Molecules and Photochemical Reactions in Biological Light Perception and Regulation

12

Lars Olof Björn

12.1 Introduction

Many photochemical reactions involved in the sensing of and regulation by light and ultraviolet radiation by organisms consist of *cis–trans* (and *trans–cis*) isomerizations. We shall start with this class of photosensors and then go on to other mechanisms. There are many more known and unknown light-sensing molecular systems than those briefly described below, but (except for the first one) I have tried to concentrate on those more widespread. As examples of light-sensing pigments with a very limited distribution, one can mention stentorin and blepharismin of certain ciliates (Lenci et al. 2001).

The term "photoreceptor" means different things to different people. In zoology it refers to cells, which respond to light, such as the rods and cones of our eyes, but to plant scientists it means a pigment molecule, such as rhodopsin or phytochrome, which absorbs light at the start of a chain of events leading to light perception or regulation of a physiological process by light. We shall use the term here in this latter sense.

"Photoreceptor" in this sense is a concept related to "photoenzyme," i.e., an enzyme active only in light. A class of photoreceptors, the cryptochromes, are thought to have evolved from certain photolyases. Another photoenzyme, NADPH-protochlorophyllide oxidoreductase, can also be regarded as a photoreceptor, helping the plant to regulate chlorophyll synthesis and chloroplast development (Beale 1999).

12.2 *Cis–Trans* and *Trans–Cis* Isomerization

Double bonds and conjugated double bond systems provide molecules with a certain rigidity. Molecular groups cannot rotate freely around double bonds or around single bonds in a continuous conjugation suite, as they can around isolated single bonds provided there is room enough. When a double bond is involved, there are two opposite torsion angles for which the energy has a minimum value and which thus represent stable conformations. Often the carbon atoms at the double bonds carry one hydrogen atom and one larger atomic group. These atoms then lie in the same plane, which is the same plane as the corresponding groups on the carbon atom at the other end of the double bond (in Fig. 12.1 this is the plane of the paper).

If the larger atomic groups are on the same side of the line through the double bond and the carbon atoms at its ends, the molecule is said to be in *cis*-configuration; if they are on opposite sides, the molecule has a *trans*-configuration. However, for larger molecules this designation may be difficult to apply, and another nomenclature has been introduced, i.e., Z- (for German *zusammen*, together) and E- (for German *entgegen*, opposite) configurations. In this system a priority is assigned for the atoms immediately attached to the double bond, such that higher priority is assigned to atoms of higher atomic number. Thus carbon atoms in the example in Fig. 12.1 have first priority, hydrogen atoms second priority.

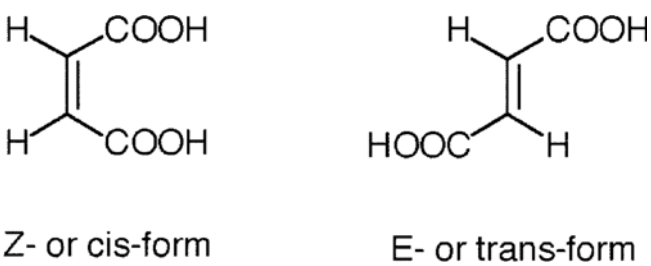

Fig. 12.1 A simple example of *cis–trans* isomerism

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When atoms of same priority are on the same side, we have a Z-configuration, otherwise an E-configuration.

By a very rough consideration, we can appreciate why *cis–trans* (E–Z) isomerizations are suitable for light sensing. A typical carbon–carbon single bond has a bond energy of 387 kJ per mole, while the typical double bond has a strength of about 610 kJ per mole. The difference is 263 kJ per mole. We can think of a rotation around a double bond to consist of the breaking of one of the bonds in the double bond, rotation around the remaining (single) bond, and reformation of a double bond. Thus one would need to add 263 kJ per mole (or 263,000/6.02 10^{23} J per molecule) to achieve the rotation. The energy of a photon is $h{\cdot}c/\lambda$ (see Chap. 1), and by equating the two energies, one obtains a typical wavelength for rotation of 455 nm, in the middle of the optical part of the electromagnetic spectrum to which the atmosphere is transparent. In rhodopsin of our rods the actual energy barrier for isomerization is 238 kJ/mol (Okada et al. 2001), but in different other photoreceptors based on *cis–trans* (or *trans–cis*) isomerization, the wavelength varies from the UV-B (for urocanic acid) to the near-infrared (for phytochrome) region. Some of the principles for this "tuning" are described in Chap. 9. In general the chromophores are bound to proteins, but we begin our account with *cis–trans* isomerization of a chromophore that is not bound to protein and is free to diffuse in the skin.

12.2.1 Simple Signaling Metabolites Undergoing *Cis–Trans* Isomerization

12.2.1.1 Urocanic Acid

Urocanic acid is present in the human skin, and photoisomerization from the *trans* to the *cis* form causes downregulation of the immune system (Chap. 24). This radiation-sensing reaction appears at first glance to be a very simple one: the pigment is structurally simple, of low molecular weight, and not protein bound. The isomerization seems to be a very simple reaction (Fig. 12.2).

But the simplicity is only apparent. The first indication of this is the fact that the action spectrum for photoisomerization is very different from the absorption spectrum of *trans-urocanic* acid. The absorption spectrum has a broadband peaking at about 280 nm, but radiation of this wavelength does not produce any photoisomerization, i.e., the quantum yield is zero at this wavelength. The quantum yield is maximal, 0.5, at 310 nm, where absorption is much weaker (also for the *cis* to *trans* isomerization, the quantum yield is about 0.5 at long wavelengths). Various theoretical explanations have been given for this strange behavior (Li et al. 1997; Hansson et al. 1997; Page et al. 2000; Ryan and Levy 2001), and the discussion is still going on. Urocanic acid is not the only substance which behaves in this way: cinnamic acid and related compounds have a quantum yield for *cis–trans*

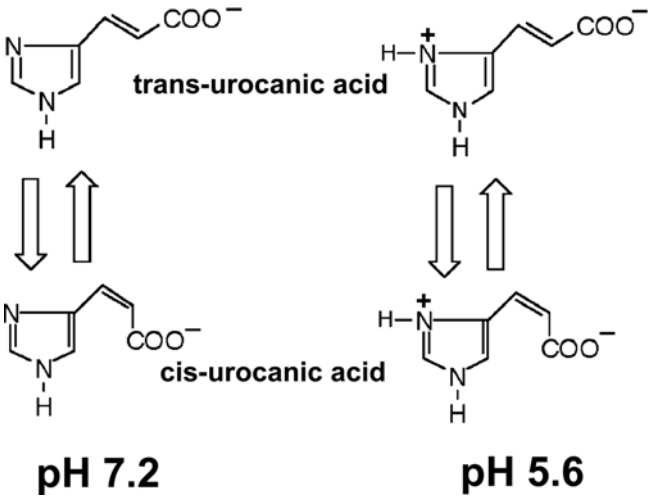

Fig. 12.2 Photoisomerizations of anionic and zwitterionic forms of urocanic acid. The *trans* form is also called E-urocanic acid, the *cis* form Z-urocanic acid

isomerization which is wavelength dependent (see the Sect. on 12.2.4 below).

Urocanic acid exists in several ionic forms depending on pH: at low pH as a cation, at slightly acidic pH in the skin as a zwitterion, and at neutral or higher pH as an anion. Spectra for both *trans* and *cis* forms peak near 280 nm in neutral solution, but near 270 nm at pH 5.6 (Morrison et al. 1980; Hansson et al. 1997; Li et al. 1997; Fig. 12.3).

Trans-urocanic acid is formed from histidine in the outermost layer of the skin, and after photoisomerization the *cis-urocanic* acid diffuses inward and acts on cells in deeper layers. The mechanism is uncertain. One view is that it interacts with a serotonin receptor (Walterscheid et al. 2006), a view that has been opposed by others (Prêle Finlay-Jone and Hart 2006).

Before we go on to proteins carrying chromophores that undergo *cis–trans* photoisomerization, a couple of other simple molecules should be mentioned.

12.2.1.2 Retinoic Acid

Retinoic acid occurs in the all-*trans* form as well as in a number of *cis*-stereoisomeric forms. All-*trans* retinoic acid binds more firmly to specific nuclear retinoic acid receptors (RARα, RARβ, RARγ) at low (nanomolar) concentrations and 13-*cis*-retinoic acid more weakly. Another group of nuclear receptors, retinoid-X receptors (RXRs), bind only to 9-*cis*-retinoic acid (Heyman et al. 1992; Teboul et al. 2008). Thus different stereoisomers can be expected to have different physiological effects, an expectation that has been experimentally confirmed. All-*trans* retinoic acid is involved in regulating the mammalian circadian clock and to influence the expression of a number of proteins within the "clock gene family" via RARα (e.g., Sherman et al. 2012), and RARα and RARγ exhibit circadian oscillation.

