Directed Evolution of Biosynthetic Pathways to Carotenoids with Unnatural Carbon Backbones

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

2006

(Defended August 19, 2005)

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ACKNOWLEDGMENTS

The opportunity to attend graduate school at Caltech has been a precious gift that I will treasure for the rest of my life. Working at this world-class institute with such talented, intelligent, motivated, and conscientious colleagues has contributed to my professional and personal development in ways I could never have imagined at the outset.

To my advisor, Frances Arnold, thank you for giving me the guidance and opportunities to develop and mature as a scientist. I have never encountered anyone who can cut directly through to the essence of an idea or concept the way you can, and I hope that some of your keen scientific vision and insight has rubbed off on me. Thank you also for always being truthful. It wasn't easy knowing that something I said, did, or wrote didn't satisfy you or come across the right way, but on the other hand, when you told me that my work was "excellent" or "just right," I knew you meant it and that really made me feel like I had succeeded.

To my other committee members, Anand Asthagiri, Linda Hsieh-Wilson, and Dianne Newman, thank you all for serving on my candidacy and thesis committees and for your insightful questions and comments.

I would like to thank the Natural Sciences and Engineering Research Council of Canada for the two postgraduate scholarships I was awarded, and the United States National Science Foundation for a grant supporting this research. I am appreciative not only of the financial contributions from these organizations, but also for the associated expressions of belief in the value of this research.

I would not have gotten very far in characterizing the carotenoids my cultures have synthesized without the dedicated assistance of Mona Shahgholi and Nathan

Dalleska. Thank you both for the hours of time you spent helping me with mass spectrometry, for answering my questions and critiquing my hypotheses, and most of all, for helping me to collect key data for this thesis.

Manish Raizada and Hyman Hartman contributed valuable comments that helped us to make the review paper reproduced in Chapter 1 significantly more focused and lucid. Gerhard Sandmann deserves many thanks for supplying me with valuable experimental guidance and eminently appropriate literature at just the right moments. I am also grateful to George Britton for his expert advice on the extraction and analysis of carotenoids from biological material. Shinichi Takaichi provided helpful assistance with the naming of the new carotenoids reported in Chapter 3.

Most of my fondest memories of Caltech involve the many wonderful students, postdocs, and visitors who have spent time in the Arnold group during my tenure here. I want to thank every one of these group members for contributing to the Arnold lab's wonderful atmosphere of interdisciplinary collaboration, for advice and ideas, and for good times at parties and other social functions.

In particular, I am indebted to Daisuke Umeno, who was my mentor and collaborator on the carotenoids project and who taught me how to clone genes, build plasmids, and "do" directed evolution. Daisuke, I am ever so grateful for your friendship, instruction, camaraderie, supportiveness, and truly unique sense of humor.

I wish to specially acknowledge several other members of the Arnold group: to Adam Hartwick, thank you for your friendship and for your ability to discuss carotenoids and their biosynthesis with me as only someone with your experience could. To Michelle Giron and Brenda Parker, thank you both for your contributions to the carotenoids

subgroup and for being such great students to mentor. To Joff Silberg, thank you for your friendship, advice, and the numerous conversations we had about everything from science and politics to family and food. To Michelle Meyer, thank you for being a wonderful officemate, for your friendship, and for all our conversations over the years. To Matt Peters, thank you for being so willing to answer (or attempt to answer) my almost incessant barrage of chemistry-related questions. To Jorge Rodriguez, thank you for your friendship and our many discussions of scientific and personal import. To Allan Drummond, thank you for your contagious passion for science and our fruitful and enlightening discussions. And to Geethani Bandara, thank you for keeping the lab running smoothly, for all your friendly and helpful advice, and for hosting such great parties at your home.

In contrast to my final year, my first four years at Caltech were not spent entirely in the lab. I am grateful to the members of the Caltech Beavers hockey team for their camaraderie and constant supply of memorable moments on and off the ice. In particular, I thank Adam Olsen and David Jenkins for their friendship and hundreds of hours of stimulating banter on the way to and from Sylmar. I would also like to thank my colleagues who served with me on the Caltech Graduate Student Council. My “GSC education” was highly complementary to my research education at Caltech, and I am appreciative of my fellow board members for helping me to improve my “soft” and leadership skills.

