an outstanding Ph.D. student beginning her third year of graduate school. She will benefit greatly from this training opportunity at NCCAT, and with her acquired expertise, she will be positioned to help the entire UCSC structural biology community.

a) Marceau A.H., Brison C.M., Nerli S., Arsenault H.E., McShan A.C., Chen E., Lee H.W., Benanti J.A., Sgourakis N.G., Rubin S.M. An order-to-disorder structural switch activates the FoxM1 transcription factor. eLife. 2019 May 28;8 PMID: 31134895 PMCID: PMC6538375

b) Guiley K.Z., Iness A.N., Saini S., Tripathi S., Lipsick J.S., Litovchick L., Rubin S.M. Structural mechanism of Myb-MuvB assembly. Proc Natl Acad Sci USA. 2018 Oct 2;115(40):10016-10021 PMID: 30224471 PMCID: PMC6176624

c) McGrath D.A., Fifield B.-A., Marceau A.H., Tripathi S., Porter L.A., Rubin S.M. Structural basis of divergent Cyclin-dependent kinase activation by Spy1/RINGO proteins. EMBO J. 2017 Jun 30, published on line. PMID: 28666995 PMCID: PMC5441720

d) Liban T.J., Medina E.M. Tripathi S., Sengupta S., Henry R.W., Buchler N.E., Rubin S.M. Conservation and divergence of C-terminal domain structure in the retinoblastoma protein family. Proc Natl Acad Sci USA. 2017 May 9;114(19):4942-4947 PMID: 28439018 PMCID: PMC5441720

B. Positions and Honors

Positions and Employment

2006-2012 Assistant Professor, Department of Chemistry and Biochemistry, University of California at Santa Cruz
2012-present Associate Professor, Department of Chemistry and Biochemistry, University of California at Santa Cruz
2017-present Professor, Department of Chemistry and Biochemistry, University of California at Santa Cruz

Other Experience and Professional Memberships

2002-present Member, American Chemical Society
2016-present Member, DNA Mechanisms in Cancer Study Panel, American Cancer Society
2016-present Member, University of California, Cancer Research Coordinating Committee

Honors and Memberships

1996-1998	National Barry M. Goldwater Scholar
1997	Elected to Phi Beta Kappa
1998-2001	National Science Foundation Predoctoral Fellow
2003-2006	Damon Runyon Cancer Research Foundation Fellowship Award
2008-2012	Pew Scholar in the Biomedical Sciences

C. Contribution to Science

1) Structure-function studies of the Rb protein family: My laboratory has a long-standing commitment to elucidating retinoblastoma protein (Rb) structure and understanding how phosphorylation controls Rb association with its binding partners. Rb negatively regulates the cell cycle by controlling entry into S phase, and the Rb protein pathway is deregulated in most if not all tumors. The best-characterized biochemical function of Rb is its capacity to bind and inhibit E2F transcription factors and recruit other repressive factors to promoters that control expression of cell-cycle genes. Rb interactions with E2F and these other factors are lost upon multisite phosphorylation by Cyclin-dependent kinases. Remarkably, we found that specific phosphorylation events drive different conformational changes in Rb that disrupt distinct protein interaction surfaces. We solved crystal structures of Rb phosphorylated at two sites and determined how they specifically regulate E2F binding. These observations have lead to a novel model in which different phosphorylation events control assembly of Rb into diverse regulatory protein complexes.

