Photoprotection, Photoinhibition, Gene Regulation, and Environment

Advances in Photosynthesis and Respiration

VOLUME 21

The scope of our series, beginning with volume 11, reflects the concept that photosynthesis and respiration are intertwined with respect to both the protein complexes involved and to the entire bioenergetic machinery of all life. *Advances in Photosynthesis and Respiration* is a book series that provides a comprehensive and state-of-the-art account of research in photosynthesis and respiration. Photosynthesis is the process by which higher plants, algae, and certain species of bacteria transform and store solar energy in the form of energy-rich organic molecules. These compounds are in turn used as the energy source for all growth and reproduction in these and almost all other organisms. As such, virtually all life on the planet ultimately depends on photosynthetic energy conversion. Respiration, which occurs in mitochondrial and bacterial membranes, utilizes energy present in organic molecules to fuel a wide range of metabolic reactions critical for cell growth and development. In addition, many photosynthetic organisms engage in energetically wasteful photorespiration that begins in the chloroplast with an oxygenation reaction catalyzed by the same enzyme responsible for capturing carbon dioxide in photosynthesis. This series of books spans topics from physics to agronomy and medicine, from femtosecond processes to season long production, from the photophysics of reaction centers, through the electrochemistry of intermediate electron transfer, to the physiology of whole organisms, and from X-ray crystallography of proteins to the morphology of organelles and intact organisms. The goal of the series is to offer beginning researchers, advanced undergraduate students, graduate students, and even research specialists, a comprehensive, up-to-date picture of the remarkable advances across the full scope of research on photosynthesis, respiration, and related processes.

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Photoprotection, Photoinhibition, Gene Regulation, and Environment

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Springer

Library of Congress Control Number: 2008936125

ISBN 978-1-4020-9281-7 (PB)
ISBN 978-1-4020-3564-7 (HB)
ISBN 978-1-4020-3579-1 (e-book)

Published by Springer,
P.O. Box 17, 3300 AA Dordrecht, The Netherlands.

www.springer.com

Cover Credit:
Cover photograph of Douglas fir on the north-facing slope of Gregory Canyon, Colorado, on a late afternoon during winter by William Adams. Scheme by Barbara Demmig-Adams and William Adams.

Printed on acid-free paper

From the Series Editor

Advances in Photosynthesis and Respiration, Volume 21

I am delighted to announce the publication, in *Advances in Photosynthesis and Respiration* (AIPH) Series, of ***Photoprotection, Photoinhibition, Gene Regulation, and Environment***, a book covering the central role of excess light in how plants monitor, and respond to environmental changes. This volume was edited by three distinguished authorities, all based in the USA, Barbara Demmig-Adams, William W. Adams III, and Autar K. Mattoo. Two earlier AIPH volumes dealt with the topics of Environment and Regulation: *Photosynthesis and the Environment* (1996; edited by Neil R. Baker, from UK); and *Regulation of Photosynthesis* (2001; edited by Eva-Mari Aro and Bertil Andersson, from Finland and Sweden). The current volume follows the 20 volumes listed below.

Published Volumes (1994–2005)

- *Volume 1:* ***Molecular Biology of Cyanobacteria*** (28 Chapters; 881 pages; 1994; edited by Donald A. Bryant, from USA);
- *Volume 2:* ***Anoxygenic Photosynthetic Bacteria*** (62 Chapters; 1331 pages; 1995; edited by Robert E. Blankenship, Michael T. Madigan and Carl E. Bauer, from USA);
- *Volume 3:* ***Biophysical Techniques in Photosynthesis*** (24 Chapters; 411 pages; 1996; edited by the late Jan Amesz and the late Arnold J. Hoff, from The Netherlands);
- *Volume 4:* ***Oxygenic Photosynthesis: The Light Reactions*** (34 Chapters; 682 pages; 1996; edited by Donald R. Ort and Charles F. Yocum, from USA);
- *Volume 5:* ***Photosynthesis and the Environment*** (20 Chapters; 491 pages; 1996; edited by Neil R. Baker, from UK);
- *Volume 6:* ***Lipids in Photosynthesis: Structure, Function and Genetics*** (15 Chapters; 321 pages; 1998; edited by Paul-André Siegenthaler and Norio Murata, from Switzerland and Japan);
- *Volume 7:* ***The Molecular Biology of Chloroplasts and Mitochondria in Chlamydomonas*** (36 Chapters; 733 pages; 1998; edited by Jean David Rochaix, Michel Goldschmidt-Clermont and Sabeeha Merchant, from Switzerland and USA);
- *Volume 8:* ***The Photochemistry of Carotenoids*** (20 Chapters; 399 pages; 1999; edited by Harry A. Frank, Andrew J. Young, George Britton and Richard J. Cogdell, from USA and UK);
- *Volume 9:* ***Photosynthesis: Physiology and Metabolism*** (24 Chapters; 624 pages; 2000; edited by Richard C. Leegood, Thomas D. Sharkey and Susanne von Caemmerer, from UK, USA and Australia);
- *Volume 10:* ***Photosynthesis: Photobiochemistry and Photobiophysics*** (36 Chapters; 763 pages; 2001; authored by Bacon Ke, from USA);
- *Volume 11:* ***Regulation of Photosynthesis*** (32 Chapters; 613 pages; 2001; edited by Eva-Mari Aro and Bertil Andersson, from Finland and Sweden);
- *Volume 12:* ***Photosynthetic Nitrogen Assimilation and Associated Carbon and Respiratory Metabolism*** (16 Chapters; 284 pages; 2002; edited by Christine Foyer and Graham Noctor, from UK and France);
- *Volume 13:* ***Light Harvesting Antennas*** (17 Chapters; 513 pages; 2003; edited by Beverley Green and William Parson, from Canada and USA);
- *Volume 14:* ***Photosynthesis in Algae*** (19 Chapters; 479 pages; 2003; edited by Anthony Larkum, Susan Douglas and John Raven, from Australia, Canada and UK);
- *Volume 15:* ***Respiration in Archaea and Bacteria: Diversity of Prokaryotic Electron Transport Carriers*** (13 Chapters; 326 pages; 2004; edited by Davide Zannoni, from Italy);
- *Volume 16:* ***Respiration in Archaea and Bacteria 2: Diversity of Prokaryotic Respiratory Systems*** (13 Chapters; 310 pages; 2004; edited by Davide Zannoni, from Italy);
- *Volume 17:* ***Plant Mitochondria: From Genome to Function*** (14 Chapters; 325 pages; 2004; edited

by David A. Day, A. Harvey Millar and James Whelan, from Australia);

- *Volume 18:* ***Plant Respiration: From Cell to Ecosystem*** (13 Chapters; 250 pages; 2005; edited by Hans Lambers, and Miquel Ribas-Carbo, 2005; from Australia and Spain).
- *Volume 19:* ***Chlorophyll a Fluorescence: A Signature of Photosynthesis*** (31 Chapters; 817 pages; 2004; edited by George C. Papageorgiou and Govindjee, from Greece and USA); and
- *Volume 20:* ***Discoveries in Photosynthesis*** (111 Chapters; 1262 pages; 2005; edited by Govindjee, J. Thomas Beatty, Howard Gest and John F. Allen, from USA, Canada and Sweden (& UK)).

In addition, *Volume 22* (**Photosystem II: The Light-Driven Water:Plastoquinone Oxidoreductase** (34 Chapters, xxvii + 16 color plates + 786 pp., edited by Thomas J. Wydrzynski and Kimiyuki Satoh, from Australia and USA) has already been published in 2005.

Further information on these books and ordering instructions can be found at <http://www.springeronline.com> under the Book Series 'Advances in Photosynthesis and Respiration'. Special discounts are available for members of the International Society of Photosynthesis Research, ISPR (<http://www.photosynthesisresearch.org/>).

Photoprotection, Photoinhibition, Gene Regulation, and Environment

This book was edited by three outstanding authorities in the areas of Photoprotection, Photoinhibition, Gene Regulation, and Environment: Barbara Demmig-Adams and William W. Adams III (both at the University of Colorado, Boulder, Colorado) and Autar K. Mattoo (Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, Maryland).

