Photoactive Proteins

11

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11.1 Introduction

Light is important for the biological function of a large number of proteins. We can group them as follows:

1. Photoenzymes
2. Light-activated and light-deactivated enzymes
3. Light-driven ion pumps
4. Light-regulated ion channels
5. Photosynthetic antenna proteins
6. Photoreceptors
7. Photoproteins and luciferases

These categories are not completely free from overlap. Many of the photoreceptor proteins also belong to group 2. They will be treated separately in Chap. 13. Photoproteins and luciferases (light-emitting proteins) will be treated in Chap. 26.

11.2 Photoenzymes and Light-Activated Enzymes

We shall in the following use the term *photoenzyme* for those enzymes which are "driven" by light, for which light can be regarded as one of the substrates in the enzyme-catalyzed reaction. For the reaction to proceed in these cases, light must be continuously applied. Occasionally the term has also been used for enzymes which become active after irradiation, but continue to be active at least for some time after irradiation has stopped. We shall treat these enzymes separately and use for them the term *light-activated enzymes*. Some enzymes can be reversibly activated or inactivated with different kinds of light. One enzyme, LPOR, belongs to both groups, photoenzymes and light-activated enzymes, because it needs light both for activation and for driving the catalytic reaction.

11.2.1 Photoenzymes

Photoenzymes have built-in chromophores which catch the light energy which is a necessary requirement for their function. Some very important photoenzymes are not often thought of as photoenzymes. They will be treated in other chapters, and we shall only mention them briefly here to point out that they are also, strictly speaking, photoenzymes. Photoenzymes have attracted attention not only for the particular processes that they catalyze but also more generally. Because they allow starting the catalytic reaction with a short flash of light, at the same time for all enzyme molecules in a sample, they allow detailed study of how enzymes function.

11.2.1.1 Plastocyanin: Ferredoxin Oxidoreductase

Plastocyanin: ferredoxin oxidoreductase is usually referred to as photosystem I. The main energy-catching chromophore is chlorophyll *a* and in some cases chlorophyll *d*. Some cyanobacteria preferentially use cytochrome c-553 as electron donor in place of plastocyanin (Ferreira and Straus 1994), while others switch between cytochrome c-553 and plastocyanin depending on the availability of iron and copper. In bacteria the corresponding photosystems use cytochromes and have various bacterial types of chlorophyll as chromophores (Azai et al. 2010; Sarrou et al. 2012).

11.2.1.2 Water: Plastoquinone Oxidoreductase

Water: plastoquinone oxidoreductase is usually referred to as photosystem II. Anoxygenic photosynthetic bacteria have related enzymes that oxidize various other substances in place of water. Energy-harvesting chromophores are various chlorophylls, carotenoids, and bilins.

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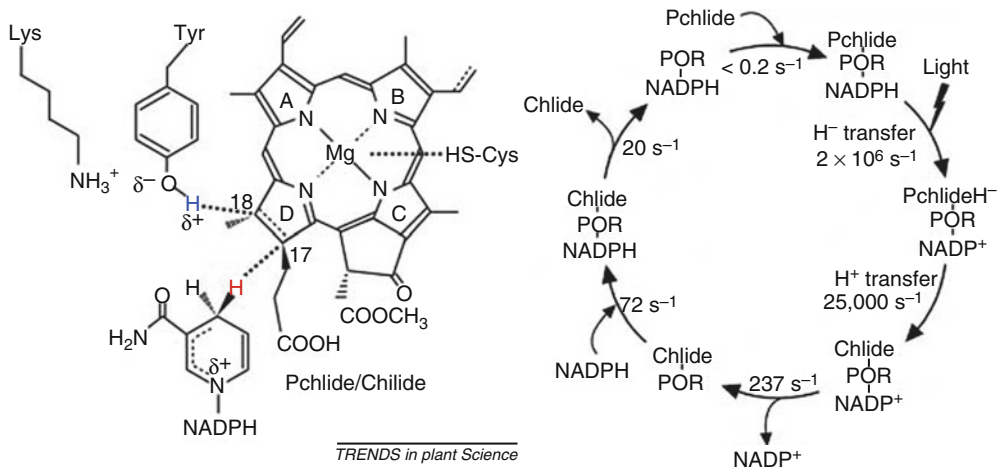

Fig. 11.1 *Left Panel*: Reduction of protochlorophyllide to chlorophyllide a by transfer of hydrogen as hydride ion from NADPH and as proton from tyrosine in LPOR (From Reinbothe et al. 2010). *Right panel*: The photocycle of LPOR according to Scrutton et al. (2012)

11.2.1.3 Light-Dependent NADPH: Protochlorophyllide Oxidoreductases (LPORs)

This is one type of enzyme that is able to reduce protochlorophyllide to chlorophyllide *a*; in angiosperms and some gymnosperms it is the only one. The other enzyme that can carry out this reaction, as well as the corresponding production of bacteriochlorophylls in bacteria, is DPOR, light-independent protochlorophyllide oxidoreductase. The two enzymes are not closely related, although some motifs are shared (Gabruk et al. 2012). DPOR is the only NADPH: protochlorophyllide reductase in anoxygenic bacteria, while many cyanobacteria and lower plants possess both (or one of them during part of the life cycle, and the other one during another phase). DPOR is thought to have, at least partly, evolved from nitrogenase (Bröcker et al. 2008, 2010). The phylogeny of LPOR among eukaryotic organisms has been studied by Yang and Cheng (2004). It is related to alcohol dehydrogenase (Masuda and Takamiya). In *Arabidopsis* there are three forms of LPOR, i.e., PORA, PORB, and PORC (Masuda and Takamiya 2004).

