BIOGRAPHICAL SKETCH

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NAME: van Eeuwen, Trevor

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

POSITION TITLE: Graduate Student

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)	END DATE MM/YYYY	FIELD OF STUDY
Fairleigh Dickinson University, Madison, NJ	B.A.	05/2015	Biochemistry
University of Pennsylvania, Philadelphia, PA	Ph.D.	03/2021	Biochemistry and Molecular Biophysics

A. Personal Statement

In addition to my previous experience in undergraduate research in cell biology and computational chemistry, I have obtained strong expertise in structural biology (cryo-electron microscopy), and protein biochemistry through my PhD training at the University of Pennsylvania. Using these techniques, my long-term research goals are to understand the underlying mechanisms of genomic instability and how they contribute to cancer. My current research focuses on understanding the structural and molecular basis of Nucleotide Excision Repair (NER) and its role in causing genome instability and disease. To date I have been involved in several research projects including my previous experience in undergraduate research has provided me with a solid basis in cell biology, computational chemistry and biophysics. These experiences have equipped me well as a technically skilled researcher capable of conducting hypothesis driven research. As an undergraduate at Fairleigh Dickinson University, I was responsible for a project under the joint direction of Dr. Gloria Anderle and Dr. Patricia Melloy that studied temperature sensitive mutants in the Mitotic Checkpoint Complex (MCC) in *Saccharomyces cerevisiae*. Using homology modeling, molecular dynamics and protein docking, I demonstrated that some surface mutants destabilize Cdc20, an essential component of the MCC, at non-permissive temperatures while other mutations disrupt binding interactions with regulatory components. I was able to complement these computational conclusions *in vivo* by generating fluorescently tagged versions of the mutant proteins and tracking protein localization through the cell cycle and comparing co-localization with other MCC components using fluorescent microscopy. This work culminated in multiple presentations at society national meeting, an award from FDU for outstanding Honors Thesis and an ACS sectional award. For my graduate training at the University of Pennsylvania, I have shifted my focus on biochemical and structural biology through cryo-Electron Microscopy (cryo-EM). Under the direction of Dr. Murakami as my PhD advisor, I have significantly contributed to structural studies on a diverse series of projects, and, through these collaborations, have obtained strong expertise in structural biology, in particular, single-particle analysis using cryo-electron microscopy, which can resolve atomic details of macromolecular complexes under physiological conditions. Most significant among these has been the characterization of the human centromeric nucleosome and the core centromeric nucleosome complex (CCNC) on human alpha satellite DNA by cryo-EM. This work has allowed us to begin to reveal the structure-function relationship of DNA and centromeres, the chromosomal regions necessary for proper chromosome segregation during mitosis.

My ongoing thesis project will address the large gaps in knowledge of how NER occurs and how it is regulated, specifically through structural characterization of NER intermediates and *in vitro* reconstitution using endogenously purified proteins. Failures along the pathway lead result in cancer, neurodegeneration and premature aging. In future work, I wish to determine how NER contributes to genomic instability in the context of cancer. The current work here will develop my career in structural biology and cancer biology as an independent scientist and mentor.

B. Positions and Honors

Positions and Employment

2014 - 2015 Laboratory Teaching Assistant, Fairleigh Dickinson University, Madison, PA

2015 - Graduate Student Research Assistant, University of Pennsylvania, Philadelphia, PA

Other Experience and Professional Memberships

2014 - 2015 Member, American Chemical Society
2014 - 2016 Member, American Society of Cell Biology
2016 - Member, American Society of Biochemistry and Molecular Biology

Honors

2013 Honors Program, Fairleigh Dickinson University
2014 Novo Nordisk Summer Research Fellowship, Fairleigh Dickinson University
2014 Gamma Sigma Epsilon, Chemistry Honor Society
2014 Phi Omega Epsilon, Senior Honor Society
2014 Scholarship, Novartis Science Research
2015 Jean Asell Duranna Award for Outstanding Research Presentation
67th Annual North Jersey Section ACS Undergraduate Research Conference
2015 New Jersey Institute of Chemists Student Award in Biochemistry
2015 FDU: University Honors Program Outstanding Research Student Award
2016 Chemistry Biology Interface (CBI) Scholar, The Wistar Institute
2016 - 2018 NIH T32 Structural and Molecular Biology Training Program, University of Pennsylvania
2017 National Science Foundation: Graduate Research Fellowship Honorable Mention

