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The Function of Natural Colorants: The Biochromes[a])

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Abstract. The colors of nature belong undoubtedly to the beautiful part of our environment. Colors always fascinated humans and left them wonderstruck. But the trivial question as to the practical application of natural colorants led soon and consequently to coloring and dyeing of objects and humans. Aesthetical, ritual and similar aspects prevailed. This function of dyes and pigments is widespread in nature. The importance of such visual-effective dyes is obvious: they support communication between organisms with the aid of conspicuous optical signals and they conceal revealing ones, when inconspicuosness can mean survival. But the purpose of natural pigments and biochromes is broader. Emergence and development of life on earth is inseparably associated with the radiating energy source, the sun. This led to the evolution of biochromes with various functions: collection and harvesting of light, transduction into chemical energy, transport of electrons or gases, photoreceptor processing of information and color discrimination, to mention only a few. Without these pigments – with their photobiological, biochemical, and physiological reactions – life, as we know it, would not have been possible. Color is essential. Several times during the evolution protecting devices and mechanisms had to be invented against the destructive and damaging influence of reactive oxygen, light and UV radiation. To this end nature employed accessory pigments as carotenoids and flavonoids. It is notable and interesting to recognize that human epidemiology and animal studies have indicated that cancer risk may be modified by changes in dietary habits or dietary components. Recent studies indicate that phytochemicals, among them the carotenoids and flavonoids, can inhibit tumor genesis at one or several stages.

1. Introduction

1.1. *Life, Light, and Color*

Light from the sun has been associated with life since the very origin of life itself. This is born out by the fact that all organisms, from bacteria to man, exhibit some form of photosensitivity to solar radiation. According to *Grotthus-Draper*'s law of absorption photobiological phenomena require specific molecules, pigments or dyes (biochromes) or systems containing them, to absorb the light energy. Photoprocesses and colors are essential for life on earth, and without these biochromes and the photophysical and photochemical interactions, life as we know it would not have been possible [1][2].

1.2. *Notation*

The terms colorants, dyes, and pigments ought to be used in the following way [3]: Colorants are either dyes or pigments, the latter being practically insoluble in the media in which they are applied. Indiscriminate use of these terms is frequently to be found in literature, but in many biological systems it is not possible at all to make this differentiation. The coloring compounds of organisms have been referred to as biochromes, and this seems to be a suitable expression for a biological colorant, since it circumvents the difficulty to distinguish between dye and pigment. Notwithstanding these objections all terms will be used when discussing color in living systems [4][5].

1.3. *Function*

The functions of biochromes can be either biochemical and metabolic or biophysical and physiological, the visual-effect functions belonging to the latter category. The connection between the two groups is made by the light-perceptor functions [6]. These three main functions are presented in *Fig. 1*

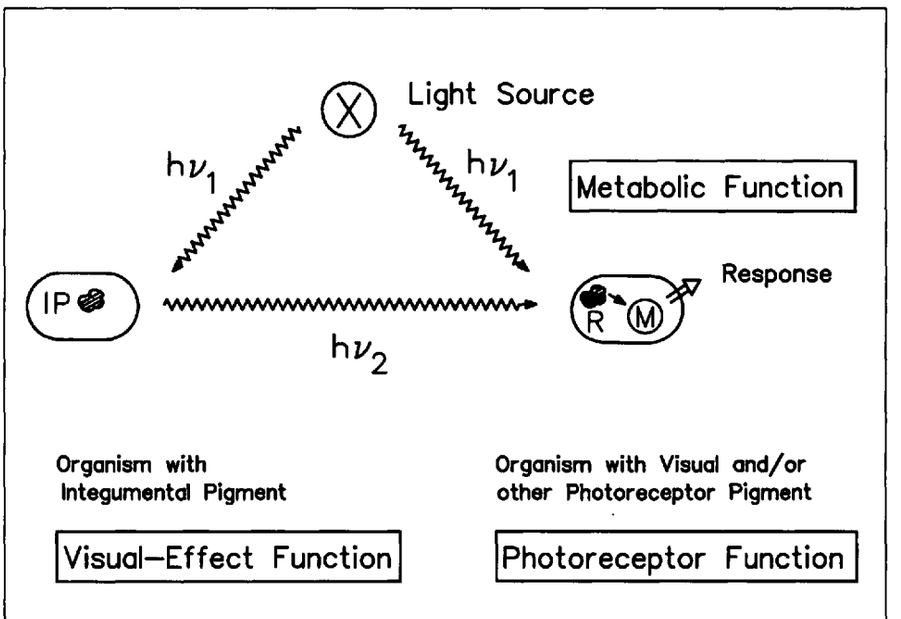

Fig. 1. *Visual-effect, photoreceptor and metabolic functions.* IP: integumental pigment, R: photoreceptor, M: metabolism.

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2. The Physical and Chemical Basis of Natural Color

2.1. *Structural Colors*

When a physical structure is capable of producing color this may be called structural color (the name 'schemochrome' has also been suggested [4]).

2.1.1. *Diffraction Gratings*

These colors are rather rare in nature. They are only observable using direct light and disappear when light becomes diffuse. The change of color with the angle of viewing is strong, and the reflection is iridescent. Examples are the surface of beetles *Serica sericea* and the indigo snake *Drymarchon corais* (color print *Fig. 38*).

Cholesteric mesophases of liquid crystals form stacks of layers. The twisting of them produces diffraction and interference. The iridescent color of scarab beetle *Lomaptera jamesi* and *Plusiotis gloriosa* belongs to this category [7].

2.1.2. *Thin Film Interference*

The majority of iridescent colors in biological systems originates from this phenomenon.There is still a color change with the viewing angle, although not as strong as with diffraction.The iridescence coined the terms 'metallic or enameld' color. Outstanding examples are the beautiful colors caused by multiple film interferences of butterfly wings and hummingbird or peacock feathers as well as mother-of-pearl. Some nocturnal animals (cat, bush baby) have a reflecting layer-structure behind the retina in their eye that gives rise to brilliant metallic reflection (*tapetum lucidum* in the choroid). The iridescent scales of butterflies, *e.g. Morpho*, contain a variety of layered structures, and the under-scales are pigmented with melanin. It is this structure of the scales with its vanes that is responsible for interference color, as shown schematically in *Fig. 2* [6][7] (color prints *Fig. 39–43*).

2.1.3. *Light Scattering*

Rayleigh scattering is observed when the responsible particles are smaller than the wavelength of light, so that good blue scattering can be seen from particles as large as 300 nm on down to sizes of 1 nm, this is also called *Tyndall* blue. Most non-iridescent blue colors in animals are *Tyndall* blues: blue eye color in humans, brilliant blue and purple areas in the faces, buttocks and genital areas of monkeys, blue color of bird feathers located on the barbules, blue color of fishes and reptiles usually based on guanine particles (color print *Fig. 44*).

When the size of the scattering particles becomes larger and eventually exceeds that of the wavelength of light, no *Rayleigh* but *Mie* scattering is observed. White or whitish scattering arises in this way: snow, fog, mist, clouds, white hair, white butterflies and moths, white and silver fish, white feathers. It should be mentioned here, that many green and purple colors originate from *Tyndall* blue and an additional yellow or red pigment, respectively [2][4][7].

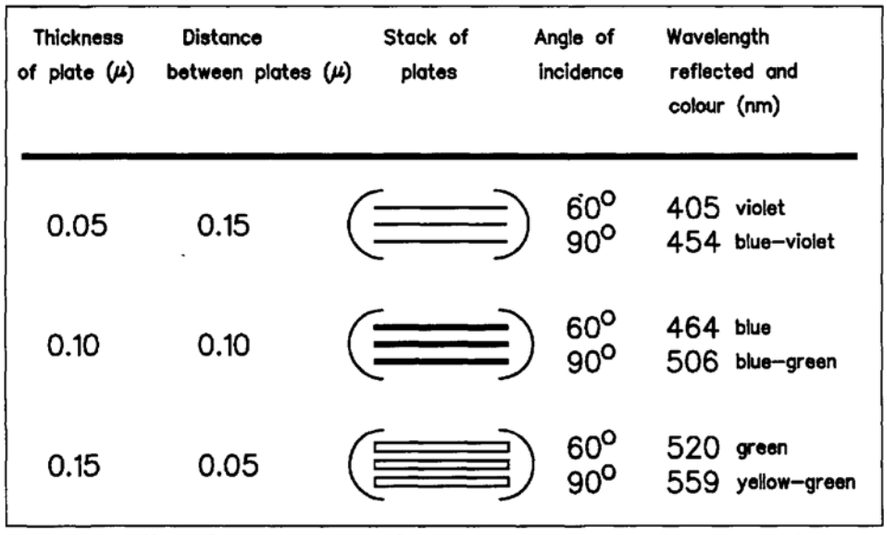


Fig. 2. *Origin of butterfly wing interference.* Structure of plates arises from the vanes in the iridescent scales (modified after *Fox* [4]).

Table. *Some Functional Localizations of Biochromes*

Localization	Function	Species
Chromatophores	Photosynthesis	Purple Bacteria
Chlorosomes	Photosynthesis	Green Bacteria
Thylakoids	Photosynthesis	Prochloron
Thylakoids	Photosynthesis	Cyanobacteria
Phycobilisomes	Photosynthesis	Cyanobacteria, Red Algae
Chloroplasts	Photosynthesis	Higher Plants, Algae
Membrane	Light-Mediated Proton Pump	Halobacterium
Stigma, Paraflagellar Body, Membrane	Phototaxis	Euglena, Chlamydomonas
Pineal Eye	Color Change, Rhythms	Amphibians, Reptiles, Birds
Eyes	Vision	Animals
Mitochondria	Respiration	Cells
Chromoplasts, Vacuoles	Visual-Effect	Higher Plants
Chromatophores	Visual-Effect	Animals

2.2. *Chemical Color*

2.2.1. *Localization of Biochromes*

The biochromes that will be discussed in the following exert their function in specialized organs, tissues, fluids, cells, organelles, and plastids. Some of these that are important for our discussion, are listed in the *Table*.

Energy Collection and Transduction:

a) Lamellae, tubes, vesicles, called chromatophores, continuous with membrane (phototrophic purple bacteria); *b*) Chlorosomes attached to but not continuous with cytoplasmic membrane (phototrophic green bacteria); *c*) cytoplasmic membrane only (*Heliobacteria*); *d*) internal thylakoid membrane structure (*Prochloron*); *e*) lamellar multilayered thylakoid membrane system especially prevalent towards the periphery of the cell (cyanobacteria); *f*) phycobilisomes (cyanobacteria and red algae); *g*) chloroplasts (higher plants and most algae); *h*) membranes (*Halobacterium*).

Sensory Transduction:

a) Stigma and paraflagellar swelling (*Euglena*); *b*) eyespot and plasma membrane (*Chlamydomonas*); *c*) plasma membrane (*Halobacterium*); *e*) extra-ocular receptors like median eye (arthropods), front organ (amphibians), parietal eye (reptiles) and pineal organ (birds and other); *f*) integumental photoreceptors (bivalves, ascidians, insects); *g*) nerve cells (*Aplysia*, echinoids, crinoids); *h*) visual organs and eyes (eye cups, compound eyes, pinhole eyes, lens eyes).

Metabolic Functions:

a) Chloroplasts (higher plants); *b*) mitochondria (respiration); *c*) blood, muscle, root nodules (oxygen transport).

Bioluminescence:

a) Bacteria (*Photobacterium*); b) light organs (luminescent animals)

Visual-Effect Functions:

a) Chloroplasts, chromoplasts, vacuoles (higher plants); *b*) chromatophores or pigments dispersed in epidermis, feathers, hair (animals).

2.2.2. *The Basic Chromophores*

The number of known classes of biochromes is surprisingly small. This may be due to the general law of biochemical austerity which governs the exploitation of materials by living organisms [6].

It has been argued that there are good reasons for this photobiological economy. Since light absorption of biochromes leads primarily to excited states that can be extremely harmful if not deactivated and lead to highly toxic species like superoxide anion or singlet oxygen, their concentration must be kept limited. One way to confine their production has been – during the course of evolution – to limit the presence of chromophore that can be photochemically active and damaging [8].

It is, therefore, understandable that many of the important biochromes are not active in their S_1 state but deactivate radiationless to the ground state. Moreover it is precisely this group of chromophores that frequently act as protecting pigments against light or agressive molecules. The most important general chemical structures are presented in *Fig. 3*.

3. The Evolution of Natural Dyes

3.1. *The Radiation Environment*

Life must have evolved by adapting to the visible part of the electromagnetic spectrum from *ca.* 380 nm to *ca.* 760 nm.

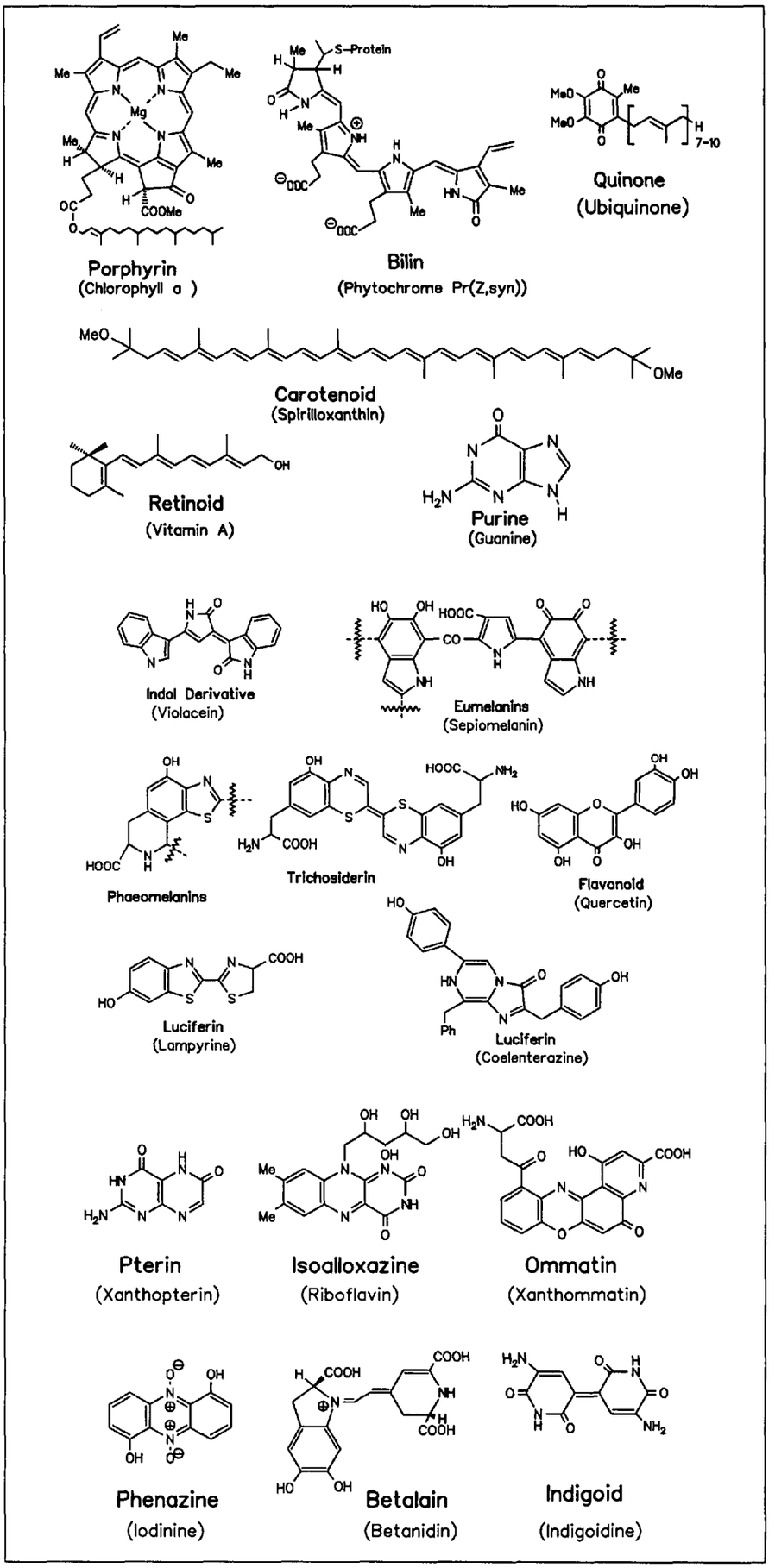


Fig. 3. *Classes of chromophores*

The solar light intensity, *i.e.*, the photon irradiance in quanta per s and cm^2, incident on the surface of the earth, is given in *Fig. 4*. UV Radiation below 300 nm is largely absorbed by ozone in the upper atmosphere. In water high light absorption and light scattering modify the situation completely. With increasing depth there is not only a considerable light intensity decrease but also a shift in the spectral distribution. The least absorbed part is green and blue-green. The light conditions are of eminent importance for the natural selection of biochromes.

Nature developed chromophores and pigments for all spectroscopically relevant wavelengths. Not only structures of chromophores were modified but wavelength adaptation was also achieved by using the modulation by the apoprotein. The apoprotein associated with the chromophore of a photoreceptor can significantly modify the wavelength of light absorbed by the latter. The wavelenght of light absorbed by the photobiologically active molecule determines the spectral response of an organism to solar radiation. This evolutionary adaptation is the basis of a 'photobiological spectrum' that contains important chromophores and dyes distributed over the whole range of biological meaningful radiation [10]. In *Fig. 5* a selection of those molecules is given displaying their absorption or emission in dependence on the solar spectrum.