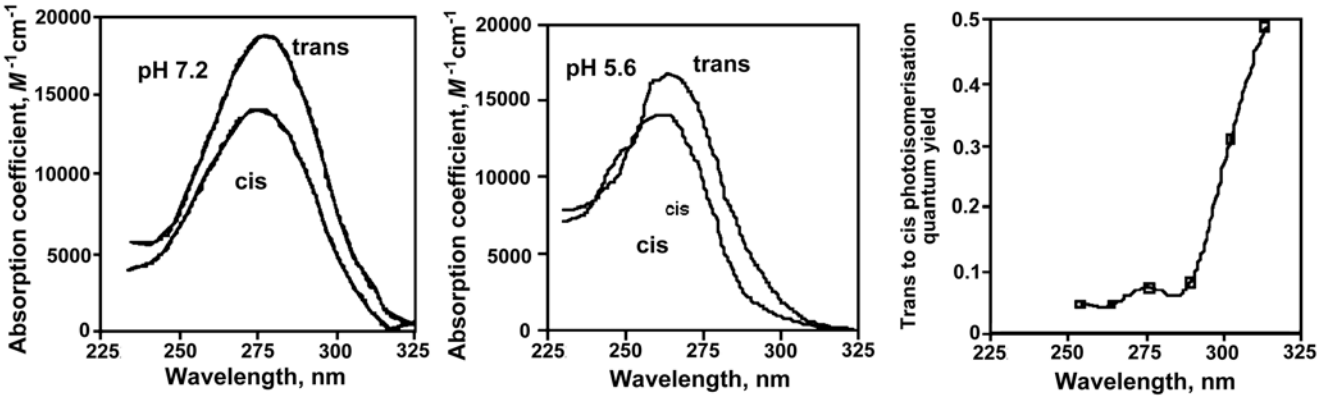

Fig. 12.3 Absorption spectra for *trans*- and *cis*-urocanic acid at pH 7.2 (redrawn after Hanson et al. 1997) and at pH 5.6 (redrawn after Morrison et al. 1980), as well as the quantum yield for *trans* to *cis* photoisomerization (from data of Morrison et al. 1984)

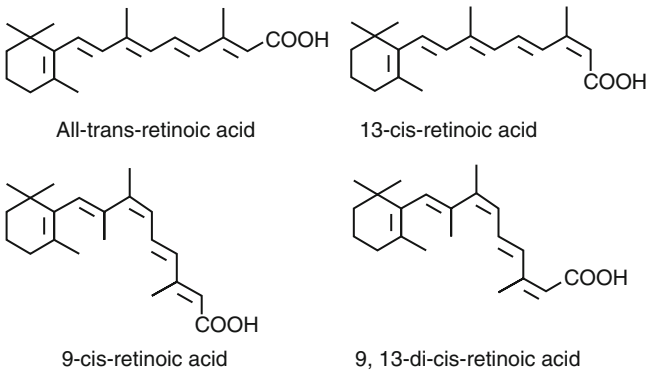

Fig. 12.4 Structures and absorption spectra of all-*trans*, 9-*cis*, and 13-*cis*-retinoic acids dissolved in methanol (Recalculated and drawn from data in Iole et al. (2005))

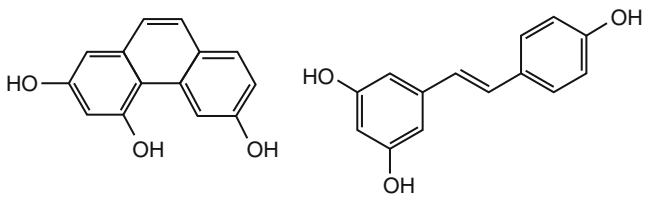

Fig. 12.5 *Cis*-resveratrol (*left*) and *trans*-resveratrol (*right*)

The different stereoisomers have different absorption spectra (Fig. 12.3), and therefore it is likely that the isomeric composition after irradiation with ultraviolet radiation depends on the spectrum of the radiation, but information about this available in the literature is very scant. Fu et al. (2003) report that irradiation of all-*trans* retinoic acid by fluorescent lamps resulted in five isomerization products (13-*cis*-RA, 11-*cis*-RA, 11,13-bis-*cis*-RA, 9-*cis*-RA, and 9,11-bis-*cis*-RA), all-*trans*-5,6-epoxyretinoic acid, and 13-*cis*-5,6-epoxyretinoic acid, see Fig. 12.4). Murayama et al. (1997) describe the light used only as 1200 lx white fluorescent light. They found that photoequilibrium in 10 mM ethanol solution is almost reached after 30 min with the following composition: all-*trans*-retinoic acid 25 %, 9-*cis*-retinoic acid 10 %, 11-*cis*-retinoic acid 10 %, 13-*cis*-retinoic acid 30 %, 9, 13-di-*cis*-retinoic acid 5 %, and unidentified compound 20 %. Several authors report that if oxygen is present, also photooxidation takes place. The situation in vivo is, however, more complicated, since retinoic acid occurs not only free but also bound to various other molecules, which probably affects phototransformation.

Ultraviolet radiation does not penetrate far into the human body, so phototransformation of retinoic acid can take place only in the skin and eyes (and in preparations containing retinoic acid applied to the skin; see Fu et al. 2003). Kunchala et al. (2000) as well as Hellmann-Regen et al. (2013), based on experiments with skin cell cultures, speculate that the light from traditional fluorescent lamps and especially compact fluorescent light bulbs may disrupt normal regulation by photoisomerization of all-*trans*- to mainly 13-*cis*-retinoic acid. As retinoic acid is applied for the treatment of acne, photoisomerization may have some effect in this context.

Retinoic acid is, however, used as a regulator throughout the animal world (Albalat 2009), and as in mammals different isomers perform different functions. Photoisomerization may have a great impact in small animals, such as sea urchin embryos (Maeng et al. 2012), hydrozoan larvae (Pennati et al. 2013), and insect larvae (Chen et al. 2010).

12.2.1.3 Resveratrol

Resveratrol is a stilbene derivative that can exist in *cis* or *trans* forms (Figs. 12.5 and 12.6). It is not synthesized by animals. Of the two stereoisomers of resveratrol, the *trans* isomer is the most stable one and the most abundant one in nature, but both coexist in different proportions in wine and foods (Álvarez Rodriguez et al. 2012) and especially in high amounts in red wine. It is considered to have a number of health-promoting effects in man (Kalantari and Das 2010). The *trans* form is in general more physiologically active, for

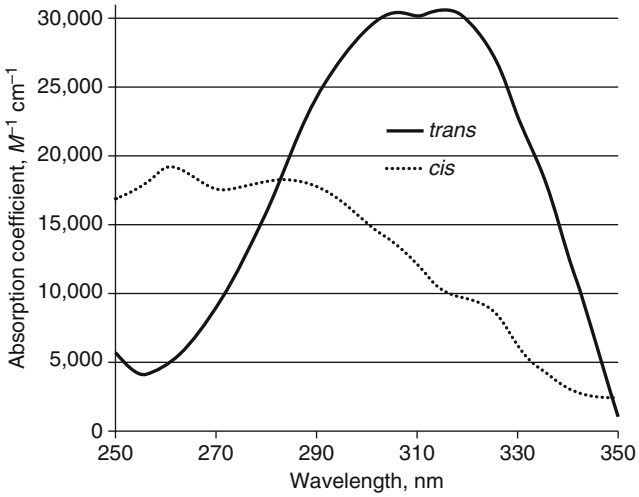

Fig. 12.6 Absorption spectra for *cis*- and *trans*-resveratrol. Calculated from data of Figueiras et al. 2011

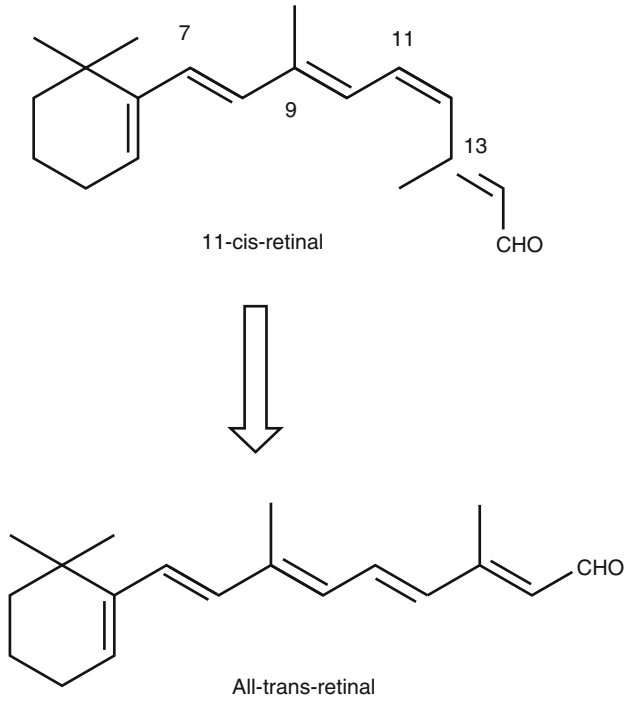

Fig. 12.7 Photoisomerization of the isolated retinal chromophore

instance, as an antiproliferative and antiinflammatory agent, than the *cis* form (Anisimova et al. 2011; Kim and Oh 2011; Rius et al. 2010), and is the form enzymatically synthesized in plants, but ultraviolet radiation partially converts it to the *cis* form (even in the plant) and so decreases the amount of the form active in the human body.

