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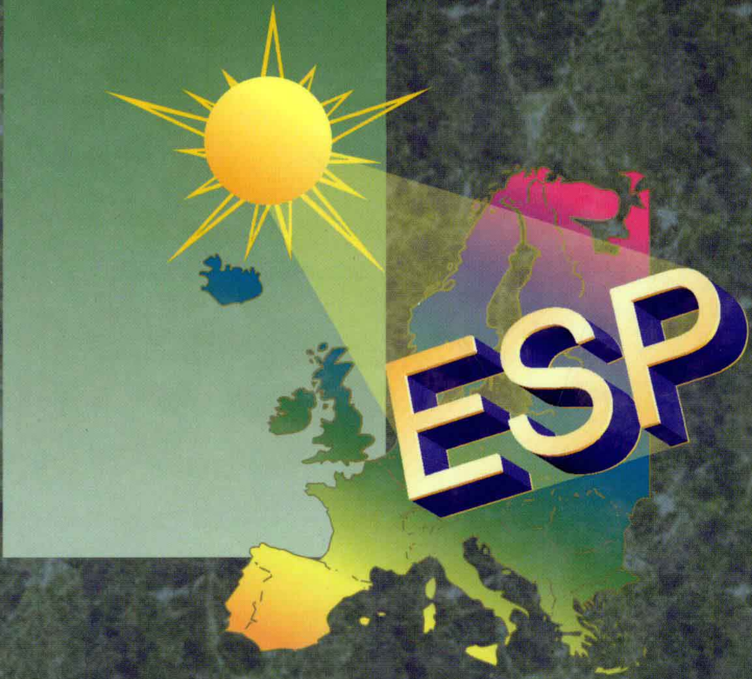

# Photoreceptors and Light Signalling

*volume editor* ALFRED BATSCHAUER

*series editors* DONAT-PETER HÄDER
*and* GIULIO JORI

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& PHOTOBIOLOGY

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# Preface for the ESP series in Photochemical and Photobiological Sciences

"It's not the substance, it's the dose which makes something poisonous!" When Paracelsius, a German physician of the 14th century made this statement he probably did not think about light as one of the most obvious environmental factors. But his statement applies as well to light. While we need light for example for vitamin D production too much light might cause skin cancer. The dose makes the difference. These diverse findings of light effects have attracted the attention of scientists for centuries. The photosciences represent a dynamic multidisciplinary field which includes such diverse subjects as behavioral responses of single cells, cures for certain types of cancer and the protective potential of tanning lotions. It includes photobiology and photochemistry, photomedicine as well as the technology for light production, filtering and measurement. Light is a common theme in all these areas. In recent decades a more molecular centered approach changed both the depth and the quality of the theoretical as well as the experimental foundation of photosciences.

An example of the relationship between global environment and the biosphere is the recent discovery of ozone depletion and the resulting increase in high energy ultraviolet radiation. The hazardous effects of high energy ultraviolet radiation on all living systems is now well established. This discovery of the result of ozone depletion put photosciences at the center of public interest with the result that, in an unparalleled effort, scientists and politicians worked closely together to come to international agreements to stop the pollution of the atmosphere.

The changed recreational behavior and the correlation with several diseases in which sunlight or artificial light sources play a major role in the causation of clinical conditions (e.g. porphyrias, polymorphic photodermatoses, *Xeroderma pigmentosum* and skin cancers) have been well documented. As a result, in some countries (e.g. Australia) public services inform people about the potential risk of extended periods of sun exposure every day. The problems are often aggravated by the phototoxic or photoallergic reactions produced by a variety of environmental pollutants, food additives or therapeutic and cosmetic drugs. On the other hand, if properly used, light-stimulated processes can induce important beneficial effects in biological systems, such as the elucidation of several aspects of cell structure and function. Novel developments are centered around photodiagnostic and phototherapeutic modalities for the treatment of cancer, artherosclerosis, several autoimmune diseases, neonatal jaundice and others. In addition, classic research areas such as vision and photosynthesis are still very active. Some of these developments are unique to photobiology, since the peculiar physico-chemical properties of electronically excited biomolecules often lead to the promotion of reactions which are characterized by high levels of selectivity in space and time. Besides the biologically centered areas, technical developments have paved the way for the harnessing of solar energy to produce warm water and electricity or the development of environmentally friendly techniques for addressing problems of large social impact (e.g. the decontamination of polluted waters). While also in use in Western countries, these techniques are of great interest for developing countries.

