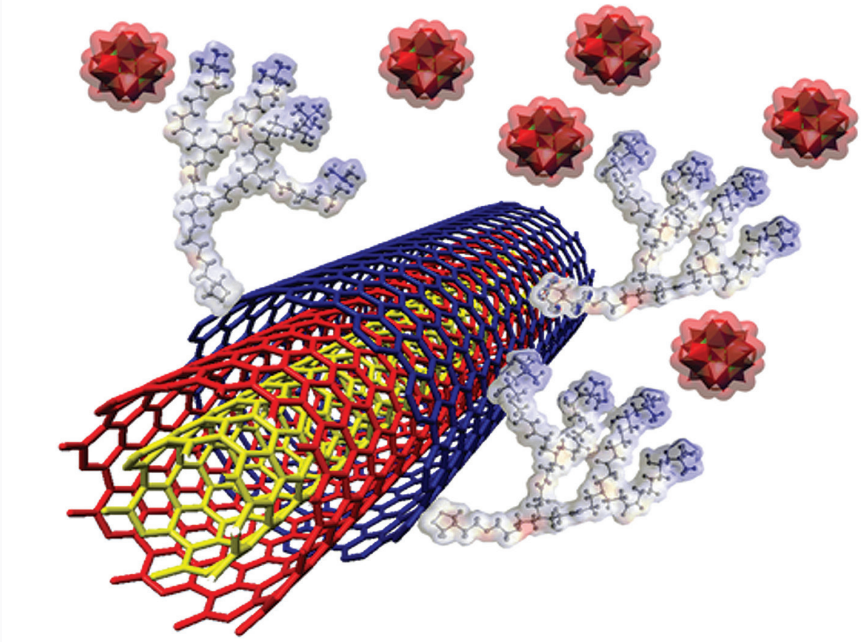
SELF-HEALING at the NANOSCALE

Mechanisms and Key Concepts of Natural and Artificial Systems

Edited by
Vincenzo Amendola • Moreno Meneghetti



Foreword by **Francesco Stellacci**

CRC Press
Taylor & Francis Group

SELF-HEALING at the NANOSCALE

Mechanisms and Key Concepts of Natural and Artificial Systems

SELF-HEALING at the NANOSCALE

Mechanisms and Key Concepts of Natural and Artificial Systems

Edited by
Vincenzo Amendola • Moreno Meneghetti

Foreword by **Francesco Stellacci**

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

© 2012 by Taylor & Francis Group, LLC
CRC Press is an imprint of Taylor & Francis Group, an Informa business

No claim to original U.S. Government works
Version Date: 2011930

International Standard Book Number-13: 978-1-4398-5474-7 (eBook - PDF)

This book contains information obtained from authentic and highly regarded sources. Reasonable efforts have been made to publish reliable data and information, but the author and publisher cannot assume responsibility for the validity of all materials or the consequences of their use. The authors and publishers have attempted to trace the copyright holders of all material reproduced in this publication and apologize to copyright holders if permission to publish in this form has not been obtained. If any copyright material has not been acknowledged please write and let us know so we may rectify in any future reprint.

Except as permitted under U.S. Copyright Law, no part of this book may be reprinted, reproduced, transmitted, or utilized in any form by any electronic, mechanical, or other means, now known or hereafter invented, including photocopying, microfilming, and recording, or in any information storage or retrieval system, without written permission from the publishers.

For permission to photocopy or use material electronically from this work, please access www.copyright.com (http://www.copyright.com/) or contact the Copyright Clearance Center, Inc. (CCC), 222 Rosewood Drive, Danvers, MA 01923, 978-750-8400. CCC is a not-for-profit organization that provides licenses and registration for a variety of users. For organizations that have been granted a photocopy license by the CCC, a separate system of payment has been arranged.

Trademark Notice: Product or corporate names may be trademarks or registered trademarks, and are used only for identification and explanation without intent to infringe.

Visit the Taylor & Francis Web site at
http://www.taylorandfrancis.com

and the CRC Press Web site at
http://www.crcpress.com

Contents

Foreword

Materials have characterized the development of mankind. From the Stone Age to the Bronze Age, from wood to the present silicon age, materials with their properties have determined what civilization could and could not dream and do. It is reasonable to ask what will come next. What new material will enable a new leap forward in civilization? At present, it is hard to believe that we will unveil a property, and consequently a material, that we cannot foresee now. The next leap forward in materials will be in moving from a "property"-dominated vision toward a "function"-dominated vision.

In nature, materials (or, better, materials assemblies) have functions more than properties. They grow, renew, and, yes, they self-repair. This book addresses all aspects of self-healing from a broad viewpoint. It starts with biological systems and then covers devices, nanomaterials, and complex assembly processes. The link is the conquer of this important function. The ability to study different systems and materials and find a common thread will give the readers an edge in understanding and mastering this important concept of self-healing materials and/or systems.

Francesco Stellacci
Constellium Professor
Institute of Materials
École Polytechnique Fédérale de Lausanne
Lausanne, Switzerland

Preface

It has been more than 30 years since Gerd Binnig and Heinrich Rohrer invented the scanning tunneling microscope at IBM Zurich in 1981, which many regard as the founding stone of science at the nanoscale. Since then, the science of nanostructures has become a mature and widely diffused research field. A common feature of well-established disciplines is the shift toward problems with high complexity, often bordering other scientific disciplines. Nanoscience is no exception to this rule and has recently expanded its frontiers in the study of more advanced systems such as, for example, nanomaterials that can self-repair.

A self-healing material is a material that is capable of repairing its components when they are damaged by wear and tear or by accidental external events. In general, self-repair represents a sophisticated strategy to increase the robustness and extend the life span of a material, especially in cases where repair or replacement of materials is economically detrimental, dangerous, or impossible. This is the case in materials that are exposed to extreme physical and chemical conditions, such as corrosive environments; high-intensity light irradiation, ionizing radiation, or high temperatures; and materials that are used in areas inaccessible to humans, such as outer space and the deep sea.

In the case of nanomaterials, however, the self-repairing ability may be needed even in ordinary conditions. For purely thermodynamic reasons, nanomaterials are generally more fragile materials than their micro- and macroscopic equivalents. In fact, nanostructures are characterized by a high surface-atom-to-bulk-atom ratio, and the creation of interfaces has high costs in energy. It thus follows that, frequently, the excellent functional properties of nanomaterials are balanced by fast degradation and structural damage during use. The creation of nanosystems that are able to repair themselves can be the solution whenever it is too expensive or not possible to create more robust materials with the same functions.

The field of self-healing materials and nanomaterials is still in its early stage, and there are very few examples of such systems. Due to this, self-healing materials are classified into systems that are capable of autonomous self-repair, i.e., that do not require any external intervention to restore the damage (and are a minority), and systems that are capable of nonautonomous self-repair, i.e., that require an external trigger to start the repair process, such as increasing the temperature or applying a magnetic field or an external force.

One of the reasons that makes the design of self-healing systems a very ambitious target is the need for expertise in different fields like material science, chemistry, engineering, and biology. Biologists' contributions are particularly valuable because nature provides sophisticated, elegant, and efficient mechanisms of damage repair from the molecular to the macroscopic scale, many of which have been studied extensively. Even if they far exceed the self-healing mechanisms used in material science in terms of efficiency and complexity, it is worth noting that biological building blocks, like proteins and nucleic acids, are of nanometric size; therefore, most

of the biological machinery takes place at the same size scale as that of nanomaterials. If nature has been successful in creating extraordinary self-repairing systems using components with nanoscale dimensions, then humans, in principle, should also be able to create self-repairing artificial nanosystems using nanomaterials. In addition, hybrid materials composed of engineered nanomaterials and biological components can provide an extra degree of freedom for designing self-repairing systems. Therefore, self-healing at the nanoscale is an area that has enormous potential for the future. Indeed, a few brilliant examples in this direction have appeared recently (and are also discussed in this book).

BASIC FEATURES OF SELF-HEALING SYSTEMS

For many years, self-repairing strategies of nanoscale materials included almost exclusively the spontaneous or externally triggered atomic-scale processes that are driven by a net gain of free energy. These are well-known processes in materials science and have been studied for a long time.

In the category of processes driven by a gain of enthalpy are the phenomena of passivation and surface reconstruction. The binding energy of individual atoms composing the material increases during passivation and reconstruction and can be considered as simple cases of autonomous self-healing systems. For example, when the surface of metals like Al, Cr, Ti, Zr, and Cu is exposed to air, a passivating oxide layer forms in order to decrease surface free energy. This layer is quite impermeable and protects the underlying metal from further oxidation. In case of a mechanical removal of a part of this layer, air oxidation of the exposed metal surface readily restores the protective film (Hultquist et al. 2001). A similar effect is observed in some systems by surface reconstruction. For instance, CdSe and CdTe semiconductor quantum dots undergo surface reconstruction in the presence of a shell of passivating ligands on the surface (Puzder et al. 2004). The interface reconstruction allows the preservation of the inner crystalline structure and composition and has great importance in opening the optical gap in these nanoparticles.

In the category of self-repair processes driven by a gain of entropy are shape memory materials that have the ability to restore their initial form after a plastic deformation. For instance, shape memory materials can recover their form after a bulk deformation on their surface integrity after slight bumps, indentations, or scratches. Shape recovery requires an external trigger, in most cases a temperature increase above a transition threshold. Therefore, these materials fall into the category of nonautonomic self-healing materials. Several metal alloys, ceramics, polymers, and hydrogels with shape memory behavior are known. Shape memory basically exploits an entropy-driven phase transition, for instance, from martensite to austenite in the case of the NiTi alloy, from tetragonal to monoclinic in the case of ZrO_2, from glassy state to rubber–elastic state in the case of polymers, and from hydrophilic to hydrophobic state in the case of hydrogels (Mather et al. 2009; Müller and Seelecke 2001).

In recent years, new systems with higher levels of complexity have appeared among self-repairing materials. These systems are based, in particular, on autonomous assembly or on responsive chemical reactions. In the first case, the damage is

repaired thanks to the self-assembling ability of some components in the material. In the second case, the repair process uses a chemical reaction between appropriate compounds included in the material that are self-activated after the damage or are activated by an external stimulus.

Some of the self-healing materials that have appeared in recent years have been inspired by natural repair strategies. Although natural and artificial self-healing systems are different in terms of complexity, sophistication, functions, and components, it is still possible to identify some general characteristics that are common in both cases (Table P.1):

1. Modularity: The system consists of several components with distinct functions.
2. Self-assembly: The different components or building blocks of the system are able to assemble autonomously.
3. Redundancy: The system is able to perform its function even when a part of it is damaged and is being repaired.
4. Transport: The damaged components are transferred from the functional site to the repair site.
5. Specialized repairing components: Some of the components of the system have functions dedicated exclusively to transport and repair of the damaged components.
6. Hierarchical structure: Repairing components have a higher level of complexity and are larger than the damaged unit. Sometimes, the repair can take place in sites other than those in which the system performs its function.

By increasing the level of complexity and efficiency of the self-healing system, the number of aforementioned characteristics that can be identified in the system also increases. To date, only a few natural repair systems have all these six properties.

TABLE P.1
Frequent Features of Self-Healing Systems

1. Modularity
2. Self-assembly
3. Redundancy
4. Transport
5. Specialized repairing components
6. Hierarchical structure

OUTLINE OF THE BOOK

The book includes contributions about selected representative examples in the diverse and multidisciplinary field of self-repairing systems. Several self-healing systems found in natural processes and others created by man-made activity are reported with a special emphasis on the key concepts, strategies, and

mechanisms at the atomic, molecular, and nanometric scales. The contributions of the book are grouped into three parts.

Part I: Natural Self-Healing Systems—covers paradigmatic self-repair systems developed by nature in living organisms.