The light-dependent formation of chlorophyll (ide) *a* has been studied for a long time. Early researchers (Schmidt 1914; Frank 1946; Koski et al. 1951) determined action spectra in search of the light-gathering chromophore. Proof that this is protochlorophyllide was obtained by Smith and Kupke (1956), who managed to extract from dark-grown seedlings a protochlorophyllide-containing protein complex that they called protochlorophyll holochrome, which was converted to a chlorophyllide *a* protein complex upon exposure to light. Later much smaller active LPORs have been isolated, having about a tenth of the molecular weight of protochlorophyll holochrome. LPORs are unique among photoenzymes in that they require absorption of a photon to become active (Sytina et al. 2008, 2011). This photon changes the conformation of the protein to be more favorable for the catalytic reaction. If the enzyme is left in darkness after activation, it remains active for hours.

Reduction of protochlorophyllide to chlorophyllide *a* requires addition of two hydrogen atoms to a double bond between carbon atoms 17 and 18. The two hydrogens are transferred in quite different ways. First a hydride ion, i.e., a proton with two electrons, is transferred from NADPH bound to the enzyme. In a second step a proton is transferred from a tyrosine residue in the protein. Then the $NADP^+$ cation is exchanged for a new proton taken up from the environment (Fig. 11.1).

The photocycle of Scrutton et al. (2012) shown in Fig. 11.1 is not completely accepted by all. Much discussion has concerned a species named I675 (after the wavelength of its absorption maximum) not shown there, which is formed very quickly. By some it is regarded as an intermediate (e.g., Sytina et al. 2012), by others as being on a side track.

11.2.1.4 Aspartate Aminotransferase from *Escherichia coli*

Aspartate aminotransferase from *Escherichia coli* has a low activity in darkness, but irradiation with blue-violet light (410–430 nm) more than doubles the activity for the wild-type enzyme. The chromophore is pyridoxal 5′-phosphate, a form of vitamin B_6. In a mutant where a lysine residue has been changed to alanine, the activity is lower than that of the wild type in darkness, but increased several hundred times in light (Hill et al. 2010). The light dependence was for a while thought to be bi-photonic, but later investigations have shown that it is a single-photon process. The activity stimulation is due to photoenhanced deprotonation. Since the effect of light is rather modest for the wild type and can be so much increased by a substitution of a single amino acid, it is not likely that light plays a great biological role in this particular enzyme. But aspartate aminotransferase belongs to a very large group of enzymes with pyridoxal 5′-phosphate as coenzyme, and it is not unlikely that more interesting photoenzymes will be found among its members.

11.2.1.5 Photolyases

As described in Chap. 22 DNA can be damaged by ultraviolet radiation. Before the role of DNA was known, Hausser and v. Oehmcke (1933) discovered that discoloring of fruit peels due to exposure to ultraviolet radiation (UV) could be prevented if the fruits were exposed to strong visible light immediately after the UV treatment (Figs. 22.11, 28.8, and 28.9). Other early publications on the subject include Blum et al. (1949), Dulbecco (1949), and Kelner (1949). They all noted a remediation of UV damage to DNA by subsequent visible light. Rupert et al. (1958) were the first to carry out photoreactivation in vitro, and they showed that the photoreactivation catalyst had a dialyzable and a non-dialyzable part.

It is now known that some of the lesions in DNA molecules (likely reasons for all the UV effects described in the works cited above) can be repaired by photoenzymes referred to as photolyases (or photoreactivating enzymes). Some photolyases can repair cyclobutane-pyrimidine dimers (CPDs) and another type (6–4) photoproducts. If (6–4) photoproducts are left unrepaired, further irradiation with UV-A can convert them to Dewar photoproducts. For some time it was believed that Dewar photoproducts cannot be photorepaired. Recently, Fingerhut et al. (2012) found that (6–4) photolyase can repair Dewar photoproducts derived from T-C (6–4) photoproducts, but not those derived from and some (but not all) Dewar photoproducts formed from T-T (6–4) photoproducts (Fig. 11.2).

Photolyases are closely related to cryptochromes (see Chaps. 13 and 14), which have evolved from photolyases. Like these they contain an FAD cofactor (as $FADH^-$ in the dark state of the enzyme ready to receive light, see Liu et al. 2013) and another chromophore that can deliver absorbed light energy to the FAD. Also light absorbed directly in the FAD can drive the enzymatic DNA repair (Figs. 11.3, 11.4 and 11.5). At least some photolyases are phosphorylated (Teranishi et al. 2013).

When the pyrimidine bases in DNA are dimerized by the action of UV, they pop out of the central part of the DNA double helix into the aquatic environment, which makes it possible for the photolyase to recognize the lesion and attach to it. The FAD is held in a folded configuration with the adenine part folded back toward the flavin part, so that both parts are close to the DNA part to be repaired. While it is the flavin that donates an electron to the lesion, it is the adenine that conducts the returned electron back to the flavin (Fig. 11.4 and 11.7, left panel).

The full cycle of CPD photolyase action is depicted in Fig. 11.5. Note the unproductive back flow of electrons, which is here depicted as slow, and therefore not very important. Not all investigators agree that it is so slow and unimportant, but significantly decreases the quantum yield of the repair. We shall therefore have reason to discuss the quantum yield later.

The antenna chromophore varies between species and therefore also the action spectra for photolyase action. In most CPD photolyases it is methenyltetrahydrofolate (MTHF) or 8-hydroxy-5-deaza(ribo)flavin. The latter occurs in both some prokaryotes and eukaryotes. In some prokaryotes it is FMN or FAD. In (6–4) photoproduct photolyase from *Agrobacterium tumefaciens*, it was found to be 6,7-dimethyl-8-ribityllumazine (Zhang et al. 2013). In many cases the antenna chromophore is unknown. The CPD photolyase of *Bacillus firmus* (Malhotra et al. 1994) action spectrum shows a single peak at 410 nm and has a folate chromophore of some kind, but the action spectrum is different from those of MTHF-containing photolyases. Figure 11.6 shows some photolyase action spectra and one photolyase absorption spectrum. It is evident that spectra can be different even with the same chromophore, due to tuning influence of the protein. Nothing is known about the ecological or evolutionary significance of the spectral differences.