12.2.2 Eukaryotic Rhodopsin

When we see the word rhodopsin, our thoughts first go to the light-sensitive pigment of the rods in our own eyes. Very similar pigments, however, are present also in our cones and in the eyes of various insects, mollusks, and other animals. Recently it has been discovered that essentially the same type of pigment also occurs in various algae (Foster et al. 1984; Hegemann and Deininger 2001; Gualtieri 2001). Several types of archaea (archaebacteria) contain a kind of rhodopsin, but the latter is sufficiently different, both with respect to the chromophore and the protein structure, to warrant treatment in a separate section (Sect. 12.2.3). Both eukaryotic and archaean rhodopsins, however, are membrane bound and have seven membrane-spanning helices in the molecule. It should also be noted that there are eukaryotes which have proteins more similar to the archaean than to typically eukaryotic rhodopsin.

The chromophore of rhodopsin is retinal (in some animals dehydroretinal; see Chap. 9). We show it first in isolated form (Fig. 12.7) to display in a simple way the phototransformation from the 11-*cis* form to the all-*trans* form, corresponding to the primary process of vision. The side chain changes from a bent to a straight form. In rhodopsin the terminal carbon atom of retinal polyene chain is covalently tethered to a lysine side chain (lysine 296) of the protein (Fig. 12.7), forming a "Schiff base" in its protonated form. The counterion for the positive charge is formed by glutamine 113. The protein forms an antiparallel β-sheet in the vicinity of the chromophore, bringing the side chain of another amino acid (position 181) in close contact to the polyene chain of retinal. In our rod rhodopsin this amino acid is glutamine; in the red- and green-sensitive pigments of our cones, it is histidine.

When light isomerizes the rhodopsin-bound retinal from 11-*cis* to all-*trans*, the polyene chain cannot at once straighten out completely, because the straight chain does not fit in the protein pocket (see Okada et al. 2001 for a more detailed review of the events than the one given here). This restraint makes the retinal store energy, like a cocked spring. About two thirds of the photon energy is thus stored in the initial phase. Probably it is mainly the proximity of serine 186 that prevents the retinal from immediately reaching its *trans* equilibrium position. This intermediate stage is termed bathorhodopsin. Within microseconds the proton on the Schiff base is translocated to glutamine 113, whereby the attractive force between the two parts of the protein disappears and the protein helices can adjust their relative positions to allow the retinal to straighten out completely. This brings the rhodopsin to a low-energy state called metarhodopsin I. From this the rhodopsin, within a millisecond, changes to metarhodopsin II. This has higher energy than metarhodopsin I, and the transformation is made possible by a simultaneous increase in entropy: the forces between different parts of the

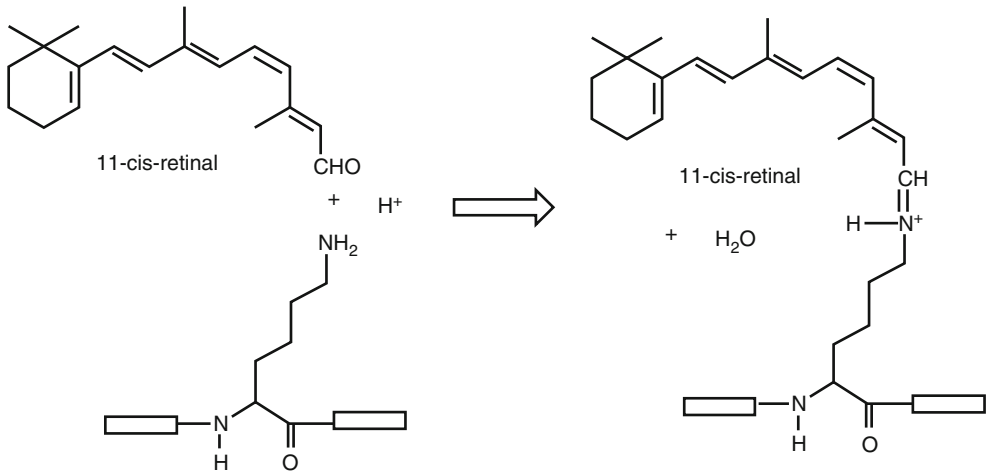

Fig. 12.8 The binding of retinal to a lysine side chain in the rhodopsin protein, forming a protonated Schiff base. Rectangles symbolize amino acids of the opsin

protein are decreased, and the different parts can move more freely with respect to each other. The process has some similarities to the melting of ice, and we may recall that the free energy change (ΔG) of a system consists of the change in total energy (enthalpy, ΔH) minus an entropy term, TΔS. In the transformation from metarhodopsin I to metarhodopsin II, the free energy is thus decreased even though the total energy increases. In this respect eukaryotic rhodopsin resembles photoactive yellow proteins (Sect. 12.2.4), but differs from archaean rhodopsins (Sect. 12.2.3). The transition also involves uptake of a proton (Fig. 12.8).

Metarhodopsin II is the "signaling state" of rhodopsin. By its formation groups are exposed which can interact with a protein called transducin, a so-called G protein, and thus make the transition from a biophysical to a biochemical phase of the signal transduction. The activated transducin activates phosphodiesterase which hydrolyzes cyclic guanosine monophosphate (cGMP). When the concentration of cGMP has fallen sufficiently, sodium ion channels in the membrane close, the electric membrane potential increases, and an electrical impulse is sent on to the nervous system.

Recently a new light-sensitive system has been discovered in vertebrates (Provencio et al. 1998; Barinaga 2002; Berson et al. 2002; Hattar et al. 2002). The start in this new development came with the study of how frogs can adjust their skin color by changing the size, shape, and position of the pigment-containing cells in their skin, the melanophores (Provencio et al. 1998). It turned out that the skin cells contained a light-sensitive pigment which was named melanopsin. The same pigment was found also in the frog's retina, as well as in mouse retinas. However, it is not in the rods (or cones), but in cells, retinal ganglion cells, inside (in the front of) the visual receptors. Some of these cells have nerve connections, not to the brain areas involved in vision, but to the suprachiasmatic nuclei where the main clock of the body (see Sect. 18.1.5) is thought to reside. The logical conclusion is that melanopsin is involved in the resetting of the biological clock by light. However, some of the melanopsin-containing retinal ganglion cells have connections to the part of the brain regulating pupil size in response to light. Melanopsin-containing cells, in contrast to the rods and cones, do not adjust their sensitivity in response to light level. They are therefore suited to record the light level, which is important, e.g., in photoperiodism and pupil size regulation. Although the melanopsins studied so far occur in vertebrates, their protein structure is more closely related to that of invertebrate opsins than to the vertebrate opsins of the rods and cones. Their photochemical reactions have so far not been studied in detail but are thought to be similar to those of rhodopsins.

12.2.3 Microbial Rhodopsins

Four types of archaean and bacterial rhodopsins are known. In contrast to the eukaryotic rhodopsins, they all contain all-trans retinal as the chromophore, and the photochemical step consists of its isomerization to 13-*cis* retinal. In some cases also the reverse reaction has some importance.

Many species within the Haloarchaea (the subdivision of Archaea formerly referred to as halobacteria) have been investigated, (Spudich 2001) but only four distinct types have been found, which are all present in species of the best investigated genus, *Halobacterium*. One of these rhodopsins, called bacteriorhodopsin (BR, Fig. 12.9), uses light energy to pump hydrogen ions out of the cells, and another one, called halorhodopsin (HR), pumps chloride ions into the cells. Both reactions contribute to making the inside of the cells negative, thus allowing the cells to accumulate cations at the expense of light energy. The light-driven export of protons also creates the proton motive force necessary for ATP synthesis and, indirectly, the free energy necessary for swimming and biochemical syntheses. BR and HR are induced only under low oxygen tension, while under high oxygen tension, the organisms can utilize oxygen for creation of the necessary free energy.

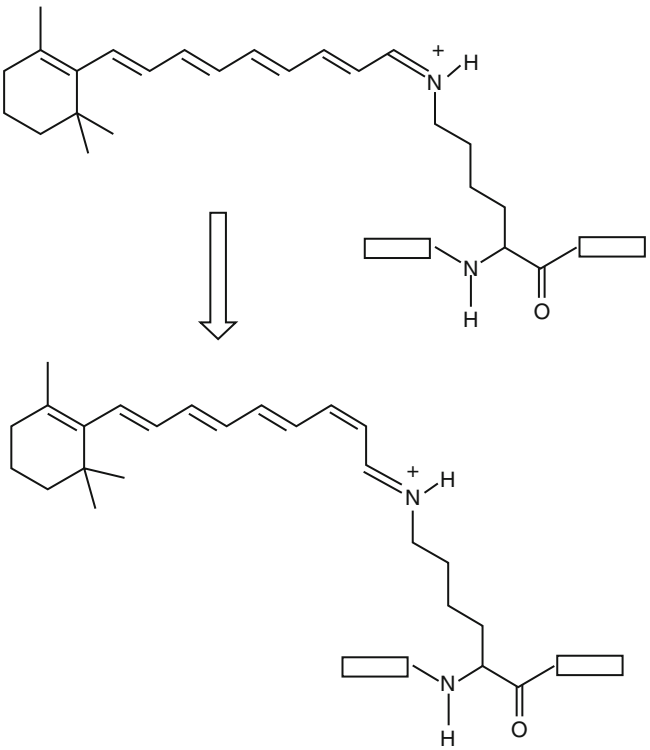

Fig. 12.9 The *trans–cis* isomerization of the chromophore in bacteriorhodopsin. As in Fig. 12.6, the *rectangles* symbolize amino acids of the opsin. The rapid *trans–cis* photoisomerization is followed by slower rearrangements of the opsin structure and movements of protons