Fig. 38. *The indigo snake* Drymarchon corais. An example for structural colors due to diffraction gratings. With kind permission of *Droemer-Knaur-Kindler* [83].

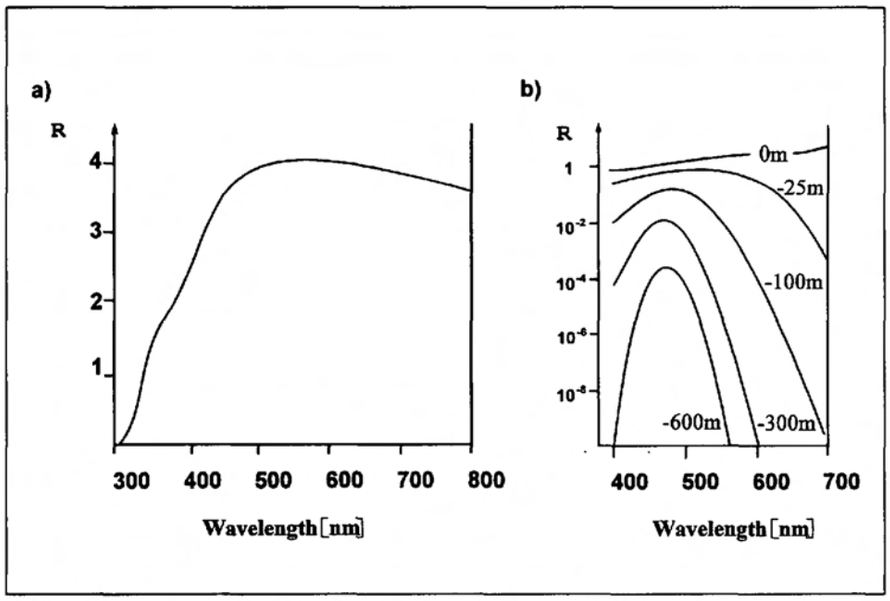


Fig. 4. a) *Radiant flux density R* (in 10^{14} q s^{-1} cm^{-2} nm^{-1}) *of solar radiation on the surface of the earth;* b) *R in various depths of a crater lake* (modified after [9])

3.2. *The Phylogeny of Biochromes*

The formation of the earth began more than 4.5 billion years ago. The first fossilized evidence of living cells is found in rocks from Australia and South Africa which date back 3.5 billion years (photosynthetic cyanobacteria, stromatolites). The early atmosphere of the primordial earth was anoxic, devoid of significant amounts of oxygen and hence constituted a reducing environment with changing amounts of H_2O, CH_4, H_2, N_2, NH_3, HCN, CO, CO_2 *etc.* Energy sources were UV radiation (UV-A, -B, -C), lightning discharges, radioactivity and thermal energy from volcanic activities. If gaseous mixtures resembling those thought to be present on the pristine earth are irradiated with UV light or excited by electrical discharges, electrons or heat (*Stanley Miller*-type of experiments, *Hodgson, Ponnamperuma*, 1968) important molecules are formed: amino acids, carboxylic acids, sugars, adenine, purines, pyrimidines, and porphyrins [1][11–13].

Porphyrins:

The cyclic tetrapyrroles are of very ancient origin. According to the abovementioned experiments it is very likely that the origin of porphyrins precedes that of life. Porphyrins have been components of living systems from the very beginning. The formation of uroporphyrin from succinic acid and glycine is thermodynamically favored. Porphyrins combine two important properties: ability to absorb light and to undergo reversible redox reactions when associated with metal ions. This might have been the start of photosynthesis [14].

Pterins and Flavins:

Much the same holds for the pteridin and isoalloxazine system. They probably existed during prebiotic evolution, too. Their importance is in their function as part of flavoproteins and prosthetic groups in redox reactions, blue light perception and DNA repair mechanisms. The removal of toxic oxygen using bioluminescent systems is also considered to have been of evolutionary value [15].

It has been speculated that flavin-mediated photoresponses (phototaxis, photophobic reactions) evolved after those that

were modulated by the quinone stentorin (a hypericin) and pterins in order to enable phototrophic bacteria and other not to move too far away from the light source [13].

DNA photolyase, containing flavins, must have played an eminent role on the primitive earth and later on, and this explains why this enzyme is stable towards oxygen whereas the bacterial luciferase is extremely sensitive. The occurrence of the 5-deazaflavin chromophore in the enzyme F_{420}-NADP oxidoreductase of methane-producing archaea gives also a hint as to the early appearance of this biochrome two billion years ago. It seems that this coenzyme F_{420} (acting as electron carrier) together with methanopterin (acting as C_1 carrier) and coenzyme F_{430} constitute a very first of redox active coenzymes which differs in many respects from that of eubacteria (*Fig. 6*) [11]. The anaerobic respiration leading from CO_2 to CH_4 can then be simply formulated as:

$$CO_2 + 4\,H_2 \xrightarrow{F_{420}} \xrightarrow[\text{Pterin}]{} \xrightarrow{F_{430}} CH_4 + 2\,H_2O$$

Carotenoids:

In the early period of life on the primordial earth organisms depended on anaerobic fermentation for the inefficient production of ATP. *Archaebacteria* contain in their membranes hydrocarbon chains most of which are derivatives of the isoprenoid compound squalene, *i.e.* they have branched chains in contrast to unbranched fatty acids of modern membranes. In addition extremely halophilic *Archaebacteria* (*Halobacteria*) possess higher C_{50} carotenoids, *e.g.* bacterioruberin (*Fig. 6*). It is, therefore, not difficult to imagine that a first primitive photoreceptor like bacterial rhodopsin may have evolved from the primitive membrane structure in which it is located [13]. The simultaneous occurrence of three bacterial rhodopsins in some *Halobacteria* suggests a possible sequence of appearance: sensory rhodopsins (responsible for phototaxis and photophobia) evolved first, then a light-driven chloride pump halorhodopsin was invented (in order to maintain a high intracellular salt concentration), and later the light-driven proton pump bacteriorhodopsin was developed (in order to pump protons across the membrane and to drive ATP synthesis). When oxygenic photosynthesis evolved one of the key functions of carotenoids was to protect aerobic photosynthetic organisms against destruction by photodynamic sensitization. Aerobic photosynthesis would not exist without the coevolution of caro-

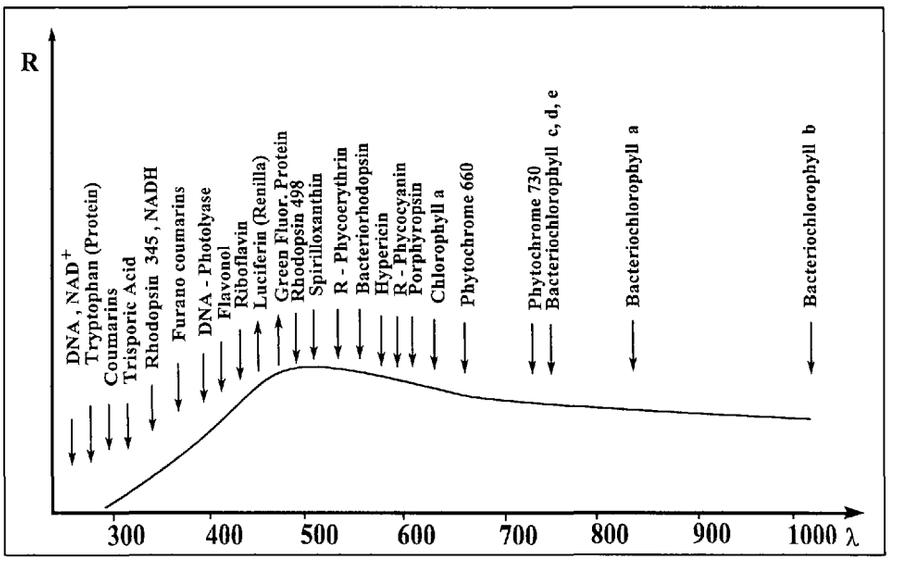


Fig. 5. *The photobiological spectrum showing important chromophores together with the solar spectrum.* R: Solar radiant flux density on the surface of the earth, λ: Wavelength [nm] (after [10])

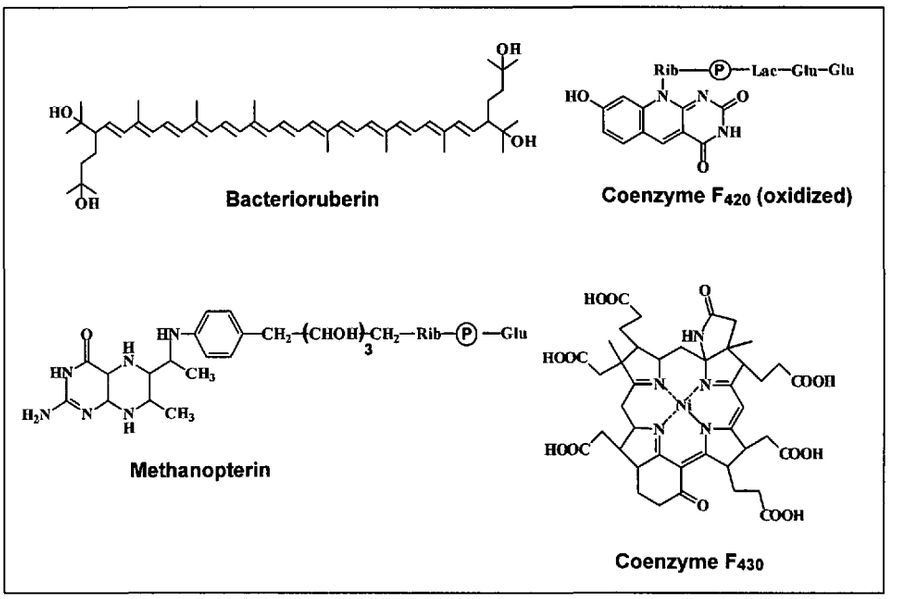


Fig. 6. *Some evolutionary ancient chromophores found in* Archaebacteria

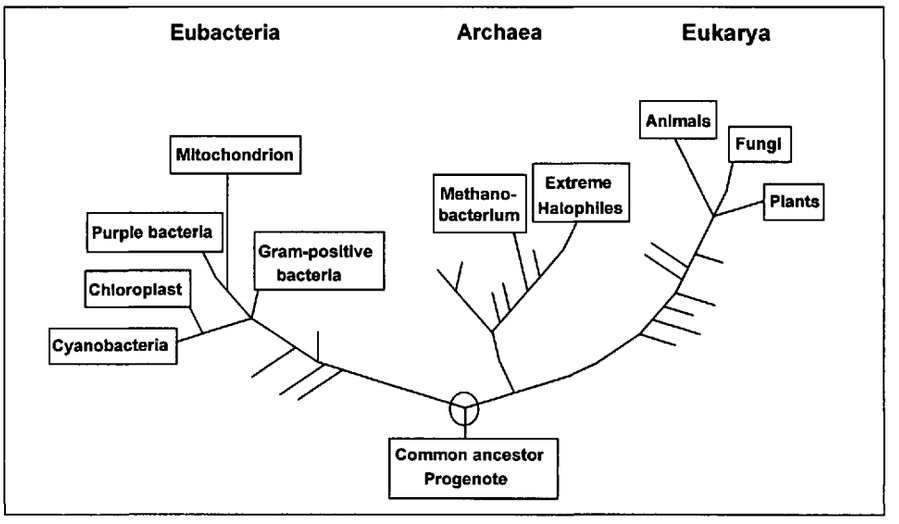


Fig. 7. *The universal tree of life as determined from comparative ribosomal RNA sequencing* [11]

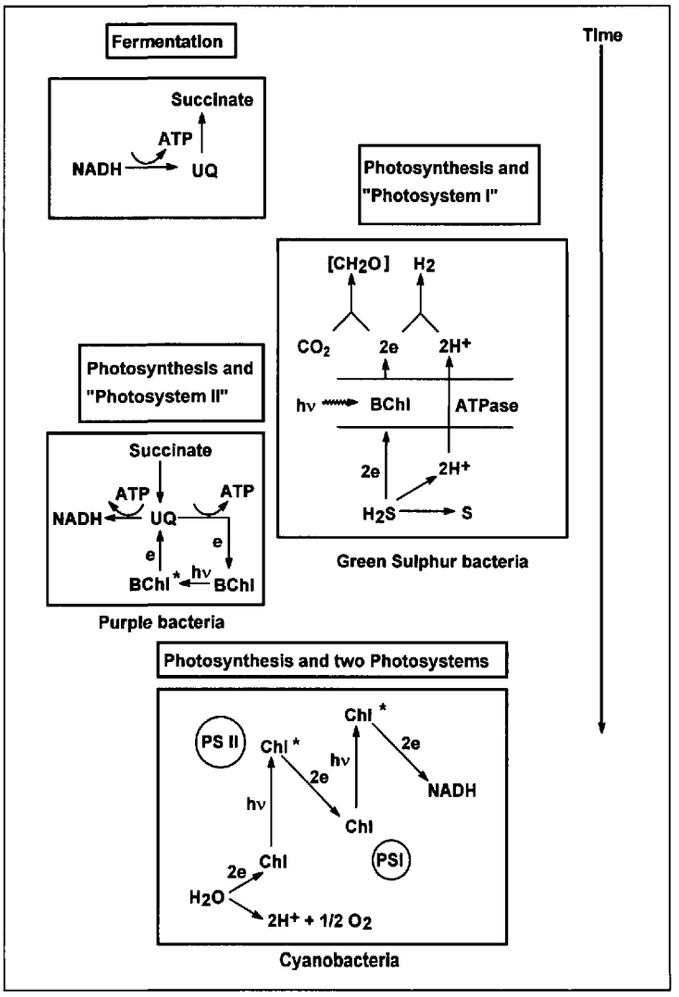


Fig. 8. *The evolution of oxygenic photosynthesis showing the development of pigments like quinones (UQ), bacteriochlorophylls (Bchl) and chlorophylls (Chl),* (modified after [12][18])

Time before present (billions of years)	Pigments	Function	Oxygen % in Atmosphere
4.5	—	—	—
4.0	Porphyrins Flavins, Purines Quinones Carotenes Retinoids	Fermentation Chemolithotrophy Anoxygenic Photosynthesis	Anoxic
3.5	Bacteriochlorophyll Chlorophyll a		
3.0		Oxygenic Photosynthesis Accessory Pigments	
2.5	Xanthophylls Chlorophyll b		0.1
2.0	Phycobilins		1
1.5			10
1.0	?		
0.5	Flavonoids Phytochrome Melanins	Photoprotection Chemical defense Photoreceptor	20
0.0		Visual-Effect	

Fig. 9. *Approximate evolution of natural pigments and biochromes*

tenoids alongside the chlorophylls in photosynthetic organelles [16].

3.3. *The Evolution of Photosynthetic Pigment Systems*

Obviously electron and proton transporting pigment systems were invented twice: the *Archaebacteria* and the *Eubacteria* developed different strategies. The eubacterial way was more successful in the long run [12].

It is very useful to discuss functional pigment systems in relation to the phylogenetic tree representing the three superkingdoms of equal status: the *Archaebacteria*, the *Eubacteria* and the *Eukaryotes* (*Fig. 7*). Rhodopsin is so widely distributed in and outside the animal kingdom that it goes back at least to the common ancestor of the *Archaebacteria* and *Eukaryotes* and probably to the common ancestor of all three superkingdoms. Rhodopsin was found to be but one member of a family of homologous receptor proteins. The gene for the mammalian β-adrenergic receptor, which senses the hormone epinephrine (adrenaline) shows clear sequence homology with the gene for bovine rhodopsin. Rhodopsin could have originated as an early experiment in photosynthesis, a role it retained only in the *Archaebacteria* [17].

Chlorophyll-based photosynthesis arose only once, early in the eubacterial line (see *Fig. 7*). Eukaryotic chloroplasts originated as photosynthetic endosymbionts [17].

The early, very first primitive organisms carried out some form of anaerobic metabolism, *e.g.*

$$FeS + H_2S \rightarrow FeS_2 + H_2$$

as a chemolithotrophic example and fermentation, *e.g.*

NADH + Pyruvic acid $\rightarrow \rightarrow$ Succinic acid

as a chemoorganotrophic example.

A highlight in metabolic evolution would have been the utilization of already available porphyrins and retinoids/carotenoids as photoreceptors in simple photosynthetic devices.

Fig. 8 gives an impression how the sequence starting with fermentation and arriving at two light reactions could have evolved in the porphyrin receptor line. The rhodopsin line of *Archaebacteria* was not pursued any more but, instead, rhodopsins became most important sensory photoreceptors in *Eukaryotes*.

The first organisms using chlorophyll

were probably eubacteria. They evolved later than the rhodopsin-based *Archaebacteria* (see below). Chlorophyll b, the xanthophylls, and the phycobilins were invented during the transition from a reducing to an oxidizing atmosphere. They all need oxygen in one or several steps of their biogenesis [18].

Flavonoids are universally present in all species of land plants. They also occur in two species of relatively advanced green algae families whose ancestors are believed to have been the progenitors of land plants over 400 million years ago. Phytochrome is considered to be a typical pigment of higher plants although some phytochrome effects have also been reported in green and red algae. Rapid evolution of flavonoids occurred during the *Devonian* period (400 million years ago) and in the *mid-Cretaceous* (130 million years ago). The original rise of flavonoid diversity was due to their importance in protection of plants against light, herbivores and phytopathogens, the *post-Cretaceous* development reflected their association with the co-evolving diverse pollinators, birds and insects [19][20] (*Fig. 9*).