Solar radiation is both a source of energy and a possible cause of damage. In Chapter 1, Nixon and Komenda describe how plant cells are able to selectively regenerate only those components affected by light damage among all those involved in the photosynthesis. In Chapter 2, Ma et al. show how self-repair is a prerequisite for the proper functioning of the visual cycle in humans, namely, a process with different stages in which the photoactive material is transported from the site of operation to the repair site and back again continuously.

In living organisms, the repair of larger systems inevitably requires the involvement of a greater number of components, which indicates a rise in the level of complexity of the repair mechanism. In Chapter 3, Sinigaglia presents an overview of the biological mechanisms of repair on an increasing size scale, highlighting the importance of the presence of a hierarchy between the different components.

Part II: Artificial Self-Healing Systems—describes various materials whose structures have been engineered at the micro- or nanoscale to obtain the self-repair ability. Also, some artificial nano or nanostructured materials that unexpectedly revealed the ability to repair themselves are described.

Chapter 4 shows that a nanosystem with only three components can reveal a complex network of interactions sufficient to allow the spontaneous repair of its functional nanostructures, as happens in the case of the self-healing of gold nanoparticles during laser irradiation. In Chapter 5, Lackinger shows that the capacity for self-assembly at the nanoscale can guarantee the self-repair ability for purely thermodynamic reasons, as happens in two-dimensional molecular monolayers.

Extracting energy from the sun with the maximum efficiency and the minimum waste of resources is one of the most appealing targets for researchers. In Chapter 6, Bonchio et al. describe the state of the art and the working principles of inorganic nanocatalysts for water splitting, which can either self-repair or withstand the harmful conditions in which water oxidation is carried out. In Chapter 7, Strano et al. present a sophisticated way to solve the problem of photodamage in photovoltaic hybrid systems, incorporating the properties of carbon nanotubes with those of natural photosystems.

In Chapter 8, Alessandri provides a comprehensive overview of nanocomposite materials in which nanostructures were introduced specifically to obtain self-healing through autonomous or nonautonomous mechanisms. In Chapter 9, Picchioni et al. describe the sound results and the interesting perspectives offered by synthetic chemistry for the construction of macromolecular and polymeric systems with functional groups that grant self-repairing ability to the system.

Change in microscopic structure can confer self-healing properties to traditional materials as well. In Chapter 10, Shchukin et al. discuss the state of the art for the use of microscopic smart containers in obtaining surfaces that can self-repair the damages of corrosion, which is one of the oldest problems in materials science. In Chapter 11, Méar et al. demonstrate the possibility of conferring the self-repairing

ability even to a traditionally fragile material like glass, when the structure of the material is modified on a very small size scale. Finally, in Chapter 12, Hager and Schubert cover the most significant strategies for the repair of polymers, ceramics, concrete, and metals by the engineering of their structure on a microscopic scale.

Part III: Frontiers of Self-Healing Systems—includes contributions on systems that were studied in recent years and that have shown good potential for developing or inspiring new self-healing nanomaterials in the future.

Nanoscale self-healing processes have been studied not only by experimental scientists, but they have also been studied using computational methods, with striking results. An excellent example is provided in Chapter 13 by Balazs et al. describing a repair-and-go system for site-specific healing at the nanoscale.

If we consider the founding event of nanotechnology, more than 50 years ago, to be Richard Feynman's famous speech "There's plenty of room at the bottom" during the annual meeting of the American Physical Society, then we soon go to the vision of nanotechnology as the discipline that would have allowed us to obtain "a hundred tiny hands" able of self-replication down to the atomic scale. In Chapter 14, Stano shows how self-healing and self-replication are connected abilities in living organisms; hence, the realization of self-healing nanomaterials could be a pivotal step in the process of fabricating self-replicating nanomachines. Moreover, in Chapter 15, Månsson et al. describe the fascinating case of a molecular machine optimized by nature that has a relevant role in a biological nanoscale self-healing process.

To date, only one type of artificial system has reached a complexity level that is comparable to nature—that is, the information technology system. In Chapter 16, Tempesti et al. discuss how this area opens interesting opportunities for the development of nanoscale electronic devices capable of self-healing.

The organization of the book in three parts not necessarily is the best possible, and some readers may prefer a cross-reading path. For example, Chapters 1, 6, and 7 are connected by the theme of the need to convert sunlight into energy by systems that are able to resist photodamage. In Chapters 3, 12, and 13, a comparison between the natural healing strategies of tissues, the best repair strategies for structural materials achieved by man so far, and the strategies that theoreticians indicate as the most promising for the development of more efficient self-repairing artificial systems is provided. Chapters 5 and 14 show that self-assembly is an effective strategy for self-repair in natural and artificial molecular systems. It is likely that the readers will be able to find even more cross-correlations and alternative reading paths through the book.

In conclusion, we hope that this book will provide a panorama of the concepts, mechanisms, and types of self-healing systems at the nanoscale in order to be a source of inspiration for the development of the self-healing systems of the future.

Vincenzo Amendola

Moreno Meneghetti

REFERENCES

Hultquist, G., Tveten, B., Hörnlund, E., Limbäck, M., and Haugsrud, R. 2001, Self-repairing metal oxides, *Oxidation of Metals*, 56(3), 313–346.

Mather, P.T., Luo, X., and Rousseau, I.A. 2009, Shape memory polymer research, *Annual Review of Materials Research*, 39, 445–471.

Müller, I. and Seelecke, S. 2001, Thermodynamic aspects of shape memory alloys, *Mathematical and Computer Modelling*, 34(12–13), 1307–1355.

Puzder, A., Williamson, A.J., Gygi, F., and Galli, G. 2004, Self-healing of CdSe nanocrystals: First-principles calculations, *Physical Review Letters*, 92, 217401.

Acknowledgments

This book was made possible thanks to the valuable work and the scientific expertise of all the contributors. We would therefore gratefully acknowledge all the contributors.

Editors

Vincenzo Amendola has been an assistant professor of physical chemistry in the Department of Chemical Sciences of Padua University since 2008. He received his PhD in material science and engineering in 2007 and his master's degree in material science in 2004, both from Padua University, working in the group of Professor M. Meneghetti. In 2007, he was a 6-month visiting PhD student at the Massachusetts Institute of Technology, as part of the group of Professor F. Stellacci, and in 2011 he was a 7-month academic visitor at Cambridge University, as part of the group of Professor A.C. Ferrari. He was honored with the Levi prize and the Semerano prize from the Italian Chemical Society. He is the author of several peer-reviewed papers, one patent, and has given invited talks at several conferences, including the first conference about *Laser Irradiation and Nanoparticles Generation in Liquids (ANGEL2010)*. He serves as a referee for several leading journals of Physical Chemistry, Material Science and Nanotechnology.

Amendola's main research topics are the laser ablation synthesis in solution (LASiS) of functional and functionalizable nanostructures, the study of plasmonic properties of nanoparticles, and, in general, the interaction of nanosecond laser pulses with matter for the generation of new materials and of new phenomena (like self-healing processes). He is currently collaborating with various research groups in Europe and Asia. More information about his research activities can be found at www.chimica.unipd.it/vincenzo.amendola.

Moreno Meneghetti received his doctor's degree in 1979 and is now a full professor of physical chemistry at the University of Padova. He focused his research interest on the optical properties of materials as tools for understanding their behavior. He used both experimental and theoretical approaches for characterizing phase transitions in conducting and superconducting organic materials focusing especially on electron–phonon couplings involving molecular units. He developed full diagonalization and non-mean field approaches to study the optical properties (vibrational and electronic) of low-dimensional solids. His research interests include nonlinear optical properties of molecular systems and, in particular, dynamics of nonlinear transmission of excited states with applications like optical limiting. Nonlinear optical properties and Raman properties of nanostructures based on carbon-like fullerenes and nanotubes were also investigated for their reactivity and for the preparation of new materials. Models for the electronic properties of single-wall nanotubes based on nonconventional methodologies are also the focus of his current research. Laser ablation of metals in solution for the production of metal nanoparticles is another aim of his research, focusing especially on the SERS effect. In particular, gold nanoparticles are used as nanobioconjugates with applications in nanomedicine for targeting and imaging at the subcell level.

Meneghetti's research activity has been published in more than 130 papers in peer-reviewed journals.

Contributors

Ivano Alessandri
Interuniversity Consortium on Science and Technology of Materials
and
Chemistry for Technologies Laboratory
University of Brescia
Brescia, Italy

Vincenzo Amendola
Department of Chemical Sciences
University of Padua
Padua, Italy

Anna C. Balazs
Chemical Engineering Department
University of Pittsburgh
Pittsburgh, Pennsylvania

Serena Berardi
Department of Chemical Sciences
University of Padua
Padua, Italy

Ardemis A. Boghossian
Department of Chemical Engineering
Massachusetts Institute of Technology
Cambridge, Massachusetts

Marcella Bonchio
Institute on Membrane Technology
Italian National Council of Research
Padua, Italy

A.A. Broekhuis
Chemical Engineering Department (Product Technology)
University of Groningen
Groningen, the Netherlands

Mauro Carraro
Department of Chemical Sciences
University of Padua
Padua, Italy

Jong Hyun Choi
Birck Nanotechnology Center
Bindley Bioscience Center
School of Mechanical Engineering
Purdue University
West Lafayette, Indiana

Daniel Coillot
Calalysis and Solid State Chemistry Unit
Université Lille Nord de France
Lille, France

Alfred J. Crosby
Polymer Science and Engineering Department
University of Massachusetts
Amherst, Massachusetts

Todd Emrick
Polymer Science and Engineering Department
University of Massachusetts
Amherst, Massachusetts

Dmitry O. Grigoriev
Department of Interfaces
Max-Planck Institute of Colloids and Interfaces
Potsdam, Germany

Martin D. Hager
Laboratory of Organic and Macromolecular Chemistry (IOMC)
Jena Center for Soft Matter (JCSM)
Friedrich-Schiller-University Jena
Jena, Germany

Moon-Ho Ham
Department of Chemical Engineering
Massachusetts Institute of Technology
Cambridge, Massachusetts

German V. Kolmakov
Department of Physics
New York City College of Technology
City University of New York
Brooklyn, New York

Josef Komenda
Laboratory of Photosynthesis
Institute of Microbiology
Trebon, Czech Republic

Markus Lackinger
TUM School of Education
Technical University Munich
Munich, Germany

and

Deutsches Museum
Munich, Germany

Jian-Xing Ma
Department of Physiology
University of Oklahoma Health Science Center
Oklahoma City, Oklahoma

Alf Månsson
School of Natural Sciences
Linnaeus University
Kalmar, Sweden

François O. Méar
Calalysis and Solid State Chemistry Unit
Université Lille Nord de France
Lille, France

Moreno Meneghetti
Department of Chemical Sciences
University of Padua
Padua, Italy

Franck Michoux
Division of Biology
Department of Life Sciences
Imperial College London
London, United Kingdom

Gennadiy Moiseyev
Department of Physiology
University of Oklahoma Health Science Center
Oklahoma City, Oklahoma

Lionel Montagne
Calalysis and Solid State Chemistry Unit
Université Lille Nord de France
Lille, France

Peter J. Nixon
Division of Biology
Department of Life Sciences
Imperial College London
London, United Kingdom

Marlene Norrby
School of Natural Sciences
Linnaeus University
Kalmar, Sweden

F. Picchioni
Chemical Engineering Department (Product Technology)
University of Groningen
Groningen, the Netherlands

Renaud Podor
Institut de Chimie Séparative de Marcoule
Bagnols-sur-Cèze, France

D. De Reus
Chemical Engineering Department (Product Technology)
University of Groningen
Groningen, the Netherlands

Joël Rossier
Reconfigurable & Embedded Digital Systems (REDS) Institute
Haute Ecole d'Ingénierie et de Gestion du Canton de Vaud
Yverdon-les-Bains, Switzerland