a) Liban T.J., Medina E.M. Tripathi S., Sengupta S., Henry R.W., Buchler N.E., Rubin S.M. Conservation and divergence of C-terminal domain structure in the retinoblastoma protein family. Proc Natl Acad Sci USA. 2017 May 9;114(19):4942-4947 PMID: 28439018 PMCID: PMC5441720
b) Liban T.L., Thwaites M.J., Dick F.A., Rubin S.M. Structural conservation and E2F binding specificity within the retinoblastoma pocket protein family. J Mol Biol. 2016 Oct 9;428(20):3960-3971. PMID: 27567532 PMCID: PMC5048593
c) Burke J.R., Hura G.L., Rubin S.M. Structures of inactive retinoblastoma protein reveal multiple mechanisms for cell cycle control. Genes Dev. 2012 Jun 1;26(11):1156-66. PMID: 22569856 PMCID: PMC3371405
d) Hirschi A., Cecchini M., Steinhardt R.C., Schamber M.R., Dick F.A., Rubin S.M. An overlapping kinase and phosphatase docking site regulates activity of the retinoblastoma protein. Nat Struct Mol Biol. 2010 Sep;17(9):1051-7. PMID: 20694007 PMCID: PMC2933323

2) Structure and function of noncanonical Cdk activators: My laboratory has characterized important biochemical functions for protein subunits of the cycln-dependent kinase (Cdk) holoenzyme. For example, the Cks subunit plays a role in regulating multisite phosphorylation. Phosphorylation of cell-cycle proteins on multiple sites generates manifold responses and intricate signaling properties, yet the enzymatic mechanisms that tune multisite phosphorylation are poorly understood. The importance of Cks is evident from the

observations that its loss causes severe cell cycle defects and lethality in model organisms and that Cks is upregulated in human cancers. We established an innovative model in which Cks targets Cdk to specific substrates primed by initial phosphorylation and thereby enhances Cdk kinetics and signal outputs. We also recently solved the structure of Spy1, which is a non-Cyclin Cdk activator important in development and found upregulated in cancer. Our structural and biochemical studies determined how Spy1 activates Cdk in a manner that is resistant to normal Cdk regulatory mechanisms.

e) McGrath D.A., Fifield B.-A., Marceau A.H., Tripathi S., Porter L.A., Rubin S.M. Structural basis of divergent Cyclin-dependent kinase activation by Spy1/RINGO proteins. EMBO J. 2017 Jun 30, published on line. PMID: 28666995 PMCID: PMC5441720
f) McGrath D.A., Balog E.R.M., Kõivomägi M., Lucena R., Mai M.V., Hirschi A., Kellogg D.R., Loog M., Rubin S.M. Cks confers specificity to phosphorylation-dependent Cdk signaling pathways. Nat Struct Mol Biol., 2013 Dec;20(12):1407-14. PMID: 24186063 PMCID: PMC4242096
g) Koivomagi M., Valk E., Venta R., Iofik A., Lepiku M., Balog E.R.M., Rubin S.M., Morgan D.O., Loog M. Cascades of multisite phosphorylation control Sic1 destruction at the onset of S phase. Nature. 2011 Oct 12;480(7375):128-31. PMID: 21993622 PMCID: PMC3228899
h) Balog E.R.M., Saetern OC, Finch W., Hoeft C.O., Thai V., Harvey S.L., Kellogg D.R., Rubin S.M. The structure of a monomeric mutant Cks protein reveals multiple functions for a conserved hinge-region proline. J Mol Biol. 2011 Aug. 19; 411(3):520-8. PMID: 21704044

3) Mechanisms of cell-cycle dependent transcription: My laboratory has determined the structure and mechanism of regulation of several transcription factors that regulate cell-cycle dependent gene expression. We have determined the assembly and regulation of the DREAM protein complex, which plays a key role in cell cycle control of transcription. Tumor cells lack the ability to exit the cell cycle into quiescence, and transformation often results from inappropriate escape of quiescence. DREAM plays important roles in maintaining cell cycle exit and tumor suppression. DREAM consists of a MuvB complex core, which associates with the p130-E2F4 complex to repress gene expression in quiescent cells. We found a direct interaction between p130 and the MuvB subunit LIN52 and solved the crystal structure of a complex to identify how DREAM is assembled upon LIN52 phosphorylation. In another study, we determined the structure of the DNA binding domain of the DREAM component LIN54 bound to DNA. More recently, we determined the molecular mechanism underlying how the FoxM1 transcription factor, which controls mitotic gene expression, is regulated.