The topic of the book, as provided by our 3 distinguished editors, is: "*Photoprotection, Photoinhibition, Gene Regulation, and Environment*"; it examines the processes whereby plants monitor environmental conditions and orchestrate their response to change, an ability paramount to the life of all plants. 'Excess light', absorbed by the light-harvesting systems of photosynthetic organisms, is an integrative indicator of the environment, communicating the presence of intense light and any conditions unfavorable for growth and photosynthesis. Key plant responses are photoprotection and photoinhibition. In this volume, the dual role of photoprotective responses in the preservation of leaf integrity and in redox signaling networks modulating stress acclimation, growth, and development is addressed. In addition, the still unresolved impact of photoinhibition on plant survival and productivity is discussed. Specific topics include dissipation of excess energy via thermal and other pathways, scavenging of reactive oxygen by antioxidants, proteins key to photoprotection and photoinhibition, peroxidation of lipids, as well as signaling by reactive oxygen, lipid-derived messengers, and other messengers that modulate gene expression. Approaches include biochemical, physiological, genetic, molecular, and field studies, addressing intense visible and ultraviolet light, winter conditions, nutrient deficiency, drought, and salinity. This book is directed toward advanced undergraduate students, graduate students, and researchers interested in Plant Ecology, Stress Physiology, Plant Biochemistry, Integrative Biology, and Photobiology."

"*Photoprotection, Photoinhibition, Gene Regulation, and Environment*" has 21 authoritative Chapters, and is authored by 57 international authorities from 16 countries. The book begins with three perspectives: Harry Yamamoto (USA) presents a random walk to and through the xanthophyll cycle (*Chapter 1*); Barry Osmond and Britta Förster (Australia) provide an account of Photoinhibition: then and now (*Chapter 2*); Marvin Edelman and Autar Mattoo (Israel and USA) discuss the past and future perspectives of the involvement of the D1 protein in photoinhibition (*Chapter 3*). These perspectives are followed by 18 chapters. In *Chapter 4*, Barbara Demmig-Adams, Volker Ebbert, Ryan Zarter and William Adams (USA) summarize characteristics and species-dependent employment of flexible versus sustained thermal dissipation and photoinhibition. Then, William Adams, C. Ryan Zarter, Kristine Mueh, Véronique Amiard and Barbara Demmig-Adams (USA) discuss details of energy dissipation and photoinhibition as a continuum of protection (*Chapter 5*). In *Chapter 6,* Fermín Morales, Anunciación Abadía and Javier Abadía (Spain) discuss photoinhibition and photoprotection under nutrient deficiencies, drought, and salinity. This is followed by a summary, by Donat-P. Häder (Germany), of photoinhibition and UV responses in the aquatic environment (*Chapter 7*); and a discussion, by Alexander V. Vener (Sweden), of phosphorylation of thylakoid proteins (*Chapter 8*). In *Chapter 9*, Hou-Sung Jung

and Krishna K. Niyogi (USA) provide a molecular analysis of photoprotection of photosynthesis. Stefan Jansson (Sweden) discusses the saga of a protein family involved in light harvesting and photoprotection (*Chapter 10*). In *Chapter 11*, a team of 10 authors (Norman Huner, Alexander Ivanov, Prafullachandra Sane, Tessa Pocock, Marianna Król, Andrius Balseris, Dominic Rosso, Leonid Savitch, Vaughan Hurry and Gunnar Öquist (Canada, Sweden and India) discuss the role of reaction center quenching versus antenna quenching in the photoprotection of Photosystem II. Then, Kittisak Yokthongwattana and Anastasios Melis (Thailand and USA) discuss, in *Chapter 12*, the mechanism of a Photosystem II damage and repair cycle involved in photoinhibition (and its recovery) in oxygenic photosynthesis. Subsequently, regulation by environmental conditions of the repair of Photosystem II in cyanobacteria is discussed by Yoshitaka Nishiyama, Suleyman Allakhverdiev and Norio Murata (Japan and Russia) in *Chapter 13*. Tsuyoshi Endo and Kozi Asada (Japan) provide, in *Chapter 14*, an understanding of the role of cyclic electron flow and the so-called water-water cycle in photoprotection, particularly around Photosystem I. This is followed by *Chapter 15* on the integration of signaling in antioxidant defenses by Philip Mullineaux, Stanislaw Karpinski and Gary Creissen (UK and Sweden). *Chapter 16*, by Christine Foyer, Achim Trebst and Graham Noctor (UK, Germany and France), deals with signaling and integration of defense functions of tocopherol, ascorbate, and glutathione. Then, in *Chapter 17*, Sacha Baginsky and Gerhard Link (Switzerland and Germany) discuss redox regulation of chloroplast gene expression. Robert Larkin (USA) provides, in *Chapter 18*, a summary of intracellular signaling and chlorophyll synthesis. Nine authors (Karl-Josef Dietz, Tina Stork, Iris Finkemeier, Petra Lamkemeyer, Wen-Xue Li, Mohamed El-Tayeb, Klaus-Peter Michel, Elfriede Pistorius, and Margarete Baier), from Germany and Egypt, discuss, in *Chapter 19*, the role of peroxiredoxins in oxygenic photosynthesis of cyanobacteria and of higher plants and pose the question of the importance of peroxide detoxification or redox sensing in the process. *Chapter 20*, by Mauro Maccarrone (Italy), reviews lipoxygenases, apoptosis, and the role of antioxidants. The book ends appropriately with *Chapter 21*, by Christiane Reinbothe and Steffen Reinbothe (Germany and France), on the regulation of photosynthetic gene expression by the environment from the seedling de-etiolation stage to leaf senescence.

A Bit of Early History – From there to here

"*It is a noble employment to rescue from oblivion those who deserve to be remembered*" (Pliny the Younger, Letters V).

In 1996, the grand young man of Photosynthesis Jack Myers wrote about the findings in his PhD thesis 65 years ago (Country boy to scientist. Photosynth. Res. 50: 195–208, 1996.) "*Cranking up for my first real experiments was an exciting day. Carefully pipette a cell sample into the Warburg vessel and let it come to temperature in darkness. Then turn on the projection lamp to give a bright light spot already measured at 38 000 foot-candles, almost 4 times as bright as sunlight. . . . That first experiment was a complete bust. There was only a short burst of the increasing pressure I expected. Thereafter, the pressure change became negative in evidence of oxygen uptake. Something was wrong. So I repeated the procedure with the same result. Only when the intensity was much reduced (1000 foot-candles, by wire screens) did I see the expected high and steady rate of oxygen evolution. Though it took a lot of confirming and polishing experiments, that was an exciting day in the life of a young photosynthetiker. I had made a discovery. I knew something unknown to anyone else in the world. That had been my romantic vision of the fruit of research. And it has not changed in the sixty years since.*" This experiment was published by Jack Myers and George O. Burr (Some effects of high light intensity in Chlorella. Jour. Gen. Physiol. 24: 45–67, 1940)—the discovery of inhibition of photosynthesis by high light, the phenomenon of photoinhibition, but without its name. Only in 1956, did Bessel Kok (On the inhibition of photosynthesis by intense light. Biochim. Biophys. Acta 21: 234–244, 1956) characterize this phenomenon in an elegant manner. Itzhak Ohad and his coworkers (N. Adir, H. Zer, S. Scochat and I. Ohad: Photoinhibition-a historical perspective. Photosynth. Res. 76: 343–370, 2003) have written a current history of photoinhibition. In addition, I mention the personal perspective by Barbara Demmig-Adams (Linking the xanthophyll cycle with thermal energy dissipation. Photosynth. Res. 76: 73–80, 2003). Photographs shown in these two latter papers and in Govindjee and Manfredo Seufferheld (Non-photochemical quenching of chlorophyll a fluorescence: early history and characterization of two xanthophyll cycle mutants of *Chlamydomonas reinhardtii*. Funct. Plant Biol. 29: 1141–1155, 2002) are worth seeing and enjoying. And while much important work on

the role of the xanthophyll cycle has been done over many years, it is only now that the nature of the role of zeaxanthin in the de-excitation of chlorophyll is being identified (see N.E. Holt, D. Zigmantas, L. Valkunas, X.-P. Li, K.K. Niyogi and G.R. Fleming: Carotenoid cation formation and the regulation of photosynthetic light harvesting. Science 307: 433–436, 2005).

There have been many books, many chapters in several books, many reviews, and an enormous number of papers in the field of '*Photoinhibition and Photoprotection*'. I do mention, for historical reasons, an edited book, published 18 years ago, in 'Topics of Photosynthesis' (Volume 9, Series Editor James Barber): David Kyle, Barry Osmond and Charles Arntzen (eds) (1987) 'Photoinhibition', Elsevier, Amsterdam (307 pages; Foreword is by Jack Myers; it has 11 Chapters, including chapters by C.B. Osmond, G. Öquist, K. Asada and N. Murata who are also authors in the current book).