The European Society for Photobiology (ESP) is an organization for developing and coordinating the very different fields of photosciences in terms of public knowledge and scientific interests. Due to the ever increasing demand for a comprehensive overview of the photosciences the ESP decided to initiate an encyclopedic series, the "Comprehensive Series in Photochemical and Photobiological Sciences". This series is intended to give an in-depth coverage over all the very different fields related to light effects. It will allow investigators, physicians, students, industry and laypersons to obtain an updated record of the state-of-the-art in specific fields, including a ready access to the recent literature. Most importantly, such reviews give a critical evaluation of the directions that the field is taking, outline hotly debated or innovative topics and even suggest a redirection if appropriate. It is our intention to produce the monographs at a sufficiently high rate to generate a timely coverage of both well established and emerging topics. As a rule, the individual volumes are commissioned; however, comments, suggestions or proposals for new subjects are welcome.

Donat-P. Häder and Giulio Jori
Spring 2002

# Volume preface

Light is one of the most important environmental factors for living organisms, providing them in the case of photosynthetic organisms with energy, and information about their surroundings such as day and night cycles. This information is then used either to change behaviour or physiology. Therefore it is not surprising that, in all kingdoms, most species are able to sense light through so-called sensory photoreceptors. However, these photoreceptors are not only able to distinguish between light on and light off, but together can also use the total information that is present in the light. This information includes (i) the irradiance, (ii) the colour or spectral distribution, (iii) the direction of light, and (iv) the polarisation of light.

In principle, the irradiance can be measured by determining how often the photoreceptor is excited during a specified unit of time. This, of course, depends on the absorption cross section of the photoreceptor and how fast it reaches its ground state after excitation. The colour, or wavelength, of the photon can be sensed either by a complex photoreceptor such as phytochrome or by the combination of different photoreceptors. The absorption spectrum of the photoreceptor (and in particular the chemical nature of its chromophore) determines whether the photon can be detected. The ability to sense the direction of light can be governed by measuring a light gradient within the cell or – in multicellular organisms – within a tissue which depends on comparing light intensities in space. The movement of organisms through areas of different light intensity can also be used to sense the direction of light by measuring changes in light intensity over time. The ability to sense the polarisation of light probably depends on a fixed orientation of the photoreceptor (e.g. at membranes).

All photoreceptors known to date consist of the following: A protein moiety and one or several chromophore(s) which are covalently or non-covalently bound to the protein. If additional photoreceptors are identified in the future, it is very unlikely that they will disobey this rule since the protein by itself is not able to absorb light (at least in the visible region) and thus needs the chromophore. In principle, the chromophore can also originate from the protein as for the green fluorescent protein although this is not a sensory photoreceptor. The chromophore, with its conjugated $\pi$-electron system, can be excited with photons of longer wavelengths, or lower energy, such as those present in the visible region (400–760 nm). The protein moiety is required to transduce the primary light signal to downstream components. A possible exception to this rule could be UV-B photoreceptors, which have not been characterised at the molecular level so far.

It might be a bit surprising that only a small number of chromophore classes have been found in photoreceptors. However, one can argue from this small number that only a few chromophores are particularly well suited for photoreceptor function. These chromophore classes are: retinals, present in rhodopsins; linear tetrapyrroles, present in phytochromes and related photoreceptors from bacteria; thiol-ester linked 4-OH-cinnamic acid, present in xanthopsins (with the photoactive yellow protein as the archetype of this family); the flavins FAD and FMN, present in cryptochromes and phototropins, respectively; and the pterin 5,10-methenyltetrahydrofolate, present as a second chromophore in cryptochromes. Whereas some photoreceptor families have a wide distribution, such as the rhodopsins that are present in Bacteria, Archea, and Eukarya, others seem to have a very limited distribution, such as the phototropins that, so far, have only been found in plants. However, very recently phototropin-like proteins were identified in Bacteria [A. Losi et al. (2002). *Biophys. J.*, **82**, 2627–26349]. Further research might change this picture even more, an example being the phytochromes, which were originally thought to be typical plant photoreceptors. In recent years, genome projects have led to the identification of photoreceptors in cyanobacteria and even in non-photosynthetic eubacteria, which are related to phytochromes. It is also likely that additional photoreceptors will be found in the future. The progress in identifying novel photoreceptors is seen, for example, in the case of the plant blue-light photoreceptors. Before 1993, none were molecularly characterised or cloned, but with the use of molecular biology and genetic methods both the cryptochromes and the phototropins were then identified within a short time period. In the meantime, interacting partner proteins had already been found, well-characterised and, for phototropin, a photocycle had been demonstrated. Shortly after the discovery of cryptochromes in plants they were also identified in animals and humans through characterisation of mutants in circadian entrainment (*Drosophila*) and from the results of genome projects (human).