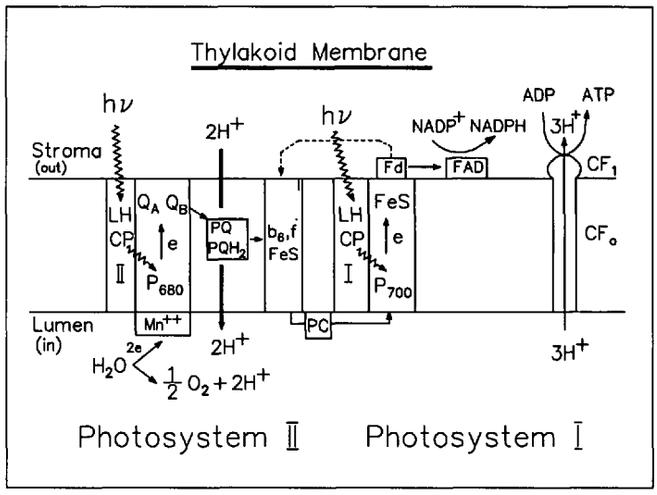


Fig. 10. *Structural model of photosynthetic thylakoid membrane in green plants and algae.* LHCP: light-harvesting chlorophyll a,b-protein, P: reaction center, PQ: plastoquinone, b_6, f: cytochromes, FeS: iron-sulphur protein, Fd: ferredoxin, FAD: flavoprotein, CF_0, CF_1: coupling factors of ATP synthase, PC: plastocyanin [21].

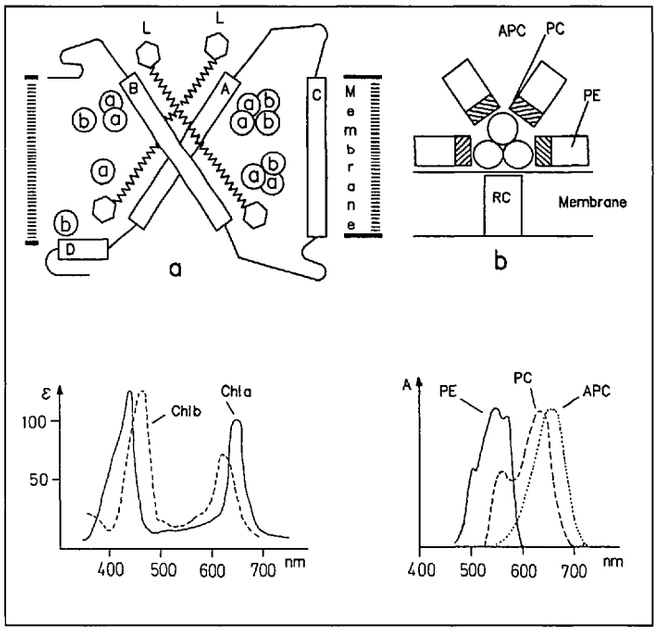


Fig. 11. *Light-harvesting complexes.* a) LHC II of higher plants with α-helices A,B,C,D, lutein carotenoids and Chl a,b. b) Phycobilisome. PE: phycoerythrin, PC: phycocyanin, APC: allophycocyanin. Absorption spectra a) Chl a,b in ether, ε [mM^{-1} cm^{-1}], b) phycobiliproteins [20][21][23].

4. The Functions

4.1. *Energy Transducing Photoreceptors*

4.1.1. *Energy Collection*

The photosynthetic membranes contain all the pigments and other components of the light-gathering apparatus. The location of these membranes within the cell differs between prokaryotes and eukaryotes. The chloroplasts of eukaryotes possess stacked thylakoids forming grana. Within the thylakoid membrane, chlorophyll molecules are associated in complexes consisting of 200–300 molecules. Only few of these are involved in photochemistry: the reaction center chlorophyll. The more numerous other chlorophylls are light-harvesting or antenna molecules. The structural model of a thylakoid membrane including all membrane complexes and units is shown in *Fig. 10*. Some of these functions are being discussed below.

The chromophoric molecules in light-harvesting complexes function as antennas, receiving radiation of defined wavelength and transfer the energy to the reaction center with high efficiency. This vectorial energy transfer is intimately dependent on the fine structure, order and symmetry of the collecting system.

The sequence of absorbing and transferring molecules is energetically in decreasing order of excitation: with decreasing distance from the reaction center the absorption maximum of transmitting clusters shifts to longer wavelengths, *i.e.* in a bathochromic way. This is nicely demonstrated by inspection of important light-harvesting complexes and systems.

Higher Plants and Green Algae:

Light-harvesting pigments are chlorophylls a and b (Chl a, Chl b). The reaction center (RC) contains Chl a. The structure of the light-harvesting complex II (LHC II) is known [22]. There are three transmembrane – helices, two of them are held together by ion pairs. In the center of the complex Chl a is in close contact with Chl b for rapid energy transfer, and with two carotenoids (lutein) that prevent the formation of toxic singlet oxygen (*Fig. 11a*). The sequence of energy transfer is from the antenna to the RC:

Chl b → Chl a → Chl a → Chl a (spec., PS II)
650 nm ~660 nm 670 nm 680 nm

Red Algae and Cyanobacteria:

Extramembrane light-collecting systems, the phycobilisomes, contain bilin pigments: phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC). The transfer of energy to the RC is precisely in that order as shown in *Fig. 11b* by the fine structure of phycobilisomes [20][21].

PE → PC → APC → APC B → Chl a (spec, PS II)
570 nm 630 nm 650 nm 670 nm 670 nm

Purple and Green Bacteria:

The pigment complexes contain bacteriochlorophylls a, b (purple), and a, c, d, e (green) [24].

The sequence of transfer in purple bacteria is [21]:

B 800–820 → B 800–850 → B 870 → RC (870)

Within the chlorosomes of green bacteria the direction of energy transfer is:

B 750 → B 790 → B 804 → RC (840)

Light-collection and harvesting depends strongly on the position of the absorption band. The different spectra of the pigments turn out to be a consequence of different conditions at those periods when they evolved (*Fig. 9*). The following hypotheses may give a plausible explanation [18].

The first photosynthetic organisms could have been planktonic *Archaebacteria* operating with bacteriorhodopsin and absorbing light (570 nm) right in the middle of the visible spectrum. *Halobacterium halobium* is an extant, present-day example.

All green and yellow light would have been absorbed. Light was used to transport protons and for the production of ATP. The chlorophylls and bacteriochlorophylls absorb strongly UV, violet, and blue light as well as red or IR light. The bacteriochlorophylls are now used by purple and green bacteria, the first chlorophyll a containing organisms were probably *Eubacteria*. Since they lived in the deep mud under water, rich in organic matter, they received only red and blue light. The green and yellow portion had been filtered out by the purple colored *Archaebacteria*. This could have led to the evolution of the green chlorophyll a (*Fig. 12*).

The phycobilins as accessory collecting pigments evolved later because chlorophyll cannot absorb green light. Chlorophyll b is the main accessory pigment in higher plants and is the result of oxidation of a short side chain in chlorophyll a. Many carotenoids have oxidized functional groups and absorb in the middle of the spectrum (like phycobilins, Chl b) and serve as accessory pigments. Only the bacteriochlorophylls explored the IR part of the spectrum with bacteriochlorophyll b going farthest (BChl b: 835–850, 1020–1040 nm). The reason is that these bacteria could utilize the spectral window in water-absorption between 970 and 1190 nm [25].

There is a tendency for organisms living deep underwater to contain a greater proportion of phycoerythrin because it is better at absorbing green light. In brown algae the carotenoid fucoxanthin is very efficient in transmitting energy to the reaction center. With its absorption maxima at 421, 446, and 475 nm it operates successfully together with chlorophyll c in harvesting light in the blue and green range [21][26].

Spirilloxanthin, a carotenoid typical of purple bacteria, has a chain of 13 conjugated double bonds. This renders the molecule an ideal light collector in the useful spectral range 450...550 nm (absorption maxima at *ca.* 460, 490, and 530 nm) [21].

All this happened probably in the transition from a reducing to an oxidizing atmosphere. The carotenoids (see *Fig. 11a*) as the lutein molecules in LHC II had a dual role: light-harvesting function and a protective function, namely to quench triplet chlorophyll excited states.

The chemical structural modifications that finally led to a changed spectral absorption were minor redox reactions and rearrangements but with the mentioned consequences. *Fig. 13* represent the basic structures in the chlorophyll series.

4.1.2. *Energy Transfer*

Electronic energy transfer can be discussed considering different limiting cases [28]. Depending on the magnitude of donor-acceptor interaction energy three cases arise, defined as strong interaction case, weak interaction case and very weak interaction case. The *Förster* mechanism, *i.e.*, the long-range transfer *via* dipole interactions is based upon the very weak interaction model.

It is now generally accepted that singlet-singlet energy transfer between chlorophylls occurs by the *Förster* mechanism. The typical distances of neighboring chromophores are 1–2 nm (10–20 Å). The rate of energy transfer obeys the equation given in *Fig. 14*.

Here k is dependent on dipole orientation, Φ_D is the donor fluorescence quantum yield, n is the refractive index of the solvent, N_0 is *Avogadro*'s number, r is the donor-acceptor distance, τ_D is the donor fluorescence lifetime, and J is the spectral overlap. Carotenoid-chlorophyll energy transfer has an efficiency which can be as high as 100% [29]. Carotenoids have an intense absorption band that is due to the transition from the ground to a second exited singlet state S_2 (symmetry ^1B_u). The first excited singlet state S_1 is not to be seen in conventional absorption spectra since the transition is forbidden (symmetry ^1A_g). However, the lifetimes of S_1 and

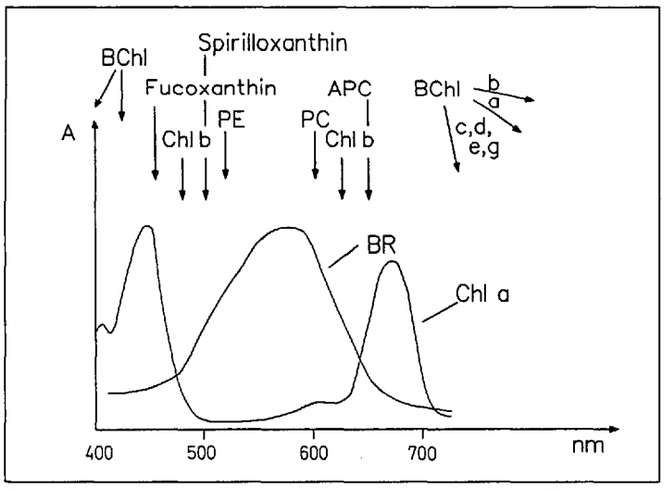


Fig. 12. *Spectral adaptation of light-harvesting pigments.* The spectral window first used and closed by bacteriorhodopsin BR had to be opened using chlorophyll (Chl) a and bacteriochlorophylls a–g. Later, in the course of oxygen evolution, BR disappeared and the window was used by accessory pigments: carotenoids, Chl b, phycoerythrin PE, phycocyanin PC and allophycocyanin APC [18].

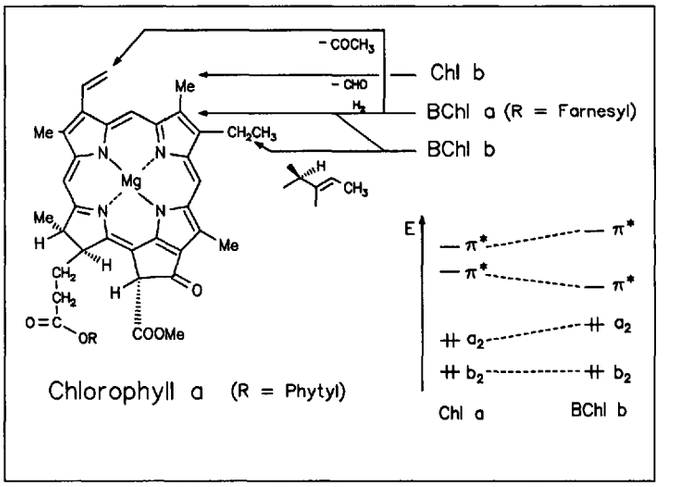


Fig. 13. *Structure of chlorophylls and bacteriochlorophylls.* Frontier orbitals of Chl a and BChl a, showing the bathochromicity in the latter chromophore [27].

S_2 are different: $S_1 \sim 10^{-11}$ s, $S_2 \sim 10^{-13}$ s. Both S_1 and S_2 states participate in the above-mentioned singlet energy transfer [30].

4.1.3. *Energy Transduction*

The third major role of chlorophylls – beside energy collection and energy transfer – is light-energy transduction, *i.e.* the transfer of an electron from a specially oriented chlorophyll molecule to an electron acceptor.

The reaction centers of purple bacteria (*e.g. Rhodobacter sphaeroides* and *Rhodopseudomonas viridis*) have been investigated using X-ray diffraction [31]. The analysis revealed that there is an approximate two-fold rotation symmetry that relates the bound bacteriochlorophylls, bacteriophaephytins and quinones. The carotenoid is the only chromophore in the RC that does not conform to this approximate two-fold symmetry. The reaction sequence after excitation of one bacteriochlorophyll b of the special pair is shown in *Fig. 15*.

The role of the carotinoid is usually the quenching of chlorophyll (the primary donor) triplet states [31]. This is true in *Rb. sphaeroides* where spheroidene, a carotenoid with 10 conjugated double bonds, accepts triplet energy in *ca.* 30 ns. The exception to this rule is *Rps. viridis*, the main carotenoid of which, 1,2-dihydroneurosporene, does not quench the triplet state of the primary donor (*Fig. 15*) [29]. It is remarkable that *cis*-configurations of carotenoids are selected by the RC for the photo-protective functions. This is attributable to the unique isomerization properties of the *cis*-molecules. On the other hand *trans*-configurations prevail in the LHC because of better energy transfer abilities [30]. The energy transduction is finally brought about by the ubiquinone which enters in reduced form the electron and proton transport chain (*Fig. 10*).

4.1.4. *Light-driven Ion Pumps*

The purple membrane of *Halobacterium halobium* contains a pigment of which retinal is the chromophore. This bacteriorhodopsin functions as a light-driven proton pump. A second retinal pigment of *halobacteria*, called halorhodopsin, acts as a chloride pump. The purpose of both pumping systems is as follows: *Halobacteria* are equipped with the regular metabolism for energy transduction as in *Eubacteria*. But upon oxygen limitation the synthesis of bacteriorhodopsin is induced. When light is present, retinal-based photosynthesis replaces oxidative metabolism [32].

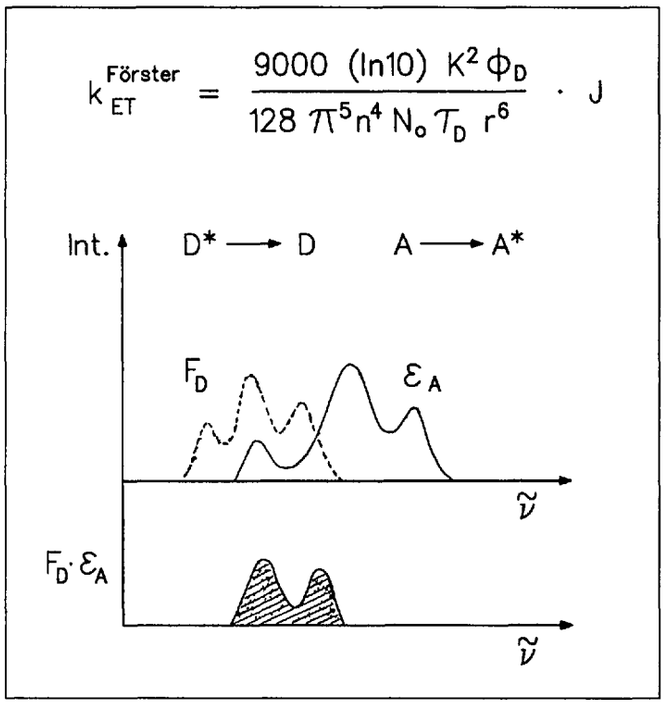


Fig. 14. *Rate of energy transfer according to the* Förster *equation and explanation of spectral overlap* J [28]

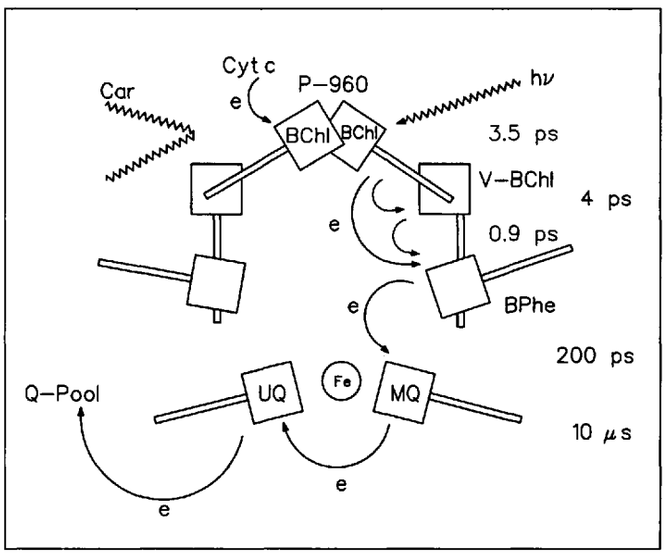


Fig. 15. *Reaction center of* Rps. viridis [21] [31]. P-960: special pair, BChl: bacteriochlorophyll b, V-BChl: 'Voyeur' molecule, Bphe: bacteriophaephytin b, MQ: menaquinone, UQ: ubiquinone, Cyt c: cytochrome c, Car: 1,2-dihydroneurosporene.