Future AIPH Books

The readers of the current series are encouraged to watch for the publication of the forthcoming books (not necessarily arranged in the order of future appearance):

- **Photosystem I: The Light-Driven Plastocyanin: Ferredoxin Oxidoreductase** (Editor: John Golbeck);
- **The Structure and Function of Plastids** (Editors: Robert Wise and J. Kenneth Hoober);
- **Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications** (Editors: Bernhard Grimm, Robert J. Porra, Wolfhart Rüdiger and Hugo Scheer);
- **Biophysical Techniques in Photosynthesis. II.** (Editors: Thijs J. Aartsma and Jörg Matysik);
- **Photosynthesis: A Comprehensive Treatise; Physiology, Biochemistry, Biophysics, and Molecular Biology, Part 1** (Editors: Julian Eaton-Rye and Baishnab Tripathy); and
- **Photosynthesis: A Comprehensive Treatise; Physiology, Biochemistry, Biophysics, and Molecular Biology, Part 2** (Editors: Baishnab Tripathy and Julian Eaton-Rye)

In addition to these contracted books, we are already in touch with prospective Editors for the following books:

- Anoxygenic Photosynthetic Bacteria. II
- Chloroplast Bioengineering
- Molecular Biology of Cyanobacteria. II.
- Protonation and ATP Synthases
- Genomics and Proteomics
- Sulfur Metabolism in Photosynthetic Organisms

Other books, under discussion, are: Molecular Biology of Stress in Plants; Global Aspects of Photosynthesis and Respiration; and Artificial Photosynthesis. Readers are encouraged to send their suggestions for these and future volumes (topics, names of future editors, and of future authors) to me by E-mail (gov@uiuc.edu) or fax (1-217-244-7246).

In view of the interdisciplinary character of research in photosynthesis and respiration, it is my earnest hope that this series of books will be used in educating students and researchers not only in Plant Sciences, Molecular and Cell Biology, Integrative Biology, Biotechnology, Agricultural Sciences, Microbiology, Biochemistry, and Biophysics, but also in Bioengineering, Chemistry, and Physics.

I take this opportunity to thank Barbara Demmig-Adams, William W. Adams III, and Autar K. Mattoo for their outstanding and painstaking editorial work. I thank all the 57 authors of volume 21: without their authoritative chapters, there would be no such volume. I owe Jacco Flipsen and Noeline Gibson (both of Springer) special thanks for their friendly working relation with us that led to the production of this book. Thanks are also due to Jeff Haas (Director of Information Technology, Life Sciences, University of Illinois at Urbana-Champaign, UIUC), Evan DeLucia (Head, Department of Plant Biology, UIUC) and my dear wife Rajni Govindjee for their constant support.

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A historical group photograph taken at the wedding of Adam Gilmore and Xiao-Ping Li (29 June, 2003). From left to right: Govindjee; Olle Björkman; Harry Yamamoto; Adam; Xiao-Ping; Krishna Niyogi; and Barry Osmond. Background, Lake Tahoe, California. Photo by Rajni Govindjee.

Govindjee is Professor Emeritus of Biochemistry, Biophysics and Plant Biology at the University of Illinois at Urbana-Champaign (UIUC), Illinois, USA, since 1999. He obtained his B.Sc. (Chemistry and Biology) and M.Sc. (Botany, specializing in Plant Physiology) in 1952 and 1954, respectively, from the University of Allahabad, Allahabad, India. Govindjee was a graduate student, first of Robert Emerson and then of Eugene Rabinowitch, receiving his Ph.D. in Biophysics from the UIUC in 1960. His honors include: Fellow of the American Association for the Advancement of Science (1976); Distinguished Lecturer of the School of Life Sciences, UIUC (1978); President of the American Society of Photobiology (1980–1981); Fulbright Senior Lecturer (1996–1997); and honorary President of the 2004 International Photosynthesis Congress (Montréal, Canada). Govindjee's research has focused on the function of "Photosystem II" (water-plastoquinone oxido-reductase), particularly on its primary photochemistry; the unique role of bicarbonate in electron and proton transport; as well as thermoluminescence, delayed and prompt fluorescence (particularly lifetimes), and their use in understanding electron transport and photoprotection against excess light. His major contribution on the topic of this book has been to use lifetime of chlorophyll a fluorescence measurements, with Adam Gilmore, to demonstrate the 'dimmer-switch' of thermal dissipation (see e.g., Gilmore et al. (1995) Proc. Natl. Acad. Sci. USA 92: 2273–2277; Gilmore et al. (1998) Biochemistry 37: 13582–13593) and monitor, with Oliver Holub and others, zeaxanthin-dependent quenching of chlorophyll a fluorescence using FLIM (Fluorescence Lifetime Imaging Microscopy) in single cells of *Chlamydomonas reinhardtii* (Holub et al. (2000) Photosynthetica 38: 583–601). Some of his reflections on the topic of 'photoprotection' may be found in Govindjee (2002) Plant Cell 14: 1663–1668; and in Govindjee and M. Seufferheld (2002) Functional Plant Biology 29: 1141–1155. Govindjee's scientific interests now include regulation of excitation energy transfer in oscillating light (L. Nedbal et al. (2003) Biochim. Biophys. Acta 1607:5–7). However, his major focus is on the "History of Photosynthesis Research" (see volumes 73 (2002), 76 (2003), and 80 (2004) of *Photosynthesis Research*), and in Photosynthesis Education. In addition to being the Series Editor of *Advances in Photosynthesis and Respiration*, he serves as the "Historical Corner" Editor of "*Photosynthesis Research*". For further information, see his web page at: http://www.life.uiuc.edu/govindjee.

Contents

Preface

Photosynthesis is integral to plant productivity, both as a source of energy and materials and as a target for feedback regulation by the demand of the whole plant for photosynthate. Photosynthesis is also strongly modulated by the environment; multiple environmental conditions result in the absorption of potentially harmful levels of excess light. Photoprotection and the phenomenon of photoinhibition of photosynthesis are thus key responses of plants to the environment as well. However, it remains unknown whether photoinhibition decreases or increases plant survival, fitness, and productivity. Similarly, interactions among different components of the photoprotective antioxidant network and their roles in cellular signaling and gene expression remain to be fully elucidated. This volume (*Photoprotection, Photoinhibition, Gene Regulation, and Environment*) brings together contributions from widely different areas in the hope of stimulating future research. Several concepts are emphasized in this volume 21 of Advances in Photosynthesis and Respiration. One is that chloroplast defenses against oxidative stress and excess reactive oxygen production are highly integrated with each other and are in communication with the cellular antioxidant network. Furthermore, it is increasingly recognized that antioxidants have a crucial role in redox sensing and signaling, in addition to their role in protecting the integrity of the chloroplast. This is highly significant since cellular redox balance participates in the modulation of a host of key responses in growth and development, such as the regulation of the cell cycle, senescence, and programmed cell death. This volume combines contributions on photoinhibition and photoprotection with those on redox signaling and gene modulation to integrate photoprotective responses into 'the bigger picture'. We bring together the full continuum of processes from protection on one hand to senescence and cell death at the other extreme.

Specific topics covered here include perspectives on the historic development of this research area. Photoprotective thermal dissipation of excess excitation energy is reviewed and its relationship to photoinhibition discussed. What role does photoinhibition play in plant survival, fitness, and productivity? Where does photoinhibition fit into the continuum of plant responses-from photoprotection by a cascade of defense mechanisms to cell death? During seasons with extreme conditions, such as icy winters or scorching, dry summers, the primary reactions of photosynthesis can become disabled. Many studies focus on the inactivation of the photosystem II core protein D1 seen during photoinhibition, and it is currently widely assumed that this inactivation represents damage. However, much of what has been assumed to be damage to chloroplast processes may, in fact, be caused by genetic programs (D. Wagner, D. Przybyla, R. op den Camp, C. Kim, F. Landgraf, K. P. Lee, M. Würsch, C. Laloi, M. Nater, E. Hideg and K. Apel, 2004, The Genetic Basis of Singlet Oxygen–Induced Stress Responses of *Arabidopsis thaliana*. Science 306: 1183–1185). It is noteworthy that the plants exhibiting the greatest degree of photoinhibition of photosynthesis are perennial evergreens adapted to extreme environments. Is photoinhibition a limitation to plant productivity, or might it be a 'talent' of stress-tolerant species? In this volume, widely different views are expressed concerning these questions, making it clear that additional work is needed to resolve them.