Data on quantum yields for photolyase repair varies among reports, from about 0.1 (Heelis and Sancar 1986) or about 0.2 (Byrdin et al 2010) to about 0.9 (Kim and Sancar 1991; Kao et al. 2005), even when the same photolyase, in

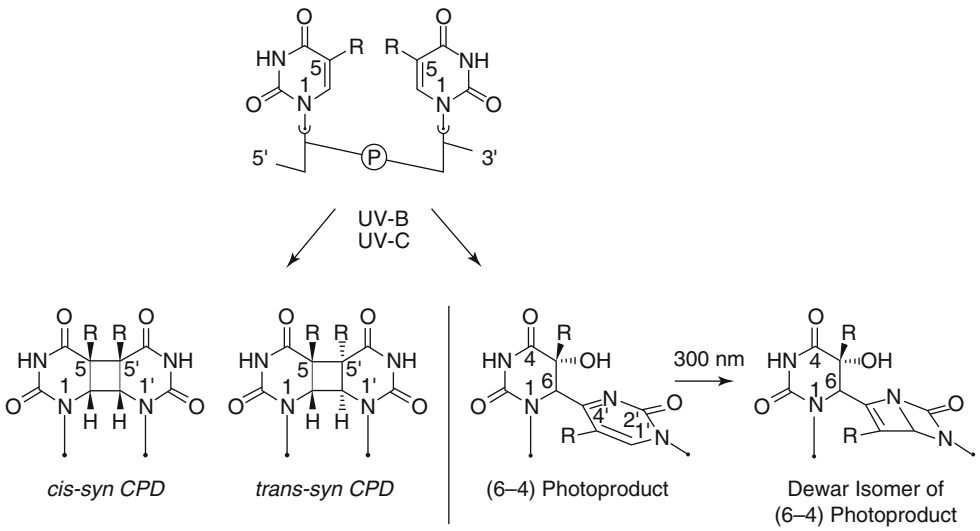

Fig. 11.2 Types of UV-induced lesions in DNA that can be repaired by photolyases (other kinds of damage that are less frequent need other systems for repair). Part of undamaged DNA-molecule on top. R denotes H in uracil (U) or CH3 in thymine (T). The cis–syn CPD is a diastereoisomer of the trans–syn CPD (From Weber (2005)

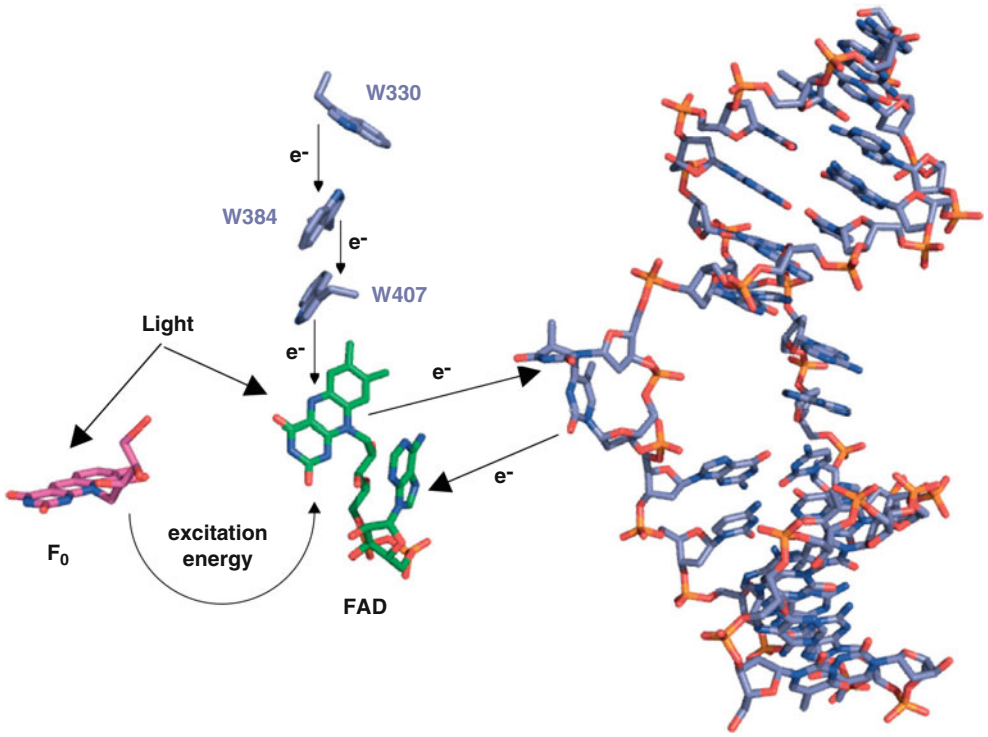

Fig. 11.3 The principle for remediation of a CPD lesion in DNA by a CPD photolyase. The structure to the right is the damaged DNA. Light is absorbed either in FAD or in the antenna chromophore. The excited FAD receives an electron from a tryptophan residue in the photolyase protein and is converted to $FAD^{-\bullet}$. The donating tryptophan regains an electron via a conductor of other tryptophan residues in the protein. The FAD semiquinone donates the odd electron to one bond in the CPD, but regains one from another bond. This results in splitting of the CPD to produce repaired DNA. Fo stands for the antenna chromophore, 8-hydroxy-5-deazariboflavin. From Benjdia (2012)