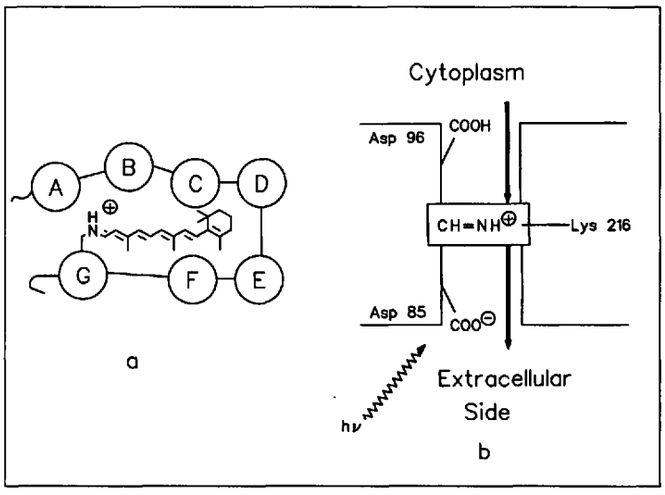


Fig. 16. a) *The seven transmembrane* α*-helices of bacteriorhodopsin.* b) *Light-induced proton translocation.* Asp: Aspartic acid (after [33]).

One absorbed photon pumps one proton across the bacterial membrane. The resulting electrochemical pH-gradient drives ATP synthesis. In order to counteract extracellular osmotic pressure the bacteria have to accumulate a large concentration of potassium chloride. This is achieved by the chloride pump halorhodopsin. The rhodopsin pigments belong to an evolutionary very old family of membrane proteins having seven transmembrane helices. The process of proton translocation is shown in *Fig. 16*. Two aspartic acid residues (Asp 96 and 85) act as donor and acceptor for protons during the light-induced transport [33].

4.2. *Photoprotection*

4.2.1. *Quenching of Excited States*

If cells of photosynthetic organisms without carotenoids (*e.g.* mutants like *Rh. sphaeroides R-26*) are illuminated in the presence of oxygen they sensitize their own death [29]. The process responsible for the killing is

$$Chl \xrightarrow{h\nu} {}^1Chl^* \rightarrow {}^3Chl^*$$
$${}^3Chl^* + {}^3O_2 \rightarrow Chl + {}^1O_2^*$$

The produced singlet oxygen will then wreak havoc in all organelles. Carotenoids protect the system efficiently provided that they are bound in proximity to the chlorophylls. Apart from transferring energy this protection is a most essential function of carotenoids in oxygenic, aerobic photosynthesis [31].

On the other hand there is also a direct interaction of carotenoids with singlet oxygen

$${}^1O_2^* + Car \rightarrow {}^3O_2 + {}^3Car^*$$
$${}^3Car^* \rightarrow Car + heat$$

There are some carotenoids which undergo rapid, light-induced changes in their concentration. In higher plants, ferns, mosses, green and brown algae these are violaxanthin, antheraxanthin, and zeaxanthin. In other algae (diatomeae, chrysophyceae, xanthophyceae, dinophyceae, and other) the two carotenoids diatoxanthin and diadinoxanthin are important.

The conversion of violaxanthin into zeaxanthin, the de-epoxidation sequence, as well as the reverse, the epoxidation sequence, is a true cycle since the forward and the back reaction sequences are catalyzed by two different enzymes. The de-epoxidation is favored under excessive light and a low pH (optimum 5.1), and it uses ascorbate. The epoxidation occurs under limiting light, a higher pH (optimum 7.5), and needs oxygen as well as NADPH. What is the function of this xanthophyll cycle?

An excess of excitation energy could potentially result in the accumulation of excitation energy. This, in turn, could lead to the damaging and destructive species singlet oxygen $^1O_2$, superoxide anion $O_2^{\cdot -}$ and hydrogen peroxide H_2O_2. However, the accumulation of excess excitation energy is counteracted: thermal dissipation of excess energy occurs directly within the system. There is strong evidence that zeaxanthin is involved in this thermal energy dissipation, thus protecting the photosynthetic apparatus against the adverse effects of excessive light. The precise mechanism, however, is not yet known. One suggestion is that the singlet excited state of chlorophyll is quenched by the carotenoid. The diverse protecting functions are summarized in *Fig. 17* and *18* [34–40].

The accumulation of large amounts of carotenoids in algae under intensive solar radiation is well-known. Snow algae occupy a unique habitat in high altitude and

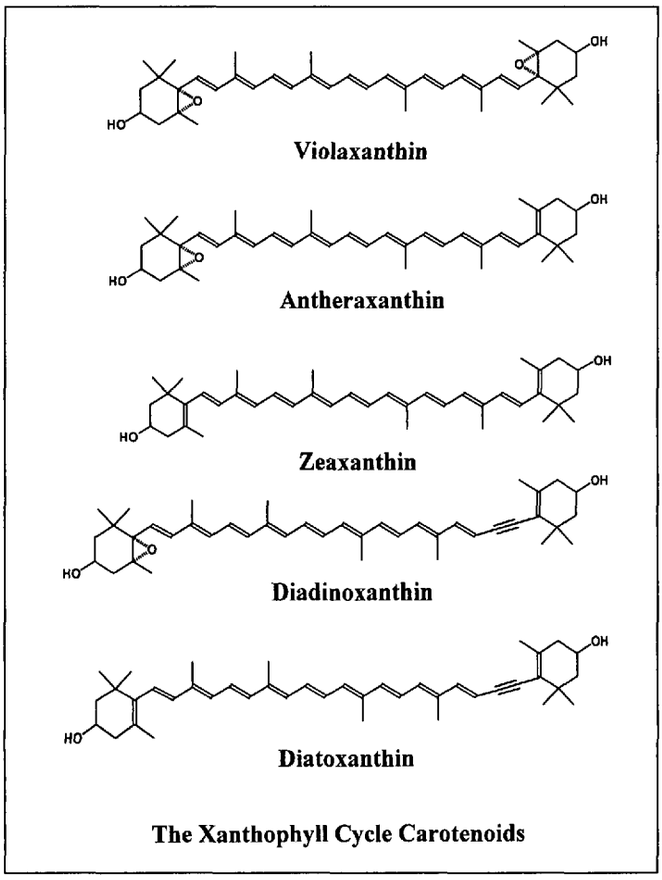


Fig. 17. *The carotenoids involved in the two xanthophyll cycles of higher plants and algae* [34][35]

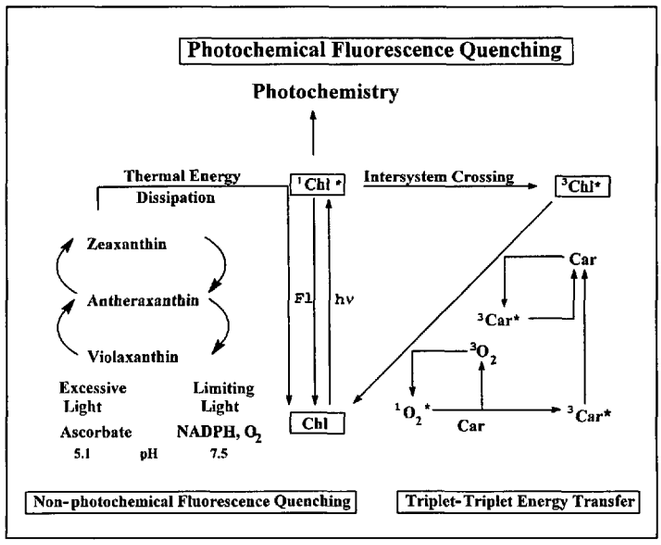


Fig. 18. *The protecting role of carotenoids.* Chl: Chlorophyll, Car: carotenoid, Fl: fluorescence [29][35].

polar environments. Unusually large accumulations of astaxanthin esters in extrachloroplastic lipid globules produce the characteristic red pigmentation typical of some snow algae, *e.g. Chlamydomonas nivalis*. Consequently, these compounds greatly reduce the amount of light available for absorption by the light-harvesting pigment-protein complexes, thus potentially limiting photoinhibition and photodamage caused by intense solar radiation [41]. Much the same occurs in *Euglena sanguinea*, a freshwater flagellate, when cells are irradiated with UV-B radiation. This suggests that the formed carotenoid is a photoprotective pigment [42].

4.2.2. *Photoreactivation*

Photoreactivation is a process in which an enzyme is activated by long-wave UV or visible radiation in order to bind and cleave dimers of pyrimidine bases, *i.e.* dimers of thymine. This photorepair of UV-induced DNA damage is a vital function that is present in microorganisms, fungi, plants, and animals including man. Irradiation with far UV-light induces formation of pyrimidine dimers. Upon absorption of light of wavelengths between 300 and 600 nm, a DNA photolyase splits the cyclobutane ring that was formed by cycloaddition of adjacent pyrimidines [8][15][26].

The enzyme isolated from *E. coli* contains both flavin ($FADH_2$) and a pterin derivative (5,10-methenyl-tetrahydrofolylpolyglutamate) absorbing at 390 nm in the protein-bound state. The *in vivo* action spectrum for photoreactivation is at 380 nm. The pterin chromophore causes a fluorescence at 470 nm. Some other repair enzymes of the DNA photolyase type contain not a pterin but two special flavin chromophores. *Streptomyces griseus* and *Methanobacterium thermoautotrophicum* possess $FADH_2$ together with 8-hydroxy-5-deazaflavin which has already been mentioned in *Fig. 6*. The action spectrum has a peak at 436 nm. The deazaflavin chromophore is an evolutionary very ancient type (at least 2 billion years old) and had been preserved throughout the plant and animal kingdom [15]. The mechanism of repair is not completely understood but a working hypothesis is given in *Fig. 19* [8].

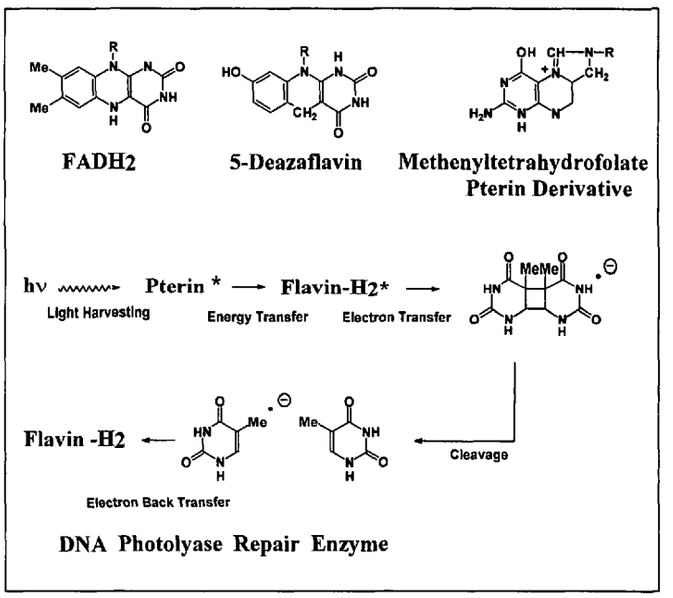


Fig. 19. *Chromophores of DNA photolyase repair enzyme.* Suggested mechanism of thymine dimer cleavage [8][15].

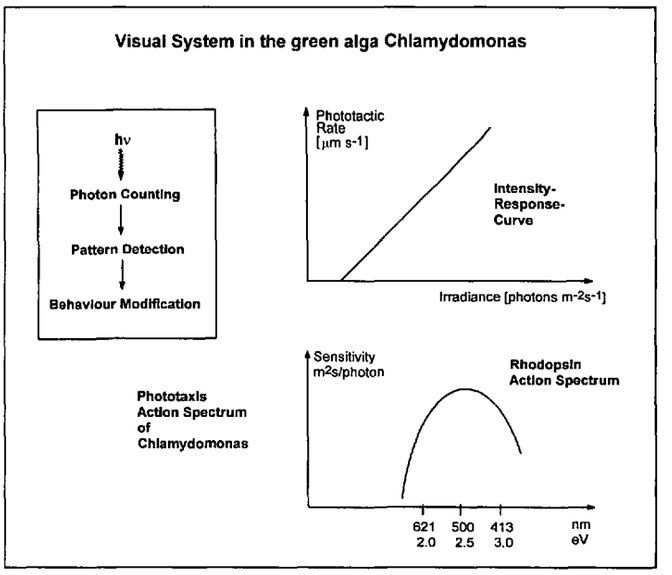


Fig. 20. *Phototaxis in the green alga* Chlamydomonas [17]

4.3. *Sensory or Signal Transducing Photoreceptors*

4.3.1. *Photomotion (Tactic, Kinetic, Phobic)*

Besides providing essential energy for life on earth, light provides signals and images that enable organisms to manage successfully in their environments [8]. The most important chromophores for sensory transducing pigments are retinal, tetrapyrrole, flavin, pterin, and hypericin pigments. Absent from this list are particularly the carotenoids and hemes. The reason is that the latter two pigment groups show efficient radiationless deactivation to the ground state without any significant photoreceptor photochemistry. On the other hand these biochromes have their own important function as precursors for retinals or as protecting dyes. One of the most important means for identifying the kinds of molecules in a photosensitive system is by obtaining the behavioral action spectrum. The *action spectrum* is the relative response that a plant or animal makes to a light stimulus of different wavelengths. The action spectrum obtained should correspond to the absorption spectrum of the molecules reponsible for that behavior [1]. For any particular light source, threshold is the lowest intensity that produces a reponse [17]. The phenomenon is shown explaining phototaxis of the green alga *Chlamydomonas reinhardtii*. Photomotion comprises *phototaxis* (stimulus-oriented directed movement relative to radiation direction), *photokinesis* (dependence of rate [ms^{-1}] on photon irradiance [mol s^{-1} m^{-2}]), and *photophobic reactions* (movement responses to changes of light intensity or photon irradiances) [12][26]. The prerequisite for a phototactic movement is the existence of a photoreceptor with corresponding pigments. A photoreceptor molecule must meet stringent requirements. It must absorb a photon with high probability and it must pass the information on

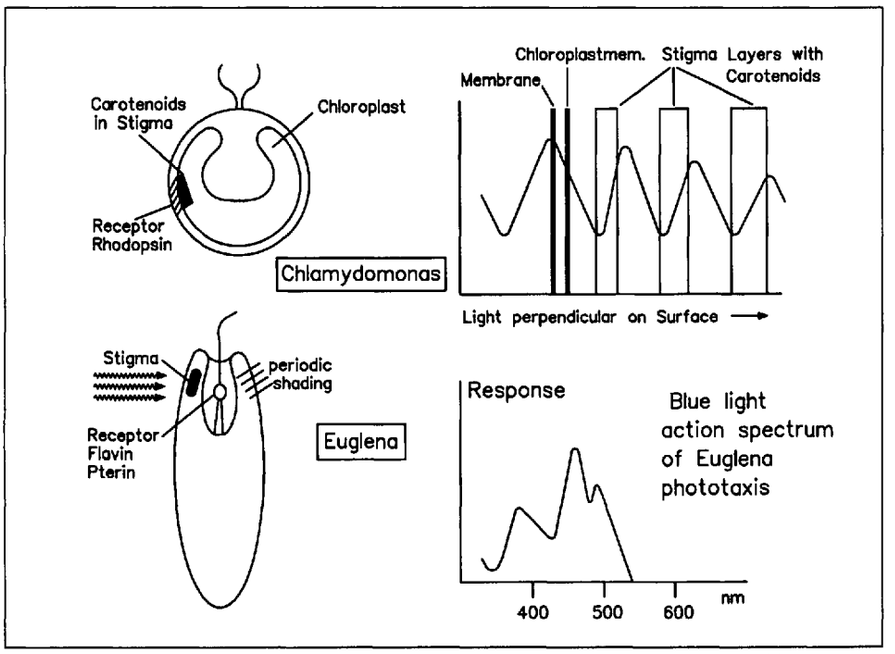


Fig. 21. *Photoreceptor apparatus of the green alga* Chlamydomonas *and the euglenoid* Euglena *and function of the eyespot (stigma)* [15][17][26][43]

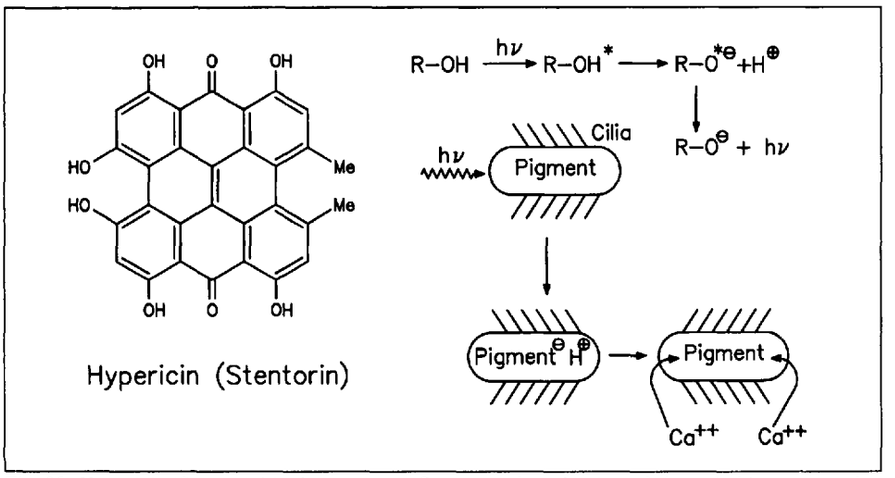


Fig. 22. *Hypericin (Stentorin) in the protozoan* Stentor *and mechanism of photophobic response,* i.e. *ciliary reversal* [45]

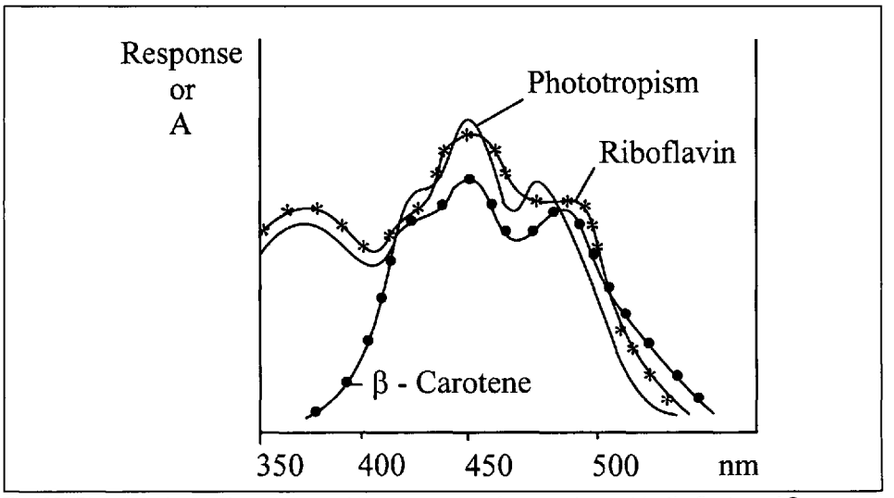


Fig. 23. *Phototropism action spectrum (*Avena, *oats) and comparison with riboflavin and β-carotene* [12]

with high efficiency. A visual photoreceptor system must also detect patterns and should modify its behavior. Thus, we have three criteria for visual photoreceptor systems: photon counting, pattern detection and behavior modification [17]. In *Fig. 20* the responses and the action spectrum for phototactic rate of *Chlamydomonas* are given, showing the rhodopsin-type action spectrum of this particular photoreceptor [17].