A wide range of environmental factors that affect photoprotection and photoinhibition is considered here: intense visible and ultraviolet light, winter conditions in temperate climates, nutrient deficiency, drought, and salinity. A molecular analysis of photoprotection is presented and the role of specific proteins in photoprotection is discussed. In addition to thermal dissipation pathways, alternative pathways for electron flow as well as the scavenging of reactive oxygen species by antioxidant metabolites (such as tocopherol, asccorbate, glutathione, xanthophylls) and enzymatic antioxidant pathways are reviewed. The peroxidation and recycling of lipids is considered together with the signaling functions of these reactions and of lipid-derived messengers. A comprehensive overview of the current understanding of signaling by reactive oxygen species and other signals that modulate gene expression is provided. Experimental approaches range from biochemical, physiological, genetic, and molecular approaches to field studies in a variety of natural environments.

This volume is suitable for advanced students and researchers interested in the general area of redox signaling and antioxidant defenses and their role in responses to the environment as well as the regulation of internal responses of all organisms. In the field of plant

biology, the areas of photosynthesis, stress physiology, gene regulation by the environment, and growth and development are particularly relevant.

We express our sincere appreciation to the 57 authors from 15 countries for their outstanding contributions to this book. We are thankful to the Series Editor Govindjee for his invitation to develop this exciting book and for his support. We are grateful to Noeline Gibson and Jacco Flipsen (both of Springer) for their cooperation in producing this book.

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William Adams, Barbara Demmig-Adams, Melanie Adams, and Robert Adams, during a trip to Barbara's native Germany during the summer of 2004. Photograph by Markus Demmig.

Barbara Demmig-Adams and **William W. Adams III** are Professors in the Department of Ecology and Evolutionary Biology at the University of Colorado at Boulder, USA. Barbara, as a native of Germany, received both her undergraduate degree (1979) in biology and chemistry and her graduate degree (1984) in plant physiology (with the late Prof. Hartmut Gimmler) from the Universität Würzburg. She subsequently spent two eventful years (1984–1986) as a postdoctoral fellow in the laboratory of Professor Olle Björkman at the Carnegie Institution of Washington's Department of Plant Biology in Stanford, California. Barbara and Olle Björkman characterized the photoprotective thermal energy dissipation that occurs in the antenna pigments of photosystem II, including the fact that this process can become sustained in evergreens under stress and lower the photon yield of photosystem II for prolonged periods. William attended the University of Kansas, receiving undergraduate degrees in biology (1981) and in atmospheric sciences (1983) as well as an MA degree (1984) in botany (investigating adaptations of epiphytic bromeliads from Mexico). This was followed by 18 months (1984–1985) of research in Reno, Nevada and Death Valley, California as the first half of his PhD work. During this time, William and Barbara began to collaborate personally and professionally across the Sierra Nevada divide that separated Reno and Stanford. Barbara returned to Würzburg in 1986 and provided the first evidence that the xanthophyll zeaxanthin, formed via the xanthophyll cycle under excess light, is involved in thermal energy dissipation. For a more detailed description of the discovery of the role of the xanthophyll cycle in thermal dissipation, see her historical minireview in Photosynthesis Research (2003) 76:73–80. While Barbara was in Germany pursuing the role of zeaxanthin, William completed another 17 months of research in Canberra and at three field sites in Australia, resulting in a PhD (1987) from the Australian National University under Professor C. Barry Osmond's mentorship. This work centered on photoinhibition in CAM plants, and included the first reports of photoinhibition and photoprotection under natural conditions in the field. With the support of a NATO postdoctoral fellowship and a fellowship from the Alexander von Humboldt Foundation, William then spent two years at the Universität Würzburg, where the personal and professional collaboration between him and Barbara Demmig became more firmly established. In the spring of 1988, Barbara completed her habilitation in plant biology (at the Lehrstuhl of Professor Otto L. Lange in Würzburg) and Barbara and William made their union official. They moved to the University of Colorado in 1989, brought two children (Robert, born in 1990, and Melanie, born in 1992) into the world, and continued their collaborative efforts on various aspects of the ecology and physiology of xanthophyll-dependent thermal dissipation. One focus of their work has been the study of unique modifications of photoprotective energy dissipation in evergreen species. They use tropical evergreens as models for the role of sustained thermal dissipation during shade-sun acclimation, and conifers and other evergreens to study its importance in acclimation during Colorado winters. These studies

include ecological, comparative, and mechanistic approaches to integrate photoprotective energy dissipation and photoinhibition into whole plant functioning. William and Barbara have also begun to evaluate the influence of foliar carbon export pathways on the acclimation of photosynthesis and photoprotective or photoinhibitory responses. In addition, Barbara has had a long-standing interest in the role of zeaxanthin and other plant protective compounds in human health (see their paper in Science (2002) 298:2149–2153). Their research has been cited frequently by colleagues, leading to their recognition as highly cited researchers in the Plant & Animal Science category by the Institute for Scientific Information (<http://isihighlycited.com/>). Furthermore, William has been honored for his efforts in teaching plant biology by University of Colorado students (Mortar Board Certificate of Recognition for Exceptional Teaching, 2000) and faculty (Boulder Faculty Assembly Excellence in Teaching Award, 2004). For additional information, see their web site: (http://www.colorado.edu/eeb/EEBprojects/Adams_ Demmig/).

Autar K. Mattoo

Autar K. Mattoo is a senior scientist with the Henry A. Wallace Beltsville Agricultural Research Center, United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Beltsville, Maryland. He is originally from Kashmir, India and received his M.Sc. in Biochemistry (1965) and Ph.D. in Microbiology (1969) from the Maharaja Sayajirao University of Baroda (India). His Ph.D. advisor was Vinod Modi. He was a postdoctoral fellow with Bruce Keech at the University of Adelaide, a visiting scientist with Bob Vickery at the University of New South Wales in Australia, and later a DAAD Fellow with Marvin Edelman at the Weizmann Institute of Science in Israel. Autar's research activities fall under two diverse programs: (1) Molecular aspects of chloroplast function with particular emphasis on the photosystem II (PS II) reaction center proteins; and (2) Regulation of ethylene biosynthesis and fruit ripening. In collaboration with Marvin Edelman, he identified the rapidly turning over D1 – 32 kDa protein as a diuron-modulated PS II protein, elucidated its complete metabolic life history, demonstrated its reversible, photo-regulated post- translational phosphorylation and palmitoylation, and showed that D1 phosphorylation is regulated by a circadian clock. His current interest is in protein kinases, chloroplast-chromoplast differentiation, and hormonal cross talk in fruit development and ripening. In the field of biotechnology, Autar continues to use molecular genetics, nutritional genomics, and transgenic approaches to produce functional foods. He has incorporated research on interfacing genetically enhanced vegetables in sustainable, alternative agriculture systems. He has published over 200 research articles, twice chaired the Gordon Research Conference on Plant Senescence, and served on the Technical Advisory Committee of the US-Israel BARD. He is a member of the Overseas Standing Advisory Committee for the Department of Biotechnology, Government of India (2004–2007). He has lectured widely in the areas of biochemistry, molecular biology, and physiology. He served as Secretary-Treasurer of the Washington Section of the American Society of Plant Physiologists (1987). He was recognized as the Beltsville Area Scientist of the Year (1998), ARS's Distinguished Senior Scientist of the Year (1998), Secretary of Agriculture's "People Making a Difference" award (1999), and USDA Secretary's Honor Award for Scientific Excellence (1999). For further information, see his web site: (http://www.barc.usda.gov/psi/vl/mattoo.htm).

Chapter 1

A Random Walk To and Through the Xanthophyll Cycle

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Summary

This is an account of my personal and professional life as a student of the violaxanthin-antheraxanthin-zeaxanthin scheme for the xanthophyll cycle in higher plants. I had no early vision of becoming a scientist, but one circumstance led to another, and what began as a random walk ultimately developed into a life-long study of the biochemistry, physiology, and function of the xanthophyll cycle. The circumstances and people with whom I shared this path are described, with special attention given to the early developments.