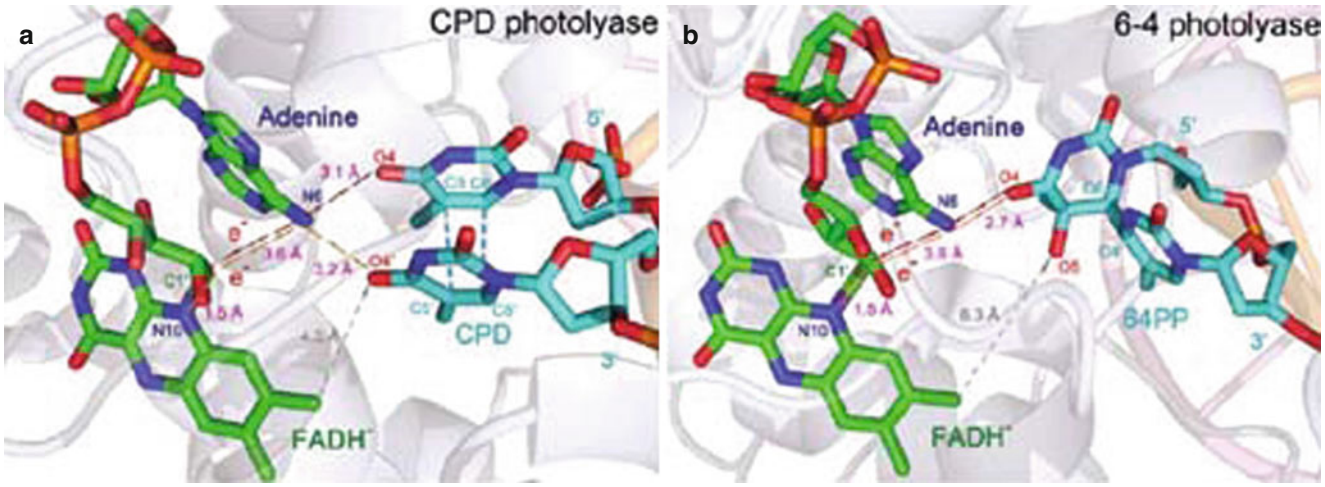

Fig. 11.4 Comparison of the enzyme structure around FAD in (**a**) CPD and (**b**) (6–4) photolyases (From Liu et al. 2011)

most cases CPD photolyase from *Escherichia coli*, is used. There can be various reasons for this. Not always is the substrate specified. Low yields can sometimes be explained by the use of an unnatural antenna chromophore. Thus Ramsey et al. (1992) obtained a quantum yield of 0.66 ± 0.03 for enzyme without antenna chromophore, 0.72 ± 0.04 for the intact enzyme with its natural 5,10-methenyltetrahydropteroylpolyglutamate cofactor, and only 0.34 ± 0.015 for the enzyme with the antenna chromophore without the polyglutamate part. The ratio between the two latter is in agreement with the finding by Lipman and Jorns (1992) that energy transfer from the antenna pigment is only half the native value if the polyglutamate part is omitted. In summary, it is likely that the quantum yield for the enzyme in the natural state is about 0.7. The structure of photolyase around the FAD chromophore is shown in Fig. 11.7. The expression of photolyase genes is often stimulated by ultraviolet radiation (e.g., Isely et al. 2009).

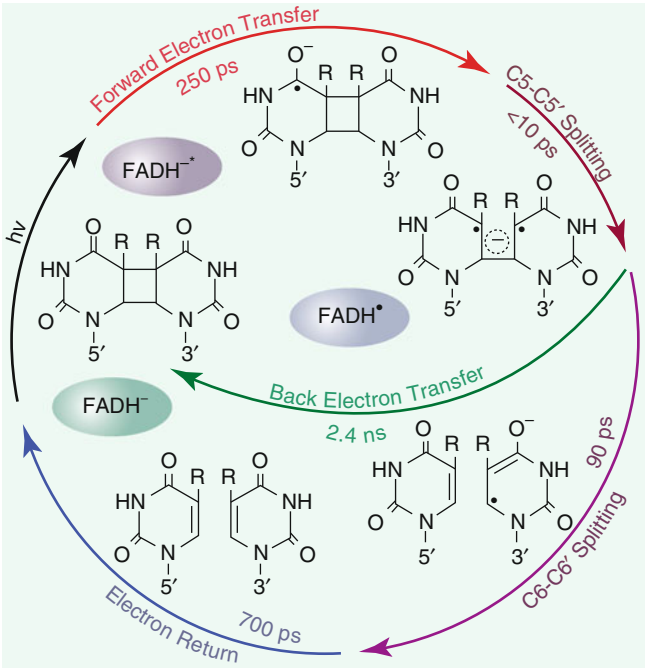

Fig. 11.5 Catalytic cycle of CPD photolyase (From Liu et al. 2011)

11.2.2 Type I Light-Activated Enzymes: Light-Induced Conformational Change

We have already mentioned above that LPOR belongs to this group, at the same time as it is a photoenzyme. The majority enzymes in this category, however, are not photoenzymes. Most of them function as photoreceptor proteins with bilin flavin chromophores.

11.2.2.1 Biliprotein Protein Kinases

Biliprotein protein kinases (phytochromes and related). These are photoreceptors and will be treated elsewhere (Chaps. 9 and 12–14).

11.2.2.2 BLUF Proteins

These will also be treated as photoreceptors (Chaps. 9 and 12–14).

11.2.3 Type II Light-Activated Enzymes: Photodissociation of Inhibitor

11.2.3.1 Urocanase

Urocanase (urocanate hydratase, EC 4.2.1.49) of *Pseudomonas putida* is an enzyme containing tightly bound NAD^+, which in the natural state is inhibited by sulfite in darkness. Irradiation with 11 W m^{-2} UV-A (ca 365 nm) dissociates off the sulfite and activates the enzyme (Hug and Roth 1971; Hug et al. 1972; Venema and Hug 1985). Experimentally the enzyme can also be activated in darkness by being brought in contact with triplet-excited indole-3-aldehyde, and light-activation can be counteracted by triplet quenchers (Venema et al. 1985). Thus it can be concluded that the photoactivation is due to triplet excitation of the NAD^+. No biological role for the light-dependency is known.