The arrangement of the pigment system (eyespot pigments and photoreceptor pigments) in the cells of *Euglena* and *Chlamydomonas* is an excellent example of combined pigment function (*Fig. 21*). In *Chlamydomonas* the eyespot (stigma) is composed of several layers of carotenoid pigment formed in the chloroplast. The carotenoid layers are spaced in such a way that the eyespot becomes a tiny mirror which is due to constructive interference of light reflected from each surface of the layers. The photoreceptor pigment, rhodopsin, used for phototaxis forms a patch in the plasma membrane over the eyespot [17][26][43].

In *Euglena gracilis* the eyespot is not part of the chloroplast, but its pigments are also carotenoids. The flagellum contains the photoreceptor pigment in its paraflagellar body. *Euglena* uses two receptor pigments: a flavin receptor for phototaxis and a pterin receptor for photophobic response and negative phototaxis [15].

A novel class of photoreceptor pigments was discovered in the protozoan *Stentor coeruleus*. These pigments, which employ hypericin as chromophore, are stentorin and blepharismin. They serve as photoreceptors for phototaxis and photophobic responses [8].The remarkable fact is that hypericin acts as photosensitizer for photodynamic action. Hypericin is also a product of higher plants, the *Hypericaceae*, and particularly of *Hypericum perforatum*. Hypericin is responsible for the toxicity of these plants towards animals (*St. John's wort*). Hypericin acts in animals as photosensitizer which brings about photooxidation and causes inflammations, oedema and even death [44]. Despite of this phototoxicity the ciliate *Stentor* has developed the capacity, *i.e.* the step-up photophobic response (light-avoiding) and negative phototaxis (away from light source), to survive the damaging radiation environment [8][10][26]. Hypericin, stentorin I and II absorb around 590–620 nm and the action spectra of *Stentor* for photophobic response are similar. The primary photochemical process may be a proton dissociation in the excited state (*Fig. 22*). The resulting pH gradient triggers a Ca^{++}

Fig. 39. *The word 'color' was written using letters of the butterfly alphabet.* Beautiful patterns on butterfly wings form letters and numbers. They have been observed and photographed by *K.B. Sandved.* With kind permission of *Sandved Photography* [82].

influx which is finally responsible for ciliary reversal [45] (color prints *Figs. 45* and *46*).

4.3.2. *Blue Light Photoreceptors (Cryptochrome)*

The assignment of blue light photoreceptors to flavins, pterins or carotenoids is a serious problem. Often the lack of a near UV peak in the action spectra has been taken as evidence that a carotene functions as receptor. This conclusion, however, is unwarranted, because flavins and flavoproteins are known that are atypical, *i.e.*, they lack a near UV peak [15].

An additional problem is that action spectra can depend on light intensity. The most probable candidates for blue light receptors are flavins and pterins.

4.3.3. *Phototropism*

A growth response involving bending or curving of a plant toward or away from an external light stimulus is called phototropism. Photoreceptor for phototropism of the coleoptiles of oats (*Avena sativa*) is a flavin (*Fig. 23*). The earlier discussed carotenes are not photoreceptors but may function as screening pigments. Since the shoot bends in the direction of irradiance differences between the flanks of an organ, screening pigments will increase irradiance differences and enhance phototropic sensitivity.

4.3.4. *Photomorphogenesis*

Three pigment systems are indispensable for the development of chloroplasts and chlorophyll: protochlorophyllid, phytochrome and blue light receptors. Protochlorophyllid is transformed into chlorophyll a in two steps. The first is a light-requiring reaction for the reduction of pyrrole ring D, the second one is esterification of the propionic acid substituent. It is remarkable that protochlorophyllid serves as the photoreceptor for its own conversion to chlorophyll a [24]. Phyto-

Fig. 40. *Colors of butterflies are caused by thin film interference.* This monarch *Danaus plexipus* is distasteful. Birds avoid them. With kind permission of *Droemer-Knaur-Kindler* [84].

Fig. 41. *The butterfly* Limenitis archippus *may be looked at as a mimic of the monarch, but it is bad-tasting, too.* An example for *Müllerian* mimicry [49]. With kind permission of *Droemer-Knaur-Kindler* [84].

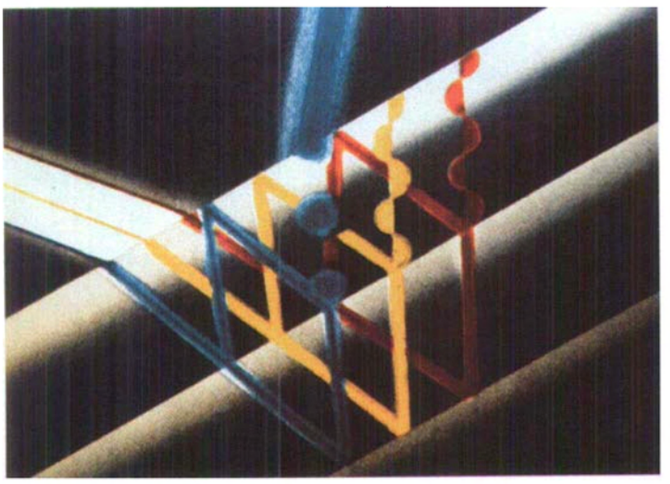


Fig. 42. *Iridescent colors of butterflies originate from the layered structure of scales.* With kind permission of *Droemer-Knaur-Kindler* [85].

Fig. 43. *Nocturnal animals enhance vision using a reflecting layer (tapetum lucidum) behind the retina.* The layer consists of golden-yellow crystalls of riboflavin [2]: the bush-baby *galago*. With kind permission of *Droemer-Knaur-Kindler* [86].

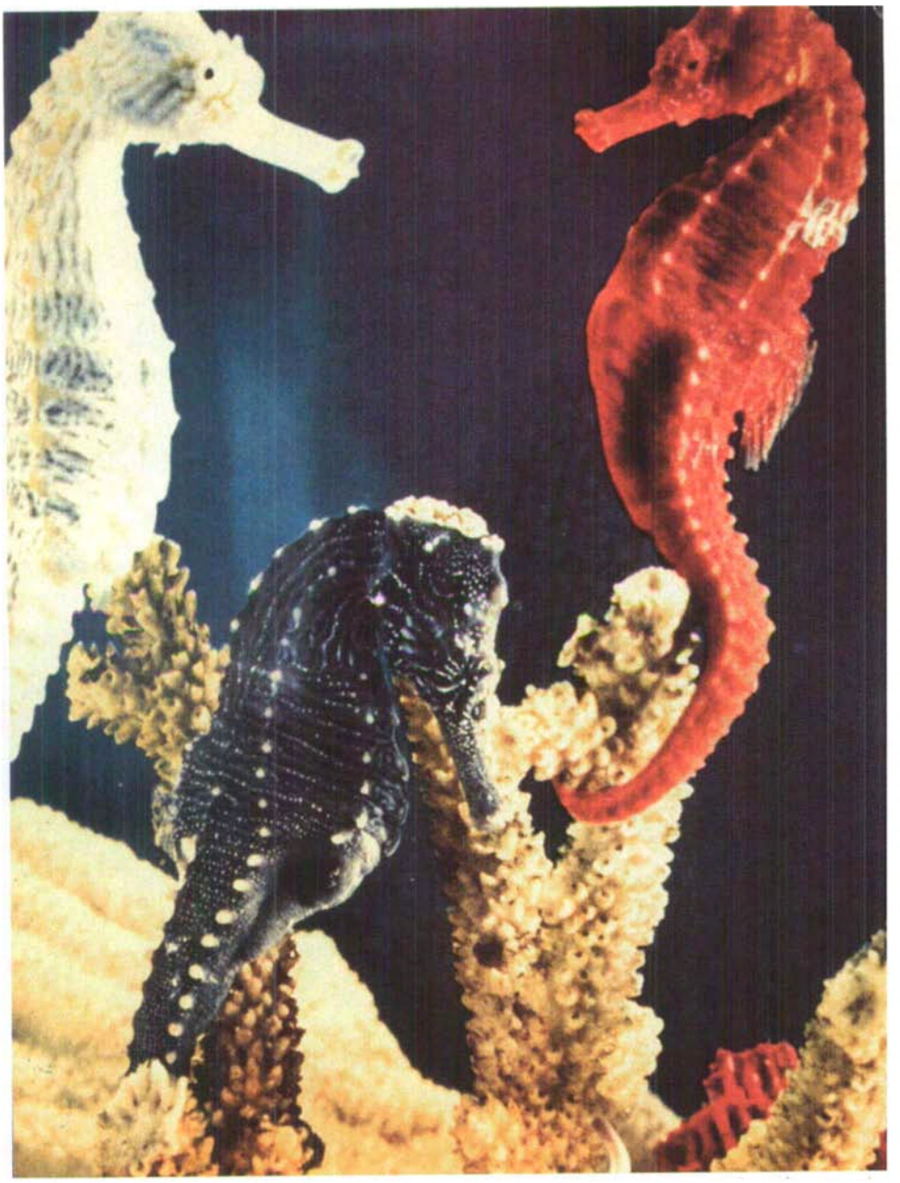


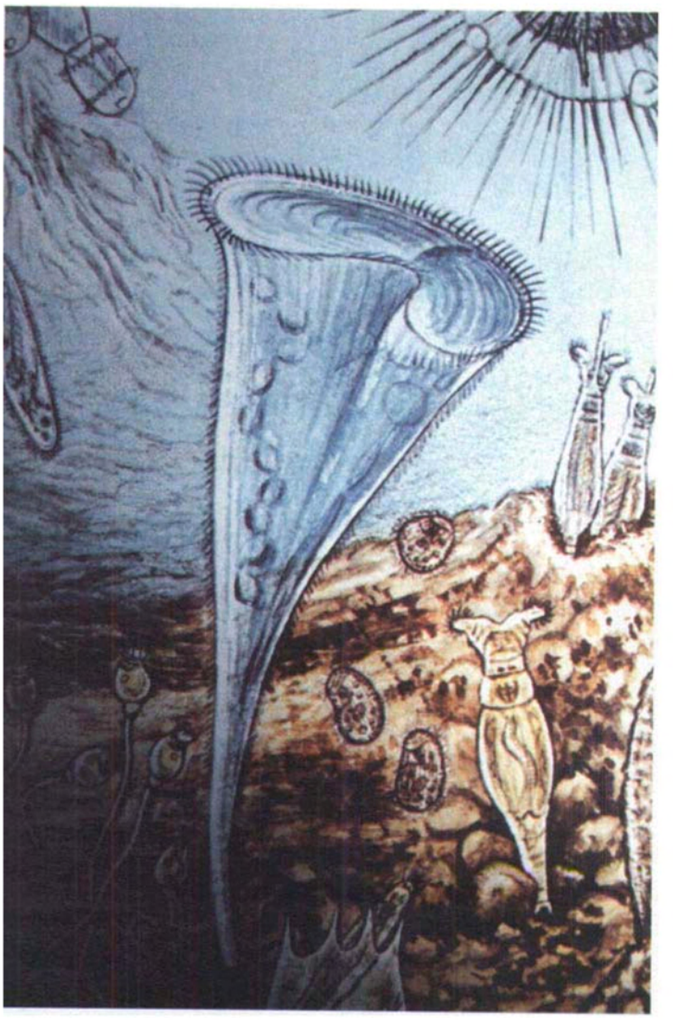
Fig. 44. *Colors of fishes (e.g. Hippocampus hudsonius) caused by light scattering based on guanine particles* (blue, silvery) *or on carotenoids* (yellow, red) *or based on both effects* (green). Melanin in melanophores contributes to dark and black [2]. With kind permission of *Droemer-Knaur-Kindler* [87].

chrome is a chromoprotein and the red light receptor in plants [12][46][47]. It has been called the 'visual pigment of plants' [47]. The photochromic phytochrome system has a conspicuous analogy to rhodopsin and bacteriorhodopsin: the primary photoprocess is a very fast (< 100 ps) (*E*/*Z*)-isomerization of the chromophore which leads to subsequent changes of the surrounding protein and triggers such important processes as pattern formation, induction of germination, induction of flowering, synthesis of chlorophyllid and many other. Since phytochrome absorbs either red or far-red light (P_r and P_{fr}), it responds to light quantity and to light quality *via* the ratio P_r/P_{fr} or P_{fr}/P_{tot}, respectively (*Fig. 24*) [12].

4.3.5. *Photoperiodism*

Many growth and developmental phenomena are controlled by the daily radiant exposure period. A critical day lenght is decisive: day lenghts longer than the critical lenght are called long-day, otherwise short-day. The photoperiodical regulation of flowering has been well investigated. One can distinguish between short-day, long-day, and day-neutral plants. The phytochrome system enables the plant to sense whether it is in light or in darkness, but the actual measuring of the time lapse between the moment the plant senses the onset of darkness and the moment it senses the next exposure to light depends on an internal clock. *Fig. 25* shows the effect of day lenghts on flowering (color prints *Figs. 47* and *48*).

In animals there are also processes that are regulated by light and correspond to diurnal circadian or longer-period rhythms. The light is detected either by eyes or by other extra-retinal and extra-ocular pho-



Figs. 45 and 46. *Hypericin is responsible for the toxicity of plants like* Hypericum perforatum (*St. Johns wort*). The same chromophore acts as photoreceptor in the protozoan *Stentor coeruleus*. With kind permission of *Ecomed* [88] and *Droemer-Knaur-Kindler* [89].

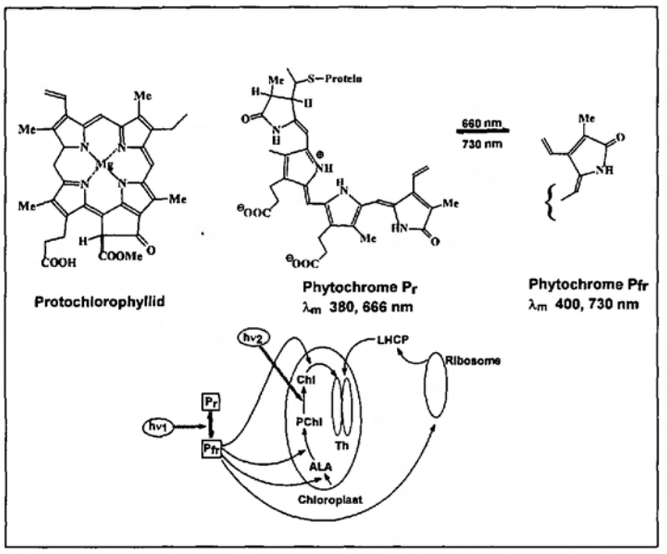


Fig. 24. *Protochlorophyllid (PChl), the precursor of chlorophyll, and the photochromic phytochrome P_r/P_{fr} system.* Control of chloroplast formation by light and two pigment system. Chl: Chlorophyll, ALA: aminolevulinic acid, Th: thylakoids, LHCP: light-harvesting chlorophyll protein [48]. Two photoprocesses are needed for formation of chloroplasts and thylakoids: excitation of P_r as well as photoconversion of PChl.