I. Introduction

Does anyone accept an autobiographical assignment such as this without some hesitancy? I appreciated the invitation to tell my story, but wondered if I had anything worth contributing. What should I say? Who would care? After some reflection, I thought (or possibly rationalized) that my story, which is best characterized as a random walk to and through the xanthophyll cycle, may give comfort to young people whose vision of what they wish to accomplish in life may not be entirely clear. The circumstances that led to the discovery of light-induced conversions among violaxanthin (V), antheraxanthin (A), and zeaxanthin (Z)–the VAZ pathway for the xanthophyll cycle–may also be of interest. Although the walk was random, with many small and uncertain steps, it almost always carried me forward and ultimately brought me to the "right" path. As a child and even through college, I had no thoughts about becoming a scientist, only an innate desire to seek a better future as my parents had done. With luck and help from many people, I have been privileged to the better life, better than I could have imagined possible as a child. I extend my special thanks to the book's editors for giving me a chance to reflect and open doors to many good memories.

II. The Beginnings

To start, I can thank my father for my good fortune at being born in the U.S. Dad, the second eldest son, had to seek his independent fortune and immigrated to Hawaii while still a teenager. I grew up in the shadows of the famous Moana Hotel on Waikiki Beach. Perhaps some readers who have visited Waikiki remember the large banyan tree in the International Market Place located cater-corner from the hotel. We lived about three hundred yards from that tree; once, I fell out of it while playing Tarzan and broke my arm. Our home was provided by the hotel because Dad, a carpenter, was on call "24-7." We were poor, but I was not aware of it; my

B. Demmig-Adams, William W. Adams III and A.K. Mattoo (eds), Photoprotection, Photoinhibition, Gene Regulation, and Environment, 1–10.

parents never complained, and all those around us were also poor. It was a happy and carefree time for me.

I was 8 years old when the Japanese attacked Pearl Harbor. Both my parents were treated as aliens even though Mom was a native-born U.S. citizen. Fortunately, we and many others in Hawaii were not sent to the "relocation camps" in which Americans of Japanese descent were detained on the U.S. mainland. In Hawaii, most of us were spared relocation largely by the actions of John Burns who, as police captain in charge of espionage for the FBI, vouched for the loyalty of Japanese-Americans in Hawaii. Burns was Delegate to Congress when Hawaii became the 50th state and later was elected Governor for three terms. He touched many lives. During the war, I carried a gas mask to school and my club house was the underground shelter Dad had built for our safety. The attack on Pearl Harbor had been led by Admiral Yamamoto; although he was no relation, I avoided problems by assuming the name Harry Chang when around soldiers on "R & R" (rest and recuperation) in Waikiki.

As a child I must have shown an interest in science because one of the best Christmas gifts I recall receiving was a Gilbert Chemistry Set. I can still picture it. It came in a red fold-out wooden case with rows of chemicals in small bottles, a simple balance, a watch glass, and spatulas. It had a manual from which I learned to make, among other things, black powder and "stink bombs." My parents weren't always pleased with the results of my experiments. These types of sets may not be available today and, if they are, their contents are probably more limited given modern concerns about hazardous substances.

III. Education

My friends are the reason I went to college. I took the entrance examination to the University of Hawaii only because they did. A few months later, I enrolled as a freshman and chose medical technology as my major because that is what a friend had selected and, much to my liking, it had a strong science emphasis. The course load was so heavy in zoology, microbiology, and chemistry that it nearly met the major's requirement for each of those fields. However, botany was not required for obvious reasons: medical technology deals with sick people, not sick plants. I didn't know then that I would spend my entire professional life happily working on plants.

The senior year in medical technology consisted of laboratory rounds at hospitals, public health laboratories, and the blood bank. During that year, I took night calls at Kuakini Hospital on alternate nights to earn my tuition for the year. Working as I did, I learned that the field of medical technology, as least at the time, offered limited economic opportunities. This important realization probably came about because by then I had a steady girl friend and was thinking about how to become a good provider. After graduating with a B.S. (1955) and completing a six-month tour of duty as a 2nd lieutenant with the U.S. Army Infantry in Ft. Benning, Georgia and Fort Riley, Kansas as part of my eight-year obligation in the Army Reserves, I embarked on the next leg of my random walk. I enrolled in the M.S. program in the Department of Food Technology at the University of Illinois at Urbana-Champaign. The selection of food technology as a field of study is not as curious as it may seem. I had worked in the Del Monte pineapple cannery for three summers prior to my senior year and was promoted each year to a better paying and more responsible position. I could see that a large food processing company offered many opportunities and thought that an advanced degree in the field would be useful. In changing to food technology, I accepted the possibility of not being able to return to Hawaii since most major food industries, except for pineapple processing, were on the mainland. It was a risk that I was willing to take. As it turned out, the greater risk was the demise of the pineapple canning industry in Hawaii, brought about by foreign competition. With one exception on the island of Maui, the canneries have all since closed.

Attending the University of Illinois was a good decision in several ways. First, I learned that, contrary to what I had assumed, changing fields of study was relatively easy. I discovered it wasn't necessary to complete all the requirements of the previous degree before starting work on a higher degree. Being the only one of three siblings to pursue graduate studies, I hadn't known any better. Next, during my first meeting to discuss my academic program with Reid Milner, Chairman of the Department of Food Technology, he casually asked if I intended to go on for the Ph.D. Me, whose parents had little schooling, who went to college only because his friends were going, and who had decided to pursue the M.S. only as a means for gainful employment? It was an unexpected and welcome expression of confidence in my potential. Thank you Prof. Milner! Finally, while pursuing the M.S., I found that I was more interested in "Why?" than "How?" and preferred fundamentals to applications. The title of my M.S. thesis was "*Kinetic Studies on the Heat Inactivation of Peroxidase in Sweet Corn.*" Peroxidase activity was used, and is

possibly still being used, as an indicator for adequacy of heat treatment (blanching) of corn prior to freezing. Blanching prevents frozen corn from developing undesirable "off" flavors that result from enzyme activity. I found that there were two types of peroxidases with markedly different heat sensitivities (Q_{10}). The inactivation of both forms followed pseudo first-order kinetics, and the stability of the heat-resistant component made heating to inactivate it almost futile. I think it was this study that awakened by interest in basic science. I have the University of Illinois, and the Department of Food Technology in particular, to thank for giving some direction to my random walk.

The University of California, Davis had a program that seemed ideal for me: a Ph.D. in Comparative Biochemistry within the Department of Food Science and Technology. I was married and had a young son by then. The three of us drove for California, pulling our worldly possessions in a U-Haul trailer. We felt like pioneers traveling cross-country with an infant, but instead of in a covered wagon, we had an aging Ford. In Lincoln, Illinois, just a hundred miles out of Champaign-Urbana, our car broke down and required, so we were told, a complete engine overhaul that strained our resources and delayed our journey by several days. Even with the repairs, we barely made it over Donner Pass at the border between Nevada and California. A few months ago we drove through that region and the incline was hardly noticeable. Was the U-Haul so heavy, the road now less steep, or the rental car that much better? Perhaps yes to all. Thinking back on it now, that trip in the summer of 1958 was a great adventure and a test of endurance. What better preparation could one have for a doctoral program?

At Davis, my research advisor was Clinton "Chi" Chichester, a student of Gordon Mckinney, both of whom were interested in the biosynthesis of carotenoids. Paul Stumpf was my academic advisor. The biosynthetic pathway for carotenoids was not yet clearly established. During my first year I worked on an early step in the pathway and published a note in *Nature* (Yamamoto et al., 1961).

IV. The VAZ Pathways Story

I owe much of what came next to Sputnik, the first satellite, which was placed into orbit by the Soviet Union in October 1957. This achievement shocked the U.S. into giving more support to science and not just to space science. I benefited from this new commitment through a National Science Foundation predoctoral fellowship that funded the balance of my doctoral program. Besides relieving me of financial worries, the fellowship allowed me more flexibility in selecting a research topic. Also as a result of Sputnik, English translations of Russian articles became available, and a paper by David I. Sapozhnikov was brought to my attention by Tommy Nakayama, a friend and colleague of Chi's (Fig. 1). Sapozhnikov et al. (1957) reported that, in leaves subjected to alternating light and dark treatments, high light induced reciprocal changes in the levels of violaxanthin and lutein. He hypothesized that the reaction was involved in photosynthetic "oxygen transfer," that is, from water to molecular oxygen. I believed the observation merited further study because, unlike most carotenoids that are metabolic end products, this system appeared to be dynamic. Also, if the cycle was indeed involved in photosynthetic oxygen evolution, it would be a very significant discovery. I felt, however, that the reported kinetics made this possibility unlikely. Furthermore, if the reaction was involved in oxygen evolution, it was not an essential pathway since oxygen-evolving organisms such as blue-green algae lack violaxanthin.