11.2.3.2 Nitrile Hydratase

Nagamune et al. (1990) found that when washed cells of *Rhodococcus sp*. N-771 were incubated in the cold (5°) and dark, their nitrile hydratase became gradually inactivated, but most of the activity could be recovered by illumination.

11.2.3.3 Cytochrome C Oxidase

This important enzyme does not need light for being active under normal conditions. But under some conditions it can be inactivated by endogenous nitric oxide, and this inhibition can be released by light. The complicated relations between the cytochrome c oxidase and nitric oxide are further treated in Chap. 26.

11.3 Light-Driven Ion Pumping by Rhodopsins

Rhodopsins acting as light-driven ion pumps occur in Archaea, Bacteria, and Eukarya. Organisms in the class Halobacteria (in the phylum Euryarchaeota in the domain Archaea) contain various rhodopsins, of which some transport ions and others have sensory functions. *Bacteriorhodopsins* transport protons (from the inside to outside the cells). The chromophore retinal is bound to a lysine residue in the protein via a protonated Schiff base. When the positively charged retinal absorbs a photon, it switches from all-trans to 13-cis configuration. This causes the pKa to drop (i.e., the dissociation constant increases) transiently, and the proton becomes less firmly bound and is released to the outside. Another proton is then taken up from the inside.

Halorhodopsin is a related light-driven pump (Schobert and Lanyi 1982) transporting chloride into cells. It undergoes a photocycle as depicted in Fig. 11.8. It has two chromophores, retinal and the carotenoid bacterioruberin. Contrary to the rhodopsin in our eyes, the retinal chromophore in the dark state is in the all-trans conformation and changes to the 13-cis form upon exposure to light. The first metastable state containing this 13-cis chromophore is called the K state, or HR_{600} (after its absorption spectrum). From this state the cycle proceeds forward to chloride transport or reverts to the original state. The HR_{600} state is a minimum energy state, so