Thomas P. Russell
Polymer Science and Engineering Department
University of Massachusetts
Amherst, Massachusetts

Andrea Sartorel
Department of Chemical Sciences
University of Padua
Padua, Italy

Ulrich S. Schubert
Laboratory of Organic and Macromolecular Chemistry (IOMC)
Jena Center for Soft Matter (JCSM)
Friedrich-Schiller-University Jena
Jena, Germany

Dmitry G. Shchukin
Department of Interfaces
Max-Planck Institute of Colloids and Interfaces
Potsdam, Germany

Alessandro Sinigaglia
Department of Histology, Microbiology, and Medical Biotechnologies
University of Padua
and
Venetian Oncological Institute
Padua, Italy

Pasquale Stano
Biology Department
University of Roma Tre
Rome, Italy

André Stauffer
Logic Systems Laboratory
Ecole Polytechnique Fédérale de Lausanne
Lausanne, Switzerland

Michael S. Strano
Department of Chemical Engineering
Massachusetts Institute of Technology
Cambridge, Massachusetts

Sven Tågerud
School of Natural Sciences
Linnaeus University
Kalmar, Sweden

Gianluca Tempesti
Department of Electronics
University of York
York, United Kingdom

C. Toncelli
Chemical Engineering Department (Product Technology)
University of Groningen
Groningen, the Netherlands

Part I

Natural Self-Healing Systems

1 Keeping the Green World Alive

The Repair Cycle of Photosystem II

Josef Komenda, Franck Michoux, and Peter J. Nixon

CONTENTS

1.1 INTRODUCTION

Oxygenic photosynthesis is a key biological process on Earth: it gives rise to much of its organic matter and is essential for the maintenance of most forms of life. During this process, light energy is converted into chemical energy, which is stored in the form of highly reduced organic compounds. The initial events of this transformation involve absorption of solar radiation by various pigments (predominantly chlorophylls [Chls] and carotenoids [Cars] but also by phycobilins in some organisms), and subsequent trapping of absorbed energy by a group of specialized pigment molecules (the so-called reaction center [RC] pigments) bound within membrane protein complexes termed photosystems or RC complexes. Excitation of these RC pigments leads to transmembrane charge separation and initiation of electron flow within the membrane to ultimately produce reduced nicotinamide adenine dinucleotide phosphate (NADPH), a source of reducing power for the cell. The electron transport chain consists of a system of electron carriers ranging from simple organic compounds

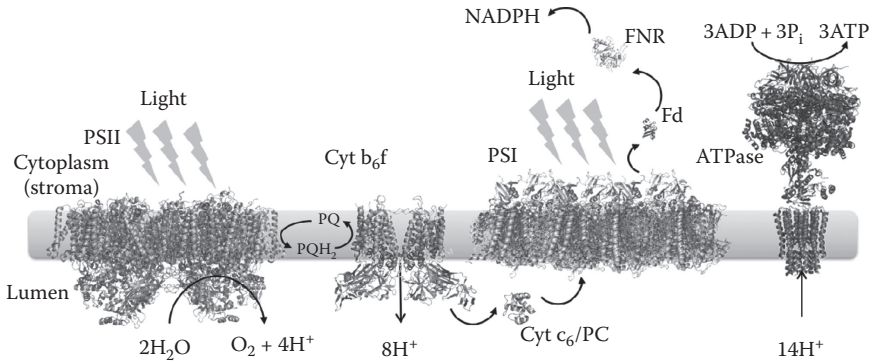


FIGURE 1.1 **(See color insert.)** Protein components of the photosynthetic electron transport chain. Electron transport from water to $NADP^+$ is driven by PSII extracting electrons from water and transferring them to plastoquinone (PQ) to produce plastoquinol (PQH_2). PSI catalyzes the reduction of ferredoxin and, subsequently, formation of NADPH via ferredoxin-$NADP^+$ reductase (FNR). Oxidized PSI is re-reduced by electrons from the cytochrome b_6f complex (Cyt b_6f) via cytochrome c_6 (Cyt c_6) or plastocyanin (PC), whereas PQH_2 produced by PSII reduces oxidized Cyt b_6f. Electron transport is coupled to the translocation of protons from the cytoplasm in cyanobacteria (or stroma in chloroplasts) to the lumen to produce a proton-motive force. In the case of Cyt b_6f, the Q-cycle operates (not shown in the figure to aid clarity) to give a stoichiometry of eight H^+ deposited in the lumen per four electrons transported to PSI. Additional translocation of protons across the membrane is mediated by cyclic electron flow around PSI (data not shown). The proton-motive force coupled to movement of protons back across the membrane through the ATP synthase (ATPase) drives synthesis of ATP from ADP and inorganic phosphate (P_i). The protein complexes shown are the crystal structures derived from various cyanobacteria.

(e.g., quinones) and small redox-active proteins up to multisubunit pigment-binding protein complexes, all of which are embedded in specialized photosynthetic membranes (termed thylakoids) found within cyanobacteria and the chloroplasts of plants and algae (Figure 1.1). Electron transfer is coupled to formation of a gradient of protons across the thylakoid membrane, which is utilized for ATP synthesis by the ATP synthase. NADPH and ATP generated by the "light reactions" are used in the "dark reactions" for synthesis of reduced organic compounds, mostly sugars, from CO_2.

In oxygenic photosynthesis, linear electron transport is driven by two RC complexes called Photosystem I (PSI) and Photosystem II (PSII). PSII has the unique ability to extract electrons from water and so plants, algae, and cyanobacteria are called oxygenic phototrophs since oxidation of water results in oxygen release. There also exist anoxygenic photosynthetic bacteria that use different electron donors but their importance for the overall photosynthetic productivity is relatively small.

1.2 STRUCTURE AND FUNCTION OF PHOTOSYSTEM II

The structure of the PSII complex from thermophilic cyanobacteria has been determined to high resolution by x-ray crystallography (Ferreira et al. 2004; Guskov et al. 2009; Umena et al. 2011). The crystallized complex contains 16 or 17 intrinsic

membrane proteins, depending on the preparation, three extrinsic membrane proteins, and a large number of cofactors including Chls, pheophytins (Pheos), β-carotenes, plastoquinones, lipids, and ions including those forming the oxygen-evolving Mn_4Ca cluster (Ferreira et al. 2004; Guskov et al. 2009; Umena et al. 2011). Unfortunately, high-resolution structural models have not yet been obtained for plant PSII, but, based on low-resolution models (Barber 2002), it is highly likely that the structures will be very similar, except for the set of extrinsic proteins on the lumenal side of the complex which differ (Enami et al. 2008).

The most important protein constituents of PSII are homologous, five transmembrane helix–containing proteins termed D1 and D2. These proteins form the so-called D1–D2 heterodimer that binds six Chls, two Pheos, and two β-carotenes. The N-termini of both proteins are exposed to the cytoplasmic side (stromal side in chloroplasts) of the thylakoid membrane, whereas the C-termini protrude into the lumen (inner part of thylakoids). D1 is synthesized as a precursor protein (pD1) with a cleavable C-terminal extension (Marder et al. 1984). The extension is present in all oxygenic phototrophs with the exception of *Euglena* and some dinoflagellates and its length is species-dependent; in plants the extension usually consists of 9 amino acid residues, whereas in cyanobacteria the extension is usually 16 residues. Removal of the extension by a cut on the carboxyl side of residue Ala344 (Takahashi et al. 1988) is required for the assembly of the functional oxygen-evolving complex (Nixon et al. 1992). The extension is removed by a specific processing endoprotease termed CtpA (Anbudurai et al. 1994), which cleaves the plant extension in a single step, whereas the cleavage in cyanobacteria seems to occur in two consecutive steps with a primary cut occurring close to the middle of the extension (Komenda et al. 2007a). The precise role of the D1 extension is not yet clear, although it is needed for optimal photosynthetic performance under high irradiance (Ivleva et al. 2000; Kuviková et al. 2005). The extension interacts with a PSII assembly factor, YCF48, required for the efficient formation of PSII (Komenda et al. 2008), so lower fitness of the extension-less cyanobacterial mutant under high irradiance might be due to an attenuation of this interaction.

The D1–D2 heterodimer is surrounded by CP47 and CP43, two homologous proteins that bind a number of Chls and β-carotenes and function as an inner or proximal light-harvesting antenna system delivering energy to the RC pigments bound to the D1 and D2 proteins. CP47 and CP43 possess six transmembrane helices and a large lumenal loop joining transmembrane helices 5 and 6 that is involved in the binding of the lumenal extrinsic proteins (in the cases of both CP47 and CP43) or in coordinating one of the Mn ions of the oxygen-evolving complex (in the case of CP43; Ferreira et al. 2004). Both proteins are located symmetrically either side of the D1–D2 heterodimer: CP47 next to D2 and CP43 next to D1 (Figure 1.2).

The PSII complex also contains a number of small membrane protein subunits that are assumed to assist the correct assembly of the complex and to optimize PSII electron flow (for review, see Müh et al. 2008). On the lumenal side of the PSII, there is an inorganic metal cluster (Mn_4CaO_5), consisting of a single Ca and four Mn ions linked by oxo bridges, which forms the oxygen-evolving center (OEC), the catalytic site at which water binds and is oxidized (Umena et al. 2011) (Figure 1.2B). The OEC is

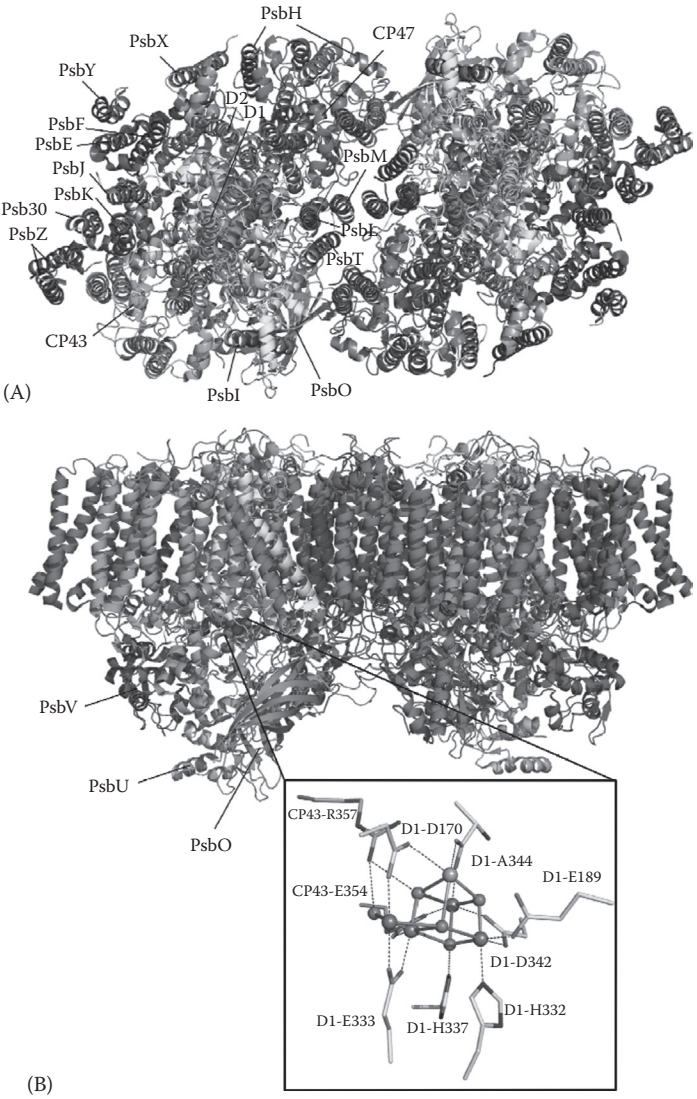


FIGURE 1.2 **(See color insert.)** Crystal structure of the PSII complex isolated from thermophilic cyanobacteria. View of the homodimeric complex from *Thermosynechococcus elongatus* from the cytoplasmic side of the thylakoid membrane (panel A) and perpendicular to the membrane normal (panel B). The 20 subunits have been annotated in panel A and color-coded: D1 (yellow), D2 (orange), CP43 (green), CP47 (red), cytochrome b-559 (purple), PsbO (violet), PsbV (dark blue), and PsbU (light blue). The remaining 11 small transmembrane subunits are shown in gray. For clarity, pigments have been omitted. The inset in panel B shows the structure of the Mn_4CaO_5 cluster involved in water oxidation, with coordinating amino acid residues, determined for *Thermosynechococcus vulcanus* (Umena et al. 2011). The Ca ion is shown in gray, the Mn ions in violet, and the oxo bridges in red. For clarity, bound waters have been omitted. The figure was created with the software Pymol (http://pymol.sourceforge.net, version 0.99) and the PDB files 3BZ1 and 3BZ2 (Guskov et al. 2009) and 3ARC (Umena et al. 2011).