Fig. 1. Photograph taken at the National Science Foundation sponsored Carotenoid Symposium in Kyoto, Japan, 1965. Clinton Chichestor is being greeted by Prof. H. Mitsuda. Tom Nakayama is in the background.

Of course, the instrumentation and analytical methods available 45 years ago were crude by today's standards. I used preparative columns packed with powdered sugar to separate the xanthophylls of saponified extracts of leaves. Saponification removed chlorophyll that these columns could not resolve from xanthophylls. To assure complete recovery of xanthophylls after saponification, the xanthophylls were washed into ethyl ether instead of petroleum ether. Safety precautions were not what they are today and I was lucky not

to have blown up the lab and myself with it. I would often return to my apartment reeking of ether. The procedures were so slow that I could obtain barely two sets of data points in a day. Despite these limitations, the very first experiment confirmed that high light induced in leaves a decrease in violaxanthin and an apparently corresponding increase in lutein. However, I was still not fully convinced that a symmetrical reactant, violaxanthin, was being converted to an asymmetrical product, lutein. Two mono-de-epoxidase reactions by different enzymes could explain such a conversion but would not be consistent with Sapozhnikov's hypothesis, which implied a single-step double de-epoxidation. Alternatively, the product could be zeaxanthin rather than lutein, leaving open the possibility of a single-step double de-epoxidation. Since the sugar column resolves pigments by normal-phase partitioning, I reasoned that zeaxanthin would, if formed, likely co-migrate with lutein, given that both molecules have similar structures and identical numbers of hydroxyl groups. Rechromatography of the sugar column's lutein band on a magnesium oxide (adsorption) column showed the product to be zeaxanthin and not lutein. The next question presented itself: does the conversion to zeaxanthin occur in one step or two? Antheraxanthin, the expected product of a two-step reaction, was found on the sugar column as a faint band between violaxanthin and lutein. These results established the currently accepted VAZ scheme: the light-induced cyclical conversion, in leaves, of violaxanthin (V) through antheraxanthin (A) to zeaxanthin (Z) (Yamamoto et al., 1962). Today, the pathway for the cycle seems obvious and can be easily demonstrated by HPLC analyses with a column that has mixed partitioning and absorption properties (Gilmore and Yamamoto, 1991). I referred to the pathway as the "violaxanthin cycle" but now "*the* xanthophyll cycle" is more commonly used. I emphasize "the" to acknowledge that other xanthophyll cycles are known, specifically the diadinoxanthin cycle in several algal species (Hager and Stransky, 1970) and the lutein epoxide cycle in mistletoe (Matsubara et al., 2001). It is uncertain whether these other xanthophyll cycles have the same biochemistry and functions as the VAZ cycle.

The VAZ scheme, in my opinion, was strong evidence against Sapozhnikov's hypothesis. Besides the very slow kinetics and incorporation of ^{18}O from O_2 on re-epoxidation of zeaxanthin (Takeguchi and Yamamoto, 1968), stepwise mono-de-epoxidation excluded a single-step removal of the epoxides of violaxanthin as might be expected for a role in oxygen evolution. Prof. Sapozhnikov acknowledged that the product of violaxanthin was zeaxanthin but, as far as I am aware from the literature, he ignored antheraxanthin and continued to suggest a role for the cycle in oxygen evolution (Sapozhnikov, 1973). Regrettably, I did not get to meet Prof. Sapozhnikov to congratulate him for his initial observation that light induces a change in the violaxanthin concentration. Following the VIIIth International Congress on Photosynthesis, which was held in Stockholm, Sweden in 1989, I went on a tour to the Soviet Union. In St. Petersburg I met a few of Prof. Sapozhnikov's former associates and learned that he had passed away in Italy in 1985 on his way to his new adopted home in Canada. Olga Koroleva gave me a photograph of his group taken in 1974; it is a wonderful photograph and I share it as a tribute to him (Fig. 2). In a different vein, I also express my appreciation to C. Stacy French, who was Director of the Carnegie Institution of Washington (Stanford, California), for his encouragement, patience, and graciousness to an aspiring graduate student. I visited his laboratory several times to learn as much as I could about photosynthesis and became friends for life.

V. Further Adventures and Advances

The notion that pursuing higher education would preclude my returning to Hawaii proved wrong. A year before completing the Ph.D., I was offered and accepted a position in the newly formed Department of Food Science and Technology in the College of Tropical Agriculture at the University of Hawaii. By then I had two children, and a secure job was attractive. For the first few years, I pursued research related to agriculture and refrained from working on the xanthophyll cycle, expecting that another student in Chi's lab would take up the work. When it became clear that no one would, I returned to the xanthophyll cycle, focusing on the biochemistry with the long-range objective of gaining insights into function. During my xanthophyll cycle hiatus (1962–65), Achim Hager made significant progress on the cycle's biochemistry. He showed that violaxanthin de-epoxidase (VDE) was localized in the chloroplast lumen and required ascorbate and low pH for activity (Hager, 1966). The cycle's transmembrane organization (Fig. 3) was established when both groups showed that the reverse epoxidation of zeaxanthin to violaxanthin occurred on the stromal side of the thylakoid at near neutral pH in the presence of NADPH and O_2 (Hager, 1975; Siefermann and Yamamoto, 1975).

Working on the xanthophyll cycle in Hawaii was not easy. Funding was limited, and there were no researchers nearby with whom I could interact that were engaged in related work on photosynthesis or carotenoid biosynthesis. Fortunately, grants from the

Fig. 2. 1974 photograph of David I. Sapozhnikov's group given to me by O. Koroleva when I visited St. Petersburg in 1989. Front row from left: I. Popova, D. I. Sapozhnikov, S. Eidelmann, O. Popova. Back row from left: E. Morkovskaja, M. Gabr, O. Koroleva, T. G. Maslova, and G. Kornjushenko.

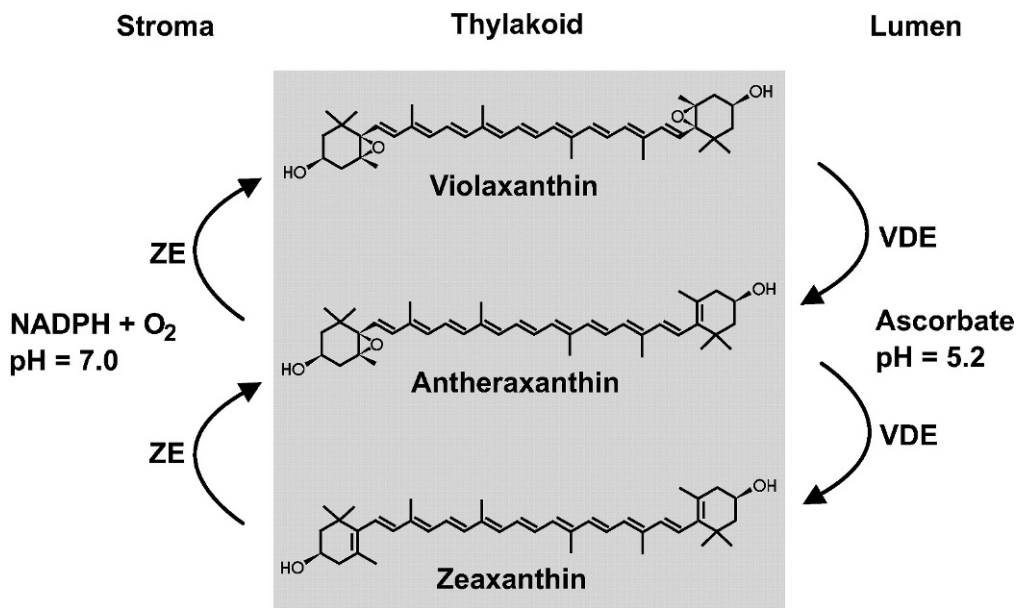

Fig. 3. VAZ transmembrane pathway for the xanthophyll cycle in higher plants.