C. Contribution to Science

1. Mitosis is the process by which cells faithfully divide their replicated chromosomes into two daughter cells. Progress through mitosis is governed by the Mitotic Checkpoint Complex (MCC). In a joint undergraduate research mentorship by Dr. Gloria Anderle and Dr. Patricia Melloy, I studied how I studied how temperature sensitive mutants affect the function of the MCC in *Saccharomyces cerevisiae.* In Dr. Melloy's lab, I used fluorescent microscopy and yeast genetics to characterize the interactions of temperature sensitive mutants of the MCC. In Dr. Anderle's lab, I used molecular dynamics and computational techniques to investigate the biophysical implications of mutations on a member of the MCC, Cdc20p. I could computationally demonstrate that some surface mutations destabilize Cdc20p at elevated temperatures, leading to mitotic arrest. I was able to support these conclusions by demonstrating that the destabilized proteins are trafficked away from the MCC for degradation *in vivo*. This work culminated in two poster presentations at society national meetings and a regional poster session award.

Posters and Citations:

   a. **van Eeuwen T.**, Luginsland J., Melloy P., Anderle, G.. (2014) Homology modeling and functional analysis of the mitotic checkpoint complex in budding yeast. *Mol Biol Cell* 25, 25 (abstract P1067). Poster. American Society of Cell Biology National Meeting; 2014 December; Philadelphia, PA. PMCID: PMC4263442.
   b. **van Eeuwen T.**, Luginsland J., Melloy P., Anderle, G.. (2015) Homology modeling and functional analysis of the mitotic checkpoint complex in budding yeast. Poster. William Paterson University 9th Annual Undergraduate Research Symposium; Wayne, NJ. First Place Cell Biology.
   c. **van Eeuwen T.**, Luginsland J., Melloy P., Anderle, G.. (2015) Studying the structure of the mitotic checkpoint complex using computational analysis and temperature-sensitive yeast mutants. *Abstracts of Papers, 250th ACS National Meeting & Exposition, Boston, MA.* Poster. Sci Ed Section 2015:1324386 CAPLUS.

2. The DNA damage response is an essential process for the survival of living cells. Transcription of some stress responsive genes after DNA damage or other stresses is regulated by Elongin. In the lab of Dr. Kenji Murakami, we were able to demonstrate a link between the *Saccharomyces cerevisiae* Elongin (Ela1-Elc1) complex and a RNA polymerase II degradation factor Def1 in transcription after DNA damage. Furthermore, we could demonstrate that Def1 not only enhanced transcription initiation and restart *in vitro* but also was required for proper regulation of stress responsive genes *in vivo*. I generated deletion mutants

of the degradation factor, Δdef1 strains, and used these to demonstrate misregulation of stress responsive genes after DNA damage and heat shock by Real-Time Quantitative Reverse Transcription PCR (qRT-PCR). I was also able to demonstrate by negative stain Transmission Electron microscopy (TEM) that endogenously purified Def1 forms amyloid fibrils *in vitro.* These findings extended our understanding of the cellular response to stress with regards to gene regulation and transcription. A part of this work resulted in a publication (Damodaren et al., 2017), and the structural study is in progress.

Citation:

a. Damodaren N, **Van Eeuwen T,** Zamel J, Lin-Shiao E, Kalisman N, Murakami K.. (2017) Def1 interacts with TFIIH and modulates RNA polymerase II transcription. *Proc. Natl. Acad. Sci. U.S.A.* 114 (50) 13230-13235. PMID:29180430.

