Probing the role of transmembrane domain interactions in Toll-like receptor signaling

by

James Isaac Godfroy III

B.S., University of Notre Dame, 2009

A thesis submitted to the

Faculty of the Graduate School of the

University of Colorado in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

Department of Chemical and Biological Engineering

2015

This thesis entitled:
Probing the role of transmembrane domain interactions in Toll-like receptor signaling
written by James Isaac Godfroy III
has been approved for the Department of Chemical and Biological Engineering

_____
Prof. Hubert Yin

_____
Prof. Theodore Randolph

_____
Prof. Amy Palmer

Date _____

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

Godfroy III, James Isaac (Ph.D., Chemical and Biological Engineering)

Probing the role of transmembrane domain interactions in Toll-like receptor signaling

Thesis directed by Prof. Hubert Yin and Prof. Theodore Randolph

Membrane proteins account for approximately 30% of the human proteome, are the therapeutic target of nearly 60% of current pharmacological agents, yet account for <1% of all solved structures. This lack of information on membrane proteins extends from the difficulty in studying transmembrane domains due to their inherent insolubility and instability outside of membrane mimetics. New methods to study these domains are critical for advancing the understanding of membrane protein signal transduction.

The Toll-like receptor (TLR) family of membrane proteins serve as critical sensors of infection for the innate immune system. As mounting evidence suggests that the transmembrane domain is a critical region in several protein families, we hypothesized that this was also the case for TLRs. Using a biochemical and biophysical approach in membrane mimetic systems, we were the first to report the role of isolated transmembrane domain interactions in TLR oligomerization. We showed that all TLR transmembrane domains were capable of homotypic interactions. We also demonstrated that the TLR2 transmembrane domain preferentially interacted with the transmembrane domains of its heterotypic signaling partners, TLR1 and TLR6.

To better understand the role of transmembrane domain interactions in biologically relevant membranes, we utilized acceptor photobleaching Förster resonant energy transfer to image the involvement of individual domains in TLR assembly in live cells. This technique is a simple, rapid approach to screen for both homotypic and heterotypic interactions in native protein environments. Results suggest that TLR2-TLR1 and TLR2-TLR6 transmembrane domains are interacting heterotypically in native cell membranes.

To better screen TLR signaling pathways for novel therapeutic intervention, we established a genetic reporter in native immune cells, monocytes and T-cells, for monitoring TLR signaling

activity. Monocytes specifically responded to TLR2 agonists and T-cells were specific for TLR5 agonists. Utilization of these cell lines allowed for screening of transmembrane domain libraries to investigate the potential of regulating TLR2 and TLR5 signaling through transmembrane domain interactions. Furthermore, they allowed for validation of small molecule therapies to treat TLR related diseases.

Dedication

To my parents for their endless support in all my endeavors.

To my friends for all the enjoyable adventures.

Acknowledgements

I would like to first thank my advisors, Hubert Yin and Ted Randolph, for the help, encouragement, and guidance on this project throughout my time in graduate school. I would also like to thank Amy Palmer and her lab for all of the assistance they have provided in my endless quest for the perfect images and FRET experiments. I would also like to thank my committee members, Joel Kaar and Stephanie Bryant, for the general support and guidance they have provided.

I also have to acknowledge all the great support staff that have significantly improved the day to day work as a graduate student. First, I would like to thank Theresa Nahreini for all her help in cell culture, flow cytometry, and the general daily discussions. I would also like to thank Joe Dragavon in the microscope core facility for his assistance with all the imaging experiments. I also can never say enough about the assistance Dominique de Vangel has provided for meeting all the graduate school deadlines and getting everything organized.

To all past and present Yin lab members, I have greatly appreciated the support in learning new techniques, running experiments, and discussing science in general. You have made my time in lab much more enjoyable with the friendly banter. I also appreciate all of the undergraduate students who have spent time with me working on various portions of this project.

I would like to thank my friends for making my time here enjoyable. I've had several great ski trips, numerous soccer games, and late night lifting sessions which have all provided me a nice distraction from lab work.

Lastly, I would like to thank the two most important people, my parents, for all their support and encouragement.