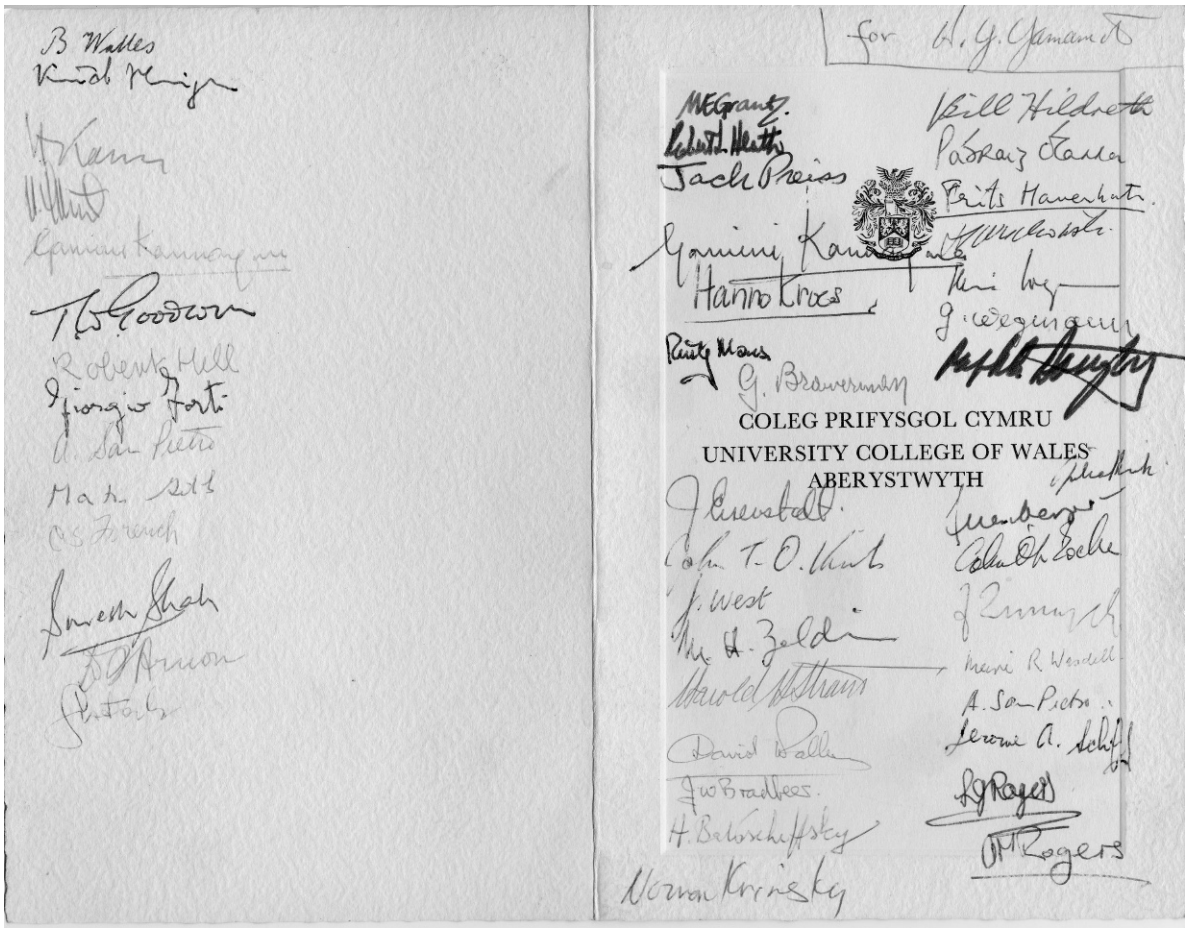

Fig. 4. Autographed banquet menu from the 1965 NATO Advanced Study Institute on the Biochemistry of Chloroplasts in Aberystwyth, Wales. In addition to names already mentioned, signatures by Trevor Goodwin, Giorgio Forti, Martin Gibbs, Norman Krinksy, Harold Strain, Jack Pries and Joseph Bradbeer, among others, are also present. How many signatures can you, the reader, recognize? It was exciting for me to be at this meeting of such notable scientists, most of whom I met for the first time.

National Science Foundation, the U.S.D.A. Competitive Grants Program, and the Department of Energy allowed me to continue research on the VAZ cycle. These grants also enabled me to travel about once a year to a major meeting on photosynthesis. Given my isolation from the mainstream of photosynthesis research, the importance of attending these meetings cannot be overemphasized. The first international meeting I was privileged to attend was the Advance Study Institute on the Biochemistry of Chloroplasts held in Aberystwyth, Wales in 1965, sponsored by the North Atlantic Treaty Organization (NATO). I believe my invitation to attend came from Trevor W. Goodwin. While looking through memorabilia in preparation for this perspective, I found the menu that I had passed around for signatures at the farewell dinner meeting (Fig. 4). I hope readers can make out the names in this marvelous collection of signatures. Among them are Robin Hill, Tony San Pietro, C. Stacy French, Dan Arnon, and many more, with apologies to those I have not mentioned.

Contact with the photosynthesis and plant biochemistry community has been an essential part of my forty-year stroll through the xanthophyll cycle and has created opportunities I might otherwise have missed. For example, in 1968 I spent my first sabbatical with Leo Vernon at the C.F. Kettering Research Laboratory in Yellow Springs, Ohio. There I met Teruo Ogawa, who was completing a postdoctorate with Leo, and with whom I became close personal friends. Teruo introduced me to the "opal glass" spectrophotometric technique perfected by Kazuo Shibata for measurement of light-scattering samples (Shibata, 1973). Upon returning to Hawaii, I applied the technique to chloroplast suspensions and found that violaxanthin de-epoxidation was detectable as a difference spectrum, with a peak at 505 nm, and could also be followed

kinetically at 505 *minus* 540 nm (Yamamoto et al., 1972). This sensitive and rapid method for *in situ* measurement of xanthophyll cycle activity in chloroplasts was key for much of the progress we made during the 30 years that followed. Early applications of the spectrophotometirc assay included the discovery of the "availability" phenomenon, the intensity-dependent fractional release of violaxanthin from the total pool (Siefermann and Yamamoto, 1974); inhibition of VDE by dithiothreitol (Yamamoto and Kamite, 1972); and epoxidation of zeaxanthin to violaxanthin (Siefermann and Yamamoto, 1975). The method was also well suited for *in vitro* studies that demonstrated the requirement of lipid for de-epoxidation of pure violaxanthin (Yamamoto et al., 1974) and the substrate stereospecificity of VDE (Yamamoto and Higashi, 1978). The spectrophotometric assay of VDE activity remains useful to this day. It was recently applied to demonstrate that monogalactosyldiacylglycerol (MGDG), the major thylakoid membrane lipid, has a limited capacity to accommodate zeaxanthin and when this capacity is exceeded, stereospecific product feedback inhibition of VDE results (Hieber et al., 2004).

The serendipitous discovery of MGDG as the optimal chloroplast lipid for *in vitro* de-epoxidation of violaxanthin proved important. While I was able to obtain de-epoxidation of violaxanthin bound in washed thylakoid membranes, the same crude VDE preparation had no activity against purified violaxanthin, as had been reported by Hager (1966). Violaxanthin is insoluble in aqueous buffer, and various attempts to suspend or solubilize violaxanthin in a form that yielded activity failed. Isomerization and decomposition of the preparation were excluded as possible reasons. In the course of these tests, I ran out of the violaxanthin preparation that I had been using and, as a matter of convenience, recovered violaxanthin from "fat plates"* that Dorothea Siefermann, then a postdoctoral researcher in my laboratory, happened to be using for analysis of chloroplast pigments. Violaxanthin that was eluted from these plates with acetone and used without further purification gave rapid and nearly complete conversion to zeaxanthin. The reason for this success was traced not to coconut oil from the plates but rather to a lipid component in the unsaponified extract that co-chromatographed with violaxanthin. C. Freeman Allen earlier had separated the lipids in chloroplasts (Allen et al., 1966) and he kindly sent me samples that he still had on hand. All of the lipid samples we received supported de-epoxidation to varying degrees. We subsequently prepared a complete set of the major chloroplast lipids and found that MGDG was the most effective, giving rapid and complete de-epoxidation of violaxanthin in about 5 minutes under optimal conditions (Yamamoto et al., 1974). These results helped define the *in vivo* substrate of VDE: the violaxanthin that is converted to zeaxanthin is free in the membrane lipid phase rather than bound to pigment proteins. Exchanges between protein-bound pigments and free pigments in the lipid phase are implied. Recently, model systems consisting of soybean phosphatidylcholine only (Grotz et al., 1999) or egg phosphatidylchloline combined with MGDG (Latowski et al., 2002) have confirmed that lipid is required for "activation" of pure violaxanthin. However, de-epoxidation in these presumably bilayer systems were relatively slow and incomplete compared to de-epoxidation in the MGDG micelle system. MGDG constitutes a much larger fraction of the total chloroplast lipid: 60% to phosphatidylcholine's 2% or less (Webb and Green, 1991) and thus the micelle system may more closely approximate the *in situ* environment of free violaxanthin. Whatever model system is employed, violaxanthin should be prepared from saponified extracts to avoid artifacts from even trace amounts of contaminating chloroplast lipid.