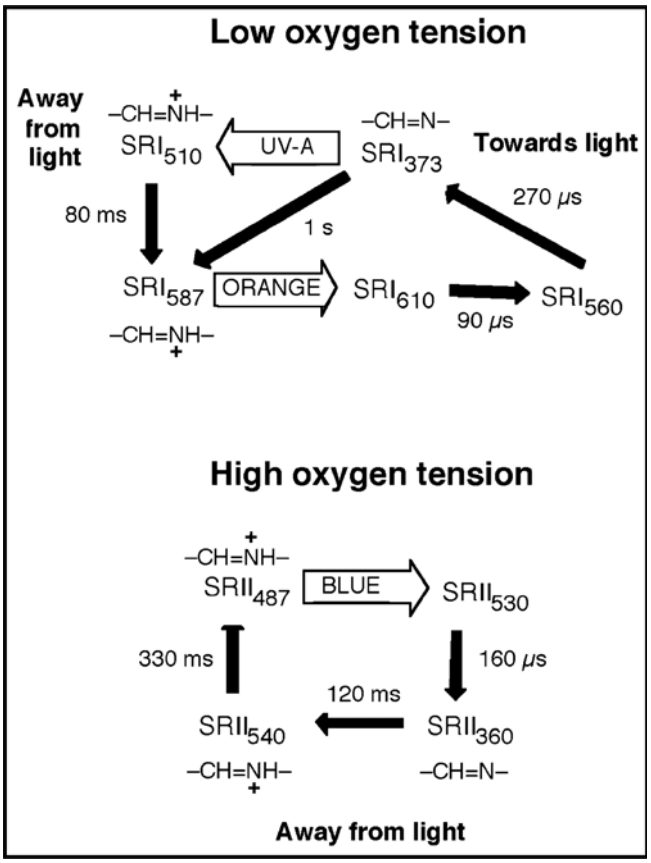

Fig. 12.10 The photocycles of sensory archaeal rhodopsins. Under low oxygen tension (*top*) *Halobacterium* and related organisms form bacteriorhodopsin and halorhodopsin, which pump ions, and sensory rhodopsin I (*SRI*). The latter is used to find a suitable light environment. As long as light is not very strong, the *orange light*-sensing reaction causes accumulation of the long-lived intermediate SRI373, which is a signaling state causing the organisms to move toward stronger light. If the UVA light becomes too strong, the UVA-sensing reaction causes conversion to SRI510, a signaling state which causes movement away from the strong light. In the presence of a high concentration of oxygen (*bottom*), only SRII is induced. The *blue-light*-sensitive reaction causes movement away from light. Like other nonoxygenic pigmented organisms, these are probably much more light sensitive in the presence of oxygen due to the possibility of formation of reactive oxygen species under illumination. As indicated in the figure, in some pigment forms the Schiff base is protonated, in other forms not (After Hoff et al. 1997, modified)

The two remaining archaerhodopsins, designated SRI and SRII (SR for sensory rhodopsin, Fig. 12.10), are used by the halobacteria to orient with respect to light. SRI is induced only under low oxygen conditions, SRII only under high.

SRI, formed under low oxygen conditions, has two signaling states. One, SRI_{373}, formed by the orange component of weak daylight, causes the cells to move toward stronger light. This is not due to direct sensing of light direction as in the topophototaxis of eukaryotic flagellates, but by modulation of the frequencies of spontaneous reversals of swimming direction. If the light becomes very strong, another signaling state, SRI_{510}, is formed under the action of the UVA component of daylight. This causes the cells to move toward weaker light.

Under high oxygen tension SRII, but not SRI (neither HR nor BR), is induced. SRI mediates only a light-avoiding signal.

SRI and SRII do not engage a signal-transmitting protein during only part of the photocycle (as eukaryotic rhodopsin engages transducin). Instead each one of them is permanently attached to its signal-transmitting protein, HtrI or HtrII, respectively. Obviously the conformational change in the rhodopsins caused by the photoisomerization of the chromophore is somehow transmitted to the signal-transmitting protein, but the details of this are not known.

12.2.4 Photoactive Yellow Proteins (PYPs, Xanthopsins)

Photoactive yellow proteins (PYPs) function as photoreceptors in purple bacteria, mediating negative phototaxis. One might think that this is too humble a function to warrant

treatment in a book like this one, but PYP happens to be one of the best-known photoreceptor pigments, and we can learn some more general principles from it. PYPs are also referred to as xanthopsins, although this term is misleading, since the proteins are not opsins. Three photoreactions shuttle the pigment between several forms, as shown in Fig. 12.11.

The PYP chromophore is *trans*-4-hydroxy cinnamic acid, and light causes photoisomerization to the *cis* form (Fig. 12.12). This initial reaction is followed by rotation of one half of the molecule with respect to the other around a single bond. Genick et al. (1997, 1998) have succeeded in following in detail the changes in the protein structure associated with these changes (Fig. 12.13). They managed to crystallize the protein and by time-resolved x-ray crystallography at low temperature captured the structure of the otherwise extremely short-lived (nanoseconds) intermediate.

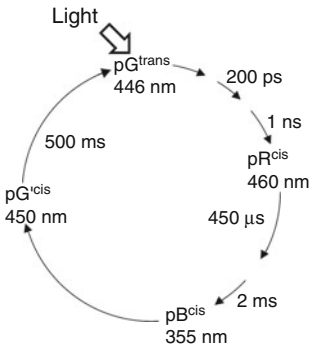

Fig. 12.11 The photocycle of PYP, simplified from Naseem et al. (2013) and other sources. There is no consensus regarding intermediates, transformation pathways, or reaction rates

By conversion to the signaling state, forces between different parts of the protein are weakened. It becomes more flexible, and the conversion can be likened to "melting," just as in the case of animal rhodopsin.

PYP is interesting also because it contains the prototype for a "PAS domain" (Pellequer et al. 1998). By this we mean a protein structure that occurs in many other signaling proteins (Taylor and Zhulin 1999), including some other photoreceptor proteins: phytochrome, phototropin (Salomon et al. 2000; Christie and Briggs 2001), and a blue-light receptor in the fungus *Neurospora* (Ballario and Macino 1997). PAS domains have been identified in proteins from all types of organisms: Archea, Bacteria, and Eucarya. It comprises a region of 100–120 amino acids. They seem to occur almost exclusively in sensors of two-component "phosphorelay" regulatory systems. The activation of a PAS protein, by either the photoconversion of a chromophore, binding of an external activator, or voltage sensing (as in some proteins regulating voltage-sensitive ion channeling), seems to involve the exposure of the PAS domain and initiation of protein kinase activity (either histidine kinase or, as in the case of phytochrome, serine/threonine kinase).

12.2.5 Phytochrome

The discovery of phytochrome is one of the classical detective stories of plant science (Butler 1980; Björn 1980b; Sage 1992). It started with the discovery that some effects of red light, such as the germination of seeds, photomorphogenesis of etiolated plants, and the inhibition of flowering in short-

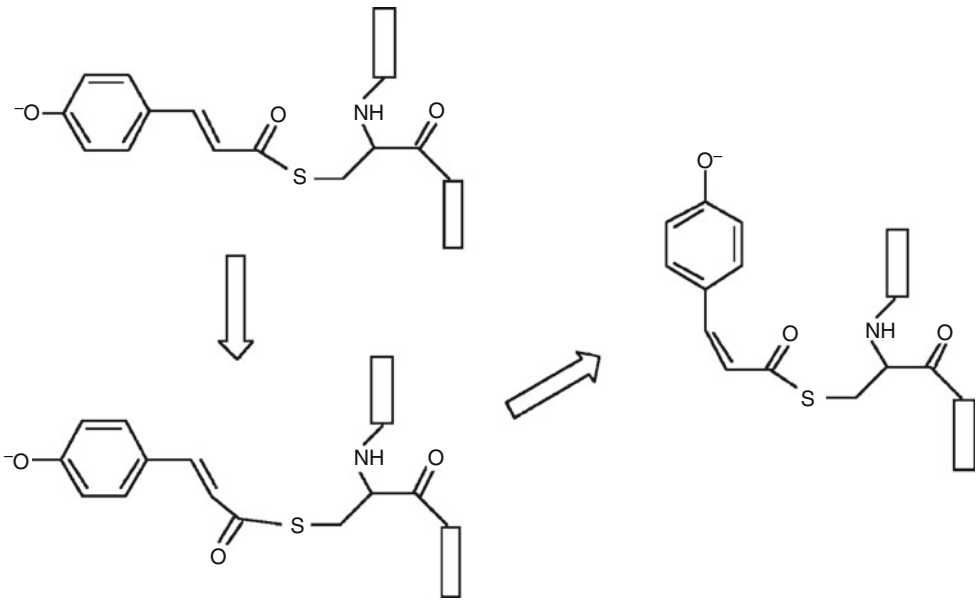

Fig. 12.12 A sketch of change of the PYP chromophore structure in two steps. The *rectangles* symbolize part of the protein. The cystine residue which forms a thioester linkage with the cinnamic acid is outlined. Only the first step requires photon absorption and at physiological temperature is complete in a few nanoseconds. The second step takes several milliseconds