stabilized by a set of extrinsic proteins that cap the cluster shielding it from the aqueous phase. Significant differences can be found in this part of PSII between plants and other oxygenic phototrophs (for review, see Roose et al. 2007a). The common protein for all groups of organisms is the 33 kDa polypeptide, the product of the *psb*O gene. Cyanobacteria and red algae contain, in addition, the 15-kDa *psb*V (cytochrome c-550) and 12-kDa *psb*U gene products. In higher plants, the PsbU and PsbV proteins are missing and their function is fulfilled by the 23-kDa PsbP and 16-kDa PsbQ subunits. Cyanobacterial homologues of PsbP and PsbQ have been identified (Thornton et al. 2004) but their precise roles in PSII function remain unclear (Summerfield et al. 2005a,b; Roose et al. 2007b).

The outer or distal light-harvesting systems also differ significantly among oxygenic phototrophs (Green et al. 2003). Large peripheral phycobilisomes, consisting of water-soluble proteins with covalently bound chromophores (phycobilins), are attached to the cytoplasmic/stromal side of the thylakoid membrane of cyanobacteria and some algal groups (e.g., Rhodophyta) (Figure 1.3). In other algae and in higher plants, the antenna complexes are embedded in the membrane and bind various types of Chl and Cars.

Despite the presence of many proteins in PSII, the cofactors involved in transferring electrons from water to plastoquinone are bound primarily by the D1–D2 heterodimer (Diner and Babcock 1996) (Figure 1.3). In brief, light energy captured by pigments within the antenna system is delivered to the Chls that constitute the primary electron donor, P680, to generate an excited singlet state, P680*, which initiates electron flow by reducing a nearby Pheo, a molecule structurally similar to Chl but lacking the central Mg ion, to form the primary radical pair $P680^{+}Pheo^{-}$. $Pheo^{-}$ then reduces the primary plastoquinone electron acceptor, Q_A, bound to the D2 protein. Unlike regular quinone molecules, Q_A is normally able to accept only a single electron due to its special protein environment. The electron is then transferred to the secondary plastoquinone electron acceptor, Q_B, bound to D1, which becomes doubly reduced after another charge separation event and is then protonated to form the plastoquinol, Q_BH_2. This molecule leaves PSII and is replaced by an oxidized plastoquinone molecule from a pool of plastoquinone molecules located in the lipid bilayer. The oxidized primary donor, $P680^{+}$, is reduced by an electron from a redox-active tyrosine residue, Tyr161 of the D1 protein, termed Y_Z (Debus et al. 1988; Metz et al. 1989), which in turn is reduced by the Mn_4Ca cluster, coordinated by aspartate, glutamate, and histidine residues in the D1 protein, including the C-terminus of mature D1, and by a glutamate residue of CP43 (Ferreira et al. 2004; Guskov et al. 2009; Umena et al. 2011) (Figure 1.2B). The Mn_4Ca cluster exists in five distinct oxidation or S states, termed S_0–S_4, where the subscript indicates the number of accumulated oxidizing equivalents. Oxidation of two molecules of water to one molecule of dioxygen occurs after formation of the S_4 state, resetting the enzyme to the S_0 state, and so occurs with a periodicity of four. The recent structural model of the cluster determined at atomic resolution has identified potential binding sites for substrate waters plus channels for the movement of incoming water molecules and exiting protons (Umena et al. 2011).

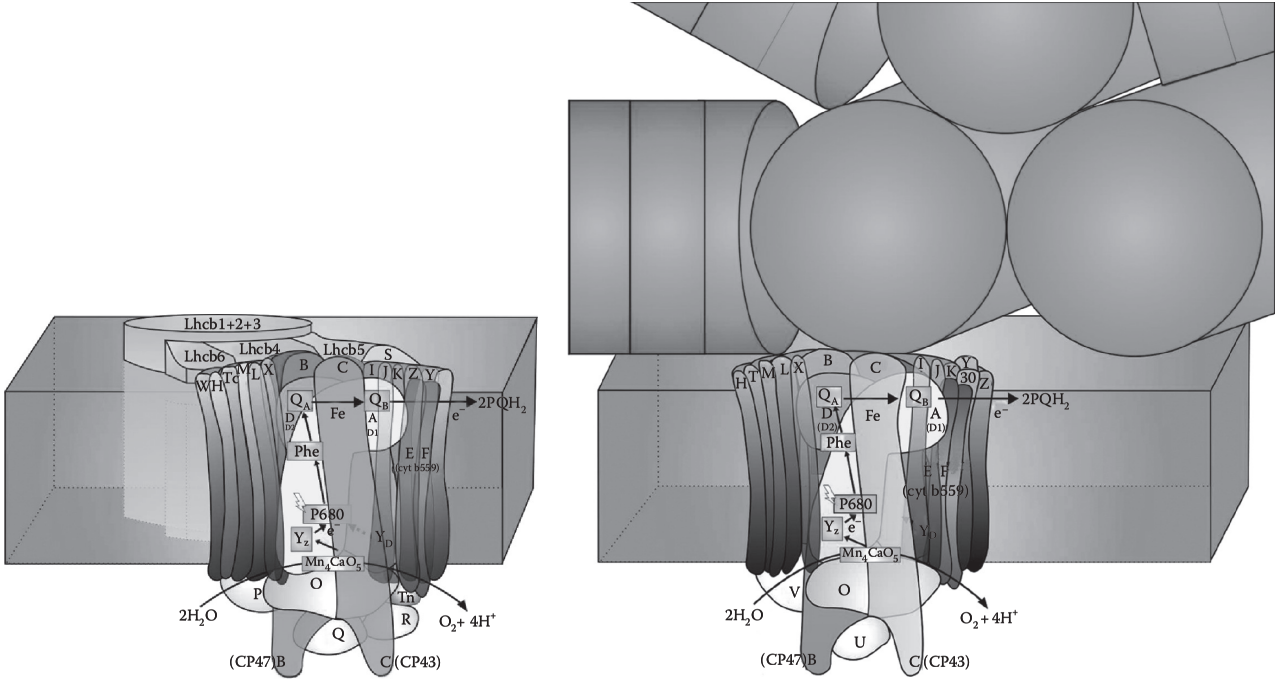


FIGURE 1.3 **(See color insert.)** Comparison of PSII in chloroplasts (left panel) and cyanobacteria (right panel). Excitation of the primary electron donor, P680, leads to stepwise reduction of the pheophytin electron acceptor (Phe) and the plastoquinones, Q_A and Q_B, located close to a non-heme iron (Fe). PQH_2 is produced after two photoacts. On the donor side, $P680^+$ oxidizes tyrosine, Y_Z, which in turn oxidizes the Mn_4CaO_5 oxygen-evolving center. Other redox-active components not involved directly in water oxidation include a second redox-active tyrosine, Y_D, located within the D2 subunit and the heterodimeric Cyt b-559 complex. Subunits are annotated so that PsbA is labeled A, and so on. Chloroplasts contain an integral light-harvesting system (Lhcb subunits), whereas cyanobacteria contain the extremely large phycobilisome (large blue–green cylinders) that docks on to the cytoplasmic surface of the thylakoid membrane. The figure was kindly provided by Dr. Jon Nield (http://www.queenmaryphotosynthesis.org//nield//).

1.3 PHOTODAMAGE TO PHOTOSYSTEM II

Despite the existence of a plethora of photoprotective mechanisms *in vivo* to prevent oxidative damage (Niyogi 1999), a characteristic feature of PSII is its relative vulnerability to photodamage. The quantum yield of photodamage has been estimated to be 7×10^{-8} in pumpkin leaves which, although a deceptively low value, still means that approximately half the PSII complexes are damaged in a leaf during a 2 h exposure to an irradiance of 1500 μmol of photons/m^2/s (Tyystjärvi and Aro 1996). Indeed, damage to PSII is thought to be an important obstacle for optimal photosynthetic performance in the field and its mitigation is a prime target for crop improvement (Murchie and Niyogi 2011).

Given the complexity of electron transfer events within PSII, it has proved difficult to determine where and how the inactivation to PSII occurs. Indeed, after almost three decades of research, there is still intense debate regarding the dominant mechanism of damage. Several mechanisms have been proposed, which differ in the specific light conditions and the functional state of PSII (reviewed by Tyystjärvi 2008; Vass 2011). The so-called acceptor-side mechanism was proposed to occur in fully functional PSII under high light conditions when the plastoquinone pool becomes overreduced. Under these conditions, the primary quinone acceptor, Q_A, becomes singly reduced or even leaves its binding site on the D2 protein if it becomes doubly reduced and protonated (Vass et al. 1992). This leads to a block in the oxidation of reduced Pheo (hence why it is termed an acceptor-side mechanism of photoinactivation), which increases the probability of charge recombination to form the triplet state of the primary electron donor, ^{3}P680, which in turn can react with triplet oxygen to generate the highly damaging singlet oxygen species (Hideg et al. 1994). Another proposed type of photoinactivation called donor side–induced inactivation is considered to be a meaningful event when the oxygen-evolving machinery is not fully functional and donation of electrons to oxidized P680 is too slow. Under these conditions, oxidative events interrupt the electron flow from oxygen-evolving complex to the primary donor P680 (Chen et al. 1992). The third proposed pathway of PSII photoinactivation occurs at extremely low light intensities when reactive singlet oxygen is generated from ^{3}P680 formed via charge recombination from the singly reduced secondary quinone acceptor, Q_B^-, and the oxidized Mn_4Ca cluster (Keren et al. 1997). All of the aforementioned mechanisms have been demonstrated to occur *in vitro* but there is still uncertainty about which one dominates *in vivo*. Recent studies showing a higher quantum yield of inactivation for blue light, a region of the spectrum that is directly absorbed by the Mn ions in the Mn_4Ca cluster, would seem to speak for a modified version of the donor-side mechanism in which the initial damaging event is the cluster itself (Hakala et al. 2005; Ohnishi et al. 2005). A similar mechanism is also assumed for inactivation of PSII by UV-B radiation (Szilárd et al. 2007). Damage to the Mn_4Ca cluster is also supported by the finding that the rate of photoinactivation of PSII is directly proportional to the light intensity (with exception of extremely low light as mentioned earlier) (Tyystjärvi and Aro 2006), which seems to be at odds with inactivation mechanisms that are dependent on a specific redox state of certain electron carriers (overreduction of acceptor-side electron carriers or overoxidation of the donor-side electron carriers).