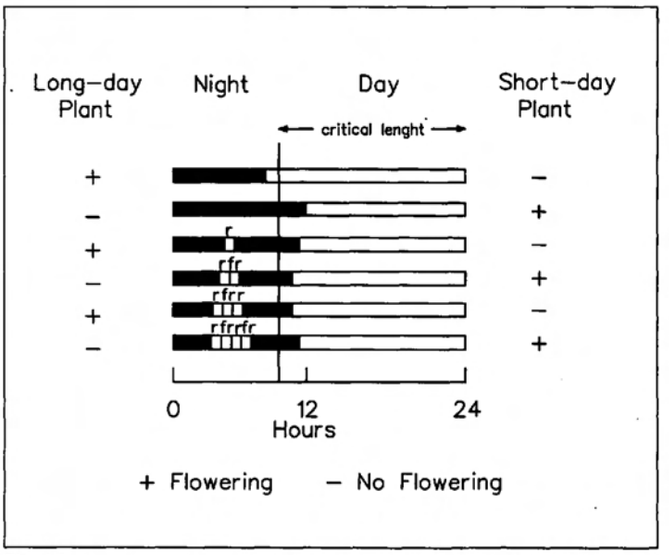


Fig. 25. *Reactions of long-day and short-day plants.* r: red flash, fr: far red flash [49]. Long-day plants flower when the night is shorter than the critical length. A long night with a red flash is seen as a short one. A second far red flash will abolish the effect of the first flash.

Figs. 47 and 48. Euphorbia pulcherrima *and* Hyoscyamus niger *are examples for short-day and long-day plants, resp.* Hyoscyamus *requires about 10 h day-length* (depending on temp.) *or more to flower.* With kind permission of *Urania*-Verlag [90] and *Ecomed* [91].

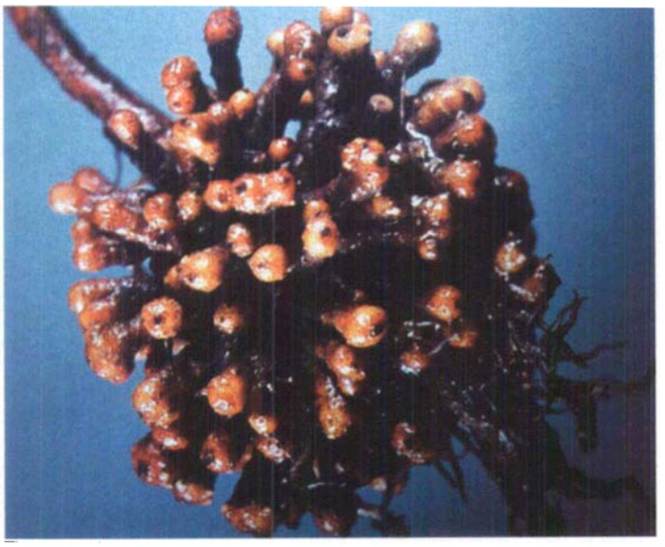


Fig. 49. *Alder trees (alnus) form root-nodules that are induced by nitrogen-fixing actinomycetes similar to legumes and* Rhizobium *where flavonoids activate nodulation genes.* With kind permission of *Urania*-Verlag [92].

Fig. 50. *Flatfishes, like* Bothus, *show excellent camouflage due to cryptic coloration.* With kind permission of *Belser*-Verlag [93].

Fig. 51. *The praying mantid* Hymenopus coronatus *lives on flowers of an orchid which is extremely similar in shape and color.* With kind permission of *Droemer-Knaur-Kindler* [94].

Fig. 52. *Pterins, not carotenoids, are responsible for yellow colors of wasps: Xanthopterin in* Paravespula germanica. With kind permission of *Droemer-Knaur-Kindler* [95].

toreceptors like median eyes, pineal gland, front organ, parietal eye or even parts of the integument.

4.3.6. *Vision*

Two basic eye structures are the refracting eye with a large single lens (vertebrates) and the compound eye with a large number of small photoreceptor units called ommatidia (invertebrates, arthropods). The photoreceptor cells are of special interest: rods and cones in vertebrates, and rhabdoms in arthropods. They contain the visual pigments, the rhodopsins, which employ retinal or structural modifications of it as photoreceptors (*Fig. 26*). Four retinal aldehydes (and the corresponding alcohols) are known: A_1–A_4 [32]. The rhodopsin class is of very ancient origin occurring in prokaryotic and lower eukaryotic organisms. It is assumed that the rhodopsin polypetide chains thread their way back and forth through the membrane with total of seven transmembrane helical segments [8][32][50]. All retinal A_1 based pigments are called rhodopsins, and those using retinal A_2 are called porphyropsins. Xanthopsins are then derived from retinal A_3 [32].

In *Fig. 26* two structural models are given; one for bovine rhodopsin (*cf. Fig. 16*), and one for the fly visual pigment which has two chromophores. The photochemistry of visual pigments and bacteriorhodopsins is involved but fairly well understood [8][32][33].

In *Halobacterium halobium* four rhodopsin pigments have been discovered, all of which reside in the plasma membrane:
Bacteriorhodopsin (photocycle, proton pump function),
Halorhodopsin (photocycle, chloride pump function),
Sensory rhodopsin I (photocycle, phototaxis function, attraction by green light, repulsion by UV light),
Sensory rhodopsin II (photocycle, repulsion by blue-green light, 'phoborhodopsin').
The function of the above mentioned two visual pigments,
Fly xanthopsin (photocycle, thermostable intermediate, sensitizer, vision function),
Bovine rhodopsin (photocycle, vision function), is shown in *Fig. 27*.

In the vertebrate retina the rod cells are responsible for scotopic or night vision, *i.e.* detection of low light intensities. In apparent contrast to the constant occurrence of rhodopsin in terrestrial vertebrates are the scotopic pigments of fishes. These pigments are either bathochromically or hypsochromically shifted from 500 nm in accordance with the nature of

Fig. 53. *In most families of* Centrospermae (*Caryophyllales*), *with the exception of the* Caryphyllaceae *and* Molluginaceae, *yellow and violet pigments, the betalains, occur instead of the usual flower pigments anthocyanins:* Bougainvillea. With kind permission of *Urania*-Verlag [96].

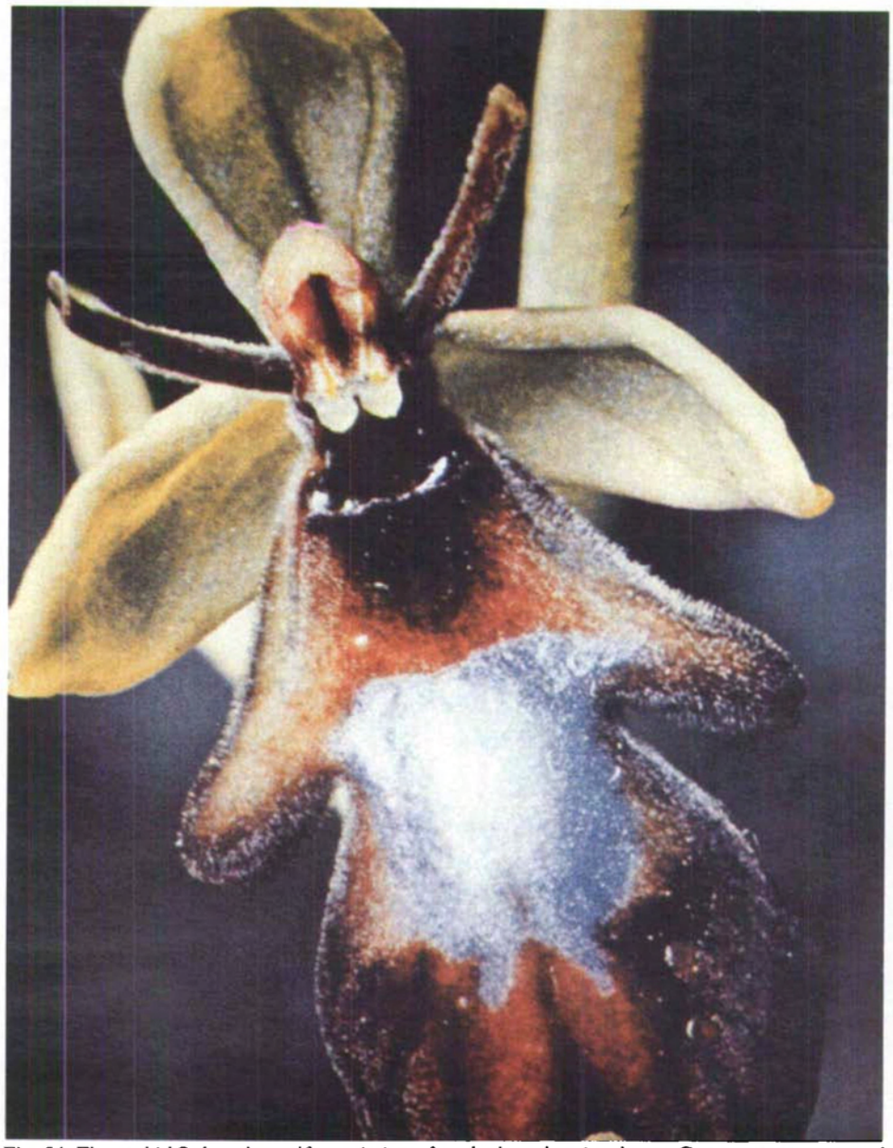
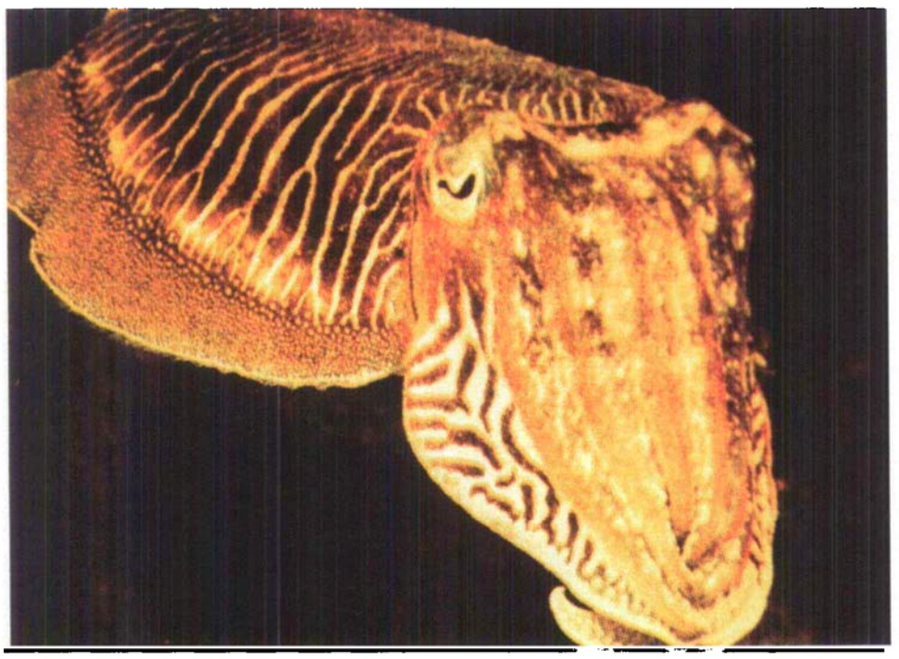


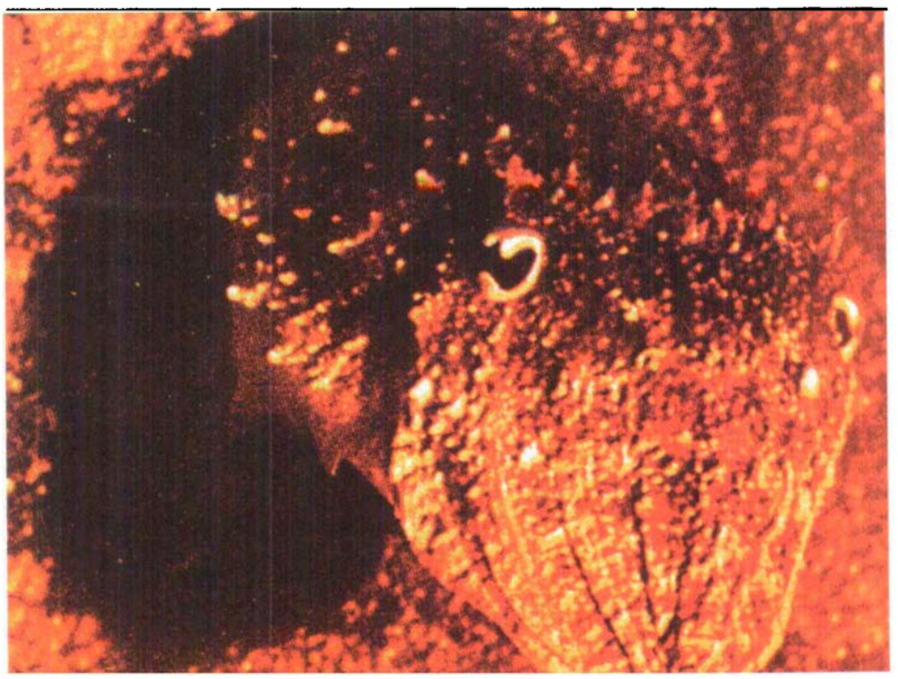
Fig. 54. *The orchid* Ophrys insectifera *mimics a female thread-waisted wasp* Gorytes mystaceus *and allures the male insects.* An example for mimicry of plants [97]. With kind permission of *O. Danesch* [98].

their photic habitat. Nature employs two methods: *a*) varying the structure of the individual opsin (opsin shift) and *b*) varying the chromophore, either A_1 or A_2 retinal. In some fishes the retina has both A_1 and A_2 pigments, the proportion changing with season (A_2 winter/autumn and A_1 spring/summer). In certain amphibia larvae have porphyropsin (*ca.* 520 nm) and later a change occurs in metamorphosis to *ca.* 500 nm [51]. The visual adaptation to water quality and water depth is shown in *Fig. 28* and reflects the light conditions shown in *Fig. 4*.

4.4. *Screening and Protection Against Radiation*

Light is used by living organisms in so many beneficial ways that it is easy to overlook the fact that light energy can also be damaging. Various natural pigments, *e.g.* porphyrins and hypericin, can act as sensitizer for light-catalyzed damage, especially in the presence of oxygen, unless there is some suitable protective mechanism [2]. The protective role of carotenoids in photosynthesis has been discussed (see *Fig. 18*). But carotenoids also play an important part in non-photosynthetic organisms, particularly in microorganisms. The mechanisms for deactivation of singlet oxygen were already mentioned. The screening effect of carotenoids in phototropism was also discussed.





Figs. 55 and 56. *Cephalopod molluscs (e.g. Sepia officinalis) are famous examples for efficient color changes.* In addition they produce a very dark brown pigment, sepiomelanin, in their defensive ink. With kind permission of *Droemer-Knaur-Kindler* [99].

Cyanobacteria (*e.g. Chlorogloeopsis*) have developed a passive UV-A sunscreen, the pigment scytonemin. It is a yellow-brown dye of cyanobacterial extracellular sheaths, and was found in species thriving in habitats exposed to intense solar radiation. High light intensities (up to 250 mmol photon $m^{-2}s^{-1}$) promoted the synthesis of scytonemin in cultures of cyanobacteria. UV-A (320–400 nm) was very effective in eliciting scytonemin synthesis. In acetone scytonemin absorbs strongly at 385 nm with a long tail extending to the IR. Other absorptions are at 250 and 280–320 nm. The dye is probably a sunscreen far more ancient than plant flavonoids and animal melanins [52].

Lichens are mutualistic symbiotic associations between ascomycetes and certain genera of green algae or cyanobacteria. The protective surface of a lichen, the upper cortex, contains compounds that absorb incident light. They function as screen and control the sun-light intensity for the algae which are located below the upper cortex. Sun-exposed lichens have larger quantities of screening compounds than species growing at shady sites. The screening pigments can reduce the transparency of the upper cortex by as much as 50%. In addition there is a selective UV absorption in some pigments (*Fig. 29*).

Usninic acid and parietin absorb considerable UV proportions. The colorless atranorin acts possibly as accessory pigment that sensitizes photosynthetic active chlorophylls [53][54].

In animals, protection against light is usually afforded by a screening layer of pigment which either absorbs all light or filters out harmful rays. Dark pigments as melanin are often utilized. The black slug *Arion ater* accumulates an amount of melanin proportional to the amount of photodynamic free porphyrin in the integument [2].

Free porphyrins occur in man when there is a deficiency in uroporphyrinogen III cosynthetase and large amounts of uroporphyrinogen I accumulate (erythropoietic porphyria). Injected β-carotene can be deposited in dermal tissues where it absorbs light and protects against photooxidation [2].

Naphthoquinones in echinoderms have also been discussed as photoprotectors. The protecting role of flavonoids during the evolution of terrestrial plants has been discussed in *Sect. 3.3* [19][55]. Flavonoids, as apigenin, naringenin or quercetin, absorb strongly around 290 and 350 nm. They efficiently screen plants and animals (guinea pig) against UV-B radiation and photooxidation [56–58].

4.5. *Accessory Pigments in Vision*

The accessory visual pigments can be classified in three groups: screening pigments, color discriminating pigments and reflecting pigments.

Screening pigments in vertebrates are melanins that are present in various tissues. Melanin absorbs stray light of all wavelength. In invertebrate eyes screening pigments in the outer zones of receptor units absorb all light that is not directed along the axis of the ommatidium. The pigments can be melanin, ommochromes and pterins. One example has been given in *Fig. 27.*

Color discriminating dyes occur in oil-droplet light filters in inner segments of receptor cells of reptiles and birds. They contain carotenoids.