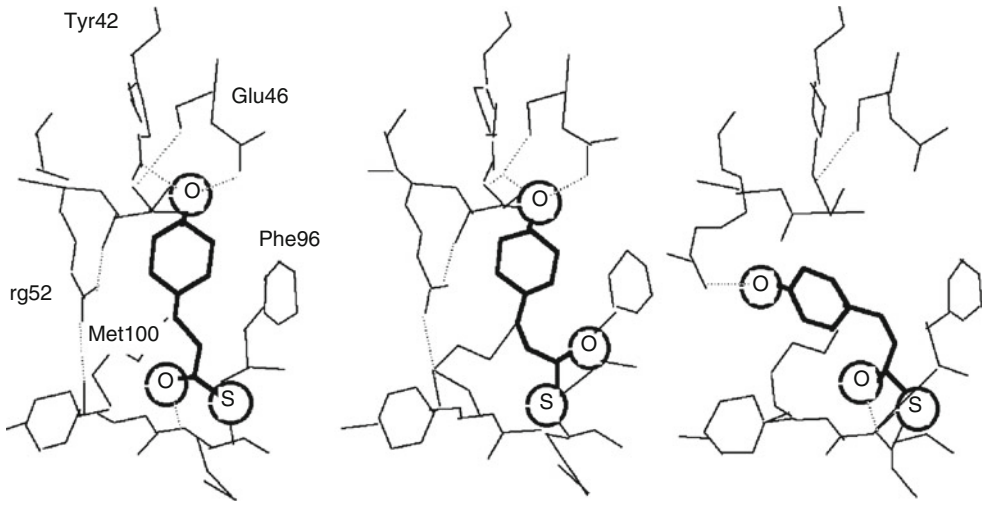

Fig. 12.13 Changes in PYP induced by light. Shown to the *left* is the region around the chromophore before light absorption. The chromophore skeleton is outlined in *bold*, with oxygen and sulfur atoms indicated. Amino acid skeletons are outlined with *thin lines. Dotted lines* stand for noncovalent interactions (hydrogen bonds). In the center is the intermediate structure a few nanoseconds after light absorption. The *trans–cis* isomerization of the chromophore has taken place, and its tail has flipped over, but the ring is still in its original position, with hydrogen bonds to the upper oxygen still intact. To the *right* is the "signaling state" reached after several milliseconds. The chromophore is still in its *cis* state, but the whole chromophore has changed position, broken the original hydrogen bonds to the ring-attached oxygen atom, and formed a new hydrogen bond. By these rearrangements both the chromophore and the PAS domain of the protein become accessible from the outside (Based on Genick et al. 1998)

day plants, could be reversed by irradiation with light of longer wavelength, the so-called far-red light (700–740 nm). By accurate action spectroscopy (see Sect. 8.5) the spectral properties of two different pigment forms were defined, and this made possible the detection in plants by absorption spectrophotometry and the subsequent purification of phytochrome.

It is now known that plants contain several phytochromes with different properties and regulatory roles. This is not the place to describe this in detail, and the reader is referred to chapters in this volume on photomorphogenesis and photoperiodism in plants (Chap. 19) and on the biological clock and its resetting by light (Chap. 18).

Phytochrome or phytochrome-like proteins have also been found in various algae, a myxomycete, cyanobacteria, and other photosynthetic and nonphotosynthetic bacteria (Schneider-Poetsch et al. 1998; Davis et al. 1999; Jiang et al. 1999; Herdman et al. 2000; Lamparter and Marwan 2001; Hubschmann et al. 2001; Bhoo et al. 2001).

Phytochrome is synthesized by the plant in the red-absorbing form, called Pr, and can be converted, via several intermediates, to the far-red-absorbing form by red light or direct daylight. It is the far-red-absorbing form that is considered to be the active (signaling) state, but in some cases one or several intermediates may be active. The reverse conversion (via another set of intermediate states) can take place under far-red light, under daylight filtered through vegetation or soil, or (with some phytochrome types and more slowly) in darkness. A pigment that changes its absorption spectrum in light (without being destroyed) is called photochromic; phytochrome is said to be photoreversibly photochromic, since the original state can be restored by another kind of light.

The chromophore in phytochrome has generally been believed to be an open-chain tetrapyrrole (see Fankhauser 2001), phytochromobilin (Fig. 12.14). Hanzawa et al. (2002) discuss other possibilities, such as the related phycocyanobilin, the same chromophore as is present in the photosynthetic antenna pigments phycocyanin and allophycocyanin of cyanobacteria and red algae. Phycocyanobilin is also the chromophore in cyanobacterial phytochrome, while those phytochromes of nonphotosynthetic bacteria that have been investigated so far have biliverdin as chromophore (Bhoo et al. 2001).

An interesting optical property of phytochrome is that conversion from Pr to Pfr or vice versa results in rotation of the transition moment corresponding to the long-wavelength absorption band with respect to the bulk of the protein. This was first shown by Etzold (1965) and Haupt (1970) by in vivo linear action dichroism and later confirmed by various methods (Sarkar and Song 1982; Kadota et al. 1982; Sundquist and Björn 1983a; b; Tokutomi and Mimuro 1989). At first it was believed that the rotation amounts to 90°, but the newer experiments and reinterpretation of the old in vivo experiments (Björn 1984) point to a smaller angle. Based on this and other evidence, Rospendowski et al. (1989) produced

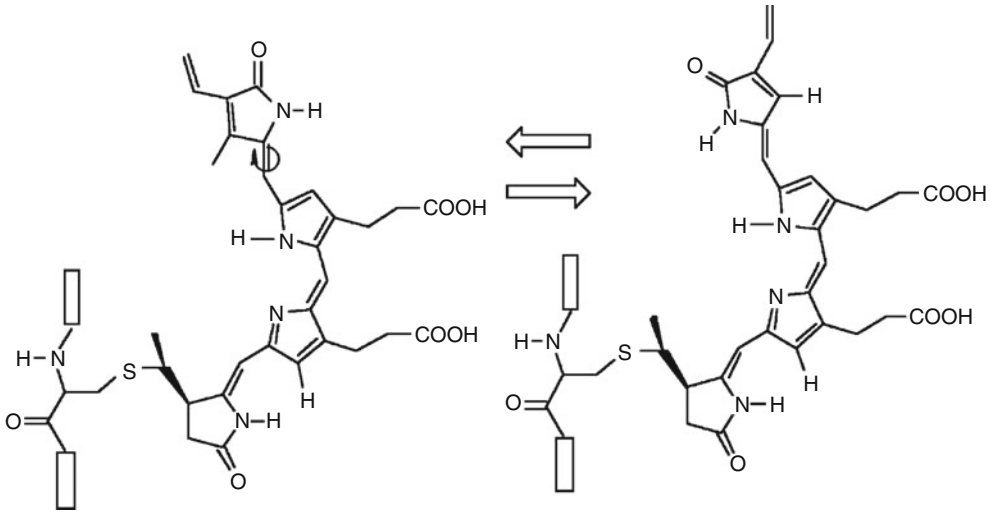

Fig. 12.14 The chromophore of higher plant phytochrome A (phytochromobilin) in the Pr (*left*) and Pfr (*right*) forms. The *rectangles* symbolize amino acid residues in the protein. The *circular arrow* indicates the double bond at which the pyrrole group to the right rotates during photoisomerization. Phytochrome B may contain the very similar phycocyanobilin as chromophore, in which the double bond and CH_2 group pointing straight up on top of the right-hand formula is replaced by a single bond and a CH_3 group, and phytochromes in nonphotosynthetic bacteria contain biliverdin

a drawing of how the chromophore moves in the protein during conversion.

Phytochrome in solution is a dimer (Jones and Quail 1986), and there is evidence that it is also dimeric in vivo. Like many other sensors it has a PAS domain (see Sect. 12.2.4.) and is an autophosphorylating protein kinase (Boylan and Quail 1996; Watson 2000).

12.2.6 Photosensor for Chromatic Adaptation of Cyanobacteria

Many cyanobacteria have the ability to adjust the amounts of the photosynthetic antenna pigments phycocyanin (red-absorbing) and phycoerythrin (green-absorbing) according to the spectrum of ambient light. This regulation process is known as chromatic adaptation, although with present-day definitions it would more appropriately be called chromatic acclimation. Long ago action spectroscopy revealed that the photoreceptor for chromatic adaptation in cyanobacteria must be a phycobiliprotein (Fujita and Hattori 1962; Lazaroff and Schiff 1962; Diakoff and Scheibe 1973; Vogelmann and Scheibe 1978), just like phytochrome. From cyanobacterial antenna pigments several photochromic chromopeptides can be prepared (Scheibe 1962; Björn 1980a; Fig. 12.15).