1.4 PHOTOSYSTEM II REPAIR CYCLE

Regardless of the mechanism of PSII photodamage, it is accepted that PSII damage results in irreversible damage to protein subunits of PSII, in particular to the D1 subunit (Adir et al. 2003). The high frequency of damage has necessitated the development of a sophisticated mechanism to repair the inactive PSII complex and fully restore its photochemical activity. Such a mechanism is termed the "PSII repair cycle." It is known to consist of a number of distinct steps but a detailed understanding of the process is still lacking (Figure 1.4). It is assumed that the inactivation of the native dimeric PSII core complex is accompanied by a monomerization of the complex. Subsequently, the CP43 antenna and the lumenal extrinsic proteins stabilizing oxygen evolution are detached from the monomer. In this way, the damaged D1 protein within PSII becomes accessible for proteolytic removal and subsequent replacement by a newly synthesized copy of D1. The last phase of the cycle consists of rebinding of CP43 and the lumenal extrinsic proteins, assembly of the Mn_4Ca cluster, reactivation of oxygen evolution, and dimerization of the complex; the precise order of the last steps remains unknown.

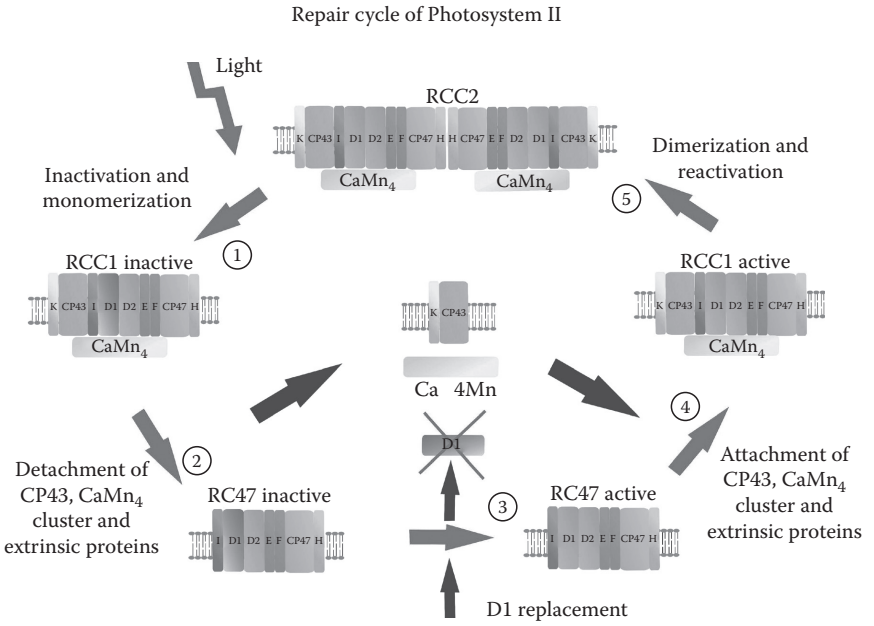


FIGURE 1.4 The repair cycle of Photosystem II. PSII repair cycle starts with light-induced inactivation of the PSII dimer, RCC2, and its monomerization (step 1). Then CP43, the $CaMn_4$ cluster, and extrinsic proteins (displayed collectively as a vanilla-colored shape) are detached (step 2) and the damaged D1 subunit is selectively replaced by a newly synthesized D1 copy at the level of the RC47 complex (step 3). Finally, CP43, Ca and Mn ions, and extrinsic proteins are reattached (step 4), and the repaired PSII monomer is dimerized with concomitant reactivation of the oxygen-evolving machinery (step 5). For clarity, many of the smaller PSII subunits have been omitted.

1.4.1 Initiation of the Repair Cycle

It is generally accepted that light-driven damage to PSII initiates the process of repair although the details remain unclear. Many early reports suggested a crucial role for the Q_B-binding pocket in signaling damage and triggering repair (Kirilovsky et al. 1988; Gong and Ohad 1991; Komenda and Barber 1995). However, relatively normal PSII repair in mutants of the cyanobacterium *Synechocystis* PCC 6803 lacking up to 20 amino acid residues in the vicinity of the Q_B-binding site argued against participation of this region (Nixon et al. 1995; Mulo et al. 1997). Instead, recent data obtained with cyanobacterial mutants having an impaired oxygen-evolving complex are in agreement with a triggering role for the lumenal part of PSII surrounding the Mn_4Ca cluster (Komenda et al. 2010), in line with the concept of direct photoinactivation of the Mn_4Ca cluster as the dominant mechanism *in vivo*. Since the cluster is coordinated by residues in D1 and CP43, we assume that damage and disruption of the cluster could lead to destabilization of the surrounding D1 structure, perturbed binding of extrinsic proteins, and even possibly an altered interaction between D1 and CP43, which may be recognized by the protease.

A key step of the PSII repair process is the selective removal of the D1 protein and its replacement by the newly synthesized D1 copy. This process can be followed experimentally by a radioactive pulse-chase method, which involves labeling of cells with radioactively labeled amino acid and subsequent chasing of the radiolabeled protein in the presence of non-radioactive ("cold") amino acid. Using this approach, the rate of protein turnover can be estimated after electrophoretic separation of membrane proteins and autoradiography. Comparable but not fully equivalent information can also be obtained by following the amount of the protein after inhibition of cellular protein synthesis. Both these approaches revealed much faster turnover/degradation of the D1 protein when compared with other PSII subunits (Edelman and Reisfeld 1978; Schuster et al. 1988). D1 turnover occurs at all light intensities and its rate is similar to the rate of PSII photoinactivation and proportional to the light intensity (Mattoo et al. 1984; Komenda and Barber 1995; Tyystjärvi and Aro 1996). Nevertheless, a detailed kinetic study of D1 degradation in *Spirodela* plants revealed that D1 degradation is a low light–associated process and irradiance as low as 5 µmol photons/m^2/s already elicits about 25% of the total D1 degradation response observed at full sunlight (about 1500 µmol photons/m^2/s) (Jansen et al. 1999).

The crystal structures of cyanobacterial PSII (Ferreira et al. 2004; Guskov et al. 2009; Umena et al. 2011) show that the D1 protein is buried within the center of the large PSII complex and is not easily accessible to the machinery that catalyzes its selective replacement. Therefore, to allow access to the protein, the neighboring inner antenna CP43 must be temporarily detached from the core complex (Barbato et al. 1992; Komenda and Masojídek 1995) and also most probably the proteins stabilizing the Mn_4Ca cluster must be at least partly relocated. How these proteins are detached and whether specific protein factors are involved in this event remain unknown. The exposed D1 protein still bound in the complex lacking CP43 (termed the RC47 complex) becomes a target of the protease (Adir et al. 1990; Komenda et al. 2006).

1.4.2 Selective D1 Degradation and the Identity of the Protease

The identity of the protease involved in selective D1 degradation remained an enigma for nearly 30 years after the first detection of fast D1 turnover (Edelman and Reisfeld 1978). To simplify the search for the protease, early studies initially focused on the use of isolated membranes (Ohad et al. 1985) and later on isolated PSII complexes in which exposure to high light intensities induced formation of specific D1 fragments (Virgin et al. 1990). The inhibitory effect of serine protease inhibitors on D1 fragmentation in various types of isolated PSII complex led to the proposal that either the inner antenna CP43 (Salter et al. 1992) or the RC complex consisting of D1, D2, and additional small polypeptides (Shipton and Barber 1991) possesses a serine protease activity participating in D1 degradation. However, later it appeared that the fragments detected in many *in vitro* studies may represent products of chemical cleavage caused by the direct action of reactive oxygen species (ROS) rather than the result of enzymatic proteolysis (Miyao et al. 1995; Lupínková and Komenda 2004). Therefore, attention has switched more recently to the analysis of physiologically relevant proteases and, in particular, homologues of the HtrA/DegP and FtsH bacterial proteases that are present in cyanobacteria and chloroplasts (Adam 1996).

Initially, an ATP-independent Deg-type serine protease (Deg2) was proposed to be responsible for the initial cleavage of damaged D1, in the loop between the fourth and fifth transmembrane helix, leading to the generation of a 23 kDa N-terminal D1 fragment (Haussühl et al. 2001) that had been detected both *in vitro* (De Las Rivas et al. 1992; Salter et al. 1992) and *in vivo* (Greenberg et al. 1987). Subsequent proteolysis of the breakdown fragments was proposed to be catalyzed by an FtsH-type protease based on the dependence of its degradation on the presence of ATP and Zn ions (Lindahl et al. 2000). However, later studies cast doubts upon the strict cooperation of DegP and FtsH during D1 degradation as inactivation of the *ftsH2* gene in the cyanobacterium *Synechocystis* 6803 (Silva et al. 2003; Komenda et al. 2006) led to the strong inhibition of D1 degradation while no such effect was observed after inactivation of all three genes coding for Deg proteases (Barker et al. 2006). Moreover, a mutant lacking FtsH2 showed stabilization of damaged D1 protein, and no evidence of fragmentation, despite loss of PSII function. This has led to the present prevailing opinion that FtsH homologues are the primary candidates for the enigmatic D1-degrading protease.

The genome of the model plant *Arabidopsis thaliana* contains a number of FtsH-related genes and two of them, FtsH2 (yellow variegated 2 or *VAR2*; Bailey et al. 2002) and FtsH5 (*VAR1*; Sakamoto et al. 2002) have also been found to be important for D1 degradation in analogy to the effect of the *ftsH2* deletion in the cyanobacterium *Synechocystis* 6803.

In chloroplasts, there have been a number of suggestions that Deg proteases play a physiological role in D1 degradation (Edelman and Mattoo 2008; Huesgen et al. 2009). Chloroplast of *Arabidopsis* contains a set of Deg proteases with Deg2 and Deg7 being present in the stroma and Deg1, Deg5, and Deg8 in the lumen. Deg2 is able to cleave the D1 protein in isolated thylakoids giving rise to the aforementioned N-terminal 23-kDa fragment (Haussühl et al. 2001) but *in vivo* is not essential for D1 degradation (Huesgen et al. 2006). Neither the lumenal Deg5 and/or Deg8, nor the

stromal Deg7 proteases are required for D1 degradation under standard growth light conditions (Sun et al. 2007, 2010a), Nevertheless, under high irradiance D1 degradation is slowed down in *deg5/deg8* or *deg7* mutants which at first sight would indicate that under extreme conditions when the FtsH-mediated pathway of D1 degradation is insufficient, Deg5, Deg7, and Deg8 may additionally help to cope with high levels of PSII damage. However, the data currently available do not exclude an indirect role for these proteases in D1 replacement, such as ensuring that sufficient ATP is available. The remaining lumenal protease Deg1 has also been suggested to participate in D1 degradation (Kapri-Pardes et al. 2007), but more recent data showed that D1 turnover was relatively unaffected in its absence; instead, Deg1 appears to be important for PSII assembly via an interaction with the D2 protein (Sun et al. 2010b). In summary, the current mutant data support the view that the chloroplast Deg proteases are not essential for D1 degradation *in vivo* but might play an auxiliary role at higher light intensities, possibly cleaving interhelical loops, to facilitate and accelerate the FtsH-mediated processive degradation of D1.