3. Centromeres are the portions of chromosomes that link sister chromatids during mitosis. These chromosomal regions are also important as they assemble kinetochores, the machinery required for proper chromosomal segregation. The chromatin at centromeres is distinguished by a special nucleosome containing the histone variant CENP-A, an additional core centromeric nucleosome complex (CCNC) and an alpha satellite DNA sequence. Under the tutelage of Dr. Murakami and through a collaboration with Dr. Ben Black, we have worked to reconstruct and characterize the structure of the centromeric nucleosome complex using single particle cryo-Electron Microscopy. Using cryo-EM, we have successfully determined several structures of the CENP-A nucleosome with alpha satellite DNA and core members of the CCNC, CENP-C and CENP-N at high resolution. These structures help reveal how the CCNC organize kinetochores and suggest an important role for alpha satellite DNA in assembling centromeric nucleosomes.

Citation(s):

a. Allu P.K., Dawicki-McKenna J.M., **van Eeuwen T.,** Slavin M., Braitbard M., Xu C., Kalisman N., Murakami K., Black B.E.. Structures of Interphase and Mitotic Forms of the Human Core Centromeric Nucleosome Complex. *NSMB* (under review).

4. The electron transport chain (ETC) is a series of membrane protein complexes in bacteria or the mitochondria or chloroplasts of eukaryotes that help establish an electrochemical gradient needed for ATP generation. The membrane protein complexes couple electron transport to generation of a proton gradient across a membrane, which is used to power ATP synthase. These chains can form modular “supercomplexes” consisting of cytochromes I-IV that can vary the activity of the ETC members. Under the direction of Dr. Murakami and in collaboration with Dr. Fevzi Daldal, we have determined the structure of a respiratory supercomplex from *Rhodobacter capsulatus*. Using single particle cryo-EM, we determined the structure of a cytochrome C-CIII-CIV supercomplex (preparing a manuscript for publication). This work demonstrates how quinol oxidation in CIII is linked to oxygen reduction in CIV and provides important structural information on potential antibiotic drug targets in the bacterial redox pathway.

5. My current thesis research in the lab of Dr. Kenji Murakami focuses on determining the structural and molecular basis of Nucleotide Excision Repair (NER). Small insults to DNA structure such as cyclopyrimide dimers or 6,4-photoproducts that result from UV exposure can be disastrous for RNA or DNA polymerases. To remove these roadblocks, a series of proteins will recognize damage, open a small single stranded bubble, excise the damaged strand and then synthesize the proper complement. Failure to due this results in the diseases *xeroderma pigmentosum,* Cockayne syndrome and Trichothiodystrophy. My thesis work focuses on constituting the individual steps of this process using purified proteins from *S. cerevisiae* and determining how these proteins function together using cryo-EM. I have already successfully reconstituted the damage recognition complex (Rad4-Rad23) with the helicases responsible for opening the single strand bubble (TFIIH) on a damaged DNA template. We have collected cryo-EM data and have a structural model for how these proteins come together that we are validating using cross-linking mass spectrometry.

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
	FAIRLEIGH DICKINSON UNIVERSITY	
2013	Calculus II	A
2013	College Writing Workshop	A
2013	Introduction to Molecules, Cell & Genes Lab	A
2013	Introduction to Molecules, Cell & Genes	A
2013	Organic Chemistry I	A
2013	Organic Chemistry I Lab	A
2013	Research in Biology	A
2013	Microbiology	A-
2013	Microbiology Lab*	
2014	Latin American Culture & Civilization	A
2014	Applied Statistics	A
2014	Organic Chemistry II	A
2014	Organic Chemistry II Lab	A
2014	Inorganic Chemistry	A
2014	Inorganic Chemistry Lab*	
2014	Cell Biology	A
2014	Cell Biology Lab*	
2014	Research in Biology II	A
2014	Research Writing Workshop: Travel	A
2014	History of Film	A
2014	American Dreams, American Tragedies	A
2014	Instrumental Analysis	A
2014	Instrumental Analysis Lab	B
2014	Biochemistry I	A
2014	Mentored Research in Biology§	
2015	Multimedia	A
2015	Global Issues	A
2015	Perspectives on the Individual	A
2015	Honors Thesis Chemistry	A
2015	Chemistry Capstone Research Experience	A
2015	Biochemistry II	A
2015	Physical Chemistry II	A
2015	Physical Chemistry II Lab	A
2015	Analytical Chemistry	A
2015	Analytical Chemistry Lab*	
	UNIVERSITY OF PENNSYLVANIA	
2015	Cell Biology	B+
2015	Macromolecular Biophysics: Principles and Methods	A
2015	Macromolecular Crystallography: Methods and Application	A
2015	Lab Rotation	A
2016	Regulation of the Genome	B+
2016	Biological Data Analysis	A
2016	Structural and Mechanistic Biochemistry	A+
2016	Physical Principles of Mechano-Enzymes	A
2016	Computation Programming in Biochemistry and Biophysics	A
2016	Lab Rotation	A

