Professional Leave Report Cover Sheet

Name: Hwan Youn

Department: Biology

College: Science & Mathematics

Leave taken: ◉ Sabbatical ○ Difference in Pay ○ Professional Leave without Pay

Time Period: ◉ Fall 2022

○ Spring

○ Academic Year

○ Other

Your report will be sent to your Dean for your PAF and to the Library Archives.

Post-Sabbatical Leave Report (for Fall 2022)

Hwan Youn, Ph.D.

Department of Biology

March 30, 2023

a) Accomplishments

1. Paper publication. I wrote and published one journal article (listed below) with one of my Fresno State graduate students.

Youn* H, and Carranza^ M. (2023) cAMP activation of cAMP receptor protein, a model bacterial transcription factor. J. Microbiol. Mar 9. doi: 10.1007/s12275-023-00028-6. Online ahead of print. Review. (*corresponding author, ^student author)

2. Data generation. During my visit in South Korea, I regularly visited the lab of Prof. Jin-Won Lee, Hanyang University and generated data which will be used for two manuscripts. Tentative titles and authors for the manuscripts are listed below.

- Rea A, Montiel C, Pacheco D, Gunasekara SM, Carranza M, Lee J-W, and Youn H. Unexpected importance of non-DNA contacting F-helix residues in the cAMP receptor protein (to be submitted to Journal of Microbiology).
- Zhou Y, Pospisil CR, Gunasekara SM, Park J, Lee J-W, and Youn H. Enhanced c-di-GMP production in a DgcZ mutant (to be submitted to Journal of Microbiology and Biotechnology).

3. Continued research collaborations with Prof. Jin-Won Lee and Dr. Sung Gyun Kang. In South Korea, I met with Prof. Jin-Won Lee (Hanyang University) on a regular basis and visited one time the lab of Dr. Sung Gyun Kang (Korea Institute of Ocean Science and Technology). In doing so, I was able to strengthen research collaborations with them. Collaborative papers are expected in the future with Prof. Jin-Won Lee on PerR, a *Bacillus* transcription factor, and with Dr. Sung Gyun Kang on a CO-sensing archaeon.

4. Multiple STEM meetings with Dr. Rin Yun. I met with Dr. Rin Yun, a STEM education expert and exchanged ideas regarding how to improve my two microbiology courses, BIOL 160 (microbial physiology) and BIOL 153 (microbial genetics). I gained valuable insights from Dr. Rin Yun which will be implemented in these courses to improve the lab components.

5. Research network. In South Korea, I met with many outstanding scientists (listed below) and had ample research communications.

- Prof. You-Hee Cho (Cha University); bacterial pathogens
- Prof. Hongbaek Cho (Sungkyunkwan University); bacterial cell envelope biogenesis
- Dr. Hyun Sook Lee (Korea Institute of Ocean Science and Technology); marine bacteria
- Dr. Hyung-Soon Yim (Korea Institute of Ocean Science and Technology); marine organisms
- Prof. Jung-Shin Lee (Kangwon National University); yeast genetics
- Prof. Soojin Lee (Chungnam National University); eukaryotic microorganisms
- Prof. Sangyun Jeong (Jeonbuk National University); Drosophila genetics

- Prof. Won-Ki Huh (Seoul National University); functional genomics
- Dr. Cheolju Lee (Korea Institute of Science and Technology); molecular proteomics
- Prof. Jeong-Mok Kim (Hanyang University); protein modifications

b) Modifications: None

c) Unaccomplished Goals: None

d) Anticipated Outcomes

1. Publication of 2 more papers. Two more manuscripts are currently being prepared and will be submitted for journal publication. The original plan was to submit these in the Spring 2023 semester; however, submissions are being delayed due to delays in data analysis. Tentative titles and authors are listed below.

- Rea A, Montiel C, Pacheco D, Gunasekara SM, Carranza M, Lee, J-W, and Youn H. Unexpected importance of non-DNA contacting F-helix residues in the cAMP receptor protein. (to be submitted to Journal of Microbiology).
- Zhou Y, Pospisil CR, Gunasekara SM, Park J, Lee J-W, and Youn H. Unexpected importance of non-DNA contacting F-helix residues in the cAMP receptor protein. (to be submitted to Journal of Microbiology and Biotechnology).

2. Submission of a grant proposal to NIH. Based on research activities performed during the sabbatical, I will submit an external grant proposal (NIH SuRE proposal) by the annual deadline (May 26, 2024).

