PREFACE

Photobiology is the scientific study of the interactions of light and living organisms. The last decades have witnessed great strides in understanding the biological effects of light from the molecular all the way up to the whole organism level. Much of the boost for advancing the science of photobiology came from the dramatic increase over this period in the incidence of melanoma and other sun-related skin diseases. In this book, current knowledge about the risks of ultraviolet radiation (UVR) on infant skin are summarized, including epidemiologic evidence regarding sun exposure in this age group. This book also explores and discusses the complex cascade of modifications induced within proteins through exposure to irradiation. Exciting new approaches to the characterization, location, tracking, and ultimately control of photoxidation within proteins are discussed as well. Moreover, the prevention for phototoxic effect is one of the important themes of photobiology. The chemopreventive action of novel organic and inorganic materials on photosensitized biomolecules damage is described in this book. Other chapters provide insights into the death and protective responses of cells treated by photodynamic therapy (PDT) as well as the relationships among gene expressions, examine the effects of natural ultraviolet radiation at zooplankton populations and communities in inland water ecosystems, and the application of folates and their role for skin homeostasis.

Chapter 1 - The modern physiology of vision has saved up many facts which contradict the accepted approach to mechanisms of primary visual perception. From H. Helmholtz time it is considered that an image of some object primarily constructed by the eye cornea and lens first unequivocally is projected to the matrix of photoreceptors (rod and cone cells), and then is exposed to processing in several layers of the nervous cells arranged behind them. Actually, at the taxonomically superior animals with developed brain including human beings, the optical image is projected to photoreceptors through the layers of nervous cells, i.e. the human retina is inverted.

The next not clear till now effect is the termination of vision at stabilization of micro oscillations of eye, so called Troxler effect. Absence of oscillations means no vision. And, at last, recently opened ultra fast reaction of photoisomerisation of rodophsine inevitably causes a question on the possible reasons and consequences of such fast reaction as time duration of the arisen potentials of photoreceptors is on 9-10 orders more.

It appears that if to consider all the specified features in common it is possible, at least at a physical level, to offer a new model of primary visual perception, which, in the first, is well

coordinated with morphological and experimental data, and secondly, eliminates the arisen contradictions.

Such model assumes, that, owing to the specified features, the eye apparatus is capable to fix the so-called "full optical field" scattered by object or optical amplitude-phase distribution of optical field, though the registered parameter is light intensity. Certainly, it is possible only at registration of scattered object fields as interferometric patterns or holograms.

Chapter 2 - The last decades have witnessed great strides in understanding the biological effects of light from the molecular all the way up to the whole organism level. Much of the boost for advancing the science of photobiology came from the dramatic increase over this period in the incidence of melanoma and other sun-related skin diseases, as well as the realization that light plays an important role in skin aging. Due to the fact that many of these conditions occur late in life, little attention has been paid to the effects of light on infant skin, with the exception of the use of violet-blue light for the treatment of jaundice in newborns. However, mounting evidence shows that a history of early sun exposure may affect the development of sun-related conditions later in life. In this chapter the authors summarize current knowledge about the risks of ultraviolet radiation (UVR) on infant skin, including epidemiologic evidence regarding sun exposure in this age group. Based on published results of studies performed using cell culture models, animal models, and human clinical trials, the authors specifically examine the effects of UVR on the biology of infant skin, as well as the known effects of blue-light phototherapy on neonatal skin. Finally, the authors identify areas for future research in this exciting field of science.

Chapter 3 - Gene polymorphism is responsible for the expression of many variants of a protein among different subjects of the same species. To be classified into polymorphism, variations of gene sequences must appear in at least 1% of the population. Such genetic variants, called alleles, though not necessarily directly pathogenic, are frequently correlated to the occurrence of diseases. For this reason, the possibility to perform wide range screening of the relevant polymorphic genes would provide a powerful tool for disease prediction.

DNA sequencing, which is the election technique to identify allelic variants, is expensive and time consuming and is not applicable to the screening of vast populations. However, if the relevant alleles are known, other molecular typing methods can offer a valuable alternative to sequencing. Several of these techniques are based on the detection of light emitted as either chemiluminescence or fluorescence. Most of them consist in ON/OFF intensity measurements, as the emission/non-emission of light is triggered by recognition of the genomic template by allele specific probes. These techniques often require preliminary purification of DNA and amplification of the polymorphic gene of interest.