A new start on an old problem has been made from the other end at the Department of Plant Biology of the Carnegie Institution of Washington. The recent work has been reviewed by Grossman et al. (2001). Kehoe and Grossman (1996) found a gene, rcaE, coding for the protein RcaE, which is necessary for chromatic adaptation. RcaE binds a

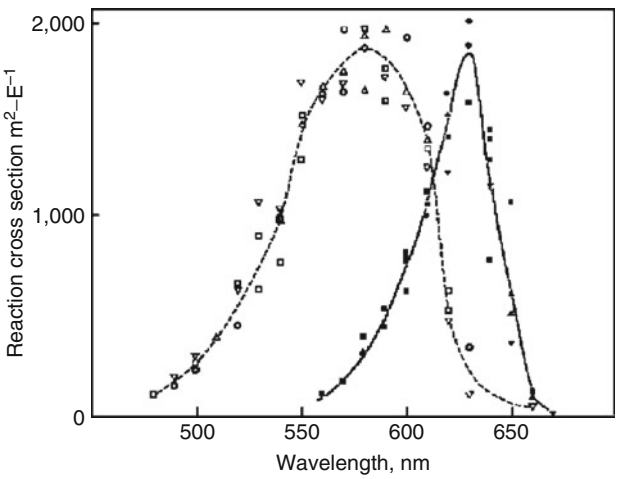

Fig. 12.15 Action spectra for conversions of phycochrome c to the short-wavelength form (*solid line* and filled symbols) and to the long-wavelength form (*dashed line*, empty symbols) (From Björn and Björn 1978). Somewhat similar action spectra were also obtained for phycochrome a, another polypeptide from phycocyanin (G.S. Björn 1980a)

tetrapyrrole chromophore covalently in a domain similar to that of phytochromes and also has a PAS domain typical for signal sensing proteins. It is believed that the chromophore is phycocyanobilin, which would fit well with the old spectral observations. According to the theory proposed by Grossman et al. (2001), there are, in addition to RcaE, two other proteins, RcaF and RcaC, involved in the signaling for chromatic adaptation. Under red light, RcaE autophosphorylates and transfers a phosphate group via RcaF to RcaC. In RcaC there are two sites that can be phosphorylated, and the one active

in this context is near the N-terminal (the role of the other site is unclear). This chain of events results in increased phycocyanin production. Green light, on the other hand, causes RcaE to change to the nonphosphorylating conformation, resulting in phycoerythrin synthesis. Kehoe and Gutu (2006) and Montgomery (2007) point out that chromatic adaptation in cyanobacteria is more complex than formerly believed and involves at least three signaling pathways: in addition to the phytochrome-type Rca system with separate effects of red and of green light, the Cgi ("controlled-by-green-light") system. Some cyanobacteria can acclimate to far-red light by a different mechanism (Gan et al. 2014).

12.2.7 Violaxanthin as a Blue-Light Sensor in Stomatal Regulation

Stomata are adjustable valves in the outer layer (epidermis) of leaves and other photosynthetic plant organs. They are designed to let sufficient carbon dioxide in from the external air without causing the plant to dry out due to outward diffusion of water vapor. Their regulatory system senses the water status, both directly in the leaves and indirectly in the rest of the plant body via the hormone abscisic acid. It also senses the internal carbon dioxide concentration. It senses light in several ways. One indirect way is via photosynthesis, since this causes the internal carbon dioxide concentration to fall. But the fastest and most dramatic light effect is blue-light specific, and there seems to be another light-sensing molecule involved than the cryptochromes and phototropins dealt with in the next section: the xanthophyll zeaxanthin (see Zeiger 2000 and Assman and Wang 2001 for reviews).

The strongest evidence for participation of zeaxanthin as a blue-light sensor of stomata is the fact that the stomata of an *Arabidopsis* mutant, npq1, which lacks a functional violaxanthin deepoxidase and therefore cannot accumulate zeaxanthin, does not show a blue-light-specific response (Frechilla et al. 1999). On the other hand, mutants defective in cryptochromes 1 or 2 or phototropin 1 have a normal response. However, there is also evidence of several independent blue-light channels for stomatal regulation (Lasceve et al. 1999) and that violaxanthin is only one of the sensors.

Violaxanthin has nine double bonds, so there are many possibilities for *cis–trans* isomerizations. Such a photoisomerization has not been directly shown, but postulated from kinetic experiments and the fact that the blue-light effect can be reversed by green light (Iino et al. 1985; Frechilla et al. 2000). The reversal spectrum has peaks at 490, 540, and 580 nm, similar to a wavelength-shifted zeaxanthin spectrum. We would like in this context to mention several experiments in the 1960s and 1970s in which blue-light effects in algae were reversed by light of longer wavelength (see Björn 1979 for a review).

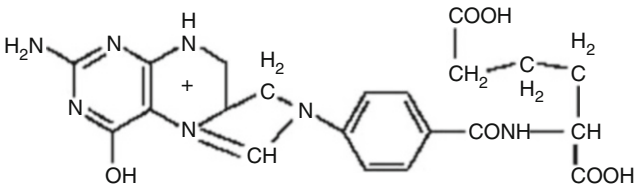

Fig. 12.16 N5, N10-methenyl-5,6,7,8-tetrahydrofolic acid, one of the chromophores in cryptochrome, probably acting as an antenna pigment for the other chromophore, FAD

12.3 Other Types of Photosensors

12.3.1 Cryptochromes

The term cryptochrome has been in use for a long time in plant physiology, as a name for the unknown blue-light photoreceptor. The name derives from the fact that it was hiding for such a long time (Björn 1980b). Now it is known that there are at least two quite different types of blue-light receptors in plants, called cryptochromes (cry1 and cry2) and phototropin. Thus the term has acquired a more restricted meaning than it used to have. On the other hand it has recently been discovered that chromoproteins similar to the plant cryptochromes are present also in other organisms than plants, including humans. These proteins are also called cryptochromes, but may have arisen independently during evolution (Todo et al. 1996). Recent reviews covering both cryptochromes and phototropins are provided by Lin (2000) and Christie and Briggs (2001). Regarding the effect of magnetic fields on cryptochromes, see Chap. 20 and Ahmad et al. (2007).

In plants cry2 represses phytochrome B action and plays a role in photoperiodism. cry1 regulates the period of the biological clock and is involved in the entrainment of the circadian oscillator (see Christie and Briggs 2001 for references).

Cryptochromes have two chromophores: 5,10-methenyltetrahydrofolic acid (Fig. 12.16) and flavin adenine dinucleotide (FAD). The latter is noncovalently bound to the protein. The role of the former is not known; probably it acts as an antenna pigment (in analogy with antenna pigments in photosynthesis) and transfers absorbed light energy to FAD, as is the case for the related photolyases.

The FAD part undergoes at least partial reduction upon illumination. The semiquinone formed by light action on cry1 has a long lifetime and seems to be able to act as a chromophore, too, giving the cryptochrome a sensitivity to green light under some circumstances. It has long been known that various blue-light effects in plants and fungi, in experiments designed to determine action spectra, are not parallel in log fluence vs. effect diagrams (see, e.g., Shropshire and Withrow (1958). The explanation may be this light sensitivity of the semiquinone or the participation

of several photoreceptors (such as the two cryptochromes, phototropin, and phytochrome) in the effects studied.

The signal transduction chains associated with cryptochromes have been difficult to elucidate, not only because more than one cryptochrome probably act in different ways but because plants have another blue-light receptor (phototropin) and because there are interactions with phytochrome. However, one signaling pathway proved surprisingly simple (Wang et al. 2001). In the dark, a protein called COP1 present in the nucleus prevents the activity of several genes by preventing the action of their transcription factors. After photoactivation of cryptochromes, their conformation is changed so they can bind to COP1 and prevent its action, thereby activating the genes.

Apart from their roles in the cell nucleus related to rhythmicity and gene regulation, cryptochromes seem to have direct effects on membranes. Thus cry1 activates an anion channel in the cell membrane and thereby influences the membrane potential. As for the mechanism of action, there is so far hardly more than speculation. Flavins are known to mediate light-driven electron transfer in other cases, but this has not been shown for cryptochromes. One indication for a role of electron transfer is the similarity between cryptochromes and photolyases. Merrow and Roenneberg (2001) speculate about relations between redox potential, cryptochromes, and the mechanism of the circadian oscillator.

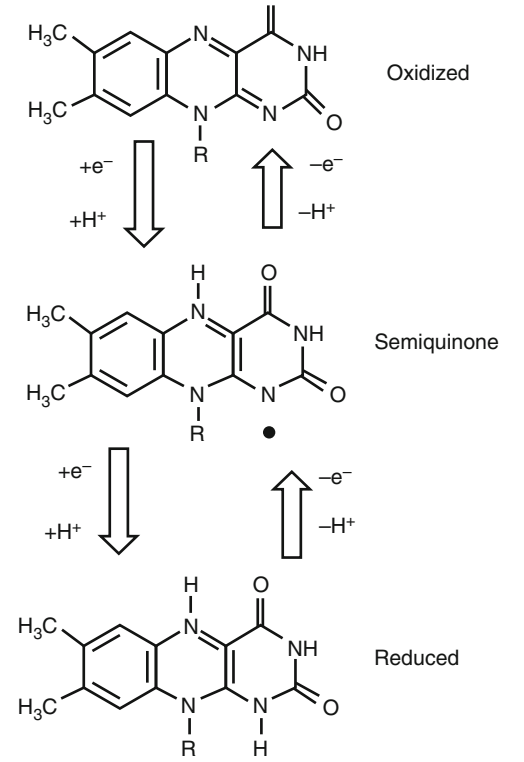

Fig. 12.17 The structures of FMN and FAD in oxidized and reduced forms and in the half-reduced (semiquinone) form

12.3.2 Phototropin

Phototropin is the photoreceptor primarily involved in plant phototropism, the phenomenon which, beginning with Darwin, has meant so much for stimulating interest in research in plant photobiology and about plant hormones. However, cryptochromes and phytochrome are also involved in the very complex phenomenon of phototropism (Galland 2001; Iino 2001). On the other hand, phototropin is involved in other blue-light reactions, such as high- and low-light-induced chloroplast movements (Sakai et al. 2001; Jarillo et al. 2001; Kagawa et al. 2001) and inhibition of hypocotyl extension growth (Folta and Spalding 2001). So far two main types of phototropin, phot1 and phot2 (Briggs et al. 2001), have been identified, of which phot1 acts primarily in phototropism, phot2 primarily in chloroplast photomovement.