3. Continued research collaborations. Collaboration with Prof. Jin-Won Lee (Hanyang University) and Dr. Sung Gyun Kang (Korea Institute of Ocean Science and Technology) will continue, and collaborative publications are expected in the future.

4. Revamping my two microbiology courses (BIOL 160 and BIOL 153). In both courses, lab components, especially regarding data generation and dissemination, will be revamped.

e) Post-Sabbatical Seminar at Fresno State (Biology Colloquium)

Title: cAMP activation of the cAMP receptor protein, a model bacterial transcription factor
Time: 3-4 pm, March 24, 2023
Place: Rm 161, McLane Hall

Enclosed are:

- Original sabbatical proposal (2 pages)
- Flyer for the post-sabbatical seminar at Fresno State (1 page)
- First page of the published journal article (1 page)

Sabbatical Leave Proposal

Hwan Youn, Ph.D., Department of Biology
September 15, 2021

Section 1. The Proposal

1. Requested period: Fall 2022 at full pay.

2. Objectives

(1) To conduct research and acquire data which cannot be accomplished at Fresno State
(2) To revise and submit 2 manuscripts for journal publication
(3) To write a grant proposal (NIH score SC3)
(4) To extend my microbiology research into anaerobic microorganisms
(5) To revamp my two lab-containing microbiology courses
(6) To strengthen research networks for potential collaborations and resources

3. Preliminary arrangements

See Table 1 below. A support letter from Dr. Jin-Won Lee, one of the hosts, is also attached.

Table 1. Preliminary arrangements of sabbatical visits.

Host Name	Host position	Host institution	Visit duration	Visit purpose
Dr. Jin-Won Lee	Full professor	Hanyang University (South Korea)	5 months (July – Dec, 2022)	Acquire essential data for 2 manuscripts & outline/develop a grant proposal
Dr. Sung Gyun Kang	Division director	Korea Institute of Ocean Science and Technology (South Korea)	2 weeks (Oct, 2022)	Acquire anaerobic research techniques
Dr. Rin Yun	Full professor	Hanbat National University (South Korea)	2 weeks (Oct, 2022)	Revamp my two microbiology CURE* courses

*CURE stands for "Course-based Undergraduate Research Experiences."

4. Planned activities

In summer 2022 and sabbatical semester (fall 2022), I will travel to South Korea and visit the labs of Dr. Lee, Dr. Kang and Dr. Yun (Table 1). Drs. Lee and Kang are my long-time friends and collaborators. I have published 7 peer-reviewed articles with Dr. Lee and 2 peer-reviewed articles with Dr. Kang.

Dr. Lee is an expert in protein chemistry and enzyme kinetics. During my stay in South Korea, I will spend 5 months in Dr. Lee's lab to do the following two series of experiments. First, I will purify wild type cAMP receptor protein (CRP) and a representative CRP mutant, and measure their *in vitro* DNA binding activities. CRP is the main protein studied in my lab and concerns one of the two manuscripts. Second, I will purify wild type DgcZ and a key DgcZ mutant and acquire *in vitro* enzyme kinetics data on the proteins. DgcZ which I started to study a few years ago is a novel enzyme synthesizing cyclic di GMP and concerns the second manuscript. So far, my Fresno State students and I have accumulated *in vivo* data on the aforementioned proteins, however the lack of straightforward biochemical (*in vitro*) data has been heavily criticized by journal reviewers, thereby having caused manuscript rejections in my publication tries. Given that, what I propose to do in Dr. Lee lab are very important for successful manuscript revision and publication. Dr. Lee's lab is perfect because he is not only an expert in the field, but also owns an instrument (a fluorescence spectroscopy coupled with anisotropy system) which allows for *in vitro* DNA binding measurement (the machine is not available at Fresno State). In addition, some of my time in Dr. Lee's lab will be allotted to outlining and developing a grant proposal (target:

NIH SCORE SC3). Then, the grant proposal ideas and plans will be shared with Dr. Lee and his research team (postdocs and PhD students) for feedback and discussion.

Dr. Kang is an expert in biological carbon monoxide (CO) metabolism and H_2 production (he published a Nature paper as corresponding author on the topic). These microorganism-specific metabolisms occur only in the absence of oxygen, and Dr. Kang is an internationally renowned scientist on such anaerobic metabolisms. During my postdoctoral period at University of Wisconsin-Madison, I intensely studied a CO-sensing protein CooA which activates anaerobic CO oxidation in a microorganism *Rhodosprillum rubrum*. However, my CooA study at Fresno State has not been very fruitful due to my outdated anaerobic techniques. I will visit Dr. Kang's lab for 2 weeks in Oct, 2022 to hone my anaerobic skills by purifying wild type CooA and CooA mutants and anaerobically culturing H_2-producing microorganism.