In this Chapter, the authors focus on the HLA-DQB1 locus of the human major histocompatibility complex (MHC), which comprises the most polymorphic genes in the human genome. This very high degree of polymorphism dictates the tissue compatibility in organ transplants and the susceptibility to several diseases, such as insulin-dependent diabetes mellitus (IDDM).

The authors introduce a novel typing method that promises to avoid purification and amplification of DNA, and apply it to the recognition of HLA-DQB1 allelic variants. The author's method is based on time-resolved fluorescence resonance energy transfer (FRET) measurements performed on a donor-acceptor dual-labelled oligonucleotide probe, which carries the donor and the acceptor at its opposite ends. The fluorescence of the FRET donor is quenched by the acceptor in a strongly donor-acceptor distance-dependent way, which makes

the donor fluorescence decay time sensitive to the conformation of the probe-target complex. Fluorescence decay time values are measured with a time-correlated single-photon counting apparatus endowed with 30 ps resolution, which allows extremely high sensitivity on conformational differences. As the apparatus can reveal single photons, the decay time can be measured even for a single probe molecule, avoiding DNA amplification.

Target oligonucleotides with sequences equal to those of the region around codon 57 of the exon 2 allelic variants were synthesized. This region was chosen because it is the most polymorphic region of DQB1 gene and because its variability is associated with susceptibility/resistance to IDDM. An oligonucleotide corresponding to DQB1-0201 allele was used as the only dual-labelled probe. This probe forms a perfect double strand with the complementary target oligonucleotide (allele 0201), whereas for any other allelic variant, probe and target sequence are slightly mismatched at sites that differ from allele to allele. This causes the probe-target hybrid conformation to be different from allele to allele and thus the distance between the donor and the acceptor bound at the ends of the probe to change. It is shown that the donor fluorescence lifetime is correspondingly different and specific for each probe-target pair, which provides a tool for precise identification of each allele. The present results indicate that the author's method can be successfully used to molecularly type even highly polymorphic genetic systems, such as the HLA system.

Chapter 4 - UV-induced photomodification of proteins has been implicated in damage to a broad range of different protein-based substrates. Protein photo-oxidation has been linked to such diverse degenerative processes as human hair and skin damage, degradation of pharmaceuticals, reduced food quality and nutrition, discoloration of natural fibres, eye lens opacification, loss of enzymatic activity and crop damage. Ongoing ozone reduction in the stratosphere, with resultant increased exposure to UVB, has generated further impetus for advancing the understanding and controlling the complex protein degradation mechanisms and pathways underlying these damage processes.

For proteins, photo-oxidative damage is generally attributable to the generation and attack of reactive oxygen species (ROS) on amino acid residue side chains and the protein backbone. Singlet oxygen and hydroxyl radicals, in particular, play a significant role in initiating protein modification.

This chapter explores and discusses the complex cascade of modifications induced within proteins through exposure to irradiation, the effect of photo-oxidation on protein substrate properties from the protein primary level through to higher order structure, and the mechanisms underpinning protein photo-oxidation. It will also discuss exciting new approaches to the characterisation, location, tracking, and ultimately control of photo-oxidation within proteins.

Chapter 5 - Contemporary data are reviewed concerning the mechanism of terminal light-dependent stage of chlorophyll biosynthesis from the dark precursor, protochlorophyllide. The application of spectral methods provided the possibility to investigate this process immediately in plants leaves. The photochemical hydrogenation of the precursor molecule catalyzed by the photoenzyme, protochlorophyllide oxidoreductase with the involvement of hydrogen donor NADPH includes two consecutive photoreactions that give rise to a branched network of dark reactions. These reactions produce a number of chlorophyll–protein complexes of the light-harvesting antenna and minor components integrated into the structure of two photochemical systems of photosynthesis. One of the branches of the reaction pathways represents the terminal light-dependent biosynthesis of pheophytin a, which is an

immediate electron acceptor of photosystem II in green plant leaves. The features of chlorophyll biosynthesis at early stages of etiolated leaf formation and in green plant leaves are considered.

Footnote: Dedicated to the memory of the author's friend and colleague, Dr. N.V. Ignatov.