1.4.3 Mechanism of D1 Replacement and Its Dynamics

FtsH homologues in cyanobacteria and chloroplasts are predicted to have two transmembrane helices close to the N-terminus, connected by a lumenal loop, followed by an AAA^+ (ATPase associated with diverse cellular activities) module and a Zn^{2+}-metalloproteinase domain both exposed to the stroma/cytoplasm (Nixon et al. 2005). Studies in non-photosynthetic systems suggest that FtsH subunits are able to assemble into either homo/hexameric complexes or hetero/hexameric complexes consisting of two types of subunit, with the N-terminal region of FtsH important for oligomerization. The FtsH complex is hypothesized to use ATP hydrolysis to pull target membrane protein substrates into an inner cavity where proteolysis occurs (Langer 2000). The FtsH-mediated proteolysis of membrane proteins in *Escherichia coli* most frequently starts from either the N- or C-terminus and continues in a processive way without generation of large polypeptide fragments (Chiba et al. 2002). Alternatively, FtsH can also cleave in membrane-exposed protein loops and processively degrade the resulting fragments (Okuno et al. 2006). With the proteolytic cavity exposed to the cytoplasmic/stromal side, the most attractive FtsH targets in the D1 structure are the long N-terminus and the loop between the fourth and fifth transmembrane helix (Ferreira et al. 2004). Proteolysis from the N-terminus is particularly attractive, as this part of the protein protrudes from the overall structure of the complex and is sufficiently long to be attacked by FtsH. Indeed, a cyanobacterial D1 protein lacking the first 20 amino acid residues is degraded very slowly (Komenda et al. 2007b), whereas mutants lacking most of the large stromal part between the fourth and fifth transmembrane helix of D1 still maintains a fast rate of its degradation (Nixon et al. 1995; Mulo et al. 1997). Moreover, D1 degradation from the N-terminus might explain why N-terminal phosphorylation of D1 in chloroplasts affects its degradation rate (Koivuniemi et al. 1995).

After removal of damaged D1 protein, insertion of the new D1 molecule must occur promptly to minimize the time when the complex is disassembled and when the probability of ROS generation is increased. Therefore, it is important that the

new D1 copy or a synthesis intermediate is available for insertion after the degradation of the "old" D1. Data from isolated chloroplasts indicate that the insertion of the new D1 copy into the D1-less PSII subcomplex occurs cotranslationally (Zhang and Aro 2002). On the other hand, the observation that cyanobacterial mutants with inhibited D1 degradation (due to the absence of the FtsH2 protease or due to the N-terminal truncation of D1; Komenda et al. 2006, 2007b) accumulate unassembled full-length D1 protein rather speaks for the quick posttranslational integration of the whole protein into the complex, although cotranslational insertion in wild type cannot be totally excluded.

Degradation of damaged D1 and insertion of the new D1 protein into the PSII complex are highly coordinated processes that seem to stimulate each other. This has been well documented in cyanobacteria where it has been shown that ongoing D1 synthesis is necessary for the maximal rate of the D1 degradation (Komenda and Barber 1995; Komenda et al. 2000), and that mutants displaying a high rate of D1 degradation, for instance, those with an impaired oxygen-evolving complex, also stimulate the synthesis of D1 (Komenda et al. 2010). The mechanism of this synchronization remains unclear.

The cyanobacterial photosynthetic membrane complexes are located in thylakoid membranes that usually form several concentric layers more or less parallel with the cellular surface. The synthesis of D1 occurs on ribosomes bound to membranes but it is not clear yet if these are regular thylakoids, a specific subpopulation of thylakoids, or even cytoplasmic membranes. Nickelsen and colleagues have recently proposed a model in which PSII biogenesis occurs in specific, so-called intermediate membranes that are in contact with both the thylakoid and cytoplasmic membranes (Nickelsen et al. 2010). These membranes might be identical to the so-called "thylakoid centers" identified by electron microscopy in proximity of the cytoplasmic membrane (Kunkel 1982; van de Meene et al. 2006). These centers may represent the site of not only PSII assembly but also PSII repair. If so, PSII complexes in need of repair would have to migrate to this region of the membrane or into its vicinity. However, there are currently no compelling data to confirm this movement. PSII seems to be unusually immobile as suggested by "fluorescence recovery after photobleaching" (FRAP) studies and only strong light of specific wavelength induced some rearrangement of the complex (Sarcina et al. 2006).

The dynamics of PSII repair is potentially even more complicated in higher plants. PSII dimers and its associated light-harvesting system are located in semi-ordered arrays within the appressed membranes of the granal stacks (Dekker and Boekema 2005), which poses logistical problems for access of the FtsH protease to damaged D1 during PSII repair. Current models suggest that phosphorylation of light-harvesting proteins and PSII core proteins, including D1, facilitates PSII repair by enhancing the mobility of damaged PSII complexes within the appressed membranes to allow migration to the granal margins where PSII repair is likely to occur (Tikkanen et al. 2008; Fristedt et al. 2009; Goral et al. 2010; Yoshioka et al. 2010). Whether D1 phosphorylation at the N-terminus plays a role in the synchronization of D1 degradation and synthesis (Rintamäki et al. 1996) is currently unclear as higher plant mutants specifically lacking this phosphorylation site have not yet been studied. In the case of cyanobacteria, D1 is not phosphorylated, even

though the phosphorylatable threonine residue is conserved, consistent with the idea that phosphorylation is a specific adaptation associated with the formation of grana.

1.4.4 Fate of Cofactors during D1 Replacement

The degradation of damaged D1 protein is accompanied by the release of cofactors such as Chls, Cars, quinones, and ions from the Mn_4Ca cluster, which then have to rebind again during, or soon after, the insertion of the newly synthesized protein. However, it is not known to what extent the cofactors present in the freshly repaired PSII are identical to the released ones and to what extent they are replaced by the new, externally delivered ones. If the cofactors are to be reused, the replacement process must occur in the vicinity of transient carriers that are able to immediately catch them and promptly deliver to the newly repaired PSII complex. Alternatively, the replacement of D1 may occur without assistance of carriers in a compartment with high local concentration of the cofactors due to their effective transport into a spatially separated compartment in which the cofactors may quickly bind to the newly synthesized D1 protein as soon as it is synthesized. Such a compartment may represent the aforementioned thylakoid centers. However, the high local concentration model cannot apply to Chl molecules as their permanent binding to proteins is essential for preventing generation of reactive singlet oxygen, which occurs when Chl remains free. The newly synthesized or released Chl molecules must therefore immediately be bound to transient carriers or to their permanent binding sites in the Chl-binding proteins including the D1 protein. The candidates for the carrier function in cyanobacteria are the so-called high light–induced proteins (HLIPS) (Dolganov et al. 1995), which are also termed small CAB-like proteins (SCPs) (Funk and Vermaas 1999). They belong to a large family of proteins, which in plants participate in light harvesting (e.g., LHCII) as well as in protection against excessive light (e.g., the PsbS subunit). All HLIPs identified in *Synechocystis* 6803 contain a putative Chl-binding site similar to that found in plant light-harvesting complexes (Funk and Vermaas 1999) and are predicted to contain carotenoids (Xu et al. 2004). Recent data on the biogenesis of the inner PSII antennae, CP43 and CP47, in the cyanobacterium *Synechocystis* 6803 show that binding of Chls and Cars to these apoproteins is able to occur before incorporation of CP47 and CP43 into larger complexes during PSII assembly (Boehm et al. 2011). It seems reasonable to assume that binding of pigments occurs during or very soon after translation of the corresponding protein but convincing experimental evidence is still missing. This binding should occur immediately after release of the Chl molecule from the last enzymes of the Chl biosynthesis pathway or there might be a transient Chl carrier(s) that deliver it to the correct protein in the membrane. There is no information about whether the newly synthesized D1 protein already contains Chl bound when it is either inserted into the D1-less RC47 complex or binds to the D2 protein during formation of new RC subcomplexes.

Assembly of the Mn_4Ca cluster requires light to drive the photooxidation of Mn^{2+} ions in a process known as photoactivation (reviewed by Burnap 2004; Dasgupta et al. 2008). The fact that D1 provides amino acid ligands to all four Mn ions and

the Ca ion means that the cluster must be assembled de novo following selective D1 replacement during PSII repair. Early kinetic models have suggested a two quantum mechanism for photoactivation in which an Mn^{2+} ion binds at a high-affinity site in an Mn-depleted PSII complex and is oxidized by turnover of the PSII RC, following absorption of the first photon, to generate an unstable Mn^{3+} state. After a dark step, thought to involve some sort of structural rearrangement, a second Mn^{2+} ion is photooxidized by turnover of the PSII RC following absorption of the second photon to give a relatively stable binuclear Mn center (Tamura and Cheniae 1987; Zaltsman et al. 1997) with an oxidation state equivalent to a super-reduced S state equivalent to S_{-3} (Burnap 2004). Binding and oxidation of two further Mn^{2+} by the PSII RC leads to an Mn_4 cluster with oxidation state (III, III, IV, IV) in the dark-stable S_1 state (Kulik et al. 2007). Mutant studies indicate that the Mn ion ligated by D1-Asp170 and D1-Glu333 in the crystal structure (Figure 1.2B) is the first one that is bound and oxidized during photoactivation (Nixon and Diner 1992; Cohen et al. 2007) and that the C-terminal region of D1 is already folded back close to D1-Asp170 in the absence of bound Mn (Cohen et al. 2007). Although assembly of the Mn_4Ca cluster can occur *in vitro*, it is likely that assembly *in vivo* is highly regulated to minimize photodamage. This could include the participation of "assembly factors" to deliver Mn^{2+} to PSII, and auxiliary proteins to synchronize C-terminal processing of D1, photoactivation of the cluster, and attachment of the lumenal extrinsic proteins. Indeed, the recently discovered Psb27 protein would appear to play such a regulatory role, although the mechanism is currently unclear (Roose and Pakrasi 2008).

1.5 CONCLUSIONS AND PERSPECTIVES

Relatively little is known about how multisubunit membrane proteins are repaired following damage (Daley 2008). The relatively high rates of damage and repair observed with PSII make it an excellent system to study fundamental aspects of protein recognition, selective protein degradation, and replacement and insertion of pigment and metal cofactors. Given that FtsH proteases, which are considered housekeeping proteases in the cell, with broad substrate range, play such an important role in D1 degradation, it seems likely that many of the lessons learned with PSII might be applicable to other complexes in the thylakoid membrane.

A combination of molecular biology and biochemistry has identified a number of auxiliary proteins that are important for PSII repair (Nixon et al. 2010). The next challenge will be to determine when and where they act in the PSII repair cycle and how they engage with PSII. It should even be possible, with the appropriate genetic background, to isolate PSII repair complexes for detailed structural studies and so gain insights at the sub-nano level.

ACKNOWLEDGMENT

We would like to thank Dr. Jon Nield (Queen Mary, University of London) for his kind generosity in providing Figure 1.4 and for fruitful scientific discussions.

REFERENCES

Adam, Z. 1996. Protein stability and degradation in chloroplasts. *Plant Mol. Biol*. 32:773–783.

Adir, N., Shochat, S., Ohad, I. 1990. Light-dependent D1 protein synthesis and translocation is regulated by reaction center II. Reaction center II serves as an acceptor for the D1 precursor. *J. Biol. Chem*. 265:12563–12568.

Adir, N., Zer, H., Shochat, S., Ohad, I. 2003. Photoinhibition—A historical perspective. *Photosynth. Res*. 76:343–370.

Anbudurai, P.R., Mor, T.S., Ohad, I., Shestakov, S.V., Pakrasi, H.B. 1994. The *CtpA* gene encodes the C-terminal processing protease for the D1 protein of the photosystem-II reaction-center complex. *Proc. Natl. Acad. Sci. USA* 91:8082–8086.

Bailey, S., Thompson, E., Nixon, P.J. et al. 2002. A critical role for the Var2 FtsH homologue of *Arabidopsis thaliana* in the photosystem II repair cycle in vivo. *J. Biol. Chem*. 277:2006–2011.

Barbato, R., Friso, G., Rigoni, F., Vecchia, F.D., Giacometti, G.M. 1992. Structural changes and lateral redistribution of photosystem II during donor side photoinhibition of thylakoids. *J. Cell Biol*. 119:325–335.

Barber, J. 2002. Photosystem II: A multisubunit membrane protein that oxidises water. *Curr. Opin. Struct. Biol*. 12:523–530.