YEAR	COURSE TITLE	GRADE
2016	Lab Rotation	A
2017	Candidacy Exam Course	A
2017	Passed Doctoral Preliminary Exam: 04-26-17	
2014	GRE GENERAL TEST	
	Verbal Reasoning: 164/170	(94%)
	Quantitative Reasoning: 160/170	(74%)
	Analytical Writing: 4.5/6.0	(82%)

*Lab grade incorporated into course grade. §Taken for no credit due to credit restriction.

BIOGRAPHICAL SKETCH

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NAME: Kenji Murakami

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

POSITION TITLE: Assistant Professor of Biochemistry and Biophysics

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
The University of Tokyo	B.Sc.	03/1999	Physics
The University of Tokyo	Ph.D.	03/2004	Biophysics
Teikyo University, postdoctoral fellow		01/2008	Biophysics
Stanford University, postdoctoral fellow		12/2014	Biophysics

A. Personal Statement

Research: I am an experienced research scientist with strong expertise in structural biology (cryo-electron microscopy), protein and transcription. Studied macromolecular complexes in cytoskeleton (Murakami et al., Cell, 2010) during my PhD and eukaryote transcription initiation (Murakami et al., Science, 2013; Murakami et al., PNAS, 2015) during my postdoc. My current focus is to understand how transcription is regulated in the context of chromatin using cryo-electron microscopy.

Teaching and Mentoring: I am involved in a lot of teaching and mentoring. I teach a cryo-EM practical class (BMB634) every spring semester. Involved in teaching electron microscopy and single-particle analysis in BMB508 and T32 training grant (since 2015 as an assistant professor), and teaching eukaryote transcription in BMB509. Involved in thesis committees, candidacy exams for graduate students in Chemistry and BMB programs. In my own lab, (1) I mentored four graduate students since Jan 2015 (as an assistant professor); two of them are forth year graduate students and they succeeded in determining remarkable cryo-EM structures (preparing manuscript for publication), while the other two are in the second year. (2) I mentored two undergraduate students as a protein biochemist; one left for a PhD program (UCLA) in 2017 and the other is leaving for PhD program this summer. (3) I also trained two postdocs through collaborations for our cryo-EM projects since 2016, and both of them have successfully determined challenging cryo-EM structures of a membrane protein of electron transfer and a centromere nucleosome (submitted for publication).

Philosophy of training and commitment to promoting diversity: I greatly value promoting diversity given my research experiences in three countries in the past (PhD training in Japan, international exchange program at Cambridge in UK during 2003, and my postdoc training at Stanford University in USA, and my lab at Upenn in USA). Interaction with a variety of colleagues with different disciplines, cultures, genders, and perspectives gives us good opportunities to work harder on explaining our rationale and alternatives than we would have otherwise, and eventually greatly helps us develop our scientific thinking skills. Thus my lab members are from many nations (Japan, USA, Korea, India, Germany including in 2015-2019) with a balanced gender mix, and are well cross-culturally competent.

B. Positions and Honors

Positions and Employment

1995-1999 Undergraduate Research, The University of Tokyo, Japan (advisor: Dr. Takeyuki Wakabayashi)
1999-2003 Graduate Research, The University of Tokyo, Japan (advisor: Dr. Takeyuki Wakabayashi)
2004-2007 Postdoctoral fellow, Teikyo University, Japan (advisor: Dr. Takeyuki Wakabayashi)
2008-2014 Postdoctoral fellow, Stanford University, CA (advisor: Dr. Roger Kornberg)

2015-present	Assistant Professor of Biochemistry and Biophysics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