Chapter 6 - The prevention for phototoxic effect is one of the important themes of photobiology. Photosensitized damage of biomolecules including DNA, proteins, and low molecular weight compounds participates in solar carcinogenesis, photogenotoxicity, and phototoxicity. Ultraviolet radiation and visible-light induce oxidation of the above biomaterials via the electron transfer from biomolecules to an excited endogenous and/or exogenous photosensitizer or the formation of reactive oxygen species, especially singlet oxygen. Chemoprevention of photosensitized biomolecules damage is important methods against the above phototoxic effects. Organic ultraviolet B absorbers and antioxidants such as vitamins are useful ultraviolet-protector. However, the oxidized products of organic materials occasionally produce secondary reactive oxygen species to damage biomolecules. To avoid this problem, organic quenchers have been studied. For examples, xanthone derivative isolated from plant can prevent photosensitized DNA damage through quenching of the excited state of photosensitizer. These molecules may be used as the preventive drugs for an adverse side-effect of photodynamic therapy. On the other hand, inorganic sunscreen such as titanium dioxide and zinc oxide particles are effective materials to shield solar UVA. Furthermore, inorganic materials can be used as an antioxidant based of their catalytic activity for decomposition of reactive oxygen species generated from the photochemical reaction.

Chapter 7 - Photochemical analysis of secondary compounds in lichens from the Venezuelan Andean snow and glacier zones (4800-5000 m) was carried out in order to determine the absorbance capacity of UV radiation in the UVA, UVB and UVC ranges and to characterize the probable UV-protective function. Spectrotometric (UV-VIS, NIR, FTIR, MS, NMR) and chromatographic (HPTLC) standardized techniques were utilized to identify the lichen compounds. UVB irradiance in the glacier zone (5000 m) revealed a value of ~ 3W m^{-2} which is sufficient to produce important biochemical and cell alterations. Of a total of 25 lichen species distributed in the glacier and snow zones, 68% showed the presence of phenolic compounds having strong absorption for UVC radiation, 96% had strong absorption for UVB radiation and 100% had strong absorption for UVA radiation. The substance groups that had the highest resistance to UVA and UVB radiation were characterized by ester bonds among both phenolic units (depsides). They were the most abundant products to be found among the lichens, whereas substances having ester and ether bonds in both phenolic units (depsidones) had a higher capacity to absorb UVC radiation. Microorganisms having adaptive UV-screening responses similar to the lichens investigated can perhaps be expected to occur on Earth-like planets containing O_2 levels $\leq 10^{-2}$ PAL and orbiting around G, F, and K stars.

Chapter 8 - Many animals sense light signals for visual and non-visual functions. Light is captured by rhodopsin-like photopigments in photoreceptor cells and is transduced to cellular light-response through a G-protein-mediated phototransduction cascade. More than 2000 kinds of rhodopsin-like photopigments have been identified thus far and they are divided into eight subgroups. Accumulated evidence suggests that displacement of counterion, an essential amino acid residue for rhodopsin-like photopigment to absorb visible light, is closely related to the evolution of vertebrate visual pigments. Four kinds of phototransduction cascades have been found thus far, such as those mediated by transducin (Gt type G protein) in vertebrate

rod and cone visual cells, by Gq type G protein in insect and molluscan rhabdomeric-type visual cells, by Go type G protein in scallop and lizard ciliary-type visual cells and by Gs type G protein in Jellyfish visual cells. Since photoreception has evolved with phototransduction cascades and has diverged in different species, the study on rhodopsin-like pigment and the signaling cascade of varied animals is important for the understanding of the diversity and evolution of animal phototransduction. Here the classification of animal phototransduction cascades are reviewed. Animal photoreceptor cells are roughly divided into two types, ciliary and rhabdomeric types. In the case of the ciliary type photoreceptors, the jellyfish (prebilaterian) Gs-mediated, vertebrate (deuterostomes) Gt-mediated and scallop (protostome) and lizard (deuterostome) Go-mediated phototransductions exhibit partial similarity, because all involve cyclic nucleotide signaling, suggesting a monophyletic origin of ciliary phototransduction among animals. The amphioxus (deuterostome) rhabdomeric-type photoreceptor cells contain the homologue of the melanopsin, the circadian photopigment in the photosensitive retinal ganglion cells of vertebrates, and have a Gq-mediated phototransduction cascade similar to protostome rhabdomeric-type visual cells. The phototransduction of amphioxus rhabdomeric photoreceptor cells represents an evolutionary link between phototransduction of the invertebrate visual cells and the vertebrate circadian photoreceptor cells. Based on these findings, the authors discuss a functional and evolutionary classification of animal phototransduction and photoreceptor cells, the rhabdomeric photoreceptor cell containing phosphoinositol signaling mediated by Gq, and the ciliary photoreceptor cell containing cyclic nucleotide signaling mediated by Gt, Go or Gs.