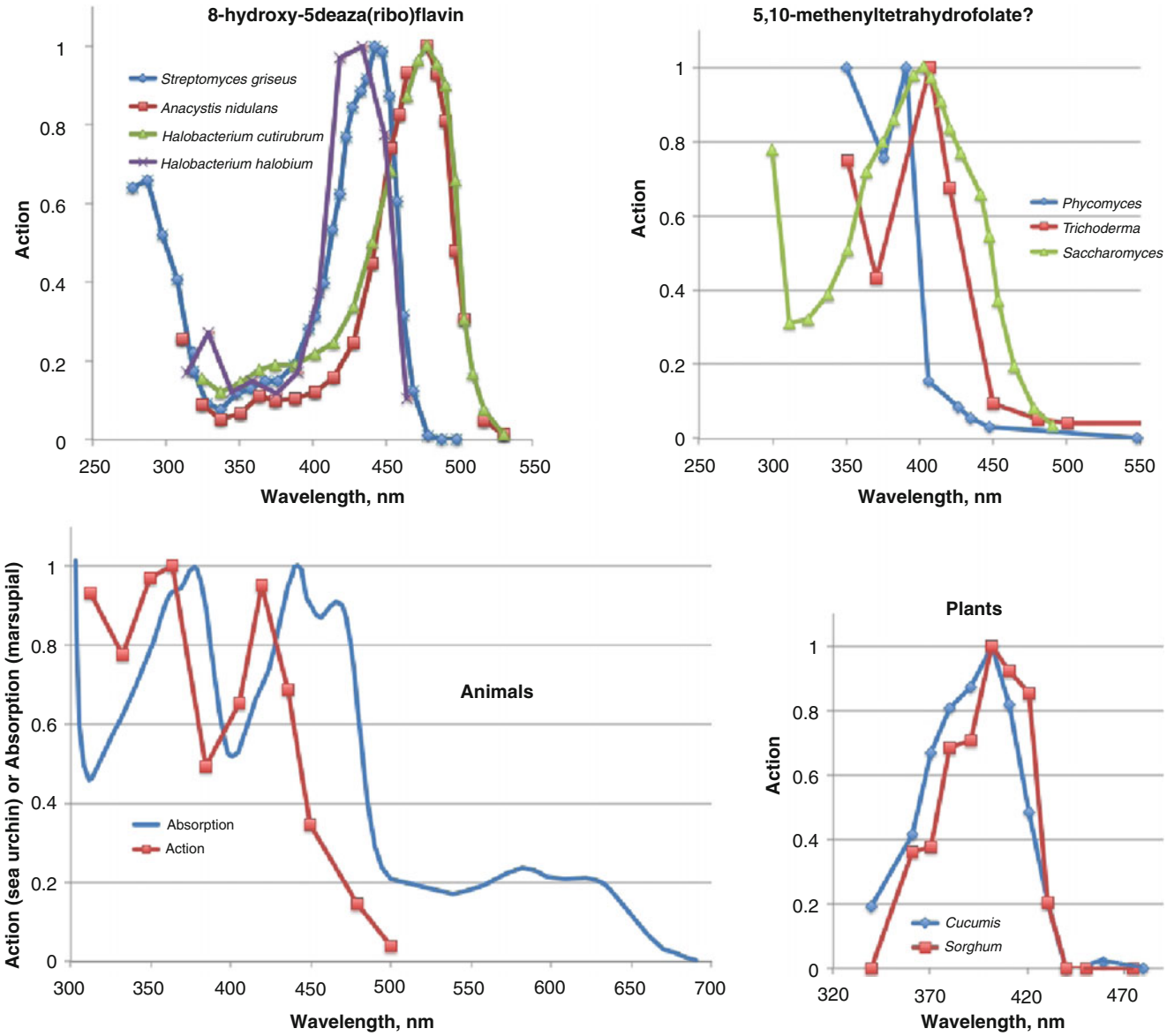

Fig. 11.6 Examples of spectra for photolyases, demonstrating the diversity. All are action spectra, except for the blue curve in the lover left panel, which is an absorption spectrum for purified photolyase. *Upper left* panel, photolyases using 8-hydroxy-5-deazaflavin or 8-hydroxy-5-deazariboflavin as antenna chromophore: *Streptomyces griseus* (bacterium, Eker et al. 1981), *Anacystis nidulans* (cyanobacterium, Eker et al. 1990), *Halobacterium cutirubrum* (archaeon, Eker et al. 1991), *Halobacterium halobium* (archaeon, Iwasa et al. 1988). *Upper right panel*, fungal photolyases probably using 5,10-methenyltetrahydrofolate as antenna chromophore: *Phycomyces blakesleeanus* (Zygomycota, Galland 1996), *Trichoderma harzianum* (Ascomycota, Sametz-Baron et al. 1997). *Lower left panel*, animal photolyases: Action spectrum for sea urchin eggs (*Hemicentrotus pulcherrimus*, Ejima et al. 1984), absorption spectrum of purified CPD photolyase from *Potorous tridactylis* (rat kangaroo, a marsupial, Yasui et al. 1994). The photolyases in this panel are thought to lack an antenna chromophore, and the active light is thus absorbed only in the FAD^- anion. *Lower right panel*, plant CPD photolyases: A dicotyledon (*Cucumis sativus*) and a monocotyledon (*Sorghum bicolor*), both from Hada et al. (2000). All spectra redrawn

proceeding from it either forward or backward in the cycle requires thermal energy. Pfisterer et al. have some interesting considerations concerning the optimal depth of the energy minimum associated with the HR_{600} state. If the energy is high in relation to the energy barrier in the forward direction, less thermal energy is needed, so the reaction is faster if the minimum energy is low. But then, on the other hand, the risk of backward reaction increases, so there is an optimum energy level for the HR_{600} state, and Pfisterer et al. find that this coincides with the energy level that the HR_{600} state actually has.

The difference between chloride pumps and proton pumps is very small. In fact, a proton pump can be converted to a chloride pump by substitution of a single amino acid (Sasaki et al. 1995). The pumping of chloride is best understood by first considering proton pumping. The retinal chromophore

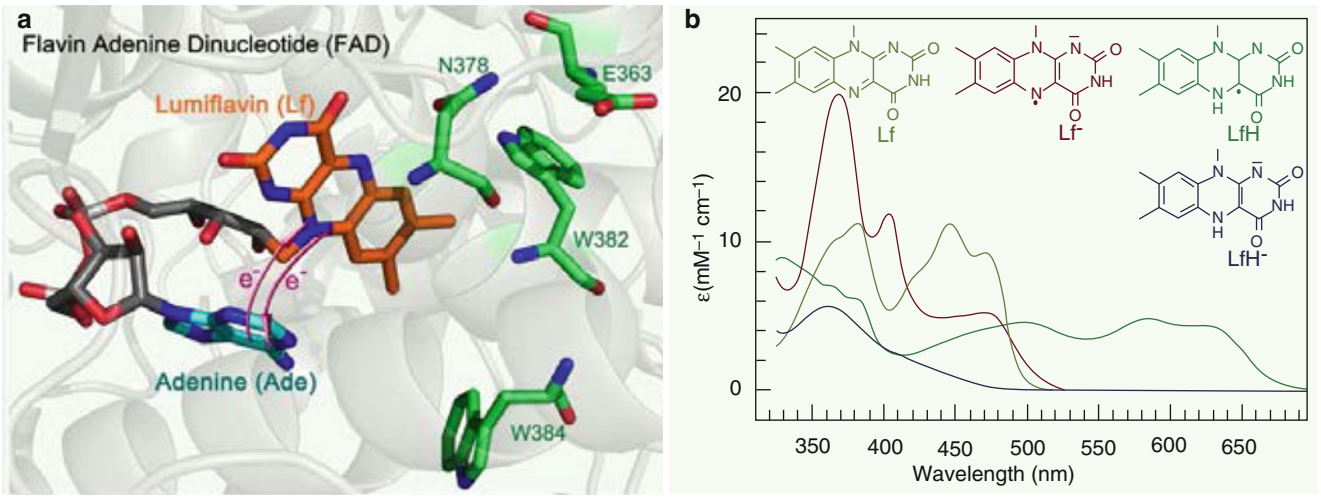

Fig. 11.7 *Left panel*: The adenine part of the FAD chromophore plays an important role in conducting electrons between the nucleotide bases in DNA and the flavin part of FAD. *Right panel*: absorption spectra for the different forms of flavin, i.e., neutral (*yellow-green*), anion (*red*), semiquinone (*green*), and protonated (*blue*). It is the anion form which is receiving light energy for repair, either directly or from the antenna chromophore (which is not shown here), and which therefore contributes to the action spectra (From Liu et al. 2013)

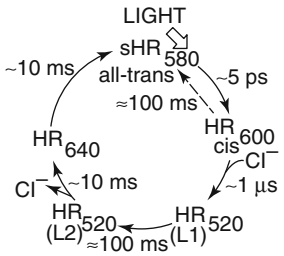

Fig. 11.8 The halorhodopsin (HR) photocycle. In darkness the halorhodopsin exists in the all-trans from shown on *top*. The trans-cis photoisomerization takes place at the 13-position. Note two different 520 nm forms (Modified after Pfisterer et al. 2009)