Honors

2015	McCABE Fellow Award, USA
2010	Kazato Research Award (JEOL), Japan
2009	Kanae Foundation Postdoctoral Fellowship, Japan
2009	JSPS Postdoctoral Fellowship, Japan
2008	Uehara Memorial Foundation Postdoctoral Fellowship, Japan

C. Contribution to Science

1. My PhD thesis was on the molecular mechanism of calcium regulation of muscle contraction using cryo-electron microscopy (supervised by prof. Takeyuki Wakabayashi in the University of Tokyo). In particular, I pursued structure determination of the actin filament by cryo-electron microscopy. Despite decades of research, little was known about how filament assembly leads to ATP hydrolysis. The inherent flexibility of the actin filament deforms the helical symmetry and hinders high-resolution structural analysis. To overcome this limitation, we developed all EM programs for data analysis in our lab to apply single-particle analysis without the use of helical symmetry and determined the actin filament structure at 6 Å resolution (Murakami et al, Cell, 2010). Comparison of the filamentous actin model with the crystal structure of the actin monomer revealed how filament assembly triggers ATP hydrolysis and subsequent phosphate release.

I also worked on muscle thin filaments using a combination of cryo-EM and other structural techniques. We discovered that a 6 kDa mobile domain of troponin is the central switch of this regulation, and we determined its structure by NMR spectroscopy as a collaboration (Murakami et al., JMB, 2005; Murakami et al., Adv. Exp. Med. Biol. 2007). In order to directly resolve its interaction with muscle thin filaments, we crystallized the troponin-tropomyosin complex and determined the structure of the head-to-tail junction of tropomyosin in a complex with a fragment of troponin (as a collaboration with Marray Stewart at MRC in Cambridge) (Murakami et al., PNAS, 2008). The crystal structure was consistent with cryo-EM structure of thin filament we have determined at 16 Å resolution, and was successfully docked into the EM density, revealing how tropomyosin homodimers bind only a single troponin and how the asymmetry functions as a calcium switch on the actin filament (Murakami et al., JMB, 2005; Murakami et al., PNAS, 2008).

a. **Murakami, K.**, Yasunaga, T., Noguchi, T.Q., Gomibuchi, Y., Ngo, K.X., Uyeda, T.Q., and Wakabayashi, T. Structural basis for actin assembly, activation of ATP hydrolysis, and delayed phosphate release. Cell, 143, 275-287 (2010).

b. **Murakami, K.**, Stewart, M., Nozawa, K., Tomii, K., Kudou, N., Igarashi, N., Shirakihara, Y., Wakatsuki, S., Yasunaga, T., and Wakabayashi, T. Structural basis for tropomyosin overlap in thin (actin) filaments and the generation of a molecular swivel by troponin-T. Proc. Natl Acad. Sci. USA, 105, 7200-7205 (2008).

c. **Murakami, K.**, Yumoto, F., Ohki, S.Y., Yasunaga, T., Tanokura, M., and Wakabayashi, T. Structural basis for calcium-regulated relaxation of striated muscles at interaction sites of troponin with actin and tropomyosin. Adv. Exp. Med. Biol. 592, 71-86 (2007).

d. **Murakami, K.**, Yumoto, F., Ohki, S.Y., Yasunaga, T., Tanokura, M., and Wakabayashi, T. Structural basis for Ca2+-regulated muscle relaxation at interaction sites of troponin with actin and tropomyosin. J. Mol. Biol. 351, 178-201 (2005).

2. During my postdoctoral work, I worked on the molecular mechanism of transcription initiation in the laboratory of Roger Kornberg at Stanford University. The proteins responsible for eukaryote transcription initiation, a set of general transcription factors (GTFs) and RNA polymerase II (pol II), associate in a so-called pre-initiation complex (PIC). Evidence for a PIC was previously obtained with nuclear extract or with partially purified GTFs assembled on immobilized promoter DNA. I succeeded in the assembly of a PIC with pure GTFs

and pol II from the yeast S. cerevisiae (Murakami et al., Mol.Cel, 2015; Murakami et al., PNAS, 2012; Murakami et al., JBC, 2013). Based on this biochemical development, we have also succeeded in tracking a pol II molecule during transcription initiation using optical tweezers, and thereby directly observing all of the major steps in initiation including promoter opening, start site scanning, and promoter escape with single base-pair resolution (a collaboration with the Steven Block lab at Stanford University, Fazal et al., Nature, 2015).

