BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

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. During my postdoctoral work, 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 for the first time. 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. As an assistant professor 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. Proc Natl Acad Sci U S A, Accepted for publication (2019).

5. As an assistant professor at the University of Pennsylvania, my laboratory also pursues transcription regulation in chromatin by cryo-EM (Allu et al., Current Biology, 2019). Recent structural studies have yielded insight on a variety of structures of nucleosome, in which nucleosomes are artificially stabilized by employing an artificial sequence (the 'Widom 601' sequence), and may not correctly reflect all of the structural features important for functions. We have determined the first structure of the centromere nucleosome with a centromere-specific natural sequence (a-satellite DNA), providing a near-atomic resolution view of the complex that is significantly different from previous structures with an artificial sequence and is critical in assembly of centromere nucleosome.

a. Allu, P. K., Dawicki-McKenna, J., 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., Current Biology, Accepted for publication (2019).

D. Research Support

Ongoing Research Support

NIH R01 GM123233 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