Barker, M., de Vries, R., Nield, J., Komenda, J., Nixon, P.J. 2006. The Deg proteases protect *Synechocystis* sp. PCC 6803 during heat and light stresses but are not essential for removal of damaged D1 protein during the photosystem two repair cycle. *J. Biol. Chem*. 281:30347–30355.

Boehm, M., Romeo, E., Reisinger, V. et al. 2011. Investigating the early stages of photosystem II assembly in *Synechocystis* sp. PCC 6803: Isolation of CP47 and CP43 complexes. *J. Biol. Chem*. 286:14812–14819.

Burnap, R.L. 2004. D1 protein processing and Mn cluster assembly in light of the emerging photosystem II structure. *Phys. Chem. Chem. Phys*. 6:4803–4809.

Chen, G.X., Kazimir, J., Cheniae, G.M. 1992. Photoinhibition of hydroxylamine-extracted photosystem II membranes: Studies of the mechanism. *Biochemistry* 31:11072–11083.

Chiba, S., Akiyama, Y., Ito, K. 2002. Membrane protein degradation by FtsH can be initiated from either end. *J. Bacteriol*. 184:4775–4782.

Cohen, R.O., Nixon, P.J., Diner, B.A. 2007. Participation of the C-terminal region of the D1-polypeptide in the first steps in the assembly of the Mn_4Ca cluster of photosystem II. *J. Biol. Chem*. 282:7209–7218.

Daley, D.O. 2008. The assembly of membrane proteins into complexes. *Curr. Opin. Struct. Biol*. 18:420–424.

Dasgupta, J., Ananyev, G.M., Dismukes, G.C. 2008. Photoassembly of the water-oxidizing complex in photosystem II. *Coord. Chem. Rev*. 252:347–360.

Debus, R.J., Barry, B.A., Sithole, I., Babcock, G., McIntosh, L. 1988. Directed mutagenesis indicates that the donor to P680 in photosystem II is tyrosine-161 of the D1 polypeptide. *Biochemistry* 27:9071–9074.

De Las Rivas, J., Andersson, B., Barber, J. 1992. Two sites of primary degradation of the D1-protein induced by acceptor or donor side photoinhibition in PSII core complexes. *FEBS Lett*. 301:246–252.

Dekker, J.P., Boekema, E.J. 2005. Supramolecular organization of thylakoid membrane proteins in green plants. *Biochim. Biophys. Acta* 1706:12–39.

Diner, B.A., Babcock, G.T. 1996. Structure, dynamics, and energy conversion efficiency in photosystem II. In: *Oxygenic Photosynthesis: The Light Reactions*, eds. Ort, D.R., Yocum, C.F., pp. 213–247. Dordrecht, the Netherlands: Kluwer Academic Publishers.

Dolganov, N.A.M., Bhaya, D., Grossmann, A.R. 1995. Cyanobacterial protein with similarity to the chlorophyll a/b binding-proteins of higher-plants—Evolution and regulation. *Proc. Natl. Acad. Sci. USA* 92:636–640.

Edelman, M., Mattoo, A.K. 2008. D1-protein dynamics in photosystem II: The lingering enigma. *Photosynth. Res*. 98:609–620.

Edelman, M., Reisfeld, A. 1978. Characterization, translation and control of the 32,000 dalton chloroplast membrane protein in *Spirodela*. In: *Chloroplast Development*, eds. Akoyunoglou, G., Argyroudi-Akoyunoglou, J.H., pp. 641–652. New York: Elsevier.

Enami, I., Okamura, A., Nagao, R., Suzuki, T., Iwai, M., Shen, J.R. 2008. Structures and functions of the extrinsic proteins of photosystem II from different species. *Photosynth. Res*. 98:349–363.

Ferreira, K.N., Iverson, T.N., Maglaoui, K., Barber, J., Iwata, S. 2004. Architecture of the photosynthetic oxygen-evolving center. *Science* 303:1831–1838.

Fristedt, R., Willig, A., Granath, P., Crevecoeur, M., Rochaix, J.D., Vener, A.V. 2009. Phosphorylation of photosystem II controls functional macroscopic folding of photosynthetic membranes in *Arabidopsis. Plant Cell* 21:3950–3964.

Funk, C., Vermaas, W. 1999. A cyanobacterial gene family coding for single helix proteins resembling part of the light-harvesting proteins from higher plants. *Biochemistry* 38:9397–9404.

Gong, H., Ohad, I. 1991. The PQ/PQH_2 ratio and occupancy of photosystem II Q_B site by plastoquinone control the degradation of the D1 protein during photoinhibition in vivo. *J. Biol. Chem*. 266:21293–21252.

Goral, T.K., Johnson, M.P., Brain, A.P., Kirchhoff, H., Ruban, A.V., Mullineaux, C.W. 2010. Visualising the mobility and distribution of chlorophyll-proteins in higher plant thylakoid membranes: Effects of photoinhibition and protein phosphorylation. *Plant J*. 62:948–959.

Green, B.R., Anderson, J.M., Parson, W.W. 2003. Photosynthetic membranes and their light-harvesting antennas. In: *Light-Harvesting Antennas in Photosynthesis*, eds. Greeen, B.R., Parson, W.W., pp. 1–28. Dordrecht, the Netherlands: Kluwer Academic Publishers.

Greenberg, B.M., Gaba, V., Mattoo, A.K., Edelman, M. 1987. Identification of a primary in vivo degradation product of the rapidly-turning-over 32 kD protein of photosystem II. *EMBO J*. 6:2865–2869.

Guskov, A., Kern, J., Gabdulkhakov, A., Broker, M., Zouni, A., Saenger, W. 2009. Cyanobacterial photosystem II at 2.9-Å resolution and the role of quinones, lipids, channels and chloride. *Nat. Struct. Biol*. 16:334–342.

Hakala, M., Tuominen, I., Keranen, M., Tyystjaervi, T., Tyystjaervi, E. 2005. Evidence for the role of the oxygen-evolving manganese complex in photoinhibition of photosystem II. *Biochim. Biophys. Acta* 1706:68–80.

Haussühl, K., Andersson, B., Adamska, I. 2001. A chloroplast DegP2 protease performs the primary cleavage of the photodamaged D1 protein in plant photosystem II. *EMBO J*. 20:713–722.

Hideg, É., Spetea, C., Vass, I. 1994. Singlet oxygen and free radical production during acceptor- and donor- induced photoinhibition. Studies with spin trapping EPR spectroscopy. *Biochim. Biophys. Acta* 1186:143–152.

Huesgen, P.F., Schuhmann, H., Adamska, I. 2006. Photodamaged D1 protein is degraded in *Arabidopsis* mutants lacking the Deg2 protease. *FEBS Lett*. 580:6929–6932.

Huesgen, P.F., Schuhmann, H., Adamska, I. 2009. Deg/HtrA proteases as components of a network for photosystem II duality control in chloroplasts and cyanobacteria. *Res. Microbiol*. 160:726–732.

Ivleva, N.B., Shestakov, S.V., Pakrasi, H.B. 2000. The carboxyl-terminal extension of the precursor D1 protein of photosystem II is required for optimal photosynthetic performance of the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Physiol*. 124:1403–1411.

Jansen, M.A.K., Mattoo, A.K., Edelman, M. 1999. D1–D2 protein degradation in the chloroplast. Complex light saturation kinetics. *Eur. J. Biochem*. 260:527–532.

Kapri-Pardes, E., Naveh, L., Adam, Z. 2007. The thylakoid lumen protease Deg 1 is involved in the repair of photosystem II from photoinhibition in *Arabidopsis. Plant Cell* 19:1039–1047.

Keren, N., Berg, A., van Kann, P.J.M., Levanon, H., Ohad, I. 1997. Mechanism of photosystem II photoinactivation and D1 protein degradation at low light: The role of back electron flow. *Proc. Natl. Acad. Sci. USA* 94:1579–1584.

Kirilovsky, D., Vernotte, C., Astier, C., Etienne, A.-L. 1988. Reversible and irreversible photoinhibition in herbicide-resistant mutants of *Synechocystis* 6714. *Biochim. Biophys. Acta* 933:124–131.

Koivuniemi, A., Aro, E.-M., Andersson, B. 1995. Degradation of the D1 and D2 proteins of photosystem II in higher plants is regulated by reversible phosphorylation. *Biochemistry* 34:16022–16029.

Komenda, J., Barber, J. 1995. Comparison of *psb*O and *psb*H deletion mutants of *Synechocystis* PCC 6803 indicates that degradation of the D1 protein is regulated by the Q(B) site and dependent on protein synthesis. *Biochemistry* 32:1454–1465.

Komenda, J., Barker, M., Kuviková, S., DeVries, R., Mullineaux, C.W., Tichý, M., Nixon, P.J. 2006. The FtsH protease slr0228 is important for quality control of photosystem II in the thylakoid membrane of *Synechocystis* PCC 6803. *J. Biol. Chem*. 281:1145–1151.

Komenda, J., Hassan, H.A.G., Diner, B.A., Debus, R.J., Barber, J., Nixon, P.J. 2000. Degradation of the photosystem II D1 and D2 proteins in different strains of the cyanobacterium *Synechocystis* PCC 6803 varying with respect to the type and level of *psbA* transcript. *Plant Mol. Biol*. 42:635–645.

Komenda, J., Knoppová, J., Krynická, V., Nixon, P.J., Tichý, M. 2010. Role of FtsH2 in the repair of photosystem II in mutants of the cyanobacterium *Synechocystis* PCC 6803 with impaired assembly or stability of the $CaMn_4$ cluster. *Biochim. Biophys. Acta* 1797:566–575.

Komenda, J., Kuviková, S., Granvogl, B. et al. 2007a. Cleavage after residue Ala352 in the C-terminal extension is an early step in the maturation of the D1 subunit of photosystem II in *Synechocystis* PCC 6803. *Biochim. Biophys. Acta* 1767:829–837.

Komenda, J., Masojídek, J. 1995. Functional and structural changes of the photosystem II complex induced by high irradiance in cyanobacterial cells. *Eur. J. Biochem*. 233:677–682.

Komenda, J., Nickelsen, J., Eichacker, L.A. et al. 2008. The cyanobacterial homologue of HCF136/YCF48 is a component of an early photosystem II assembly complex and is important for both the efficient assembly and repair of photosystem II in *Synechocystis* sp. PCC6803. *J. Biol. Chem*. 283:22390–22399.

Komenda, J., Tichy, M., Prasil, O. et al. 2007b. The exposed N-terminal tail of the D1 subunit is required for rapid D1 degradation during photosystem II repair in *Synechocystis* sp. PCC 6803. *Plant Cell* 19:2839–2854.

Kulik, L.V., Epel, B., Lubitz, W., Messinger, J. 2007. Electronic structure of the Mn_4O_xCa cluster in the S_0 and S_2 states of the oxygen-evolving complex of photosystem II based on pulse ^{55}Mn-ENDOR and EPR spectroscopy. *J. Am. Chem. Soc*. 129:13421–13435.

Kunkel, D.D. 1982. Thylakoid centers: Structures associated with the cyanobacterial photosynthetic membrane system. *Arch. Microbiol*. 133:97–99.

Kuviková, S., Tichý, M., Komenda, J. 2005. A role of the C-terminal extension of the photosystem II D1 protein in sensitivity of the cyanobacterium *Synechocystis* PCC 6803 to photoinhibition. *Photochem. Photobiol. Sci*. 4:1044–1048.

Langer, T. 2000. AAA proteases: Cellular machines for degrading membrane proteins. *Trends Biochem. Sci*. 25:247–251.

Lindahl, M., Spetea, C., Hundal, T., Oppenhaim, A.B., Adam, Z., Andersson, B. 2000. The thylakoid FtsH protease plays a role in the light-induced turnover of the PSII D1 protein. *Plant Cell* 12:419–431.