Chapter 9 - Exposure to natural ultraviolet radiation has risen in southern Patagonia due to atmospheric ozone depletion. The available literature describes how natural ultraviolet radiation affects the ecosystems, and in aquatic environments ultraviolet radiation can penetrate the water column under conditions of low concentration of substances such as dissolved organic carbon which provide a screen effect by absorbing wavelengths corresponding to natural ultraviolet radiation. The present chapter is a description of literature about the effects of natural ultraviolet radiation in zooplankton populations and communities in the inland water ecosystems of Chilean Patagonia (38–51°S). At the population level, two patterns have been observed: first, the effects in species with a low tolerance to natural ultraviolet radiation exposure, which corresponds with transparent species; and second, the effects in species with photoprotective substances that are tolerant to high levels of natural ultraviolet radiation. On the community scale it has been observed that under high exposure to natural ultraviolet radiation only tolerant species are present, and under oligotrophy it was observed that few species are tolerant, mainly calanoid copepods, which are dominant in zooplankton assemblages.

Chapter 10 - For decades, it has been widely accepted that folates play a central role in cell metabolism, cell turnover and DNA repair. Despite the abundant food supply, insufficient folate status has been named one of the most frequent vitamin deficiencies in the industrialized world. Accordingly, a number of epidemiological studies correlated inadequate folate supply with an increased incidence for certain diseases.

Human skin represents the outermost barrier of the body. The epidermal compartment undergoes a life-long renewal process and the skin is constantly exposed to environmental factors, such as ultraviolet (UV) irradiation. Until recently, however, only very little was known about the effects of folates on human skin and its importance for tissue homeostasis was certainly underappreciated.

A number of both *in vitro* and *in vivo* studies were published that now help to elucidate the function folates play in the skin, particularly during the process of photo-aging. Altogether, these studies demonstrated that topically applied folic acid is bio-available for human skin, that its cellular up-take is significantly increased upon UV irradiation and that topical application improves epidermal regeneration *in vivo*, to name a few effects. Although many questions still need to be addressed the available literature appears to support the notion that folates play an important role for skin homeostasis and that this crucial vitamin serves to facilitate the cellular response following solar irradiation.

Chapter 11 - The mechanism of the cytotoxic effect of photodynamic therapy (PDT) with respect to gene profile has not been fully elucidated. The authors used DNA microarrays to analyze the time courses of transcriptional responses of lung cancer cells (RERF) at 0, 30, 60, 120 and 240 min after PDT using a water-soluble photosensitizer (ATX-S10•Na(II)). Apoptotic cell death was prominent and loss of ~ 40% of cells was seen at 120 min and 240 min after PDT using a 670-nm laser. Approximately 1300 genes in 10K tested genes responded to PDT with a more than 1.5-fold induction rate. Most of the up-regulated genes (~ 700) were revealed to be closely related to energy metabolism and protein synthesis. Immediately after PDT, genes related to activities of ATP synthase and NADH dehydrogenase were significantly up-regulated. In addition, genes related to translation in protein synthesis, ribosomal proteins and proteasome were overexpressed both at 0 min and 240 min after PDT. Expression levels of heat shock protein-related genes gradually increased over a period of 240 minutes. Further characterization of the PDT- induced gene expression profiles obtained here may lead to identification of novel biomarkers and shed light on the cytotoxic mechanism of PDT.

Chapter 12 - Many animals such as insects, reptiles, amphibians and marine vertebrates and invertebrates are endowed with bright colors and contrasting patterns intended to warn other animals that they are venomous and such is the case also insofar as the brown yellow coloration of the Oriental hornet. The present work, however, suggests that in the Oriental hornet this is not the only purpose of these bright pigments. The Oriental hornet *Vespa orientalis* (Hymenoptera, Vespinae) correlates its flight activity with the insolation. The Oriental hornet cuticle bears yellow-colored stripes composed of yellow granules. The yellow granule contains xanthopterin. This array of yellow granules maximizes the ability of the extensively conjugated xanthopterin to absorb a wide range of visible light extending up to UV light. Photovoltaic properties of yellow cuticle evince that the potential difference between darkness and UV illumination is sufficient for ATP production from ADP. This unique photovoltaic behavior of yellow cuticle suggests that it may act as an organic solar cell.