Phototropin, like the cryptochromes, is a flavoprotein, and it also has two chromophores per molecule. In the phototropin, however, both chromophores consist of covalently bound flavine mononucleotide (FMN, Fig. 12.17). The protein part is quite different from that of the cryptochromes. The chromophore-binding regions are the so-called PAS domains, designated LOV1 and LOV2 (Salomon et al. 2000; Christie and Briggs 2001). (LOV stands for *l*ight, *o*xygen, or *v*oltage regulated.) The properties of these two domains can be investigated separately using molecular biology techniques. The absorption spectra of both show a striking (and expected) similarity to the action spectra for phototropism determined long ago (Fig. 12.18).

Upon illumination the chromophores, in both the LOV1 and LOV2 domains, undergo a spectral shift (Fig. 12.19); the absorbance in the blue region decreases, while that in the UVA part of the spectrum changes only little. Illumination also causes quenching of the fluorescence. In darkness both LOV1 and LOV2 return to the original state with halftimes at room temperature of 11.5 and 27 s, respectively. By amino acid substitution it has been made likely that the spectral change is caused by the formation of a bond between cystine (Cys39) and the FMN chromophore, with simultaneous reduction of the flavin (Fig. 12.20). This was confirmed by other methods (Crosson and Moffat 2001). The C-terminal end of phototropin is a serine/threonine kinase, which supposedly is activated by a conformational change resulting from the light-induced change in the chromophore region. The detailed structure of the LOV2 domain has now been determined and compared to other PAS domains (Crosson and Moffat 2001).

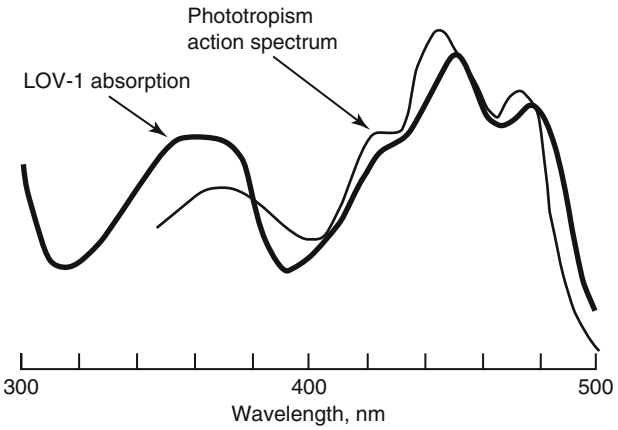

Fig. 12.18 Comparison of the action spectrum for phototropism of oat coleoptiles determined by Thimann and Curry (1961) with the absorption spectrum of the LOV1 protein domain of phototropin of an oat mutant determined by Salomon et al. (2000). The spectra for both LOV1 and LOV2 of the native oat are very similar, although not identical

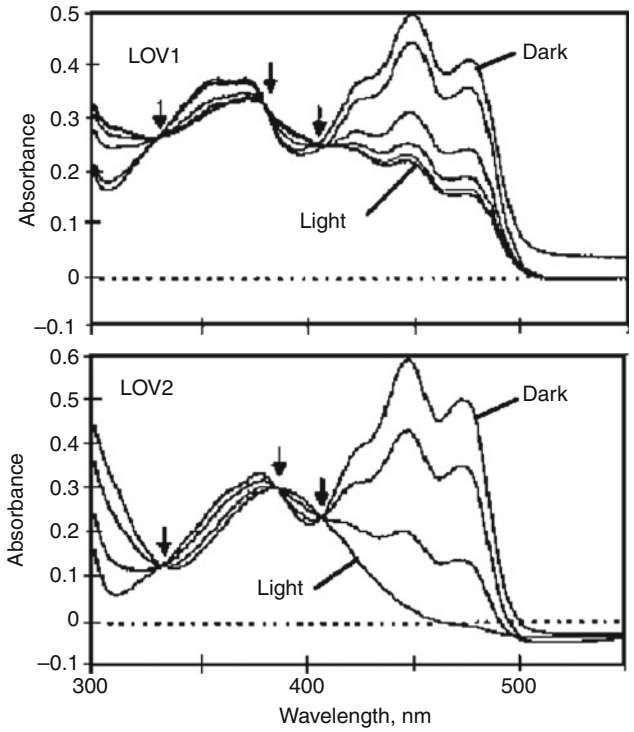

Fig. 12.19 Light-induced spectral changes taking place in the LOV1 and LOV2 domains of phototropin upon illumination. The *arrows* point to isosbestic points (wavelength positions with unchanged absorbance) (Reprinted, slightly modified, with permission from Salomon et al. 2000)

Red-light effects and blue-light effects in plants have traditionally been investigated by different sets of researchers, and there have been red-light meetings and blue-light meetings. It has become more and more difficult to uphold such a segregation as more and more interactions between the

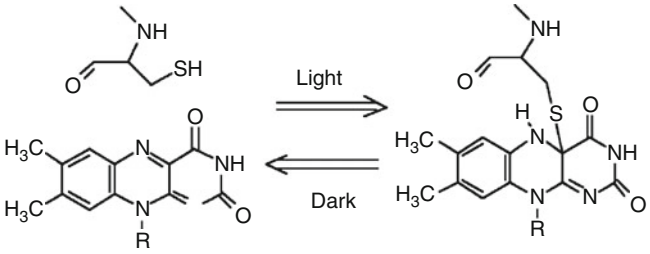

Fig. 12.20 The proposed light-induced, dark-reversible bonding between FMN and the cysteinyl residue in phototropin (Redrawn and simplified from (Crosson and Moffat 2001))

signaling channels have been discovered (Mohr 1994; Neff and Chory 1998; Parks et al. 2001). Nozue et al. (1998) even found a protein in a fern that possesses both phytochrome and phototropin properties.

12.3.3 The Plant UV-B Receptor UVR8

Ultraviolet B radiation (280–315 nm) affects plants and other organisms in many destructive and inhibitory ways (Chap. 22).

But plants also exhibit specific regulatory effects of UV-B radiation, and it has long been realized that plants possess a UV-B-specific photoreceptor, but only recently has it been characterized at the molecular level. This photoreceptor differs from all others enumerated above by having no non-amino acid chromatophore (Christie et al. 2012; Wu et al. 2012). The UV-B absorption is achieved by a large number of aromatic amino acids in the protein, of which several tryptophan residues are so closely positioned that the π-orbitals overlap. Absorption of UV-B radiation around 290 nm causes the protein to be monomerized from the dimeric state and translocated from the cytoplasm to the nucleus, where it interacts with a protein called COP1 and affects genes (O'Hara and Jenkins 2012). The photoreceptor is widespread among plants. The green alga *Chlamydomonas* has a similar protein (Christie et al. 2012) that probably has the same function. Related proteins with other functions occur in other organisms, including animals. One of these is RCC1, a regulator of DNA replication (Dasso et al. 1992; Dasso 1993) and mitosis. It thus appears that the UVR8 photoreceptor is specific for green plants, but has an ancient ancestry. Also some cyanobacteria have a receptor for UV-B radiation (Portwich and García-Pichel 2000).

The strong absorption band of UVR8 in the UV-B region (Fig. 12.21) is due mainly to 14 tryptophan and 10 tyrosine residues per monomer, with some contribution also from 8 phenylalanine residues. Of the tryptophan residues, 13 are located in the central core. Some of the tryptophan residues are so close that their pi orbitals overlap. Three of them (W233, W285, and W337), close to the monomer surface

interacting with the other monomer in dimer formation, are particularly important for the function, and W285 is indispensable.

The UVR8 dimer is kept together by electrostatic interactions between positively charged arginine and negatively charged amino acids on the opposite monomer (Fig. 12.22). Interactions occur also between arginine and the pi electron clouds of the tryptophan residues. When UV-B radiation is absorbed, these pi electron orbitals are altered, and the interactions are no longer strong enough to keep the monomers together.

Recently Wu et al. (2014) have performed calculations that indicate that the primary reaction initiated by UV-B radiation is a transfer of electrons from tryptophan 233 via tryptophan 285 to arginine 338 coupled to proton transfer from tryptophan 233 to aspartic acid 129, leading to disruption of salt bridges, starting at arginine 338.