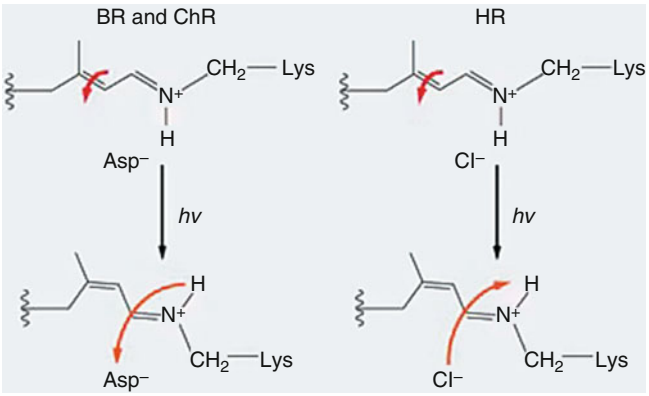

Fig. 11.9 Light-induced changes in proton-pumping (*left*) and chloride-pumping (*right*) rhodopsins. The light-induced trans-to-cis isomerization changes the position of a proton bound to the Schiff base nitrogen in such a way that it becomes less strongly bound and is transferred to an aspartate residue and from there via glutamate residues to the extracellular space. In the case of chloride pumping, the proton charge pulls the chloride along with it when the proton moves. The state after the initial isomerization corresponds to the HR_{600} state in Fig. 11.8, while the L1 state in Fig. 11.1 results from the chloride movement (From Zhang et al. 2011)

is attached to the protein via a so-called Schiff base linkage to a lysine residue (Fig. 11.9). When light absorption in the retinal chromophore causes its trans-to-cis isomerization, a proton bound to the lysine nitrogen comes into a new electric environment which allows it to be transferred to an aspartate residue in the protein. This in turn results in conformational change of the protein and transfer of the proton via glutamate residues to the space outside the cell.

The energy captured by light-induced proton or chloride pumping can be utilized for synthesis of ATP and also for driving other kinds of ion transport. Proton pumping rhodopsins have been found in halobacteria, eubacteria (Wang et al. 2012; Riedel et al. 2013), green algae (Wada et al. 2011), and fungi (Waschuk et al. 2005). *Proteorhodopsins* constitute a particularly widespread type of proton pump (Beja et al. 2000; de la Torre et al. 2003; Giovannoni et al. 2005; Frigaard et al. 2006; Bamann et al. 2013). Its operation in light has been shown to increase bacterial survival when organic carbon is scarce (Gomez-Consarnau et al. 2007, 2010; DeLong and Béjà 2010). Organisms living at different depths possess different spectral types of proteorhodopsin (Man et al. 2003). Also a kind of rhodopsin called *xanthorhodopsin* is quite widespread among different bacteria. Xanthorhodopsin is characterized by having a carotenoid (salinixanthin) antenna to collect extra light energy to the rhodopsin part (Lanyi and Balashov 2008). It sometimes occurs in the same organism together with other light-driven proton pumps (Kang et al. 2010; Riedel et al. 2013).

Recently a third kind of light-driven ion pump called KR2 (Fig. 11.10) has been discovered (Inoue et al. 2013). Under

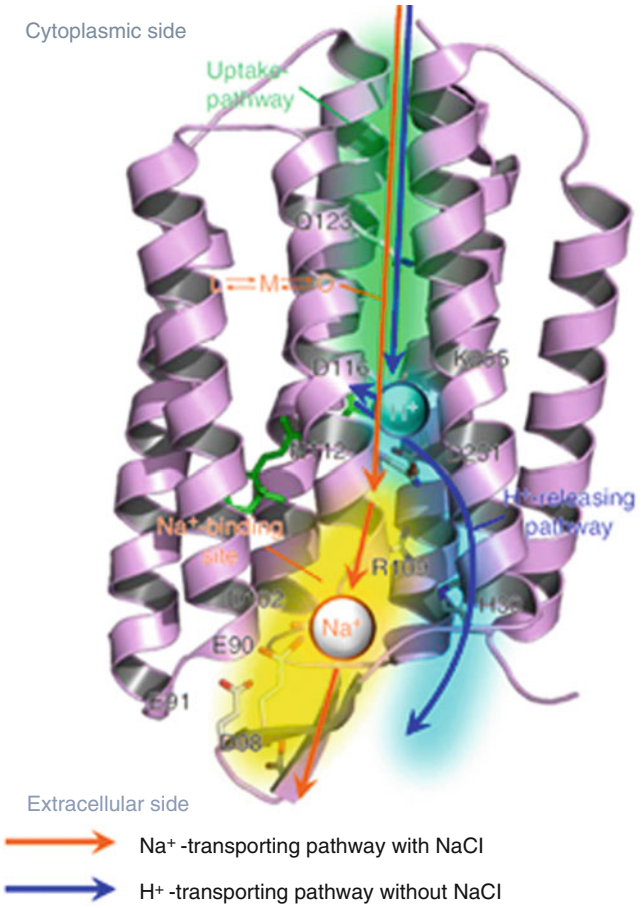

Fig. 11.10 Model of the light-driven sodium ion pump KR2 (From Inoue et al. (2013), with permission from Nature Publishing Group)

natural condition it transports sodium ions out of cells in the marine flavobacterium *Krokinobacter eikastus*. In the absence of its natural substrate sodium ions, the protein can pump the smaller lithium ions, but when presented only with larger cations like potassium, it switches to pumping protons.

For a time it was thought that a bacteriorhodopsin was a light-driven sodium pump (MacDonald et al. 1979), but later it has been clarified that the light-induced sodium ion transport observed was an indirect phenomenon: light-driven proton pumping followed by proton-sodium antiporting (Luisi et al. 1980).