Reflecting pigments serve to increase the sensitivity of the eye. In some cases a reflecting layer lies behind the receptor layer of diurnal and nocturnal animals. In bush-baby (*galago*) the dye is riboflavin, emitting light at 520 nm, and in the deep sea fish *Malacosteus* the pigment is astaxanthin [2][39] (color print *Fig. 43*).

4.6. *The Metabolic Transport Functions*

Electron, ion (proton), and gas (oxygen) transport phenomena are indispensable metabolic processes. Many pigments are involved and have been adapted to these specific functions. Electron and proton transport were discussed in the context of photosynthesis (*Fig. 10*). The respiratory chain in the mitochondria is similar. As an example of the versatile transport function of an involved dye, the quinone pool with the Q-cycle is shown in *Fig. 30.*

Q-Cycles play a part in both photosynthetic electron transport and in the respiratory chain. Using this cycle ubiquinone or plastoquinone is able to carry two protons across the membrane while transferring one electron to cytochrome.

The vital, oxygen-transporting blood pigment of most animals is hemoglobin, in muscles it is the related myoglobin. Leghemoglobin has been indentified in leguminous plants. There its presence is confined to root-nodule cells containing the symbiotic nitrogen-fixing bacteria.

A very interesting function of flavonoids has been found in this process of nitrogen fixation [11]. One of the most important plant bacterial interactions is that between leguminous plants and bacteria of the genera *Rhizobium*, or between other plants, *e.g.* alder trees (*alnus*) and actinomycetes. Infection of the roots of a leguminous plant with *Rhizobium* leads to the formation of root nodules. The oxygen for *Rhizobium* is carefully controlled by the O_2-binding protein leghemoglobin. In the process of gene expression the Nod D gene encodes a regulatory protein that controls expression of other nod genes. Following interaction with inducer molecules, the conformation of the Nod D-protein changes (*Fig. 31*). This presumably initiates transcription of other nod genes. The inducers are in most cases flavonoids that are secreted by the roots of leguminous plants in order to trigger

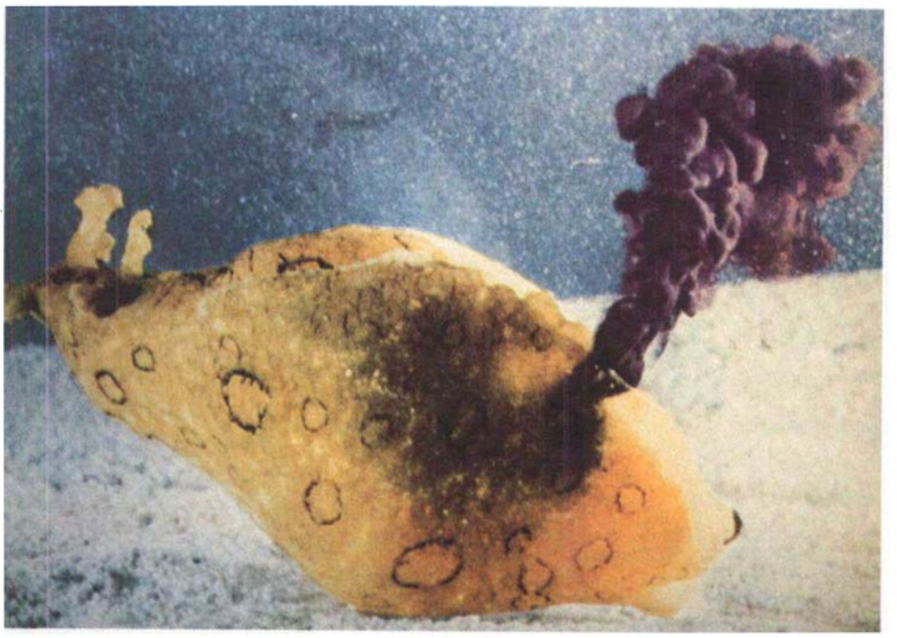


Fig. 57. *The sea hare* Aplysia dactylomela *uses a defensive purple ink which contains the tetrapyrrole biliprotein aplysioviolin, that is the methyl ester of phycoerythrobilin* [2]. With kind permission of *Droemer-Knaur-Kindler* [100].

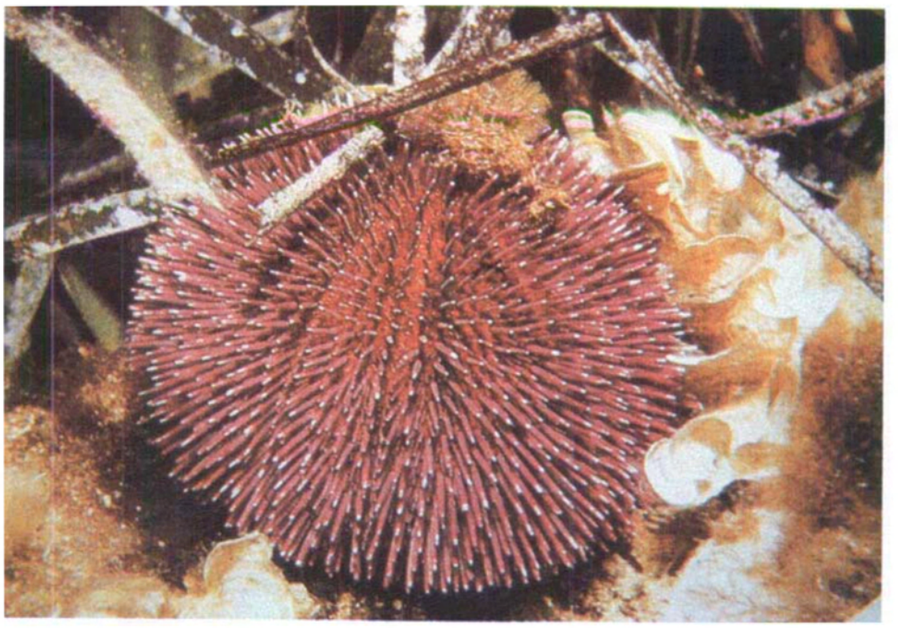


Fig. 58. *This purple sea urchin* Sphaerechinus granularis *is colored by 1,4-naphthoquinones.* With kind permission of *Belser*-Verlag [101].

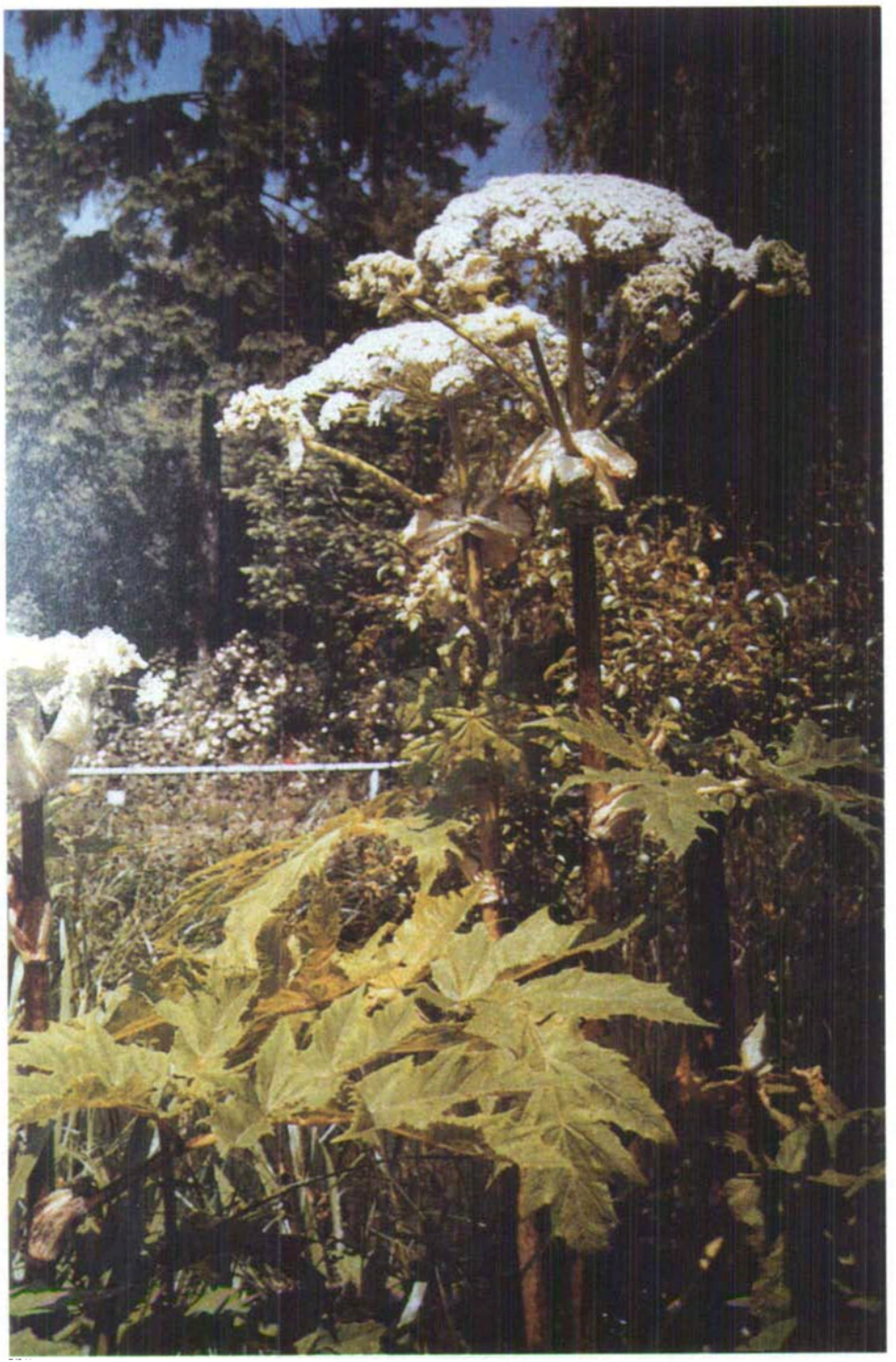


Fig. 59. *The furocoumarines of* Heracleum mantegazzianum *are well-known for their photosensitizing properties.* With kind permission of *Urania*-Verlag [102].

gene expression [11][60] (color print *Fig. 49*).

4.7. *Bioluminescence*

Bioluminescence, the 'cold light', has been observed in marine bacteria, dinoflagellates, some fungi and particularly in animals. The light, which is emitted, is used in courtship displays, shoaling and communication, differentiation of the sexes, finding and attracting prey, distracting predators and camouflage. Since light in the depths of water tends to be blue-green (*Fig. 4*), it is understandable that marine bioluminescence is blue-green as a rule [2][61–64].

The chromophores (two of them are shown in *Fig. 3*) stem from various classes of heterocyclic and aliphatic compounds. The mechanism may be generalized as

luciferin (substrate) + luciferase (enzyme) + O_2 → product * → product + hν

This dependence on oxygen has led to the opinion that during evolution bioluminescence had been adopted as a convenient mechanism to detoxify the system of molecular oxygen [15].

Later, other applications evolved, *e.g.* camouflage. An impressive example for camouflage is bioluminescence of fishes (or symbiotic bacteria in fishes) that is directed downward and intensity-adapted to the surface-light intensity. Thus, seen from below, the fish vanishes in surface-light [9]. The dinoflagellate *Gonyaulax polyedra* displays the interesting phenomenon of circadian rhythms of bioluminescence and aggregation ('red tides') [65].

The real mechanism of production of excited and emitting states can be rather involved as is shown for bioluminescence of *Renilla* (an anthozoan *coelenterate*) and *Photobacterium* (*Fig. 32*).

4.8. *Visual-Effect Functions*

One important function of integumental pigments is in the contrasting roles of crypsis (camouflage) and semasis (advertisement). A classification of integumental color schemes is given in *Fig. 33* [6].

The aim and purpose of cryptic colors is to conceal. This can be done by adaptation to environmental color and shapes, by countershading and shape-disruption (somatolysis). A species may also imitate an inconspicuos object (mimesis) (color prints *Figs. 50* and *51*).

Sematic coloration is either repulsive or attractive. Aposematic colors warn the viewer that the colored species is either dangerous or unpalatable. Episematic colors attract the sexual mate or allure insects and birds for pollination and seed dispersal [2] (color prints *Figs. 52* and *53*).

Pseudosematic colors mimic the sematic or aposematic pattern of another species (*Fig. 34*). This mimicry can comprise [66–68]:

a) *Müllerian* mimicry (model and mimic are dangerous or unpalatable, color prints *Figs. 40* and *41*),
b) *Batesian* mimicry (the mimic is undangerous and palatable),
c) *Peckham*'s mimicry (the mimic is the predator or deceiver that has an advantage by cheating and misleading, also called aggressive mimicry, color prints *Figs. 51* and *54*).

Many animals change their appearance often very rapidly, in response to environmental changes. These physiological or chromomotor color changes occur in reptiles, amphibians, fishes, and many invertebrates. There are two principal mechanisms that are regulated by the pituitary gland and the pineal gland. The one mechanism consists of aggregation and dispersion of pigment granules (*e.g.* mel-

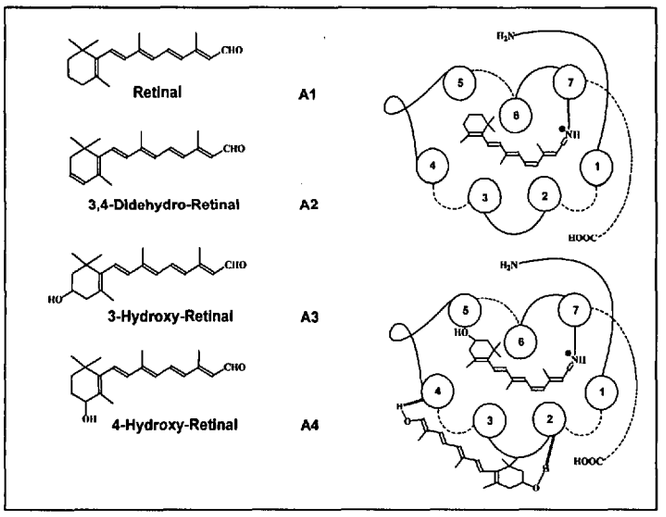


Fig. 26. *The four retinal chromophores A_1–A_4.* a) Model of the seven α-helices of bovine rhodopsin, b) model of fly visual pigment with 11-*cis*-retinal A_3 and a sensitizing chromophore all-*trans*-retinol A_3.

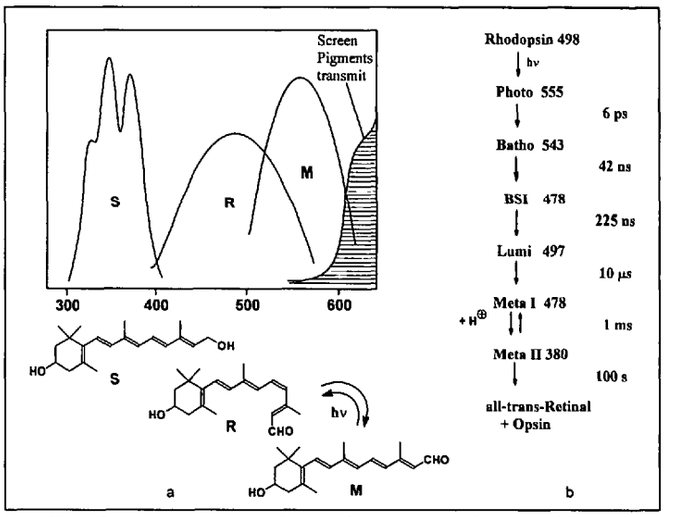


Fig. 27. a) *Fly photoreceptors, R: visual pigment, M: thermostable intermediate which is photoreconverted to R by red stray light, S: sensitizer for UV light;* b) *Photoreaction and sequence of bovine rhodopsin* (after [32])

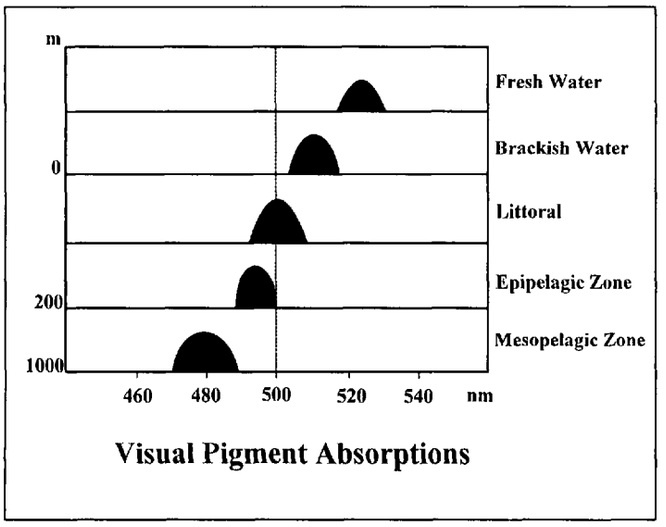


Fig. 28. *Wavelengths for visual pigments of teleosts in freshwater and seawater in various depths* (after [9]). Littoral: zone of the sea where light gets to the sea bed. Pelagial: open water zone with epipelagic zone (0–200 m) and mesopelagic zone (200–1000 m).