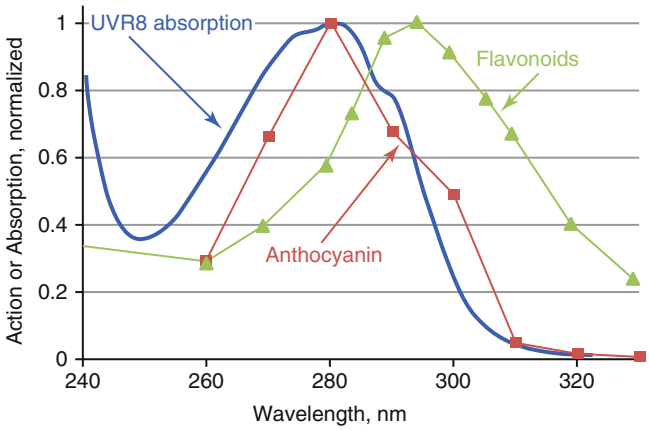

Fig. 12.21 Examples of action spectra for processes thought to be mediated by a special UV-B receptor: (induction of flavonoid synthesis (Beggs and Wellmann 1995) and of anthocyanin synthesis (Takeda and Abe 1992) compared to the absorption spectrum for UVR8 (Christie et al. 2012)

12.3.4 The Orange Carotenoid Protein, ORP

The orange carotenoid protein (OCP) is a cyanobacterial photoactive protein binding a keto-carotenoid molecule (Fig. 12.23). Its role is to adjust the photosynthetic apparatus to the fluence rate and protect the organism against excessive light. Plants, algae, and cyanobacteria have various mechanisms for protection against excess light which may cause damage by the so-called non-photochemical quenching (NPQ). In plants and algae the lowering of pH inside the thylakoids caused by strong light triggers this photoprotection. But the OCP functions in a completely different way, independently of changes in pH or redox potential (Berera et al. 2013). The same protein acts as light sensor, signal propagator, and quencher of excess energy. It is most

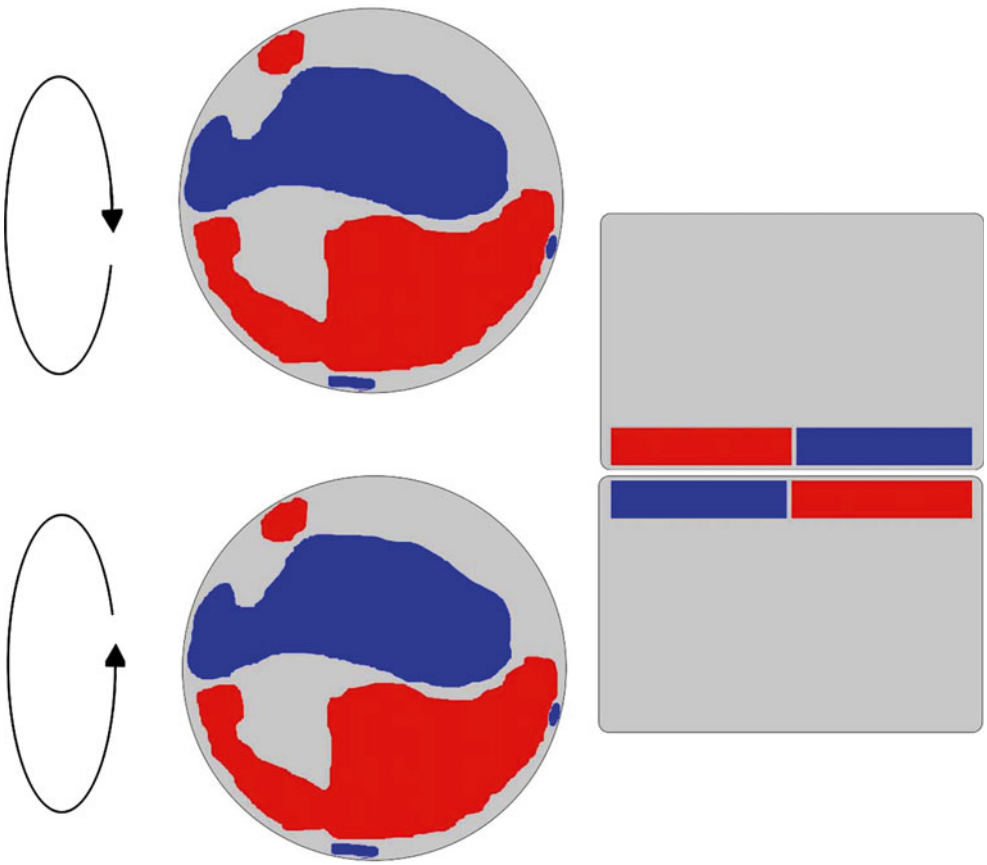

Fig. 12.22 Sketch of UVR8 monomers (*left*) with positive charges indicated by *blue* and negative charges by *red* and a dimer (*right*) showing how opposite charges hold the dimer together

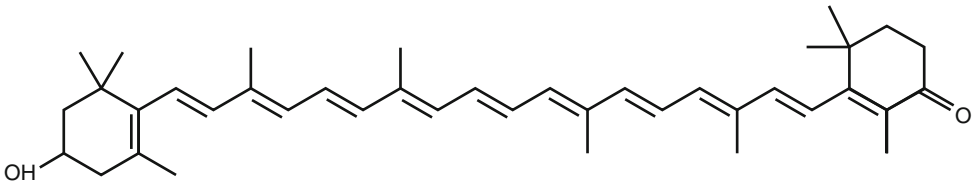

Fig. 12.23 The xanthophyll 3′-hydroxyechinenone present in OCP

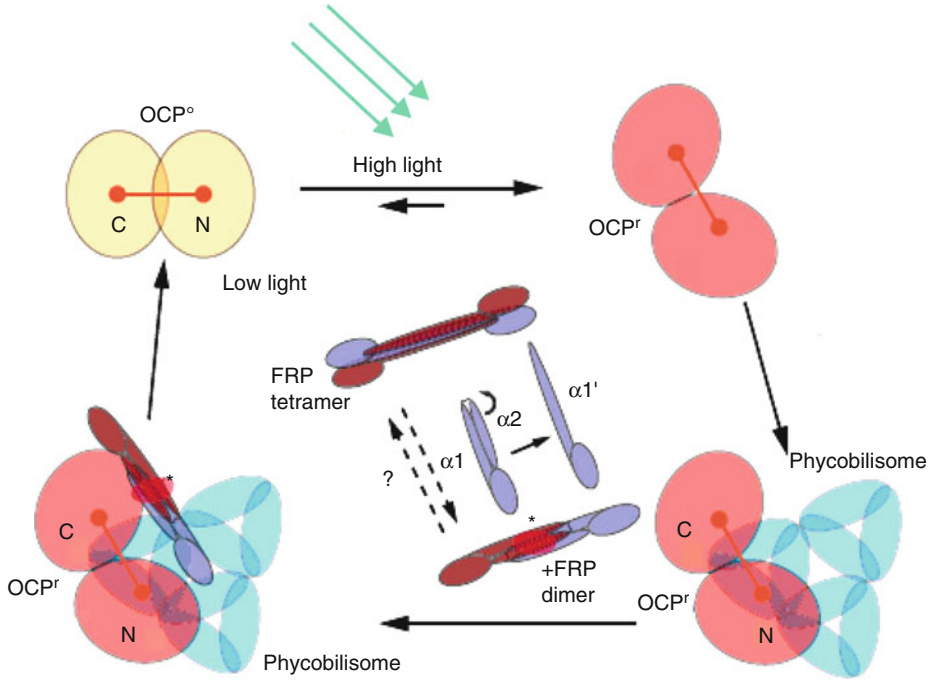

Fig. 12.24 Light-induced transformation of OCP from the OCP° (*low light*) state to the OCP^r (*high light*) state and following attachment to phycobilisomes, and FRP detatchment and return to the OCP° state in low light. From Sutter et al. (2013)

sensitive to blue-green light. When it is isolated from the organism, it has an orange color in its weak-light state, but shifts to red upon irradiation with strong light. In the organism, it is free in solution in its dark form. Upon exposure to strong light, it undergoes a large conformational change and attaches to phycobilisomes (cyanobacterial light-harvesting protein complexes on the thylakoid membranes, Fig. 12.24). Excess energy is channeled from the phycobilisomes to the OCP and is then degraded to heat. When energy in this way is channeled away from the phycobilisome, its fluorescence decreases, which provides a convenient way to monitor the changes in its energy state.

The quantum yield for change of the OCP from its low-light state to the protective state is only about 0.03 (Wilson et al. 2008), which makes sense, since it is only in strong light that excitation energy should be degraded to heat. When light is absorbed in the carotenoid molecule, there is a redistribution of charges which affects the interactions with the protein, so that the latter changes conformation. This exposes new parts of the molecule that have affinity for the phycobilisome. A second protein, called FRP (for "Fluorescence Recovery Protein) is required to release it again when incident fluence decreases.

Gwizdala et al. (2011) showed that it is necessary for the OCP to have contact with the central part (the core) of the phycobilisome to be able to channel energy away. OCP has no effect on the peripheral parts (the rods).

Although all cyanobacteria do not possess OCP, it is represented in all major morphological subsections and phylogenetic clades (Kirilovsky and Kerfeld 2013; Kerfeld and Kirilovsky 2013).

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