While writing this book, a novel blue-light receptor was described [M. Iseki et al. (2002). *Nature*, **415**, 1047–1051], which mediates the photoavoidance response in the unicellular flagellate *Euglena gracilis*. This blue-light receptor is a flavin-containing adenylyl cyclase and thus represents the third class of blue-light receptors identified within one decade.

Photobiology and research on photoreceptors and light-signalling is an interdisciplinary field using a broad range of methods such as action spectroscopy, various methods for protein purification, the whole range of molecular biological and genetic methods, and uncountable numbers of spectroscopic methods from absorption and fluorescence spectroscopy to X-ray diffraction for solving the structure of photoreceptors. Intimate knowledge of the structure and function of photoreceptors can thus only be reached through the combined effort of scientists from physics, chemistry and biology.

As outlined above, some photoreceptors have been known for many decades whereas others have been identified very recently. It is thus not surprising that the depth of knowledge and understanding of photoreceptor function, structure and signalling is quite different for the various photoreceptors. For example, rhodopsins and xanthopsins are already very well understood at the atomic level, whereas structural data still seems far away for other photoreceptors. In contrast, the structure and the photocycle of photoactive yellow protein is very well known but, still, the physiological role of this photoreceptor is not well understood.

Such differences in our knowledge of the structure, photochemistry, signalling and physiological responses of the different photoreceptors is, of course, also reflected in the twelve chapters of this book. However, I believe that this is not a disadvantage but reflects the current status of photoreceptor and light-signalling analysis, and demonstrates the broad range of experimental approaches towards one goal, which is the full understanding of photoreceptor function all the way down to the atomic level.

The chapters of this book cover all known photoreceptors, with the exception of the above-mentioned *Euglena* blue-light receptor and those candidates for which photoreceptor function has not unambiguously been shown. Examples for such candidates exist in fungi.

I am aware that much more knowledge about photoreceptors and light signalling will be available after publication of this book, due to the very fast progress in this field. Consequently, the authors have updated their chapters even during editing so that most of the very recent results are included. I'm very happy and grateful for the involvement of the authors in making it possible for all of the chapters to be written by leading experts in their respective fields. I thank the authors for the time they have invested in writing their chapters and in answering the burning questions from the editor.

Finally, it is my hope that this book will not only be of worth to experts but that it can also attract biology, chemistry and physics students to this fascinating and interdisciplinary research field.

Alfred Batschauer

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# Abbreviations

B, bathorhodopsin
BphP, bacteriophytochrome photoreceptor
BR, bacteriorhodopsin
BSI, blue-shifted intermediate
BV, biliverdin IX-a
CCA, complementary chromatic adaption
CD, circular dichroism spectroscopy
cDNA, complementary DNA
cFR, constant far-red light
Chop1, channel opsin 1
Cop, chlamyopsin
CpH1, cyanobacterial phytochrome 1
Cry (or CRY), cryptochrome
E-PYP, PYP from *Ectothiorhodospira halophila*
FL, full length
FMN, flavin mononucleotide
FR, far-red light
FTIR, Fourier-transform infrared
FTR, Fourier-transform Raman spectroscopy
GFP, green fluorescent protein
GPCR, G-protein-coupled receptor
Gtbc, bc heterodimer subunit of Gt
Gtα, α subunit of Gt
Gt, transducin (retinal G-protein)
GUS, β-glucuronidase
HIR, high irradiance response
HOOP, hydrogen out-of-plane
HR, halorhodopsin
Htp, halobacterial transducer protein
L, lumirhodopsin
LADS, lifetime-associated difference spectra
LFR, low fluence response
MI, metarhodopsin I
MII, metarhodopsin II
NMR, nuclear magnetic resonance spectroscopy
PϕB, phytochromobilin
PAS, photoacoustic spectroscopy
PBD, photothermal beam deflection
PC, phosphatidylcholine
PCB, phycocyanobilin
PE, phosphatidylethanolamine
PEB, phycoerythrobilin

PEC, phycoerythrocyanin
Pfr (or $P_{fr}$), far-red-adsorbing state of phytochrome
Phot (or PHOT), phototropin
Phy (or PHY), phytochrome
Pr (or $P_r$), red-adsorbing state of phytochrome
PS, phosphatidylserine
PSB, protonated Schiff base
PYP, photoactive yellow protein
R*, light-activated rhodopsin
R, rhodopsin
RK, rhodopsin kinase
ROS, rod outer segment
RPE, retinal pigment epithelial cells
SB, Schiff base
SDM, site-directed mutagenesis
SPR, surface plasmon resonance spectroscopy
SR, sensory rhodopsin
TG, thermal grating
VLIR, very low fluence response
Vop, volvoxopin

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# Archeabacterial phototaxis

**Martin Engelhard, Georg Schmies and Ansgar A. Wegener**

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