a) Marceau A.H., Brison C.M., Nerli S., Arsenault H.E., McShan A.C., Chen E., Lee H.W., Benanti J.A., Sgourakis N.G., Rubin S.M. An order-to-disorder structural switch activates the FoxM1 transcription factor. eLife. 2019 May 28;8 PMID: 31134895 PMCID: PMC6538375
b) Guiley K.Z., Iness A.N., Saini S., Tripathi S., Lipsick J.S., Litovchick L., Rubin S.M. Structural mechanism of Myb-MuvB assembly. Proc Natl Acad Sci USA. 2018 Oct 2;115(40):10016-10021 PMID: 30224471 PMCID: PMC6176624
c) Marceau A.H., Felthousen J.G., Goestch P.D., Iness A.N., Lee H.W., Tripathi S.M., Strome S., Litovchick L., Rubin S.M. Structural basis for LIN54 recognition of CHR elements in cell cycle-regulated promoters. Nat Commun. 2016 Jul 28;7:12301. PMID: 27465258 PMCID: PMC4974476
d) Guiley K.Z, Liban T.J., Felthousen J.G., Ramanan, P., Litovchick L., Rubin S.M. Structural mechanisms of DREAM complex assembly and regulation. Genes Dev. 2015 May 1 29(9) 961-974. PMID: 25917549 PMCID: PMC4421984

List of published work: http://www.ncbi.nlm.nih.gov/pubmed?cmd=PureSearch&term=Rubin+Seth

D. Research Support

Current Research Support

NIH/NCI 1 R01 CA228413 (PI :Rubin, MPI: Sage) 12/1/2018 – 11/30/2023
Molecular basis of tumor suppression by Cdk4/6 inhibition
The goals of this project are to understand the mechanisms by which Cdk4/6 inhibition leads to tumor suppression.

NIH/NIGMS R01 GM127707 (PI :Rubin) 2/1/2019 – 1/31/2023
Structural Mechanisms of FoxM1 Regulation
The goals of this project are to understand the structural mechanisms by which FoxM1 is inhibited and activated during the cell division cycle.

NIH/NIGMS 1 R01 GM124148 (PI :Rubin) 5/1/2018 – 3/31/2022
Structural Mechanisms Controlling Cell-Cycle Gene Expression
The goals of this project entail structure-function studies of the DREAM and Myb-MuvB transcription factor complexes, which regulate cell-cycle dependent gene expression.

Alex's Lemonade Stand Foundation (PI: Rubin) 10/01/2017 – 09/30/2019
Innovation Grant : Targeted Degradation of Proliferative Transcription Factors in Pediatric Cancers
The goals of this project are to test the hypothesis that E2F transcription factor degradation arrests retinoblastoma cells and identify molecules that bind E2F and can be developed into proteolysis targeting chimeras.

University of California 587167 (PI: Rubin) 7/01/2018 – 6/30/2021
Tobacco-Related Disease Research Program: Determinants of lung cancer cell response to Cdk4/6 inhibition
The major goals of this project are to identify new determinants of the efficacy of Cdk4/6 chemical inhibitors such as palbociclib in lung cancer cells.

Recently Completed Research Support

NIH/NCI 5 R01 CA132685-10 (PI: Rubin) 6/1/2013 – 3/31/2018
Molecular Mechanisms Regulating the Retinoblastoma Pocket Proteins
The major goals of this project are to characterize the structure of the Rb tumor suppressor protein and its homologs p107 and p130. Mechanisms for regulation of pocket proteins by phosphorylation are also under investigation.

American Cancer Society RSG-12-131-01-CCG (PI: Rubin) 2012-2016
Research Scholar Grant: Mechanism and Function of Multisite Phosphorylation in Cell Cycle Control
Project Goals: This project determined the role of the Cks protein in stimulating multisite phosphorylation of Cyclin-dependent kinases. We studied enzymatic mechanisms and substrate specificity as well as a specific function of Cks in stimulating Wee1 phosphorylation at mitotic entry.