11.4 Proton Pumping Using Bacteriochlorophyll-Based Photosystem

Also plants, algae, and various photoautotrophic bacteria pump protons across membranes using their photosystems. What is less known is the great importance of bacteria which are not photoautotrophic but pump protons using a very slimmed-down photosynthetic system that is easily transferred horizontally between bacterial strains. These bacteria, referred to as aerobic anoxygenic photoheterotrophic bacteria, are doing essentially the same as the organism pumping protons with rhodopsins (which strictly speaking are also aerobic anoxygenic photoheterotrophic bacteria), but are doing it using a different molecular machinery. Both kinds use the proton pumping for producing ATP and for helping in assimilating organic carbon from the environment. Aerobic anoxygenic photoheterotrophic bacteria are genetically very diverse (Richie and Johnson 2012). They were first discovered in the ocean but have later been found in the most diverse habitats exposed to sunlight, e.g., soil crusts (Csotonyi et al. 2010a, b) and high-altitude wetlands (Dorador et al. 2013) and saline lakes (Jiang et al. 2009). A particularly important group of aerobic anoxygenic photoheterotrophic bacteria is the so-called *Roseobacter* clade (Buchan et al. 2005).

11.5 Channelrhodopsins: Light-Gated Ion Channels

Kenneth Foster and coworkers were the first to realize that rhodopsins act as photoreceptors in green algae (Foster and Smyth 1980; Foster et al. 1984). *Channelrhodopsins* are type I (microbial type) rhodopsins that mediate phototaxis and other light-induced phenomena. Different types have been found in a large number of species (Yawo et al. 2013). The best investigated ones are the first discovered ones, channelrhodopsin 1 (ChR1, Nagel et al. 2002) and channelrhodopsin 2 (ChR2, Nagel et al. 2003) in *Chlamydomonas reinhardtii*. They differ not only spectrally (absorption and action peaks at 550 nm and 460 nm, respectively) but also in their ionic selectivity: ChR1 conducts exclusively protons, while ChR2 conducts small mono- and divalent metal and organic cations as well. In contrast to the rhodopsins dealt with in the previous section (light-driven ion pumps), they are light-gated cation channels.

Channelrhodopsins (as well as some of the proton-pumping rhodopsins) have found a practical application in "optogenetics" and related areas, i.e., techniques to manipulate neurons cells with light (Nagel et al. 2005; Dugué et al. 2012; Lin 2012).

11.6 Optogenetics

11.6.1 Introduction

The meaning of the term "optogenetics" has shifted over time (Dugué). We shall here follow Deisseroth (2011) and use it in the sense of "the combination of genetic and optical

methods to achieve gain or loss of function of well-defined events in specific cells of living tissue," but include in this chapter also some methods which do not fit into this definition.

11.6.2 Early Optical Methods for Recording Cell Processes

The first optical method for following what is going on in living cells was absorption spectrophotometry. Fortunately some of the molecules participating in respiration (cytochromes, cytochrome oxidase, flavoproteins, NAD) have prominent absorption spectra which change with oxidation level. This was exploited by pioneers as Keilin, Warburg, and Lundegårdh. Also for studies of the photosynthetic process, absorption spectrophotometry became important at an early date, but in vivo fluorescence, too, has provided crucial information.

In 1951 Strehler and Arnold (1951) attempted to demonstrate photophosphorylation in a suspension of chloroplast fragments by measuring light from added firefly extract containing luciferin and luciferase (Chap. 26). They failed to do that, but in the process discovered "delayed light emission," the light emitted by reversal of photosynthetic processes (Chap. 26). This in itself has become a valuable, noninvasive method for the study of photosynthesis. Later people have studied ATP generation cloning the luciferase gene into organisms and adding luciferin, but this method cannot be used while cells are still alive.

In 1985 the calcium indicating protein aequorin was cloned and expressed in a foreign organism (Inouye et al. 1985; Prasher et al. 1985). Aequorin is a protein from the jellyfish *Aequorea victoria* that emits light when it encounters calcium ions. Very quickly this was exploited for measurement of calcium release into the cytosol.

A dream for neurobiologists, and especially for scientists studying the function of the brain, has been to activate or inactivate specific neurons and groups of neurons. With the development of optogenetics this dream has come true. Although there are other methods, we shall focus here on those which employ natural or modified photoactive proteins.

Another opsin that has been used in optogenetics is channelrhodopsin 2 (Boyden et al. 2005; Li et al. 2005). It can be expressed in mammalian neurons and when irradiated with blue light (450–490 nm) causes depolarization. It reacts rapidly enough to cause individual spikes, reaching their maxima within a couple of milliseconds after the onset of light, and they do this in a very reproducible manner.

Channelrhodopsin 2 can also be used in the reverse way. Berglund et al. (2013) fused it to a luciferase in such a way that the luciferase part remained outside the cell when the channelrhodopsin was incorporated into the cell membrane. When the luciferin (called coelenterazine) for this particular luciferase appears in the external medium, the luciferin emits light.

11.6.3 Examples of Modern Developments

A recent general overview is provided by Packer et al. (2013).

11.6.3.1 Light Switch for Pain

Optovin is a small molecule containing the chromophore rhodanine. It can be attached specifically, via cysteine, to an ion channel called TrpA1 which mediates the sensation of pain in vertebrates. It allows the remote control of animal behavior by violet light (Kokel et al. 2013).

11.6.3.2 Restoration of Vision

Promising animal experiments for restoring vision in blind individuals have been done by many groups, including Doroudchi et al. (2011). These, as several other researchers, used virus to introduce channelrhodopsin 2 into the defunct receptor cells. Channelrhodopsin 2 has the advantage of producing the required transport of sodium ions resulting in fast (within 50 ms) depolarization of the membrane in a more direct way than the natural system. The light effect is directly on the ion channel, while the natural system employs intermediate messengers. As long as only the natural channelrhodopsin 2 is used, vision is monochromatic with maximum sensitivity in the violet region. A recent thorough description of this field is provided by Natasha et al. (2013).

11.6.3.3 UV-B-Triggered Protein Secretion

Chen et al. 2013 modified the plant UV-B receptor UVR8 to create a system by which protein secretion can be controlled by UV-B and which can be used for studying protein trafficking in neurons.

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