Chapter 13 - Within the field of photobiology, the mechanism of phototransduction has been a subject of intense investigation. Phototransduction is regulated by the light-sensitive interaction among visual pigment-coupled receptor proteins, such as rhodopsin, in the retina. There are some reports that the conformation of rhodopsin is influenced by the composition of phospholipids in the lipid bilayer membrane. Very recently, the authors reported the distribution of retinal phospholipids based on *in situ* analysis with vacuum type imaging mass spectrometry. However, there has been no *in situ* analysis of retinal phospholipids under natural conditions with atmospheric pressure. The authors recently developed an atmospheric pressure matrix-assisted laser desorption/ionization (AP-MALDI)-based imaging mass spectrometry (IMS) system. This system enables us to perform structural analyses using a

tandem mass spectrometer, as well as to visualize phospholipids and peptides with high spatial resolution in frozen sections. In the present study, the authors used the AP-MALDI-based IMS system to visualize and identify phospholipids in mouse retinal sections. From a spectrum obtained by raster-scanned analysis of the sections, peaks with high intensities were analyzed by tandem mass spectrometry (MS/MS) analysis. As a result, six diacyl-phosphatidylcholine (PC) species, i.e., PC (16:0/16:0), PC (16:0/18:1), PC (16:0/22:6), PC (18:0/18:0), PC (18:0/18:1), and PC (18:0/22:6), were identified. The ion images revealed different distributions on the retinal sections: PC (16:0/18:1), PC (18:0/18:0), and PC (18:0/18:1) were distributed in the inner plexiform, PC (16:0/16:0) in the inner plexiform layer, inner segment and outer segment, and both PC (16:0/22:6) and PC (18:0/22:6) in the inner and outer segments. The AP-MALDI-based IMS system demonstrated a zonal distribution of PC species on the retinal sections. Therefore, this approach may be useful for analyzing the phototransduction mechanism through phospholipids and the association of phospholipids in such diseases.

Chapter 14 - Exposure to ultraviolet light (UV) leads to a rapid elevation of nitric oxide ($NO^{\bullet}$) and superoxide ($O_2^{\bullet-}$) in irradiated cells. $NO^{\bullet}$ competes with superoxide dismutase (SOD) for $O_2^{\bullet-}$ to form peroxynitrite ($ONOO^-$), which increases the oxidative stress and reduces $NO^{\bullet}$ bioavailability. The balance between $NO^{\bullet}$ and $ONOO^-$ plays significant roles in regulation of gene expression at both the translational and transcriptional levels. In mammalian cells, three nitric oxide synthases (NOS) – neuronal NOS (nNOS, NOS1), inducible NOS (iNOS, NOS2) and endothelial NOS (eNOS, NOS3) catalyze L-arginine (L-Arg) to generate $NO^{\bullet}$ in response to UV-irradiation. Depending on the type of cells and physical properties of the irradiation, the patterns of $NO^{\bullet}$ and $ONOO^-$ productions can be very dynamic. The production of $NO^{\bullet}$ and $ONOO^-$ leads to the phosphorylation of the alpha subunit of the eukaryotic initiation factor 2alpha (eIF2α) by two eIF2α kinases (EIF2AK) - the dsRNA-dependent protein kinase-like endoplasmic reticulum (ER) kinase (PERK, EIF2AK3) and the general control nonderepressible protein kinase 2 (GCN2, EIF2AK4). The activation of EIF2AKs and phosphorylation of eIF2α inhibit global protein synthesis, which leads to the activation of transcription factor - nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Additionally, production of $NO^{\bullet}$ and $ONOO^-$ generates oxidative and nitrosative stresses that induce signaling pathways that further modulate transcriptional activation. The combined effect of translational and transcriptional regulation ultimately determines aspects of cell physiology, such as growth and death.

Chapter 15 - Bacterial infections in orthopedics are resistant to conservative therapies, and patients must therefore undergo invasive treatments and prolonged administration of antibiotics, resulting in a significant decrease in quality of life. A novel strategy for overcoming the current status is therefore needed.

Photodynamic therapy (PDT), a therapeutic modality for proliferating diseases combined with tumor-seeking photosensitizers and low-powered laser irradiation, achieves a successful outcome in clinical use.