When I think about the path of my professional life, two of my teachers stand out as having profoundly influenced my choice to pursue a career in engineering. Charles Ledger and Fraser Simpson, my junior high school mathematics teachers, were truly in a

class of their own. They ignited my passion for math and problem-solving with their contagious enthusiasm and love for the subject as well as their innovative teaching methods (dissatisfied with the textbooks of the day, they designed their own curriculum and somehow convinced the school board to let them teach it). I might have pursued the same career path had I not been taught by Mr. Simpson and Mr. Ledger, but I doubt I would have done so with quite the same vigor.

To J.R., I am much obliged to you for your sage guidance and for improving my self-awareness. Your attentive ear and knowledgeable advice have assisted me through many of life's trials and have helped me to tame my worries. I am no doubt a better person to myself and others because of you.

My parents, Lili and David Tobias, deserve special recognition for their love and guidance and for encouraging me to explore my curiosity about the world in myriad ways. Mom and Dad, the lessons you worked so hard to teach me have served me well: finish your homework before you play, do it right the first time, respect other people and their property, help others in need, and wait for things to go on sale. Thank you for giving me just the right amount of freedom and responsibility when I was growing up so that I could learn to be independent but not get into too much trouble.

To Mikael, thank you for being the best brother anyone could ask for. I think part of our special bond is rooted in our choice of careers, which are quite different but also appealing to both of us. This allows us to live vicariously through each other, so that I get a taste of the life of a musician and you can experience a bit of life as a scientist. Although we haven't lived in the same city for some time now, I can't imagine life without you and I am grateful for our e-mails, telephone calls, and visits.

To my dearest Jill, thank you for your boundless love, compassion, understanding, and support. I feel the bond of our marriage grow stronger every day, and to be your husband is the ultimate privilege. Thank you for cheering me up when my experiments weren't working, for bringing me dinner at the lab when I could take only a short break, and for waiting for me all those times when I needed "just another 15 minutes" before I could come home. Thank you also for celebrating my successes and milestones with me, but more importantly, for insisting that these moments be celebrated when I didn't feel up to it at first. For all you have contributed, I feel like we have completed this thesis together. As we embark on the next chapter of our lives, I look forward to all the new experiences, moments, and memories that I will have the honor of sharing with you.

ABSTRACT

Over the course of evolution, nature continually discovers new small molecules through the alteration of biosynthetic enzymes and pathways by mutation and gene transfer. Hundreds of these natural products have proven indispensable to medicine, culture, and technology, greatly contributing to increases in the length and quality of human lives. Chemists have found that the "chemical space" surrounding natural products is especially rich in functional molecules, and synthesis of natural product analogs has uncovered many with new or improved properties.

Inspired by nature's search algorithm, we and others have conducted our own evolution of carotenoid biosynthetic pathways in the laboratory. Chapter 1 comprehensively reviews the motivations, accomplishments, and challenges of this research area as of early 2005, and describes in detail how biosynthetic routes to dozens of new carotenoids have been established.

To expand the number of carotenoid backbones beyond the C_{30} and C_{40} carbon scaffolds that give rise to the ~700 known natural carotenoids, we subjected a carotenoid synthase, the enzyme responsible for carotenoid backbone synthesis, to directed evolution. Chapter 2 describes the evolution of the C_{30} carotenoid synthase CrtM from *Staphylococcus aureus* for the ability to synthesize C_{40} carotenoids. This work also resulted in novel carotenoids with C_{35} backbones. We later found that some of the CrtM mutants generated in this laboratory evolution experiment, as well as several second-generation variants, are also capable of synthesizing unnatural C_{45} and C_{50} carotenoid backbones when supplied with appropriate prenyl diphosphate precursors.

Chapter 3 describes the creation of full-fledged pathways to carotenoid pigments based on the C_{45} and C_{50} scaffolds. Coexpression of the carotenoid desaturase CrtI from *Erwinia uredovora* resulted in the biosynthesis of at least 10 new C_{45} and C_{50} carotenoids with different systems of conjugated double bonds. We also present evidence of an unnatural asymmetric C_{40} carotenoid pathway beginning with the condensation of farnesyl diphosphate (FPP, C_{15}PP) and farnesylgeranyl diphosphate (FGPP, C_{25}PP). In addition to clarifying how CrtM and CrtI achieve their product specificities, this work also sheds light on the molecular mechanisms used by evolution to access new chemical diversity and the selective pressures that have shaped natural product biosynthesis.

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