Lupínková, L., Komenda, J. 2004. Oxidative modifications of the photosystem II D1 protein by reactive oxygen species: From isolated protein to cyanobacterial cells. *Photochem. Photobiol*. 79:152–162.

Marder, J.B., Goloubinoff, P., Edelman, M. 1984. Molecular architecture of the rapidly metabolized 32-kilodalton protein of photosystem II—Indications for COOH-terminal processing of a chloroplast membrane polypeptide. *J. Biol. Chem*. 259:3900–3908.

Mattoo, A.K., Hoffman-Falk, H., Marder, J.B., Edelman, M. 1984. Regulation of protein metabolism; coupling of photosynthetic electron transport to in vivo degradation of the rapidly metabolized 32-kDa protein of chloroplast membranes. *Proc. Natl. Acad. Sci. USA* 81:1380–1384.

Metz, J.G., Nixon, P.J., Rögner, M., Brudvig, G.W., Diner, B.A. 1989. Directed alteration of the D1 polypeptide of photosystem II: Evidence that tyrosine-161 is the redox component, Z, connecting the oxygen-evolving complex to the primary electron donor, P680. *Biochemistry* 28:6960–6969.

Miyao, M., Ikeuchi, M., Yamamoto, N., Ono, Y. 1995. Specific degradation of the D1 protein of photosystem II by treatment with hydrogen peroxide in darkness: Implications for the mechanism of degradation of the D1 protein under illumination. *Biochemistry* 34:10019–10026.

Müh, F., Renger, T., Zouni, A. 2008. Crystal structure of cyanobacterial photosystem II at 3.0Å resolution: A closer look at the antenna system and the small membrane-intrinsic subunits. *Plant Physiol. Biochem*. 46:238–264.

Mulo, P., Tyystjärvi, T., Tyystjärvi, E., Govindjee, Mäenpää, P., Aro, E.-M. 1997. Mutagenesis of the D–E loop of photosystem II reaction center protein D1. Function and assembly of photosytem II. *Plant Mol. Biol*. 33:1059–1071.

Murchie, E.H., Niyogi, K.K. 2011. Manipulation of photoprotection to improve photosynthesis. *Plant Physiol*. 155:86–92.

Nickelsen, J., Rengstl, B., Stengel, A., Schottkowski, M., Soll, J., Ankele, E. 2010. Biogenesis of the cyanobacterial thylakoid membrane system—An update. *FEMS Microbiol. Lett*. 315:1–5.

Nixon, P.J., Barker, M., Boehm, M., de Vries, R., Komenda, J. 2005. FtsH-mediated repair of the photosystem two complex in response to light stress in the cyanobacterium *Synechocystis* sp. PCC 6803. *J. Exp. Bot*. 56:347–356.

Nixon, P.J., Diner, B.A. 1992. Aspartate 170 of the photosystem II reaction center polypeptide D1 is involved in the assembly of the oxygen-evolving manganese cluster. *Biochemistry* 31:942–948.

Nixon, P.J., Komenda, J., Barber, J., Deak, Z., Vass, I., Diner, B.A. 1995. Deletion of the PEST-like region of photosystem II modifies the Q_B-binding pocket but does not prevent rapid turnover of D1. *J. Biol. Chem*. 270:14919–14927.

Nixon, P.J., Michoux, F., Yu, J., Boehm, M., Komenda, J. 2010. Recent advances in understanding the assembly and repair of photosystem II. *Annals Botany* 106:1–16.

Nixon, P.J., Trost, J.T., Diner, B.A. 1992. Role of the carboxy terminus of polypeptide D1 in the assembly of a functional water-oxidizing manganese cluster in photosystem II of the cyanobacterium *Synechocystis* sp. PCC 6803—Assembly requires a free carboxyl group at C-terminal position 344. *Biochemistry* 31:10859–10871.

Niyogi, K.K. 1999. Photoprotection revisited: Genetic and molecular approaches. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. 50:333–359.

Ohad, I., Kyle, D.J., Hirchberg, J. 1985. Light-dependent degradation of the Q_B protein in isolated pea thylakoids. *EMBO J*. 4:1655–1659.

Ohnishi, N., Allakhverdiev, S.I., Takahashi, S. et al. 2005. Two-step mechanism of photodamage to photosystem II: Step 1 occurs at the oxygen-evolving complex and step 2 occurs at the photochemical reaction center. *Biochemistry* 44:8494–8499.

Okuno, T., Yamanaka, K., Ogura, T. 2006. An AAA protease FtsH can initiate proteolysis from internal sites of a model substrate, apo-flavodoxin. *Genes Cells* 11:261–268.

Rintamäki, E., Kettunen, R., Aro, E.-M. 1996. Differential D1 dephosphorylation in functional and photodamaged photosystem II centers. Dephosphorylation is a prerequisite for degradation of damaged D1. *J. Biol. Chem*. 271:14870–14875.

Roose, J.L., Kashino, Y., Pakrasi, H.B. 2007b. The PsbQ protein defines cyanobacterial photosystem II complexes with highest activity and stability. *Proc. Natl. Acad. Sci. USA* 104:2548–2553.

Roose, J.L., Pakrasi, H.B. 2008. The Psb27 protein facilitates Mn cluster assembly in photosystem II. *J. Biol. Chem*. 283:4044–4050.

Roose, J.L., Wegener, K.M., Pakrasi, H.B. 2007a. The extrinsic proteins of photosystem II. *Photosynth. Res*. 92:369–387.

Sakamoto, W., Tamura, T., Hanba-Tomita, Y., Sodmergen, Murata, M. 2002. The VAR1 locus of *Arabidopsis* encodes a chloroplastic FtsH and is responsible for leaf variegation in the mutant alleles. *Genes Cells* 7:769–780.

Salter, A.H., Virgin, I., Hagman, A., Andersson, B. 1992. On the molecular mechanism of light-induced D1 protein degradation in PSII core particles. *Biochemistry* 31:3990–3998.

Sarcina, M., Bouzovitis, N., Mullineaux, C.W. 2006. Mobilization of photosystem II induced by intense red light in the cyanobacterium *Synechococcus* sp. PCC7942. *Plant Cell* 18:457–464.

Schuster, G., Timberg, R., Ohad, I. 1988. Turnover of thylakoid photosystem II proteins during photoinhibition of *Chlamydomonas reinhardtii. Eur. J. Biochem*. 177:403–410.

Shipton, C.A., Barber, J. 1991. Photoinduced degradation of the D1-polypeptide in isolated reaction centers of photosystem-II—Evidence for an autoproteolytic process triggered by the oxidizing side of the photosystem. *Proc. Natl. Acad. Sci. USA* 88:6691–6695.

Silva, P., Thompson, E., Bailey, S. et al. 2003. FtsH is involved in the early stages of repair of photosystem II in *Synechocystis* sp. PCC 6803. *Plant Cell* 15:2152–2164.

Summerfield, T.C., Shand, J.A., Bentley, F.K., Eaton-Rye, J.J. 2005a. PsbQ (Sll1638) in *Synechocystis* sp. PCC 6803 is required for photosystem II activity in specific mutants and in nutrient-limiting conditions. *Biochemistry* 44:805–815.

Summerfield, T.C., Winter, R.T., Eaton-Rye, J.J. 2005b. Investigation of a requirement for the PsbP-like protein in *Synechocystis* sp. PCC 6803. *Photosynth. Res*. 84:263–268.

Sun, X., Fu, T., Chen, N., Guo, J., Ma, J., Zou, M., Lu, C., Zhang, L. 2010a. The stromal chloroplast Deg7 protease participates in the repair of photosystem II after photoinhibition in *Arabidopsis. Plant Physiol*. 152:1263–1273.

Sun, X., Ouyang, M., Guo, J., Ma, J., Lu, C., Adam, Z., Zhang, L. 2010b. The thylakoid protease Deg1 is involved in photosystem II assembly in *Arabidopsis thaliana. Plant J*. 62:240–249.

Sun, X., Peng, L., Guo, J., Chi, W., Ma, J., Lu, C., Zhang, L. 2007. Formation of DEG5 and DEG8 complexes and their involvement in the degradation of photodamaged photosystem II reaction center D1 protein in *Arabidopsis. Plant Cell* 19:1388–1402.

Szilárd, A., Sass, L., Deák, Z., Vass, I. 2007. The sensitivity of photosystem II to damage by UV-B radiation depends on the oxidation state of the water-splitting complex. *Biochim. Biophys. Acta* 1767:876–882.

Takahashi, M., Shiraishi, T., Asada, K. 1988. COOH-terminal residues of D1 and the 44-kDa CPa-2 at spinach photosystem II core complex. *FEBS Lett*. 240:6–8.

Tamura, N., Cheniae, G. 1987. Photoactivation of the water-oxidizing complex in photosystem II membranes depleted of Mn and extrinsic proteins. 1. Biochemical and kinetic characterization. *Biochim. Biophys. Acta* 890:179–194.

Thornton, L.E., Ohkawa, H., Roose, J.L., Kashino, Y., Keren, N., Pakrasi, H.B. 2004. Homologs of plant PsbP and PsbQ proteins are necessary for regulation of photosystem II activity in the cyanobacterium *Synechocystis* 6803. *Plant Cell* 16:2164–2175.

Tikkanen, M., Nurmi, M., Kangasjarvi, S., Aro, E.M. 2008. Core protein phosphorylation facilitates the repair of photodamaged photosystem II at high light. *Biochim. Biophys. Acta* 1777:1432–1437.

Tyystjärvi, E. 2008. Photoinhibition of photosystem and photodamage of the oxygen evolving manganese cluster. *Coord. Chem. Rev*. 252:361–376.

Tyystjärvi, E., Aro, E.-M. 1996. The rate constant of photoinhibition, measured in lincomycin-treated leaves, is directly proportional to light intensity. *Proc. Natl. Acad. Sci. USA* 93:2213–2218.

Umena, Y., Kawakami, K., Shen, J.-R., Kamiya, N. 2011. Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature* 473:55–60.

van de Meene, A.M.L., Hohmann-Marriott, M.F., Vermaas, W.F.J., Roberson, R.W. 2006. The three-dimensional structure of the cyanobacterium *Synechocystis* sp. PCC 6803. *Arch. Microbiol*. 184:259–270.

Vass, I. 2011. Molecular mechanisms of photodamage in the photosystem II complex. *Biochim. Biophys. Acta*. doi:10.1016/j.bbabio.2011.04.014.

Vass, I., Styring, S., Hundal, T., Koivuniemi, A., Aro, E.-M., Andersson, B. 1992. Reversible and irreversible intermediates during photoinhibition of photosystem II: Stable reduced Q_A species promote chlorophyll triplet formation. *Proc. Natl. Acad. Sci. USA* 89:1408–1412.

Virgin, I., Ghanotakis, D.F., Andersson, B. 1990. Light-induced D1-protein degradation in isolated photosytem II core complexes. *FEBS Lett*. 287:125–128.

Xu, H., Vavilin, D., Funk, C., Vermaas, W. 2004. Multiple deletions of small Cab-like proteins in the cyanobacterium *Synechocystis* sp. PCC 6803. *J. Biol. Chem*. 279:27971–27979.

Yoshioka, M., Nakayama, Y., Yoshida, M. et al. 2010. Quality control of photosystem II. FtsH hexamers are localized near photosystem II at grana for swift repair of damage. *J. Biol. Chem*. 285:41972–41981.

Zaltsman, L., Ananyev, G.M., Bruntrager, E., Dismukes, G.C. 1997. Quantitative kinetic model for photoassembly of the photosynthetic water oxidase from its inorganic constituents: Requirements for manganese and calcium in the kinetically resolved steps. *Biochemistry* 36:8914–8922.

Zhang, L., Aro, E.-M. 2002. Synthesis, membrane insertion and assembly of the chloroplast-encoded D1 protein into photosystem II. *FEBS Lett*. 512:13–18.