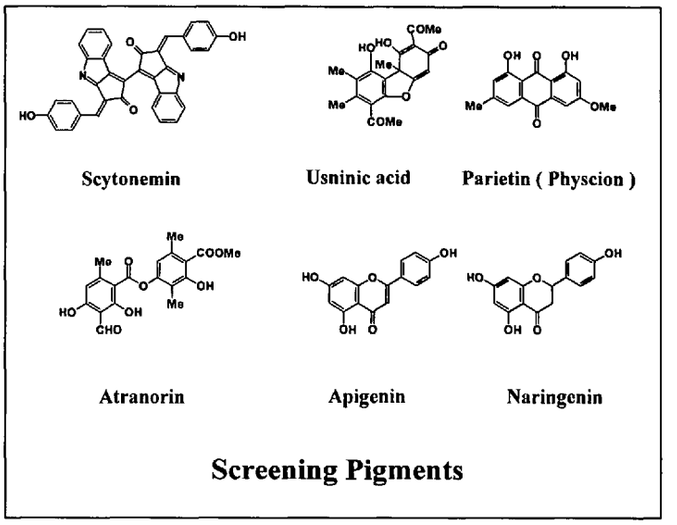


Fig. 29. *Screening pigments*

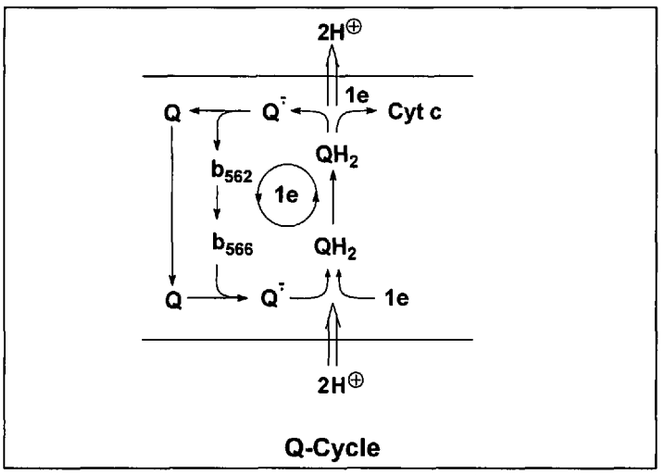


Fig. 30. *Q-Cycle of the respiratory chain.* Ubiquinone Q carries *two* protons across the membrane and transfers *one* electron. The other electron is always in the cycle. $Q^{\cdot-}$: semiquinone radical, b_{562}, b_{566}: heme b in cytochrome b (after [59]).

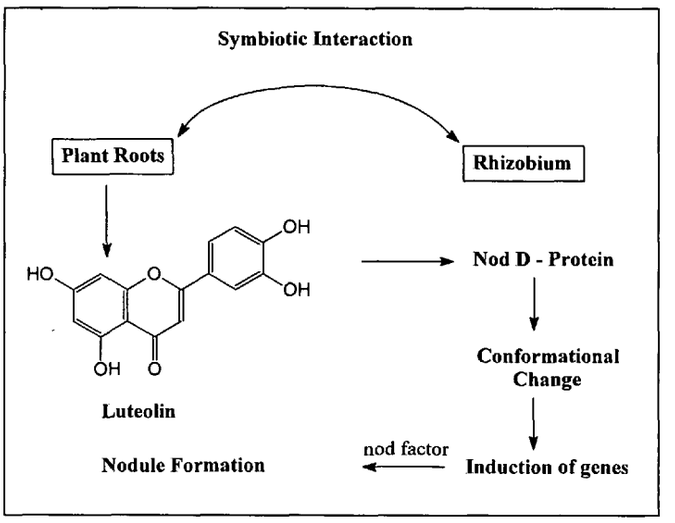


Fig. 31. *Molecular signalling between roots of leguminous plants and Rhizobia in the soil* (after [60])

anin), and is utilized by the chameleon as an example.

The other mechanism, used by cephalopods (squid, octopus), expands or contracts the whole chromatophore with the aid of radial muscle fibres [2][9] (color prints *Figs. 55 and 56*).

4.9. *Chemical Defense*

Many dyes of organisms have destructive properties towards the tissues of other species and may, therefore, used for defensive or protective purpose (*Fig. 35*). Many quinones are produced as defense chemicals, antibacterial and antiviral compounds. Examples are the benzoquinones of the *bombardier beetle*, the naphthoquinone juglone of the *walnut tree* and hypericin of *St. John's wort* [2]. The function of many pigments of fungi, however, is not yet understood [69]. *Aspergillus flavus* produces the toxic aflatoxins [70]. *Chromobacterium violaceum* synthesizes the antibiotic pigment violacein [71]. In plants the phytoalexins play the part of defense compounds that are produced only when the cell is being attacked. Phytoalexins belong to various classes, among them the flavonoids and isoflavonoids. Isoflavonoids have been studied mainly because of this ability. The furocoumarines act against herbivores. They have strong photosensitizing properties (color prints *Figs. 45* and *57–59*).

4.10. *Vitamin, Antimutagenic and Anticarcinogenic Functions*

Many of the so far discussed classes of biochromes have contributed to the metabolism and life functions of man. In particular they afford vital compounds as vitamins, coenzymes, prosthetic groups *etc.* Among the water- and liposoluble vitamins, that can be ranked among the various biochrome classes, the following have to be mentioned: A (retinol), E (tocopherol), K (menaquinone), Q (ubiquinone), B_2 (riboflavin, folic acid), B_{12} (cobalamine).

Epidemiological studies indicate that the frequent and high intake of fresh vegetables and fruits is associated with lower cancer incidence and that high plasma levels of ascorbic acid, α-tocopherol, β-carotene, vitamin A and certain phytochemicals are inversely related to cancer incidence [72]. Obviously humans ingest large numbers of naturally occurring antimutagens and anticarcinogens in food. The US Food and Drug Administration has cautiously acknowledged that the 'publicly available evidence does indicate that diets rich in fruits and vegetables, which are low in fat and high in vitamin A (as β-carotene), vitamin C and fiber are associated with a decreased risk of several types of cancer [73]'. Among the phytochemi-

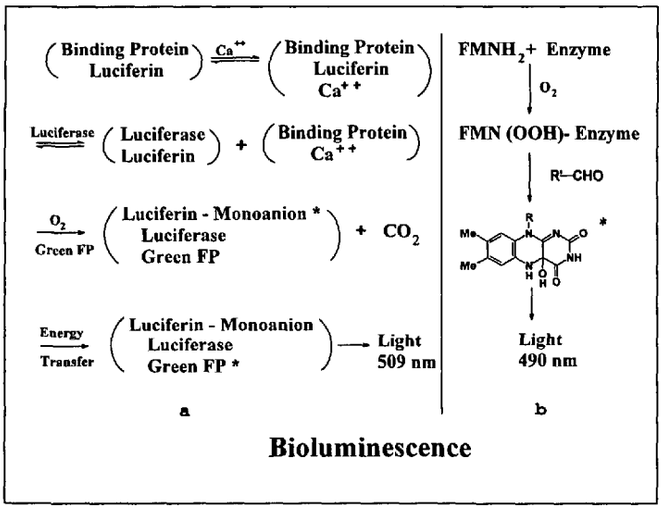


Fig. 32. a) *Renilla bioluminescene*, b) *bacterial luminescene*. (after [25][64]). FP: fluorescent protein.

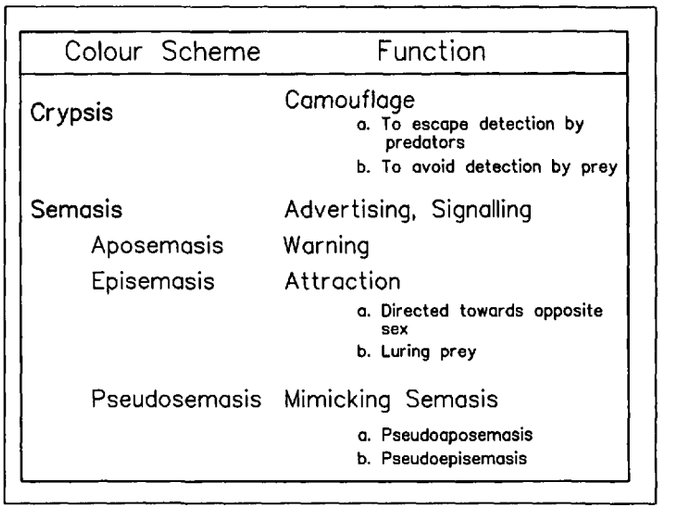


Fig. 33. *Classification of integumental color schemes* (after [6])

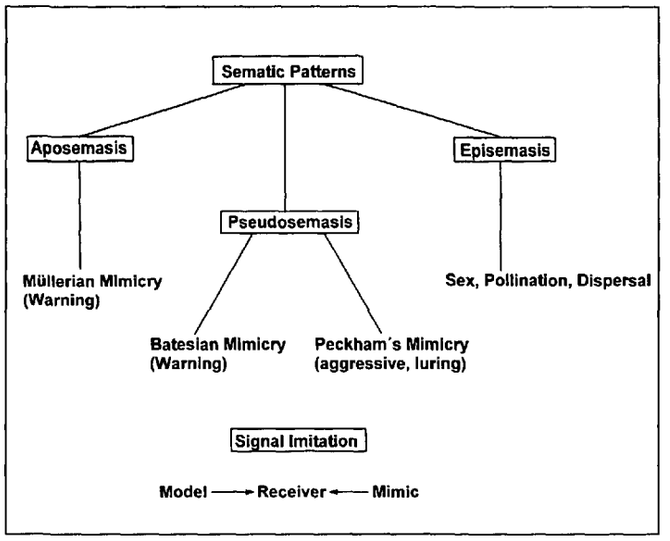


Fig. 34. *Mimicry and sematic patterns*

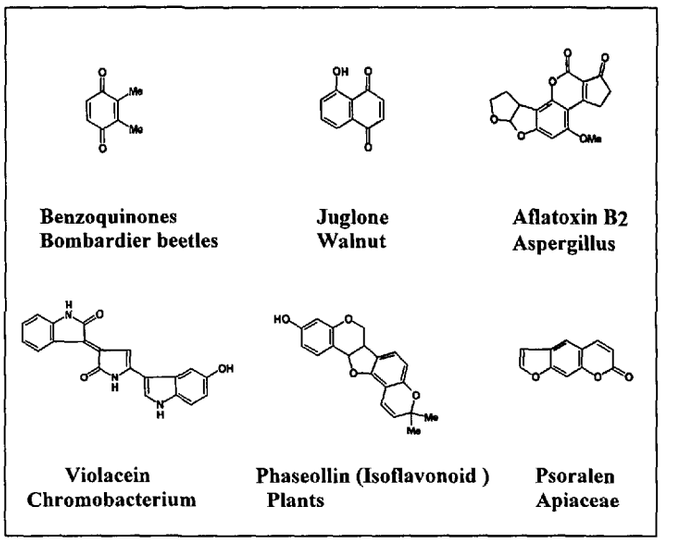


Fig. 35. *Defense chemicals*

cals that show inhibitory effect on chemically induced carcinogenesis are [72][74]:
Vitamins (A, E, C)
Carotenoids (β-carotene)
Chlorophyll
Flavonoids (quercetin, rutin, tangeretin, nobiletin)
Gallotannins
Ellagitannins

The mode of interaction of these dyes and phytochemicals is speculative but they intervene at one or the other stage of the carcinogenesis (*Fig. 36*). In cell cultures of carcinogen-initiated fibroplasts, a number of retinoids and carotenoids can reversibly inhibit progression to the transformed state. The retinoids acted during the promotion stage as chemopreventive, not as chemotherapeutic, agents, reversibly suppressing transformation after initiation but before expression of the transformed phenotype. The anticarcinogenic action of retinoids and carotenoids is closely correlated with enhanced gap-junction cell-to-cell communication and with increased synthesis of the gap-junction protein, connexin [77] [78].

Retinoids are of particular interest in oncology. They exert their antitumor activity through inhibition of cell proliferation, induction of cell differentiation and suppression of oncogene expression. In animal experiments, retinoids have preventive and therapeutic effects on premalignant and malignant lesions [79–81]. A possible machanism for vitamin A (retinol) and tretinoin (all-*trans*-retinoic acid) activity is given in *Fig. 37* [80].

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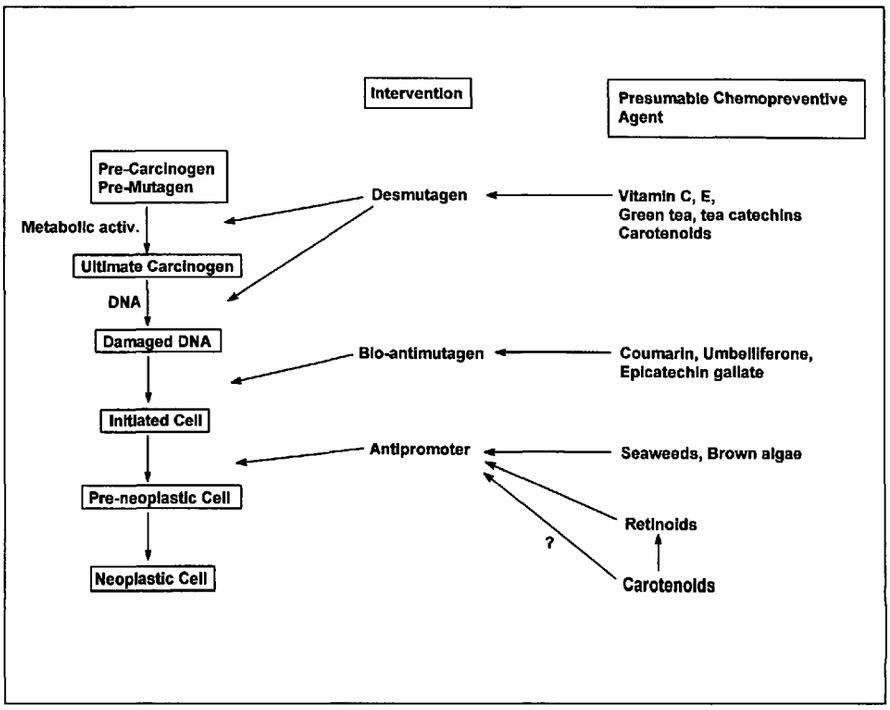

Fig. 36. *Multistage carcinogenesis model and possible points of intervention by chemopreventive phytochemicals, pigments, and vitamins and some presumable agents* [72–78]

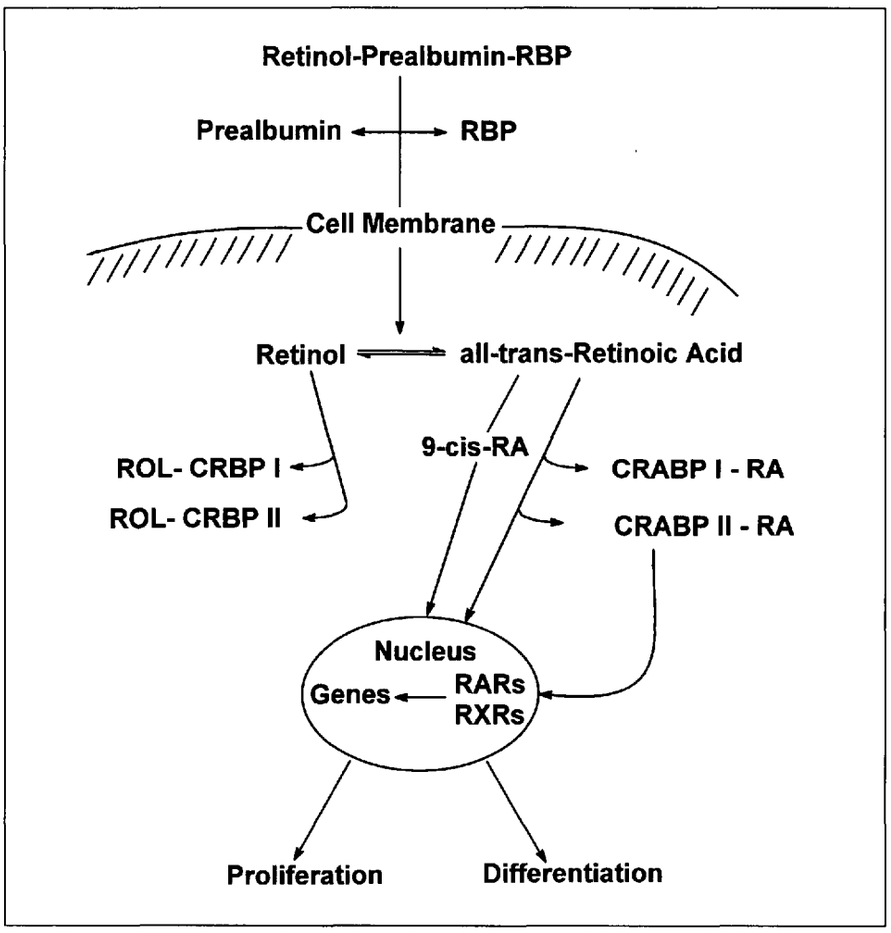

Fig. 37. *Vitamin A activity and possible mechanism for cell proliferation and cell differentiation.* CRBP: cellular retinol binding protein, CRABP: cellular retinoic acid binding protein, RBP: retinol binding protein, RAR: retinoic acid receptor α, β, γ, RXR: retinoid X receptor, RA: retinoic acid, ROL: retinol [79–81].

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