

# **The Regulation of Sleep and Circadian Rhythms: The Role of Melatonin and Adenosine in Zebrafish**

Thesis by

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In Partial Fulfillment of the Requirements for  
the degree of  
Doctor of Philosophy



CALIFORNIA INSTITUTE OF TECHNOLOGY

Pasadena, California

2015

(Defended April 27, 2015)

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For Veronica

&

My Parents

## Acknowledgements

This PhD has been quite the winding adventurous road, and I would not have been able to get to the end of it, if it hadn't been for the help and support of many people.

First and foremost I'd like to sincerely thank my PhD supervisor David Prober. Over time, I've learnt to respect his logical and organized style of working and his ethic of hard work. I've learnt by example, how to take the un-certainty in science with equanimity and how to keep trying something new even if nothing has worked yet. With these lessons under my belt, I believe I'm well on my way to becoming a skillful independent scientist.

I'd like to thank my thesis committee members, Marianne Bronner, Paul Sternberg and Viviana Gradinaru, for their positivity, support and suggestions over the years, especially during those years when it seemed like I'd never graduate...

I'd like to acknowledge my previous mentors Veronica Rodrigues and Mani Ramaswami for their belief in me and for honing my abilities as I took my first tentative steps into the world of scientific research.

I'd like to thank the members of the Prober lab, especially Daniel Lee and Grigoris Oikonomou for their positivity and help and advice during periods of crisis, Chanpreet Singh, for his infective enthusiasm for science and the next experiment he's up to, Audrey Chen for her calm composure and reasoned opinions. My thanks to Eric Mosser for his help with the Melatonin project, and Cindy Chiu for conversations and tools. I'd like to thank Viveca Sapin for being the lab 'mom' and Jae Engle and Brett Niles for their help with the mountains of genotyping over the years. I'd like to thank Jason Schwer, Kenna Mollinder and Alex Mack for their efforts and care with the fish, and especially Kenna for conversations and coffee during times of need. I'd also like to thank my zebrafish for being the wonderful animals they are...

I'd like to thank my friends and lab mates Justin and Wendy, with whom I started this journey and who have been around for support, brainstorming sessions and encouragement and

without whom I cannot even imagine having come to this ending point. You guys have been my lifeline for the last 6 years... so thank you.

I'd like to thank the members of the Mazmanian, Grdinaru and Sternberg labs for being such wonderful neighbors. I could drop by for anything from a tube of amplitaq to coffee creamer and they were nothing but nice to me.

I would like to express my gratitude for my parents Manisha and Vasant, for inspiring and nurturing my dream to do scientific research and for their love and support through the ups and downs of my journey with phone calls and visits especially when things seemed too difficult.

I would like to thank my fiancé Alok for his unconditional love and support (and cheese cake) these last years. I've turned into a grumpy monster so many times during these past years and he's always managed to talk or feed me out of it.

A big thank you to my sister Anita for moving to the west coast so we could be on the same time zone, and then for her willingness to give me pep talks on demand.

I'd like to express my heartfelt gratitude for my wonderful 'local families' Asha and Mahindra Mehta and Shailee, Setoo and Suchi as well as Vasudev and Vrinda Bhandarkar and Mallika, who always kept their doors open for me to recover after weeks of experiments. I've felt like a part of their families for these last 6 years, and their love and care (and food) has made my journey possible. I'd also like to thank Raju and Rupal Desai and the Mehta sisters for their generous support during my TIFR days, which has helped bring me to Caltech.

Thank you all for making this great journey worthwhile!

## Abstract

Sleep is a highly conserved behavioral state whose regulation is still unclear. In this thesis I first briefly introduce the known sleep circuitry and regulation in vertebrates, and why zebrafish is seen as a good model to study sleep-regulation. I describe the existing two-process model of sleep regulation, which posits that the two processes C (circadian) and S (homeostatic) control timing of sleep-wake behavior. I then study the role melatonin plays in the circadian regulation of sleep using zebrafish. First, we find that the absence of melatonin results in a reduction of sleep at night, establishing that endogenous melatonin is required for sleep at night. Second, melatonin mutants show a reduction in sleep in animals with no functional behavioral rhythms suggesting that melatonin does not require intact circadian rhythms for its effect on sleep. Third, melatonin mutants do not exhibit any changes in circadian rhythms, suggesting that the circadian clock does not require melatonin for its function. Fourth, we find that in the absence of melatonin, there is no rhythmic expression of sleep, suggesting that melatonin is the output molecule of process C. Finally, we describe a connection between adenosine signaling (output molecules of process S), and melatonin. Following this we proceed to study the role adenosine signaling plays in sleep-wake behavior. We find that first, adenosine receptor A1 and A2 are involved in sleep- wake behavior in zebrafish, based on agonist/antagonist behavioral results. Second, we find that several brain regions such as PACAP cells in the rostral midbrain, GABAergic cells in the forebrain and hindbrain, Dopamine and serotonin cells in the caudal hypothalamus and sox2 cells lining the hindbrain ventricle are activated in response to the A1 antagonist and VMAT positive cells are activated in response to the A2A agonist, suggesting these areas are involved in adenosine signaling in zebrafish. Third, we find that knocking out the zebrafish adenosine receptors has no effect on sleep architecture. Finally, we find that while the A1 agonist phenotype requires the zfAdora1a receptor, the antagonist and the A2A agonist behavioral phenotypes are not mediated by the zfAdora1a, zfAdora1b and zfAdoraA2Aa, zfAdora2Ab receptors respectively.

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