Dr. Yun is a prominent STEM expert who gets regularly featured in Korean newspapers. I have a personal connection with him, and will spend 2 weeks in Oct, 2022 in his lab. During daily visits, I will have a lot of informal conversations and attend formal STEM events with him. The know-hows gained from Dr. Yun will then be applied to my two microbiology courses (Microbial Physiology and Microbial Genetics) to revamp CURE projects.

During my stay in South Korea, I also expect to meet with scientists and give invited seminar presentations (as I did in my previous Fall 2014 sabbatical). The people I am likely to meet are: Dr. You-Hee Cho (Cha University, expert on *Pseudomonas aeruginosa*), Dr. Sang Hee Lee (Myongii University, expert on structure-based drug design) and Dr. Kwan Soo Ko (Sungkyunkwan University, expert on multidrug resistance among bacteria). In-person interactions with these people will strengthen my international research networks and may lead to future collaborative projects.

5. Timeline for main tasks:

- 2 manuscripts – acquisition of required data — July 15, 2022 – Dec 15, 2022
- 2 manuscripts – revision and submission — Sep 15, 2022 – Feb 15, 2023
- NIH SCORE SC3 grant proposal – outlining, developing — July 15, 2022 – Dec 15, 2022
- NIH SCORE SC3 grant proposal – writing — Spring 2023 semester
- NIH SCORE SC3 grant proposal – submission — by May 25, 2023 (NIH cycle II)

Section 2. Benefits to the faculty

(1) Opportunity to produce and analyze research data that is not possible at Fresno State
(2) Opportunity to acquire new research skills required for studying anaerobic microorganisms
(3) Opportunity to write, revise, and submit research manuscripts
(4) Opportunity to plan, write, and submit an NIH grant proposal
(5) Opportunity to revamp two microbiology courses
(6) Opportunity to strengthen international research networks

Section 3. Benefits to the university

(1) Enhancing the reputation of the college and university in academic excellence and student preparation
(2) Promoting global connections with academic institutions
(3) Improving microbiology curriculum in the Biology Department
(4) Enhancing the international recognition of Fresno State (I will introduce and advertise Fresno State to South Korea academic institutions and research centers I visit).
(5) Potentially initiating international exchange programs between Fresno State and South Korea academic institutions

Section 4. Previous Leave

I had a sabbatical leave in Fall 2014 and my report is attached to this proposal.

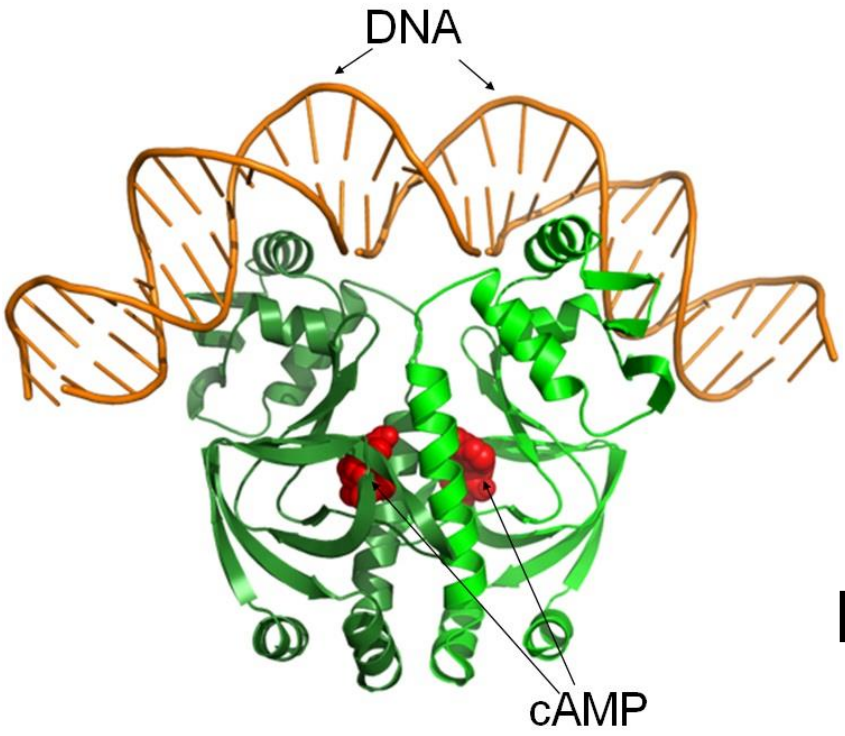
FRESNO STATE

Department of Biology Colloquium Seminar Series presents…

"cAMP activation of the cAMP receptor protein, a model bacterial transcription factor"

Date:
24 March 2023
Friday

Time:
3:00 – 4:00 PM (PDT)
In person: MCL 161



Biology Team Presentation:

Dr. Hwan Youn
&
Marcus Carranza

If you need a disability-related accommodation or wheelchair access, please contact Maria Rochin at the Department of Biology at (559) 278-2001 or e-mail mrochin@csufresno.edu at least **one week prior to the event**.