We used the pH-dependent binding of VDE to the thylakoid membrane and to MGDG to obtain the partial C-terminal sequence (Rockholm and Yamamoto, 1996), which was then used to clone the gene and express the VDE protein (Bugos and Yamamoto, 1996). The complete sequence showed that VDE was a lipocalin enzyme, the first identified in plants** (Bugos et al., 1998; Yamamoto et al., 1999). This finding confirmed conclusions drawn 20 years earlier–before lipocalins were known to exist–that the shape of the VDE active center resembled a deep well (Yamamoto and Higashi, 1978). The cloned VDE carried out the forward VAZ reaction, providing strong evidence that the reaction could be catalyzed by a single enzyme with mono-de-epoxidase function. Evidence that a single gene product accounted for de-epoxidation was shown by Niyogi et al. (1998), in which a deletion mutation in *Arabidopsis* inhibited all de-epoxidase activity. The cysteine rich domain in the N-terminal sequence and highly charged domain in the C-terminal sequence explained, respectively, the DTT inhibition (Yamamoto and Kamite, 1973) and the pH-dependent membrane binding of VDE (Rockholm and Yamamoto, 1996).

My walk through the xanthophyll cycle took several administrative detours from 1980–82, 1982–86, and 1994–96 as Acting Associate Dean of Research, Chair of the Department of Plant Molecular Physiology, and Director of the Hawaii Agricultural Experiment Station, respectively. During the second of these, another chance occurrence caused me to refocus on

research. It was popular for a time to hold small, informal bi-national conferences in Hawaii. One such conference, on photoinhibition, was held in Honolulu in 1985. At that time the subject was outside of my field of interest, but I attended on invitation from David Fork, whom I knew from visits to Carnegie during my days as a graduate student. One report by Olle Björkman caught my attention. He showed the kinetics of chlorophyll fluorescence quenching resulting from photoinhibition, which I recognized as being similar to the kinetics of violaxanthin de-epoxidation. After the meeting, I wrote a research proposal, including a request for a pulse-amplitude modulated fluorometer (PAM)*** that I would need to investigate the possible connection between photoinhibition and zeaxanthin formation. The grant proposal was successful but I was "scooped" by publication of a seminal paper by Demmig et al. (1987) that reported the correlation between non-photochemical quenching (NPQ) and zeaxanthin formation. Barbara Demmig had, in fact, noted the possible correlation a few years earlier but had difficulty convincing others of its reality. (For an interesting account of the events surrounding her important discovery, see Demmig-Adams, 2003.) Later, Adam Gilmore showed by a modeling technique that antheraxanthin also contributed to NPQ as effectively as zeaxanthin (Gilmore and Yamamoto, 1993). It is now common practice to express de-epoxidation as the de-epoxidation state (DES), or $(Z + A)/(V + A + Z)$, in conjunction with NPQ. The question of whether the correlation is a direct or indirect effect was recently answered with evidence that zeaxanthin is a direct quencher of excess energy (Ma et al., 2003).

Advances in research often result from the coupling of new analytical instrumentation or methods with the efforts of talented and dedicated individuals. This is certainly the case for contributions my laboratory made regarding the xanthophyll cycle and its relationship to NPQ. In terms of technology, the 505-nm absorbance change associated with de-epoxidation, the MGDG model system, and the HPLC method for resolution of zeaxanthin and lutein made significant differences in our research. The 505-nm change and development of the PAM provided an exceptional opportunity to examine xanthophyll cycle activity and NPQ simultaneously in chloroplasts. This application made it possible to show that although de-epoxidation and NPQ were both induced by light-dependent low pH, the protons for each were localized in different domains of the membrane (Mohanty and Yamamoto, 1996). I have not understood why the relatively simple opal-glass technique for the 505-nm change has not found more use, especially since it can be used simultaneously with NPQ measurement. In contrast, the HPLC method we developed for analysis of plant pigments is in wide use today (Gilmore and Yamamoto, 1991). As with the identification of the 505-nm change and the development of the MGDG model system, we arrived at this method somewhat by circumstance. Thayer and Björkman (1990) had reported an HPLC method that separated lutein and zeaxanthin but the column they used was no longer available. Based on my previous experience in separating lutein and zeaxanthin by sequential partitioning and absorption columns, we looked for a column that had both of these properties. ODS-1 was identified as a possibility because of its light carbon loading and non-endcapping of active silyl groups. The column performed as we hoped.

Although the mechanism of quenching has largely been resolved, numerous questions about the xanthophyll cycle remain. The physiology of the cycle is not well understood. The pool size of violaxanthin and the fraction of the pool that is active in the cycle vary among plant species and growth conditions (Demmig-Adams et al., 1999, this volume). There is a growing body of evidence that the cycle's operations may be related to more than just NPQ (Yokthongwattana and Melis, this volume). If the cycle has multiple functions, how are these functions regulated? Mutant studies suggest that the cycle in not essential for photosynthesis (Jung and Niyogi, this volume) and yet, as far as I am aware, all wild-type plants have the xanthophyll cycle. Why has nature retained this complex, apparently multifunctional system if it is not of some critical advantage? Did the system provide the adaptability to light environments needed for terrestrialization over multiple generations? Is it simply coincidental that the dominant photosynthetic life forms in the ocean and on land have xanthophyll cycles, the diadinoxanthin cycle and the VAZ cycle, respectively? The xanthophyll cycle has been related to photoprotection of plants against sudden and prolonged light stress (Verhoeven et al., 2001) and to improved plant fitness, as indicated by seed production, under fluctuating light intensities of the natural environment (Külheim et al., 2002). Interestingly, only half of the VAZ cycle (to antheraxanthin) appears to be present in a few Rhodophyceae (Aihara and Yamamoto, 1968) and in *Mantoniella* (Goss et al., 1998). Are these species less fit? We recently proposed that zeaxanthin functions as a messenger in a signal-transduction network that operates in the lipid phase of the chloroplast membrane to explain the cycle's multifunctional capabilities (Hieber et al., 2004). As one who has been involved with the xanthophyll cycle for nearly 45 years, I am surprised at how the questions seem never to end.

Fig. 5. Millie and I seated for lunch in Bagan during a recent tour of Myanmar.

VI. Many Thanks to Many

The cycle was the vehicle through which I entered the world of photosynthesis, traveled world wide, and made many good friends. I thank the photosynthesis community, the granting agencies, students, postdoctoral researchers, and colleagues for making my journey such a joy. I have also been blessed with recognition from peers through two awards, the Samuel Cate Prescott Award for Research in 1969 from the Institute of Food Technologists and the Charles Reid Barnes Life Membership Award in 2003 from the American Society of Plant Biologists. I extend special thanks to Govindjee, who offered me encouragement at every stage. Most importantly, I thank my family, especially my wife, Millie, for being understanding and supportive of my "obsession" for these many years. Now that I am retired, we have more time to spend together (Fig. 5). The question that I asked so many years ago as to why higher plants have retained the cycle remains unanswered. To all who will be continuing the walk, I look forward to learning what you find. I hope you enjoy the journey as much as I have!

Notes

*Fat (Egger) plates are Kieselguhr G plates that are dipped in hydrogenated coconut-oil/hexane solution and dried prior to use (Egger, 1962; also see Yamamoto, 1985). The plates, equivalent to a C_{18} endcapped HPLC column, resolve chlorophylls and carotenoids, except for lutein from zeaxanthin, in unsaponified chloroplast extracts. Inasmuch as lutein concentration is not normally affected by light treatments, Egger plates are an inexpensive method for tracking xanthophyll cycle activity.

**Lipoclains are a family of proteins that transport small, hydrophobic molecules such as retinol and porphyrins.

***I met Ulrich Schreiber, the developer of the PAM, at the 1971 International Congress of Photosynthesis meeting in Stresa, Italy. He approached me to discuss my paper on the incorporation of ^{18}O from O_2 into antheraxanthin and violaxanthin (Takeguchi and Yamamoto, 1971). I always appreciated his expression of interest, which was offered long before much was known about fluorescence quenching, let alone its relationship to the xanthophyll cycle.

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