a. Fazal, F.*, Meng, C.*, **Murakami, K.***, Kornberg, R.D., Block, S.M. Real-Time Observation of the Initiation of RNA Polymerase II Transcription. Nature, 525: 274-277 (2015). (* equally contributed)

b. **Murakami, K.**, Mattei, P.J., Davis, R.E., Jin, H., Kaplan, C.D., Kornberg, R.D. Uncoupling promoter opening from start site scanning. Mol. Cell, 59, 133-138 (2015).

c. **Murakami, K.**, Calero, G., Brown, C.R., Liu, X., Davis, R.E., Boeger, H., and Kornberg, R.D. Formation and Fate of a Complete, 31-Protein, RNA polymerase II Transcription Initiation Complex. J. Biol. Chem. 288, 6325-6332 (2013).

d. **Murakami, K**., Gibbons, B.J., Davis, R.E., Nagai, S., Liu, X., Robinson, P.J., Wu, T., Kaplan, C.D., Kornberg, R.D. Tfb6, a previously unidentified subunit of the general transcription factor TFIIH, facilitates dissociation of Ssl2 helicase after transcription initiation. Proc. Natl Acad. Sci. USA, 109, 4816-4821 (2012).

3. Our success in the functional and homogeneous assembly of the complete transcription pre-initiation complex (see section 3) enabled structural studies of the entire transcription initiation machinery. In the first of such studies, we have determined the structure of the PIC in the closed state by cryo-electron microscopy(cryo-EM) at 15 Å resolution (Murakami et al., Science, 2013) and sub-nanometer resolution (~8 Å) using an independent analysis pipeline coupled to a more powerful electron detector (Murakami et al., 2015, PNAS, 2015).

a. **Murakami K*,** Tsai K-L*, Kalisman N, Bushnell DA, Asturias FJ, Kornberg RD. *Structure of an RNA polymerase II preinitiation complex*. Proc Natl Acad Sci U S A, 112, 13543–13548 (2015).

b. **Murakami, K.***, Elmlund, H.*, Kalisman, N.*, Bushnell, D.A., Adams, C.M. Azubel, M., Elmlund, D., Levi-Kalisman, Y., Liu, X., Levitt, M., and Kornberg, R.D. Architecture of an RNA Polymerase II Transcription Pre-Initiation Complex. Science, 1238724 (2013). (* equally contributed)

4. At the University of Pennsylvania, my laboratory has leveraged out expertise in biochemistry and cryo-EM to study transcription regulation including Mediator, the central co-activator of transcription, especially in response to stress using cryo-EM (Tsai et al., Nature, 2017) (Damodaren et al., 2017)(Fujiwara and Murakami, 2019).

a. Tsai, K-L., Yu, X., Gopalan, S., **Murakami, K.**, Conaway, R.C., Conaway, J.W., and Asturias, F.J. Atomic models of Mediator and holoenzyme: implications for the Mediator transcription regulation mechanism. Nature, 544(7649):196-201 (2017).

b. Damodaren, N., Van Eeuwen, T., Zamel, J., Lin-Shiao, E., Kalisman, N., and **Murakami, K**. Def1 interacts with TFIIH and modulates RNA polymerase II transcription. Proc Natl Acad Sci U S A, 114, 13230–13235 (2017).

c. Fujiwara, R., and **Murakami, K**. In vitro reconstitution of yeast RNA polymerase II transcription initiation with high efficiency. Methods, S1046-2023(18)30298-6 (2019).

d. Fujiwara, R., Damodaren, N., Welusz E.J., and **Murakami, K**. The capping enzyme facilitates promoter escape and assembly of a follow-on pre-initiation complex for re-initiation. Under revision for publication in Proc Natl Acad Sci U S A,

D. Research Support

Ongoing Research Support

NIH R01 09/01/17-08/31/22
The Mechanism of Transition from Transcription Initiation to Elongation
Role: PI

McCABE Fellow Award 07/01/15-6/30/18
The Mechanism of Transition from Transcription Initiation to Elongation
Role: PI