Journal of Microbiology
https://doi.org/10.1007/s12275-023-00028-6

REVIEW

cAMP Activation of the cAMP Receptor Protein, a Model Bacterial Transcription Factor

Hwan Youn[1] · **Marcus Carranza**[1]

Received: 27 December 2022 / Revised: 9 February 2023 / Accepted: 13 February 2023

Abstract

The active and inactive structures of the *Escherichia coli* cAMP receptor protein (CRP), a model bacterial transcription factor, are compared to generate a paradigm in the cAMP-induced activation of CRP. The resulting paradigm is shown to be consistent with numerous biochemical studies of CRP and CRP*, a group of CRP mutants displaying cAMP-free activity. The cAMP affinity of CRP is dictated by two factors: (i) the effectiveness of the cAMP pocket and (ii) the protein equilibrium of apo-CRP. How these two factors interplay in determining the cAMP affinity and cAMP specificity of CRP and CRP* mutants are discussed. Both the current understanding and knowledge gaps of CRP-DNA interactions are also described. This review ends with a list of several important CRP issues that need to be addressed in the future.

Keywords CRP · cAMP affinity · cAMP specificity · CRP* · DNA binding

Introduction

Escherichia coli prefers glucose as a carbon and energy source, and consequently does not metabolize other carbon sources in the presence of glucose. This phenomenon is known as catabolite repression (Magasanik, 1961). If glucose is absent, *E. coli* produces a signaling molecule cAMP which binds and activates the cAMP receptor protein (CRP). The cAMP-bound CRP then acquires a high-affinity DNA binding and subsequent transcriptional regulation (Busby & Ebright, 1999; Harman, 2001). If glucose is present, no cAMP is made, and CRP remains as apo-protein which is inactive in DNA binding.

CRP is a model bacterial transcription factor and currently both apo-CRP and cAMP-bound CRP structures are known (McKay & Steitz, 1981; Parkinson et al., 1996b; Popovych et al., 2009; Schultz et al., 1991; Seok et al., 2014; Sharma et al., 2009). CRP is a homodimer, and each subunit consists of an N-terminal cAMP-binding domain and a C-terminal DNA-binding domain which are connected by the C-helix which is also the main dimerization interface (Fig. 1A and B). Importantly, the cAMP-binding site in the N-terminal domain is far away from the DNA-contacting site in the C-terminal domain: for example, the α carbon distance between Glu72 (a cAMP-contacting residue) and Arg180 (a DNA-contacting F-helix residue) is > 30 Å in the structure of active cAMP-bound CRP. Therefore, the transfer of the cAMP-binding signal from the cAMP-binding site to the DNA-binding F-helix is allosteric and occurs via a series of conformational rearrangements in the C-helix, D-helix, β4/β5 loop, and hinge region (Fig. 1A–D). The active and inactive CRP structures were compared to generate a paradigm in the cAMP-induced activation of CRP that is also consistent with numerous biochemical studies of CRP and CRP* mutants, a group of CRP mutants that commonly possess a measurable amount of CRP activity in the absence of cAMP. The current paradigm is presented below in the main section.

CRP binds cAMP with high affinity and is specific to cAMP in that only cAMP can effectively activate CRP. The cAMP affinity and cAMP specificity of CRP are dictated by the effectiveness of the cAMP pocket and the protein equilibrium of apo-CRP (Youn et al., 2008). How these two factors interplay in determining the cAMP affinity and cAMP specificity of both CRP and CRP* mutants are discussed below.

CRP binds DNA through the F-helix, the recognition helix of the helix-turn-helix DNA-binding motif. A comprehensive picture of the interactions between the F-helix of CRP and DNA is provided below. The transcriptional activity of CRP

✉ Hwan Youn
hyoun@csufresno.edu

[1] Departments of Biology, California State University Fresno, Fresno, CA 93740, USA

Published online: 09 March 2023